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A systematic look at an old problem

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As life expectancy increases, a systems-biology approach is needed to ensure that we have a healthy old age.

The continuing increase in life expectancy, which in many countries advances by several hours per day, is one of humanity's most astonishing successes. But as the population ages, new approaches are required to unravel the complex biology of ageing and understand its links with frailty and disease.

The increase in human life expectancy over the past ten years has taken both scientists and the population generally by surprise. Until recently, demographers were confidently predicting that once the gains made by reducing mortality in early and middle life had reached completion, growth in longevity would stop and we would see the fixed reality of the ageing process. This has not happened[1]. In much of the developed world, life expectancy continues to increase at the rate of five or more hours per day; in some developing countries, which have some catching up to do, the rate is even faster.

Precisely why life expectancy is still rising (Fig. 1), and where this process might end, is some thing we need urgently to discover. Think of it this way. You woke up this morning to what is effectively a 29-hour day. Twenty-four of those hours, you will use now; the other five will be put by for later. The challenge posed by population ageing translates into ensuring that these extra hours will be as good as possible, free from high-cost dependency, when in time we come to use them.

Meeting this challenge requires research that is neither overly simplistic nor overwhelmed by the apparent complexities of the ageing process. Lord Rayleigh, the 1904 Nobel laureate in physics, asserted that one should "neither seek nor avoid complexity" in finding appropriate solutions to problems. This approach is the cornerstone of a long-term effort to tackle the challenge of an ageing population using an intensely multidisciplinary approach known as 'systems biology'[2] — essentially, the study of interactions between the components of a biological system (see Box 1). Ageing is a highly complex biological problem that benefits greatly from systems biology. Equally, ageing will surely demonstrate the worth of systems science.

Not all scientists have been ready to engage with the complexity of ageing, however. Some have suggested that ageing is too complicated for serious scientific study, or that it is like a slow-motion car crash — everything just gets wrecked. There are even those who declare, rather strangely, that "there is no such thing as ageing — old age is associated with disease, but does not cause it"[3].

At the opposite extreme are those who see ageing merely in terms of some favourite mechanism — a simple matter of the erosion of telomeres (the protective structures at the ends of chromosomes), oxidative damage by 'free radicals', or the dysfunction of mitochondria (the energy-generating organelles within the cell).

The discovery that single gene mutations can cause major increases in lifespan in animals such as the nematode *Caenorhabditis elegans* prompted many to think that the genetic control of ageing lay in a simple programme that evolved, perhaps, for the altruistic purpose of

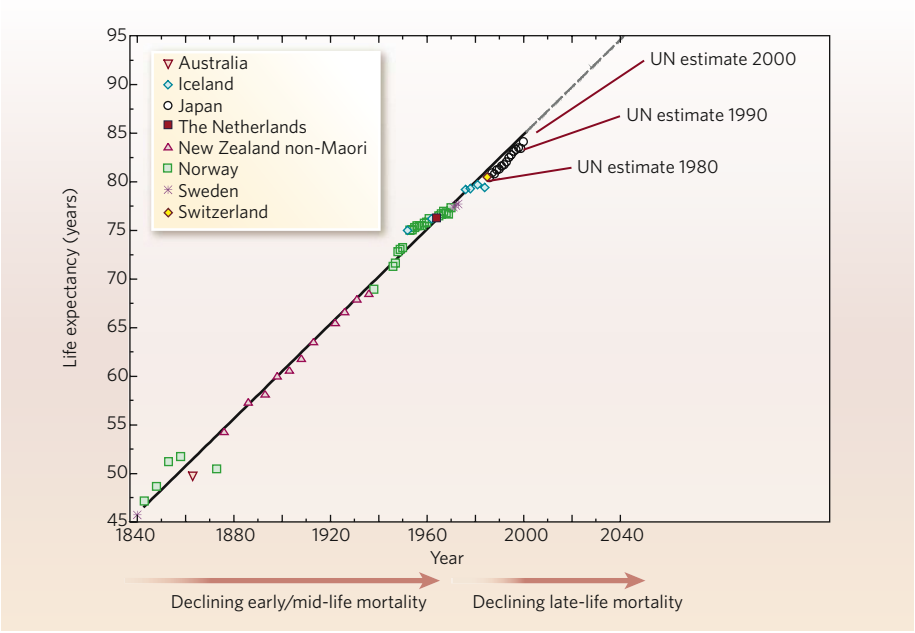

Figure 1 | Life expectancy around the world has increased steadily for nearly 200 years. The graph (adapted from ref. 1) shows the life expectancy in the then longest-living country. During the nineteenth and early twentieth centuries, the increase was driven mainly by improvements in sanitation, housing and education, causing a steady decline in early and mid-life mortality, which was chiefly due to infections. This trend continued with the development of vaccines and then antibiotics. By the latter half of the twentieth century, there was little room for further reduction in early and mid-life mortality. The continuing increase is due almost entirely to a new phenomenon: the decline in late-life mortality.

bringing life to a timely close, thereby creating necessary living space for progeny.

The idea that ageing is programmed was dismissed long ago by evolutionary gerontologists who recognized that natural selection could not, and would not, bring about such a fate (except in very special circumstances)[4,5]. Even for those who spurned such logic, the idea of programmed ageing began to seem rather odd when it was found that the genes that affect longevity do so not by changing the timing of a mechanism for self-destruction, but by adjusting hundreds of mechanisms for maintenance and repair[6,7]. If ageing were programmed, this would be a clumsy way to do it.

Bridging the simple and the complex

Clear consensus now exists that ageing is caused by the gradual, lifelong accumulation of a wide variety of molecular and cellular damage[8]. At the heart of the genetic determination of lifespan is the extent to which the organism's genome invests in survival. Because life is usually 'nasty, brutish and short', it serves no purpose to squander resources on maintenance and repair in an attempt to last indefinitely — this idea that the body must be expendable is the core of the 'disposable soma' theory[5,9].

But if ageing is a matter of things falling apart, can research realistically hope to achieve anything useful? The answer is emphatically yes — there is plenty of evidence that it is possible to intervene in the underlying causative mechanisms. Indeed, the malleability of the ageing process, as revealed by demography, derives precisely from the fact that it seems to be possible to slow the rate at which damage accumulates. Human longevity continues to increase when further gains from reducing mortality earlier in life are negligible because nowadays we reach old age, on average, in better condition than ever before.

Our understanding of the ageing process has advanced to a point at which it can be summarized relatively simply: molecular and cellular damage eventually results in frailty and disease (Fig. 2). But the devil is in the details. A great deal of complex biological research is needed to understand precisely which factors underlie our increasing longevity, and how far 'healthy ageing' is attainable. Even if, as some fear, obesity and sedentary lifestyles combine in the future to slow, or even reverse, the increase in life expectancy, we still need this understanding because unhealthy living usually drives people to early graves through pathology that is, to a significant degree, age-related.

Can't beat the systems

It was recently suggested that 'robustness' is one of the fundamental characteristics of biological systems, and that building a solid theoretical foundation of biological robustness is a key challenge for systems biology[10]. This is unquestionably true, but so is the converse: it is the pervasive vulnerability of living systems to damage, which erodes the functionality of the mechanisms underpinning robustness, that lies at the heart of ageing and disease. If the general tendency of natural selection has been towards greater robustness, then understanding ageing is the way to secure insights into the limitations and trade-offs that make robustness imperfect.

An enormous range of faults arises regularly in molecules, cells and tissues (Box 2). For each of these there is evidence that the relevant lesions do indeed accumulate during ageing, but for none of them do the faults seem sufficiently numerous to cause the systematic deterioration and loss of function that characterizes the senescent phenotype. Furthermore, because nearly all the mechanisms are intrinsically stochastic (subject to the laws of chance), there is marked variability from molecule to molecule, cell to cell, and individual to individual[11]. It is this combination of multiplicity and stochasticity of mechanisms that means there can be little expectation of a satisfactory understanding of ageing without adopting an integrative approach for exploring the synergy and interactions of the different mechanisms.

The need for a systems understanding is shown by the large volume of data on the possible contribution of oxidative damage to the mechanisms that underpin ageing. The suggestion that reactive oxygen species (commonly known as free radicals) cause ageing is already half-a-century old, and there is plenty of evidence to support this idea. Comparative studies have shown a strong association between the longevity of a species and the capacity of its cells to withstand oxidative stress[12]. But how

Box 1 | What is systems biology?

Systems biology can be viewed in a number of ways, as follows:

1. As a discipline or **field of study** in its own right, involving the quantitative analysis of interactions between elements of biological systems. There is an emphasis on complexity and large data sets, which are typically produced by a variety of high-throughput genomic, proteomic and metabolomic techniques.

2. As a set of multidisciplinary **methodologies**, in which the emphasis is placed on cycles of iteration between experimental data collection and computational or mathematical modelling. These lead to further development of theory, which in turn motivates new experimental investigations.

3. As an **integrative approach**, offering an alternative to the 'reductionist' approach that is seen by many to have dominated the research agenda for years.

4. As an **organizational phenomenon** involving the bringing together, in exceptionally close working partnerships, of scientists from diverse disciplinary backgrounds, particularly the biological, engineering and mathematical sciences.

Most initiatives in systems biology include several of these features. **T.B.L.K.**

Box 2 | Damage that may contribute to ageing

DNA damage (genome instability)
- Somatic mutations (copying errors, imperfect repair)
- Telomere shortening
- Chromosome rearrangements
- Mitochondrial-DNA mutations
- Gene disruption by viruses, transposons etc
- Aberrant epigenetic modifications

RNA damage
- Transcription errors
- Aberrant splicing

Protein damage
- Misfolding
- Synthesis errors
- Aberrant post-translational modifications
- Aberrant aggregation
- Impaired protein turnover (catabolism)

Membrane damage
- Oxidation

Additionally, there may also be disruption through stochastic variation in gene expression, cell-fate determination, differentiation, damage segregation during cell division, cell migration and cell death. **T.B.L.K.**

do we account for the fact that some long-lived species, notably naked mole rats, have exceptionally high levels of oxidative damage[13]; that transgenic disruption of key components of antioxidant defences does not necessarily affect lifespan[14]; and that dietary supplementation with antioxidants seems to have little or no effect? It seems a fair bet that answering these questions will require a much more systematic look at the complex network of reactions through which reactive oxygen species are generated and by which the cell defends itself.

Metabolic regulation

One of the most exciting areas of progress in ageing research is the discovery of metabolic factors that influence longevity. These include genes that affect insulin-signalling pathways[15], such as *daf-2* in *C. elegans*, the action of proteins known as sirtuins[16], or externally imposed changes in food supply (dietary restriction). In each case, the route to altered longevity seems to have some impact on the way the cell's maintenance systems are controlled.

Given the centrality of resource allocation in understanding how trade-offs are mediated between maintenance, growth and reproduction, it is not surprising that metabolic factors can modulate the level of maintenance. The scope of such effects merits study from an evolutionary perspective. In the case of *C. elegans*, an evolved metabolic plasticity is evident in the alternative developmental pathway that, when nutrients are scarce, generates the long-lived 'dauer' larva, a stress-resistant dispersal form[17]. This innate plasticity provides the basis for several of the most dramatic life-extension mutants in this species. In mice, quantitative modelling of the evolutionary energetics of reproduction and maintenance suggests that the life-extending effects of dietary restriction might be adaptive — as a means to wait out times of famine — but only under certain conditions[18]. The crux of the matter is whether enough energy can be diverted from reproduction to maintenance to make a physiologically important difference and whether, in a world where mortality pressure is high anyway, it actually boosts darwinian fitness. For humans, in whom reproduction is proportionately much less costly than in mice, the same logic suggests that dietary restriction is unlikely to postpone ageing[19].

Despite the evidence that metabolic regulation can have large effects on longevity, at least in small animals, it is important to note that it affects principally the rate of ageing and not, apparently, its nature. This preservation of the intrinsic complexity of the underlying molecular and cellular pathology is strikingly revealed in *C. elegans*, for example, where there is a large degree of stochastic variation within any given experimental regimen[20–22]. This provides a deeper challenge for the systems biology of ageing: to deliver the potential to intervene in the ageing process in ways that can specifically enhance the quality of later life. It seems highly unlikely that the ageing process itself will be abolished any time soon so, even if metabolic interventions are found to extend the years of healthy life in humans, we still need to grapple with the problem of age-related disease.

Ageing and disease

Age is by far the biggest risk factor for a wide range of clinical conditions that are prevalent today. One might therefore presume that a major effort is being made to understand the ways in which ageing renders the elderly more vulnerable to pathology. Nothing could be further from the truth. There is a large number of medical research institutes around the world, many with a focus on one or more of the major age-related diseases — cancer, heart disease, arthritis or dementia. Yet only a tiny fraction of these carries out any research on the intrinsic contribution from the ageing process itself.

Given that ageing is driven by damage, and that this is also true for the many age-related diseases, there must be considerable overlap between the underlying causative pathways. In cases of 'normal' brain ageing, for example, in which an older person's cognition remains essentially intact, autopsies reveal almost as many neurofibrillary tangles and amyloid plaques as are seen in patients with Alzheimer's disease[23]. This suggests that these lesions, which are taken as diagnostic for Alzheimer's, are far more closely connected with intrinsic ageing than is commonly thought, prompting questions about the role of underlying systems properties of the ageing brain.

Common factors, such as oxidative stress, are implicated in several age-associated diseases and, as in ageing itself, there may be important synergy between mechanisms. For instance, an accumulation of dysfunctional mitochondria will give rise to a decline in energy production, which in turn will lead to a decline in the efficacy of cell maintenance systems such as protein turnover, resulting in pathogenic protein aggregates, and so on. This may explain the association between mutations in mitochondrial DNA and neuronal death in the substantia nigra of brains in patients with Parkinson's disease[24,25].

One set of disorders in which the connections with ageing are particularly striking are the inherited human 'progeroid' syndromes, such as Werner's syndrome, in which the mutation of a gene coding for a DNA-maintenance enzyme causes early onset of multiple pathology. Such conditions, and their corresponding models in the mouse, highlight the complexity that needs to be understood. For example, in mice with a mutation in the *Ercc1* gene, increased DNA damage was recently discovered to cause the altered expression of more than a thousand genes, including downregulation of metabolic factors and upregulation of antioxidant and DNA repair pathways[26]. Although at first sight it seems surprising that the pattern of altered gene expression in this short-lived mutant was the same as that reported in long-lived genetic mutants or diet-restricted mice, it is entirely plausible from a systems perspective that widespread damage should trigger pathways that invoke heightened protection against such damage. Thus, the cycles of cause and effect are complex and can have different origins.

The connection between ageing and disease is profoundly important in cancer. Total cancer

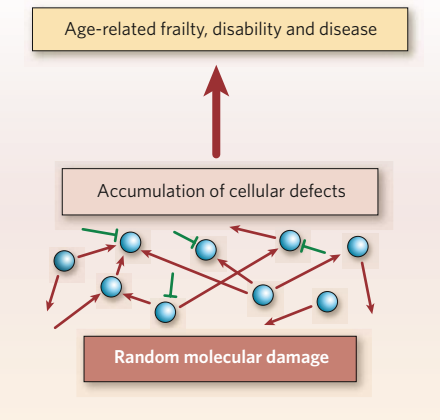

Figure 2 | The mechanisms of ageing. The ageing process is driven by the lifelong accumulation of molecular damage, leading to age-related frailty, disability and disease, and eventually to death. Although individual instances of damage are essentially random, the overall rate at which damage accumulates is regulated by a complex array of maintenance and repair pathways, which in turn may be modulated by metabolic factors. This scheme readily accommodates genetic influences on longevity (via the setting of maintenance functions) as well as contributions from environmental and lifestyle factors, which can influence exposure to damage and the capacity to withstand it.

Box 3 | Case study: systems biology of cellular senescence

An extensively studied model of cellular ageing is the cultured human diploid fibroblast, which divides only a finite number of times before entering a state of 'replicative senescence' (this number is known as the Hayflick limit). Although senescence is commonly attributed to simple telomere erosion, there is remarkable cell-to-cell heterogeneity in division potential.

There is evidence that the rate of telomere shortening is strongly affected by oxidative stress, and that an important source of damage-inducing 'free radicals' (reactive oxygen species) is the intracellular population of mitochondria, particularly those that are themselves damaged by random mutation. As a result, a mathematical model was developed that showed how the heterogeneity of cell senescence can be explained by the synergy of multiple mechanisms (oxidative damage, telomere shortening and the stochastic nature of mutation to mitochondrial and nuclear DNA)[31].

These modelling predictions prompted the experimental study of a role for mitochondrial dysfunction in senescence[32], something previously unexplored. Confirmation of this role has opened a new vista on the complex interactions underlying ageing in dividing cells. It has also provided a wealth of fresh and challenging data that will re-enforce the development of more detailed and realistic models, driving the process of discovery through a cyclic interaction between modelling and data. **T.B.L.K.**

incidence rises steeply with age and, across species, scales with longevity. The core of the connection is, of course, damage — how cells guard against it, and how they respond to it when it arises. Long-lived species invest in better maintenance, which delays both ageing and cancer. On the other hand, when damage does occur, it seems that cancer and ageing sit on opposite sides of a see-saw[27]. Deleting damaged cells too readily can protect against cancer but accelerates other forms of age-related pathology that are linked to cell loss. As we continue to discover an ever-increasing variety of types of damage-induced cellular senescence and probe their connections with cancer[28], we will need to build this knowledge into a systems framework.

Implementing systems approaches

Some substantive commitment will be needed to realize the huge potential benefits of addressing the challenge of ageing from a systems perspective. As in systems biology generally, scientists from biology, bioinformatics, computing science, mathematics, statistics and engineering must be enabled to build enduring partnerships. We have seen the creation of some systems-biology institutes, which provide physical co-location, but, although they are useful as exemplars, it is by no means obvious that in the longer term such ventures will be necessary or even optimal. Systems approaches need ultimately to be incorporated into the working practices of a majority of scientists.

Designing experiments that can simultaneously combine the contributions of different mechanisms to the ageing process is not easy (see Box 3). However, provided that experiments are planned with a view to making firm connections with other data, it is possible to structure an accumulation of knowledge in integrative models and in well-curated data archives[29]. For example, the Biology of Ageing e-Science Integration and Simulation (BASIS) system[30] provides a facility, supported by web services, for building, synthesizing and simulating mechanistic models using the Systems Biology Markup Language (SBML). An excellent example of a large-scale project in integrative systems biology is the Human Physiome Project. As further examples accrue, the momentum is expected to build.

The advances of recent years in understanding the mysteries of ageing are spectacular, but in truth we have only scratched the surface of this extraordinarily difficult problem. The intrinsic nature of the ageing process is essentially one of systems degradation. Only by systematically probing the complex mechanisms underlying ageing and its associated diseases can we transform our dramatic past successes in postponing death into a future in which ageing will, hopefully, lose some of its sting. ■

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