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Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

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TWENTY YEARS OF ILLEGITIMATE RECOMBINATION

NAOMI FRANKLIN (1967) described "normal" recombination events as "those which occur with regularity and relatively high frequency between homologous DNA segments." Such recombination, familiar to us all, occurs with high fidelity and results in crossing over and gene conversion. Molecular mechanisms of homologous recombination are understood in at least outline form (DRESSLER and POTTER 1982).

FRANKLIN (1971) described "extraordinary" or "illegitimate" recombination, on the other hand, as being "rare, haphazard, and not obviously dependent upon genetic homology." Such recombination is typically termed "nonhomologous," indicating that *extended* regions of base sequence homology are not present at the crossover point. Examples of illegitimate recombination include the DNA rearrangements leading to deletions and duplications, and specialized transducing phages. This issue of GENETICS marks the 20th anniversary of a paper by FRANKLIN (1967) in which she investigated the relationship of illegitimate to homologous recombination. This *Perspectives* will consider how our understanding of illegitimate recombination—especially that which relates to formation of spontaneous deletions—has matured during these two decades.

The goal of FRANKLIN's 1967 experiments was to determine whether spontaneous deletions are generated using the enzymatic machinery of homologous recombination. Her experiments were made possible by the isolation of mutants in which homologous recombination is abolished (CLARK and MARGULIES 1965; HOWARD-FLANDERS and THERIOT 1966). FRANKLIN's experiments were simple and direct: the frequency of spontaneous deletions was measured in recombination-proficient and recombination-deficient backgrounds. Her results were equally direct: a recombination deficiency had no effect on the frequency of spontaneous deletions. Similar results have been obtained subsequently by numerous investigators (see, for example, INSELBURG 1967; SPUDICH, HORN and YANOFSKY 1970; MÜLLER-HILL and KANIA 1974; GHOSAL and SAEDLER 1979; FOSTER *et al.* 1981).

FRANKLIN proposed two general models to explain deletion formation. First, "slipped mispairing" during DNA replication might cause regions of the DNA to be bypassed. FRANKLIN's formulation of this model, like all others since, derives from the work of STREISINGER *et al.* (1966). His appealing and persuasive models account for the formation of frameshift mutations in bacteriophage T4 but are applicable to a wide variety of DNA arrangements. Second, FRANKLIN proposed that errors of DNA breakage and reunion might lead to deletions. In this model, enzymes that break and join DNA as part of their normal functions would do so on sequences that share little or no homology.

How have our thoughts concerning illegitimate recombination changed in these years? Surprisingly little. Contemporary models are more explicit, and specific enzymatic activities have been implicated in the deletion process. Yet, FRANKLIN's 1967 models remain the more general formations of today's specific ideas. The difference between "legitimate" and "illegitimate" recombination, however, has become vague. Illegitimate recombination is less frequent than normal recombination, but it is certainly not haphazard. Illegitimate recombination, furthermore, *does* depend upon base sequence homology, but this was not apparent using the genetic techniques available to FRANKLIN.

The importance of base sequence homology to deletion formation was recognized only when techniques of DNA sequencing became available. FARABAUGH *et al.* (1978) were the first to demonstrate that the break points of spontaneous deletions are not random. Rather, deletion termini are usually located within pairs of fortuitous, short, direct repeats (5–10 base pairs). The material between the repeats is deleted, and the resulting chromosome contains a single copy of what originally was the repeat. Similar results have been obtained by many investigators. The association of direct repeats with deletion termini in prokaryotes is very striking and contemporary models always in-

volve base pairing at some level to account for this.

If homologous sequences participate in forming a deletion, why then is *recA*$^+$ function, which is essential for all homologous recombination, not required for spontaneous deletion? Obviously, *recA*-independent mechanisms exist for these sequences to interact. GLICKMAN and RIPLEY (1984) argue that base pairing between inverted repeats, or involving both direct and inverted repeats simultaneously, can generate structural intermediates for deletions, leading either to enzymatic removal of a single-strand loop or to replication across a cruciform structure formed transiently in single-stranded DNA. Inverted repeats of the transposon Tn*10* have dramatic effects on the frequency of Tn*10* excision (FOSTER *et al.* 1981); Tn*10* excision is a deletion process that is unrelated to transposition. Certain deletions could be generated by site-specific recombination. Sequence repeats in this case might reflect protein-DNA recognition, rather than direct base pairing alone. However, the immense variety of deletion termini argues that, except in special circumstances, site-specific recombination is not responsible for most spontaneous deletions.

In fact, some deletions *do* depend upon *recA*$^+$ for their formation. In an elegant series of experiments, ALBERTINI *et al.* (1982) investigated the involvement of short homologies and general recombination in the formation of *lacI* deletions. Deletion termini in this system are often located within typical direct repeats. Mutational disruption of these repeats reduces the frequency of the corresponding deletion. Deletions in this system depend upon *recA*$^+$ function; such events occur at much lower frequencies in *recA*$^-$ than in *recA*$^+$ strains. Surprisingly, deletions obtained in a *recA*$^-$ background have their termini located at direct repeats that are identical to those utilized in *recA*$^+$ cells. The *recA*$^+$ function, therefore, must facilitate (but not be required for) some step in the deletion process.

What other enzymatic activities might be involved in deletion formation? DNA polymerases, copying an *Escherichia coli lacZ* template *in vitro* and unassisted by any other proteins, frequently generate deletions, often between short direct repeats (KUNKEL 1985a,b). DNA topoisomerases can also covalently join DNA molecules *in vitro*. DNA topoisomerases modify the topological states of DNA by catalyzing specific types of coupled breakage/rejoining reactions (reviewed by WANG 1985). Type I topoisomerases catalyze transient single-strand breaks, whereas type II topoisomerases catalyze transient double-strand breaks. *E. coli* DNA gyrase (a type II topoisomerase) catalyzes illegitimate recombination of DNAs *in vitro* (IKDEA, MORIYA and MATSUMOTO 1981; IKEDA, AOKI and NAITO 1982). IKEDA *et al.* propose that such recombination occurs when two covalent DNA-gyrase complexes exchange subunits before the rejoining step. T4 DNA topoisomerase (another type II enzyme) also catalyzes illegitimate recombination *in vitro* (IKEDA 1986). In neither case do these *in vitro* recombinations require *recA*$^+$ function.

Are these *in vitro* reactions relevant in *in vivo* deletions? The answer is probably yes. MARVO, KING and JASKUNAS (1983) describe a series of deletions arising *in vivo* that are very similar to the recombinants isolated *in vitro* by IKEDA *et al.* and present a "gyrase cascade model" to account for their structures. They suggest that sequence repeats at deletion termini arise because the protruding single strand of a DNA-gyrase complex is more likely to be rejoined to a second DNA-gyrase complex with which it shares base-sequence homology. An unresolved problem with this idea is that deletion termini should correspond to DNA gyrase cleavage sites (MORRISON and COZZARELLI 1979). The sequences around deletion crossover points occasionally but inconsistently confirm this (MARVO, KING and JASKUNAS 1983).

Deletions affecting bacteriophage M13 directly suggest the involvement of a type I DNA topoisomerase. The M13 gene *II* protein has enzymatic activities that are similar to those of a type I DNA topoisomerase (MEYER and GEIDER 1982). Gene *II* enzyme initiates DNA replication by introducing a site-specific nick at the M13 origin of replication and terminates replication by cleaving a newly synthesized strand and sealing it to form the circular viral molecule. Spontaneous deletions near the M13 origin of replication depend upon gene *II* for their formation. The termini of these deletions are located within typical direct-repeat sequences, but one endpoint is always located precisely at the site of gene *II* nicking (MICHEL and EHRLICH 1986). Such structures demonstrate convincingly that site-specific enzymatic activities can be involved in certain types of deletions.

It emerges from this discussion that at least two and perhaps several pathways exist for spontaneous deletion in prokaryotes. The short homologous sequences found at deletion termini can interact in either a *recA*-dependent or *recA*-independent manner. Features that distinguish these two pathways are unknown. Inverted repeat structures can facilitate the deletion of nearby sequences, but the details of this process are unclear. Which of the *in vivo* pathways involve DNA topoisomerases is unknown. Some of these uncertainties might be clarified if a wider variety of mutations affecting the deletion process were available. Mutants selected because they exhibit either altered frequencies or altered termini of spontaneous deletions would be valuable. Such mutants would identify components of the deletion process and possibly indicate the relationship of DNA deletion to replication, recombination and repair. Mutants affecting excision of the transposon Tn*10*, for example, have implicated genes involved in methylation-directed mismatch repair as

being important for certain types of deletion (LUNDBLAD and KLECKNER 1985).

Are deletions in eukaryotes formed by mechanisms similar to those in prokaryotes? DNA topoisomerase I has been implicated in the eukaryotic deletion process. The crossover points leading to excision of SV40 from the mammalian chromosome correspond to topoisomerase I cleavage sites (BULLOCK, CHAMPOUX and BOTCHAN 1985). The sequence features of deletion termini in eukaryotes, however, are much less striking than those in prokaryotes. Sequence repeats, if present, are generally quite small. NALBANTOGLU *et al.* (1986) observe repeats that are 2–5 base pairs long at the termini of five spontaneous deletions affecting the *aprt* locus of CHO cells. R. PULAK and P. ANDERSON (unpublished results) observed repeats that are at most 4 base pairs long at the termini of 16 spontaneous deletions affecting a nematode myosin heavy chain gene. ROTH, PORTER and WILSON (1985) have summarized the repeat sizes associated with a large number of published eukaryotic illegitimate recombinations. Their summary indicates that crossover points contain on average slightly more homology than that predicted by completely random breakage and rejoining. ROTH and WILSON (1986) demonstrate that the slight bias toward sequence repeats at points of illegitimate recombination can be explained by a minor role for short sequence homologies in the DNA joining reaction. Thus, deletions in eukaryotes are similar to those in prokaryotes, but the DNA repeats are much less striking. This could indicate either a fundamental or superficial difference in their mechanisms of formation.

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