Spectrum

# Giant chromosomes and the Z-DNA story

Chromosomes — those microscopic tangled threads upon which life's message is written — are made of DNA and protein, and they are at the very core of genetics. Recently, new techniques for extracting chromosomes, developed by Dr Ron Hill of CSIRO's Division of Molecular Biology, have led to the clarification of some previously controversial findings concerning the existence of Z-DNA — an unusual form of the DNA molecule.

It was towards the end of last century that biologists noticed that certain insects, among them that famous little fruit fly *Drosophila*, contained relatively enormous chromosomes (3 $\mu$m wide and 500 $\mu$m long while a typical one measures 0·5 $\mu$m by 5 $\mu$m) in large cells of some of their organs, especially the salivary glands. In the 1930s, with the development of new techniques for extracting, fixing, and staining them, these giant chromosomes captured the enthusiasm of geneticists who dubbed them 'the lair of the gene'.

The reason for the excitement was the detection of light and dark bands on the chromosomes. These bands were thought to be the physical manifestations of genes. Also, the bands allowed one chromosome to be distinguished from another, and chromosomal mix-ups — where one chromosome breaks and rejoins with another — would be observed.

Later, scientists realised that these giant chromosomes come about by repeated replication of DNA without the separation of the new daughter strands of the molecule. The result is a chromosome of many layers made of a thousand or more DNA molecules aligned in parallel. Biologists call them 'polytene' chromosomes — the name meaning literally 'many threads'.

Not only are the chromosomes banded, they may also contain regions where they 'puff out'. Since the 1950s, studies of chromosome puffs have helped us to investigate the way in which selected genes are switched on at certain times.

In the last few years have come attempts to understand the molecular architecture of chromosomes, and how the chromosomal proteins organise and control the functioning of the DNA. But, despite the sophistication of our modern studies, the method of extracting the polytene chromosomes from cells, and then stabilising them, has not changed since the 1930s. It relies on squashing the cells in 45% acetic acid, which disrupts the membranes of the cell and the nucleus and at the same time 'toughens' and fixes the chromosomes.

The process is ideal for observing the structures microscopically, but the obvious worry is that the acid extraction treatment may alter the very complex material that is under study. Any studies, based on extracting the polytene chromosomes, that purport to show the structure and arrangement of DNA in the living cell must be viewed with the knowledge that the acidic pH of the acetic acid is not a natural environment for a cell structure.

This is precisely what led Dr Hill to develop a new approach to the preparation of polytene chromosomes from *Drosophila*. He suspected that, to preserve the molecular architecture of a chromosome, not only should the pH be at a physiological level, but so too should the ionic composition and concentration within the extraction medium.

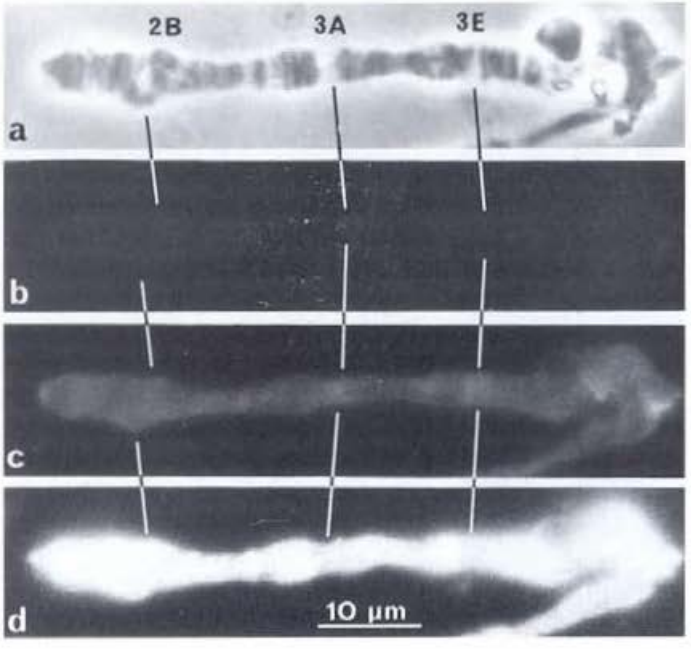
Accordingly, Dr Hill has developed a microsurgical operation that allows him to cut open, literally, the nucleus of a cell and pull out the 'giant' (3 $\mu$m wide) chromosomes. The nucleus, at about 20 $\mu$m diameter, is large by comparison with nuclei of many other animal cells, but still represents a difficult object in which to make a surgical incision. Not surprisingly, operation of the micro-manipulator is highly skilled.

The instrument is attached to a microscope through which the operator monitors his progress. Despite attempts to set up a similar procedure in the United States, only Dr Hill's group at CSIRO has, to date, succeeded in routinely isolating the giant *Drosophila* chromosomes without exposure to acid.

Use of the micro-manipulator allows extraction of 'natural' chromosomes from the cell. When they are out, the scientist can put them into a medium of physiological pH and ionic composition, simulating their natural intracellular environment. Dr Hill's studies of such 'natural' chromosomes are already producing important findings on the molecular organisation of genes and chromosomes.

The normal structure of DNA, the genetic material, elucidated in 1953 by Crick and Watson at Cambridge University, is a right-handed double helix. Much surprise was therefore generated when, in early 1980, scientists at the Massachusetts Institute of Technology and the California Institute of Technology announced the discovery of a remarkable left-handed DNA helix, the so-called Z-DNA.

**These pictures, taken through a light microscope, indicate that acid may have a role in producing Z-DNA. The top one shows a polytene chromosome from *Drosophila*, extracted without the use of acid. The lack of fluorescence after the chromosome was exposed to anti-Z-DNA antibodies (photo b) shows that the antibodies have not found any Z-DNA. However, after 5 seconds' exposure to 45° acetic acid (c), the presence of some Z-DNA is suggested. The bottom picture was taken after 30 seconds' exposure to acid, and Z-DNA is apparent everywhere.**



**Dr Hill manoeuvres the micro-manipulator while watching his progress through the microscope. In this way he can literally cut open the nucleus of a living cell.**

As well as being left-handed, the helix also differs in other ways. For example, it has 12 base pairs (the organic compounds that are letters in the genetic alphabet) in each turn of the helix, compared with 10 in the usual form.

The Z-DNA form was first discovered in crystals of DNA, but in 1981 scientists found it in structures from living organisms, namely the giant polytene chromosomes of *Drosophila*. They did so by raising antibodies against the Z-DNA, making those antibodies fluorescent, and then observing that they attached, or bound, to parts of the fruit fly's chromosome.

However, a controversy quickly arose when laboratories in three different countries each observed different patterns of binding of the antibodies to the chromosomes.

The polytene chromosomes used were isolated by means involving the classical squashing-in-acid techniques. Dr Hill, in Sydney, repeated some of these experiments using chromosomes isolated by micro-manipulation and without acid fixation.

Judging by the degree of fluorescence that he observed, almost no antibody bound to the DNA, which suggested that very little of the chromosome was in the Z-DNA form. And yet these were the same type of chromosomes, from the same organism, in which the overseas researchers had demonstrated the presence of Z-DNA, albeit in differing patterns along the chromosome.

A possible explanation lay in the difference in the methods used to prepare the chromosomes. Was the acetic acid, and the correspondingly low pH, used by the American and European groups changing the conformation of DNA into this 'new' form?

To find out, Dr Hill took one of the chromosomes he had isolated under 'natural' conditions — at neutral pH — and exposed it to 45% acetic acid for 5 seconds. Then he treated it with the fluorescent anti-Z-DNA antibody, which would attach to any Z-DNA and reveal its presence by fluorescing.

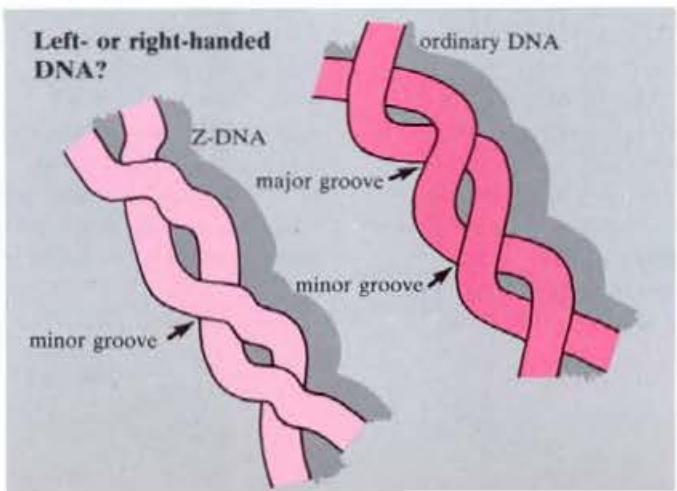
You've probably guessed that, following this treatment with acid, he then detected the presence of Z-DNA. If a chromosome was exposed to 45% acetic acid for 30 seconds even more fluorescence revealed the presence of yet more Z-DNA, leading to the inescapable conclusion that this exciting new form of our genetic material was not necessarily present *in vivo*, but was more likely an artefact brought about by the preparation procedures used.

Furthermore, Dr Hill found that he could change the pattern of binding along the chromosome by changing the time of its exposure to acid, thereby providing an explanation for the paradoxical difference in the patterns seen by the various overseas scientists.

Since Dr Hill first performed this experiment in 1983, scientists at the Max Planck Institute in Göttingen, West Germany, have succeeded in repeating the work. However, not yet having mastered Dr Hill's micro-surgical procedure, they used chromosomes from the midge *Chironomus* sp., which are ten times larger than *Drosophila*'s giant chromosomes. But it's reassuring to know that they came to essentially the same conclusions.

Of course, it is still interesting and important that DNA can adopt this other form, but whether the point has physiological significance is by no means proved.

**Z-DNA, as well as being twisted in the opposite sense to ordinary DNA, has a slightly different physical structure. This causes a zig-zagging in the sugar–phosphate backbone of the molecule.**




Interestingly, Z-DNA is more immuno-reactive than its normal form; that is to say, it stimulates the production of antibodies against itself more readily. This observation may have relevance in certain unpleasant and chronic human diseases — the so-called auto-immune diseases — where the body makes antibodies to attack its own cells, failing to distinguish them from foreign matter.

In the condition of systemic lupus erythematosus, antibodies against the patient's own DNA are a common finding, and it has long puzzled scientists why and how this happens. Could parts of the DNA be changing into the Z form? Nobody knows for sure.

Some biologists have also suggested that local regions of the DNA molecule may rewind into the Z form when small parts of the chromosome open up for a gene to be 'read' — that is, transcribed into messenger RNA. Dr Hill has proposed various molecular mechanisms by which regions of the DNA double helix could change rapidly into the Z-DNA form and then back to the more usual form, and has experimental evidence to support some of these ideas.

Whatever the final answers, Dr Hill's work, and his hypothesis that most Z-DNA found in polytene chromosomes may just be what happens to normal DNA when exposed to acid, has put the cat among the genetic pigeons.

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On the status of the Z-DNA question for animal chromosomes. R.J. Hill. *BioEssays*, 1984, **1**, 244–9.

The preparation of polytene chromosomes for localisation of nucleic acid sequences, proteins and chromatin conformation. R.J. Hill, M.R. Mott, and D.M. Steffensen. *International Reviews of Cytology*, 1986 (in press).