Finally, the most apparent drawback is the time required for each computation. Whereas a simple desktop computer can solve the seven-city instance of the Travelling Salesman Problem in less than a second, Adleman took seven days[1]. The use of DNA chips[2] or other approaches may eventually lead to automation, which would save considerable amounts of time, but fundamental DNA computing technology needs to advance far beyond its current bounds before it can be made practical.

DNA computing has its advantages, though. One is its massive parallelism — that is, brute-force algorithms can search through quadrillions of molecules at the same time and find a correct solution, akin to *in vitro* selection[3]. Another is miniaturization. And once the procedures are under control, the raw materials cost less too. "Here's nature's toolbox," commented Adleman[7], "a bunch of little tools that are dirt cheap; you can buy a DNA strand for 100 femtocents."

The near future

Now is an exciting time in the field of DNA computing, as there is so much that has not been tried. In June, over 120 molecular biologists, computer scientists, mathematicians and chemists from around the world gathered in Leiden[8] to discuss the latest in DNA computing technology.

Clearly a next step is automation. McCaskill and colleagues in Germany have constructed a 'microflow reactor' on which they propose to solve a 20-bit satisfiability problem in an hour and a half[8]. One could also construct a microfluidic device consisting of gated channels so small that only one molecule can pass through at a time[9], vastly improving readout[8]. And a team led by Adleman recently solved a 6-variable, 11-clause satisfiability problem using a 'dry' computer consisting of thin, gel-filled glass tubes[8].

As for DNA chips, their future in DNA computing looks bright as well, because 'universal' DNA chips could contain every possible DNA sequence of a given length (probably about 8–12 base pairs). Hagiya and colleagues in Tokyo are finding creative uses for single-stranded DNA molecules that fold into intra-strand 'hairpins'[8,10]. Winfree, Seeman and colleagues — responsible for construction of beautiful assemblies with DNA, such as a DNA nano-cube[11] — have proposed the assembly of even more ordered structures that show patterned algorithmic supramolecular self-assembly[8,11–13]. Even a handful of mathematicians have lent a hand, proposing faster and more efficient algorithms tailored to the needs of DNA computing[8].

Whether or not nucleic acid computers ultimately prove feasible, they have already contributed to multi-disciplinary science by causing us to question the nature of computing and to forge new links between the biological and computational sciences. For example, it has led us to focus on the nature of biological DNA computations, such as the assembly of modern genes from encrypted building-blocks in the genomes of some single-celled ciliates (FIG. 5)[14]. After all, our bodies already contain millions of complicated, efficient, evolved molecular computers called cells.

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TIMELINE

Hayflick, his limit, and cellular ageing

Jerry W. Shay and Woodring E. Wright

Almost 40 years ago, Leonard Hayflick discovered that cultured normal human cells have limited capacity to divide, after which they become senescent — a phenomenon now known as the 'Hayflick limit'. Hayflick's findings were strongly challenged at the time, and continue to be questioned in a few circles, but his achievements have enabled others to make considerable progress towards understanding and manipulating the molecular mechanisms of ageing.

To set Hayflick's discoveries in context, we need to go back to 1881 (TIMELINE, overleaf), when the German biologist August Weismann[1] speculated that "death takes place because a worn-out tissue cannot forever renew itself, and because a capacity for increase by means of cell division is not ever-lasting but finite". This concept, which was almost entirely forgotten by the time Hayflick began his work, was later challenged by the French Nobel-prize-winning surgeon Alexis Carrel, who suggested that all cells explanted in culture are immortal, and that the lack of continuous cell replication was due to ignorance on how best to cultivate the cells. Carrel's view was based on his and Albert Ebeling's work, done at the Rockefeller Institute in New York City, in which they claimed that chick heart fibroblasts grew con-
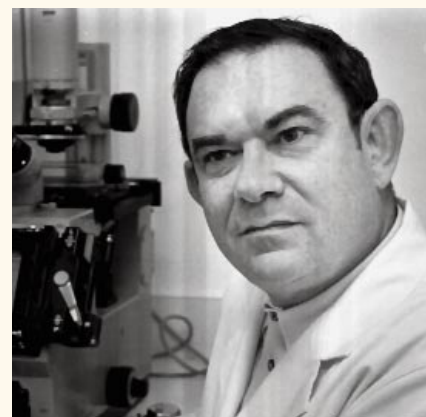

Figure 1 | **Leonard Hayflick in 1988.**
(Photograph: Peter Argentine.)

tinuously for 34 years[2]. This led to the general idea that all vertebrate cells could divide indefinitely in cell culture. However, Carrel's original observations could not be reproduced by other scientists[3,4], and may have been due to an experimental error[4]. The cells were fed with a daily extract of chick embryo tissue extracted under conditions that permitted the addition of fresh living cells to the culture at each feeding[3]. It has been suggested that Carrel knew about this error but never admitted it[5,6], but even if this explanation is untrue, no one has ever confirmed Carrel's work.

The Carrel experiments were of great importance because, if valid, they meant that normal cells freed from *in vivo* control mechanisms could function normally and, apparently, forever. However, reports were beginning to emerge of difficulties in long-term cell culture when Leonard Hayflick (FIG. 1) and Paul Moorhead entered the field. They brilliantly got to the heart of the matter, demonstrating finite replicative capacity of normal human fibroblasts and interpreting the phenomenon as ageing at the cellular level[3,4]. These initial observations sparked Leonard Hayflick's passion — which has lasted his entire career — to overturn the central dogma that all vertebrate cells grown in culture are immortal. But even today, there are sceptics. One is Harry Rubin[7], who stated: "The concept of a genetically predetermined number of human fibroblast replications, and its implied extension to other cells, is based on an artefact resulting from the damage accumulated by the explanted cells during their replication in the radically foreign environment of cell culture." Rubin is not alone in his opinion, and perhaps the truth lies somewhere in between. Nevertheless, the Hayflick limit is now generally accepted.

How Hayflick found his limit

After obtaining his Ph.D. in 1956 from the University of Pennsylvania, Hayflick spent two years with one of the leading personalities in tissue culture at that time, Charles M. Pomerat, at the University of Texas in Galveston. In 1958, Hayflick was recruited to run the Wistar Institute's cell-culture laboratory and also to initiate research on the possible viral aetiology of human cancer. He intended to expose normal human embryonic cells to cancer-cell extracts, in the hope of observing cancer-like changes in normal cells. When the normal cells no longer grew (FIG. 2), Hayflick thought he might have made a mistake in preparing the culture medium or washing glassware, or made some other technical oversight. He was assuming that Carrel was correct, and that cells could propagate indefinitely if provided with appropriate conditions. After

> "The largest fact to have come from tissue culture in the last fifty years is that cells inherently capable of multiplying will do so indefinitely if supplied with the right milieu *in vitro*."

all, it had been 60 years since Ross Harrison had started the field of cell culture, and normal cultured cells were thought to be immortal. For Hayflick to propose that a cell-division counting mechanism could be involved in ageing was a completely new idea. But Hayflick was young and ambitious, and a series of carefully conducted experiments over about three years convinced him that the failure of his normal cells to replicate indefinitely was not due to technical errors.

In 1961, working with the talented cytogeneticist Paul Moorhead, Hayflick did a series of experiments that challenged Carrel's views. Hayflick and Moorhead showed that populations of cultured normal human fibroblasts doubled a finite number of times, after which the cells stopped dividing and entered what Hayflick termed the phase III phenomenon[3]. He called the primary culture phase I; the ten or so months of luxuriant growth, phase II; and the period when cell replication diminished and ultimately stopped, phase III (FIG. 3). These initial experiments showed that the previous interpretation — that all cells are immortal — was incorrect. The principle behind these experiments was simple: Hayflick and Moorhead mixed equal numbers of normal human male fibroblasts that had divided many times (cells at the fortieth population doubling) with female fibroblasts that had divided only a few times (cells at the tenth population doubling). Unmixed cell populations were kept as controls. When the male 'control' culture stopped dividing, the mixed culture was examined and only female cells were found. This showed that the old cells 'remembered' they were old, even when surrounded by young cells, and that technical errors or contaminating viruses were unlikely explanations as to why only the male cell component had died[3].

Hayflick was convinced that normal cells have a finite capacity to replicate, and appreciated that their behaviour differed profoundly from that of cultured cancer cells (for example, HeLa cells) and transplantable tumours, which are immortal. It was this insight that originated the concept of immortalization of

normal cells[3,4]. The experiment with mixed cells further assured Hayflick and Moorhead that culture artefacts could not explain their observations. They submitted a paper describing their findings to the *Journal of Experimental Medicine* but Peyton Rous, one of the journal's editors, was not easily persuaded. After the paper had been peer-reviewed, Rous included the following statement in his covering letter: "The largest fact to have come from tissue culture in the last fifty years is that cells inherently capable of multiplying will do so indefinitely if supplied with the right milieu *in vitro*." The article was not accepted. Fortunately, the editors of *Experimental Cell Research*, where the paper was published[3] in 1961, were less swayed by the dogma of the day. This work and subsequent studies (TIMELINE) changed the tenor of research, eventually leading Sir Macfarlane Burnett, Nobel laureate from Australia, to coin the phrase "the Hayflick limit" for the first time in his book *Intrinsic Mutagenesis*, published[8] in 1974.

Hayflick's enduring impact

The durability and importance of Hayflick's work are reflected in its citation history. Between 1961 and 1999 this paper was cited about 3,000 times. Of the roughly 70 million scientific papers published since 1945, only one in every 135,000 has been cited as many times or more than this paper. Eugene Garfield[9], editor of *Current Contents*, stated
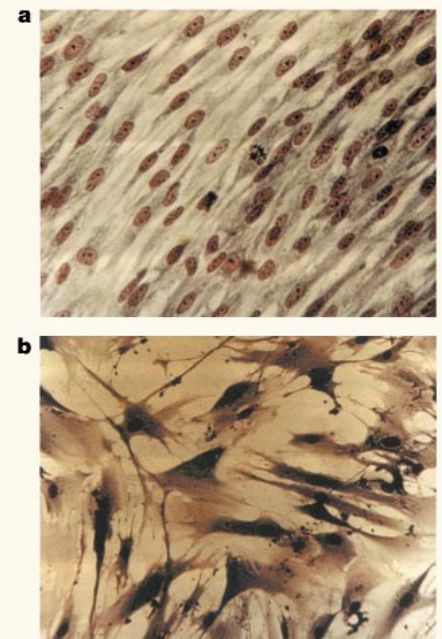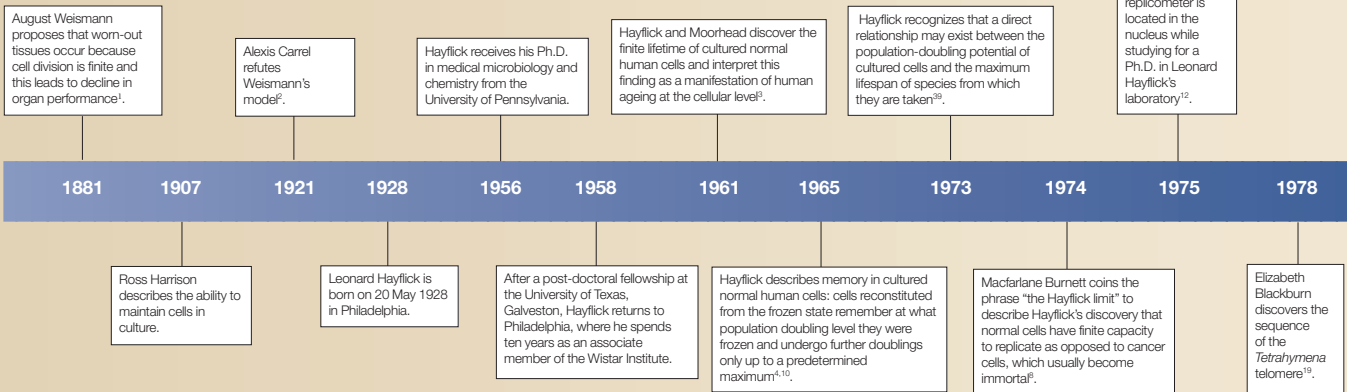
Figure 2 | **Young and old human diploid cells (strain WI-38). a** | Young cells in phase II at population doubling 20. **b** | Old cells in phase III at population doubling 55.

Timeline | **Hayflick and his limit**

August Weismann proposes that worn-out tissues occur because cell division is finite and this leads to decline in organ performance[1].

Alexis Carrel refutes Weismann's model[2].

Hayflick receives his Ph.D. in medical microbiology and chemistry from the University of Pennsylvania.

Hayflick and Moorhead discover the finite lifetime of cultured normal human cells and interpret this finding as a manifestation of human ageing at the cellular level[3].

Hayflick recognizes that a direct relationship may exist between the population-doubling potential of cultured cells and the maximum lifespan of species from which they are taken[39].

Woodring Wright shows that the replicometer is located in the nucleus while studying for a Ph.D. in Leonard Hayflick's laboratory[12].

1881	1907	1921	1928	1956	1958	1961	1965	1973	1974	1975	1978

Ross Harrison describes the ability to maintain cells in culture.

Leonard Hayflick is born on 20 May 1928 in Philadelphia.

After a post-doctoral fellowship at the University of Texas, Galveston, Hayflick returns to Philadelphia, where he spends ten years as an associate member of the Wistar Institute.

Hayflick describes memory in cultured normal human cells: cells reconstituted from the frozen state remember at what population doubling level they were frozen and undergo further doublings only up to a predetermined maximum[4,10].

Macfarlane Burnett coins the phrase "the Hayflick limit" to describe Hayflick's discovery that normal cells have finite capacity to replicate as opposed to cancer cells, which usually become immortal[5].

Elizabeth Blackburn discovers the sequence of the *Tetrahymena* telomere[19].

in 1983: "By studying accelerated ageing under glass, as Hayflick calls it, we can learn a great deal about changes in ageing cells that could contribute to functional losses throughout our bodies. Therefore, it is not surprising that research on tissue culture in ageing research is one of the most active ageing research fronts."

A cellular counting mechanism

The existence of a counting mechanism is implied by two of Hayflick's observations: first, that normal cultured human fetal cells only undergo a specific number of population doublings, and second, that cryogenically preserved cells can 'remember' how many times they have divided when they have frozen[10]. Although this mechanism has been referred to as a clock or timing mechanism, the replicative limit of normal cells is actually related to rounds of DNA replication, and not to the passage of time. Hayflick suggested the term "replicometer" be used to designate the putative molecular event counter[11]. So what is the molecular basis of the replicometer? In 1975, a doctoral student in Hayflick's laboratory, Woodring Wright, showed that the replicometer was located in the nucleus[12].

Telomeres and telomerase

In the early 1970s it was realized that the properties of DNA replication prevent the cells from fully copying the ends of linear DNA, called telomeres. Because of the nature of lagging-strand synthesis, DNA polymerase cannot completely replicate the 3′ end of linear duplex DNA. This was referred to as the end-replication problem (FIG. 4) in 1972 by one of the discoverers of the double helix, James Watson[13]. At around the same time, Alexey Olovnikov, a Russian theoretical biologist, had heard a lecture in which Hayflick's work was discussed. Olovnikov entered a Moscow subway station while wondering how normal cells might have a limited capacity to replicate, and, as the train stopped, he had a flash of insight. Olovnikov saw an analogy between the train representing the DNA polymerase and the track representing the DNA. If the train replicated the DNA track underneath the car, the first segment of DNA would not be replicated because it was underneath the engine at the start[14]. This was analogous to the end-replication problem described by Watson. Olovnikov realized that this repeated shortening of the DNA molecule at each round of DNA replication might explain Hayflick's finding that normal cells can replicate only a specific number of times. Although published in both Russian and English[15,16], Olovnikov's ideas languished in the literature until the golden era of molecular biology emerged in the late 1970s.

The presence of telomeres at the tips of chromosomes had been noted at least since a lecture given by Hermann Muller[17] in 1938 and the work of Barbara McClintock[18]. However, the function of these structures in cell replication was unclear. There was evidence that telomeres prevented the ends of chromosomes from fusing to each other and

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Box 1 | **Hayflick's achievements**

During his distinguished career, Hayflick has made several fundamental observations and is often credited with starting the field of cellular gerontology — the study of ageing at the cellular level. Hayflick, who is now a professor of anatomy on the faculty of the University of California, San Francisco, was Editor-in-Chief of *Experimental Gerontology* for 13 years, president of the Gerontological Society of America, chairman of the Scientific Review Board of the American Federation for Aging Research, and a founding member and chairman of the executive committee of the Council of the National Institute on Aging, NIH. He has received more than 25 major awards, is a fellow of the American Association for the Advancement of Science, an honorary member of the Tissue Culture Association, and author of over 225 scientific papers, reviews and the popular book *How and Why We Age* (Ballantine Books, New York, 1995).

Hayflick's achievements extend beyond cellular gerontology: he is also an accomplished microbiologist and was appointed Professor of Medical Microbiology at the Stanford University School of Medicine, Stanford, California, in 1968. He developed the first normal human diploid fibroblast cell strains. One of these, called WI-38, is still the most widely used and highly characterized normal human cell strain in the world[10]. He described the extraordinary sensitivity of cultured normal human fibroblasts to human viruses and suggested that these cells could be used for virus isolation, identification and vaccine production. He was the first to produce a vaccine (oral polio vaccine) from these cells[44]. WI-38 cells, or similar human-cell strains, are used today for the manufacture of most human virus vaccines throughout the world[45], including rubella and the Salk polio vaccine. Over 750 million virus vaccine doses have been produced on WI-38 or similar diploid cell strains. Hayflick established international standards for the production of human biologicals in passaged cells, which are still used today by the biotechnology industry[46].

Hayflick is also known for discovering the cause of primary atypical pneumonia in humans. This type of pneumonia was thought to be of viral origin, but Hayflick showed that it is caused by *Mycoplasma pneumoniae*, a member of the smallest free-living class of microorganisms[47]. *Mycoplasma pneumoniae* was first grown by Hayflick on a medium that he developed[48].

Timeline boxes:

Carol Greider discovers telomerase[26].

Jerry Shay and Geron scientists show that telomerase is present in all cancer-derived cell lines and in 90% of primary human cancers[28].

Roslin Institute scientists claim that the cloned sheep, Dolly, has shorter telomeres than an age-matched control[41]. Is Dolly a sheep in lamb's clothing?

The future — pharmaceutical companies are developing lead compounds for inhibiting telomerase in cancer cells. Methods for modulating telomere length in normal cells may have medical applications for treating age-related disease[43].

1981   1985   1990   1994   1998   1999   2000

Hayflick and colleagues transform a normal human cell population to an immortal cell line with a chemical carcinogen and radiation[40].

Calvin Harley shows that telomeres shorten as cultured normal cells approach the Hayflick limit[22].

Woodring Wright and Geron scientists show that ectopic expression of telomerase in normal fibroblasts and epithelial cells bypasses the Hayflick limit[31], showing that the telomeres are the cellular replicometer.

Advanced Cell Technology scientists report cows derived by nuclear transfer from populations of senescent donor somatic cells[42], and that the telomeres are restored to at least normal lengths.

that they allowed chromosome ends to attach to the nuclear envelope in some species. Fast-forward to 1978, when Elizabeth Blackburn, working in Joseph Gall's group, found that the telomeres of the ciliated protozoan, *Tetrahymena thermophila,* consisted of a simple sequence of hexameric repeats of the nucleotides TTGGGG[19]. The telomeres in human cells also consist of thousands of repeats, but in mammals the sequence is TTAGGG[20]. Once this sequence was known, the length of human telomeres could be measured. The first hints that human telomeres might shorten appeared in 1986, when it was shown that telomere lengths are not the same in all tissues[21]. These studies culminated in the demonstration that telomeres shorten as normal human fibroblasts divide in culture[22]. These initial observations and others[23–25] supported the concept that telomere attrition limits normal cell proliferation in culture.

If short telomeres limit the rate of cell growth, there had to be a solution to the telomere problem in immortal organisms, in the germline cells of higher organisms and in

Figure 3 | **Hayflick's three phases of cell culture.** Phase I is the primary culture; phase II represents subcultivated cells during the period of exponential replication. Phase III represents the period when cell replication ceases but metabolism continues. Cells may remain in this state for at least one year before death occurs.

cancer cells. The solution originated again in studies with *Tetrahymena* by Carol Greider, a graduate student in Elizabeth Blackburn's laboratory[26]. Greider and Blackburn discovered the enzyme — telomerase — that synthesizes and elongates telomeres. Telomerase was later found in extracts of immortal human cell lines[27] and in most human tumours[28]. Telomerase contains an RNA template on which the new telomeres are made. This RNA component was cloned a few years later[29] and subsequently the catalytic portion of the enzyme was cloned[30].

However, the idea that telomere shortening causes cell senescence has only recently been demonstrated[31]. Introduction of the telomerase catalytic protein component into normal human cells resulted in telomerase activity[31]. Normal human cells stably expressing transfected telomerase can maintain the length of their telomeres, and exceed their maximum lifespan by more than fivefold. So the normal longevity-determination mechanism of telomere shortening in human cells can be circumvented — evidence for the role of telomere shortening in cell senescence and that of telomerase expression in cell immortality.

This discovery has profound theoretical and practical implications that include the immortalization of normal human cells for the production of commercially important proteins[32]. As there are sensitive methods for detecting telomerase in a single cell, the telomerase assay is a potential diagnostic tool for the detection of cancer cells in clinical specimens[33]. Telomerase inhibitors might be found that could, perhaps, be used for treating cancer[34].

From cells to the ageing organism
These observations on telomere biology in

cells propagated in culture have yet to be shown to be directly relevant to ageing of organisms. However, it is an attractive hypothesis that the replicative potential of human cells with an intrinsic capacity for replacement may be set to allow for normal growth, development, repair and maintenance, but not to allow the many divisions needed for cancer. Many cells (even in tissues noted for division) are not completely senescent — even in centenarians. But this does not contradict the role of senescent cells in ageing. Although cells can grow out of tissues obtained from elderly donors, this does not mean there are no senescent cells in that specimen. In fact, only a minority of cells in any tissue are likely to be senescent. However, the presence of some senescent cells may interfere with the function of otherwise normal somatic tissues[35,36].

Hayflick (BOX 1) proposes that telomere shortening may be the molecular equivalent of longevity determination[37]. Hundreds of physiological, molecular and behavioural changes in normal cultured human cells herald the approach of the Hayflick limit. These changes represent increasing molecular disorder, and all compromise the internal milieu, leading to loss of cell function. Hayflick suggests that the number of population doublings that a normal cell can undergo may be the *in vitro* expression of maximum potential longevity. This is never reached *in vivo* owing to the hundreds of molecular disorders that, *in vitro*, mark the approaching loss of replicative capacity, and, *in vivo*, increase vulnerability to disease and death[11].

Future challenges
Hayflick's initial observations on cellular replicative senescence have focused attention
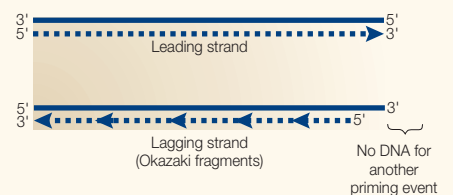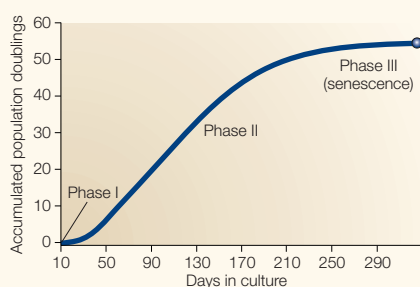

Figure 4 | **The end-replication problem.** During DNA replication the leading strand is synthesized as a continuous molecule that can potentially replicate all the way to the end of a linear template. The lagging strand is made as a discontinuous set of short Okazaki fragments, each requiring a new primer to be laid down on the template, that are then ligated to make a continuous strand. The lagging strand cannot replicate all the way to the end of a linear chromosome, as there is no DNA beyond the end for a priming event to fill in the gap between the last Okazaki fragment and the terminus. This leaves a 3′ overhang. The leading strand is also probably processed to leave a 3′ overhang.

on telomere biology and its role in human ageing and cancer. Cancer cells need to maintain telomeres if they are to divide indefinitely, and reactivation of telomerase usually solves this problem. Hayflick's idea that replicative senescence might be a barrier to tumorigenesis challenges us to determine whether this is true in all multicellular organisms and, if so, to what extent. For example, it is well established that short-lived organisms such as the inbred mouse have much longer telomeres and a higher incidence of cancer compared with humans. Are shortened telomeres serving an anti-cancer role in humans but not in mice[38]?

As almost all cells and tissues, with the exception of post-mitotic cells such as neurons and cardiomyocytes, show progressive shortening of telomeres with increased age, organ failure may sometimes occur in chronic diseases of high cellular turnover. Although the ageing process is complex and cannot be explained solely on the basis of telomere biology, there is a growing consensus that we need to understand telomeres and telomerase with regard to ageing of organisms and cancer. The challenge is to determine whether or not telomere biology leads to an increase in vulnerability to ageing and to learn how to intervene in these processes. Hayflick believes that the most important question is rarely addressed: "Why are old cells more vulnerable to pathology than are young cells?".

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Cancer: looking outside the genome

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The 'gene-centric' approach has produced a wealth of information about the origins and progression of cancer, and investigators seek a full compilation of altered gene expressions for tumour characterization and treatment. However, the cancer genome appears to be far more unstable than previously thought. It may therefore be prudent to augment gene-level approaches with supra-genomic strategies that circumvent the genomic variability of cancer cells.

The idea that cancer may one day be fully unravelled at the molecular level largely dictates how investigations into the biology and treatment of cancer are conducted. Indeed, as Zhang *et al.*[1] have stated, "much of cancer research over the past 50 years has been devoted to the analyses of genes that are expressed differently in tumour cells as compared with their normal counterparts". A prevailing paradigm asserts that cancer arises from specific gene mutations, and that it may eventually be treatable by reversing these