100 YEARS AGO

***Mankind in the Making, Anticipations* and *The Food of the Gods*. By H. G. Wells. Three books of his more especially claim to forecast the future of our race… [M]en of energy — men of science, engineers, doctors, and so forth — will shape policy and administration. The result will be marvellous efficiency, such as is rarely if ever seen now. There will be no king. Monarchy will have given place to the New Republic. Royalty is connected with all things out of date, with aristocratic privileges, ridiculous costumes and decoration. Therefore it must go… The class that supplies unskilled labour, the old servile class, will tend to disappear. The invention of machines capable of performing more cheaply all the work that has hitherto fallen to the unskilled will make such men unnecessary. Peasant proprietors and all small land-holders must pass away. They represent stagnation, and there is room only for go-ahead adaptable people. Those who fail to adapt will fall into the abyss, the great sink in which wallow all those who are unfitted for the new conditions. The people of the abyss are to be encouraged to extinguish themselves, to practise what would commonly be called vice without offspring resulting.**

From *Nature* 29 December 1904.

50 YEARS AGO

"Commonwealth Oceanographic Conference." It arose from arguments that the change of emphasis in oceanography, from exploration and survey to research directed towards the precise understanding of the basic physical and biological processes, makes it necessary to devote more effort to theoretical and experimental work… The only practical thing is for oceanographers to learn how the water movements are governed by atmospheric pressure, wind and climate so that they can use the meteorological data, supplemented every now and then by their own upper- and lower-water observations… It would no doubt be wrong to suggest that oceanographers took up the subject because they found the mathematical and physical sciences too difficult and unattractive; but they have been rather slow in applying the precise techniques of these sciences to marine research… To increase our knowledge the subject must be made attractive to men who do not mind facing up to the difficulties of fluid mechanics.

From *Nature* 25 December 1954.

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Molecular biology

Hairpins at split ends in DNA

Marjorie A. Oettinger

What do changing colours in corn kernels, mutations in houseflies and the variability of antibodies and of a T cell's antigen receptors in the vertebrate immune system have in common? A great deal, it turns out.

There once would have seemed to be little similarity between the random movement of transposable genetic elements within a fly, or a corn plant, or a flower petal, and the carefully orchestrated events by which antigen-receptor genes are assembled. The jumping of 'transposons' from one site to another in a genome can alter gene expression, giving rise to mutations that are seen as, for example, a colour change. By contrast, in the second reaction, a defined series of breakage and rejoining events within a chromosome brings together sequence elements in new combinations, for the express purpose of generating diversity in the receptors (antibodies and T-cell receptors) that detect foreign antigens. Nonetheless, a case for a mechanistic connection has been building for some time, and on page 995 of this issue, Zhou and colleagues[1] reveal a remarkable similarity in the steps by which the DNA is broken — a similarity that may extend to the structures of the enzymes responsible for this cleavage.

Transposition by the *hAT* family of mobile DNA elements (named after the *hobo* transposon from fruitflies, the maize *Ac/Ds* elements made famous by Barbara McClintock, and the *Tam* elements of snapdragon) involves the excision and complete removal of the DNA sequence of the transposable element from one site in the genome. The excised DNA sequence then jumps into another site. These reactions are mediated by a transposase — an enzyme encoded by the transposable element itself. The removal of the element creates breaks in the DNA that must be repaired to maintain the integrity of the genome. Tell-tale inverted duplications of a few base pairs of DNA are often found at the excision sites[2].

Meanwhile, in the V(D)J recombination reaction that assembles antigen-receptor genes, the RAG1 and RAG2 enzymes break both strands of DNA precisely at the border between protein-coding gene segments and their flanking recombination signal sequences. When the coding segments are rejoined in new combinations, inverted repeats are also sometimes found at the junction[2].

The presence of inverted repeats at both excision sites and coding joints led to speculation that the transposition of *hAT* elements and V(D)J recombination might involve broken DNA intermediates of the same unusual structure: covalently sealed hairpins, in which the two separate strands of the DNA helix are joined together at the tip[2,3]. If such structures were generated during breakage, and then reopened off-centre before repair, inverted repeats would be generated.

When hairpin intermediates were first proposed for plant transposons, it was suggested that two separate single-strand breaks were made in the DNA, one on the top strand and one on the bottom, followed by a ligation step that joined the broken ends together[2]. But when hairpins were first physically detected during V(D)J recombination, it became clear that they are made by a more direct route[4]. Biochemical analysis with purified RAG1 and RAG2 proteins revealed that the hairpins arise as a necessary part of V(D)J cleavage. In a first step, a single-strand nick is introduced into the DNA, precisely at the border between coding and signal sequences (Fig. 1a). This single nick leaves a chemically reactive hydroxyl group at the 3′ end of the top strand, which then attacks the opposite DNA strand in a transesterification reaction, yielding a hairpinned coding flank and a blunt, 5′-phosphorylated signal end.

As V(D)J recombination and transposition have been studied and compared, the parallels between them have mounted[5], raising the question of whether the vertebrate immune system developed its unusual mechanism of generating diversity from a primordial transposon. In this case, it would be the excision step that was harnessed, not the step in which the released element jumps into another site. The DNA removed from the chromosome during V(D)J recombination is not moved to another site; rather, the ends are usually joined together to form a circular piece that is eventually lost from the

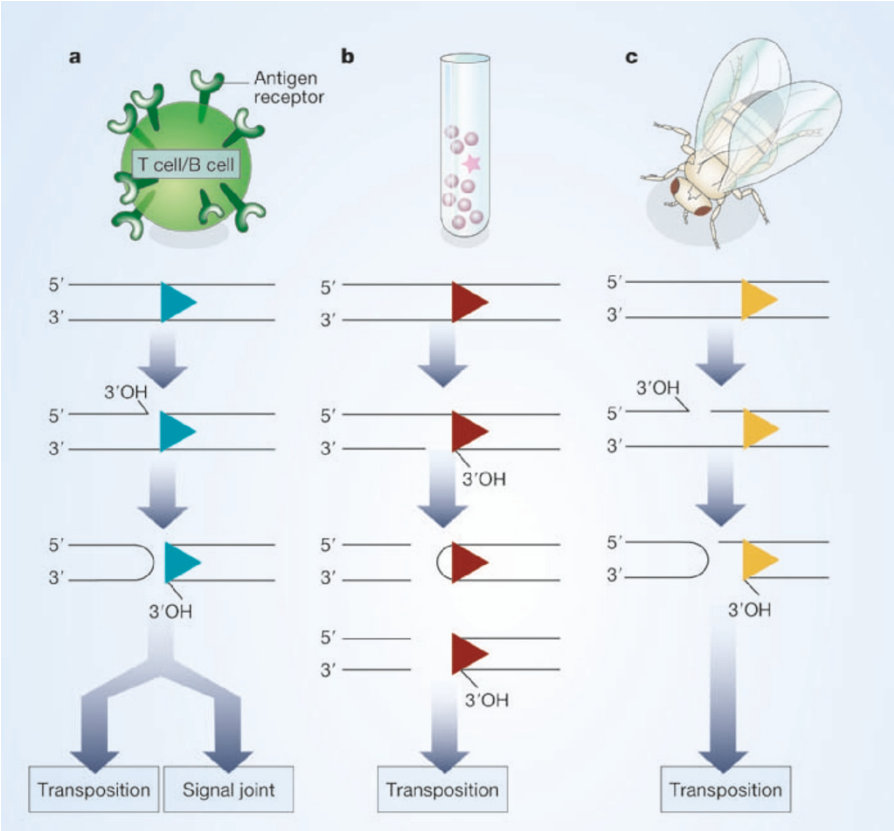

Figure 1 **Hairpin formation in transposition and V(D)J recombination. The DNA-breakage steps in** a, **V(D)J recombination, which generates diversity in antigen-receptor genes;** b, **the random transposition of the bacterial Tn*10* or Tn*5* genetic elements, which can lead to altered gene expression (represented by the change from circles to stars); or** c, **the transposition of *Hermes* elements from houseflies, as just deduced by Zhou *et al.*[1].** a, c, **In the first steps of Hermes- and RAG-mediated cleavage, a nick is introduced on the top strand of the DNA, either one nucleotide 5′ to the end of the transposition element (yellow triangle) or precisely at the border between the protein-coding sequence and the recombination signal sequence (blue triangle).** b, **By contrast, Tn*5* or Tn*10* transposition is initiated by a nick on the bottom strand. A hairpin intermediate is generated on the flanking DNA in the Hermes- and RAG-mediated reactions, and on the end of the transposon sequence in Tn*5*/Tn*10* transposition. Following DNA breakage, the excised elements can be transposed to a new site** (b,c). **In V(D)J recombination** (a), **the signal ends are normally joined to each other in a 'signal joint', making them unavailable for transposition.**

cell. This is not unknown in the transposon world, but with other transposons it is a rare event, whereas with V(D)J recombination it is the main pathway.

Despite these distinctions, there are similarities, and striking ones[5]. The unusual genomic arrangement of the *RAG* genes, found side-by-side in the genome and lacking introns (non-coding sections) within their protein-coding regions, is more like that found for bacterial or viral genes than for genes in higher eukaryotes. The RAGs have the ability to act as a transposase *in vitro*[6,7] and in yeast[8], even if they do so rarely in developing immune cells[9]. And some other transposable elements do use hairpin intermediates[10,11]. However, the arrangement is different: the hairpin is formed on the ends of the transposable element rather than on the flanking DNA (Fig. 1b). Is there a transposon that does it like the RAGs?

Zhou and colleagues[1] have now provided the missing link, showing that the housefly *Hermes* element, a member of the *hAT* family, does leave hairpinned DNA behind when it cuts itself out of DNA *in vitro*, as suggested[2]. As with the breaks generated by RAGs, the hairpin is on the DNA flanking the excised element (Fig. 1c), and arises from a top-strand nick that is converted directly to a hairpin (presumably by the same type of one-step transesterification, but this awaits demonstration).

And the similarities don't end there. Like the RAGs and members of the retroviral integrase superfamily[12], the Hermes protein contains a catalytic triad of acidic amino acids (a DDE motif) that is crucial for the protein's activity. Hitherto, RAG1 was the outlier in the superfamily, with a far greater separation in its amino-acid sequence between the second D (aspartate) and E (glutamate) residues than has been found in other family members. But Hermes shares this wider separation, hinting that there is some connection between this spacing and the generation of a hairpin on the flanking DNA. Zhou *et al.* also suggest that the DDE motif of *hAT* transposases and RAG1 lies in an RNAseH-like fold, which is common to members of the retroviral integrase superfamily[13]. That points to still closer connections between all these enzymes.

But why a hairpin? The prevalence of this intermediate suggests some biological importance, but precisely what that is remains unclear. One possibility is that a hairpin intermediate provides a molecular solution to the problem of breaking DNA by using an enzyme with a single active site. It has been suggested that the structural alterations required to achieve the chemistry of the first hydrolysis step and a subsequent intramolecular transesterification pose less difficulty than catalysing two separate hydrolysis events on opposing strands[14]. Another possibility is that the hairpin somehow aids in the resolution of the break, perhaps attracting appropriate repair factors or ensuring the proper rejoining of paired ends. As the details of how the hairpin is generated and processed are uncovered, answers to this question may be obtained.

Other questions include: what determines whether the excised fragment is transposed or the ends are harmlessly circularized? Will *hAT* elements be excised in mammalian cells and the ends rejoined, or have RAG-specific mechanisms evolved to promote V(D)J recombination and suppress RAG-mediated transposition? Are the same host factors required to process the hairpin DNA and heal the broken chromosome in both cases? Are there structural features of the Hermes and RAG proteins that explain why the hairpin is made on the flanking DNA rather than the recognition sequence? Through the efforts of Zhou and colleagues, we have a new opportunity to advance our understanding of two distinct — but remarkably similar — processes. ■

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