Royal Netherlands Academy

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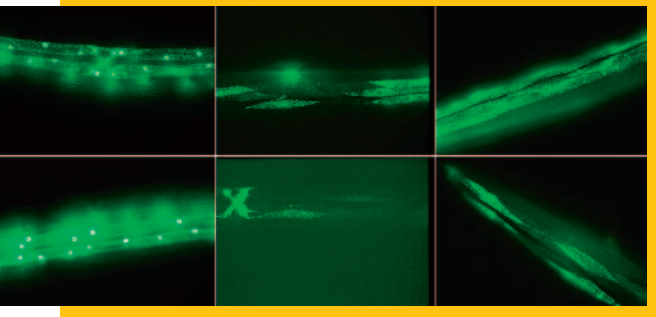

Dr H.P. Heineken Prize

for Biochemistry and Biophysics

Andrew Z. Fire

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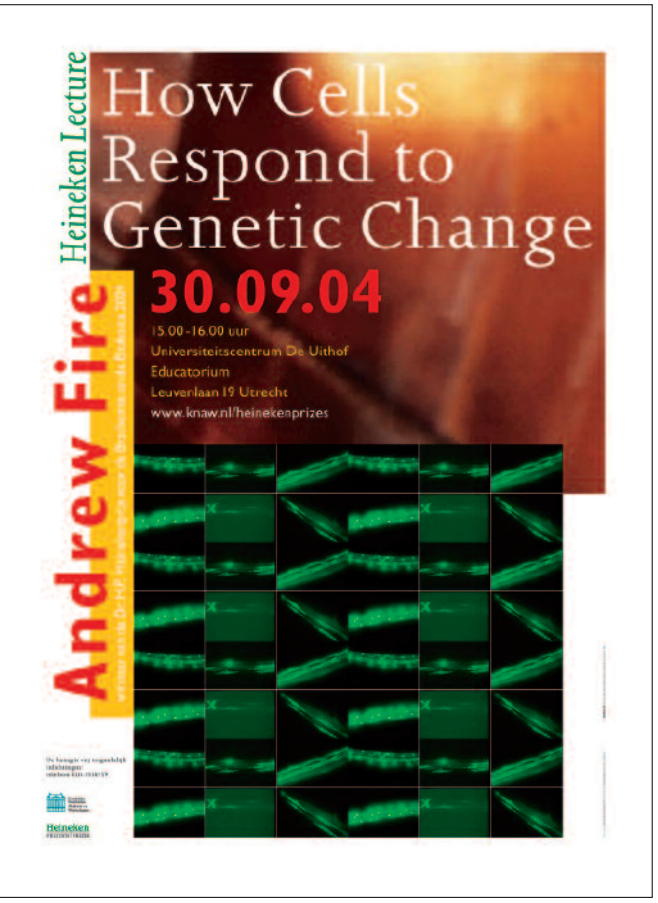

Dr H.P. Heineken Prize for Biochemistry and Biophysics

Dr Andrew Z. Fire delivered his Heineken Lecture
on September 30, 2004 at the Utrecht University.

Royal Netherlands Academy of Arts and Sciences

Heineken Lectures 2004

Amsterdam, 2005

Dr H.P. Heineken Prize for Biochemistry and Biophysics

Andrew Z. Fire

Heineken
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Royal Netherlands Academy of Arts and Sciences

Koninklijke Nederlandse Akademie van Wetenschappen

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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

Andrew Fire and his research

The research

The Royal Netherlands Academy of Arts and Sciences has awarded the Dr H.P. Heineken Prize for Biochemistry and Biophysics 2004 to Andrew Z. Fire for his 'discovery of RNA interference'. Andrew Fire has discovered that introducing double-stranded RNA in a cell will shut down any given gene. His discovery has far-reaching implications. Protein synthesis, the basis for virtually all biological processes, is controlled by the genes, but without the messenger, RNA (ribonucleic acid), the instructions – the genetic code – would never reach the ribosome's, the cellular particles where proteins are produced. Genes are expressed (as geneticists put it) only when RNA has delivered the instructions. An RNA molecule normally carries a copy of one of the two strands of DNA of a particular gene, but experiments conducted in the mid-eighties showed that the other strand of RNA (known as the antisense RNA) is sometimes capable of inhibiting RNA activity.

Fire in cooperation with his colleague Dr Craig Mello discovered in 1998 that double-stranded RNA is very effective at 'interference', i.e. at blocking protein synthesis. RNA interference takes place outside the laboratory as well, however, and not only in *C. elegans*, the roundworm that Fire uses in his research. It has been shown to be a well-preserved evolutionary mechanism that plays a key role in the natural development of all fungi, flora and fauna, and that is, for example, of huge importance in an organism's defence against viral infection.

This fundamental understanding of natural processes has led to a powerful new technology for identifying the function of genes. Although researchers have described a large number of complete genomes in the past few years, they are far from knowing the purpose of the individual genes. Using the new technology, they can explore the effect of silencing or inhibiting a single gene at cellular level and in that way uncover the role of that gene. Researchers are now also attempting to inhibit specific genes in living organisms. There is growing hope and indeed the expectation that the discovery of RNA interference will lead to new treatments against cancer, genetic disorders and viral diseases.

The laureate

Andrew Z. Fire was born in Santa Clara County, California (USA) in 1959. He majored in mathematics at the University of California, Berkeley, where he obtained his degree in only three years, but genetics were to become his life's work. At the age of 19 he went to the Massachusetts Institute of Technology in Cambridge, Massachusetts, to work in the laboratory of Professor Philip Sharp (a later Nobel Prize winner) on a new area of cell biology: the biochemistry that underlies gene expression in mammalian cells. After obtaining his

Ph.D. (the subject of his 1983 dissertation was the genetics of adenoviruses), Fire left for Cambridge in the United Kingdom, where he worked with one of the fathers of molecular biology, Nobel Prize winner Professor Sidney Brenner, on the DNA of the *C. elegans* worm, which has played a key role in his research since then.

Between 1986 and 2003, Andrew Fire was a member of staff of the Carnegie Institution of Washington, Department of Embryology (Baltimore, USA), where he supervised dozens of students, Ph.D. candidates and post-docs. In 2003, he moved his laboratory to Stanford University School of Medicine (Departments of Pathology and Genetics); in addition, he is an adjunct professor at Johns Hopkins University in Baltimore. Fire has been the recipient of many scholarships and prizes, for example the Maryland Distinguished Young Scientist Award (1997) and the National Academy of Sciences Award in Molecular Biology (2003, together with Dr Craig Mello). His colleagues praise his creativity and originality.

Key publications

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Presentation address for the Dr H.P. Heineken Prize for Biochemistry and Biophysics

Professor Rob Kaptein

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

The way in which proteins are synthesized in living cells was well understood by the nineteen nineties. As proteins are coded for by genes, segments of DNA, this process is often called gene expression. Its basic principle and the way it is controlled were already well known. It therefore came as a surprise when a novel regulatory mechanism called RNA interference or RNAi was discovered in 1998. Double-stranded RNA (or ribonucleic acid) inhibits gene expression when its sequence corresponds with that of the gene. First discovered in the roundworm, *C. elegans*, it was soon found to be a universal mechanism in eukaryotic organisms. It plays an important role in the organism's defense against viral infection. It also turned out to be an extremely useful tool in the hands of molecular biologists. And potentially, it provides a new way to cure disease.

Andrew Fire was instrumental in the discovery of RNA interference and it is for this reason that he has been awarded the Dr H.P. Heineken Prize for Biochemistry and Biophysics. At the age of 45, he is actually the youngest recipient of the Dr H.P. Heineken Prize.

Scientists had noted as early as the mid-nineteen eighties that anti-sense RNA could affect gene expression, but the results were sometimes erratic. For instance, it was observed that both anti-sense and sense RNA were able to silence genes in *C.elegans*. Andrew Fire, in collaboration with Craig Mello, then came up with the brilliant notion that the actor was in fact double-stranded RNA, and it was soon confirmed that dsRNA indeed produced a much stronger gene-silencing effect. His discovery led to the famous *Nature* article of 1998, which is generally regarded as the birth announcement of RNA interference.

Based on a natural process, RNAi soon developed into a powerful new technology for knocking out genes. Previously this had to be done by means of mutation, but injecting dsRNA is obviously much simpler. By making use of RNA libraries and high-throughput screening techniques, the method can now be used to analyze gene function on a genome-wide scale. It has become a major technology for the identification and validation of drug targets.

The discovery of RNAi, and the subsequent discovery of small interfering RNAs, or siRNAs, provided the basis for discovering a novel class of regulatory RNA molecules, the micro-RNAs or miRNAs. These are found in all plants and animals and play a crucial role in development. Their mechanism of action shares many components with the RNAi machinery.

It is truly amazing how quickly both the concept of RNAi as a novel regulatory mechanism and its use as a tool in molecular genetics were adopted by the scientific community. In 1998, only fifteen papers on RNAi were published; by 2003, that number had leaped to 1000. In 2002, the journal *Science* named RNAi 'the technology of the year'. A year later, *Fortune* dubbed it 'the next billion dollar breakthrough' in biotechnology. Whether RNAi can be used directly to cure disease remains to be seen, but as a biomedical technology it is already extremely important.

Singling out a person by awarding them a prestigious prize does not do full justice to the way science actually works: scientific breakthroughs are normally the result of the efforts of many different scientists. David Baulcombe, who conducted careful research on gene silencing in plants and Craig Mello with his anti-sense RNA work in *C.elegans* both made major contributions. Many other research groups helped to establish RNAi as a universal mechanism. Nevertheless, the real breakthrough came when Andrew Fire suggested that it was dsRNA that caused the effect. This was a huge mental leap at the time, and it is for this reason that the jury has awarded this year's Dr H.P. Heineken Prize to Dr Fire.

Today, the Academy is honoring a highly creative scientist who laid the foundations of a new and important field of research. We are convinced that Dr Fire will go on vigorously pursuing his work on the gene expression and developmental biology of the *C.elegans* worm. Dr Fire, on behalf of the jury I would like to congratulate you warmly on your Dr H.P. Heineken Prize for Biochemistry and Biophysics, which I hope will prove to be major incentive for your further scientific research.

How Cells Respond to Genetic Change or Catching Up with Change in the Subway and in the Genome: A Bedtime Story

Dr H.P. Heineken Prize for Biochemistry and Biophysics 2004

Andrew Z. Fire

The Dr H.P. Heineken Prize for Biochemistry and Biophysics 2004 was awarded to Dr Andrew Z. Fire for his discovery of RNA interference.

Nothing endures but change

Heraclitus

Nature abhors change. Nature requires change. Somewhere in the middle, there is a compromise. To maintain an identity, the successful species needs to maintain a genetic character, which distinguishes it as unique.

If one were to make random changes in a blueprint or a circuit or a subway system, the likely result would be increased chaos. Consider a map of a city's transit lines. Many of us would dearly like to change just a few key aspects of our local transit map. We would certainly be pleased if we could have a streetcar stop just across the street from home, then deliver us in order directly to the coffee shop, office, grocery store, and back home. In reality, a random change (a closed station, an unintended diversion of a section of track, or a simple miscommunication on the intended route of the 9:15 tram) is unlikely to provide such a pleasant surprise. Much more likely, such a change would create great hardship in the daily ritual.

At the same time, change is inevitable. Maybe a track has to be moved to accommodate an attractive building project. Maybe the people demand additional trains on the orange subway line to avoid the massive traffic jams on the morning streets. Maybe a flood makes part of a track impassable for a few days. As in Biology, the localized change evokes a response from the rest of the system. Some people will have their morning commute disrupted; some people will now have a streetcar running at 11pm on their quiet street. These people will complain to the local transit authorities. Some people may have an easier time... a developer who can now build a big building, a researcher who can now get to work in 22 minutes instead of 24. The 'system' responds in various ways. Local transit officials will hear the complaints of unhappy commuters, might hear the accolades of happier commuters, and (at least in some of our cities) might see nice campaign contributions from the accommodated developer. As part of the transit authority job, temporary changes with a net positive response (however that is calculated) will be attractive targets for incorporating into the permanent map and schedule. Changes that generate too many complaints will somehow disappear from the plan. At an extreme end, if the transit authorities cannot work as well as those in the next town, people will vote them out of office or move away.

If this all sounds straightforward and simple, consider the job description for the unfortunate transit official responsible for maintaining a working system. That official needs to make frequent changes based on only limited information on how the public will respond. Too little change will make the system unable to keep up with changes in the city. Transit and traffic will grind to a halt and you will be the next unemployed public official. 'One too many' unpopular changes (even temporary), will also mean that you are out of a job. Of course, one learns by experience and experimentation in such a job, but very carefully. A few rules (i.e. don't demolish the front yard of a prominent newspaper columnist or television reporter) can save a tremendous number of headaches.

The art of progress is to preserve order amid change and to preserve change amid order

Alfred North Whitehead

Confronted with rapidly changing conditions in the local and global environment, each biological species faces its own challenges in allowing and promoting beneficial changes without opening the door to chaos.

The simplest models of evolution require no discretion in the generation and handling of genetic changes. These models, which form the backdrop for much of biological thought, invoke a completely random mechanism for generating genetic change. Biological selection here is key: changes that are harmful to the organism will be lost in the population, while changes that promote survival and propagation will be retained. This type of selection is certainly a driving force in day-to-day/year-to-year biology. Nonetheless, it is clear that the organism and species can do slightly better than to randomly change the blueprint at each generation and then throw out the many individuals for which the resulting change was (not surprisingly) less-than-beneficial. Essentially, we can define a biological role for a general mechanism to distinguish (even very imperfectly) between positive and negative genetic change. By pre-filtering genetic changes with such a mechanism, the organism and species have a distinct advantage in dealing with a changing world. Likewise, one can imagine advantages to the organism and species in controlling the processes by which genetic changes that occur. Processes that tend to lead the most frequently to changes with negative effects would be minimized, while those that have some possible beneficial effect might be expected to be tolerated. The potential benefit afforded by pre-selecting the more benevolent changes will drive the appearance of concrete mechanisms. Thus, we should not be surprised to observe processes by which organisms will limit and bias the results of their own genetic change.

Numerous theoretical and experimental approaches have been taken towards identifying and understanding nonrandom mechanisms for generation and filtering of prospective of genetic changes.

While of great general interest in biology, carrying out these studies presents substantial challenges. Specific genetic changes are relatively rare under normal growth conditions for most organisms. Potentially beneficial genetic changes are much rarer, often requiring several rounds of neutral or disadvantageous change before something of potential use is derived. The scarcity of events that can be studied makes the experimental observation of genetic change in real time a rather daunting prospect. Still, much creative work has gone toward indirect studies of natural and laboratory populations. These studies have clearly demonstrated the non-random character of genetic mutations but have only begun to identify the underlying mechanisms.

They always say time changes things, but you actually have to change them yourself

Andy Warhol

I will refer to the mechanisms used by cells and organisms to follow genetic change as 'genetic surveillance'. For the purposes of this lecture, you can think of the genome as the blueprint of a cell and of an organism, with the epigenome representing (roughly) the erasable pencil marks made on the blueprint (chromatin modification) and to a set of temporary copies (RNAs) for parts of the complete blueprint that have been pieced together delivered to the relevant building and demolition departments of the cell. As we will see, changes in both the genome and the epigenome are subject to vigorous surveillance by the cell.

Many of the efforts in the field of genetic surveillance have rather fortuitous origins. Starting in the 1970s, many groups who sought to understand or harness biological processes began to use transgenesis as an experimental tool. The basic idea is that one manipulates the genome (DNA) and/or epigenome (in those days in the form of RNA populations) by introducing genes or gene products that have been specifically modified in isolation. From the perspective of a 'developmental biologist', the initial hope of this particular endeavor was to generate a one-to-one correspondence between genetic state, epigenetic state, and organismal form. In the early days, the premier method for achieving such changes was microinjection: a piece of DNA could be taken from the organism and manipulated in a controlled situation (often using an easily cultured bacterium as a host). Following injection of the DNA back into the organism (and particularly back into the nucleus of a germ cell... a cell destined to yield sperm or eggs), the expectation is that we now have an equivalent organism to the original with just one extra (and potentially slightly modified) gene that may or may not affect overall development of the animal.

Most of experimental biology is carried out using a small number of 'model systems': organisms with a long history of detailed analysis and a loyal cadre of interested biologists. Transgenesis experiments have been carried out in a wide variety of biological systems: bacteria, fungi, protozoans, plants, fruit flies, fish, frogs, mice, as well as my favorite research organism, the nematode (roundworm) *Caenorhabditis elegans*. In each case, the initial result of transgene manipulation was an encouraging verification of the basic principle behind the work. DNA could indeed be taken out of the organism, manipulated extensively in a test tube and using bacteria as a surrogate host, and put back into a living organism. Not only was the manipulated DNA apparently welcomed back as part of the genome for the organism, it also appeared in certain cases (and only in certain cases, an important caveat) to function in a similar manner to the DNA that was originally extracted. Although the behavior of the re-introduced DNA was not perfectly analogous to what one had started with, the results were 'close enough' to contribute specific details to the understanding the roles of several individual genes to the developmental process.

Despite a good degree of success in experiments described above, there was also a degree of puzzlement. Why did the injected materials often function less well than their cellular equivalents? There was no shortage of trivial explanations for this. Perhaps the original gene that was extracted from the worm (or fly or fish) actually lacked some key piece of information that would allow appropriate expression. Perhaps something in the manipulations that were carried out by the scientist was responsible for the incomplete activity.

In retrospect, a different explanation appears rather attractive: perhaps the organism was able to identify the injected material as 'foreign' or 'unwanted'.

Experimental manipulations that work less effectively than expected can be considered a nuisance. Developmental biologists wanted their purified DNA and RNA to work as efficiently as the native genes. Many experiments could be done using the limited activity that could be observed, but the results of these were always clouded by the shadow of incomplete expression. As a 'side project' (essentially the scientist's word for an off-hours hobby), some of the labs involved in this began to study the mechanisms that limited expression of the foreign materials. Initially the motivations for this were clearly technical: more efficient utilization of injected genetic materials would allow more effective characterization of genetic roles in development (and more efficient biotechnology). As the analysis of 'Gene silencing' progressed, however, the work took on a life of its own. One set of observations, made first with the fungus *Neurospora crassa* and later with plants, worms, and flies essentially drove this transition. The observation was a bizarre extension of the original silent transgene observations: not only was the newly introduced DNA (and later RNA) silenced as a result of some mysterious process; the original gene, in it's natural context in the genome, was also subject to silencing. From the perspective of mechanism, this greatly deepened the mystery. Not only was the injected material subject to some type of surveillance response... the surveillance response somehow found its way around the cell and shut off anything that matched the injected material in structure or sequence. With these observations, the silencing process acquired an additional name: 'Co-suppression' (to indicate the concurrent silencing of the injected material and its cellular counterpart). Additionally, the sheer mystery of how the cell could know how to match the injected material with a corresponding cellular sequence began to draw interest from diverse scientific fields.

It is only the wisest and the stupidest that cannot change
Confucius

The subject of the Heineken Prize citation is a number of experiments that were carried out in the summer of 1997. In almost a decade of intensive analysis preceding 1997, much had been learned about the process of co-suppression in plants and fungi. Each biological system had been observed to use multiple mechanisms to silence newly added DNA. Co-suppression in plants had been shown to involve an RNA trigger and to invoke responses both at the RNA level (producing degradation of classes of RNA that were closely related in sequence to the original 'trigger') and at the DNA level (producing a change in the state of the chromosome which under certain conditions could block expression. In a series of elegant experiments, several different labs had shown a strong connection between gene silencing (and co-suppression in particular) and antiviral responses in plants.

This provided an attractive explanation for the role of the gene silencing apparatus that was causing annoyance to countless developmental biologists: the introduction of a large segment of foreign genetic information into cells is most commonly observed as a result of virus infection. From the cell's perspective, this is not a harbinger of good things

to come. Change in the form of this much foreign information is almost certainly going to be trouble, and the best the cell can do might be to shut off the incoming information, anything that looks like that information, and (if needed to keep a foreign sequence from spreading) to temporarily or permanently shut off everything in the cell.

Working with the nematode *C. elegans*, we have been the beneficiaries of a considerable confluence of scientific background and technical possibilities. A strong gene silencing apparatus in *C. elegans* was suggested from the earliest DNA transformation experiments of Stinchcomb, Shaw, Wood, Jefferson, and Hirsh: they could introduce DNA constructs heritably into *C. elegans* but these constructs frequently failed to express the expected protein component. Co-suppression was also relatively easy to observe in *C. elegans*; our experiments 'happened' into examples of co-suppression as unexpected 'false positive' results were obtained in early attempts to do targeted gene disruption. Although not actually disrupting the gene, extra copies of unc-22 fragments produced an effect that was unquestionably a result of specific down-regulation of the endogenous (native) unc-22 gene. Most puzzlingly, a piece of the unc-22 gene could turn down its cognate cellular gene even under circumstances where the two were not linked genetically. Because these experiments involved the introduction of DNA into the animal, they were subject to a great degree of uncertainty as to whether the injected DNA, an RNA copy, or some other molecule was responsible for the silencing. Even as we suspected an RNA transcript might be responsible for the silencing, it was difficult to further characterize the nature of this transcript... to describe the situation bluntly, transgenes and their cellular activities are very messy. This made our job (and the job of those carrying out similar experiments using transgenes in other systems) very difficult... in my lab the result was that we greatly scaled back our efforts in this field following the publication of the descriptive paper on co-suppression-like phenomena in 1991.

The next contribution to the field of 'unexpected gene silencing phenomena in *C. elegans*' came from researchers at Cornell University. Su Guo, a graduate student working with Ken Kemphues had proposed the possibility of injecting negative strand copies (or 'antisense' RNA) to silence a gene that she had been studying for several years. She found surprisingly effective and specific gene silencing following injection of a population of RNA that had been prepared to contain antisense molecules; more surprisingly, she found substantial degree of silencing following injection a corresponding 'sense' RNA preparation. Her work was successful both in the characterization of her 'gene of interest' and that it provided the first examples in which synthetic RNA could trigger gene silencing in this system.

The results from Guo and Kemphues produced a great amount of excitement in the *C. elegans* community. At least for early embryonic This technique was then more broadly applied in a number of labs including Jim Priess' lab at the Fred Hutchinson Cancer Research Center. Craig Mello was completing his postdoctoral fellowship in the Priess lab at the time and took a strong interest both in the applications of this new technology and in the underlying mechanism.

As Mello began his own research group at the University of Massachusetts, he and his students began to inactivate large numbers of genes to assess their effects on embryonic development. In other experiments, Mello and members of his group began to dissect the

strange phenomenon for which they chose a new name 'RNA interference'. Their studies demonstrated a surprising potency and persistence of the RNA interference effect: most striking in that they could observe certain effects in several generations of animals following injection, and in that experiments initiated by a new graduate student (Sam Driver) in which the injected material could be effective even if delivered into the incorrect place in the animal. These results brought the field in *C. elegans* to a critical scientific cadence: in this case a point where no existing experimental paradigm could explain the observations.

Many of the early observations on RNA-mediated silencing were discussed at a reverse genetics workshop organized and chaired by Craig Mello at the 1997 *C. elegans* meeting. The meeting was held on the shores of a picturesque lake in Madison, USA. The room for this workshop had no view of the lake, however, and was as crowded as the New York subway at rush hour. I was sitting on the floor with only minimal elbowroom. Craig described the basics of sense and antisense RNA delivery and the remarkable ability to produce loss-of-function phenotypes. I recall Mike Krause also commenting during this session on the ability to generate postembryonic phenotypes from antisense RNA.

The results discussed in the RNAi workshop were as puzzling to me as to anyone else. Numerous natural RNA molecules had been followed in *C. elegans* by Geraldine Seydoux to assay stability. Most of these were substantially less stable than the mysterious silencing trigger. This apparent contradiction could have been a result of 'mistaken identity'. What if the trigger of silencing was not the bulk of the material in the synthetic RNA preparations (single stranded sense and antisense molecules that I would have predicted only a very limited stability for), but instead some type of contaminant that was formed during synthesis of the sense or antisense molecule?

The meeting officially ended at 12:30pm on Sunday June 1, 1997. With a bit of time left before departure I took a short walk around the meeting area with Jonathan Hodgkin, a research scientist at the Medical Research Council Lab in Cambridge UK. Jonathan had been a colleague and one of my many mentoring figures during my postdoctoral studies in Cambridge. As we began to discuss some of the more interesting aspects of the meeting, he asked me how I thought there could be both antisense and sense effects. Following a 'fuzzy logic' derived from my graduate work on sometimes very impure RNA populations, I suggested that the interfering principal might be double stranded RNA, present as a contaminant in both preps. Jonathan was skeptical.

Upon returning to Baltimore, I began to consider testing this hypothesis. SiQun Xu was an extremely talented molecular biology technician in my laboratory who had trained as a dentist in China. SiQun, perhaps relying on his skills as an acupuncturist, was among the most skilled individuals in the art of injecting molecules into the tiny *C. elegans* roundworms. Back in the lab, he produced standard sense and antisense RNA preparations from several different genes. The first injections of Sense, Antisense, and mixed RNAs were carried out on the 6th of June, producing somewhat encouraging results when the worms were examined early the next week: the mixture of sense and antisense strands appeared to be significantly more effective than either individual strand in producing interference. Another experiment early the following week included a titration of the RNA preparations

and produced a much stronger effect when the crude sense, antisense, and double stranded RNAs were compared, again the double stranded RNA was active at much lower doses. Additional experiments were done over the course of the next few weeks, with several important controls carried out for specificity and for the ability to interfere with numerous gene targets. A more stringent purification of single stranded RNAs in early July produced an even more striking result: purified sense and antisense RNAs resulted in almost all of the interference activity being lost; the two preps mixed together showed strong interference activity. The result was exciting but not definitive. Maybe this was some sort of non-specific effect of the double stranded RNA (which was renowned in other systems for it's ability to prime immune-like responses). Numerous conversations and experiments ensued which included notably Steve Kostas (who provided a 'perfect' *C. elegans* strain for specificity analysis, Mary Montgomery (who showed that the effect of double stranded RNA injection in the early embryo was to destabilize target mRNAs), and Craig Mello (who did numerous experiments over the summer to address specificity and kinetics as well as transport of the double stranded RNA). Finally, in September, we were ready to submit a publication.

As you have seen and certainly will see over time, our publication was not the first or the last word in the story of RNA-triggered gene silencing. Having taken all-too-much time in this lecture to describe the early days of the phenomenon, I must say (as one says to a toddler at the end of a particularly exciting bedtime story)... 'There is more to the story, but that is for another night.' One then assures that the next chapter in the story will be very interesting... In this case, certainly the promise is valid: the seven year old explosion of research into RNAi has made a very busy and exciting time for the many who have applied a skilled trade. At the same time it has been wonderful to learn from the successive waves of researchers from other disciplines (Biochemistry, Pharmacology, Genetics, Biophysics, Chemistry, Biotechnology...) who have made substantial contributions as they ply their own trades. The voices telling the next set of RNAi stories will be as engaging as they are diverse. But that is for another night.

Even as one sighs and walks away from another bedtime story to a sleepy toddler, there is a small voice that asks a question or two, drawing the storyteller back into the room for just a few more minutes. Two questions (one historical and one scientific) would be at the forefront were I listening to the story for the first time. The historical question: without the lucky coincidences of technical developments and unexpected observations in *C. elegans*, would we now know anything about this mechanism? The second question: how does the underlying biology support the useful trick of injecting double stranded RNA and getting a 'designer' worm?

As it turns out, the answers are rolled together as one. In particular, two threads from the literature on transgene silencing in plants and protozoa provide an excellent window into both the course of scientific discovery and the significance of results in this field. Several groups studying plant co-suppression had begun to pursue the very difficult task of describing exactly the structure of DNA and RNA that were present during the silencing response. Those groups (Sijen and Kooter here in the Netherlands, Waterhouse, Graham and Wang in Australia, and Grierson and colleagues in the United Kingdom) had repeatedly

found a common aspect to the most active of silencing triggers: an inverted repeat structure formed during acquisition of the foreign DNA that is not seen in DNA for genes that are normally expressed. Combined with similar results for slightly different silencing systems (Trypanosomes [Ngo, Tschudi, Gull, Ullu]; and a weed [Bender and Fink]), these studies implicated some aspect or product of this unusual structure in gene silencing. At the same time, work from Ratcliff, Harrison, and Baulcombe led to a refinement of models for the relationship between viral defense and RNA-triggered gene silencing. Ratcliff et al. proposed that the most potent triggers of gene silencing would be the ones, which looked the most like viral genomes. The strident example of a structural feature that is needed only for replication of viruses is double stranded RNA. Viruses with an RNA genome (such as influenza), need to go through a point in their life cycle at which the genome is, at least transiently double stranded. Independently of the evolving experimental data, Ratcliff et al. proposed that 'it may be possible to increase the incidence of gene silencing by ensuring that transgene transcripts have features, such as double-strandedness, that resemble explicative forms of viral RNA'. The 'inverted repeat' DNA configurations observed by the numerous groups listed above would be predicted from the known laws of physical chemistry to produce double stranded RNA. As such, one can easily imagine the connection: that the fortuitous production of double stranded RNA by inverted repeat transgenes (and perhaps by other genes with aberrant transcription patterns) could indeed trigger a cell to believe it had acquired a viral infection.

I am very pleased to have been given the opportunity to tell this story as part of such a splendid event. Given the many contributions to the field of gene silencing from several continents, I am particularly honored to have contributions from my group so recognized. Certainly, as I have described, the progress of the field has involved a diverse cast of thousands, any number of whom could justifiably be here describing an equally key contribution to the field. I have also many colleagues, students, friends and family to thank for the events leading to my visit here. The friends and family know who they are. In particular, I would like to mention the scientific colleagues in my lab and elsewhere who have been engaging collaborators and advisors and have thus contributed to our work on gene silencing. They are Joohong Ahnn, Donna Albertson, Rosa Alcazar, Arash Aryana, Phil Beachy, Daniel Blanchard, Jimo Borjigin, Sydney Brenner, Donald Brown, Natasha Caplen, Ilil Carmi, Lihsia Chen, Darryl Conte, Victor Corces, Donald Court, Dennis Dixon, Sam Driver, Susan Dymecki, Chen Ming Fan, Nina Fedoroff, Jamie Fleenor, Joe Gall, Lia Gracie, Iva Greenwald, Alla Grishok, Marnie Halpern, Yixian Zheng, Brian Harfe, Susan White Harrison, Edward Hedgecock, Jonathan Hodgkin, Jenny Hsieh, Mei Hsu, Verena Jantsch-Plunger, Steve Johnson, Richard Jorgensen, William Kelly, Cynthia Kenyon, Doug Koshland, Stephen Kostas, Mitch Kostich, Michael Krause, Sondra Lazarowitz, Rueyling Lin, Kelly Liu, Caroline Macarah, Jay Maniar, Steve Mcknight, Craig Mello, David Miller, Don Moerman, Mary Montgomery, Richard Morgan, Peter Okkema, Richard Pagano, Robert Palmer, Julia Pak, Morgan Park, Susan Parrish, Ronald Plasterk, Jim Priess, Mandith Sarkissian, David Schwartz, Eric Selker, Geraldine Seydoux, Ky Sha, Phillip Sharp, Allan Shearn, Michael Shen, Titia Sijen, Femke Simmer, Maxine Singer, Allan Spradling, John Sulston, Hiroaki Tabara, Lisa Timmons, Robert

Waterston, Harold Weintraub, John White, Siqun Xu, and Judith Yanowitz. Biological research is not cheap, particular when (like me) you drop many test tubes.

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The universe is change

Marcus Aurelius Antoninus

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).

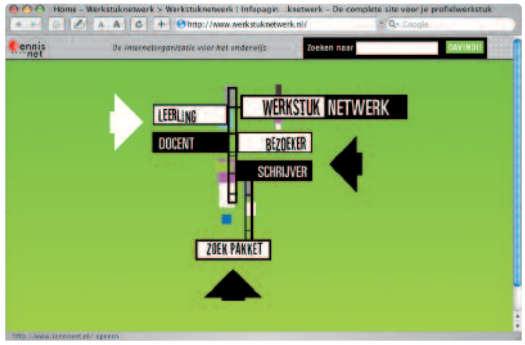
Secondary School Project about the Heineken laureates

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

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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).

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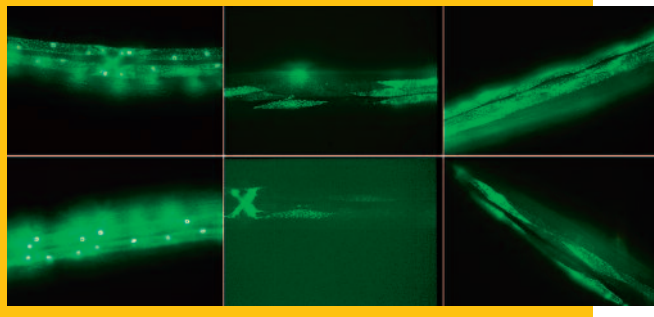
How Cells Respond to Genetic Change

The genetic landscape faced by a living cell is constantly changing. Developmental transitions, environmental shifts, and pathogenic invasions give the genome a dynamic character. Experiments in which foreign RNA or DNA are deliberately introduced into cells allow us to study general responses to genetic change and have enabled us to characterize a number of chemical features that alert the cell to unwanted genetic activity. One of these features is double-stranded RNA. Absent during 'normal' gene expression, double-stranded RNA is an essential component in the life cycle of most viruses and many other unwanted invaders. By avoiding the production of double stranded RNA during most normal gene expression, the cell can use this chemical signature as a signal to invoke protective mechanisms. Professor Andrew Fire's lecture shows that the mechanism by which cells protect themselves from unwanted RNA is interesting as a fundamental biological process, as a basis for effective genetic tools, and as an example of a structure-based defense mechanism at a subcellular level.



Photomontage of Dr Andrew Fire's research