When is the gene not DNA?

Professor Jack Heinemann
Lecturer in Genetics
University of Canterbury, New Zealand

In 2003 as part of the Royal Society's 50th anniversary observations of a series of papers that proposed a structure for deoxyribonucleic acid, or DNA, I wrote an article called "When did the gene become DNA?"[1] For many, DNA was proven to be the gene when its structure was solved. This is because the structure of DNA, a double helix, suggested a way that the molecule could be resynthesised generation after generation using the DNA molecule itself to guide the connections being formed between the units of a new molecule.

In other words, the molecule fit our expectations of how biological information could be duplicated and passed on. This process was dubbed by some as 'self-replication', although it is not literally accurate because more than just an existing molecule of DNA and a pile of unused components is needed to synthesise another molecule of DNA.

What some realised at the time, but a few generations since seem to have forgotten, is that the existence of one way to replicate and pass on information does not make it necessarily the only way. And these other ways, neglected for so long, have turned out to be very important.

Briefly back to DNA

DNA won the title of the gene just after the middle of the last century in a series of knock-out matches between protein chemists and emerging molecular biologists (Judson, 1996; Portugal and Cohen, 1977). Some credit the pioneering Rockefeller University group lead by Oswald Avery (Avery et al., 1944) for the fundamental evidence that genes were DNA, others credit the UK-based research 'collaboration' of Watson, Crick, Wilkins and Franklin, with the intellectual finale that equated the terms gene and DNA (Sayre, 2000). The issue of who deserves credit aside -

"The important thing to us was that the gene had the characteristics that it had to have. And that's why Watson and Crick were so tremendously significant to us, as genetic thinkers. Because their structure had embedded in it the properties of the gene" said pioneering molecular biologist and Nobel laureate Salvador Luria (quoted in Judson, 1996).

After the dust of the little war settled, the molecular biologists lost interest in the question of what matter genes could be composed of and got down to the work of describing the genes that were made of DNA (Heinemann, 2004). In fact, there is overwhelming evidence that genes are made of DNA. Multi-billion dollar projects, like the genome sequencing projects, are based on this simple truth.

Nevertheless, there is, and has always been, scope for asking the question: Are *all* genes DNA? Perhaps to suggest again after 60 years that genes may be made of protein (sometimes) or the interaction of several different kinds of macromolecules (other times) may appear as heretical to a few, but to not do so risks hindering progress in the life sciences.

[1] I heavily plagarise from that article here.

DNA self-replication refers to the metaphor of DNA acting as a ‘guide’ or template in the reaction that polymerizes a complementary strand of DNA during replication (Godfrey-Smith, 2000). The metaphor is unnecessary as a tool to understand the biochemistry of replication, but has been powerful at establishing DNA as the central molecule of genetics (Kay, 2000). Evidence that the metaphor is decorative rather than substantive is that it is not invoked for similar reactions that do not involve DNA.

That said, the template idea fitted existing expectations, arising largely from Nobel laureate Linus Pauling’s development of the chemical concept of complementarity, that genes should be molecules that produced primary structures after their own pattern. But there are higher order patterns that also could be guided on the molecular level. Our fixation with template-type gene has, in my opinion, stymied the search for the material form of genes that are not DNA (Weld and Heinemann, 2002).

Epigenes

There are many examples of non template-type genes, but they are not called genes. They are arbitrarily called epigenes because they are not based exclusively on DNA (Campbell, 1998; Jablonka and Lamb, 1995; Klar, 1998; Lewin, 1998; Strohman, 1997). It comes as no surprise to developmental biologists and cancer researchers that the sequencing of the genomes has been of less benefit to understanding genetics than it was suppose to be, because these groups have traditionally recognized that all that is genetic is not just DNA (Doerfler et al., 2001; Jones and Laird, 1999; Patterson, 2002).

One particularly dramatic example of a non-DNA gene is the agent that causes mad cow disease (in cows, of course), scrapie (in sheep) and Creutzfeldt-Jakob disease (in people). Each of these are neurodegenerative disorders that can be infectiously transmitted by, so far as we know, a protein rather than DNA. The proteins that cause these diseases are called prions, for "proteinaceous infectious particles". This gene may also be template-type, because the structure of the prion polypeptide influences the structure of the non-prion form of the same protein, and it is the conformation that is infectious (Campbell, 1998; Keyes, 1999; Prusiner, 1998).

But other non-DNA genes are also not template-type. For example, the group of molecules that interact to transmit the expression state of the λ virus produce a self-specifying pattern, but do not produce a particular pattern of subunits in a larger molecule, as the nucleotides do in a molecule of DNA (Heinemann, 2002; Heinemann and Roughan, 2000; Weld and Heinemann, 2002). These genes have relevance not just to cancer researchers, but to everyday geneticists. For example, we used a non-template-type gene to help us investigate the biochemistry of DNA transfer between organisms (Christie and Vogel, 2000; Heinemann, 1999) (Figure).

The experiment involved mixing two different bacteria under conditions where they could be expected to exchange DNA by a natural process called conjugation. One bacterium was already infected by the DNA element under study. The other was infected by a virus.

At the start of the experiment, the virus was in a type of hibernation maintained by the interaction of molecules operating in a circuit. That circuit was composed of DNA and various enzymes necessary for making proteins (as specified by DNA genes), and one of those proteins which is called the cI ('see one') protein. This circuit can self-replicate because the output of the circuit was its parts interacting in a new circuit.

What was lacking in the cell with the virus was the DNA gene for a second protein, called RecA, that could, under certain circumstances, establish a second, mutually exclusive circuit (Ptashne et al., 1982).

By the end of the experiment, the viruses had been roused and this new phenotype passed to each of their offspring. The only way to have broken the virus's hibernation was to have interrupted the first circuit, in effect switching it off, and turning on the alternative circuit. The protein necessary for switching the circuit off, RecA, was only found in the *first* bacterium! Therefore, the protein must have transferred between the two bacteria.

This experiment demonstrated that not just DNA but also proteins transfer between organisms in the process of horizontal gene transfer. But the point here is that what demonstrated the transfer was the protein's ability to toggle between two different epigenes.

The DNA element under study in the first bacterium transferred the switching protein as well, awakening the virus in the second bacterium. Once the circuit had been turned off, it remained off for many virus generations. So many killer viruses were produced in this chain-reaction resulting from the transfer of a protein that a clear area, defining a zone of death, on a film of bacteria spread on a nutrient base could be seen.

The effect of the RecA protein was to re-activate a latent virus in the recipient organism. The virus was genetically altered: it and its offspring continued to infect and kill bacteria even though at the start of the experiment the virus was reproducing in a way that did not cause the death of its host. Important traits can be influenced by molecules other than DNA. These molecules may be highly infectious, in the same way as viruses. The protein in the experiment I did disappeared very quickly, but it caused a virus to change from benign to deadly (to the bacteria).

Reflection

DNA deserves all the attention it gets as the gene, but it should not replace the gene. The value of labeling genes as DNA, and DNA the gene, has the generic benefits and costs that come with labels.

> "Labels, categories, nomenclature and taxonomies usually help to organize scientific thought but can also delay the reexamination of fallacious traditions, thus becoming self-fulfilling prophecies" (Zuckerman and Lederberg, 1986).

The genome sequencing projects are brilliant examples of self-fulfilling prophecies: as long as genes are defined as the material being described by sequencing, then sequencing is the way to discover all genes! The DNA sequencing paradigm (Thieffry and Sarkar, 1998) could not have discovered prions, circuits like I used in my experiments or other epigenes, from the description of the order of subunits in a DNA molecule (Heinemann, 2004; Heinemann and

Roughan, 2000). The experimental paradigm that back in the middle of the last century discovered DNA could be a gene was also the paradigm that discovered prions and other epigenes. Sequencing as a gene discovery tool only finds genes made of DNA. This reminds me of biochemist Erwin Chargaff's complaint that "by its claim to be able to explain everything (molecular biology) actually hinders the free flow of scientific ideas" (quoted in ref. Judson, 1996). The gene deserves more than circular reasoning as its raison d'etre, precisely because it needs to be better understood.

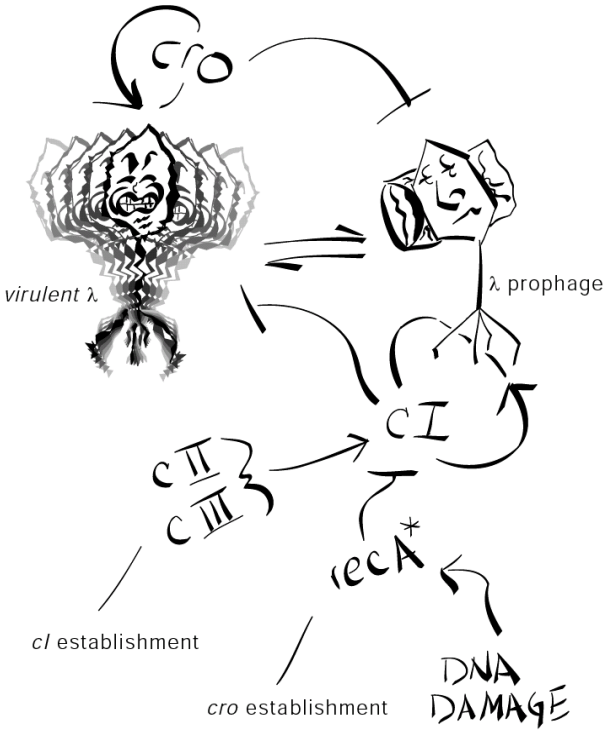

Figure: Two competing circuits that determine a heritable trait in a virus of bacteria. "Virulent λ" reproduce and kill their host bacterium. λ prophage are the same virus (identical DNA genomes to virulent) but in a benign state. That state is maintained by a circuit called the cI circuit. Under environmental conditions that lead to DNA damage (e.g., caused by UV radiation), the action of RecA protein switches the phage into the virulence under control the cro (crow) circuit.

Provided by Professor Jack Heinemann, Lecturer in Genetics, School of Biological Sciences, University of Canterbury, New Zealand http://www.biol.canterbury.ac.nz/people/heinemann.shtml

University of Canterbury Research Profile: Genetics and molecular biology of prokaryotic and eukaryotic microorganisms; horizontal gene transfer, particularly conjugation; effects of stress, particularly induced by antibiotics; evolution and risk assessment; influence of language on science, eugenics.

Research interests: Biochemical and genetic characterization of horizontal gene transfer, particularly as it relates to:

- The evolution of virulence and antibiotic resistance in microbes;
- Risks of genetically modified/engineered organisms;
- General yeast, bacteria and bacteriophage genetics.

Bibliography

Avery, O.T., MacLeod, C.M., and McCarty, M. (1944). Studies on the chemical nature of the substance inducing transformation of Pneumococcal types. J Exp Med *79*, 137-158.
Campbell, A.M. (1998). Prions as Examples of Epigenetic Inheritance. ASM News *64*, 314-315.
Christie, P.J., and Vogel, J.P. (2000). Bacterial type IV secretion: conjugation systems adapted to deliver effector molecules to host cells. Trends Microbiol *8*, 354-360.
Doerfler, W., Hohlweg, U., Muller, K., Remus, R., Heller, H., and Hertz, J. (2001). Foreign DNA Integration--Perturbations of the Genome--Oncogenesis. Ann NY Acad Sci *945*, 276-288.
Godfrey-Smith, P. (2000). The replicator in retrospect. Biol Phil *15*, 403-423.
Heinemann, J.A. (1999). Genetic evidence for protein transfer during bacterial conjugation. Plasmid *41*, 240-247.
Heinemann, J.A. (2002). Are DNA sequences too simple as intellectual property? Reply to Gene patents: are they socially acceptable monopolies - essential for drug discovery? Drug Discov Today *7*, 23-24.
Heinemann, J.A. (2004). Challenges to regulating the industrial gene: Views inspired by the New Zealand experience. In Challenging Science: Science and Society Issues in New Zealand, K. Dew, and R. Fitzgerald, eds. (Dunedin: Dunmore).
Heinemann, J.A., and Roughan, P.D. (2000). New hypotheses on the material nature of horizontally transferred genes. Ann New York Acad Sci *906*, 169-186.
Jablonka, E., and Lamb, M. (1995). Epigenetic inheritance and evolution, the Lamarckian dimension (Oxford and New York: Oxford University Press).
Jones, P.A., and Laird, P.W. (1999). Cancer epigenetics comes of age. Nature Genet *21*, 163-167.
Judson, H.F. (1996). The Eight Day of Creation (Cold Spring Harbor Laboratory, N.Y.: Cold Spring Harbor Laboratory Press).
Kay, L.E. (2000). Who wrote the book of life? A history of the genetic code (Stanford: Stanford University Press).
Keyes, M.E. (1999). The prion challenge to the 'central dogma' of molecular biology, 1965-1991. Stud Hist Phil Biol Biomed Sci *30a*, 1-19.
Klar, A.J.S. (1998). Propagating epigenetic states through meiosis: where Mendel's gene is more than a DNA moiety. Trends Genet *14*, 299-301.
Lewin, B. (1998). The mystique of epigenetics. Cell *93*, 301-303.
Patterson, M. (2002). Wake-up call for genome scanners. Nat Rev Genet *3*, 9.
Portugal, F.H., and Cohen, J.S. (1977). A Century of DNA (Cambridge: MIT Press).
Prusiner, S.B. (1998). Prions. Proc Natl Acad Sci USA *95*, 13363-13383.
Ptashne, M., Johnson, A.D., and Pabo, C.O. (1982). A genetic switch in a bacterial virus. Sci Amer *247*, 128-140.
Sayre, A. (2000). Rosalind Franklin and DNA (W.W. Norton & Company).
Strohman, R.C. (1997). The coming Kuhnian revolution in biology. Nature Biotech *15*, 194-200.
Thieffry, D., and Sarkar, S. (1998). Forty years under the central dogma. Trends Biochem Sci *23*, 312-316.
Weld, R., and Heinemann, J.A. (2002). Horizontal transfer of proteins between species: part of the big picture or just a genetic vignette? In Horizontal Gene Transfer, C.I. Kado, and M. Syvanen, eds. (London and San Diego: Academic Press), pp. 51-62.
Zuckerman, H., and Lederberg, J. (1986). Postmature scientific discovery? *364*, 629-631.

Ends