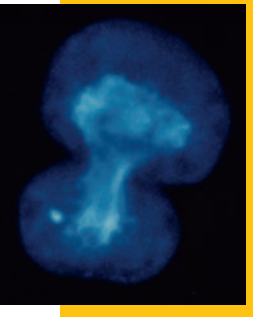
Royal Netherlands Academy

of Arts and Sciences

Heineken Lectures 2004

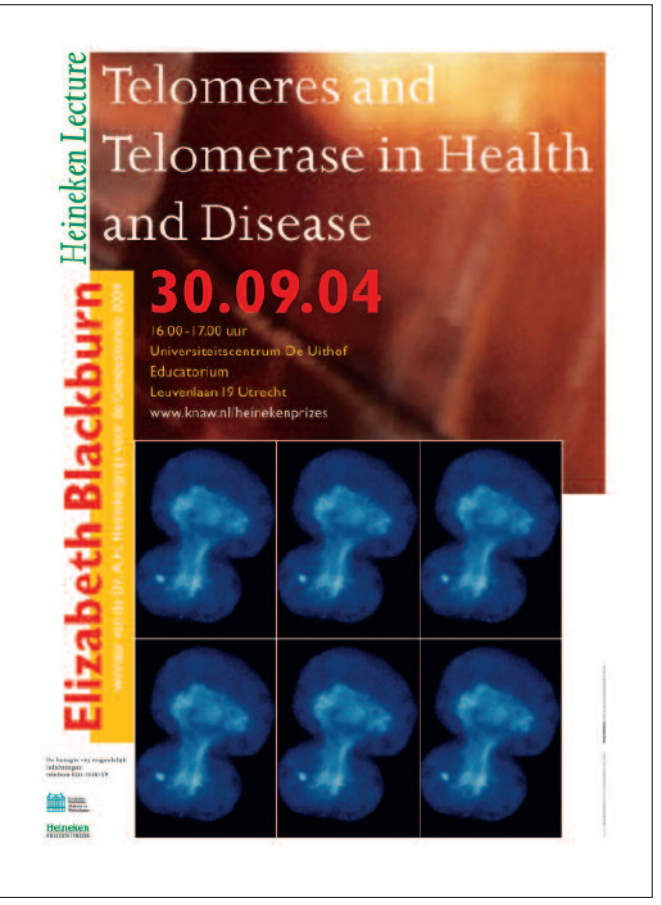


Dr A.H. Heineken Prize

for Medicine

Elizabeth H. Blackburn

Heineken Lectures 2004



Dr A.H. Heineken Prize for Medicine

Professor Elizabeth H. Blackburn delivered her Heineken Lecture on September 30, 2004 at the Utrecht University.

Royal Netherlands Academy of Arts and Sciences

Heineken Lectures 2004

Amsterdam, 2005

Dr A.H. Heineken Prize for Medicine

Elizabeth H. Blackburn

Heineken
PRIJZEN | PRIZES

Royal Netherlands Academy of Arts and Sciences

Koninklijke Nederlandse Akademie van Wetenschappen

The Heineken Lectures series for 2004 also includes
Dr H.P. Heineken Prize for Biochemistry and Biophysics 2004, Andrew Z. Fire
Dr A.H. Heineken Prize for Environmental Sciences 2004, Simon A. Levin
Dr A.H. Heineken Prize for History 2004, Jacques Le Goff

To order publications from the Heineken Lectures series for 2004,
please contact the secretariat of the Academy's Communications Department at
+31 (0)20 551 0726.

Contents

Preface

Address by Willem Levelt, President of the Royal Netherlands Academy of Arts and Sciences, on the occasion of the presentation of the 2004 Heineken Prizes on October 1, 2004.

Both in the sciences and the arts, history provides us with a looking glass that helps us to focus on the real pioneers. It is much easier for us now to recognize the epoch-making contributions of such pioneers as Huygens and Newton, Lavoisier and Pasteur, the Humboldts or Darwin than it was for their contemporaries. A contemporary of Huygens would have had a hard time telling his lasting wave theory from his failing mechanistic theory of gravity. The buzzing genius of Newton spent much more time on alchemy than on the laws of gravity and optics. Who, then, could distinguish the really lasting contributions of these men from their many dead ends? I am deliberately ignoring the abysmal public condemnations of church or state officials concerning some of the loftiest scientific or scholarly insights of their subjects. Neither will I elaborate on the ideologically motivated, unremitting support by church or state of demonstrably false theories such as Lysenko's Lamarckianism or present-day church and often state-supported creationism. Systematic disinformation of the general public on the achievements of the arts and sciences is always looming. History's looking glass cannot be dispensed with.

Admittedly, however, history can be slow in its filtering exercise. It took no less than 34 years for Father Mendel's trailblazing genetic discoveries to become recognized by the scientific community, in fact only after others, in particular Hugo de Vries, rediscovered the same laws. These laws never came to the attention of Charles Darwin, a missed opportunity to integrate genetics into evolutionary biology. Here it was the scientific community itself that was to blame. Mendel did publish the details of his experiments and theoretical analysis in the 1866 proceedings of his local scientific academy in Brünn, but nobody took any notice of them.

The story is hardly different for the arts. In 1723, the town officials of Leipzig, due to appoint a new Thomas cantor, clearly preferred Telemann and Graupner over Johann Sebastian Bach. We would have known better, wouldn't we? Similarly, the town council here in Amsterdam took down *The Oath-swearing of Claudius Civilis* that Rembrandt had painted for the new town hall, rolled it up, and returned the masterpiece to him. This in fact led to its disfigurement, because Rembrandt then had to cut the painting down in order to find another buyer. The council clearly preferred the far less controversial town hall contributions by Flinck, Lievens and Jordaens.

Clearly, major achievements in science and the arts are by no means recognized as a matter of course, either within the scientific and artistic communities themselves or by society at large. One major function of awards such as the Heineken Prizes is to breed consensus. But consensus on what? Here prizes can serve quite different purposes. There are awards, such as dissertation prizes, whose function it is to make the scientific community aware of talented upstarts. Clearly, our laureates today are not in need of such career prizes.

All of them are established experts of great repute in their own professional communities. As a rule, major awards such as the Heineken Prizes are never career prizes. They rather fulfil one or both of two other functions.

The first one is to highlight a particular landmark empirical contribution. If the Dr H.P. Heineken Prize for Biochemistry and Biophysics had been around in Georg Mendel's time, he would no doubt have received it for his 1866 paper, probably in the presence of His Majesty King William III. And the reason would not have been the excellence or even the outstanding nature of this particular work, but rather the fact that it is fundamental to the field. That is the case for Professor Andrew Fire's discovery of RNA interference, for which he today receives the Dr H.P. Heineken Prize for Biochemistry and Biophysics. It is also the case for Professor Elizabeth Blackburn's identification of the structure of telomeres and her discovery of the enzyme telomerase, which will today be honored by the award of the Dr A.H. Heineken Prize for Medicine. In this respect, these two Heineken Prizes are like the Nobel Prizes for Science, which recognize unique breakthrough contributions. In fact, we are proud to say that in many cases, the juries of our Academy's Heineken Prizes have been well ahead of the Swedish Academy's committees in identifying such landmark contributions.

The second function is to highlight a landmark theoretical contribution. Some scientific contributions are fundamental without being discoveries in the strictly empirical sense. Newton experimentally discovered the spectral dispersion of light. In contrast, his breakthrough theory of universal gravity was not an empirical finding, but a theoretical reformulation of fundamental mechanical physics. Today's Dr A.H. Heineken Prize for Environmental Sciences recognizes Professor Simon Levin's contributions to fundamental theory, the theory of ecosystem dynamics.

For obvious reasons, however, these two types of landmark contributions, the empirical and the theoretical, rarely appear as pure cases. The experimentalist is always theoretically motivated and the only way for the theorist to stay honest is to remain in close contact with empirical work. The two are inseparably interwoven in the study of history. Professor Le Goff's theoretical reformulation of medieval history emerges from a host of groundbreaking empirical studies. The Dr A.H. Heineken Prize for History recognizes this innovative two-pronged approach.

Works of art are too, in their way, empirical contributions. The artist is a discoverer and each work is, to some extent, an experiment in triggering some intended perspective in the eye of the beholder. Mr Daan van Golden receives the Dr A.H. Heineken Prize for Art for his ability to create a contextual perspective on the work of art.

Where should such consensus be established? First of all in the professional communities themselves. A Heineken Prize tells the laureate's peers: 'this work is fundamental'. A modern scientific peer community is usually quite able to recognize excellence. But it can still take years before it reaches consensus on which new insights are essential to the blueprint of their science.

Second, but equally important, is to reach consensus in the larger community, which cares, or should care, about the contributions of science and scholarship to society.

As Simon Levin expressed it in a recent interview, 'Public interest is on the macro scale'. He was, of course, referring to macro scale effects in the environment, such as the maintenance of biological diversity, but there is a more general issue here. The public at large is not so much interested in telomerase or RNA interference, but rather in questions such as 'Will it give us a cure for cancer or for AIDS?'. And here there is a major gap to bridge. Professor Blackburn, in a recent interview, gave the example of Gleevec, an effective treatment of leukemia. There was a 30-year gap between the discovery of the chromosomatic disorder in this type of leukemia and the development of an effective drug. There is, as a rule, no linear pathway from knowledge to treatment.

But the gap is even wider than this example suggests. In many cases it is simply counterproductive to go for a cure or an application that is understandably wanted by the general public, for the simple reason that at the outset the scientist doesn't know what potential knowledge is relevant to the case at hand. Eventually, there is only one way for the scientist to proceed. It is to sit down and dissect the system, whether it is a chromosome, a cell, a layered system in the environment, or a state of affairs in medieval history. The process of discovery is entirely self-governed. It has its own logic. To be successful, it should not be deterred by public pressure, by a push for quick solutions. As Professor Blackburn put it recently, 'We weren't looking to cure cancer and yet it turns out that the enzyme telomerase is one of the most frequently found characteristics of cancer cells. That was not expected'.

At the same time, the scientist has a responsibility to explain this state of affairs to the general public, time and again. Why is it that we are spending public funds in this indirect, detached fashion? The Heineken Prizes invite the general public to regard these laureates as model cases. Each, in his or her own way, has made a major effort to inform the general public, to explain the relevance of their work for our living environment, for our health care and for our understanding of ourselves as human beings. If their outstanding example helps to shape public opinion, these prizes will have been money well spent.

Willem J.M. Levelt
President of the Royal Netherlands Academy of Arts and Sciences

Elizabeth Blackburn and her research

The research

The Royal Netherlands Academy of Arts and Sciences has awarded the Dr A.H. Heineken Prize for Medicine 2004 to Professor Elizabeth H. Blackburn for identifying the structure of chromosome ends (telomeres) and discovering the enzyme telomerase. Elizabeth Blackburn has been given the sobriquet 'Queen of the Telomeres' because virtually everything we know about the form and function of the ends of chromosomes began with her. Before Blackburn, all we knew was that telomeres (from the Greek for 'end' and 'part') became shorter with each cell division. They are hence regarded as a kind of clock, which is in any event partly responsible for the natural ageing process. Since Blackburn proved that telomeres have a unique structure that protects genetic material, however, they have also been compared to the ends of shoelaces: they are a marker that ensures that a chromosome does not 'unravel'. It was also Elizabeth Blackburn who identified what telomeres are made of: a short, simple DNA sequence repeated over and over again, the sequence being slightly different in each organism.

Blackburn's research group made the spectacular discovery that, although telomeres become shorter during cell division – to the point that cells are no longer capable of dividing – they in fact also replicate, and do so in a way entirely different from the rest of the chromosomal DNA. Ordinary DNA makes an exact copy of itself using the enzyme DNA polymerase; a telomere, on the other hand, copies an RNA sequence, a process known as reverse transcription. It does so using an enzyme baptised telomerase, which has the effect of lengthening it. Telomerase has been found in some specialised cells and in stem cells. These cells are capable of 'supplementing' their telomeres and 'rejuvenating' themselves.

But although telomerase has been found to be crucial to normal cell growth, it also plays a role in the uncontrolled, menacing type of cell growth. Eighty to ninety percent of all cancer cells have lengthened telomeres and contain a relatively large quantity of telomerase. Blackburn has even said that cancer cells are 'addicted to telomerase', and found that reducing the quantity of telomerase is enough to kill off cancer cells within a few days. Is telomerase the source of eternal youth or is it a murder weapon? Researchers are in any event already working on new cancer medications based on Blackburn's discoveries.

The laureate

Elizabeth H. Blackburn was born in Tasmania, Australia, in 1948. She studied biochemistry at the University of Melbourne and received her Ph.D. in molecular biology from Cambridge in the United Kingdom in 1975. She then moved to the United States, her present home. She became a U.S. citizen in September 2003.

From 1975 to 1977, Blackburn did her post-doctoral work at Yale University, where her research included the DNA structure of the telomeres of the *Tetrahymena*, a single-cell pond-dweller (and parasite). She continued her career on the West Coast, joining the Department of Molecular Biology at the University of California in San Francisco in 1993. She is currently a professor in the Department of Biochemistry and Biophysics, where she has her laboratory (see http://biochemistry.ucsf.edu/~blackburn). Blackburn has been the recipient of many awards and marks of honour, including an honorary doctorate from Yale University, the California Scientist of the Year Award (1999) and t he General Motors Cancer Research Foundation Alfred P. Sloane Award (2001). She has taken on many executive positions (for example as the president of the American Society for Cell Biology) and has an even longer list of lectures, articles and contributions credited to her name.

After accepting President Bush's invitation to join his Council on Bioethics in 2001, Blackburn regularly took part in the public debate on therapeutic cloning and stem cell research, which she – unlike the Bush administration – advocates. The unexpected news that the White House had not renewed her membership of the Council led to protests in the foreign and American media and to considerable discussion of the role of politics in science.

Key publications

Blackburn, E.H. and J.C. Gall. 1978. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in *Tetrahymena*. J. Mol. *Biol*. 120: 33–53.

Chan, S. and E.H. Blackburn. 2003. Telomerase and the ATM homolog Tel1p protect telomeres from fusing to a double-stranded break. *Molec Cell* 11:1379-1387.

Greider, C.W. and E.H. Blackburn. 1985. Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. *Cell* 43: 405-413.

Greider, C.W. and E.H. Blackburn. 1989. A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. *Nature* 337: 331-337.

Kim. M.M., M.A. Rivera, I.L. Botchkina, R. Shalaby, A.D. Thor, and E.H. Blackburn. 2001. A low threshold level of expression of mutant-template telomerase RNA is sufficient to inhibit human tumor cell growth. *Proc. Natl. Acad. Sci. USA* 98:7982-7987.

Kirk, K.E., B.P. Harmon, I.K. Reichardt, J.W. Sedat and E.H. Blackburn. 1997. Block in anaphase chromosome separation caused by a telomerase template mutation. *Science* 275: 1478-1481.

Lin, J., D.L. Smith and E.H. Blackburn. 2004. Mutant telomere sequences lead to impaired chromosome separation and a unique checkpoint response. 15: 1623-1634 (Published online before print in *MBC in Press*, January 23, 2004).

Ly, H., L. Xu, M. Rivera, T.G. Parslow and E.H. Blackburn. 2003. A role for a novel 'trans-pseudoknot' RNA-RNA interaction in the functional dimerization of human telomerase. *Genes Dev* 17: 1078-1083.

McEachern, M.J. and E.H. Blackburn. 1995. Runaway telomere elongation caused by telomerase RNA mutations. *Nature* 376: 403-409.

Yu, G.-L., J.D. Bradley, L.D. Attardi and E.H. Blackburn. 1990. *In vivo* alteration of telomere sequences and senescence caused by mutated *Tetrahymena* telomerase RNAs. *Nature* 344: 126-132.

Presentation address for the Dr A.H. Heineken Prize for Medicine

Professor Peter C. van der Vliet

Delivered on the occasion of the presentation of the 2004 Heineken Prizes on October 1, 2004

To build and maintain an organism like the human body, cells must divide. Before cells can divide, the genetic material – that is the DNA present in our chromosomes – must replicate so that the daughter cells have the same genetic make-up. The problem with this crucial process is that the replication mechanism shortens the ends of the chromosome every time it replicates. Dr Elizabeth Blackburn has solved this problem by proposing that the ends of the chromosomes, the telomeres, have a unique structure that replicates by means of a special mechanism, a discovery that has important implications for medicine. For her pioneering work in identifying the structure of telomeres and for discovering the enzyme telomerase, the jury has proposed to award Dr Blackburn the 2004 Dr A.H. Heineken Prize for Medicine.

Dr Elizabeth Blackburn's name is almost synonymous with telomeres and telomerase. Much of what we know about the form and function of telomeres began with her discovery that the ends of linear chromosomes protect DNA from degradation. She found that these ends are specialized structures that distinguish natural chromosome ends from artificial ones, caused for instance by DNA breaks. She subsequently discovered that telomeres consist of a short, simple DNA sequence repeated over and over again. She then went on to show that telomeres were not replicated like the rest of the chromosomal DNA, but were elongated by an RNA-dependent DNA polymerase, called telomerase. This enzyme uses an RNA template to add repeats to the telomeres, thereby elongating them, a surprising departure from the models of that time.

Over the past two decades, the study of telomeres has become a central component of biology, with major new findings coming from the Blackburn lab. Most interestingly, Dr Blackburn's group showed that telomerase is vital to normal cell growth and therefore governs the life span of cells. These studies are important to our understanding of cancer. Whereas normal cells lose their telomeres because they lack telomerase, cancer cells contain a large quantity of telomerase, which enables them to elongate their chromosome ends and continue cell division, a hallmark of cancer. Telomerase has therefore become a prime target for novel therapeutic cancer intervention. Many drugs are now being developed to kill cancer cells by inhibiting telomerase, thereby preventing elongation of their telomeres.

Dr Blackburn has received over forty awards and served on many editorial boards and committees. One of these is the President's Council on Bioethics, a committee advising President Bush on ethical issues related to advances in biomedical science. Why do I mention

this particular one? Because in March this year, Dr Blackburn heard that the White House had, unexpectedly, not renewed her membership of this committee following her public criticism of the Bush administration's views on stem-cell research. It was an event that caused quite an uproar among scientists in the US, as it was the latest example of the administration's penchant for filling advisory panels only with members who supported its own viewpoints, a dangerous development. Controversial issues need open scientific debate in which all viewpoints are considered, even unwelcome ones. For science, above-board information, a broad spectrum of views and a critical attitude are essential. Policy decisions should be based on unbiased scientific data and arguments. When a friendly person and prominent scientist such as our prizewinner has to stand up in public to defend these principles, something is deeply wrong.

But we should not give the impression that such things occur only in the United States, and that the Netherlands is immune to manipulation for political ends. It can happen here as well. We are glad that our Minister of Education, Culture and Science is aware of these risks and has asked the Academy to advise her on the matter of independent scientific advice on policy issues. The viewpoints and attitude expressed by our prizewinner will set a good example of how to act when scientific integrity is threatened.

In summary, the work carried out by Dr Blackburn has not only led to the discovery of new phenomena in biology, but it also has vast medical implications. Dr Elizabeth Blackburn therefore has the uniqueness that makes her a truly outstanding winner of the 2004 Dr A.H. Heineken Prize for Medicine.

Telomeres and Telomerase in Health and Disease

Dr A.H. Heineken Prize for Medicine

Elizabeth H. Blackburn

Professor Elizabeth H. Blackburn received the Dr A.H. Heineken Prize for Medicine 2004 for identifying the structure of chromosome ends (telomeres) and discovering the enzyme telomerase.

Cells go to a great deal of trouble to protect their genetic information. Nowhere is that clearer than in the case of telomeres, which are special parts of the chromosomes that carry all our genetic information in the cells in our bodies. Telomeres protect the ends of chromosomes. Functional telomeres are essential to the stable maintenance of chromosomes and for genomic stability. Left unchecked, telomeres would gradually wear away with each cell division. Such loss of telomere function can promote cancer and may occur in human aging. It is probably not an exaggeration to say: lose a telomere, lose the chromosome.

The special nature of telomeres was recognized in early work by McClintock and by Muller, working separately with maize and with fruit flies in the 1930's[1]. McClintock's cytological discovery that normal chromosome ends lack the 'stickiness' of chromosome breaks converged with Muller's inference, based on his inability to isolate chromosomal terminal deletions, that chromosome ends (the 'terminal genes') have a protective function. Both geneticists had understood that what Muller later called 'telomeres' function to prevent chromosome fusions. This cytogenetic work, starting from the 1930's, preceded any knowledge that the genetic material is DNA.

Various analogies have been made about telomeres, but my favourite metaphor is that of the telomeres as the little plastic tip on the ends of your shoelace. If you don't have that plastic tip, the shoelace end becomes frayed, and the shoelace doesn't do its job properly. Similarly, a telomere is like that little plastic tip. It keeps the end of the chromosome from getting frayed and not doing its job properly.

Like the caps of a shoelace, telomeres prevent the chromosomal DNA strands from wearing away, protecting the genome against potential loss of genetic information.

How do we look experimentally at the molecular characteristics of telomeres and, in particular, make conclusions about how the chromosome fulfils the function of allowing full replication of the ends? We originally used a pond organism that lives in pond scum, called *Tetrahymena*. The *Tetrahymena* cell is covered with cilia that look like little hairs as seen under high magnification. This is such a tiny creature that about a hundred of them would fit on the head of a pin. But this organism happens to have a very large number of short, linear chromosomes and hence, a large number of telomeres. Because it has so many telomeres, it was an excellent source of telomeres, and then later, of telomerase.

At the molecular ends of the chromosomal DNA, the telomeres, is the telomeric DNA. With relatively rare exceptions, telomeric DNA in eukaryotes is made of repeated units; a tiny DNA sequence is repeated over and over at the ends of the chromosomes. In work done as a postdoctoral fellow in Joe Gall's laboratory at Yale University, I identified the first example of telomeric DNA sequences: I found that in *Tetrahymena*, the repeat unit is TTGGGG, not very different from the repeat unit of our telomeres or the telomeres of vertebrates, which is TTAGGG, just one base different. One also finds a few variations such as in some budding yeasts, which bring us Heineken beer, among other good things. Thus, telomeres have the same general form in most eukaryotic organisms; that is, telomeric DNA consists of an array of tandemly repeated short sequences (repeat units), generally with short clusters of guanine (G) residues on the strand oriented 5' to 3' toward the chromosomal terminus (the G-rich strand). Each eukaryotic species has a characteristic telomeric repeat sequence common to

the ends of all its chromosomes. While the majority of telomeric DNA is duplex, the G-rich strand of telomeres protrudes beyond the duplex telomeric DNA repeats forming a 3' terminal overhang during at least some part of the cell cycle.

Later, I will describe how we discovered the enzyme that not only makes this telomeric DNA sequence but also decides what the sequence of that telomeric DNA will be.

Telomeres have another function besides 'capping' the end of the chromosome. The normal DNA replication machinery has a problem replicating the very end of the chromosomal DNA. This DNA replication machinery, which replicates all the rest of the chromosomal DNA very accurately and completely, is a complicated assemblage of proteins including several protein enzymes. Each of the two DNA strands is copied to make its complementary strand. Thus, the single, long chromosomal DNA molecule is replicated into two DNA molecules that become the two new chromosomes in two new cells once the cell divides. However, because of the peculiarity of the way the replication machinery is constructed, the DNA has to be made in short pieces when copying one of the two parental DNA strands. The particular way in which this happens prevents the very end of the chromosomal DNA from being copied. This has been called the DNA end-replication problem. It was predicted to lead to gradual loss of DNA over many cell divisions: a little shortening every time the DNA replicated, each time a cell divided. This, furthermore, was predicted to cause cells eventually to go into senescence, once too much DNA became lost from the chromosomal ends. However, since life has continued for countless generations, something must exist to allow DNA to make up for this deficiency.

In the mid 1980's, in my laboratory at the University of California, Berkeley, I and my then-graduate student Carol Greider discovered telomerase, an enzyme that lengthens telomeres and protects the chromosome ends. We did not just stumble over it. For some years, results had been accumulating with telomeric DNA that could not be readily explained by current models for DNA replication. Therefore, I had decided to search in cells for a new enzyme.

First, I had discovered that the telomeric TTGGGG repeat tracts on minichromosomes in ciliates were heterogeneous in the number of repeats present on different DNA molecules. There was no reason, based on known DNA replication or even recombination mechanisms, for predicting that[2]. Second, we had discovered that telomeric TTGGGG repeat tract DNA became added to various sequences in ciliate minichromosomes as a result of new telomeres forming on chromosomes, during development of the somatic nucleus[3]. David Prescott's laboratory had simultaneously discovered a similar thing for a different ciliated protozoan too, one belonging to an entirely different group of ciliates. Third, Bernards and colleagues, by following a trypanosome gene located near a telomere, had found that the telomeric DNA on that chromosome end gradually grew longer as trypanosome cells multiplied[4]. Fourth, we had found that yeast telomeric TG1-3 repeat DNA was added directly to the ends of Tetrahymena T2G2 repeat telomeres that had been introduced and maintained in yeast[5]. Finally, an intriguing observation that Barbara McClintock told me about in 1983 was that she had noted a maize mutant stock that had lost the normal capacity for broken maize chromosome ends to 'heal', which broken ends were normally capable of doing

early on plant development[6]. This suggested to me that there might be some kind of deliberate process by which cells heal broken ends. Our ciliate work, which had examined new telomeres being formed from ends broken in the course of a developmentally controlled reshuffling of the genetic material that occurs in ciliates as part of their life cycle, had also suggested that telomeres could be added to those newly cut ends. I wondered: was a new enzyme that could extend telomeric DNA at work in cells?

We discovered that telomerase is that enzyme. It is a specialized ribonucleoprotein reverse transcriptase that contains an intrinsic RNA subunit, a small portion of which provides the template from which new DNA is copied. Thus, telomerase action can make a DNA molecule longer. Before I discuss what purpose this serves, I want to mention a couple of most intriguing things about telomerase.

First of all, this enzyme copies RNA into DNA[7]. This was a surprise because the old idea had been that, at least in normal cells, DNA was always copied into RNA. It was known that entities like retroviruses, of which the HIV virus, which causes AIDS, is the most notorious member, could copy RNA into DNA. But that was not thought to be a normal process in normal cells. And yet the enzyme telomerase is very much a part of normal cells, and indeed in most cells of most eukaryotes.

Second, telomerase is a very interesting enzyme because it acts via a collaboration between RNA and the protein. Most enzymes – the catalysts that carry out life's chemical reactions – are made of protein. But the enzyme telomerase, which replenishes the DNA at telomeres and protects them, is a chimera of a protein called reverse transcriptase (also found in the HIV virus), and an RNA, the type of molecule thought to have been the earliest enzymes on earth. It is thought that eons ago, RNA was likely the catalyst that carried out life's enzymatic reactions, then proteins took over those functions in most cases. But telomerases are peculiar enzymes because many experiments suggest that the RNA is helping telomerase perform its enzymatic reaction[8]. These intriguing observations prompt me to speculate that perhaps this RNA-protein collaboration of telomerase may even represent a relic of an ancient world in biology.

What does telomerase accomplish for cells? It adds DNA to the ends of telomeres, replenishing and lengthening them. Thus, telomerase provides telomeric DNA with a special way of replicating. The action of telomerase usually elongates chromosomal termini bearing pre-existing telomeric DNA (although in some circumstances a non-telomeric broken end can also be elongated), thereby replenishing DNA loss incurred through incomplete replication and/or degradation through nuclease action. Hence, the action of telomerase counteracts telomeric shortening. The presence of active telomerase itself also promotes cell division, as I will discuss later.

The tandem telomeric DNA repeats are present in a range of numbers at the ends of chromosomes. This is because the consequence of telomerase action at the end of the chromosome is that as a cell divides in the presence of telomerase, the telomeric DNA becomes a little longer as telomerase adds DNA and then, sometimes, a little shorter, as the DNA is incompletely replicated or is chewed away by a nuclease, an enzyme that sometimes attacks the end of the DNA. Thus, telomeric DNA is not all the same length.

Before a telomere reaches a dangerously shortened state, which makes it prone to fusing because the telomere is not able to assume a protective structure, the telomere in danger usually sends a signal to the cell to say: 'Stop dividing; there is a problem here.' Usually the cell tries to fix the potentially uncapped telomere. But if it fails, telomere fusion is one of the drastic consequences that can ensue. It is drastic because it leads to genomic instability. Interestingly, in the absence of telomerase, we found that this can occur in a low fraction of telomeres, even though they are long[9]. This finding and other data suggest that telomerase may help protect the telomere by binding it, even when it is not elongating it[10].

In healthy cells with active telomerase, the lengthening and shortening processes are kept in a kind of balance. Despite the fact that the DNA gets a little bit longer or shorter, on average it stays replenished, although the balance between lengthening and shortening of telomeric DNA can be influenced by many factors, genetic or environmental.

We and others have learned a great deal about this balance. So far, I have described only a very simple part of what occurs. But built into telomeres is their ability to maintain their own length, which we call telomere length 'homeostasis,' a term familiar to biologists and those who study medicine. 'Homeostasis' implies actively keeping things in a certain, rather stable, state. By studying not only the DNA and telomerase but also the proteins of the telomere, we are starting to get a picture of how the telomere does this.

The telomeric DNA repeats serve as a molecular scaffold for the binding of telomeric proteins, a complicated set of proteins that clothe the telomeric DNA in an elaborate complex. The conserved sequence of telomeric DNA within a species is dictated by its need to interact with multiple components, including these telomeric structural proteins. The known telomeric structural proteins fall into two general groups: those that bind DNA directly, either to duplex or single stranded telomeric DNA, and their interacting partners. Our lab and others' data support a model I call the 'two-state' model for telomeres. This model posits that this DNA-protein complex can switch between two states. A short telomere is in a state, which is welcoming to telomerase, allowing it to access the telomeric DNA end and act on the telomere, making it longer. Once it is longer, the telomeric protein assemblage on that chromosome end will have a higher probability of switching into a state that is inaccessible to telomerase, blocking its action. This state probably has a higher-order structure, whose details we still do not understand. Any of the telomeres still accessible to telomerase can get longer, thereby increasing their probability of switching into the inaccessible state, and becoming blocked to telomerase. The consequence is that because telomerase is not acting on it, any blocked telomere will then get progressively shorter as cells keep dividing, increasing the probability that it will switch back into the accessible state.

According to this model, built into the telomeric structure and its interaction with telomerase is an ingenious scheme that ensures homeostasis. We have found much evidence of various kinds for this model. I have come greatly to admire this strategy of the telomere. At first, I thought that telomerase appeared to be a very clumsy solution to the DNA replication problem, with its property of repetitive addition and loss. But now it is apparent that the telomere has built into itself the ability to self-correct – to become elongatable when it is short, and un-elongatable when it is long. Such properties, which can keep the telomere

at a favorable average length, are the essence of a homeostatic mechanism. Much work by others and us shows that maintaining this length within a well-defined range depends upon many different factors, including the states and amounts of the telomeric proteins and of telomerase. All these factors set the length of the telomere in a complicated way. In the absence of functional telomerase, the telomeres progressively shorten and cells eventually cease dividing, as was predicted. This was first observed by us in *Tetrahymena*, which is normally immortal. It becomes 'mortal' in the absence of functional telomerase, as does yeast.

How does this play out in the cells in our bodies? Even when we are adults, many cells in our bodies keep dividing, self-renewing themselves. Examples are cells in our hair follicles, our skin, the lining of our intestine, and cells of blood and our immune system, which are needed to fight off invading pathogens. All these tissues keep on self-renewing through much, if not all, of adult life, and these cells therefore have to keep dividing. I will describe one example of what happens in human blood cells. Bob Frenck and Kevin Shannon in the Pediatrics Department of the University of California, San Francisco, analyzed the average length of the telomeres in white blood cells, which have some active telomerase, and in which telomerase can be readily activated. The telomere length changed in a complicated fashion over time in these cells. The telomeres were found to shorten fastest when humans are very young, with the average length dropping precipitously even before kindergarten age. Then, from that time to old age, there was a general decline in telomere length, whose significance is unknown[11]. These observations emphasize that in cells that have telomerase, there is a battle going on between lengthening and shortening activities, which is played out in a complicated way, such that it's very hard to predict telomere length from first principles. As I mentioned, there are many cells in the adult human body that do apparently have telomerase activity throughout much of life, and so their telomeres are likely to have complicated sets of rules determining their length.

A most telling example of the importance of having enough telomerase throughout life was seen by human clinical genetic studies. This work answered the question: how do human beings respond when telomerase is only present at half-normal dosage? Telomerase RNA has been found to be mutated in families with non-X-linked dyskeratosis congenita[12]. In family members affected with this form of dyskeratosis, death occurs in early adulthood to middle age, primarily from progressive bone marrow failure. Telomeres become shorter than normal. The causative mutation is in the telomerase RNA gene. Affected individuals have one mutated hTER gene copy (allele) inherited from one parent. The other gene copy, inherited from the other parent, is wild type. The disease is a haploinsufficiency. We found that, in one such family, the mutation, identified by Vulliamy and colleagues, is like putting a stiletto into the heart of the enzyme: it alters a vital sequence at the core of the universally conserved RNA structure in telomerase[13]. In summary, what was learned was that a full human lifespan requires both telomerase RNA alleles to be functional.

Variations in telomere maintenance in humans may turn out to have other effects in vivo as well. In human blood cells, mean telomere length can be measured in people, and Cawthon and colleagues found an association between telomere length in blood and mortality in elderly people: those people with shorter telomeres in their white blood cells

had higher mortality rates from cardiovascular disease, infections, and from all causes of mortality in aggregate[14].

One kind of cell that we definitely do not want to keep on replicating is cancer cells. Human cancer cells very often have high levels of telomerase. This is a sort of two-edged sword[15], because while the presence of telomerase does allow these advanced cancer cells to keep on growing, the presence of telomerase also may, early in cancer progression, paradoxically have protective effects against cancer.

The high telomerase in cancer cells also appears to confer additional properties on the cells, which we do not fully understand. Remarkably, in addition to replenishing telomeres and hence allowing cell division, we now find that telomerase also appears to promote aspects of cancer progression such as metastasis[16].

It has become clear that telomeres and telomerase profoundly affect how the chromosome does its job, which is not only to contain genes in our genetic blueprint (and the blueprints of all organisms), but also to stay in one piece, thus ensuring the faithful transmission of the genetic information from cell to daughter cell and from generation to generation. Telomeres and telomerase have created wide interest. This interest seems justified, because telomerase is also vital to a long and healthy life. Even normal cells need a little telomerase, to counteract telomere shortening and keep their telomeres capped and intact. On the other hand, in abnormal cells, such as in more than 80% of cancers, an overabundant supply of telomerase allows the cells to become immortal and never die. In this lecture, I have described both these Dr Jekyll and Mr. Hyde faces of telomerase.

I will conclude by remarking that we certainly were not starting out to study aging or cancer when we originally began studying telomerase and telomeres in the lowly pond scum organism Tetrahymena. I personally was motivated early in my scientific research career simply by curiosity about how things work at a fundamental level in cells. I think the important message is that we need to leave a lot of space in our planning for how scientific research will be done at universities and research institutes to allow study of things that might turn out to have unexpected potential applications. At the time we discovered telomerase, we certainly didn't think of what we were doing as attacking a medical problem. In fact, I would wager that it's probably true that many things that are now known to be true in biology were not dreamed of thirty, or ten, or even two years ago.

Thus, things emerge in unexpected ways in biology. I think this is because of the very great complexity inherent to biological systems. Nowhere is that complexity more apparent than in the human body. For example, telomerase is a two-edged sword. Making use of the growing body of understanding about telomeres and telomerase for human health is going to require more work and a better understanding of their status, meaning, and function in cells. And while on the one hand we have learned an enormous amount about telomeres and about many aspects of their biology, on the other hand, I am humbled when I think about how many mysteries of life still remain unsolved, how much we have to learn, and of course, how much thought we are going to have to put into applying these very complex pieces of knowledge we are obtaining to human health.

Literature cited

1 McClintock, 1941; McClintock, 1942; Muller & Herskowitz, 1954; Muller, 1938
2 Blackburn & Gall, 1978
3 Blackburn, Flacks, Orias & Pan, 1982
4 Bernards, Borst, Lincke & Michels, 1983
5 Blackburn & Szostak, 1982
6 McClintock, personal comm., 1983
7 Blackburn & Greider, 1989; Attardi, Blackburn, Bradley & Yu, 1990
8 Blackburn, Gilley & Lee, 1995; Blackburn & Prescott, 1997
9 Blackburn & Chan, 2003
10 Blackburn, DeRisi, Smith & Smith, 2003
11 Blackburn, Frenck Jr & Shannon, 1998
12 Bessler, Dearlove, Dokal, Goldman, Marrone, Mason & Vulliamy, 2001
13 Blackburn, Comolli, James, Smirnov & Xu, 2002
14 Cawthon, Kerber, O'Brien, Smith & Sivatchenko, 2003
15 see Bishop, Blackburn, Wang & Zhu, 1999
16 Bagheri, Blackburn, Debs, Ginzinger, Kashani-Sabet & Nosrati, 2004

References

Bernards, A., Michels, P.A., Lincke, C.R. & Borst, P. 1983. Growth of chromosome ends in multiplying trypanosomes. *Nature* 303: 592 - 597.

Blackburn, E.H. & Gall, J.G. 1978. A tandemly repeated sequence at the termini of the extra chromosomal ribosomal RNA genes in *Tetrahymena*. *J Mol Biol* 120: 33 - 53.

Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenko, A. & Kerber, R.A. 2003. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 361: 393 - 395.

Chan, S.W. & Blackburn, E.H. 2003. Telomerase and ATM/Tel1p protect telomeres from non homologous end joining. *Mol Cell* 11: 1379 - 1387.

Comolli, L.R., Smirnov, I., Xu, L., Blackburn, E.H. & James, T.L. 2002. A molecular switch underlies a human telomerase disease. *Proc Natl Acad Sci USA* 99: 16998 - 17003.

Frenck, Jr., R.W., Blackburn, E.H. & Shannon, K.M. 1998. The rate of telomere sequence loss in human leukocytes varies with age. *Proc Natl Acad Sci USA* 95: 5607 - 5610.

Gilley, D., Lee, M.S. & Blackburn, E.H. 1995. Altering specific telomerase RNA template residues affects active site function. *Genes Dev* 9: 2214 - 2226.

Greider, C.W. & Blackburn, E.H. 1989. A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. *Nature* 337: 331 - 337.

McClintock, B. 1941. The stability of broken ends of chromosomes in Zea mays. *Genetics* 26: 234 - 282.

McClintock, B. 1942. The fusion of broken ends of chromosomes following nuclear fusion. *Proc. Natl. Acad. Sci. USA* 28: 458 - 463.

Muller, H.J. & Herskowitz, I. H. 1954. Concerning the healing of chromosome ends produced by breakage in *Drosophila melanogaster*. *Am. Nat.* 88: 177 - 208.

Muller, H.J. 1938. The remaking of chromosomes. *Collecting Net* 13: 182 - 198.

Nosrati, M., Li, S., Bagheri, S., Ginzinger, D., Blackburn, E.H., Debs, R.J. & Kashani-Sabet, M. 2004. Antitumor activity of systemically delivered ribozymes targeting murine telomerase RNA. *Clin Cancer Res* 10: 4983 - 90.

Pan, W.C., Orias, E., Flacks, M. & Blackburn, E.H. 1982. Allele-specific, selective amplification of a ribosomal RNA gene in *Tetrahymena* thermophila. Cell 28: 595 - 604.

Prescott, J. & Blackburn, E.H. 1997. Functionally interacting telomerase RNAs in the yeast telomerase complex. *Genes Dev* 11: 2790 - 2800.

Prescott, J. & Blackburn, E.H. 1997. Telomerase RNA mutations in Saccharomyces cerevisiae alter telomerase action and reveal non-processivity in vivo and in vitro. *Genes Dev* 11: 528 - 540.

Shampay, J., Szostak, J.W. & Blackburn, E.H. 1984. DNA sequences of telomeres maintained in yeast. *Nature* 310: 154 - 157.

Smith, C.D., Smith, D.L., DeRisi, J.L. & Blackburn, E.H. 2003. Telomeric protein distributions and remodeling through the cell cycle in Saccharomyces cerevisiae. *Mol Biol Cell* 14: 556 - 570.

Szostak, J.W. & Blackburn, E.H. 1982. Cloning yeast telomeres on linear plasmid vectors. *Cell* 29: 245 - 255.

Vulliamy, T., Marrone, A., Goldman, F., Dearlove, A., Bessler, M., Mason, P.J. & Dokal, I. 2001. The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita. *Nature* 413: 432 - 435.

Yu, G.L., Bradley, J.D., Attardi, L.D. & Blackburn, E.H. 1990. In vivo alteration of telomere sequences and senescence caused by mutated Tetrahymena telomerase RNAs. *Nature* 344: 126 -132.

Zhu, J., Wang, H., Bishop, J.M. & Blackburn, E.H. 1999. Telomerase extends the lifespan of virus- transformed human cells without net telomere lengthening. *Proc Natl Acad Sci USA* 96: 3723 - 8.

Heineken Lecture Program 2004

The Heineken Lectures were presented on 28 September and 30 September 2004.

Simon Levin
laureate of the Dr A.H. Heineken Prize for Environmental Sciences 2004
Heineken Lecture *The Ecology of Complexity, and the Complexity of Ecology*
Royal Tropical Institute, Amsterdam

Daan van Golden
laureate of the Dr A.H. Heineken Prize for Art 2004
Heineken Lecture *Red Or Blue, Some Words Of Artful Wisdom*
De Ateliers, Amsterdam

Jacques Le Goff
laureate of the Dr A.H. Heineken Prize for History 2004
Symposium *The Other Middle Ages*
De Balie, Amsterdam

Andrew Fire
laureate of the Dr H.P. Heineken Prize for Biochemistry and Biophysics 2004
Heineken Lecture *How Cells Respond to Genetic Change*
Utrecht University, Utrecht

Elizabeth Blackburn
laureate of the Dr A.H. Heineken Prize for Medicine 2004
Heineken Lecture *Telomeres and Telomerase in Health and Disease*
Utrecht University, Utrecht

Audience and publicity for the Heineken Lectures in 2004

The Heineken Lectures are intended for a broad audience. Students, scientists, Academy members, but also laymen who are interested in the field of study or the research associated with one or more of the Heineken Prizes can attend the Heineken Lectures free of charge.

In previous years, the laureates gave their Heineken Lectures during the course of a single Academy session at the Trippenhuis Building in Amsterdam, the headquarters of the Royal Netherlands Academy of Arts and Sciences. Starting in 2002, the Heineken Lectures were given at different locations throughout the Netherlands in order to reach a broader audience. In 2004, the Heineken Lectures were not only delivered at different locations, but also on different dates, drawing more people than ever before. More than seven hundred people attended one or more of the Heineken Lectures.

The large number of attendees is partly the result of a major campaign launched in 2004 to generate more publicity for the Heineken Prizes, and in particular for the Heineken Lectures. The campaign, run by the Royal Netherlands Academy of Arts and Sciences and Heineken International, consisted of leaflets, announcement posters, free tickets, the website Heinekenprizes.org, a special issue of the Academy's quarterly magazine *Akademie Nieuws*, and a booklet with more information about the Heineken Prizes and the laureates in 2004.

Between April and October of 2004, the Heineken Prizes website of the Royal Netherlands Academy of Arts and Sciences, www.knaw.nl/heinekenprizes, provided updated information on the 2004 Heineken Prizes. The site now offers a detailed review of the event, with information on the background and organization of the prizes, the nomination procedure, and the laureates, as well as press information (including photos and documentation).

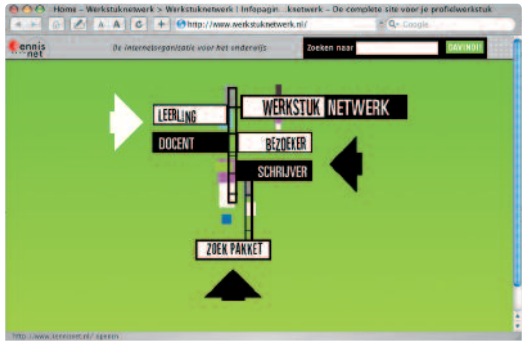
Secondary School Project about the Heineken laureates

In 2004, the Royal Netherlands Academy of Arts and Sciences initiated a secondary school project on the 2004 Heineken Prizes on *Kennisnet*, the Internet organization for primary, secondary and vocational education in the Netherlands.

The Royal Academy hired a professional teaching organization to develop five kits that help secondary school students write papers on the work and research of the five laureates of the 2004 Heineken Prizes. The kits cover the fields of Biochemistry and Biophysics (RNA-interference), Medicine (telomerase), Environmental Sciences (ecological systems) and History (the way an average person in the Middle Ages looks upon the world around him). The fifth kit is about the life and work of Dutch artist Daan van Golden. The information provided in the kits was written by Dutch university students enrolled in a variety of different programs.

By offering secondary school students kits like these, the Royal Academy is helping to acquaint them with top scientists and top scientific research. The hope is that they will then have a better idea of what they would like to study after graduation. From October to December 2004, almost three thousand students, teachers and other people inspected the Academy's kits.

The five kits can be found on the website www.werkstuknetwerk.nl.



General information

The Heineken Prizes: five prizes for outstanding contributions to the arts and sciences

Every two years the Dr H.P. Heineken Foundation and the Alfred Heineken Fondsen Foundation award four prizes – a cash gift of 150.000 USD and a crystal symbol – to scientists in the disciplines of Biochemistry and Biophysics, Medicine, Environmental Sciences and History for outstanding contributions to their field of study and one prize for the performing arts to a Dutch artist (50.000 EUR).

The selection of the winners for the Heineken Prizes has been entrusted to the Royal Netherlands Academy of Arts and Sciences. The Academy's Arts and Sciences Divisions have appointed special committees to carry out this task. The jury of the Dr A.H. Heineken Prize for Art consists of three members of the Academy complemented by experts in the particular artistic field.

The Academy also organized the 2004 Heineken Lectures. Four laureates were asked to lecture on their work to a broad audience at different locations.

List of Heineken laureates

Dr H.P. Heineken Prize for Biochemistry and Biophysics

1964 Erwin Chargaff
1967 Jean L.A. Brachet
1970 Britton Chance
1973 Christian de Duve
1976 Laurens L.M. van Deenen
1979 Aaron Klug
1982 Charles Weissmann
1985 Bela Julesz/Werner E. Reichardt
1988 Thomas R. Cech
1990 Philip Leder
1992 Piet Borst
1994 Michael J. Berridge
1996 Paul M. Nurse
1998 Tony J. Pawson
2000 James E. Rothman
2002 Roger Y. Tsien
2004 Andrew Z. Fire

Dr A.H. Heineken Prize for Art

1988 Toon Verhoef
1990 Marrie Bot
1992 Carel Visser
1994 Matthijs Röling
1996 Karel Martens
1998 Jan van de Pavert
2000 Guido Geelen
2002 Aernout Mik
2004 Daan van Golden

Dr A.H. Heineken Prize for Medicine

1989 Paul C. Lauterbur
1990 Johannes J. van Rood
1992 Salvador Moncada
1994 Luc Montagnier
1996 David de Wied
1998 Barry J. Marshall
2000 Eric R. Kandel
2002 Dennis J. Selkoe
2004 Elizabeth H. Blackburn

Dr A.H. Heineken Prize for History

1990 Peter Gay
1992 Herman van der Wee
1994 Peter R.L. Brown
1996 Heiko A. Oberman
1998 Mona Ozouf
2000 Jan de Vries
2002 Heinz Schilling
2004 Jacques Le Goff

Dr A.H. Heineken Prize for Environmental Sciences

1990 James E. Lovelock
1992 Marko Branica
1994 BirdLife International (Colin J. Bibby)
1996 Herman E. Daly
1998 Paul R. Ehrlich
2000 Poul Harremoës
2002 Lonnie G. Thompson
2004 Simon A. Levin

Colophon

P.O. Box 19121,
1000 GC Amsterdam,
the Netherlands
Telephone: +31(0)20-5510700
Fax: +31(0)20-6204941
E-mail: knaw@bureau.knaw.nl
www.knaw.nl

ISBN 90-6984-434-6

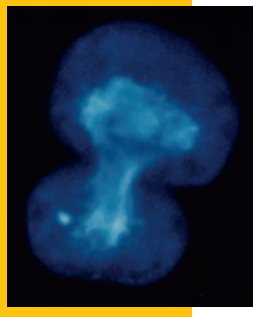
The Heineken Prizes website, www.knaw.nl/heinekenprizes,
has more information on the background and organization of the Heineken Prizes,
the nomination procedure, the laureates and their research.
The site also provides press information (including photos and documentation).

Editing
Royal Netherlands Academy of Arts and Sciences
Photography
Maarten Hartman
Graphic design
Design Studio Hans Kentie BNO
Colour separations
United Graphics
Printing
Boom Planeta Graphics
Binding
J. Voetelink & Zoon bv

Telomeres and Telomerase in Health and Disease

To build and maintain an organism like the human body, cells must divide. Before cells can divide, the genetic material – that is the DNA present in our chromosomes – must replicate so that the daughter cells have the same genetic make-up. The problem is that the replication mechanism shortens the ends of the chromosome every time it replicates. Dr Elizabeth Blackburn has solved this problem with her research on the ends of the chromosomes, known as telomeres. Telomeres protect and stabilize the ends of chromosomes. They consist of simple DNA sequences which bind protein factors and secure each end of every chromosome. Telomeric DNA has a special way of replicating: the telomeres are elongated by an enzyme called telomerase, which adds repeats to the telomeres. Without telomeric DNA and its special way of replicating, chromosome ends dwindle away.

Dr Blackburn has explored molecular features of telomerase, which plays key roles in cancer in two ways. First, lack of adequate telomerase can lead to loss of telomere functions; such a loss may promote the development of cancer. Second, late-stage cancer cells are effectively immortal and resistant to cell death. As most cancers progress, telomerase activity goes into high gear. New results produced by Dr Blackburn's laboratory show that telomerase promotes metastasis of cancer as well as cancer cell proliferation. In her current work, Dr Blackburn is exploring new strategies for cancer treatment that can be translated into rational cancer therapies. She has, for example, shown that tumor cell growth can be blocked by altering the action of telomerase in two different ways.



Blue cell, an image from research by Prof. Elizabeth Blackburn