A systems-level approach to the evolution of ageing

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Abstract

Ageing as a biological process is ubiquitous in life. In humans, ageing and its related conditions are revealed with improvements in health care conditions. Evidence for ageing is also apparent in most other organisms including unicellular species. Many of the pathways and mechanisms involved in ageing are evolutionarily conserved across the tree of life which provides an exceptional opportunity to study simpler organisms and extend the results to more complex forms of life. There is a rapidly growing body of data from organisms of varying levels of complexity, but there is a shortage of attempts in coherently making use of these data. A systems-level approach is necessary to bridge the gap between different biological levels, integrate the available information, and enable the synthesis of unifying hypotheses. Also, given the evolutionary nature of the question at hand (i.e. ageing), a successful hypothesis needs to be able to account for evolutionary considerations. In this thesis, I take a theoretical approach and try to explain a number of aspects of ageing from a systems-level perspective in an evolutionary context. Among the topics that will be covered are the following: (i) intra-islet pancreatic beta-cell dynamics, (ii) antioxidant defence system in pancreatic beta-cells, (iii) metabolic evolution of the glucose homeostatic system, and (iv) asymmetric damage segregation in unicellular organisms. In (i), I investigate the dynamics of beta-cell number within pancreatic islets and link the results to pathophysiology of diabetes and its various stages. In (ii) and (iii), I provide a unifying hypothesis for the paradoxical and unequivocal observation that metabolically active beta-cells have a weak antioxidant defence system and interestingly, that they are particularly weak in females. In (iv), I show how asymmetric segregation of damage at the time of mitosis is a fundamental step toward ageing and then evaluate whether and by how much asymmetry is optimal in a given organism under certain environmental conditions. I use a variety of techniques including deterministic and stochastic modelling in this thesis. The shared essence of these projects is an attempt to put data of various sources together in a unifying, systems-level evolutionary framework in order to better understand some aspects of the ageing process and its consequences.

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Chapter 1: Introduction

1.1 Ageing: mechanisms and theories

1.1.1 Basic biology and mechanisms of ageing

An accepted definition of ageing is the deterioration of tissues, cells, and cellular substructures over time that is manifested as an increase in intrinsic mortality and a decrease in reproductive rate (Rose 1991). In order to survive and reproduce, every organism needs to protect itself against sources of damage and repair any damage that occurs to its physical structure and physiological mechanisms. There are several sources of damage which can be broadly classified as extrinsic or intrinsic. Extrinsic sources originate from interaction with the environment and include factors such as heat, cold, radiation, infection, toxins and predation. Levels of extrinsic factors are known to correlate with longevity between and within species. Comparative studies of senescence in birds and mammals suggest that the rate of senescence has been modified by evolution in response to the potential life span allowed by extrinsic mortality factors (Ricklefs 2010). Within species differences in lifespan are apparent between populations that have been subject to different levels of protection, for example individuals of mainland populations are generally shorter lived than island populations subject to lower levels of predation (Kirkwood and Austad 2000). Intrinsic damage consists of any damage that occurs as a result of intra-organismal biological processes (Ricklefs 1998). A well-known and highly conserved process is free oxygen radical production as a result of oxidative phosphorylation and glucose metabolism (Harman 1956). If not properly neutralized, these radicals can cause significant cellular damage and are widely considered a principal mediator of ageing (Harman 1956, Paiva et al. 2005; Harman 2009). Thinking of biological entities as physicochemical systems with certain turnover rates, intrinsic damage also includes

the damage that occurs as a result of stochastic failure of one or more elements of a complex system over time (Gavrilov & Gavrilova 2004, 2006). Damage, regardless of its source, accumulates within the organism because with time the organism's protective mechanisms deteriorate and/or repair mechanisms lose their functionality (Hayflick 1998). In particular, age-related impairment of DNA base excision repair pathways has been shown to have a fundamental role in age-related neurodegenerative diseases such as Alzheimer's disease (Coppedè & Migliore 2010).

Protective mechanisms include simply avoiding the source of damage or attempting to remove the source. The immune system is the main organism-level protective system. At the intracellular level, the antioxidant system is a major protective investment that reduces the threat posed by reactive oxygen species (see below). Other such systems are toxin removal systems whose importance in ageing has been highlighted (Gems & McElwee 2005). Repair of damage that does occur operates at all biological levels: at the molecular level DNA damage is repaired by several mechanisms including base excision repair for the replacement of base lesions - the most common damage to DNA, nuclear excision repair, homologous and non-homologous end joining; damage to proteins is addressed with turnover involving proteases and the proteosome; damaged cellular components such as mitochondria are removed by processes such as autophagy and damage to cells by digestion and lysis by macrophages. The final resort, when all other less costly repair mechanisms fail, is death and silent disappearance of the cell/organism by well orchestrated processes such as apoptosis, autophagy or cellular senescence. Whether or not senescence and death are adaptive and a last resort repair mechanism, is still debated (Bergamini 2006; Kültz 2005).

Inevitably damage does occur and age-related increases in somatic mutation and DNA damage (Gensler & Bernstein 1981; Promislow 1994), telomere loss (Kim et al. 2002), mitochondrial DNA mutations resulting in impaired bioenergenesis (Wallace 1999), and impairment of the protein degradation machinery resulting in damaged protein accumulation (Terman & Brunk 2004; Carrard et al. 2002; Soti & Csermely 2003) are some of the mechanisms that may act individually or in concert to cause ageing (Kirkwood et al. 2003). Sources of damage such as oxidative stress may cause multiple types of damage including somatic mutation and DNA damage, and telomere loss, which is a good example of two or more mechanisms acting together (von Zglinicki 2002). Since many of the above mechanisms exist in unicellular and multicellular organisms, as well as eukaryotes and prokaryotes (Ackermann et al. 2003; Stewart et al. 2005), it is not surprising that ageing is universal in all kingdoms of life.

In addition to the oxididative stress theory of ageing other attempts have been made to provide a unifying framework to embody the molecular mechanisms of ageing. The network theory of ageing proposed by Kowald and Kirkwood in 1994, was an early systems approach linking multiple molecular mechanisms. It describes the reactions of free radicals, antioxidants and proteolytic enzymes, with the error propagation loops within the cellular translation machinery. This theory predicts that more investment in repair and maintenance is positively correlated with longevity. It supports the prediction of the oxidative theory of ageing that increased reactive oxygen species (ROS) production and/or inadequate antioxidant defence can destabilise an otherwise stable translation system. The model also supports the idea that caloric restriction delays ageing via a reduction of ROS generation (Kowald &

Kirkwood 1994). The theory is built upon the idea that multiple ageing mechanisms act in parallel (Kirkwood & Kowald 1997). Support for this theory comes from empirical results showing an increase in the fraction of inactive proteins with age, a significant rise in protein half-life with age, an increase in the amount of damaged mitochondria with age, and a drop in the energy generation per mitochondrion with age (Kowald & Kirkwood 1996). As a second example, a thermodynamics view of ageing states that ageing is the result of a decreased capacity of biological systems to degrade energy and maintain sufficient *specific entropy production*. According to this school of thought, senescence is a steady state characterised by two major features: a higher level of damage and a lower global biochemical activity (Toussaint et al. 1991; Toussaint et al. 1995; Toussaint et al. 2002). A number of predictions of this idea have been tested experimentally (Toussaint et al. 1992).

1.1.2 Evolutionary theories of ageing

There are several theories that focus on evolutionary origins of ageing (Kirkwood 2008). If ageing is controlled by genes, then it could evolve like any other genetically regulated phenotype. Several mutations are known to accelerate or prevent ageing. The first empirical support for the idea of ageing being seen and acted upon by natural selection came from single gene mutations that significantly increase life span in the nematode *Caenorhabditis elegans* (Friedman & Johnson 1988). More longevity genes are being discovered. Very recently, it was shown that an isoform of DAF16 (FOXO homologue), a major target of the insulin signalling pathway, regulates longevity (Kwon et al. 2010). There are theories that consider ageing as a programmed phenomenon and there are others that do not. The altruistic theory of ageing from the former category and the mutation accumulation theory, antagonistic pleiotropy, the

disposable soma theory, and the network theory of ageing from the latter category will be briefly discussed here.

Altruistic ageing: This theory suggests that organisms have a program that has evolved specifically to cause ageing and the reason for the evolution of such an apparently counterintuitive program by which the organisms gives up survival is because ageing and death can increase one's inclusive fitness (i.e. kin selection), group fitness, or population fitness. The support for this theory is mainly from recent experiments in yeast *Saccharomyces cerevisiae* in which ageing seems to be an adaptive process that benefits small subpopulations of closely related mutants through kin selection (Herker et al. 2004; Fabrizio et al. 2004). It should be noted that *Saccharomyces cerevisiae* live as clonal populations, satisfying the main requirement for kin selection to act (Hamilton 1964). Proposed examples of ageing-related benefits at the group or population level include population stabilisation, enhanced genetic diversity, shortening of the effective generation cycle, and acceleration of the pace of adaptation (Fabrizio et al. 2004). In particular, altruistic suicide is best known in the context of programmed cell death (PCD) in multicellular individuals, which is understood as an adaptive process that contributes to the development and functionality of the organism. My colleagues and I have recently argued that it is not straightforward to import the paradigm of altruistic cell death from multicellular organisms to explain active death in unicellular lineages (for a complete review see Nedelcu et al. 2010). Active death could be co-opted into an altruistic trait under conditions in which kin/group selection can act, but such conditions might not have been sufficiently common to promote the early evolution of ageing/suicide as an altruistic trait.

Mutation accumulation: This theory, proposed by Medawar (Medawar 1952), is based on the observation that the force of natural selection decreases in old age due to the enhanced value of offspring born in early life and also to the inevitable loss of individuals in late life. Consequently, mutations with detrimental late effects will be neutral to natural selection and will not be actively protected against, ageing ensuing as the inevitable consequence (Partridge & Gems 2002).

A number of attempts have been made to test the predictions of the mutation accumulation theory with mixed success. For example, the deleterious effects of inbreeding are expected to increase with age under mutation accumulation, but not under antagonistic pleiotropy (see below). Snoke and Prominslow (2003) carried out a large-scale quantitative genetic analysis of age-specific mortality and fertility in virgin male Drosophila melanogaster which was consistent with this prediction. The theory also predicts an age-related increase in additive genetic variance for fitness traits. Going against this prediction, genetic analysis of mortality in D. melanogaster showed that mortality curves of different genotypes are roughly parallel (Prominslow et al. 1996). Consistent with the prediction of the mutation accumulation theory, the genetic variability of mortality in male D. melanogaster increases greatly at very late ages (Hughes & Charlesworth 1994).

Antagonistic pleiotropy: this theory proposed by Williams (Williams 1957) and later formulated mathematically by Charlesworth (Charlesworth 1994), suggests that there are genes with beneficial effects (for reproduction or survival) early in life and with deleterious effects at old ages. Natural selection favours these genes because of their

early effects which contribute proportionally more to fitness. Ageing ensues as an inevitable consequence of the late effects. The trade-off between reproduction and longevity is a major prediction of this theory. While the mutation accumulation theory is based on mutations that occur randomly and are not passed on across generations (because they occur late in life), antagonistic pleiotropy assumes genes (with pleiotropic effects) that are under the force of natural selection (because of their early beneficial effects) and are actively maintained in the population (Le Bourg 2001). Williams suggested, as an example, that there may be a gene that increases the fixation of calcium in bones. Such a gene may have positive effects at a young age (decreasing the risk of bone fracture and subsequent death), but negative effects in later life, because of increased risk of osteoarthritis due to excessive calcification. This automatically creates a trade-off between early positive effects and late negative effects. In the wild, the negative effects of such a gene would be hidden from natural selection, because most animals would die long before the negative effects of the gene could be observed. In protected laboratory experiments, however, both positive and negative effects may be demonstrated. It was recently suggested that p53 represents a case of antagonistic pleiotropy (Ungewitter & Scrable 2009). This tumor suppressor confers protection against cancer (and death) by interrupting the abnormal proliferation of cells. The same effect on normal stem cells, however, can lead to impaired tissue homeostasis and accelerated ageing. If it could be shown that the deleterious effects of p53 on ageing are not attributable to genes that can modify p53 activity but are evolving independently, then we could consider p53 as an example for antagonistic pleiotropy. Finally, Charlesworth (1996) suggests that the involvement of both polymorphic alleles and rare mutations in pathogenesis of Alzheimer's disease is consistent with both mutation accumulation and antagonistic pleiotropy theories.

Disposable soma: This theory was proposed initially by Kirkwood and later developed with Holliday (Kirkwood 1977; Kirkwood & Holliday 1979) is built upon the concept of resource allocation optimisation. Resources are always limited in nature and thus only finite amounts can be allocated to physiological functions such as growth, reproduction and maintenance. The result is that trade-offs exist where for example more investment in reproduction and growth will retract from repair mechanisms and maintenance of soma. The function of the soma is to support the organism through its expected (not maximal) life span, which is not long in the wild given the extrinsic hazards that are the most common cause of death. Limited investment in maintenance and repair, augmented by a pressure to reproduce before the accumulated risk of death reaches critical levels, makes the organism prone to ageing if it lives long enough (Kirkwood 1977; Kirkwood & Austad 2000). The disposable soma theory provides a mechanism for the reproduction-longevity trade-off and provides a complementary theory based in physiology to the former two theories based in genetics. Several lines of evidence support the disposable soma theory, the most important of which is the observation that longer-lived mammalian species tend to have higher levels of cellular protection against stress. Kapahi et al (1999) compared cellular resistance of primary skin fibroblasts from eight mammalian species with a range of life spans. Cell survival was measured after exposure to a variety of oxidative and non-oxidative stresses and significant positive correlations were found between cellular resistance and longevity. An important part of cellular maintenance is prevention and repair of DNA damage. Poly(ADP-ribose) polymerases (PARPs) are involved in DNA base-excision repair and other repair

pathways. Mammalian longevity has been correlated in a number of studies with the abundance and activity of this maintenance system (Beneke & Bürkle 2007).

1.2 From molecules to individuals: different levels of complexity

Ageing can be studied at different levels. At the population level, one may study ageing by looking at actuarial life contingency tables and mortality curves (Gompertz 1825). The Gompertz law provides a relationship between mortality rates and the chronological age. It does not, however, deal with underlying mechanisms of ageing. Attempts have been made to explain the exponential growth of mortality that is suggested by Gompertz law by lower level events and processes. For example, it has been shown that a simple model of death as a result of an exponentially rare escape of abnormal cells from immunological removal translates into mortality curves similar in shape to those predicted by Gompertz law (Shklovskii 2005). Another curious example of such between-level links concerns the progressive accumulation of insoluble cross-linked proteins with age. These aggregates are known to impede various cellular functions and the slope of the Gompertz curve has been suggested to be a measure of the rate of insoluble protein accumulation (Hallén 2008).

One can also focus on sub-individual levels, i.e. organ/tissue ageing. This is important as the effect of ageing of different tissues on whole organism ageing is most probably not linear, even though it is possibly cumulative. For example, the rate of accumulation of DNA damage in liver and kidney cells with age in mice is inversely proportional to maximum achievable lifespan, suggesting a complex relationship between organismal and organ ageing (Su et al. 1984). In spite of some recent comparative attempts (Wang et al. 2009), it is not clear how the ageing “state” of the

organism can be represented by a function of the ageing states of its individual tissues/organs. The transcriptional profiles of ageing in Drosophila melanogaster across seven tissues was investigated recently and the age-related genes showed clear tissue-specific patterns (Zhan et al. 2007). AGEMAP, a gene expression database recently developed for ageing in mice, describes expression changes for more than 9,000 genes in 16 mouse tissues as a function of chronological age. According to this database, some tissues have a more significant contribution than others to organismal decline. Tissues were classified into one of three ageing processes: (1) a pattern common to neural tissues, (2) a pattern for vascular tissues, and (3) a pattern for steroid-responsive tissues (Zahn et al. 2007). Databases like AGEMAP will hopefully make it possible in future to describe the ageing state of the organism as a function, no matter how complex, of the ageing state of its tissues.

Most studies of mechanisms of ageing are focused at the level of the cell or below. With advanced molecular techniques, it is now feasible to dissect signalling pathways to their individual molecular components and study the role of each in the ageing process. One of the most important challenges in this approach concerns the involvement of one pathway, or part of that the pathway, in more than one cellular process. As a result, in order to see the role of a given molecule in ageing, one would need sufficient amount of knowledge about the effects of that molecule on other processes. The P53 pathway, for example, is known to be involved in several important cellular processes. It protects mammalian cells both from cancer and ageing. However, constitutionally active P53 can also induce ageing (Matheu et al. 2008). Such apparently contradictory observations may partly be explained by the overlap between different pathways and cellular processes. Clearly most if not all

molecules act in complex networks rather than pathways. The same issue applies when one wants to gain insight into evolutionary origins of ageing from a cellular/molecular perspective. Ageing has been proposed to be an inevitable and accidental by-product of selection acting on other processes (Kroemer 1997; Ameisen 2004). Alternatively, it can be a direct result of natural selection acting on the ageing process itself as an adaptive process (see evolutionary theories of ageing above).

Metabolic pathways are now recognized as regulators of longevity. This is consistent with the disposable soma theory of ageing, which considers resource allocation trade-offs resulting from limited resource availability as the root of ageing. Most of central metabolic pathways are somehow involved in nutrition sensing (insulin/insulin-like signalling pathway) and eventually affect cellular maintenance systems (Partridge & Gems 2002). Genes that regulate these pathways include those affecting insulin signalling (e.g. *daf-2* in *C. elegans*) and those coding proteins known as sirtuins (Longo & Kennedy 2006). Dietary restriction experiments, in which a restricted caloric intake is imposed on the animal, exert many of their effects through directional manipulation of metabolic pathways. When nutrients are scarce, *C. elegans* switches to an alternative developmental pathway and generates stress-resistant, long-lived forms known as dauer larva (Riddle et al. 1981). The fact that metabolic pathways are involved in the ageing process and longevity is without doubt. However, their importance under natural conditions and how longevity may be enhanced through harmless manipulations of such pathways in a given organism under certain environmental conditions is a matter of debate (Shanley & Kirkwood 2000). Taken together, as one moves from a higher level approach (e.g. population) to lower levels

(e.g. molecules), more insight is gained into detailed mechanisms at the cost of a sacrifice in evolutionary understanding of the ageing process.

As complex systems, biological systems have multiple levels. Molecules are at a lower level than cells because a cell can be broken apart into molecules but not vice versa. Three main approaches to dealing with different biological levels may be recognised: (i) top-down: starts from organisms and organs down to molecules, (ii) bottom-up: starts from molecules and attempts to integrate them in pathways, then in organelles, cells, tissues and so on to organs and organisms, (iii) middle-out approach: starts at any level for which there is sufficient understanding to identify a causal chain. When a satisfying interpretation of the available data has been achieved at the chosen level, the investigation would move out to other levels. The optimal approach, as claimed in this thesis, is one that bridges between approaches at different levels and brings them all together in a unified framework. This is a key aim of systems biology (Kirkwood 2008).

Also, multiple scales can be identified in biological systems. The functions exhibited by a complex system can be local or global, and the degree of locality may be quantified. For example, vision occurs at a larger scale than transmission of visual electric signals from the eye to the brain. Several processes of the latter type occur on a smaller scale to make vision (a larger scale phenomenon) possible. Vision is the product of communication between several neurons and their signalling. Ironically, the organism "sees" but its neurons do not. In their recent review of the cardiac Physiome project, Bassingthwaighte et al (2009) discuss three important reasons why multiscale analysis is an essential component of systems biology. Firstly, complex

systems (e.g. heart) are inevitably multiscalar, composed of elements of diverse nature, constructed spatially in a hierarchical fashion. Not only do the components determine the behaviour of the system as a whole, but also the system exerts certain constraints on the behaviour of its components. A middle-out approach seems appropriate in this situation where a single level of causation cannot be easily assumed. Secondly, in systems analysis, one needs to be able to discover at which level each function is integrated. For example, a cardiac rhythm does not make any sense at any level below the cell. On the other hand, ventricular fibrillation is a function integrated at the level of the tissue and even of the whole organ. And the pump function is an organ-level property. As the authors mention, higher-level emergent properties "do not 'emerge' blindly from the molecular events; they were originally guided by natural selection and have become hard-wired into the system". The third reason concerns the genetic differential effect problem. This concept refers to the fact that most gene-level events (e.g. mutations) do not have phenotypic effects. Biological systems are robust, meaning that genetic events are effectively buffered by back-up systems. Moreover, phenotypic effects of mutations "reveal simply the consequences of the difference at the genetic level; they do not reveal all the effects of that gene that are common to both the wild and mutated gene" (Bassingthwaighte et al 2009).

1.3 Theoretical approaches in ageing

Theory and experiment have been an inseparable pair since biology was born as a science. Even in the very past, when empirical approaches were confined mostly to pure observation without intervention, and mathematics was in its early ages, the combination of theory and experiment was not nonexistent. Ironically, the philosophy

by which observations are explained and further extended to yield more general statements can be thought as a parallel theoretical tool. With the advent of more sophisticated experimental and theoretical tools, the theory/experiment tool gained a more solid and fruitful shape. Let us briefly review some examples of this pathway to complexity.

Gompertz law, introduced above, was one of the earliest attempts to explain ageing in a generic theoretical framework (Gompertz 1825). Many more attempts have been made ever since to tackle this complex phenomenon with and without explicit connection to underlying mechanisms. Hamilton (1966, 1996) used a theoretical approach to quantify the force of selection and show that senescence is inevitable, i.e. survival and fertility decline with age. He derived expressions for the change in fitness with respect to age-specific mutations. This result has been widely used in the evolutionary theory of ageing ever since. An important assumption behind Hamilton's result is the additivity of the effects of mutations on mortality and feritiliy. Not known at the time, the results of several demographic and epidemiological analyses of risk factors have found that proportional effects are more common than additive effects. Empirical results of Promislow and Tatar (1998) are an important example of such studies. Alternative indicators may therefore be derived that can result, in some circumstances and over some age ranges, in an increasing force of selection with age. As mentioned, Hamilton's well-known conclusion depends on how we define the association between age and the indicators of the model. Hamilton's indicators are decreasing functions of age. Baudisch (2005) and Vaupel et al (2004) have used alternative parameterizations of mutational effects to derive plausible indicators that can increase with age.

Theoretical approaches addressing molecular mechanisms can be arbitrarily classified into two broad categories of bioinformatics and dynamical modelling.

Bioinformaticians use high throughput data (genomics, transcriptomics, proteomics, metabolomics, etc) to gain insight into the links between genes, proteins, and modules (Raghothama et al. 2005). Both causal (e.g. positive and negative feedback loops) and solely correlational relationships (co-expression of a number of genes) may be sought using this approach (de Magalhães & Toussaint 2004). Reverse engineering (defined as "the process of analyzing a subject system to identify the system's components and their interrelationships and create representations of the system in another form or at a higher level of abstraction" (Chikofsky & Cross 1990)) of ageing is in theory possible (D'haeseleer et al. 2000), but the number of experiments required to fully understand ageing is at present beyond our technology (Wagner 2001; Krupa 2002). In a complex system such as the ageing molecular network, in which every element depends on at most K other elements, the number of experiments required to find the causal network depends exponentially on K, emphasising the importance of the interactions between network elements (Krupa 2002).

Dynamical modeling can further be divided to deterministic and stochastic modeling techniques (Yashin et al. 2007; Portugal et al. 2008). Deterministic models are based on ordinary or partial differential equations that approximate the collective behaviour of a large number of units (molecules, cells, etc) in a deterministic way. If the structure of the model, the value of the parameters involved in the model, and the initial conditions are fully known, one can predict the behaviour of the model with complete accuracy. Population genetics models, for example, frequently use matrix

algebra and age-specific transitions embedded within a transition matrix to model the evolution of a population over time. Work in this area has benefited from the population genetics toolkit for studying ageing developed by Charlesworth (1980). In stochastic models, the extrinsic (e.g. environmental effects) and intrinsic (e.g. low number of molecules) noise are taken into account and introduce variability in the behaviour of the model. Typically, several simulations are run using similar parameter values and initial conditions and the average of the simulations is regarded as the model outcome. New methods such as cellular automata (agent-based simulation technique) have enabled us to study spatially structured systems. Using the latter technique, one can monitor the behaviour of individual elements over time. Cellular automata models may be deterministic or stochastic.

Modeling can be used in ageing sciences at different levels, from populations to molecules. In this thesis, we will see examples of deterministic and stochastic models at different levels.

1.4 Systems biology of ageing

As it is hard to come up with a single definition of systems biology, it is also difficult to summarize what systems biology of ageing is expected to yield. A practical definition, provided by Wikipedia (http://en.wikipedia.org/wiki/Systems_biology), suggests systems biology as a biology-based interdisciplinary study field that focuses on complex interactions in biological systems, using a new perspective (holism instead of reduction). Kirkwood (2008) proposes four categorically different definitions for systems biology:

(i) A field of study "involving the quantitative analysis of interactions between elements of biological systems"

(ii) A set of multidisciplinary methodologies focusing on "cycles of iteration between empiricism and theoretical modelling.

(iii) An integrative approach as an alternative to the traditional reductionist approach.

(iv) An organizational phenomenon that brings together scientists from diverse disciplinary backgrounds.

In a complex system with several layers of interactions between components, the behaviour of the system as a whole may not always be a linear sum of the behaviour of the components. "Emergent properties" are systems-level, complex behaviours that emerge from relatively simple interactions between lower level components. One of the targets of systems biology is to investigate how such emergent properties relate to the features of lower level units and their interactions. Molecular systems biology, for example, focuses on networks that originate from interconnection of genes, proteins and metabolites whose dynamic interactions generate, as an emergent property of the system, the corresponding function. A molecular systems biologist is interested in intracellular biochemical parameters, such as the expression level of gene products or the affinity between two or more proteins that must have a permissible range that gives robustness against perturbations to the system. Identification of such parameters in different organisms and systems is an interesting field of systems biology research. One recent example concerns the yeast G1-to-S transition network (Alberghina et al. 2009). In this particular example, different emergent properties have been shown to be generated from the same network by changing the strength of its interactions, not only

by altering expression level, but also through mono and multi-site phosphorylation/dephosphorylation. Noise and stochasticity make predictions along the pathway from lower to higher level behaviour less trivial and more challenging, and we know noise and stochasticity are inherent in all biological systems.

Subcellular components age and lose their functionalities; cells age as a result, followed by tissues and organs. The individual, our traditional evolutionary unit, will be affected by all these ageing processes and eventually dies. Ultimately, groups, populations, and the species will be affected. How much do we know about mechanistic details of ageing at the lower levels? The answer is we know a fair amount. However, known details are extremely scattered and using commonly used tools cannot conveniently be merged into a unifying theory. At least two important factors play a role here. The first factor is the numerical complexity of the involved mechanisms. Thinking of an organism as an extremely complex machine composed of many small parts, each prone to various sources of insult and failure, there will be an enormous number of ways the whole system may deteriorate with time as a result of injury to its parts. What makes the situation even more complicated is the cumulative nature of damage. Apart from damages that are incompatible with life (e.g. severe karyotypic changes that kill the organism early in life), most types of damage (e.g. oxidative damage) accumulate with time. Not a single *unit* of damage can be identified as the cause of system failure in most instances. Therefore, we are dealing with an additional dimension: time. Do we know how subcellular ageing is related to population level phenomena? We are currently far from it. The second factor is stochasticity. In the case that the mechanisms follow deterministic rules, one could, at least theoretically, analyse all possibilities and potentially come up with a

comprehensive explanation, no matter how complicated it would be. However, there is intrinsic stochasticity in nearly all ageing mechanisms. This stochasticity creates variability from molecule to molecule, cell to cell, and individual to individual (Finch & Kirkwood 2000). The combination of multiplicity and stochasticity makes any non-integrative approach deficient in some respect.

Evolution is now regarded an inseparable part of biology. When it comes to the individual-level and higher, evolutionary arguments become increasingly more powerful. Thinking of reproductive success as the ultimate goal of natural selection, all cellular and subcellular processes may be thought of as an outcome of selection to enhance reproductive success, unless certain things go beyond the ability of natural selection. Ageing is no exception. Do repair and maintenance processes stop functioning at their maximal capacity, not because they wear and tear, but because the reproductive success of the evolutionary unit demands so (Mitteldorf 2010)? Whether optimal resource allocation (as proposed by the disposable some theory of ageing) is the root of ageing is an important question. The major issue, however, applies to a larger scale and supersedes detailed mechanisms. The concept of optimal resource allocation may not be required to explain the evolutionary advantageous deceleration in maintenance/repair system functions. Ideas like this are what I will work on in most parts of this thesis. The role of systems biology in the question at hand is manifest given that we will be dealing with biochemical processes at the subcellular level, cell-cell interactions, whole body homeostasis, and reproductive fitness of the organism.

Let us review a few examples of recent advancements in systems biology of ageing and age-related diseases. Boudovsky et al (2007) made the first attempt towards

constructing a longevity network based on protein-protein interaction data (329 longevity-associated proteins). The network was found to be scale-free with an extremely high contribution of hubs (proteins with the highest number of partners) to the network connectivity. Most hubs tuned out to be related to at least one major age-related disease (e.g. atherosclerosis, cancer, Alzheimer's disease). Human age-related disease proteins and longevity-associated proteins together with their direct binding partners form scale-free networks. Signalling proteins are abundant in these networks. Interestingly, pathways associated with cell-cell and cell-extracellular matrix interactions and focal adhesion are overrepresented (Wolfson et al. 2009). An extracellular age-related network comprising both extracellular proteins and glycosaminoglycans was built recently. Among overrepresented biological processes in this network, ''response to wounding'' and ''tissue regeneration'' were related to extracellular events, which are impaired upon ageing (Chautard et al. 2010).

Recent systems biology studies have identified an intricate relationship between the ageing process and genetic diseases. A human disease-ageing network was constructed to study the relationship among ageing genes and genetic disease genes (Wang et al. 2009). A total of 1,438 genes were included. Protein-protein interactions, disease-gene associations, ageing-gene associations, and physiological genetic disease classification information were integrated into a single comprehensive network. It was found that disease genes are much closer to ageing genes than expected by chance. The authors suggested a novel classification of diseases according to their relationships with ageing. Age-related diseases (i.e. those with statistically significant overlapping with ageing: cancer, neurological diseases, endocrine diseases, nutritional disease, and developmental disease) turned out to be in a central position of the

protein-protein interaction network whereas non-age related disease genes had more peripheral positions. Centrality was quantified by a network property called mean closeness centrality. An important result of this study was that ageing genes make a significant contribution to associations among diseases, especially among type I diseases. The authors used this result to address the well-known question of why natural selection has not been able to remove all disease genes over the course of millions of years of evolution. Since the force of natural selection declines with age (Kirkwood 2002), by remaining close to ageing genes, disease genes can avoid negative selection. Interestingly, cancer genes did not make a significant contribution to associations among most of diseases by the closeness analysis in protein-protein interaction network.

An interesting example of how systems biology may cause a substantial improvement in our understanding of the ageing process can be demonstrated by theories of menopause (Rashidi & Shanley 2009). A long postreproductive lifespan is a characteristic feature in the life history of human females. The mother and grandmother hypotheses, the most prominent evolutionary theories of menopause, propose that the altriciality of human infants, early age of weaning, high maternal mortality, the supportive role of grandmothers in childcare, and intergroup female transfers made an early cessation of reproduction advantageous in human females. On the other hand, ovarian exhaustion (the progressive loss of oocytes beginning before birth) has been long recognised as the proximate cause of menopause, without much attention to fine details. Unfortunately, our proximate knowledge about menopause is severely detached from our evolutionary explanations. Assuming that menopause is the outcome of an evolutionary process, and is mechanistically caused by ovarian

exhaustion, one would like to see how the two processes are linked through evolutionary time. Most efforts have to date been focused on fitting curves to the few data available, rather than trying to explain why the dynamics of oocyte depletion follows a particular pattern. A systems biology approach should ideally link together the ovulation physiology, ovarian dynamics, and life history. In a recent review, we mention the outstanding issues and how they may be overcome using newer tools (Rashidi & Shanley 2009).

In brief, ageing is a process that takes place at the systems level, and is closely connected to other systems-level processes such as cell death pathways and diseases. The phenomenology of the ageing process follows a segmental and mosaic distribution, as all tissues and organs do age at different rate. Therefore, understanding what happens to the organism as suborganismal processes go on during the ageing process requires knowledge about the structure and dynamics of the complex interaction network that binds all the involved components together. The process of ageing cannot be satisfactorily understood without taking into consideration other important phenomena such as age-related diseases, genetic diseases, and cell death pathways.

1.5 Aims of thesis: overview of chapters

Unicellular organisms age, as do we humans. There is a high level of evolutionary conservation along the tree of life in the events leading to ageing. This provides an exceptional opportunity to study simpler systems and extend the results to more complex forms of life. There is a large amount of data available on ageing in organisms and suborganismal entities of varying levels of complexity. However, there

is a shortage of attempts in coherently explaining the rapidly growing data that emerges from various empirical fields such as cellular and molecular biology. A systems-level approach is required to bridge the gap between different levels. Also, given the evolutionary nature of the question at hand, a successful hypothesis needs to be able to account for evolutionary considerations. In the following projects, I take a theoretical approach and try to explain a number of aspects of ageing from a systems-level perspective in an evolutionary context. Two common themes can be identified in each chapter: (i) a systems-level approach consisting of data mining, assembling data, and hypothesis generation, and (ii) evolutionary thinking. Case studies will range from fundamentals of ageing (e.g. asymmetric damage segregation) to consequences of ageing (e.g. type 2 diabetes).

1.5.1 Pancreatic beta-cell mass regulation

Beta-cells are at the core of pancreas endocrine physiology. By secreting insulin in response to rises in plasma glucose concentrations, they effectively maintain short-term glucose homeostasis. Although short-term homeostasis is achieved by higher average insulin secretion rates per beta-cell without significant changes in the beta-cell mass (number), regulation of the mass is crucial in the long-term (Bonner-Weir 2000; Kasuga 2006; Bernard et al. 1999). Disturbances in this process seem to be responsible for chronic disease states such as glucose intolerance and diabetes (Laybutt et al. 2003; Bouwens & Rooman 2005; Rhodes 2005). Mechanisms of long-term beta-cell mass regulation have not been identified completely, hindered in part by the difficulty of in-vivo measurement but also by a lack of sufficient mathematical modelling studies. The available models (Finegood et al. 1995; Topp et al. 2000) have made significant contributions but recent experimental observations together with an

increase in our mechanistic understanding render them in need of revision. Considering that type 2 diabetes is an age-dependent disease, I develop a deterministic, analytical model of beta-cell mass regulation which takes into account recent discoveries in pancreas endocrine physiology.

1.5.2 Metabolic evolution and beta-cell antioxidant defences

Pancreatic beta-cells are unique among other cells in possessing an extremely weak antioxidant defense system (Grankvist et al. 1981; Lenzen et al. 1996; Malaisse et al. 1982; Tiedge et al. 1997; Zhang et al. 1995). Furthermore, beta-cell defenses against oxidative stress in both mice and humans are weaker in females than in males (Cornelius et al. 1993; Tonooka et al. 2007). These observations need an evolutionary explanation given that oxidative stress in beta-cells has an important contribution to the pathogenesis of type 2 diabetes (Tanaka et al. 2002; Chang-Chen et al. 2008). Using a systems-level approach to the glucose homeostatic system and beta-cell physiology, I provide a satisfactory evolutionary explanation for the puzzle. The hypothesis involves interesting connections between physiology and ecology, pregnancy, brain evolution, mammalian evolution and stress response evolution. Both micro- and macroevolutionary aspects of the glucose homeostatic system are covered by the hypothesis.

1.5.3 Evolution of asymmetric damage segregation

Ageing is ubiquitous in all kingdoms of life, hence the origins of ageing may well be sought in unicellular organisms. Asymmetric segregation of irreversibly damaged cytoplasmic macromolecules has been shown to be a major contributor to unicellular ageing (Ackermann et al. 2003). This is because with symmetric distribution of

damage at mitosis, the population either vanishes (if the rate of damage accumulation is sufficiently high) or never senesces (if the rate of damage accumulation is sufficiently low). With asymmetry, we can distinguish between an aged daughter cell (the one which inherited more damage) and a young one (the one which inherited less damage) (Ackermann et al. 2007). Irreversible protein aggregates cannot be handled by the cell and are transferred across generations if their carriers survive to the time of mitosis. The segregation strategy, however, seems to fit a continuum, ranging from near complete symmetry (Minois et al. 2006) to near complete asymmetry (Aguilaniu et al. 2003), rather than an all-or-none pattern. Furthermore, some single-celled organisms can change their segregation strategy in response to environmental changes (Aguilaniu et al. 2003). Our knowledge about the proximate mechanisms of asymmetric segregation has substantially improved over the last few years, but the ultimate evolutionary cause that determines the way damage segregates is not known. Using deterministic and stochastic models, I fill this knowledge gap. The models include concepts such as resource investment tradeoffs, evolutionary optimisation, and costs of asymmetry, and implications of the model extend to the transition to multicellularity.

Chapter 2: An evolutionary model of intra-islet beta-cell dynamics

2.1 Introduction

Beta-cell physiology and its central role in glucose homeostasis have been studied at the cellular level for several decades. Molecular pathways that enable beta-cells to sense blood glucose levels, synthesise insulin, and secrete it have been identified. A large network of genes and proteins are involved in these pathways and substantial convergence, divergence, synchrony and several other interesting network-related phenomena can be recognised in glucose-insulin networks. These properties make the system a suitable target for bioinformatic analysis and cell-level systems biology. A recently begun initiative in this context is an attempt by the Competence Center for Systems Physiology and Metabolic Diseases (http://www.ccspmd.ethz.ch/about/scientific_programs/beta_cell_consortium) to define the surface glycoproteome of beta-cells to elucidate sensing, integration and transduction mechanisms that are key to beta-cell biology and the development of predictive preclinical models. There are several beta cell-level approaches to abnormalities in glucose homeostasis, and particularly diabetes (Nolan & Prentki 2008):

1. Abnormalities in upstream components of beta-cell glucose sensing/signalling (from beta-cell membrane to nucleus): G protein-coupled receptor (GPR)-39 is a transmembrane receptor that acts as a Zn(++) sensor. Within the endocrine pancreas, GPR-39 is mainly located on beta-cells. In a recent experiment, GPR-39(-/-) mice had decreased plasma insulin response to oral glucose, expression of Pdx-1 was reduced and their purified isolated islets secreted less insulin in response to glucose stimulation than islets from wild-type mice. GPR-39 is a potential target for treatment of diabetes (Holst et al. 2009). Beta-cells have high capacity for nutrient sensing. The drawback is their reduced protection to nutrient toxicity. This potentially explains

why in susceptible individuals, chronic fuel oversupply (known as glucolipotoxicity) results in beta-cell failure and type 2 diabetes. Both fatty acids and amino acids interact with glucose signalling in beta-cells. While glucose entry into most other types of cells in the body is reduced (insulin resistance) under fuel overload conditions (a beneficial adaptive process to prevent tissue dysfunction from fuel overload) (Vannucci et al. 1997; Kraegen et al. 2001; Krebs & Roden 2004; Tremblay et al. 2007), beta-cells have to prevent hyperglycaemia and hyperlipidaemia and to protect all tissues in the body from fuel surfeit toxicity and potential death. Therefore, they do not protect themselves by blocking uptake of excess nutrients. Rather, they are there to secrete as much insulin as required. Interestingly, the recent search for type 2 diabetes genetic factors has identified more polymorphisms in genes involved in beta-cell growth and function than in genes related to insulin action (Frayling 2007; Sladek et al. 2007).

The glucose sensing/signalling pathway is evolutionary conserved. For example, AMP-activated protein kinase (AMPK) is a widely conserved Ser/Thr-specific protein kinase, homologous to Saccharomyces cerevisiae Snf1, and involved in nutrient sensing in lower organisms. This enzyme is also involved in glucose homeostasis in mammals (Rutter et al. 2003). Given this conservation, significant insight into both beta-cell physiology and pathology may be achieved by looking at less complex organisms.

2. Abnormalities in downstream components of beta-cell glucose sensing/signalling: This involves impairments in insulin synthesis/secretion in response to the received information. Enhanced function (insulin synthesis and

secretion) is part of the healthy compensatory response of beta-cells to nutrient overload. Pancreatic beta-cell failure due to excess nutrients is more likely to occur if there is an underlying genetic (Sladek et al. 2007) susceptibility defect. This is evident by comparing the diabetes resistant ZF with the diabetes prone Zucker diabetic fatty (ZDF) rat (Prentki & Nolan 2006). Both animals are similarly hyperphagic and insulin resistant, but the ZDF has a minor defect in its insulin promoter. It is only the ZDF rats that develop severe diabetes (Griffen et al. 2001).

3. Abnormalities in beta-cell survival/proliferation: Survival of beta-cells in situations of chronic fuel oversupply and insulin resistance undoubtedly depends on a healthy compensatory response involving expansion of beta-cell mass, thus decreasing the work of each beta-cell (Prentki & Nolan 2006). We will discuss this in more detail below.

Similarly, beta-cell level diagnostic and therapeutic approaches to diabetes focus on one or more of the mentioned mechanisms. Let us briefly review some of the available strategies:

1. Upstream and downstream components of beta-cell glucose sensing/signalling (from beta-cell membrane to nucleus): Insulin secretagogues (e.g. sulfonylurea drugs such as glyburide, glimepiride) are a major group of glucose lowering agents known for decades. They stimulate insulin secretion by beta-cells through various mechanisms. A very recently identified potential insulin secretagogue is GPR40. GPR40 is a G protein-coupled receptor regulating free fatty acid-induced insulin secretion. Studies in transgenic mice overexpressing the hGPR40 gene under control of the mouse insulin II promoter showed that these animals had higher levels of

glucose-induced insulin secretion suggesting that pharmacological activation of GPR40 may be beneficial for the treatment of type 2 diabetes (Nagasumi et al. 2009).

2. Beta-cell survival/proliferation: Treatment with various growth factors such as glucagon-like peptide-1 (GLP-1), betacellulin (BTC), hepatocyte growth factor (HGF), and epidermal growth factor (EGF) and forced expression of beta-cell transcription factors such as Pdx-1, NeuroD, and MafA resulted in the regeneration of beta-cells in vivo. These strategies, however, are not at the time of writing ready for clinical application. The cWnt signalling pathway is known to be involved in beta-cell growth and function. Recent studies have shown that R-spondin-1 (Rspo1; an intestinal growth factor) exerts its effects through activation of the cWnt pathway, and may thus be used in future for potential therapeutic purposes (Wong et al. 2010).

These approaches, however, do not encompass the whole spectrum of diagnostic and therapeutic methods that are currently available. For example, new biomarkers that serve as signatures of the diabetic state and to predict disease are an active field of research. They can be targets for small molecule modulators and biologicals. A combination of metabolomics and transcriptomics may link known metabolites and genes to relevant biochemical pathways. In a recent study (Connor et al. 2010), urinary NMR-based metabolomic and liver, adipose, and muscle transcriptomic results were used to discriminate between the diabetic db/db and control db/+ mice. A total of 24 distinct pathways were identified that were altered in the diabetic model. Several of these pathways were involved in lipid metabolism, gluconeogenesis, mitochondrial dysfunction and oxidative stress, as well as protein and amino acid metabolism.

Another approach, which is related to the one we take here, is looking at the total beta-cell number/mass in the pancreas both before and during the course of diabetes. For at least two reasons, a cell-level approach is not able to grasp the mass parameters. First, pancreatic islets are heterogeneous cell populations, meaning that beta-cells may differ in functional details. In order to see what happens at the level of the islet (or more generally, pancreas), one has to consider the effect of intercellular heterogeneity on common physiopathologic properties of cells. The second reason concerns the mechanisms that regulate beta-cell mass dynamics. Intracellular events are not the sole regulating mechanism. Adjacent beta-cells interact with each other and these interactions modify two crucial determinants of beta-cell mass dynamics, that is, beta-cell proliferation and survival. Taken together, the cell-level network cannot provide a comprehensive and sufficiently predictive understanding of diabetes pathophysiology. It needs to be expanded, and the next level to be investigated is the islet level.

A model of postnatal pancreatic β-cell growth in humans is now emerging from studies in both humans and rodents. Although some events at some stages are difficult to confirm in humans, it is thought to happen as follows. Cell differentiation gives rise to the initial β cells of an organism during embryogenesis, and β-cell proliferation proceeds at a high rate during late embryogenesis (Bernard-Kargar & Ktorza, 2001), but begins to decline postnatally, while β-cell apoptosis occurs at very low rates (Scaglia et al. 1997). There is a transient burst of β-cell replication just after birth, followed by a transitory rise in β-cell neogenesis (Bonner-Weir 2000). In the later phase of this burst, there is a modest increase in apoptosis associated with substantial

pancreatic remodelling (Scaglia et al. 1997). The early burst in β-cell growth and minor apoptosis result in a marked increase in β-cell mass during the early years of life. Throughout childhood and adolescence, the rates of β-cell replication, neogenesis and apoptosis drop markedly. In adults, β-cell proliferation and apoptosis rates are very low so as to compensate for each other (Bonner-Weir 2000; Lingohr et al. 2002) and, as β-cell size stays relatively constant, an optimal β-cell mass can be maintained. It was suggested in mice that, postnatally, proliferation is the main process contributing to β-cell renewal rather than neogenesis (Dor et al. 2004). During later adulthood, β-cell mass may decrease as apoptosis slightly outweighs β-cell growth (Rhodes 2005).

As mentioned above, until recently, little attention has been paid to beta-cell mass regulation at the tissue level, which has somewhat hindered our progress to a full understanding of the complex pathophysiology of type 2 diabetes (Bonner-Weir 2001). Type 2 diabetes is the result of an eventual failure of beta-cells to adapt their mass appropriately (in response to the increased insulin demand due to worsening insulin resistance), following a chronic successful compensatory phase (Weir & Bonner-Weir 2004). Five stages can be identified in the progression of diabetes, each of which is characterized by different changes in beta-cell mass, phenotype, and function. In stage 1 (compensation), insulin secretion increases to maintain normoglycemia in the face of insulin resistance and/or decreasing beta-cell mass. Differentiated function is maintained with intact acute glucose-stimulated insulin secretion (GSIS). Stage 2 is a stable state of beta-cell adaptation with loss of beta-cell mass and disruption of function (diminished GSIS and beta-cell dedifferentiation). In this stage, glucose levels start to rise. Stage 3 is early decompensation. In this

transient unstable period glucose levels rise relatively rapidly. Stage 4 (full-blown diabetes) follows stage 3, and is characterized as stable decompensation with more severe beta-cell dedifferentiation. Finally, we have stage 5 (severe decompensation) during which profound reduction takes place in beta-cell mass. With treatment, type 2 diabetic patients may move from later to earlier stages. Stage 5 is the only irrevresible stage during the course of diabetes (Weir & Bonner-Weir 2004). A study of non-diabetic and diabetic patients matched for obesity, revealed that the relative β-cell volume and, therefore, the presumptive β-cell mass is decreased in both obese and lean humans with type 2 diabetes compared with their non-diabetic age- and weight-matched counterparts (Butler et al. 2003). Interestingly, humans with impaired fasting glucose already have a decreased relative β-cell volume, suggesting that this is an early process that is probably a key factor in the development of type 2 diabetes (Butler et al. 2003).

MRI technology has been used to image in vivo beta-cell mass in order to track disease progression (Medarova & Moore 2009). Endeavors to find a biological structure specific for beta-cells led to the discovery of potential candidates that have been tested for noninvasive imaging. Among them is the monoclonal antibody IC2. The anti-IC2 monoclonal antibody has binding properties exclusively to insulin-producing beta-cells. IC2 can thus be used as a useful marker for noninvasive functional imaging of native beta-cells. Experiments with a radioisotope-chelated IC2 structure on pancreas showed that the tracer specifically bound to the beta-cell surface and could be detected by nuclear imaging (Saudek et al. 2008). Another molecule, which is expressed almost exclusively by beta-cells, is the vesicular monoamine transporter type 2 (VMAT2). VMAT2 can be noninvasively imaged with positron

emission tomography (PET). Although promising, this technique is still in its early stages and cannot be used for clinical evaluation of beta-cell mass during the course of diabetes (Ichise & Harris 2010).

Beta-cells of fully diabetic patients show a low proliferation rate, a low insulin secretion rate, and a high apoptosis rate. The mechanisms of the events eventually leading to type 2 diabetes are poorly understood. During the course of type 2 diabetes, there is a gradual rise in beta-cell apoptosis rate due to chronic hyperglycaemia (Maedler et al. 2001). Specifically, chronic hyperglycaemia triggers insulin receptor substrate-2 (IRS-2) proteosomal degradation, leading to reduced beta-cell survival (Lingohr et al. 2006).

Proliferation, apoptosis, and function are strongly interrelated phenomena in all cells and for understanding the complexity of the relations involved, mathematical modelling is of great potential use. Here, I develop a mechanistic mathematical model for the process of beta-cell mass regulation by considering the islet, rather than individual beta-cells, as the population unit. The model is based on a trade-off between proliferation and function (insulin secretion), which gives the model an evolutionary shape. The number of beta-cells in the islet and beta-cell mass are used interchangeably herein.

2.2 Previous models

The model presented here is the first one on intra-islet beta-cell dynamics. Finegood et al (1995) and Topp et al (2000) have related models on beta-cell dynamics in the whole pancreas. Finegood et al (1995) used the idea that any biological mass

dynamics, whether in normal or pathological conditions, can be modelled as a birth/death process. They model the balance of cell proliferation, cell growth, and cell death. For proper parametrization, they first analysed the data available on determinants of beta-cell mass dynamics in normal Sprague-Dawley rats. A birth/death process was then built based on the data. The model gave authors some insight into the contribution of processes such as neogenesis and cell death, which could not be measured in the lab at the time. In a later model, Topp et al (2000) developed a more sophisticated mathematical model that included insulin and glucose dynamics along with beta-cell mass dynamics. The model was composed of three ordinary differential equations, each representing one of the three dynamical processes mentioned. Glucose and insulin dynamics were fast relative to beta-cell mass dynamics. A stability analysis of the model revealed two stable fixed points (representing physiological and pathological steady states) for normal parameter values. Mild hyperglycaemia pushed the system to the physiological steady state with a high beta-cell mass. More severe glycaemia moved the system toward the pathological steady state with reduced beta-cell mass. The authors observed the emergence of a bifurcation point (with increasing glycaemia) at which progressive defects in glucose and/or insulin dynamics aggrevated the glycaemia faster than the adaptation of the beta -cell mass (which could otherwise correct the glycaemia). As mentioned, neither of these models considered the effects within the islet, e.g. the effects of intercellular contacts between beta-cells on their proliferation, function, and durvival. The present model is the first attempt to fill this gap.

2.3 The model

A system of two coupled ordinary differential equations is developed and explored using the concepts of dynamical systems. Notations and definitions are provided in Table 2.1. Note that since the volume of the islet is assumed to be constant, insulin concentration within the islet is the number of insulin moles with the islet.

Table 2.1. Notations and definitions used in the model

I : insulin concentration within the islet (mole; constant islet volume)
s : insulin secretion rate per beta-cell (unit: mole × t^{-1})
β : beta-cell mass (number) in the islet (dimensionless)
D : rate of intra-islet insulin loss (unit: t^{-1})
r_{max} : beta-cell proliferation rate in the absence of any intercellular contacts (unit: t^{-1})
μ_{min} : beta-cell death rate in the absence of any intercellular contacts (unit: t^{-1})
c : magnitude of the effect of intercellular contacts on beta-cell proliferation (unit: t^{-1})
c' : magnitude of the pro-survival effect of insulin (unit: mol × t^{-1})
c'' : magnitude of the effect of intercellular contacts on beta-cell death rate (unit: t^{-1})

Insulin dynamics (Eq. 1): Insulin synthesis occurs by beta-cells. The insulin mRNA is translated as a single chain precursor called preproinsulin, and removal of its signal peptide during insertion into the endoplasmic reticulum generates proinsulin. Proinsulin consists of three domains: an amino-terminal B chain, a carboxy-terminal A chain and a connecting peptide in the middle known as the C peptide. Within the endoplasmic reticulum, proinsulin is exposed to several specific endopeptidases which excise the C peptide, thereby generating the mature form of insulin. Insulin and

free C peptide are packaged in the Golgi into secretory granules which accumulate in the cytoplasm. When the beta cell is appropriately stimulated, insulin is secreted from the cell by exocytosis and diffuses into islet capillary blood. A first-order kinetic process (including a constant synthesis rate and beta-cell mass) is used to model insulin synthesis. There are two mechanisms for insulin loss: biochemical degradation and diffusion out of the islet. Once secreted from beta-cells, insulin diffuses through the interstitial space and finds its way to the capillaries (Bendayan 1993). Given the concentration dependence of typical diffusion processes, a simple first-order kinetic process is also used to model insulin loss.

$$\frac{dI}{dt} = s\beta - DI \qquad \textbf{Eq. 1}$$

Beta-cell mass dynamics (Equations 2-5): An islet is a heterogeneous population of individuals (Pipeleers 1992). For simplicity, however, I assume intra-islet homogeneity. Beta-cell dynamics depends on apoptosis, necrosis, proliferation, and neogenesis. These are the only sources of input or output from the total beta-cell pool. Apoptosis, or programmed cell death, is a genetically regulated and active mechanism by which a cell commits suicide. Necrosis, on the other hand, is unprogrammed and occurs due to severe insults. While necrosis leads to uncontrolled release of intracellular content into the surrounding microenvironment, thus triggering the immune response, apoptosis does not stimulate the immune system. This is owing to regulated packaging of the cellular contents in what we know as apoptotic bodies. Apoptotic bodies are inert and will be safely absorbed by neighboring cells when released from the dying cell.

Neogenesis refers to proliferation and then differentiation of non-beta-cells into beta-cells. New beta-cells could potentially originate from (i) ductal cells, (ii) already differentiated pancreatic cell (i.e., exocrine, acinar or ductal) or extra-pancreatic cells, and (iii) an islet precursor cell. Neogenesis from duct epithelium is the most currently described and the best documented process by which progenitor cells can differentiate into endocrine cells. During fetal life, and in the neonatal period, intense beta-cell differentiation is the major contributor to beta-cell mass expansion. Two mechanisms are proposed. The first one involves the emergence of cells budding from the duct epithelium, and expressing islet hormones, especially insulin. According to a second mechanism, that has been described only during the fetal life, the source of beta-cells comes from a pool of proliferating cells expressing cytokeratin (CK) and located near the ductal tree (Paris 2004).

Necrosis was ignored in the model due to scarcity of data as was neogenesis due to my particular interest in postnatal/adult life when proliferation is the main source for new beta-cells (Dor et al. 2004). A simple birth-death process was used to model beta-cell mass dynamics within the islet; beta-cells proliferate and die at certain rates (r and μ, respectively).

$$\frac{d\beta}{dt} = r\beta - \mu\beta = \beta(r - \mu) \qquad \textbf{Eq. 2}$$

Intercellular contacts between beta-cells are known to inhibit beta-cell proliferation and decrease their survival. E-cadherin is known to mediate homotypic cell adhesion between beta-cells and has also been implicated in a number of cellular processes, including proliferation, apoptosis, and differentiation. In an interesting study on the growth of insulin-secreting MIN6 cells configured as three-dimensional islet-like

clusters (pseudoislets), pseudoislet formation was associated with an increased expression of cyclin-dependent kinase inhibitors and a concomitant downregulation of Ki67, suggesting an overall reduction in cellular proliferation. Apoptosis was upregulated in islet-like structures (Luther et al. 2005). Insulin, on the other hand, is a growth factor that promotes beta-cell survival (Aikin et al. 2006; Navarro-Tableros et al. 2004). This is achieved by inhibiting apoptosis via the autocrine activation of the Akt signalling pathway (Aikin et al. 2006; Johnson et al. 2006). We can formulate these observations into the following equations:

$$r = r_{\max} - c\beta \qquad \textbf{Eq. 3}$$

$$\mu = \mu_{\min} + \frac{c'\beta}{I} + c''\beta \qquad \textbf{Eq. 4}$$

where $r_{\max}$ is beta-cell proliferation rate in the absence of any intercellular contacts, and c models the magnitude of the effect of intercellular contacts on beta-cell proliferation rate. $\mu_{\min}$ is beta-cell death rate in the absence of any intercellular contacts, c' models the pro-survival effect of insulin (insulin availability per beta-cell), and c'' shows the magnitude of the effect of intercellular contacts on beta-cell death rate.

After substitutions, equations 2-5 can be summarized as

$$\frac{d\beta}{dt} = \beta[r_{\max} - c\beta - (\mu_{\min} + \frac{c'\beta}{I} + c''\beta)] = \beta(r_{\max} - \mu_{\min}) - \beta^2(c + c'') - \frac{c'\beta^2}{I} \qquad \textbf{Eq. 5}$$

2.4 Results

Equations 1 and 5 describe a two-dimensional nonlinear dynamical system. One can find the fixed points of the system by making the two equations zero and solving for β and I. Doing this yields

$$\begin{cases} \beta^* = \dfrac{r_{\max} - \mu_{\min} - \dfrac{c'D}{s}}{c + c''} \\ I^* = \dfrac{sr_{\max} - s\mu_{\min} - c'D}{D(c + c'')} \end{cases} \qquad \textbf{Eq. 6}$$

The local Jacobean of the system can be found at the fixed point. The eigenvalues of the Jacobian determine local stability of the fixed point. The Jacobian at the fixed point is

$$J(I^*, \beta^*) = \begin{bmatrix} -D & s \\ c'(\dfrac{\beta^*}{I^*})^2 & r_{\max} - \mu_{\min} - 2\beta^*(c + c'') - \dfrac{2c'\beta^*}{I^*} \end{bmatrix} \qquad \textbf{Eq. 7}$$

Both eigenvalues can be shown to be real because

$$\Delta = [r_{\max} - \mu_{\min} - 2\beta^*(c + c'') - \frac{2c'\beta^*}{I^*} + D]^2 + 4sc'(\frac{\beta^*}{I^*})^2 > 0$$

The sum and the product of the eigenvalues are

$$S = \frac{\mu_{\min} - r_{\max} - D}{2}$$

$$P = D(r_{\max} - \mu_{\min} - \frac{c'D}{s})$$

Taken together, the following two cases can be observed:

(i) $r_{\max} < \mu_{\min} + \frac{c'D}{s}$ (Figure 2.1 and 2.2): The fixed point is a local saddle and the values of both β and I at the fixed point are negative. This means, in particular, that β decreases to zero. From a biological point of view, the outcome of this situation is disappearance of islets and full-blown diabetes (unless there is sufficient inter-islet heterogeneity and compensation by healthier islets).

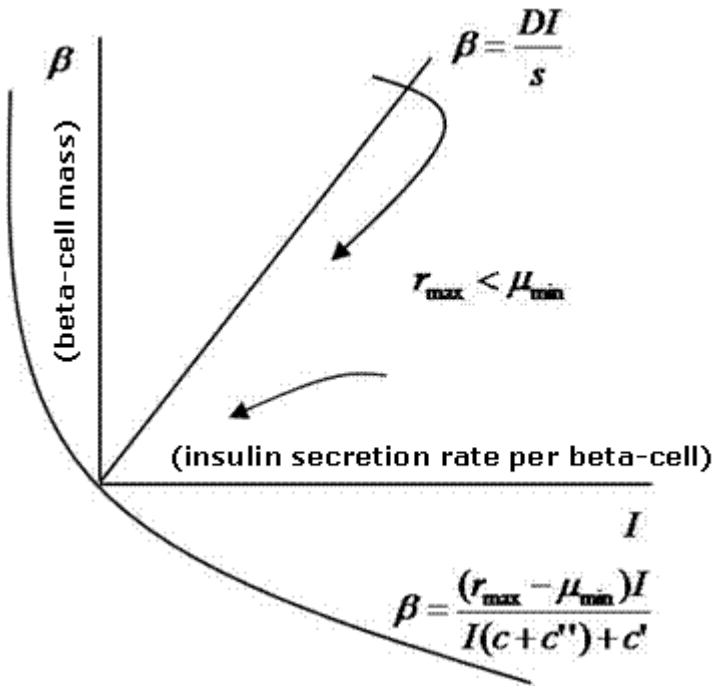

Figure 2.1: Steady state analysis when $r_{\max} < \mu_{\min}$ (case (i)). Both beta-cell mass and insulin levels approach zero with time.

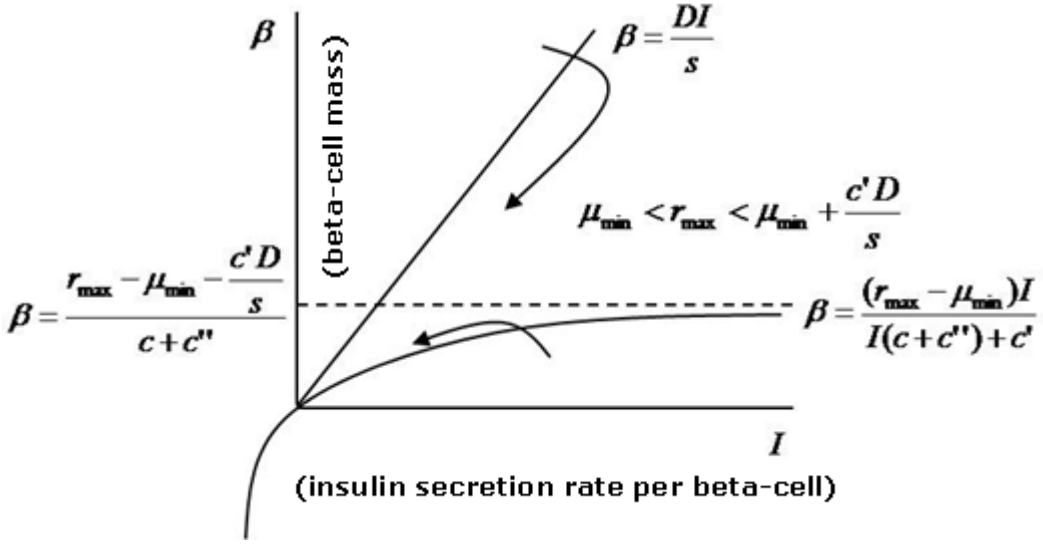

Figure 2.2: Steady state analysis when $\mu_{\min} < r_{\max} < \mu_{\min} + \frac{c'D}{s}$ **(case (i)).** Both beta-cell mass and insulin levels approach zero with time.

(ii) $r_{\max} > \mu_{\min} + \frac{c'D}{s}$ (Figure 2.3 and 2.4): The fixed point is locally stable and the values of both β and I at the fixed point are positive. This case represents a biologically more interesting situation. The islet contains a stable number of beta-cells and a stable amount of insulin.

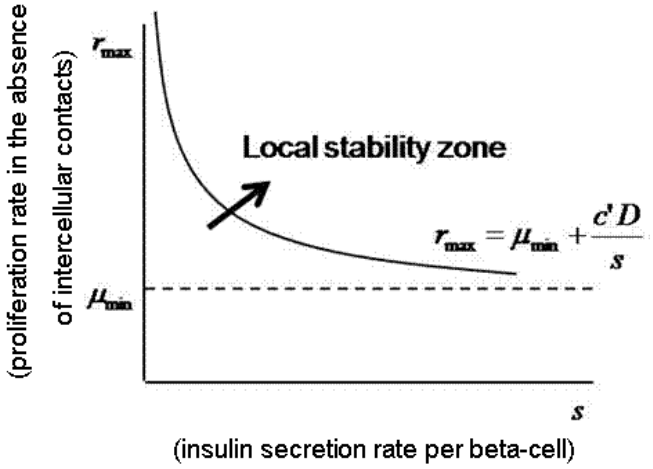

Figure 2.3: Steady state analysis when $r_{\max} > \mu_{\min} + \frac{c'D}{s}$ **(case (ii)).** There is a biologically relevant steady state for both beta-cells mass and insulin levels.

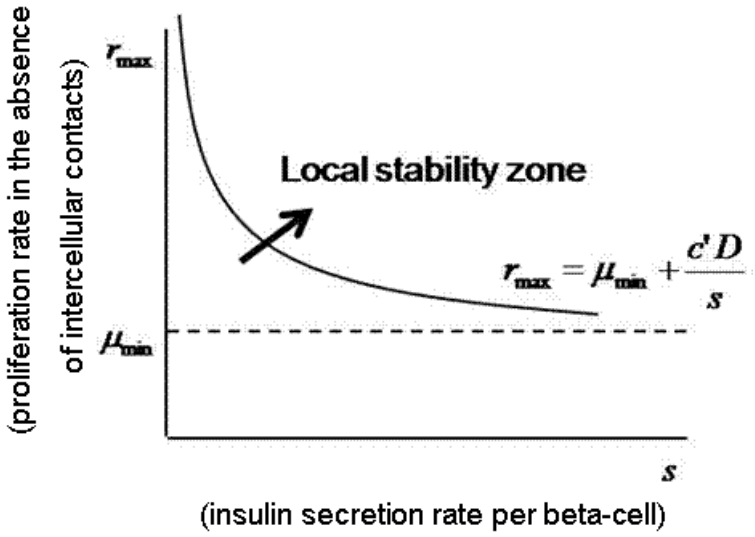

Figure 2.4: Steady state analysis for different values of r_{max} and s. When the values of r_{max} (proliferation rate in the absence of intercellular contacts) and s (insulin secretion rate per beta-cell) are above the shown curve, there is a biologically relevant steady state for both beta-cell mass and insulin levels.

The next important question concerns the determinants of the stable beta-cell mass in case (ii). r_{max} is inversely related to s. Let us represent this relationship by

$$r_{max} = f(s)$$

in which f decreases with s. This is due to the regulatory role of c-Myc, which promotes proliferation at the cost of insulin secretion (Demeterco et al. 2002). There are several lines of evidence supporting the existence of this trade-off. Mature beta-cells have low c-Myc expression (Jonas et al. 1999; Katić et al. 1999), which is consistent with a low proliferation rate (Bonner-Weir 2000). The state of

differentiation of beta-cells is inversely correlated with their proliferation rate (Philippe et al. 1987). Highly proliferative beta-cells of foetal islets do not show glucose-induced insulin response (an index of maturity/differentiation) (Bonner-Weir 2001). Glycaemia, the most potent stimulator of beta-cell proliferation, acts by inducing c-Myc overexpression which in turn stimulates beta-cell proliferation and impairs insulin gene expression (Jonas et al. 2001; Laybutt et al. 2002). The latter occurs by binding of c-Myc to the E-box element in the insulin promoter and inhibiting NeuroD-mediated transcriptional activation (Kaneto et al. 2002). Finally, the pancreatic and duodenum homeobox factor-1 (Pdx1), an essential factor for insulin gene expression, suppresses c-Myc and results in a low proliferation rate (Chen et al. 2007).

Since $r_{\max}$ is constrained by values of s, the biologically relevant outcome of evolution in case (ii) may or may not occur, depending on the shape and parameters of the function f. Figure 2.5 shows a few of the several possibilities.

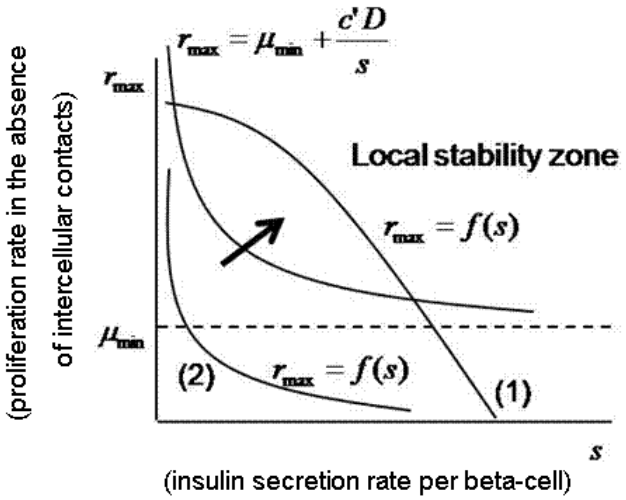

Figure 2.5: Steady state analysis for different patterns of relationship between r_{max} and s. For a given relationship between r_{max} (proliferation rate in the absence of intercellular contacts) and s (insulin secretion rate per beta-cell), one can see whether the outcome of evolution is a biologically relevant stable steady state. Shown are curve 1, part of which lies above the arrowed curve, and curve 2, all of which lie below the arrowed curve. Only in the former can the outcome of evolution be a biologically relevant stable steady state.

The first derivative of β in terms of s determines the steady state value of β for different values of s. We have

$$\frac{d\beta^*}{ds} = \frac{\frac{df}{ds} + \frac{c'D}{s^2}}{c + c''}$$

where $\frac{df}{ds}$ is negative and is calculated at s. Depending on the behaviour of $\frac{df}{ds}$, $\frac{d\beta^*}{ds}$ can have different behaviours (Figure 2.6), which one classify in the following three categories:

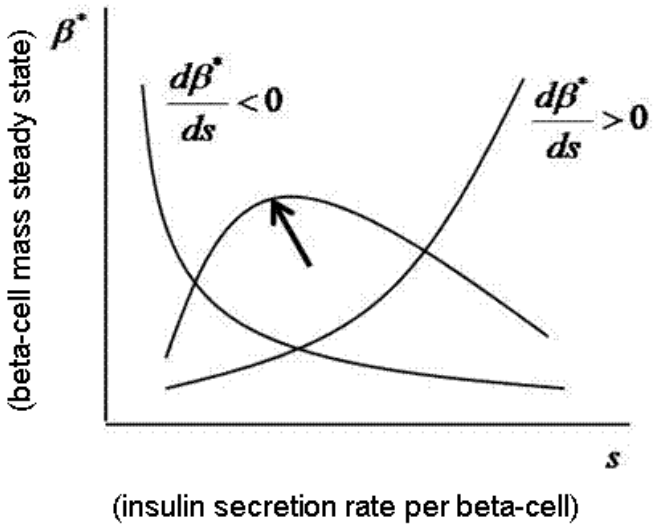

Figure 2.6: Steady state value of beta-cell mass versus s . When $\frac{d\beta^*}{ds}$ is positive for small values of s (insulin secretion rate per beta-cell) and negative for larger values of s , there is an intermediate value of s for which the steady state beta-cell mass in maximal.

(a) $\frac{d\beta^*}{ds}$ is always positive (Figure 2.6): In this case, more insulin secretion (per beta-cell) rewards larger beta-cell mass. Although more insulin secretion is associated with slower proliferation, the pro-survival effect of insulin can compensate and result in a larger beta-cell mass.

(b) $\frac{d\beta^*}{ds}$ is always negative (Figure 2.6): In this case, more insulin secretion (per beta-cell) results in smaller beta-cell mass. Beta-cell proliferation rate is more important than insulin secretion for beta-cell mass in this situation.

(c) $\frac{d\beta^*}{ds}$ is initially (for small values of s) positive and then negative (Figure 2.6):

In this interesting case, maximal beta-cell mass is achieved with intermediate rates of insulin secretion per beta-cell (arrow in Figure 2.6). Too high rates of insulin secretion seriously compromise proliferation and too high rates of proliferation seriously compromise insulin secretion.

2.5 Discussion

The present model is the first mechanistic model of intra-islet beta-cell dynamics. The next step towards a better understanding of beta-cell dynamics is to include the heterogeneity that exists both within and among islets. There are intercellular differences in the secretory activity of glucose-exposed beta cells, both in terms of glucose sensitivity and of amplitude. Heterogeneity also exists in cellular metabolic and biosynthetic functions (Karaca et al. 2009; Van Schravendijk et al. 1992). Two subpopulations of beta-cells can be identified in the pancreas. Highly responsive beta-cells express functional beta-cell markers and are highly responsive to various insulin secretagogues, whereas weakly responsive or nonresponsive beta-cells represent the main population in diabetic pancreas. An increase in highly responsive beta-cells has been shown to be associated with gain of function that follows sustained glucose overload (Karaca 2009). From a physiological point of view, it is important to note that functional consequences of heterogeneity need to be overcome by certain mechanisms in order for pulsatile and coordinated insulin secretion from pancreas to take place. Through computational modelling, it was proposed recently that cholinergic neural ganglia can serve as an islet-synchronizing agent. Specifically, periodic pulses of acetylcholine released from cholinergic neurons are able to coordinate the activity of a population of simulated islets, even if only a fraction of

these are innervated (Fendler et al. 2009). There is a rich innervation of the pancreas by preganglionic vagal neurons (Ahren et al. 1986; Berthoud & Powley 1990; Brunicardi et al. 1995; Kirchgessner & Gershon. 1990). These autonomic nerves synapse onto intrapancreatic ganglia. These ganglia are often found in the proximity of islets and provide innervation (Coupland et al. 1958; Morgan & Lobl, 1968; Persson-Sjogren et al. 2001).

Case (i), studied above, corresponds to full-blown diabetes in which islets become depleted of beta-cells and consequently insulin levels diminish. Based on my calculations, one straightforward condition for this to occur is a sufficient elevation in baseline apoptosis rate of beta-cells. Indeed, stress-induced apoptotic beta-cell death is a hallmark of type 2 diabetes (Szabadkai & Duchen 2009). Alternatively, an impaired balance between proliferation and insulin secretion is predicted by the model to be able to result in case (i). In support of this prediction, the primary progenitor of type 2 diabetes is now presumed to be progressive beta-cell dysfunction, which appears early in the clinical course (Campbell 2009). The combination of excessive levels of fatty acids and glucose leads to decreased insulin secretion, impaired insulin gene expression, and beta-cell death by apoptosis, which probably have distinct underlying mechanisms (Poitout et al. 2009).

Case (ii) represents a milder form of the conditions that exist in case (i). In this case, the islet approaches a steady state regarding beta-cell mass and insulin. As long as new interfering conditions such as medications and new diseases are not added to the scenario, the model predicts that the steady state is stable. This situation corresponds to stable diabetes. The patient may suffer from hyperglycaemia and its complications

(which might make the steady state less stable or unstable and shift it to a new point), but the situation is at least transiently stable. Under what conditions can case (ii) progress and result and convert to case (i)? A rise in baseline beta-cell apoptosis rate ($\mu_{\min}$) may facilitate this transition. A sufficiently severe immune assault, resulting for example from an acute inflammation, can increase beta-cell apoptosis rate and mediate the transition from stable diabetes to case (ii). The same process is active in type 1 diabetes (Rabinovitch 1998; Rabinovitch & Suarez-Pinzon 2007; Mandrup-Poulsen 1996). Morphological studies have uncovered an intra-islet inflammatory process in type 2 diabetes, characterized by the presence of cytokines (e.g. interleukin 1-beta), immune cells, and beta-cell apoptosis, among others. Importantly, increased numbers of macrophages are detectable very early in high fat-fed mice islets, before the onset of diabetes. These immune cells are most likely attracted by islet-derived chemokines, produced in response to metabolic stress, and under the control of interleukin-1 beta. Modulation of intra-islet inflammatory mediators, in particular IL-1 beta, has been suggested as a strategy to prevent insulitis in type 2 diabetes (Böni-Schnetzler 2008). Insulitis has been suggested to contribute to the decrease in beta-cell mass in type 2 diabetes (Donath et al. 2009). The present model predicts that if insulitis is added to a stable diabetes scenario, a bifurcation may take place and convert the case to unstable and progressive hyperglycaemia, eventually resulting in full-blown diabetes.

How can the model presented here and its results be applied in clinical setting? As the first attempt to model intra-islet beta-cell dynamics, there is still much to be included and explored in the model. The model can be more sophisticated (and possibly with more explanatory power) if it includes molecular pathways that mediate the three

main functions related to beta-cells: proliferation, apoptosis, and insulin secretion. There may well be additional trade-offs between the three (I only considered the trade-off between proliferation and insulin secretion) with significant effects on beta-cell dynamics. In its current form, and to my best knowledge, the model takes advantage of the most compelling evidence to explain the phenomenon of our interest. The generic structure of the model makes it flexible enough to become more sophisticated as new data emerge.

Chapter 3: Metabolic evolution suggests an explanation for the weakness of antioxidant defences in beta-cells

3.1 Introduction

3.1.1 Beta-cells and glucose homeostasis

Beta-cells are major constituents of the endocrine pancreas and are localized within specialized structures (the islets of Langerhans) throughout the pancreas (Figure 3.1). Beta-cells are believed to have first evolved as scattered insulin-producing cells in the intestinal tissue of primitive proto-chordates (> 500 million years ago). During the evolution of the hagfish and lampreys (~ 500 million years ago), our most primitive vertebrate species of today, they formed the endocrine pancreas within the abdominal cavity (Madsen 2007). Embryonic development of the endocrine pancreas involves sequential, cell-specific expression of transcriptional factors that determine the fate of individual cell types within the foetal pancreas (Habener et al. 2005). For example, expression of pancreatic duodenal homeobox-1 (PDX-1) and MafA (activators of insulin gene transcription) during gestation (8-21 weeks in humans) is associated with beta-cell differentiation and insulin production (Kaneto et al. 2007; Lyttle et al. 2008).

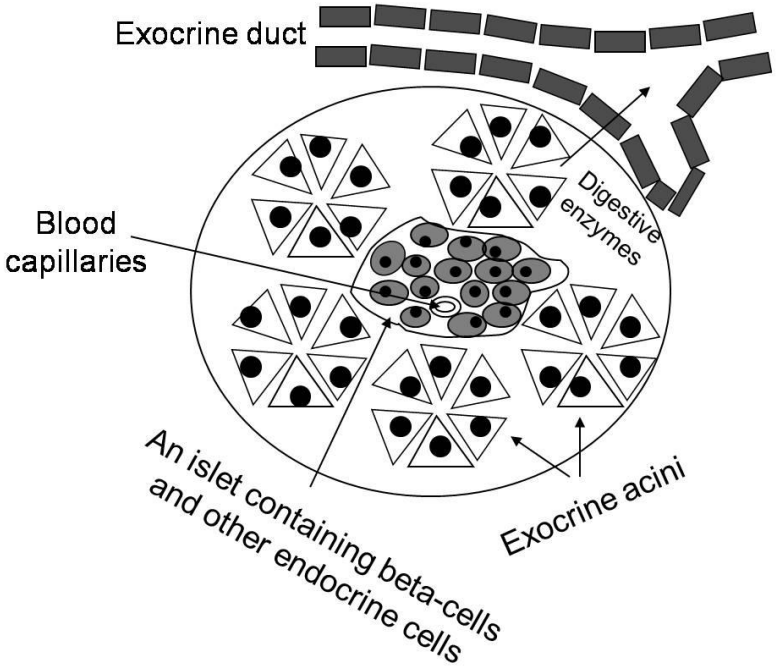

Figure 3.1: Schematic histology of the pancreas. Pancreatic islets are collections of endocrine cells (including beta-cells) scattered in between the exocrine acini.

Beta-cells play a central role in whole-body glucose homeostasis. The enormously complicated organism-level process that results in glucose homeostasis can be simplified, for the sake of the present discussion, as a feedback loop between plasma glucose and insulin (Figure 3.2). A rise in blood glucose, e.g. after a meal, is converted to a stimulatory signal inside beta-cells, leading to increased insulin synthesis and secretion. Following exocytosis and entry into the bloodstream, insulin promotes, via insulin receptors on the cell membrane, glucose disposal in various tissues such as the liver and skeletal muscles (for exceptions see below). Consequently, plasma glucose is robustly maintained within a narrow range (~70-100 mg/dl in humans), thus providing exclusively glucose-dependent organs such as the brain with a reliable energy supply. Uncorrected deviations from homeostasis can have detrimental outcomes, most notably seizure (with hypoglycaemia) and diabetes

(with hyperglycaemia). Maintenance of glucose homeostasis is contingent upon proper beta-cell function, well-regulated beta-cell mass (i.e. number), and appropriate peripheral response to insulin.

Figure 3.2: Main elements of the glucose homeostatic system. Beta-cells sense the blood glucose and secrete insulin which in turn reduces blood glucose and maintains homeostasis.

Figure 3.3 provides a simplified overview of glucose-induced insulin secretion in beta-cells and insulin-induced glucose disposal in other cell types in the body. With a few exceptions including beta-cells, the brain and the placenta, glucose disposal depends on insulin availability in the vicinity of the cell (Watve & Yajnik 2007). Binding of insulin to insulin receptors on the surface of the cell activates an intracellular signaling pathway among the effects of which is the transport of glucose receptors to the cell membrane allowing for glucose entry (Fröjdö et al. 2009). Impairment in this signaling pathway is known as insulin resistance and is a central feature of an increasingly common constellation of pathologies collectively known as metabolic syndrome. In beta-cells, the ATP-controlled potassium channels on the cell surface close following the rise in cellular ATP concentrations that are produced by glucose metabolism. The consequent depolarization of the cell membrane promotes calcium entry to the cell via calcium channels. Increased amounts of calcium in the cell cause release of previously synthesized insulin. Glucose also regulates insulin synthesis at both transcriptional and post-transcriptional levels (Andrali et al. 2008).

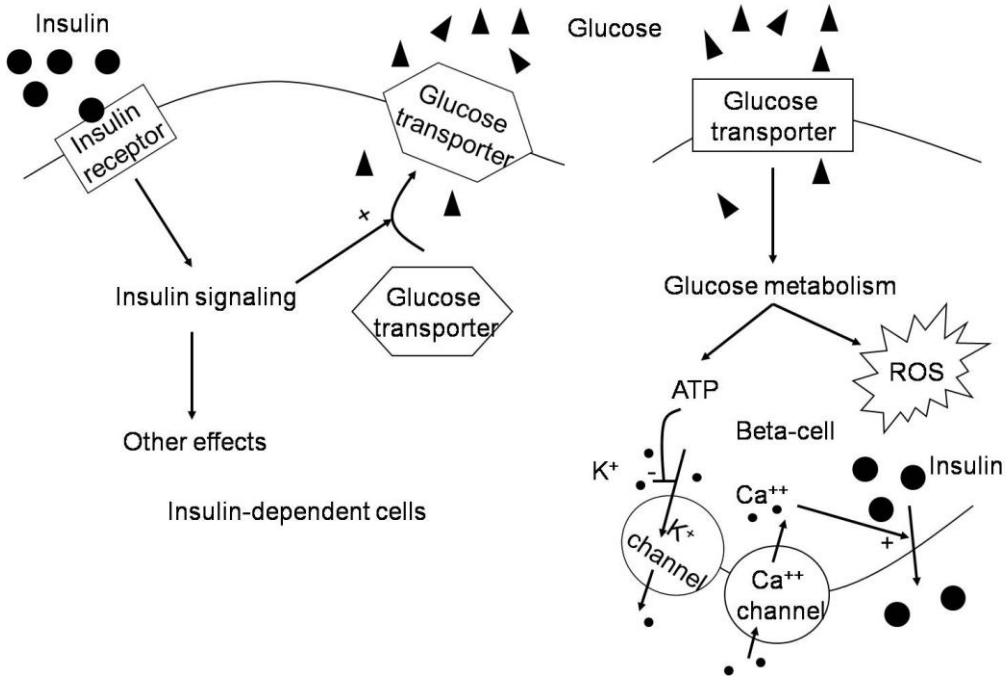

Figure 3.3: Glucose-induced insulin secretion in beta-cells and peripheral insulin-induced glucose disposal. The ATP generated following glucose metabolism results in calcium entry into the beta-cell and insulin secretion. In peripheral tissues, one effect of insulin signaling is the translocation of glucose transporters to the cell membrane and glucose disposal.

3.1.2 Reactive oxygen species: effects on beta-cells

Glucose metabolism in the cell leads to the generation of reactive oxygen species (ROS) which negatively affect the insulin synthesis/secretion machinery via several mechanisms (Wiederkehr & Wollheim 2006). Insulin gene transcription is regulated by a number of transcription factors, most notably PDX-1 and RIPE3b1a/MafA. ROS negatively affect both of these transcription factors (Harmon et al. 2005; Kaneto et al. 2005; Poitout et al. 1996; Read et al. 1997; Robertson et al. 2003). Fusion of insulin vesicles with the cell membrane depends on intracellular concentrations of calcium, which rise as a result of calcium flow into the cell through calcium channels. Calcium

channels open following the closure of ATP-dependent potassium channels and membrane depolarization. Any event that reduces cellular ATP levels may result in the closure of potassium channels. In particular, superoxide ions activate uncoupling protein-2 (UCP-2)-mediated proton leak and lower ATP levels, impairing glucose-mediated insulin secretion (Krauss et al. 2003).

The effects of transiently elevated glucose concentrations on ROS generation are opposite to those of prolonged glycaemia. Glucose acutely suppressed ROS formation in rat beta-cells by increasing cellular NADH levels (Martens et al. 2005). However, this effect was limited. ROS production quickly surpassed its rate of elimination, leading to intracellular ROS accumulation (Gurgul et al. 2004; Pi et al. 2007; Tiedge et al. 1997). That beta-cells show different behaviors under conditions of acute versus prolonged stress is probably a more universal finding applicable to other systems, and can be evolutionarily explained by considering what is "good" for the organism. Homeostatic systems evolve to allow the organism to resist transient environmental noise; the organism should not change its strategies when the same environment is likely to be encountered shortly after a transient perturbation. However, when the environment seems to be deviating, for long, from its current status, adherence to a non-flexible homeostatic system can be detrimental. Physiological systems are neither too fragile nor too strictly homeostatic. My hypothesis (see below) only concerns prolonged stress conditions.

3.1.3 Antioxidant defences in beta-cells versus other cell types

Given their slow turnover (Teta et al. 2005) and their central role in glucose homeostasis, beta-cells are expected to possess an efficient defence system that

protects them against various insults, including ROS. The three main antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Chance et al. 1979). SOD converts superoxide (O_2^-) molecules to less toxic molecules of H_2O_2, which may then be converted to oxygen and water by the enzymes catalase (CAT) and glutathione peroxidase (GPx) (Pi et al. 2007; Rhee et al. 2005). In human beta-cells, the expression pattern of the three main antioxidants is SOD>CAT>GPx (Tonooka et al. 2007). The same situation holds true in human liver cells (Asikainen et al. 1998), but antioxidants in human lung cells seem to have a different expression pattern: CAT>GPx>SOD (Asikainen et al. 1998). Antioxidant expression is cell-specific and different cell types may differ in their antioxidant responsiveness to intracellular redox changes (Asikainen et al. 1998; Jornot & Junod 1993).

Comparative studies have demonstrated that antioxidant defences are remarkably weaker and less responsive to oxidative challenge in beta-cells than in other tissues such as liver, kidney, brain, lung, skeletal muscle, heart muscle, adrenal gland and pituitary gland (Grankvist et al. 1981; Lenzen et al. 1996; Malaisse et al. 1982; Tiedge et al. 1997; Zhang et al. 1995). For example, GPx is expressed at very low levels and has almost undetectable activity in both human (Robertson & Harmon 2007; Tonooka et al. 2007) and rodent beta-cells (Grankvist et al. 1981; Tiedge et al. 1997). Catalase activity in human beta-cells is at the same level as, if not even lower than, in rat beta-cells (Sigfrid et al. 2003). Catalase gene expression is nearly undetectable in mouse beta-cells (Lenzen et al. 1996) and in rats is approximately 5% of the liver (Tiedge et al. 1997).

The weakness of antioxidant defences in beta-cells is clinically relevant. Several lines of evidence point to oxidative stress as a causative factor in the pathogenesis of type 2 diabetes. Compared to healthy controls, islets of type 2 diabetic patients contain significantly higher levels of oxidative stress markers (Chang-Chen et al. 2008), possibly because beta-cell function and turnover are highly susceptible to the adverse effects of prolonged glycaemia. The insufficiently countered oxygen radicals generated over time as a result of chronic exposure to glycaemia are believed to damage beta-cell function and turnover mechanisms (Tanaka et al. 2002). Taken together, the lack of a strong (antioxidant) defence system has been an increasingly likely suspect for beta-cell sensitivity to ROS (Kajimoto & Kaneto 2004; Lenzen 2008; Robertson et al. 2003).

3.1.4 Beta-cell antioxidant defences: gender differences

Beta-cell antioxidants have lower levels of activity in female mice than in males (Cornelius et al. 1993). Humans are no different. Tonooka et al (2007) performed one of the few studies on human beta-cell antioxidants. They observed that GPx protein expression was remarkably low and GPx activity was almost undetectable in the islets. Furthermore, they demonstrated under varying glucose concentrations that GPx protein expression was significantly lower in islets from females (Tonooka et al. 2007). There are no studies, to our knowledge, that have looked at gender-specific patterns in beta-cell antioxidant expression in species other than mice and humans.

Beta-cells seem to be rather unique among other cells in this regard. Indeed, females are generally better protected against oxidative stress (Proteggente et al. 2002). For example, mitochondria from female rats had higher antioxidant gene expression than

those from males in one study. Ovariectomy removed this difference, which re-appeared following estrogen replacement therapy (Borrás et al. 2003). Among tissues where ovariectomy causes a significant reduction in antioxidant expression and activity are bones (Muthusami et al. 2005) and the liver (Oztekin et al. 2007).

The proximate mechanisms underlying this gender difference are not clear. Estrogen has a phenol ring containing a hydroxyl group which may be donated. This structure offers estrogen a possible chain-breaking antioxidant mechanism of action, like that of vitamin E (Subbiah et al. 1993; Sugioka et al. 1987). However, physiological concentrations of estrogen are not high enough to exert significant effects on the cellular redox state. Indirect effects such as membrane stabilization are a more likely mechanism for the antioxidant properties of estrogen at physiological concentrations (Paroo et al. 2002). Beta-cells do not seem to follow the same response pattern to sex hormones as other cell types. It is androgens, rather than estrogen, that provide some protection for beta-cells, by reducing mega-islet formation (Rosmalen et al. 2001) and apoptosis (Morimoto et al. 2005).

3.1.5 Observations to be explained, questions to be answered

Taken together, the evidence prompts the following questions: Is there some advantage in possessing a reduced antioxidant status that outweighs the high price that is paid in terms of cellular vulnerability to ROS? Does the difference between the sexes have its origin in some sex-specific evolutionary factor? I examine the possibility that an answer to the first question might lie in a regulatory role for ROS in the essential function of beta-cells, i.e. insulin secretion. I also consider the idea that the weaker antioxidant system in females might be related to pregnancy. First I link the effects of ROS on beta-cells to organism-level physiological changes that occur

during the stress response. Finally, a resource allocation argument in the context of pregnancy and reproductive success of the organism will be used to complete the hypothesis.

3.2 The hypothesis

3.2.1 The stress response and pregnancy

With a few exceptions, namely the brain and placenta, glucose disposal in cells depends on insulin availability in the micro-environment (Peters et al. 2002; Watve & Yajnik 2007). Therefore, decreased insulin sensitivity does not affect insulin-independent cell types which become preferential destinations for the net extra glucose available in the blood in insulin resistant states. Corticosteroids (released during the stress response) and tumor necrosis factor- α (released during pregnancy) cause impairments in the insulin signaling pathway that leads to insulin-induced glucose disposal, thus making stress conditions and pregnancy two important insulin resistant states (Kirwan et al. 2004; Qi & Rodrigues 2007). Both acute and chronic stress activate the hypothalamic-pituitary-adrenal (HPA) axis and result in glucocorticoid release into the bloodstream. In spite of differences in upstream events, the response elicited by both types of stress is qualitatively similar (Chowdrey et al. 1995; Harbuz et al. 1992; Lightman 2008).

The average plasma glucose levels in pregnancy are lower than in non-pregnant women. This is mostly due to increased plasma volume in the first trimester and increased foeto-placental glucose utilisation in late gestation. Also, the glucose set-point is lowered by prolactin, which promotes glucose oxidation in beta-cells by up-regulating the expression of glucose transporters on the cell surface and increasing the

activity of glucokinase (glucose sensor) (Weinhaus et al. 1996). This resetting takes place in the first trimester (Butte 2000; Mills et al. 1998) and ensures that the mother will be able to avoid glycaemia as insulin sensitivity declines during the course of pregnancy (see below).

During the third trimester the average diurnal plasma glucose shows a gradual rise, well within the normal range owing to the above protective mechanisms (Parretti et al. 2001) (Figure 3.4). Decreased insulin sensitivity (Yamashita et al. 2000) and increased fasting hepatic glucose production (Catalano et al. 1992), resulting in increased postprandial glucose, are responsible for this change. Insulin resistance of pregnancy results from impaired insulin signalling due to well-known post-receptor defects (Catalano et al. 2002; Kirwan et al. 2004; Saad et al. 1997; Shao et al. 2000), mainly in skeletal muscles and adipose tissues (González et al. 2002). In particular, insulin sensitivity is blunted by up to 45–70% during the third trimester (Butte 2000; Catalano et al. 1991; Catalano et al. 1993), which is associated with a compensatory rise of fasting plasma insulin (Assel et al. 1993; Catalano et al. 1993). Tumour necrosis factor-α plays the role of classical stress hormones (see above) in mediating insulin resistance in pregnancy (Kirwan et al. 2004).

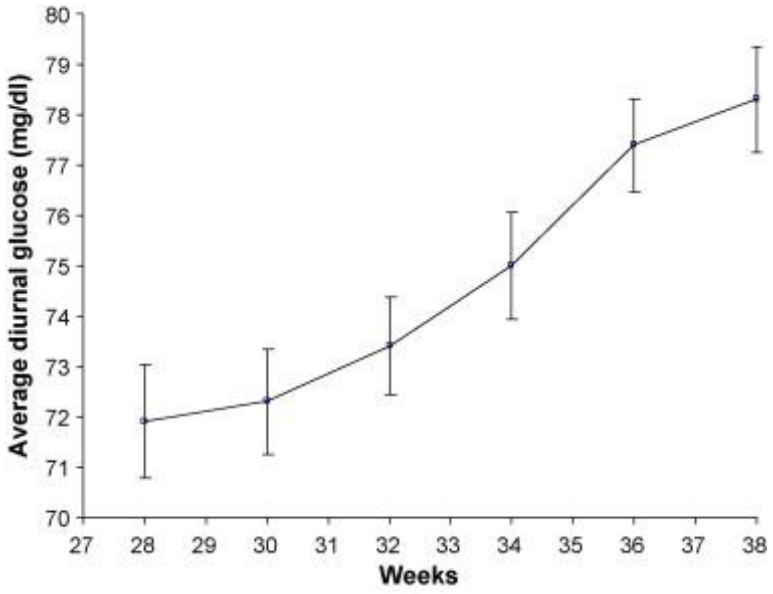

Figure 3.4: Mean diurnal glucose concentrations in the third trimester. Data were collected from 51 women with normal glucose tolerance who delivered term live-born infants without evidence of congenital malformations (Adapted from (Parretti et al. 2001))

The process of beta-cell mass regulation undergoes some alteration during pregnancy resulting from the interplay of several factors, most importantly glucocorticoids and lactogens (Arumugam et al. 2008). Glucocorticoids are an essential component of normal pregnancy with pluripotent effects on decidualisation, implantation, and placental development (Arcuri et al. 1996; Malassiné & Cronier 2002). They have, however, some undesired effects on both the mother and foetus if not counteracted. The placenta protects the foetus from high levels of maternal cortisol through its enzyme 11β-hydroxysteroid dehydrogenase type 2, which converts cortisol to inactive cortisone (Shams et al. 1998). On the maternal side, cortisol concentrations double in late gestation leading to increased beta-cell apoptosis and decreased proliferation (Freemark 2006; Ranta et al. 2006; Weinhaus et al. 2000). The effects of lactogens on

beta-cells are exactly opposite to those of cortisol (Arumugam et al. 2008; Fujinaka et al. 2007), and may therefore function to counteract the detrimental effects of corticosteroids on the glucose homeostatic system. The net result may be enhanced beta-cell mass during gestation.

Table 3.1 summarizes the changes that occur during pregnancy in glucose homeostasis and beta-cell physiology. For the purpose of my hypothesis, it is to be noted that plasma glucose rises within the normal range during pregnancy, particularly in the third trimester (Parretti et al. 2001). Beta-cells respond by secreting more insulin to compensate.

Table 3.1: Summary of the changes that occur in glucose homeostasis in pregnancy

Beta-cell mass regulation	-Late gestational increase in glucocorticoids increase beta-cell apoptosis and decrease beta-cell proliferation. -Lactogens decrease beta-cell apoptosis and increase beta-cell proliferation. -Net result: increased beta-cell mass during gestation	(Freemark 2006; Ranta et al. 2006; Weinhaus et al. 2000) (Arumugam et al. 2008; Fujinaka et al. 2007) (Sorenson & Brelje 1997)
Glucocorticoid metabolism	-Glucocorticoids are important for decidualisation, implantation, and placental development but have undesired effects if not counteracted. -The placental 11ß-hydroxysteroid dehydrogenase type 2 converts cortisol to inactive cortisone and protects the foetus. -Attenuated HPA axis stress responsiveness is another protective mechanism.	(Arcuri et al. 1996; Malassiné & Cronier 2002) (Shams et al. 1998) (Weinstock 1997)
Insulin sensitivity	-Post-receptor defects in insulin signaling mainly in skeletal muscles and adipose tissues -Blunting of insulin sensitivity more than 50% in the 3rd trimester -TNF-α plays the role of stress hormones	(Catalano et al. 2002; González et al. 2002; Kirwan et al. 2004; Saad et al. 1997; Shao et al. 2000) (Butte 2000; Catalano et al. 1991; Catalano et al. 1993; Yamashita et al. 2000) (Kirwan et al. 2004)
Glucose[a]	-Prolactin lowers the glucose set-point by up-regulating the expression of glucose transporters and glucokinase (glucose sensor) activity in beta-cells (1st trimester)[b] -Increased fasting hepatic glucose production, increased plasma volume in the 1st trimester and increased foetoplacental glucose utilization in late gestation -Net result: lower plasma glucose levels than in non-pregnant women, with a gradual rise in the 3rd trimester	(Butte 2000; Mills et al. 1998; Weinhaus et al. 1996) (Catalano et al. 1992) (Parretti et al. 2001)
Insulin	-Increased metabolic clearance of insulin, due partly to placental insulinase[c] - Compensatory rise of fasting plasma insulin	(Catalano et al. 1998; Posner 1973) (Assel et al. 1993; Catalano et al. 1993)

[a]The maternal plasma glucose level is strongly correlated to utero-placental glucose consumption, glucose transfer to the foetus and foetal plasma glucose (Hay, 1995; Hay and Meznarich, 1989).

[b]This mechanism protects the mother against glycaemia as insulin sensitivity declines in the course of pregnancy.

[c]This mechanism may aid ROS in reducing plasma insulin (see text).

3.2.2 Hypothesis: ROS in beta-cells have an adaptive function in stress conditions including pregnancy

Natural selection strongly favours strategies that maximize the production of offspring and is therefore expected to have acted, in female mammals, to tune physiological systems to optimize the chances of a successful pregnancy. Since the placenta is an insulin-independent tissue, maternal insulin resistance can help divert more glucose to the foetus (Watve & Yajnik 2007). The gradual increase in maternal average plasma glucose following the development of insulin resistance is also explained by the necessity to meet the increasing nutritional requirements of the growing foetus. In support of this, note that utero-placental glucose consumption, glucose transfer to the foetus and foetal plasma glucose are all directly and strongly related to maternal plasma glucose (Hay 1995; Hay & Meznarich 1989).

However, if the above argument is correct, then why is the compensatory hyperinsulinaemia unable to return glucose back to the set-point, thereby maintaining homeostasis at a relatively constant glucose level? There are two possibilities. Either at some point, beta-cells are simply unable to increase the rate of insulin secretion further, perhaps because they are exhausted or because insulin resistance progresses faster than they can compensate for by augmenting insulin secretion. Or, there is some reason, possibly adaptive, why beta-cells do not increase insulin secretion rate further.

There are powerful arguments against the first of these possibilities. Firstly, insulin resistance develops and progresses very slowly. Secondly, reduced plasma glucose lessens the metabolic load that would otherwise be imposed on beta-cells in insulin resistant states. Finally, no signs of beta-cell exhaustion or failure have been described

in a normal pregnancy. The second possibility requires beta-cells to be prevented somehow from secreting insulin at the level required to return glucose back to the set-point. I suggest that ROS are suitable candidates for playing this inhibitory role and that antioxidants are therefore expressed at a sufficiently low level to allow for ROS to perform their important regulatory function during pregnancy. The function of ROS in reducing the plasma insulin is aided by an increased metabolic clearance of insulin with advancing gestation. The precise mechanisms of this alteration in insulin clearance are unclear, but seem to involve the placental insulinase (Catalano et al. 1998; Posner 1973). A simple mathematical model clarifies the hypothesis (see Appendix).

3.2.3 Summary of the hypothesis

From an evolutionary perspective, pancreatic beta-cells and their weak protection against oxidative stress represent an intriguing puzzle. The hypothesis states that beta-cells evolved their weak antioxidant protection to allow for reactive oxygen species (ROS) to exert a regulatory function directly linked with reproductive fitness of the organism (Rashidi et al. 2009a; Rashidi et al. 2009b). The hypothesis throws new light on the trade-offs that are involved in the evolutionary optimization of metabolism. The use of this kind of evolutionary physiology approach has already provided insights into the underpinning of important features of human ageing and age-related diseases. For example, there are interesting inferences to be made about the phenomenon of the human menopause and whether or not it is functionally distinct from the generalized reproductive senescence that occurs in other species (Rashidi & Shanley 2009). If my hypothesis concerning beta-cells is correct, it may

yield new ways to think about the pathogenesis of a common age-related disorder, type 2 diabetes. Now, I discuss further the implications of the hypothesis.

3.3 Looking into the past: co-evolution or coincidence?

Beta-cells first evolved as scattered insulin-producing cells in the intestinal tissue of primitive proto-chordates such as amphioxus (>500 million years ago) to sense gut glucose. During the evolution of the hagfish and lampreys (~500 million years ago), our most primitive vertebrate species of today, they formed the endocrine pancreas to sense blood glucose. This was concurrent with the evolution of the most primitive (i.e. reptilian) brain. It has been suggested that the formation of the endocrine pancreas and brain is an example of within-organism co-evolution, where beta-cells "provided an extra degree of freedom for the brain to evolve without having to *think* of getting energy supply" (Madsen 2007).

Interestingly, cortisol and corticosteroid receptor evolution took place 450–500 million years ago (Bury & Sturm 2007; Ortlund et al. 2007), coinciding with the evolution of brain and the endocrine pancreas. While it is highly unlikely that simultaneous evolution of beta-cells, brain, and stress response was a random coincidence, the present hypothesis links the three in a co-evolutionary manner. Evolution of the endocrine pancreas was not enough to protect the brain when the organism was stressed. Stress-induced insulin resistance allowed for preferential diversion of glucose to the brain during stress, provided that beta-cells lost part of their antioxidant defence. I therefore suggest that a weakening of antioxidants in beta-cells occurred as brain, corticosteroid response, and beta-cells in the endocrine pancreas co-evolved.

According to my hypothesis, further reduction of the antioxidant defence in beta-cells occurred in females when placental mammals evolved approximately 100 million years ago (Kitazoe et al. 2007). Since both the initial and the later (sex-specific) reduction in the antioxidant status of beta-cells were associated with increased fitness of the whole organism, beta-cells (particularly in females) retained their weak defence to the present day.

3.4 Predictions

Table 3.2 summarizes the proposed life history explanation of the otherwise puzzling vulnerability of beta-cells and the predictions made by the hypothesis. It is proposed that beta-cells in female placental mammals lost even more of their antioxidant capacity in order to allow for proper nourishment of the foetus through the placenta during pregnancy. An intriguing prediction is that females and males were less different in terms of beta-cell antioxidants before the placenta evolved. A comparative study of a mammal species and a non-mammal vertebrate species can readily test this prediction though it should be noted that, among mammals, comparative studies are complicated by the need to take into account the well-established differences between species in the general strength of the maintenance and repair systems. Also, the current state of beta-cell structure and physiology in mammals is predicted to be evolutionary 'younger' in females than in males. Finally, if beta-cells first lost part of their antioxidant system for the sake of the brain during stress, one could predict the existence of better protected beta-cells before the brain evolved. This period, which was probably relatively short in evolutionary terms, was the time of intestinal beta-cells that monitored gut glucose.

Table 3.2: The proposed evolutionary history of beta-cells

Time	Species	Evolutionary features
> 500 million years ago	Proto-chordates	No brain No cortisol/corticosteroid receptors Beta-cells in the intestinal tissue Beta-cells sensed gut glucose Beta-cell antioxidants normal Beta-cells in males equal to females
450-500 million years ago	Vertebrates	Evolution of the brain Evolution of cortisol/corticosteroid receptors Evolution of the endocrine pancreas Beta-cells sensed blood glucose Reduction in beta-cell antioxidants Beta-cells in males equal to females
~100 million years ago	Placental mammals	Evolution of the placenta Further loss of antioxidants in beta-cells

3.5 Natural selection: master of trade-offs

If the hypothesis is correct, the model shows how antioxidant expression in beta-cells is optimized through an evolutionary trade-off which sacrifices some of the protection against ROS in order to facilitate the metabolic demands of stress in general and of pregnancy in particular. Expression of antioxidants needs to be above a certain minimum to neutralize the ROS produced as a by-product of glucose metabolism and to avoid their deleterious effects on cell survival, proliferation and function (i.e. glucose homeostasis). At the same time, antioxidant expression needs to be kept low enough to allow for the regulatory role of ROS. This explanation fits well the disposable soma theory where an evolutionary trade-off between investments in survival and reproduction is effected by optimising the respective allocations of

resources at the cellular level (Kirkwood & Holliday 1979). Given the importance of the evolving brain (in non-pregnant states) and the growing foetus (in mammals), it might have been worth investing less in somatic maintenance (e.g. antioxidant protection) and accept higher risks of oxidative stress-related problems (e.g. reduced beta-cell proliferation, survival and function), yet provide the brain and foetus with a reliable energy supply and avoid an otherwise inevitable compromise in times of stress. This can be achieved by insulin resistance-induced re-allocation of energy provided that the compensatory hyperinsulinaemia is unable to return glucose back to the set-point. Given that ROS have negative effects on insulin synthesis/secretion, I believe antioxidants in beta-cells are expressed at a sufficiently low level to allow for ROS to perform their important regulatory function during stress (including pregnancy). One would expect the evolutionarily most important tissues in the body to be insulin-independent. Taken together, antioxidants in beta-cells are expressed at a level sufficiently high to maintain the soma in a tightly regulated homeostatic environment, but low enough to contribute to the organism's fitness under stress and also to facilitate reproductive success.

Without the existence of such a trade-off, it is hard to understand why natural selection would not have enhanced the weak beta-cell antioxidant system in females. In support of the hypothesis, it is notable that androgens (rather than oestrogen) provide some protection for beta-cells, by reducing mega-islet formation (Rosmalen et al. 2001) or apoptosis (Morimoto et al. 2005). The major drawback to a weak antioxidant system is a higher susceptibility of cells to oxidative damage, as occurs when there is sufficiently severe and prolonged exposure of beta-cells to an environment with high insulin demand. This, however, is much more commonly a

problem in today's world with abundant food, obesity, physical inactivity, and increasing life expectancy. In our evolutionary past, such factors would have been seen only rarely and they would not, therefore, have been important factors in shaping the evolution of the system and its regulatory balance (Kitano et al. 2004). Furthermore, completion of pregnancies would have allowed the temporary departure from homeostasis to be corrected before glucose levels could reach the diabetic range, even though pregnancy was more commonly experienced during an individual woman's lifetime in an era of much higher fertility than exists today. One can speculate that natural selection might be on its way to make further modifications to beta-cell physiology in response to the rather sudden changes that have recently occurred in our life styles.

Why did natural selection select for organisms which had beta-cells with permanently reduced antioxidant defences, rather than those with beta-cells that were capable of tuning down their defence mechanisms only when encountering stress? In doing the latter, the organism could probably enjoy a lower life-time risk for development of type 2 diabetes because beta-cells would be more resistant to oxidative stress. It certainly requires more work before one can conclusively answer the question. Nevertheless, one can speculate that owing to a permanently weakened beta-cell antioxidant system, organisms were able to avoid the possible costs of a facultative antioxidant system, which could become significant if stress (e.g. pregnancy, infection) was a sufficiently frequent problem at the time of the evolution of the system.

For the following reasons, pregnancy has been an excellent opportunity for evolution to aid the mother in producing high-quality offspring. First, the relationship between the mother and offspring is never closer than it is in pregnancy. Second, pregnancy is a relatively long period of metabolic relationships between two genetically related individuals from successive generations. The maternal disadvantages associated with poorly protected beta-cells are offset, according to the hypothesis, by the resulting increments in offspring fitness. Adapting to pregnancy is indeed a prominent feature of mammalian evolution. Evolution of beta-cell antioxidant defences is a naive example of the link between antioxidant protection and reproductive success.

I have discussed how pregnancy might have contributed to the evolution of a weaker beta-cell antioxidant system in female mammals. The above observations suggest the possibility of the HPA axis neonatal programming having some impact on beta-cell antioxidant responsiveness. Experimental evidence is to date lacking on this possibility. Although neonatal programming may have a fine-tuning effect on beta-cell antioxidants, I believe this mechanism has not significantly influenced the evolution of beta-cell antioxidants. The proximate mechanism that down-regulates beta-cell antioxidants in females is not known at the moment. The time point during development (e.g. foetal period, perinatal period) when this modification occurs is also unclear. Sex hormones and changes in the intra-uterine environment are good candidates for future investigations. The connection between stress, pregnancy and beta-cell antioxidants becomes even more complicated when considering the reduction that occurs in HPA responsiveness to emotional and physical stressors in pregnancy (Neumann et al. 1998). It has been suggested that this mechanism might

have evolved to protect the mother and her foetus from excess corticosteroids (Weinstock 1997).

3.6 Clinical implications

Insulin resistance mediates the link between the signal (i.e. stress) and the appropriate response evoked in the stressed system, by an appropriate reallocation of energy resources (Figure 3.5). The ensuing glycaemia is sensed by beta-cells which, owing to the regulatory role of ROS, only partially correct it if the signal is sufficiently strong. The net additional amount of glucose which becomes available in the blood can subsequently be directed to insulin-independent tissues, the foetus in pregnancy and the brain otherwise. When the stress is removed, the system switches back to its original steady state. Pathology arises as a result of a failure in this last step (e.g. due to stress chronicity or severity). Type 2 diabetes is the ultimate outcome of an extremely complex interaction between various genetic and environmental factors that cause insulin resistance and beta-cell dysfunction. Therefore, the marginally higher prevalence of the metabolic syndrome and type 2 diabetes among males (Isomaa et al. 2001) is not incompatible with the weaker antioxidant system in female beta-cells.

The weakness of antioxidant defences in beta-cells along with the definitive contribution of oxidative stress to the pathogenesis of type 2 diabetes has provoked the idea of helping beta-cells better protect themselves against ROS to prevent development and progression of diabetes. This idea has been successfully tested in the lab (Yamamoto et al. 2008). Over-expression of thioredoxin, an antioxidant protein, in mouse beta-cells prevented progression of type 2 diabetes. This was associated with suppression of the ROS-induced reduction of PDX-1 and MafA expression in

beta cells. However, ignoring the evolutionary history of beta-cells and the adaptive mechanisms that drove their evolution may have undesired outcomes. In a system which has evolved under the influence of several trade-offs, trying to modulate one feature might leave the organism unfit in other functions. Without considering the evolutionary history of beta-cells, any attempt aimed at improving beta-cell sensitivity to ROS may result in compromised stress responsiveness and reduced reproductive success. It is in the nature of hypotheses that they do not prove that what they assert is the truth. Nevertheless, it is important that they should throw new light on existing data and necessary that they should be both plausible and amenable to experimental test. I believe that testing my hypothesis will lead to interesting and potentially valuable new insights into the factors that lie behind an important age-related condition.

There are a number of mechanisms by which the system tries to resist chronic stress. Glucocorticoid secretion has a circadian rhythm. After each pulse of secretion, there is a refractory period during which the HPA axis is not activated by mild stressors (Windle et al. 1998). Therefore, with increased frequency of pulsatility due to chronic stress, the animal will spend more time in stress hyporesponsiveness (Windle et al. 2001). Stress responsiveness is under both genetic (Windle et al. 1998) and epigenetic control. It is known that the HPA axis can be programmed by early life events (Levine 1967). Perinatal exposure to stress makes animals more stress responsive in adulthood. The underlying mechanism seems to be related to increased frequency of HPA pulses and glucocorticoid pulse amplitude (Shanks et al. 1995; Shanks et al. 2000).

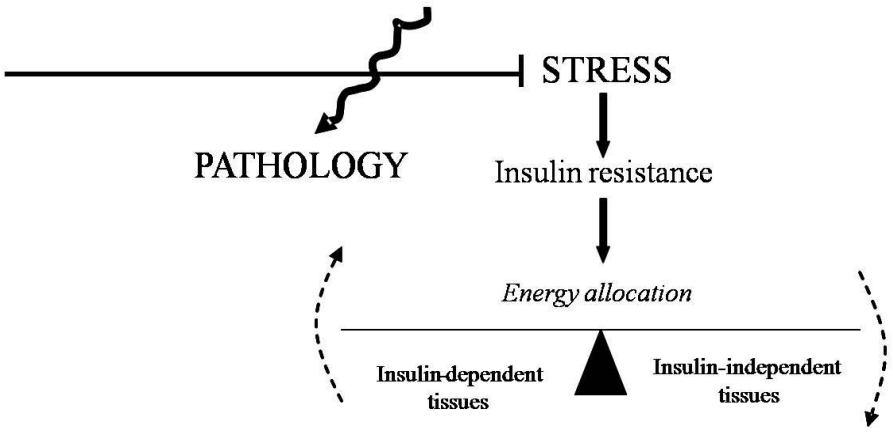

Figure 3.5: The relation between stress and energy allocation. Insulin resistance mediates the link between stress and response. Glycaemia-induced ROS signal beta-cells of the presence of insulin resistance, beta-cells partially correct the glycaemia, and energy is re-allocated to different tissues as appropriate. The system fails to return to the original steady state if the stress persists for a long time.

3.7 Beyond glucose homeostasis

Further examples exist of reproductive success being enhanced at the expense of reduced protection against ROS. Birds that have higher reproductive success also have lower levels of antioxidant expression (Wiersma et al. 2004). In zebra finches, the number of eggs laid correlates negatively with resistance to oxidative stress (Alonso-Alvarez et al. 2006). As mentioned above, the disposable soma theory is built on such trade-offs.

Oxygen radicals act in many systems as second messengers in signal transduction (Finkel 2003). Relevant to protein misfolding and protein aggregation diseases (e.g. Alzheimer disease), it has been shown that ROS are signals generated by misfolded

proteins in the endoplasmic reticulum that cause cell death (Malhotra et al. 2008). In addition, protein misfolding in the endoplasmic reticulum has been suggested to be a causative upstream event in insulin resistance (Ozcan et al. 2006). Mitochondrial oxidants act as facilitators of communication between the mitochondria and the cytosol compatible with an evolutionary adaptation following the ancient incorporation of mitochondria into eukaryotic cells (Nemoto et al. 2000). Why natural selection has so frequently chosen potentially dangerous molecules such as ROS for intracellular signalling may be explained by the fact that these molecules are inevitable by-products of cellular metabolism. By using ROS as signalling molecules, the system did not need to invent and pay additional costs for a distinct regulatory system.

3.8 Robustness, homeostasis, and allostasis

Robustness is an inherent, fundamental feature of complex biological systems and a milestone in the course of their evolution. Complete elimination of environmental perturbations, as occurs in homeostasis, is not always advantageous. Rather, maintenance of function in unstable environments frequently requires temporary or permanent departure from homeostasis. Under such circumstances, the fitness advantages of changing may outweigh those of remaining the same (Kitano 2004). The concept of robustness is being incorporated, using firm mathematical definitions, into our current understanding of fitness in biological systems (Kitano 2007). Natural selection appears to have used ROS, which tend to impair homeostasis in many systems, as regulatory molecules in beta-cells that help the organism appropriately re-allocate the available energy resources under stress conditions. Corticosteroid

response to stress and ROS-regulated beta-cell reaction to the resulting insulin resistance act in harmony to protect the brain and foetus against hypoglycaemia.

Homeostasis means remaining stable by staying the same, whereas allostasis refers to the process of remaining stable through change. Both homeostasis and allostasis are important for maintaining the stability of an organism (Fisher & Reason 1988). Allostasis makes the organism capable of actively adjusting in the long run to both predictable and unpredictable events. The costs of this long-term adaptation to stress, referred to as "allostatic load", is manifested as pathology (McEwen & Wingfield 2003). The term "glucose allostasis" has recently emerged in the literature to explain the process by which prolonged environmental stress and the adaptations they provoke lead ultimately to severe insulin resistance and type 2 diabetes (Stumvoll et al. 2004). Stumvoll et al (2003) suggest that glycaemia is a signal to inform beta-cells of the presence of insulin resistance, that is to say, the organism is likely to be facing prolonged stress. There is strong observational evidence for the involvement of allostasis in the pathogenesis of type 2 diabetes (Stumvoll et al. 2003). My hypothesis demonstrates how beta-cell defence mechanisms might have evolved to confer the organism the ability to respond to stress in an optimal manner. The role of pregnancy as a common stress condition in the past 100 million years in shaping the evolution of the glucose homeostatic system should not be underestimated.

3.9 Appendix: a model for the regulatory role of ROS

Without losing generality, insulin and glucose are measured from their original pre-pregnant set-points. The homeostatic value of glucose is therefore assumed zero and a specific value G for glucose means G units above the homeostatic set-point (units are

chosen arbitrarily). Let $I_{required} = kG$ represent the units of insulin required to remove G units of glucose from the blood. Thus, k provides a measure of insulin resistance. To account for the inhibitory effect of glucose-induced ROS on insulin, let $I_{actual} = (k-rG)G$ be the actual units of insulin following the exposure to G units of glucose. Here, $-rG$ represents the inhibitory effect of glucose-induced ROS production on insulin synthesis/secretion. The emergence of the quadratic term $-rG^2$ is consistent with the observation that if glucose exposure exceeds a certain level, insulin secretion becomes compromised, levels off, and eventually declines due to ROS-induced glucotoxicity (Brownlee 2003), as shown in Figure 3.6 (left). We add g units of glucose to the system at each step and simulate the model over time (Figure 3.6B right).

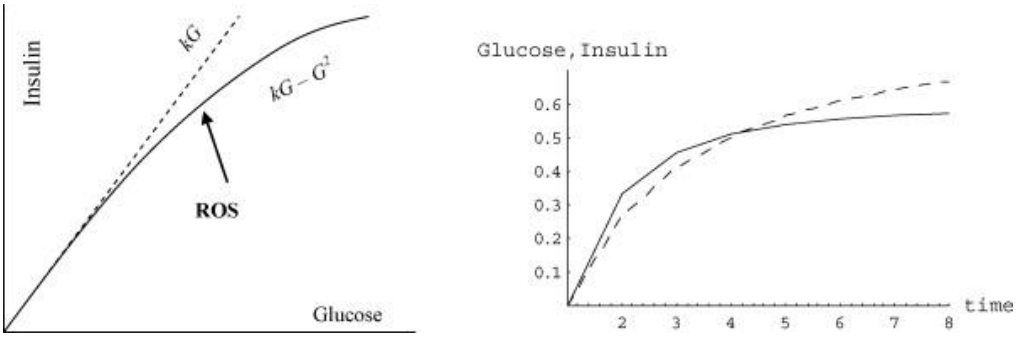

Figure 3.6: The effect of ROS on glucose homeostasis. *Left*: Hypothetical insulin-glucose curves with (solid) and without (dashed) ROS. *Right*: Departure from homeostasis due to the effect of ROS (units chosen arbitrarily).

If r is zero (sufficiently strong antioxidant system in non-exhausted beta-cells), $I_{required} = I_{actual}$ and perfect homeostasis always takes place. Glucose concentration returns back to the set-point (thus lying on the horizontal axis), by proportionately adjusted insulin secretion. With larger values of r, however, the system gradually departs from homeostasis and glucose levels rise without beta-cells being exhausted.

If the desired degree of departure from homeostasis is known, one can theoretically work out how large an *r* is needed. Given the amount of ROS required, the strength of antioxidant system can eventually be estimated.

Chapter 4: A central role for stochasticity in evolution of asymmetric damage segregation: modelling and simulation

4.1 Introduction

All living cells contain an essential complement of molecular components which may acquire faults through errors in synthesis or processing or through the impact of damage, e.g. by oxidation, radiation or heat. In order to maintain viability, damage must be kept from accumulating beyond a level which threatens cellular survival. Sooner or later any individual cell will die, and so the continuation of life depends also on a cycle of cell growth and division, during which new molecular components are synthesised and new and old components are then divided among the daughter cells. Cell division itself acts as a mechanism to inhibit the accumulation of damage, provided that the dilution of damaged components with new ones is sufficient to prevent a generation-to-generation increase in the level of damage. Where cells divide symmetrically to form two essentially identical daughters, it is simply required that damage should not accumulate faster than it is diluted (or removed by repair). It may however be possible to enhance the maintenance of a lineage by segregating damage asymmetrically so that one daughter cell remains relatively free from damage, while the other is burdened with the majority of damaged components from the parent. There are many examples where cell division occurs asymmetrically, especially in multicellular organisms but also in unicellular organisms like the yeast *Saccharomyces cerevisiae*. There has been growing interest in the possible benefits of asymmetric damage partitioning, particularly in relation to the intrinsic biology of cellular ageing (Ackermann et al. 2003; Johnson & Mangel 2006; Kirkwood 2005). Experimental data and *in silico* models suggest that damage segregation in unicellular organisms is a strategy that favours rapid growth (Watve et al. 2006; Evans &

Steinsaltz 2007), helps the single-celled organism cope with damage (Johnson & Mangel 2006; Ackermann et al. 2007; Fredriksson & Nyström 2006), and allows the lineage to withstand higher levels of damage before entering clonal senescence (Erjavec et al. 2008).

An important aspect of how damage arises and may affect cells concerns the role of intrinsic chance. Most individual instances of damage arise randomly even though the level of exposure to damage and rate of repair may be regulated. The segregation of damage at cell division may also be random, unless it is subject to some form of either constraint or control. This raises important questions of how stochastic variation not only affects the partitioning of damage but may also interact with the forces of natural selection that might have acted to influence the evolution of adaptive strategies for damage segregation. Stochastic effects are more pronounced in smaller systems (Spudich & Koshland 1976) so there is also a relationship with size both of the cell itself and also of the 'unit' of damage. For example, proteins that are damaged and escape degradation tend to form relatively small numbers of aggregates (Carrió & Villaverde 2003; Maisonneuve et al. 2008), which may thus show larger statistical fluctuations during segregation, in terms of the proportions concerned, than if the same damaged proteins persisted without aggregation. The effects of stochasticity have not been sufficiently taken into account in previous work.

Using a stochastic model, I examine the evolution of strategies and their consequences to segregate damage, taking account of another fundamental principle in living systems: the need to allocate resources between cellular maintenance and reproduction. My analysis suggests that a continuum of segregation strategies can

evolve in unicellular species and offers a link between the ecological circumstances under which organisms evolve and the intracellular events that contribute to the optimal level of segregation asymmetry. I highlight the central role of stochasticity in evolution of asymmetric damage segregation and explain a number of interesting experimental observations.

4.2 Previous models

Of the few previous modelling attempts on the subject of asymmetric damage segregation in unicellular organisms, I briefly mention the two most relevant and recent ones here. The model developed by Ackermann et al (2007) shows that the differential ageing of the parent and daughter cell (resulting in an old parent and a rejuvenated daughter cell) readily evolves as a strategy to cope with damage that accumulates due to vital activities. In this model, the authors assume a survival function, $s(D)$, which determines the probability that a cell with damage D (inherited at birth from the parent) survives to next mitosis. The authors show that asymmetric distribution of damage is favoured over symmetry unless the survival function has substantial negative curvature. The model produces similar results when damage affects fertility rather than survival. In order to support their assumption about the curvature of the survival function, the authors used data from the bacterium *Caulobacter crescentus* and show that the survival function is linear to slightly concave up, thus supporting the prediction on evolution of asymmetry. Finally, the authors make an analogy between (ageing) parent/ (rejuvenated) offspring in unicellular organisms and soma/germ distinction in multicellular lineages. The multicellular parent retains the whole damaged soma, while the offspring is made from undamaged fresh germline.

Erjavec et al (2008) developed another interesting model to investigate potential advantages of asymmetric damage segregation in a unicellular lineage. The endpoint in their model was entry to clonal senescence, i.e. they were interested in knowing the conditions that delay clonal senescence. In addition to the level of damage segregation asymmetry, the authors also considered size asymmetry at the time of mitosis. The authors included in their model a critical number of intact proteins required for cytokinesis. Furthermore, they assumed that damaged proteins do not have any intrinsic toxicity. They only prolong the time required for the cell to accumulate the critical amount of intact proteins to undergo mitosis. Erjavec et al. show that asymmetric damage segregation is advantageous in a system producing different-sized progeny only at high damage propagation rates. In contrast, asymmetric damage segregation enhances the fitness of a system of equal-sized progeny regardless of damage propagation rates. These findings suggest that asymmetry in damage distribution at mitosis is more universally advantageous than thought before, and should thus be expected to be more common.

4.3 Structure of the Model

I assume a population to comprise initially 1,000 unicellular organisms. There is no spatial or hierarchical structure within the population and organisms do not interact directly with one another. However, competition for the limited resources which are available in the environment increases the risk of death of organisms and provides a mechanism to put an upper limit, subject to stochastic changes, to the size of the population. The rate of resource replenishment is assumed to be limited and above the threshold required for survival of 1,000 individuals. Therefore, although the

probability of the population reaching a very large size is not zero, it becomes smaller and eventually approaches zero as the population expands beyond its initial size of 1,000. The environment is otherwise stable and does not undergo perturbations. The population grows by cellular proliferation and declines by death of individual cells. Proliferation is a time-dependent process in this model. The chances that a cell proliferates are minimal when the cell is formed and increase with time. Two daughter cells arise from a cell proliferation and divide all the materials that exist in their mother between each other. A daughter cell may differ from its mother in genetic and/or non-genetic materials (see below). Extrinsic death occurs because of the competition for limited resources and depends in this model on the population size; the risk per cell of extrinsic death is greater at larger populations. The probability of intrinsic death increases with accumulated damage in the cell. Cell death is assumed to occur by an apoptotic-like mechanism which removes the cell without affecting other cells.

By damage, I refer throughout the model to irreversible non-genetic damage. The damage status of each cell at a given time is defined as the total number of units of damage that has accumulated in the cell until that time. One unit of damage is defined as all damaged molecules that inevitably have the same fate, that is to say, because of being within an aggregate are all inherited by one of the daughter cells. Damage accumulation in the cell, like other processes in this model, is a stochastic process. The cells are assumed to have already identified via evolution the optimal portion of resources to invest in maintenance and reproductive functions. The population therefore remains homogenous in terms of the efficiency of either function and the parameters controlling the rates of damage accumulation and proliferation are

assumed similar for all cells. Nevertheless, I investigate the effects of different resource allocation strategies on the outcomes of the model in the analysis step.

The way damage is segregated at division between the two daughter cells is considered to be a genetically coded trait. I assume, for simplicity, that the organisms are haploid asexual and a single gene in a single locus is responsible for the segregation trait. Segregation can be completely symmetric in which case each unit of damage is inherited by a given daughter cell by a probability of 0.5. It can be completely asymmetric, and all damage units segregate into one of the daughter cells. It might also lie anywhere between complete symmetry and complete asymmetry, e.g. each damage unit having 0.75 chance of ending up in one daughter cell and 0.25 chance of ending up in the other. The segregation allele is subject to mutations. Mutations occur randomly and may increase or decrease the value assigned to the allele.

4.4 Methods

4.4.1 Events and formulations

There are five events in the model: *i*) extrinsic death, *ii*) proliferation, *iii*) damage accumulation, *iv*) intrinsic death, and *v*) mutation. Let us focus on a particular cell (cell *i*). Suppose the latest event in the population has occurred at time *t*, measured from the time of formation of cell *i*. Also assume that cell *i* is the cell to undergo the next event in the population and that event is to occur at time $t + \Delta t$. I will later explain how I determine the cell which is to experience the next event and also how I calculate the time interval between two successive events. Let $D_n(t)$ denote the number of units of damage that exist in cell n ($n = 1, 2, \ldots, N(t)$) at time t. $N(t)$ is the

size of the population (the number of living organisms in the population) at time t. The probability, $P_{i,D}(t + \Delta t)$, of cell i having D units of damage at time $t + \Delta t$ is equal to the probability, $P_{i,D-1}(t)$, of the cell having $D - 1$ units of damage at time t multiplied by the probability that damage accumulation (event *iii*) is the event chosen (among all five possible events) to occur at time $t + \Delta t$ in cell i plus the probability, $P_{i,D}(t)$, of the cell having D units of damage at time t multiplied by the probability that an event other than damage accumulation is chosen to occur at time $t + \Delta t$ in cell i. In other words

$$P_{i,D}(t + \Delta t) = P_{i,D-1}(t) \times P(\text{next event is } iii) + P_{i,D}(t) \times P(\text{next event is not } iii)$$

$$= P_{i,D-1}(t) \times h_3(t) / h_T(t) + P_{i,D}(t) \times (1 - h_3(t) / h_T(t))$$

$$= (P_{i,D-1}(t) - P_{i,D}(t)) \times h_3(t) / h_T(t) + P_{i,D}(t) \qquad (1)$$

where

$$h_T(t) = h_1(t) + h_2(t) + h_3(t) + h_4(t) + h_5(t)$$

is the total hazard at time t and $h_k(t)$ (k = 1, 2, 3, 4, 5) is the hazard or propensity of event k at time t. The hazard function for a given event divided by the sum of all hazard functions (other events) represents the probability of occurrence of that event as the next event. The value of a hazard function, in biochemical terms) depends on the number of reactants and the rate constant for the specific reaction. More generally, reactants in our context can be molecules, cells, organisms, etc. As explained below, the hazard value for intrinsic death (event *iv*) is cell-specific. In order to avoid confusion and for more clarity, however, I drop the otherwise extra index i from $h_4(t)$.

I now define the hazard functions for each of the five events. Since I am assuming that the optimum resource allocation strategies are established in the population and thus cells are investing constant amounts in maintenance/repair and reproductive functions, I consider simple constant hazard functions $h_2(t) = m$ and $h_3(t) = d$ for proliferation (event *ii*) and damage accumulation (event *iii*), respectively. The hazard function for mutation is also assumed to be time-independent constant, $h_5(t) = p$. By intrinsic death (event *iv*), I refer to death due to accumulated damage. The simplest case of an ascending function in the form $h_4(t) = D(t) \times \mu_i$ is assigned to intrinsic death, where $D(t)$ is the number of units of accumulated damage in the cell at time t and μ_i is a constant parameter. The risk of extrinsic death (event *i*) increases as the population grows. I assume the hazard function for this event to be similar for all organisms and for a given cell increase linearly with the population size, i.e. $h_1(t) = N(t) \times \mu_e$, where μ_e is a constant parameter. If proliferation and extrinsic death had been the only events in the model, the total hazard of death across the population would have become $N(t) \times N(t) \times \mu_e$, reminiscent of the quadratic term that serves to restrict the growth of the population in classical deterministic models of population growth (May 1976). The simplistic assumptions made to define hazard functions can be relaxed and more sophisticated functions may be selected without affecting the general patterns of the results. Taken together, equation (1) can be written as

$$P_{i,D}(t + \Delta t) = P_{i,D}(t) + (P_{i,D-1}(t) - P_{i,D}(t)) \times d / (N(t)\mu_e + m + d + D_i(t)\mu_i + p) \qquad (2)$$

Equation (2) describes the likelihood that cell *i* contains a given amount of damage at the time of next event. A properly designed dice, for example, may now be used to determine the time course of damage in the cell. Since the level of damage in a given

cell is known by equation (2), we can easily follow that cell until it dies or proliferates, owing to the fact that intrinsic death is the only damage-dependent event in the model.

4.4.2 Implementation and simulation

Simulation of the stochastic model was carried out using the Gillespie algorithm for coupled biochemical reactions (Gillespie 2007). Originally developed to simulate reactions within cells where the number of reactants are small, the algorithm has proved useful in simulating stochastic systems that extend beyond single cells. The main advantage of the Gillespie algorithm is that it does not require the presence of large numbers of reactants which is an essential assumption in continuous deterministic models that use systems of differential equations. Events are modelled in this algorithm as reactions, and each reaction is assigned a parameter (e.g. μ_e) using which the hazard function for that event can be calculated at each instant of time. Events and their corresponding hazard functions for the present model are provided in Table 1. None or at most one of the possible events (here extrinsic death, proliferation, damage accumulation, intrinsic death, or mutation) can occur to a cell at a given time.

Table 4.1. Events and their hazard functions for one cell

No.	Event	Definition	Parameter	Hazard (propensity)
i	Extrinsic death	death due to cell-cell competition for limited resources, due to release of toxic materials by neighboring cells, etc	μ_e	$N \times \mu_e$
ii	Proliferation	cell division (mitosis)	m	$m \times t$
iii	Damage	accumulation of one unit of damage	d	d

iv	Intrinsic death	death due to the accumulated damage	μ_i	$D \times \mu_i$
v	Mutation	mutation in the segregation coefficient	*p*	*p*

t: chronological age of the cell measured from its formation; *N*: population size; *D*: accumulated damage in the cell

The Gillespie algorithm is a variant of a dynamic Monte Carlo method and is based on the following steps:

1) Initialization: definitions and the initial conditions required for simulation of the algorithm are set in this step. A value σ (segregation coefficient) was assigned to the gene coding for the segregation trait. σ is allowed to range between 0.5 (complete symmetry; a given unit of damage goes into a given daughter cell with a probability of 0.5) and 1.0 (complete asymmetry; all damage segregate to one of the daughter cell cells). Initially, σ was set at 0.5 for all cells. I made this assumption to investigate the conditions in which selection for asymmetry would cause σ to depart from 0.5. It was assumed that initially no damage existed in the cells. In order to model mutations, random numbers were taken from a normal distribution. The mean and standard deviation of this distribution were set at zero and 0.01, respectively. Depending on the sign of each mutation, the segregation trait can become more or less asymmetric. The initial size of the population was set at 1,000.

2) Monte Carlo step: the time to the next event is a random number drawn from an exponential distribution with parameter equal to the total hazard in the system. The exponential distribution is without memory, i.e. the state of the system at any given time depends, in a probabilistic manner, only on its state at the previous time point. Now I come back to the question of how the next event and the cell to experience that event are chosen. First, I choose the cell. The probability that cell *j* ($j = 1, 2,\ldots, N$) is selected for the next event is

$$h_{T,j} / (h_{T,1} + h_{T,2} + \ldots + h_{T,N})$$

, where $h_{T,k}$ is the total hazard of events for cell *k*, if it is chosen for the next event (k = 1, 2,…, N). After the cell has been chosen, it is straightforward to choose the next reaction based on the hazard of each reaction relative to the total hazard in the selected cell, i.e. $h_j / (h_1 + h_2 + h_3 + h_4 + h_5)$ for event j (j = 1, 2, 3, 4, 5). If the next event to occur is proliferation (event *ii*), then the number of units of damage that segregates at division into a given daughter cell should be determined. I chose segregation of damage to occur in a probabilistic manner and according to a binomial distribution with parameters D (number of units of damage in the dividing cell) and σ.

3) Update: hazard functions are updated after each event.

4) Iterate: the algorithm is repeated from step 1 until there remains no cells in the population or the experimenter stops the algorithm.

The simulations were continued until the average σ stayed within an interval of length 0.05 for at least 1,000 generations. This process typically required monitoring the population for approximately 200,000 generations.

Cells gradually move on the fitness landscape and the distribution of different segregation strategies as the population reaches the steady state approximately reflects the successfulness of each strategy. In order to monitor the evolution of segregation strategies with time, σ is averaged over the population. Using the mean of σ, rather than its median, as a measure of central tendency represents a "worst- case scenario" approach for evolution of asymmetry because it turns out that after the population has settled into a steady state, segregation strategies tend to have negatively skewed

distributions where median is larger than the mean. The code for the simulation algorithm was written in Python 2.5 and simulation results were plotted in Mathematica 5.2.

4.4.3 Regression analysis

In order to determine the role of resource allocation trade-offs in the evolution of asymmetry, I focused on two parameters of the model: m as a positive correlate of reproductive investments and d as a negative correlate of maintenance investments. Together with μ_i as a measure of damage severity, m and d were included in a regression model. A total of 100 triplets of the form (m, d, μ_i) were randomly selected from the parameter space (the unit cube) and used for analysis. An arbitrary criterion was used to classify the outcome of evolution in two categories symmetry and asymmetry. This was done according to the population-averaged σ; values above 0.8 were defined as asymmetry and those under 0.8 were regarded as symmetry. Although smaller values could also be considered as the cut-off point, I chose a rather high cut-off point in order not to misinterpret symmetric cases as asymmetric. This definition represents another "worst-case scenario" for the evolution of asymmetry in the present study. The outcome of evolution was the dependent variable in the regression model to be predicted by a set of appropriately chosen predictors. Linear combinations of the three parameters as well their first-order interaction terms (e.g. $d \times m$) were considered as predicting variables. The simplest model which yielded $R^2 >$ 90% was selected. The predictors that exist in this model will therefore explain more than 90% variation in the outcome of evolution.

4.4.4 Evaluation of fitness effects of asymmetry and their determinants

In order to determine the effects of asymmetry on individual fitness, simulations were repeated without mutations ($p = 0$). Therefore, the population is initially genetically homogenous and remains homogenous over time. The population was followed for one population doubling, and the required time was used as a measure of growth rate and hence fitness. Next the three potential determinants of asymmetry (m, d, μ_i) were manipulated and the results were compared. The average of 1,000 simulations was used for each fitness measurement.

4.5 Results

In this model, there is stochasticity in the segregation of low numbers of damage units, in determining inter-event time intervals, and in determining the next event to occur at each time step. As a result, the population-averaged σ moves along noisy trajectories (Figure 4.1A), as the population size approaches and eventually fluctuates around the carrying capacity of the environment (Figure 4.1A, inset). When the force of selection for evolution of asymmetry is not sufficiently high (i.e. the effects of asymmetry on fitness are not significant), segregation strategies undergo neutral evolution by random drift and the population-averaged segregation coefficient will approach 0.75. In spite of stochasticity, σ consistently approached a plateau throughout simulations. The plateau level was affected by choice of parameter values (see below), but was rather robust to noise-induced differences between successive runs of the model with similar parameters and initial conditions (Figure 4.1A). In previous models which allowed for segregation traits to arise as an emergent property of evolution (Ackermann et al. 2007), the outcome of evolution for all cells was complete asymmetry ($\sigma = 1$). Using the same approach but within a stochastic framework, I observed the evolution of strategies including but not limited to

complete asymmetry. The steady state distribution of segregation strategies in the population was a left-skewed distribution with a long tail towards symmetric strategies (Figure 4.1B), indicating considerable variation. This variation persisted regardless of the number of generations for which the population was followed. The observation that steady state distributions for segregation strategies are left-skewed reflects selection for asymmetry.

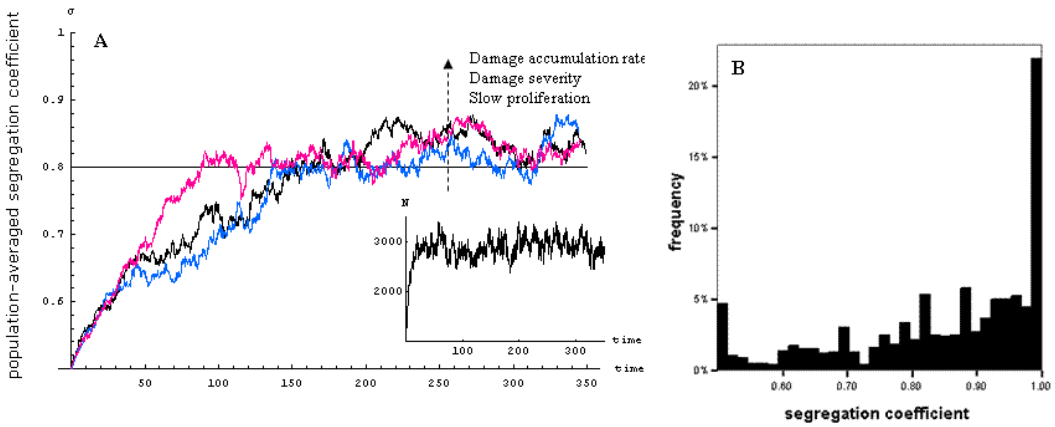

Figure 4.1: Simulation results. (A) Three runs with identical initial conditions (explained within the text) and parameter values ($\mu_e = 0.001$, $m = 0.7$, $d = 0.8$, $\mu_i = 0.2$, $p = 0.01$). The parameters denote extrinsic death rate, proliferation rate, damage accumulation rate, and intrinsic death rate, respectively. The population-averaged segregation coefficient approaches a plateau. Damage severity, damage accumulation rate, and the proliferation rate are determinants of the level of the plateau. The inset demonstrates stochastic fluctuations of the population size (N) around its mean over time. **(B)** Histogram of segregation coefficients at steady state ($\sigma = 0.84 \pm 0.13$), exhibiting considerable variation. The average of three simulations was used to produce the histogram.

In binary logistic multivariate regression analysis various combinations of d, μ_i, and m were considered as continuous potential predictors of asymmetry. Asymmetry was the dependent variable and the outcome of evolution for the population. Asymmetry was coded as 1 (population-averaged segregation coefficient greater than 0.8) or zero (population-averaged segregation coefficient less than 0.8) in the binary logistic regression model. The simplest model (i.e with the smallest number of predictors and lowest order of interaction terms) which was capable of explaining more than 90% variation of the outcome of evolution ($R^2 = 0.91$) was composed of d, m, and the interaction term $d \times \mu_i$. Specifically, large values of d ($P = 0.008$) and $d \times \mu_i$ ($P = 0.016$) and small values of m ($P = 0.009$) were significant predictors of asymmetry. Figure 4.2 shows the parameter values for which the outcome of selection is asymmetry. In particular, the distance between neighboring curves in Figure 4.2A, 4.2B or 4.2C is a measure of the sensitivity of selection to the missing parameters in that panel. Therefore, selection is most sensitive to d. The interaction term $d \times \mu_i$ in the regression model shows that the sensitivity of selection to d increases with increasing values of μ_i. Similarly, the sensitivity of selection to μ_i increases with increasing values of d. This can be seen in Figure 4.2B and 4.2C where the distance between neighboring lines increases with increasing values of μ_i and d, respectively. In summary, high rates of damage accumulation (i.e. high values of d) and severe damage with sufficiently detrimental effects on survival (i.e. high values of μ_i) promote the evolution of asymmetry, whereas rapid proliferation (i.e. high values of m) reduces the selection pressure.

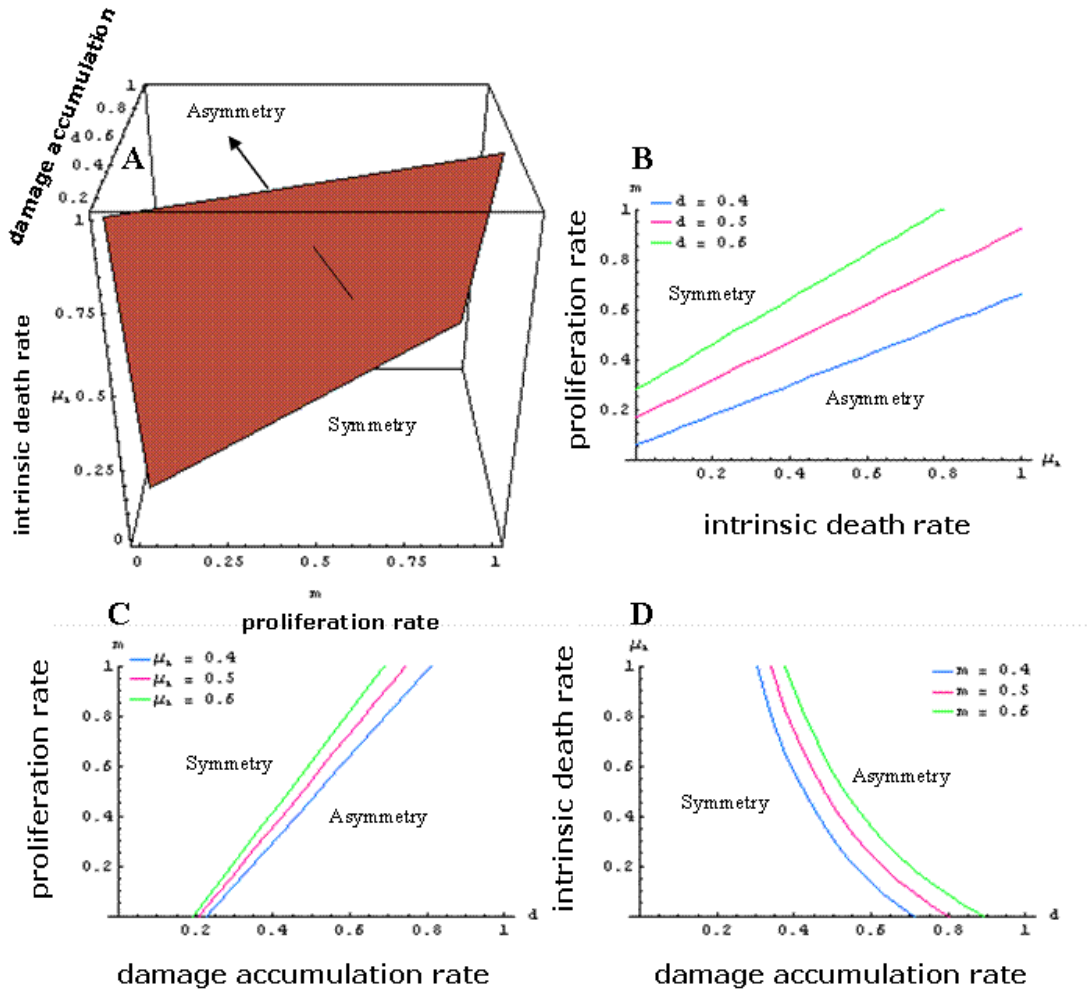

Figure 4.2: Increase of the asymmetric trait from rarity. (A) The three-dimensional panel A separates the region in which the corresponding parameter values allow for evolution of asymmetry from the region where the corresponding parameter values do not allow for evolution of asymmetry. **(B-D)** Panels B-D are two-dimensional slices the three-dimensional surface in panel A. The missing parameter in panels B-D is given three fixed values. Parameter values below the lines in panels B and C, and to the right of the curve in panel D, allow for evolution of asymmetry. Increases in d (damage accumulation rate; panel B) and μ_i (intrinsic death rate; panel C) and decreases in m (proliferation rate; panel D) lead to expansions of the region for asymmetry at the cost of reductions in the region for symmetry. The distance between two neighboring curves in each panel is a measure of the sensitivity of selection to the missing variable in that panel. Selection is therefore most sensitive to d. Also, the sensitivity of selection to d increases with increasing values of μ_i and its sensitivity to μ_i increases with increasing values of d.

Figure 4.3 shows the effects of asymmetry on fitness. There are no mutational events and the growth rate of a genetically homogenous population (measured by its population doubling time) would show the fitness of the particular segregation strategy used by the individuals in that population. Using parameter values that promoted the evolution of asymmetry in mutation-prone and thus heterogeneous populations, asymmetry has clear beneficial effects on individual fitness (curves A and D). These effects were significantly diminished (curve B) or even completely abolished (curve C) when using parameter values that reduced the force of selection for asymmetry in mutation-prone populations. A significant proportion of individuals in mutation-prone populations do not follow the fitness-maximizing level of asymmetry (as predicted by individual fitness) after the population has reached steady state distributions of segregation strategies (compare Figure 4.1B and curve A in Figure 4.3 with similar parameter values and initial conditions). In particular, the proportion at steady state of individuals with a completely asymmetric strategy ($\sigma = 1$) never exceeded 25% in my simulations.

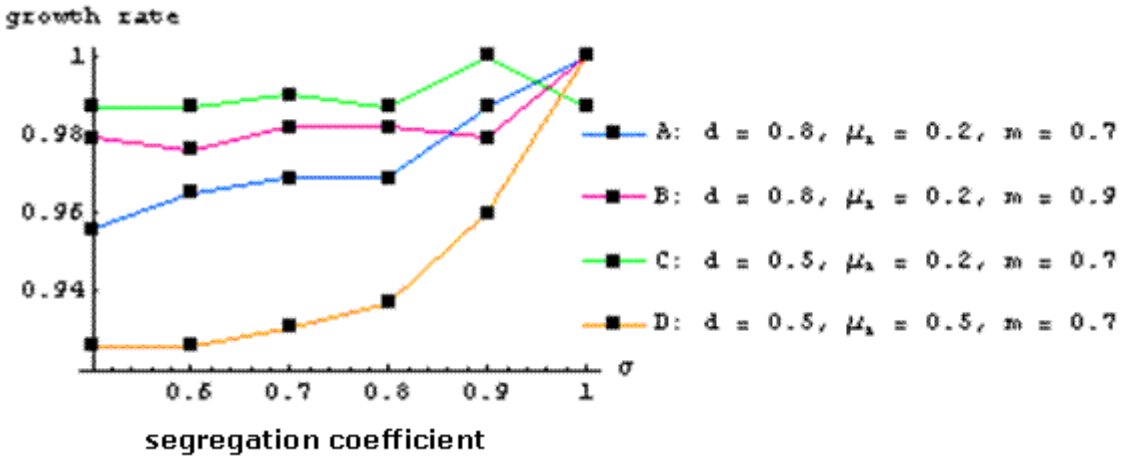

Figure 4.3: Fitness effects of asymmetry as measured by growth rates of genetically homogenous populations determined through simulations without mutations. Growth rates in each curve are normalized by the highest rate. **(B vs. A)** Increasing *m* reduces the effects of asymmetry on fitness. **(C vs. A)** Decreasing *d* reduces the effects of asymmetry on fitness. **(C vs. D)** Decreasing μ_i reduces the effects of asymmetry on fitness. The steady state distribution of segregation strategies for the same four sets of parameters as in Figure 4.3 is shown in Figure 4.4.

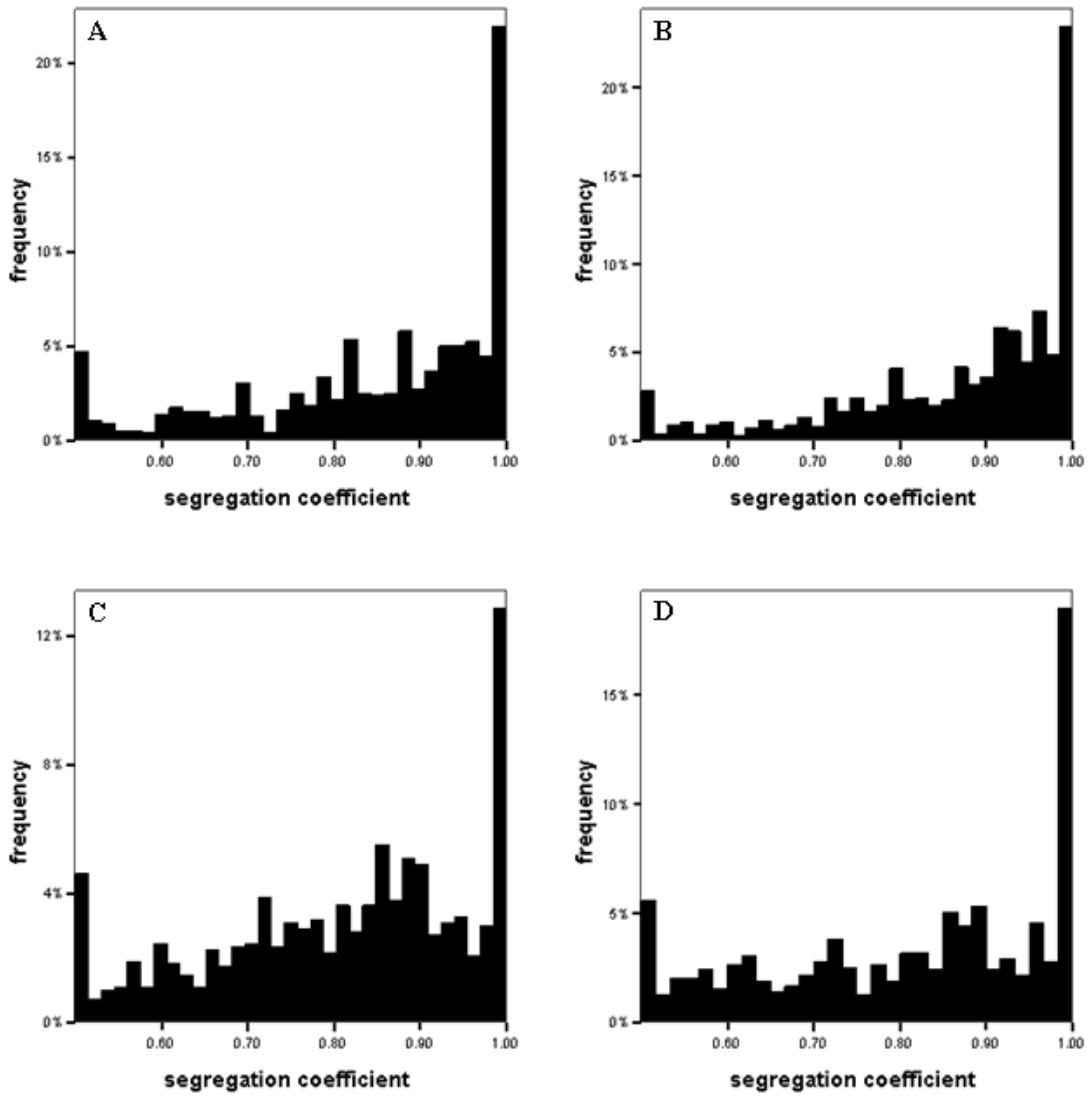

Figure 4.4: Steady state distribution of segregation strategies for the same four sets of parameters as in Figure 4.3 (A: d = 0.8, μ_i = 0.2, m = 0.7; B: d = 0.8, μ_i = 0.2, m = 0.9; C: d = 0.5, μ_i = 0.2, m = 0.7; D: d = 0.5, μ_i = 0.5, m = 0.7). (B vs. A) Increasing m (proliferation rate) in this case does not have a significant effect on the steady state distribution of strategies because for high values of d (damage accumulation rate), selection is much more sensitive to d and μ_i (intrinsic death rate) than to m. **(C vs. A)** Decreasing d reduces the frequency of the completely asymmetric strategy (σ = 1.0) and decreases the skewness of the histogram. **(C vs. D)** Decreasing μ_i has a similar effect to decreasing d.

4.6 Discussion

The results of the present study are original in several aspects. The outcome of evolution in previous deterministic models has been $\sigma = 1$ (Ackermann et al. 2007). My results represent a more realistic scenario in which the outcome of evolution depends on both organismal and ecological parameters and is not limited to complete asymmetry. *S. cerevisiae* and *Schizosaccharomyces pombe* (*S. pombe*) are examples of unicellular organisms that stand in two completely different positions on the asymmetry scale. While the former organism segregates damage using a segregation coefficient of approximately 0.75 (Aguilaniu et al. 2003), segregation of damage is very much symmetric ($\sigma \approx 0.55$) in the latter (Minois et al. 2006). The present model is capable of accounting for this range. This study is also the first demonstration of the effects of the remarkable stochasticity that might have strongly influenced one of the most likely evolutionary origins of ageing, i.e. asymmetric segregation of damage (Kirkwood 2005; Lindner et al. 2008). Instead of having a single strategy (the optimal strategy) gradually moving to fixation we had a wide range of strategies at steady state. These less fit strategies spanned the symmetry-asymmetry continuum and persisted with time. As a result, complete asymmetry was never a strategy followed by more than a quarter of the population. The trivial explanation for this interesting observation is that mutations play a scattering role and as soon as natural selection has removed less fit strategies from the population, new mutations have brought about the same strategies in other individuals. The second possible explanation for why the percentage of individuals with complete asymmetry does not get arbitrarily large (even though asymmetry might be the best strategy under certain conditions) concerns the high risk of damage-induced death in half the progeny of completely asymmetric individuals. Specifically, if the rate of damage accumulation is high enough,

individuals with highly asymmetric strategies are at serious risk of losing half of their progeny whereas less asymmetric individuals may manage to do better. Finally, given the risk of death depends on damage and different strategies lead to different amounts of damage in their followers, the fitness of one strategy may depend on the frequency of that strategy as well the frequency of other strategies in the population (frequency-dependent selection).

I determined the contribution of key factors such as proliferation rate, damage accumulation rate, and damage severity to the evolution of asymmetry. Without sufficiently rapid accumulation and severe effects on survival, damage cannot generate enough pressure to promote the evolution of a new trait, i.e. asymmetry. Likewise, rapid proliferation allows the cell to free itself of damage by passing it to the next generation before damage can reach critical levels to threaten survival. Finally, the model provided in this study provides a generic framework into which additional parameters may be incorporated in future. Examples of such parameters are environmental perturbations, repairable forms of damage, sexual reproduction, spatially structured populations, direct cell-cell interactions, and resource flux into the environment.

4.6.1 Asymmetry and trade-offs in resource allocation

Limited resource availability generates a well-known trade-off between investments in reproduction and maintenance/repair (Stearns 1976; Fischer et al. 2009). More investment in reproduction by a binary dividing unicellular organism translates to more rapid proliferation, whereas more investment in maintenance/repair functions reduces the rate of damage accumulation. I showed that rapid proliferation and low

rates of damage accumulation reduce the selection pressure for asymmetry. Taken together, it can be expected that the trade-off between reproductive and maintenance investments would render the selection forces less sensitive to the exact investment strategy. Also, since damage accumulation is the product of the incoming stress and the strength of cellular defence/repair systems, the impact of alterations in the environmental stress on asymmetry will be major and dominant (Figure 4.5).

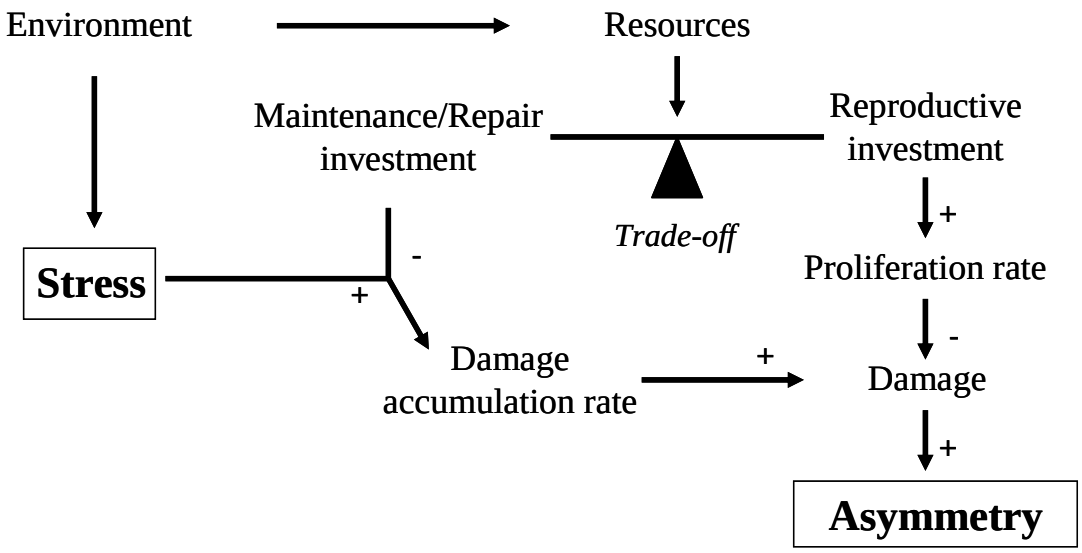

Figure 4.5: Schematic diagram showing various trade-offs affecting the evolution of asymmetry. There is a trade-off between investments in reproduction and maintenance/repair, and the model predicts that investment in either function acts to reduce the selection pressure for asymmetry. Selection will then be less sensitive to the exact investment strategy. Since damage accumulation is the product of the incoming stress and the strength of cellular maintenance/repair systems, the impact of alterations in the environmental stress on selection pressure will be major and dominant.

In support of this prediction, treatment of *S. cerevisiae* with paraquat (an oxidizing agent) increased the asymmetry in segregation of carbonylated (a type of irreversible oxidative damage) proteins (Aguilaniu et al. 2003) (Figure 4.6A). The long-term role of the ecological niche on evolution of segregation strategies with different levels of asymmetry remains to be seen.

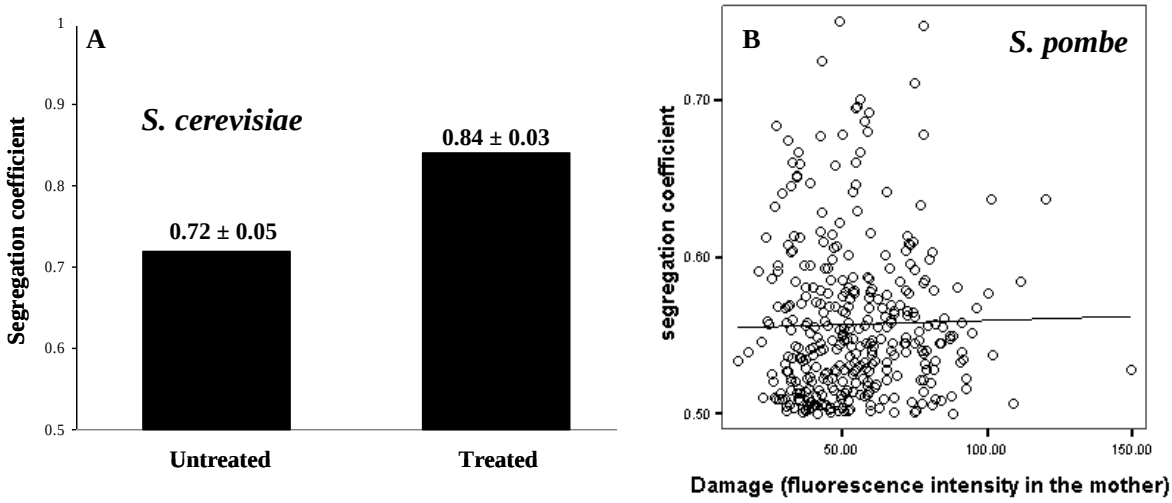

Figure 4.6: Experimental evidence for different levels of segregation asymmetry. **(A)** Treatment of *S. cerevisiae* with paraquat (400 µg/ml) for 20 minutes increased the asymmetry in segregation of carbonylated proteins (adapted from (Aguilaniu et al. 2003)) **(B)** In a total of 347 mitoses monitored in an experiment on *S. pombe*, there was no correlation (r = 0.021; *P* value = 0.692) between the segregation coefficient and the level of carbonylated proteins that existed in the mother cells undergoing mitosis (adapted from (Lindner et al. 2008)).

If asymmetry is a way to resist deleterious environmental circumstances, then mutations that promote asymmetry will be favoured in harsh environments. For the same reason, asymmetry might be an alternative strategy to heavy investments in

maintenance functions, thus providing a higher proportion of resources for reproduction. If this prediction is correct, one could then expect the asymmetric *S. cerevisiae* to have smaller maintenance investments and thus be less stress resistant than the symmetric *S. pombe*. Making such comparisons between these two species is not easy, given that their ancestors diverged more than 500 million years ago and are therefore evolutionarily rather distant (Heckman et al. 2001; Hedges 2002; Lum et al. 1996; Sipiczki 2000). Nevertheless, the existing evidence supports my prediction. Cadmium (Cd^{2+}) is a heavy metal which exerts its toxicity to the cell through reactive oxygen species generation, among other mechanisms (Vallee & Ulmer 1972). The subsequent oxidative stress can damage cellular components. Yeast responds to the Cd^{2+} stress by enhancing the activity of cellular defence mechanisms. Enhanced synthesis of glutathione (GSH) and phytochelatins are examples of such mechanisms to immobilize the toxic metal ions (Mehra & Winge 1991; Perego et al. 1997; Perego & Howell 1997). Interestingly, *S. pombe* has been shown to use both GSH and phytochelatins to resist Cd^{2+} stress, whereas *S. cerevisiae* only uses the latter. Consequently, *S. pombe* shows much higher Cd^{2+} tolerance than *S. cerevisiae* (Bae & Chen 2004). The segregation coefficient used by *Candida albicans* was identified very recently. This organism uses an asymmetric strategy ($\sigma = 0.65$) for segregation of irreversibly damaged proteins (Fu et al. 2008). By the same lines of arguments as above, we may predict higher maintenance investments made by *Candida albicans* than by *S. cerevisiae*. Indeed, the former organism is known to enjoy a significantly higher heavy metal resistance than the latter (Weissman et al. 2000; Wang & Chen 2006). However, one should keep in mind the very different ecological conditions in which *Candida albicans* (a pathogenic fungus adapted to host defences) and *S. cerevisiae* have evolved. Further experimental evidence for predictions of the model

concerning the effects of asymmetry on evolution of resource allocation trade-offs requires more comparative research.

4.6.2 Ecological aspects for the evolution of asymmetry

In variable environments, genes that enhance phenotypic plasticity may be favoured over ones that result in a fixed response (Fischer et al. 2009; Agrawal 2001). Given the role of damage in promoting the evolution of asymmetry, one would expect in unstable environments that cells that are capable of increasing asymmetry in response to high levels of damage would be fitter than those adopting a constant segregation strategy (provided that the costs associated with plasticity are not too high). This facultative strategy offers an explanation for the observation that transient treatment of *S. cerevisiae* with oxidative stress increased the asymmetry in damage segregation (Figure 4.6A) (Aguilaniu et al. 2003). In contrast, *S. pombe* does not change its symmetric segregation strategy with varying levels of damage. In a total of 347 mitoses observed in an experiment on *S. pombe*, there was no correlation ($r = 0.021$; $P = 0.692$) between σ and the level of carbonylated proteins that existed in the mother cells undergoing mitosis (Figure 4.6B) (Minois et al. 2006). The presented model would support a prediction that *S. cerevisiae* evolved in a relatively unstable environment, while the environment in which *S. pombe* evolved has probably been more stable.

The ability to respond adaptively to environmental changes requires a certain level of complexity. When change is rare, populations may persevere by relying on rare and unpredictable mutations that arise over generations. Plasticity is not a superior strategy under these conditions (Pigliucci 2005). When change is frequent relative to

the lifetime of an organism, however, signalling pathways that convert environmental cues to an appropriate response may evolve. In effect, the resulting phenotypic plasticity regulated by signalling embodies within one organism the equivalent change one would expect to arise from mutations selected under more static environmental conditions. A signalling pathway, for example, would contain a component which detects macromolecular damage (resulting from environmental insults) and would then activate a downstream component which, through interacting with the cytoskeleton, could eventually orient the mitotic spindle and alter the way damage is segregated at division. One such pathway can be represented as damage-heat shock protein-glsA/rsp1p-cytoskeleton. Heat shock proteins, a large family of highly conserved and constitutively synthesized molecular chaperons, assist refolding and degradation of misfolded proteins, and may mediate the upstream part of the pathway (Bardwell & Craig 1984; P. Liang & MacRae 1997; Kültz 2003; Kültz 2005). Hsp70 (DnaK) and Hsp104 (ClpB) are two widely studied chaperons that bind to irreversibly damaged (e.g. carbonylated) proteins (Erjavec et al. 2007; Zimmerman et al. 2004; Mogk et al. 1999; Hartl 1996; Barnett et al. 2005). On the downstream side, there are certain J domain proteins (e.g. rsp1p in *S. pombe* and glsA in the simple multicellular organism *Volvox carteri*) that form a bridge between heat shock proteins and the cytoskeleton (Zimmerman et al. 2004; Miller & Kirk 1999). Thereby, the signal (i.e. damage) can be converted to the appropriate response, i.e. the asymmetry level which maximally benefits the organism.

4.6.3 Costs of asymmetry

Repair of reversibly damaged macromolecules and degradation of irreversibly damaged ones are two principal ways used by cells to protect themselves against

deleterious effects of damage on survival. There are significant costs associated with possessing efficient repair/degradation systems (Kültz 2005). For instance, several steps in protein degradation and protein chaperoning depend on the hydrolysis of ATP, including the activity of 26S proteasome and Hsp70 (Babbitt et al. 2005; Hartl & Hayer-Hartl 2002). Chaperones such as Hsp70 are expressed at a high level and maintained at concentrations up to more than 1% of total cellular protein (Söti & Csermely 2007). Although chaperones have other functions as well in the cell, they are key players in detection of protein damage. The costs associated with maintaining the integrity and therefore the fitness of the cell have to be taken from the total energy budget of the cell and are therefore inevitably detracted from growth/reproductive capacity of the cell. Furthermore, these costs have to be paid by the same cell. Consequently, it would be worth investing in less costly alternatives if available. Asymmetry-generating mechanisms might be a suitable choice because the cytoskeleton, the primary requirement for asymmetric segregation of damage, has already had to evolve in order to provide the scaffolding for the cell. The same is true for many molecular chaperones that have several different functions in the cell. Furthermore, asymmetry is a way to get help from one's close relatives (e.g. one's daughter cells) in handling damage. A comprehensive understanding of how cells allocate their energy budget to maintenance and reproductive functions is not possible without considering asymmetry and its contribution to fitness. Chapter 5 describes a sophisticated analytical model for the evolution of asymmetry with the costs considered within the model.

The intricate trade-offs that can be thought (as a result of this new perspective) to accompany the cells throughout their evolution may provide an explanation for the

observation that *S. pombe* opts for degradation rather than asymmetry for handling irreversible damage. One reason concerns the potential costs of evolving appropriate protein-protein interactions for the sophisticated asymmetry-generating machinery to work even though the raw materials for the system might have already evolved for other purposes. Selection in my model was only for, not against, asymmetry because asymmetry was assumed to be at no additional costs to the cell. The other reason underlying the *S. pombe* preference for segregation symmetry may be related to the size symmetry of this organism at division. If the size of the two daughter cells was to be kept equal (or near equal), asymmetric segregation of damage would pose an additional cost, other than its own associated costs, to the limited energy budget of the cell. The cell would then need to invent a likely different mechanism for asymmetric segregation of normal proteins in order to fill the daughter cell that received less damage. The important assumption behind this hypothesis is of course, that size symmetry *per se* was a major goal for the cell to keep unaltered.

4.6.4 Future directions

In this study, I showed that, at least in unicellular organisms, asymmetry and its associated consequences need to be taken into account when thinking about the fitness of cells. In particular, mechanisms responsible for generating segregation asymmetry may affect the way a cell chooses to allocate its metabolic budget to various functions. Furthermore, the trade-off between reproductive and maintenance investments renders the evolution of asymmetry sensitive to environmental factors. It was also shown that evolution of asymmetry is under the strong influence of stochasticity. I hypothesize that the variation of ageing phenotypes in multicellular organisms (Kirkwood et al. 2005) may have arisen through millions of years of

natural selection from the stochasticity that existed in the evolutionary origin of ageing. Whether and how the failure of model populations to achieve homogenously fit steady states (due to the continued presence of less fit strategies) can be linked to variations in the course of ageing in higher organisms is a potential area for future research.

For multicellular organisms, embryonic development represents a stage where phenotypic plasticity has important and potentially lifelong effects and it is highly likely that an asymmetric segregation strategy will play a role. Recent evidence suggests that damaged proteins targeted for proteasomal degradation are asymmetrically distributed during mitosis in human embryonic stem cells (Fuentealba et al. 2008). Furthermore, there is emerging evidence for asymmetric segregation of damage during adulthood in higher eukaryotes. Irreversibly damaged proteins in *Drosophila melanogaster* neuroblasts and intestinal crypts of patients with protein folding disease are transported to accumulate in aggresomes at the microtubule organizing centre and then asymmetrically distributed to one of the daughter cells (Rujano et al. 2006). How asymmetric damage segregation which had already evolved in unicellular organisms for the benefit of the cell has been adapted by nature at transition to multicellularity remains to be seen. More discussion will follow in Chapter 5.

Acknowledgements

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Chapter 5: An analytical model for the evolution of asymmetric damage segregation considering possible costs of asymmetry

5.1 Introduction

The ability of all forms of life, from simple unicellular organisms to complex eukaryotes, to continue to survive is closely linked to their ability to detect errors that occur in their macromolecules and then repair them. From an evolutionary perspective, the primary function of the maintenance systems is to provide a sound internal state as the organism grows and reproduces. The external environment, however, is not under control of the organism. Indeed, the environment is typically highly variable, harsh and stressful. One inevitable product of such environments for the organism is cellular damage which, if not properly countered, threatens the survival of the lineage in unicellular forms of life and the integrity of tissues in multicellular organisms. Unrepaired genetic damage is particularly dangerous because of its direct flow to future generations. Non-genetic damage (e.g. protein damage) is important too. When a cell divides at mitosis, all of its cytoplasmic materials, including damaged macromolecules are distributed during cytokinesis between the two daughter cells. Depending on its reparability, all or parts of damage may be further transferred to the next generation.

How can a cell deal with damage and maximise its Darwinian fitness in environments with various sources of stress? Irreversible protein damage is a serious problem because the multitude of costly repair mechanisms that exist in the cell is of no use in this regard. The way this damage is inherited by the daughter cells is also important. Maximal dilution occurs with symmetric distribution of damage at division. In order to be effective, symmetry needs to be accompanied by sufficiently rapid proliferation

(Rashidi 2008). Otherwise, both daughter cells (i.e. the lineage) would suffer the same risk of accumulated damage rising eventually to a lethal level. Thus, symmetry is expected to be an efficient strategy to cope with irreversible damage when the environment is not too harsh, the cell is sufficiently rich in repair mechanisms, and/or proliferation is sufficiently fast. The advantage of making do with symmetry is that it does not require a separate and potentially costly mechanism to evolve. The disadvantage concerns the requirement for sufficient investment in maintenance (i.e. efficient repair) and/or reproduction (i.e. rapid proliferation). As an example, damage inheritance in the unicellular fission yeast *Schizosaccharomyces pombe* (*S. pombe*) is strikingly symmetric. The "old" daughter cell in this organism receives on average only 55% of maternal carbonylated proteins (Minois et al. 2006).

In contrast, asymmetric segregation of damage favours the survival of a lineage by specifically promoting the survival of the daughter cell that inherits less damage. Sufficient asymmetry (one daughter cell is born with no damage in the extreme case) may guarantee lineage survival and compensate for slow reproduction and/or inefficient maintenance systems. Not surprisingly, asymmetric inheritance is prevalent in all kingdoms of life, from yeast to higher eukaryotes (Aguilaniu et al. 2003; Macara & Mili 2008; Rujano et al. 2006). The main issue with asymmetry is a potential need for specific mechanisms including new genes, molecules or interactions to evolve. It is not trivial to see what proportion of resources the cell is best to invest in maintenance, reproduction, and possibly asymmetry when resources are limited. Resource limitation causes a multitude of trade-offs at different levels (subcellular, cellular, organismal) to emerge (Fischer et al. 2009; Stearns 1976). Mathematical modelling is an alternative to the classical experimental approach in the study of these

trade-offs. Here I investigate and model the evolution of non-genetic damage segregation in unicellular organisms.

5.2 The model

5.2.1 Basic assumptions

The model is based on a single haploid asexual cell which founds a genetically homogenous colony. Regarding damage accumulation and degradation, I study 3 genes, each with one quantitatively identified locus. The quantitative value (s, Δ, g) of the genes (e.g. corresponding to their expression level) determines the strength of the particular traits they produce. The traits are protein synthesis rate, protein damage rate, and protein damage degradation rate, respectively. Since colonies are assumed genetically homogenous, I ignore rare mutations that might occur to the three genes of our interest. I am interested in damage only to the non-genetic materials inside the cell (e.g. proteins) and only irreversible types of damage (e.g. protein carbonylation (Stadtman 2006)). Proteins are synthesised at a constant rate s. A constant proportion of the existing proteins are assumed to become irreversibly damaged at rate Δ. While repair is the principal way by which cells handle reversible forms of damage, there are generally three ways to cope with irreversible damage: *i)* degradation (e.g. by the ubiquitin/proteasome system), *ii)* exocytosis, and *iii)* simply living with damage. With the latter, and if the cell survives to reproduce, damage may be diluted between the two daughter cells such that each inherits only part of the damage. The proportion of damage received by each daughter cell affects their chances for survival and thus their fitness (Ackermann et al. 2007; Evans & Steinsaltz 2007; Fredriksson & Nyström 2006; Watve et al. 2006). This proportion may also influence the survival of the colony (Erjavec et al. 2008). For example, with sufficient asymmetry in segregation

of damage, the daughter cells in each generation which are born with relatively little damage can guarantee the survival of the lineage. The level of segregation asymmetry is assumed to be a heritable trait and rare mutations are not considered. I combine strategies *(i)* and *(ii)*, collectively referred to as "degradation". Reproduction takes place by cell division during which damaged particles in the mother cell are distributed between the two daughter cells. The timing of cell division depends on the amount of undamaged materials (in this model, native proteins) in the cell (Erjavec et al. 2008). When the number of native protein molecules reaches a certain threshold, the cell divides. If damage (D) increases beyond a fixed threshold D^*, the cell dies. Death occurs by an apoptosis-like process and does not affect neighbouring cells.

Fitness (of a cell) is defined as the number of descendants it produces per unit of time. The unit of time is arbitrary (as long as it is constant for comparison purposes) and can be defined large enough for cells to reproduce. I assume that the most primitive unicellular organisms had more symmetric damage segregation strategies than the more recently evolved cells. Therefore, the default strategy is assumed to be symmetric and I try to find triplets of the form (s, Δ, g) for which asymmetry pays. As a specific case, if all members of a colony that segregate damage symmetrically and descend from a given triplet survive, asymmetry will be associated with no fitness advantage and will thus not evolve. I develop the model in three successive steps. First, I assume that degradation mechanisms have not yet evolved. Next, I relax this assumption. In these two steps, the cell does not have to pay any costs (metabolic, energetic) to evolve asymmetry. In the third step, asymmetry is associated with a fixed cost detracted from resources invested in maintenance and/or reproduction.

5.2.2 Structure of the model

There are 3 continuous-time processes (protein synthesis, protein damage accumulation, and damage degradation) and 3 instantaneous events (cell division, damage segregation, and cell death) in the model. The continuous-time processes are embedded in and modelled as the following 2 ordinary differential equations:

$$\begin{aligned} dp/dt &= s - \Delta p \\ dD/dt &= \Delta p - gD \end{aligned} \qquad (3)$$

Proteins (p) are synthesised at a constant rate (s) and are irreversibly damaged by a first-degree kinetic process with constant Δ. Damaged proteins (D) are also degraded by the same kind of process with constant g. The cell divides when normal proteins reach a threshold p^* unless damage has already reached a threshold D^* in which case the cell dies before division. Without loss of generality, let $p^* = 1$. Time (t) is measured from the time the cell separates from the mother cell and forms a new individual. In order to give our cells a chance to reproduce, I assume $s > \Delta$. Without this assumption, the cell dies before it can divide. Segregation of damage at division is assumed to occur according to a segregation coefficient σ, which is a heritable trait. A proportion σ of damage segregates to one of the daughter cells and the rest of the damage goes to the other cell. To set the initial conditions, note that $D(0)$ is the amount of damage that a daughter cell inherits from its mother. Since aggregation of damaged proteins makes them more stable (and so makes their turn-over slower than that of normal proteins), I do not consider the inheritance of normal proteins (Carrió & Villaverde 2003; Maisonneuve et al. 2008). In other words, let $p(0) = 0$.

5.3 Results

5.3.1 No degradation

In this case, corresponding to $g = 0$, the degradation mechanisms have not yet evolved or are negligibly primitive. The system has no fixed points (i.e. stable or unstable steady states) and the amount of accumulated damage increases in an explosive manner. The only chance for survival is rapid reproduction such that before damage reaches fatal levels, the cell has already divided.

To examine the fate of the population and the advantages of asymmetry, let us look closely into the behaviour of the model. It can be shown that the necessary and sufficient condition for survival of a cell (until division) is

$$D(0) < 1 + D^* + \Delta^{-1} sLn(1 - \Delta / s) \qquad (4)$$

The accumulated damage during a cell cycle is constant:

$$x = -1 - \Delta^{-1} sLn(1 - \Delta / s)$$

and (2) can be rewritten as:

$$D(0) + x < D^*$$

Define $d(0)$ to be the initial amount of damage in the founder cell. With that, three possibilities can be considered:

(i) $x < d(0)$: In this case, and as long as the cells follow a symmetric segregation strategy, the accumulated damage at the time of cell division declines with advancing generations and there is no death in the

population. Therefore, asymmetry cannot be associated with any fitness advantage in this situation.

(ii) $d(0) < x < D^*/2$: In this case, and as long as the cells follow a symmetric segregation strategy, the accumulated damage at the time of cell division grows with advancing generations to $2x < D^*$. Again, symmetry offers the highest possible fitness and there is no selection pressure for asymmetry.

(iii) $x > \max\{d(0), D^*/2\}$: In this case, and with symmetry, the accumulated damage at the time of cell division grows with advancing generations to $2x > D^*$. If the younger daughter cells receive more than a proportion $\max\{(D^* - x)/D^*, d(0)/(d(0)+x)\}$ of maternal damage, the population will disappear at some point. Sufficient asymmetry enhances fitness and prevents the population from vanishing.

5.3.2 With degradation

Now I evaluate the case where cells already possess degradation mechanisms and hence $g > 0$. The cell cycle duration and total damage accumulated in the cell at time of division, if the accumulated damage is not high enough to have already killed the cell are given by

$$T = -\Delta^{-1} Ln(1 - \Delta/s)$$
$$D_T = \Delta(g-s)/g(g-\Delta) + (D(0) + s/(g-\Delta) - s/g)(1-\Delta/s)^{g/\Delta}$$

The analysis of the system shows that with sufficiently long cell cycle duration, the dynamics of the accumulated damage in a cell follows one of the following 3 patterns:

(i) Damage increases to a finite maximum. This case occurs when $D(0) = 0$.

(ii) Damage decreases to a minimum. This case occurs when $D(0) > s\Delta / g(\Delta - g) > 0$.

(iii) There is an initial decline in damage after which damage increases to a finite maximum. This is the case for any condition that does not satisfy *(i)* and *(ii)*.

The system has one steady state $(p, D) = (s/\Delta, s/g)$, which is stable (Figure 5.1).

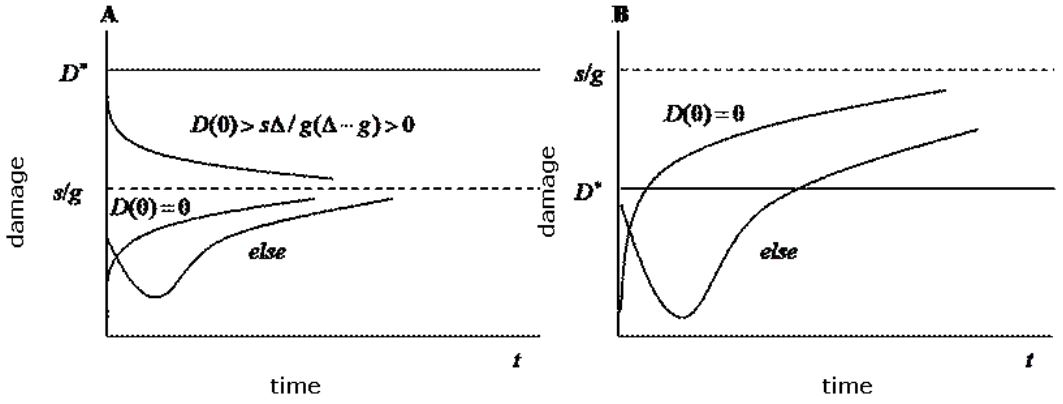

Figure 5.1: The stable steady state of the system with three typical trajectories. The diagram shows the situation in the presence of damage degradation mechanisms. All trajectories are attracted to the steady state, shown by the dashed line. (A) The ratio of maintenance to growth investment is sufficiently high ($g/s > 1/D^*$). (B) The maintenance/growth ratio is low ($g/s < 1/D^*$). D^* is the death threshold for damage.

Symmetry offers the highest possible fitness (all cells survive) when $s < gD^*$. Also, symmetry is the best strategy (all cells survive) if both of the following two conditions hold (see Appendix for detailed analysis):

$$gD^* < s < \Delta(1+D^*)/4$$
$$(\Delta - \sqrt{\Delta^2 - 4s\Delta/(1+D^*)})/2 < g < \min\{\Delta, (\Delta + \sqrt{\Delta^2 - 4s\Delta/(1+D^*)})/2\} \quad (5)$$

5.3.3 Costs of asymmetry

In the previous two sections, I have implicitly assumed that asymmetry has no cost (in terms of available resources or energy budget of the cell) for the cell, that is, the molecules that may provide an asymmetry-generating mechanism have already evolved in the cell for other purposes and asymmetry is either a costless by-product of their existence or the costs to the cell of the new interactions that need to be created between those molecules are negligibly small. Now, I relax this simplistic assumption and analyse a case in which the total energy budget of the cell is allocated in an optimal fashion to three categories of physiological functions: maintenance (required budget: e_m), reproduction (required budget: e_r), and asymmetry (required budget: e_a). There is no explicit mathematical formula to deal with this in a general way, so for tractability I only compare symmetry with complete asymmetry ($\sigma = 1$). In other words, I do not calculate the fitness conferred by partially asymmetric segregation strategies (e.g. $\sigma = 0.8$).

I make two further assumptions: *(i)* the rate of damage accumulation is inversely related to maintenance investments (under constant environmental conditions), and *(ii)* the cell cycle duration is inversely related to reproductive investments (Figure 5.2). Let us represent these two relationships with $g(e_m)$ and $f(e_r)$, respectively. Therefore, the amount of damage accumulated (and added to the initial damage the cell is born with) during a cell cycle will be $g(e_m)f(e_r)$.

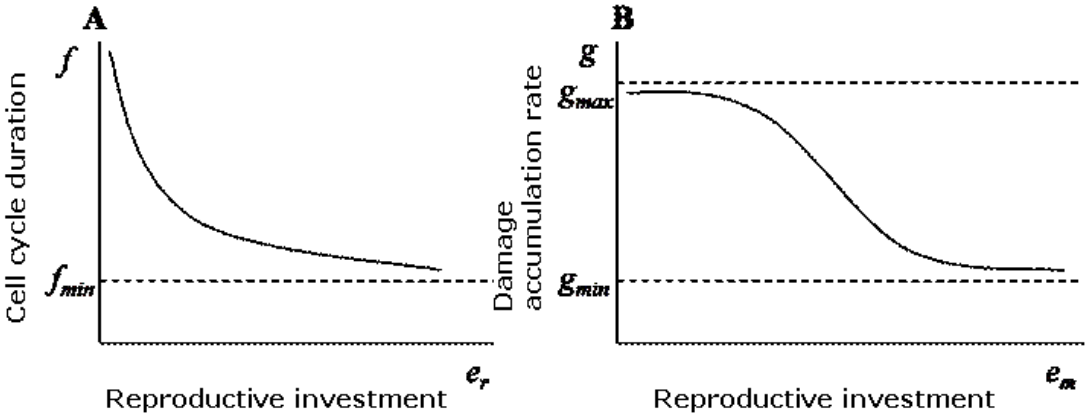

Figure 5.2: Fundamental relationships stemming from physiological trade-offs.

A cell invests in reproduction and maintenance. (A) The proliferation rate increases and thus the cell cycle duration decreases with increasing reproductive investment. The cell cycle can become infinitely long (with little investment in reproduction), but due to physicochemical constraints it cannot be shorter than a certain minimum. (B) The rate of damage accumulation decreases with increasing maintenance investment. The environment is assumed as a finite source of stress such that damage accumulation cannot be infinitely fast, even when maintenance investment is minimal. A minimum rate of damage accumulation is inevitable, irrespective of the level of maintenance investment.

It can be shown that with a symmetric segregation strategy, every cell in the population survives to reproduce if

$$g(e_m) f(e_r) < \min\{D^* / 2, D^* - D(0)\}$$

Consequently, the highest possible fitness with symmetry is achieved by

$$e_m(opt) = \min\{e_m : g(e_m) f(e_r) < \min\{D^* / 2, D^* - D(0)\}\}$$

(Figure 5.3) and the fitness (population size at time *t*) associated with this optimal strategy will be

$$\text{fitness}_{max} = 2^{t / f(e - e_m(opt))} \qquad (4)$$

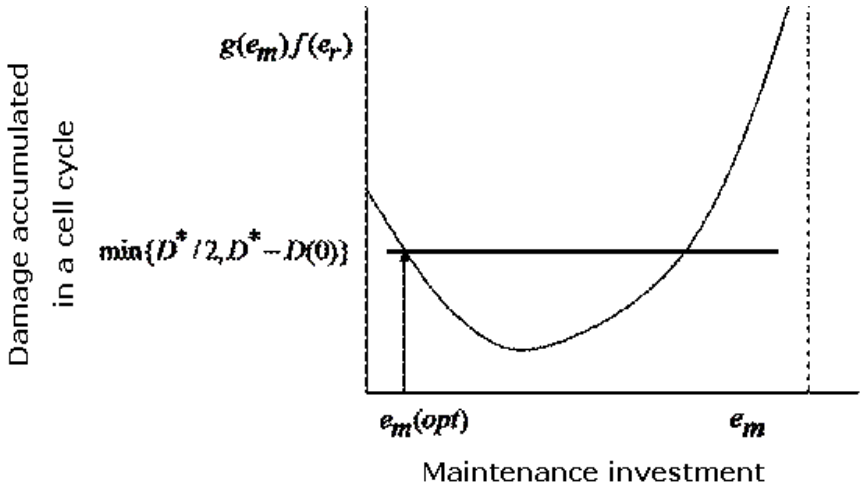

Figure 5.3: The condition in which symmetry offers its highest possible fitness advantage. Maintenance investment needs to be set at the lowest value for which the amount of damage accumulated during a cell cycle is less than a certain minimum (shown by the solid horizontal line).

With (complete) asymmetry, the amount of damage in a given cell increases to the time of division and then completely segregates to one of the daughter cells. As a result, one can trace the original cell through generations. When damage reaches the fatal threshold D^*, the cell dies. Therefore, a cell lives for a certain number, *l*, of cycles that depend on its initial damage, the fatal damage threshold, and the amount of damage accumulated in each cell cycle. We have

$$l = [(D^* - D(0)) / g(e_m) f(e_r)]$$

, where [u] is the largest integer smaller than or equal to u. For example, if the accumulating damage kills an initially damage-free cell (i.e. its old daughter cell in this example) during its second cell cycle, l will be 1 and the population size at any time (after the first cell cycle is completed) will be 2. Compared to the symmetric case discussed above, this is a considerably low fitness value. When l = 2 (i.e. damage kills a granddaughter of an initially damage-free cell), it can be shown that

$$N(n+1) = 2(N(n) - N_d(n)) \qquad (5)$$

, where $N(n)$ and $N_d(n)$ denote the total number of living cells and the number of cells that die in generation n, respectively. Further analysis shows that $N_d(n)$ is a Fibonacci number and hence

$$N_d(n) = (\varphi^{n-1} - (1-\varphi)^{n-1}) / \sqrt{5} \qquad (6)$$

, where $\varphi = (1+\sqrt{5})/2$. Substituting (6) into (5) and with some algebraic calculations we have

$$N(n) = 2(\varphi^{n+1} - (1-\varphi)^{n+1}) / \sqrt{5}$$
$$N(0) = 1, N(1) = 2$$

$N(n)$ in this formula generates delayed (i.e. without the first term in the classical sequence) Fibonacci numbers. There is no general formula for l > 2. The sequences corresponding to l > 2 are Fibonacci-like sequences in which each term is the sum of its previous l terms, and the first l terms of the sequence are increasing non-negative powers of 2. The growth rate of such sequences is initially higher than that of the simple power sequence derived in (4), which will eventually overtake the Fibonacci-like sequences. Therefore, asymmetry pays at small carrying capacities. When asymmetry is rare, it remains rare if the carrying capacity of the population is

sufficiently large. It should be emphasised that I have only considered $\sigma = 1$ as asymmetry. These results might be different with intermediate levels of asymmetry. This is important because although there is currently no known mechanism that generates a well-regulated submaximal level of asymmetry, the fidelity of damaged macromolecules in following complete segregation asymmetry may not be complete. This imperfectness becomes particularly important when the number of independently behaving damaged molecules is small (e.g. aggregation of damaged proteins) and the infidelity of segregation is considerable. The effects of this type of stochasticity are shown in Figure 5.4. In such conditions, a mechanism which is meant to produce complete asymmetry will actually lead to intermediate levels of asymmetry.

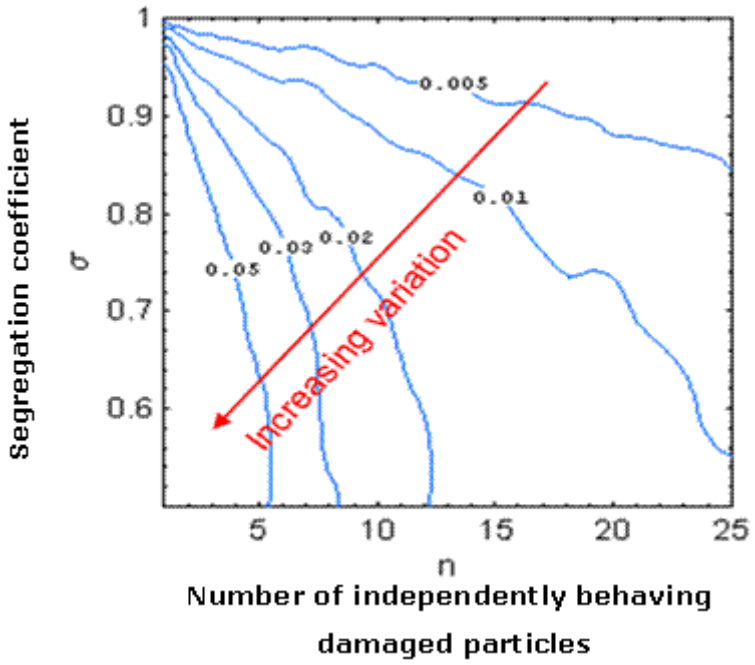

Figure 5.4: The effects of particle number and segregation fidelity on damage variation. Variance of the proportion of premitotic maternal damage received by d_1, the daughter cell into which damaged particles segregate with a higher probability, is higher for smaller values of n (the number of independently behaving damaged particles in the mother cell immediately before mitosis) and σ, the segregation

coefficient. Points located on a given curve exhibit the same level of variance in damage, represented by the number on the curve.

Figure 5.5 compares symmetric cases with asymmetric ones for the relationship between e_m and fundamental properties of the system (i.e. cell cycle duration and accumulated damage per cell cycle). The costs of asymmetry are inevitably taken from resources that could otherwise be allocated to reproductive and/or maintenance functions.

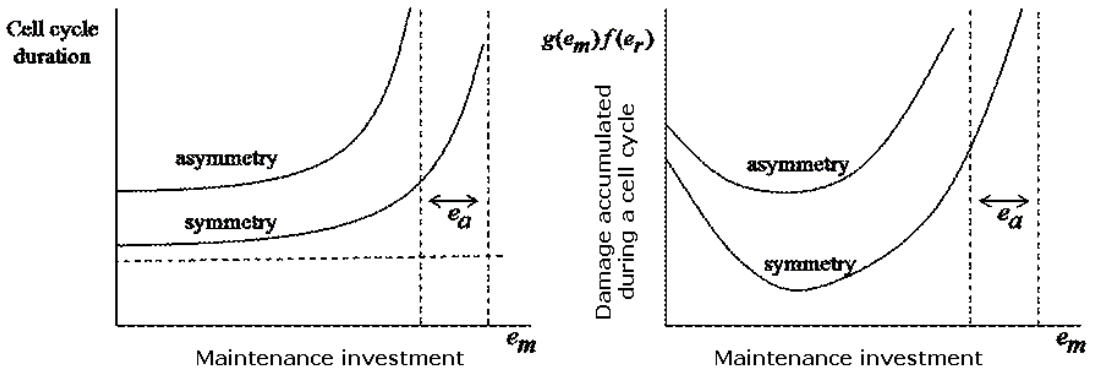

Figure 5.5: Comparison between symmetry and asymmetry for the relation between maintenance investments and fundamental properties of the system (i.e. cell cycle duration and the accumulated damage per cell cycle). With asymmetry, there are lower amounts of resources available for maintenance/reproduction investment. With asymmetry, the cell cycle duration curve is shifted to the left (left panel) and the damage accumulation curve is transformed and shifted to the left (right panel).

5.4 Discussion

5.4.1 General implications of the results

In the first section of the model, I showed that before degradation evolves, symmetry is an optimal strategy unless damage accumulation during a cell cycle is significantly high. This case is possible with small investments in reproduction, which prolong the cell cycle, and in stressful environments. If resources in such environments are low, cells will not be able to increase the energy allocated to reproduction and the damage caused by stress will accumulate during the (relatively) long cell cycle to dangerous levels. Asymmetry is the only solution in these conditions. In the second section of the model, cells had already evolved degradation mechanisms. As expected, asymmetry evolves less readily in this case. In particular, symmetry is an optimal strategy when the ratio of maintenance to growth investment is sufficiently high (i.e. $g/s > 1/D^*$). When this is not true, the condition derived in (5) opposes the evolution of asymmetry. It should be noted that there might be other cases in which symmetry is the optimal strategy. I only derived the conditions leading to two major categories of such cases.

The third section of the model was the most general situation and considered the possibility of asymmetry being associated with certain costs. Potential costs of asymmetry have not yet been identified. Many components of the known asymmetry-generating mechanisms have other functions in the cell. For example, heat shock proteins, a large family of highly conserved and constitutively synthesised molecular chaperones, assist refolding and degradation of misfolded proteins, and may mediate the upstream part of the pathway leading to asymmetric segregation of damage (Bardwell & Craig 1984; Kültz 2003; Liang & MacRae 1997). Two well-known heat

shock proteins that bind to irreversibly damaged proteins are Hsp70 (DnaK) and Hsp104 (ClpB) (Barnett et al. 2005; Erjavec et al. 2007; Hartl 1996; Mogk et al. 1999; Zimmerman et al. 2004). At least some elements required for asymmetry have therefore already evolved and hence natural selection could simply exploit them for a novel purpose without needing to pay additional costs. However, new molecular interactions might have been needed to be established and the costs associated with these inventions remain to be seen. Distal parts of the pathways that move damaged molecules (using the cytoskeleton) seem to be more specifically linked to the asymmetry mechanism. Examples of such components are certain J domain proteins (e.g. rsp1p in *S. pombe* and glsA in the simple multicellular organism *Volvox carteri*) that form a bridge between heat shock proteins and the cytoskeleton (Miller & Kirk 1999; Zimmerman et al. 2004). The costs associated with evolution of these molecules may have well been significant. It has been estimated that the average yeast protein can change its expression only by 0.5% without a change in energy costs visible to natural selection (Wagner 2005; Wagner 2007). The constraint introduced by the limited available energy budget on changes in gene expression become particularly significant at large effective population sizes, where rapid proliferation is tightly coupled to an efficient energy metabolism (Fay et al. 2004; Townsend et al. 2003). Recent studies on budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) suggest that even single amino acid replacements (which might generate asymmetry here) can be subject to natural selection on the basis of their material costs (Bragg and Wagner 2009). I showed that the carrying capacity of the population is a critical determinant of early stages of evolution of asymmetry, i.e. when asymmetry was rare. With abundant resources in the environment, for example, we do not expect high selection pressures for evolution and spread of asymmetry when it is rare.

The condition derived for evolution of asymmetry when it is costly is not uncommon. It is reminiscent of a well-known evolutionary question on origin of life. In competition between a Malthusian replicator (capable of template-mediated self-replication) and a one-member hypercycle (capable of both template-mediated and enzyme-mediated self-replication), large carrying capacities oppose the evolution of Malthusian replicators when they are initially rare (Michod 1999).

5.4.2 Links to ageing

The intrinsic biology of cellular ageing is closely linked to asymmetric damage partitioning (Ackermann et al. 2003; Johnson & Mangel 2006; Kirkwood 2005). Damage segregation in unicellular organisms is a strategy with several potential advantages including rapid growth (Evans & Steinsaltz 2007; Watve et al. 2006), improved damage handling (Ackermann et al. 2007; Fredriksson & Nyström 2006; Johnson & Mangel 2006), and more resistance against clonal senescence (Erjavec et al. 2008). Interestingly, neither symmetry nor asymmetry is universal. *S. cerevisiae*, *Candida albicans*, and *S. pombe* use, on average, segregation strategies $\sigma = 0.75$, $\sigma = 0.65$, and $\sigma = 0.55$, respectively (Aguilaniu et al. 2003; Fu et al. 2008; Minois et al. 2006). In Chapter 4, I developed a stochastic model to account for this range. Three parameters, namely d (damage accumulation rate), μ_i (damage-induced death), and m (proliferation rate), were found to be correlated to asymmetry. The simplest model which was capable of explaining more than 90% variation of the outcome of evolution was composed of d, m, and the interaction term $d \times \mu_i$. Specifically, large values of d and $d \times \mu_i$ and small values of m were significant predictors of asymmetry.

The model was stochastic and did not include costs of asymmetry. The following points summarise the results of the stochastic model:

1. The outcome of evolution depends both on organismal and ecological conditions. A significant proportion of individuals in mutation-prone populations do not follow the fitness-maximising level of asymmetry (as related to individual fitness) after the population has reached steady state distributions of segregation strategies.
2. High rates of damage accumulation and severe damage with sufficiently detrimental effects on survival promote the evolution of asymmetry. Mutations that promote asymmetry are particularly favoured in harsh environments.
3. Rapid proliferation reduces the force of selection for asymmetry.
4. Asymmetry might be an alternative strategy to heavy investments in maintenance functions.

Here I provided analytic proofs and considered asymmetry costs. The total energy budget available to the organism (the cell in unicellular organisms) is limited. Accordingly, the disposable soma theory of ageing is built on the trade-offs resulting from resource limitation and concerns the evolutionarily optimised balance between cellular investment in reproduction and maintenance/repair (Kirkwood & Holliday 1979). With high rates of extrinsic mortality in nature, it is not beneficial to put more resources into maintenance functions than are needed for the organism to survive to the time of reproduction. Due to this submaximal maintenance investment, the organism accumulates damage, declines in its physiological functions, and thus ages. While investment in growth, reproduction, maintenance, and repair directly affect the

investor, investment in asymmetry does not. It only makes sense when one considers the investor's related kin. The offspring and next generations, rather than the same cell, benefit from asymmetry investment. Nevertheless, the costs (if any) of asymmetry have to be paid by the same cell and from its total energy budget. Perhaps the disposable soma theory needs to be expanded to include asymmetry costs.

5.4.3 Transition to multicellularity

The results of the model developed here cannot be immediately extended to multicellular organisms. One important reason concerns the definition of fitness. In unicellular forms of life, the cell is the whole organism and so the fitness of the cell is the same as the fitness of the organism. The well-being of the cell is equal to the well-being of the organism and strategies that improve cell survival and/or reproduction are selected for in these simple organisms. This is not the case in multicellular organisms. All cellular strategies have to be tuned during the course of evolution and aligned with the benefit of the organism. Cell-level selfishness in a multicellular organism leads to disruption of cooperative behaviour and pathology (e.g. cancer (Michor et al. 2003)). Cells might even be sacrificed in order for the individual to survive and reproduce, as is thought to occur when intestinal stem cells preferentially undergo apoptosis following low-dose irradiation (Potten 2004). In spite of these issues, asymmetry has found its way into all kingdoms of life. Stem cell division is one example. Damaged proteins targeted for proteasomal degradation are asymmetrically distributed during mitosis in human embryonic stem cells (Fuentealba et al. 2008). Stem cell division in adulthood is the same. Irreversibly damaged proteins in *Drosophila melanogaster* neuroblasts and intestinal crypts of patients with protein folding disease are asymmetrically distributed to one of the daughter cells (Rujano et al. 2006).

Damage may act as a cell fate determinant by at least two ways. Firstly, it reduces the chances for survival. Secondly, it might attract signalling molecules. The generated signal may then activate certain metabolic pathways and eventually lead to altered cellular decisions such as growth and differentiation. Has damage and its asymmetric segregation been utilised by evolution at early stages of multicellularity to promote cellular differentiation and division of labour? Is the efficiency and activity of the maintenance/repair system regulated during embryogenesis in a way that damage levels change at specific times and at specific locations within the growing embryo? How much of the difference between somatic and germ-line protection against damage (e.g. oxidative) can be explained so? These are some of the questions that should be addressed in future research.

5.5 Conclusions

I developed a simple model for evolution of asymmetric non-genetic damage segregation in unicellular organisms and investigated the conditions in which asymmetry might be a beneficial strategy to evolve. The main components of the model were protein synthesis, damage accumulation, and damage degradation. The energy requirements of growth, maintenance, and possibly asymmetry were incorporated into the model. I suggest that asymmetry is a fundamental fitness modulator and if sufficiently costly, needs to be considered as part of the trade-offs that arise as a result of resource limitation. The most obvious application of my results concerns the evolutionary origin of ageing and yeast is the best known but only one target for testing the predictions of the present model. The model may be extended to include any form of non-genetic damage. Preferential segregation and accumulation

of extrachromosomal rDNA circles (ERC) in the mother cell have been proposed to contribute to yeast mother cell-specific ageing (Sinclair & Guarente 1997). One can choose to think of ERCs as damaged molecules and then use the idea of mother cell bias in asymmetric segregation (Shcheprova et al. 2008) to apply the model. Changes that occur to the asymmetry-related mechanisms at the transition to multicellularity are another interesting direction for future research.

5.6 Appendix

Here I show that with conditions introduced in equation (3) in the text, all cells survive under symmetry, that is to say, symmetry is the best strategy. By looking at Figure 5.1B, we realize that the condition for survival of the cell is

$$D_T < D^*$$

Considering the equation for D_T derived in section 3.2, we have

$$D(0) < s\Delta / g(\Delta - g) + [D^* + \Delta(g - s) / g(\Delta - g)][s/(s - \Delta)]^{g/\Delta}$$

If the right-hand side of the above equation is larger than D^*, it can easily be seen that everybody will survive. For this to happen we need

$$[s\Delta / g(\Delta - g) - D^*]\{1 - [s/(s - \Delta)]^{g/\Delta}\} + [\Delta/(\Delta - g)][s/(s - \Delta)]^{g/\Delta} > 0$$

, which then gives

$$s\Delta / g(\Delta - g) - D^* < [\Delta/(\Delta - g)][s/(s - \Delta)]^{g/\Delta}\{[s/(s - \Delta)]^{g/\Delta} - 1\}^{-1} \qquad (7)$$

The right-hand side of the above equation is larger than 1 for $g < \Delta$. For (7) to hold, we now only require

$$s\Delta / g(\Delta - g) - D^* < 1$$

Equivalently, we need

$$g^2 - \Delta g + s\Delta/(1 + D^*) < 0$$

This requires (for the above equation to have real roots)

$$s < \Delta(1 + D^*)/4 \qquad (8)$$

and

$$(\Delta - \sqrt{\Delta^2 - 4s\Delta/(1 + D^*)})/2 < g < (\Delta + \sqrt{\Delta^2 - 4s\Delta/(1 + D^*)})/2 \qquad (9)$$

Putting (8) and (9) together with $g < \Delta$, we have the conditions derived in (3). Note that these are sufficient, but not necessary, conditions.

Chapter 6: Conclusions and future work

In this thesis, I tried to show a systems-level approach to the evolution of ageing and age-related conditions. The approach was theoretical and spanned the range from unicellular to multicellular organisms, simple to complex systems, and cell-level to population-level. I used a variety of techniques including stochastic modelling, dynamical systems, and comparative physiology.

In chapter 2, I attempted to address a serious ignorance in the study of beta-cell dynamics. The massive literature that exists on beta-cell proliferation and death (both during normal conditions and the course of diabetes) and intrinsic and extrinsic factors that regulate life/death properties of beta-cells contains little information on how beta-cell dynamics may be regulated within an islet. Therefore, I developed a rigorous mathematical model of intra-islet beta-cell dynamics. The model takes advantage of the latest relevant data, and is sufficiently generic to include the new data that become available. I demonstrated that as a physically closed system with birth and death processes, the islet can be studied using evolutionary concepts. Implications of the model, once missing parameter values are known through empirical work, would readily extend to long-term disturbances in glucose homeostasis, e.g. type 2 diabetes. Predictions of the model cover cases of stable and unstable diabetes. It is a major challenge to keep diabetic patients metabolically stable and avoid unprecedented deterioration of glucose homeostasis. Such deteriorations, mathematically known as bifurcations and hysteresis, can be studied in the flexible framework provided by the model.

The main theme of chapter 3, which was on beta-cell antioxidant defences, was hypothesis generation based on comparative data. Given the frequently observed conservation of metabolic and homeostatic pathways, a parsimonious explanation for nontrivial observations that does not require assumptions about too many new evolutionary inventions is always preferred. In chapter 3, I linked together, under a single evolutionary explanation, a number of unexplained observations on the glucose homeostatic system, examples being the weakness of beta-cell antioxidant defences compared to other tissues which is further exaggerated in females. A unifying theory was developed with the idea that beta-cell antioxidants are tuned to an optimal level so as to maximise the fitness of the organism. The optimisation is the best solution given costs and benefits of a potential adaptation. In order to develop the theory, molecular information about the production of ROS in beta-cells, ROS handling, and the effects of ROS on beta-cell physiology were explored and then combined with organism-level glucose homeostasis. This process involved considering the few insulin-independent tissues in the body, namely the brain and placenta (in female placental mammals). Given the importance of the brain and placenta in the evolution of vertebrates, and mammals in particular, the theory had to deal with co-evolution of glucose homeostasis, brain, placenta, beta-cells, and the stress response. And hence we can see organism-, and even higher-, level insights resulting from the theory. The major outcome of theory is a proposed history for the evolution of beta-cells since their emergence until their current status. The theory may be tested once data, containing information about beta-cell antioxidant status, become available, especially from extant species representative of the time when vertebrates and/or mammals first evolved. One way to test the predictions of the model in the lab would be through

manipulation of beta-cell antioxidant gene expression and looking at the fitness of the organism in the long term.

Chapter 4 was about the evolutionary origins of asymmetric damage segregation in unicellular organisms. The origins of ageing may well share the same route which gives the modelling work in chapter 4 particular importance. A number of recent studies have been done on evolution of damage segregation, and the results are definitely illuminating. However, the simplicity of most available models does not allow for successful explanation of all available data. The current literature contains valuable information about the asymmetry of damage segregation in *Candida albicans*, budding yeast, and fission yeast. The three organisms seem to follow three different strategies for damage segregation and a model to link these differences to adaptive evolution is lacking. Also, the intrinsic stochasticity that results from the small number of entities at the cellular level has not been incorporated in previous models. Using a simulation approach, I developed models of damage segregation in a generic unicellular organism and found the optimal segregation strategy that is expected to evolve under given conditions. Potential determinants of the optimal segregation strategy were both related to cellular processes (proliferation, death due to damage accumulation) and environmental factors (damage accumulation). The optimal strategy is the one that maximises Darwinian fitness under the given conditions. This approach has not been used in previous work. Future directions for research in this field include obtaining information on segregation strategies used by other unicellular organisms, the ecological conditions under which they have evolved, and the intrinsic cellular processes that determine their reproduction and sensitivity to damage. Once this information is available, one can easily test the prediction of the

model regarding the optimal strategy that is expected to evolve under the conditions of one's interest.

Chapter 5 presents an explicit analytical solution to the problem of asymmetric damage segregation. Costs of asymmetry were ignored in chapter 4 because the parameter space to be spanned would otherwise be too large. The advantage of this simplistic assumption, of course, was the simplicity of the model and the convenience with which the results in a stochastic context could be conceived. A more rigorous treatment including fundamental physiological trade-offs and potential costs of asymmetry was provided in chapter 5. The most important missing information to be available before quantitative predictions can be made from the model is data on costs of asymmetry. The signalling pathways involved in damage segregation use energy, and energy has to come from the cell's total energy budget. However, the significance of the costs relative to the total available budget is not known. Specifically, if the costs are not significant and do not seriously affect the cell's energy-determined trade-offs, a simpler version of the model developed in 5.3.1 and 5.3.2 may be applied. I speculated on what might have happened at the transition to multicellularity and how unicellular damage segregation strategies might have been used/co-opted at the transition into the developmental timeframe of the multicellular body. A better understanding would be feasible through comparative genomics studies that look at the conservation of genes and pathways across major evolutionary transitions. Higher degrees of conservation, in structure or function, would increase the likelihood of the segregation strategies being co-opted from the unicellular to the multicellular world.

In conclusion, the models and concepts developed in this thesis can be considered as case studies on application of an evolutionary systems-level approach to different aspects of ageing. The toolkits developed and used in these projects are by no means the only available tools and the ageing features that were studied are only a few of the many areas in which ageing research may be carried out. Limitations of this thesis are mostly related to the lack of experimental data to make more plausible biological assumptions, back up the results, and test the predictions. This highlights the importance of an interdisciplinary ageing research group. Nevertheless, as new data emerge in laboratories, the predictions of the models may be tested. Depending on the fit, the models can be refined if necessary.

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Appendix: publications

1. Nedelcu AM, Driscoll WW, Durand PM, Herron MD, Rashidi A. On the paradigm of altruistic suicide in the unicellular world. *Evolution* 2010 (in press).
2. Rashidi A, Kirkwood TBL, Shanley DP. Evolution of asymmetric damage segregation: a modeling approach. In: Breitenbach M, Laun P, Jazwinski M (Eds) Aging Research in Yeast. Springer Verlag 2010 (in press).
3. Rashidi A, Kirkwood TBL, Shanley DP. Metabolic evolution suggests an explanation for the weakness of antioxidant defences in beta-cells. *Mech Ageing Dev* 2009; 130: 216-221.
4. Rashidi A, Shanley D. Evolution of the menopause: life histories and mechanisms. *Menopause International* 2009; 15: 26-30.
5. Rashidi A, Kirkwood TBL, Shanley DP. On the surprising weakness of pancreatic beta-cell antioxidant defences: an evolutionary perspective. In: Pontarotti P (Ed). Evolutionary Biology: Concept, Modeling, and Application. Springer, Berlin-Heidelberg. 2009, pp 109-126.
6. Rashidi A. Modelling the origins of ageing [Featured article of the month (9 Jan 2009) in British Society for Research on Ageing]: http://www.bsra.org.uk/e-lifespan/frontline/modelling-origins-ageing
7. Rashidi A. On evolution of menopause. *Maturitas* 2008; 59: 283-284.
8. Rashidi A. Comment on: Macara IG, Mili S. Polarity and differential inheritance-universal attributes of life? *Cell* 2008; 135: 801-812. http://www.cell.com/comments/S0092-8674(08)01392-5.
9. Rashidi A. On adaptive onset hypothesis (AOH) of menopause. *Maturitas* 2007; 58: 208-209.