MEETING REPORT

THE MOLECULAR BASIS OF RECOMBINATION
A REPORT OF THE MEETING OF THE BRITISH BIOPHYSICAL SOCIETY HELD AT THE UNIVERSITY OF SUSSEX, MARCH 24–25, 1970

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The British Biophysical Society Symposium on the molecular basis of recombination was held at The University of Sussex on March 24th – 25th, 1970. The meeting was organised by Professor N. Symonds (Brighton).

Bacteriophage λ

Dr. P. Brachet (Paris) reviewed current knowledge of recombination of the DNA of phage λ. The genetics of this phage have recently been reviewed (W.F. Dove, Ann. Rev. Genet. 2 (1968) 305–340). This is probably the system in which the most detailed information on recombination mechanism is available.

Beside the general recombination system of the host bacterium (the rec system) there are three known mechanisms of recombination of λ.

(i) *The red system*

This system can promote recombination at any point on the λ genome and allows generalized recombination during lytic development. Mutants of bacteriophage λ which have lost the ability are termed recombination deficient (red^-) mutants. Enzymologically it is found that red^- mutants lack either an exonuclease (normally produced in λ infected cells) or an antigenically related protein called β or both. The red system promotes recombination in the λ genome about 100X more efficiently than does the rec system.

(ii) *The int system*

This catalyses a very specific recombination process between sites on the bacterial chromosome and the λ chromosome. Such recombination is necessary for integration of a prophage and for its release. The specificity of this process is such that the int system catalyses recombination only at the site on the λ chromosome that interacts with the bacterial chromosome. Recombination between two λ genomes can be catalysed but the int enzyme is more efficient at catalysing recombination between the phage and the bacterial chromosome.

The int function is required for both integration and excision. Recently xis mutants of λ have been identified. These define a new gene required in some, as yet, unknown way for excision but not for integration.

(iii) *The ter function*

This is much less well defined than the other recombination mechanisms. It is argued that to cut out the linear DNA molecules with unique end sequences that constitute λ chromosomes from either concatenates or circular molecules there must be a specific nuclease capable of recognising the base sequences defining the ends and cutting at these points. If two λ chromosomes are lined end to end, as in a double lysogen, they can constitute a structure analogous to a concatenate. From this the hypothetical nuclease (terminator) could cut a λ genome partially derived from each prophage.

No mutants lacking specifically this terminator function have been isolated but evidence for the

operation of such a system has been provided by studies of the patterns of recombination that still occur in the absence of rec, red and int systems.

Bacteriophage P2

Dr. G. Lindahl (Stockholm) discussed recombination in phage P2, a system showing marked resemblances to that for λ but also intriguing differences.

He first showed that the Campbell model (originally proposed to explain integration of λ) by which a linear bacteriophage DNA molecule adopts a circular form and integrates by recombination with the chromosome, could be applied to P2. In this case a number of chromosomal attachment sites on the bacterial chromosome are known but all seem to recombine with the same region of the phage chromosome. Thus the gene order is the same in all prophages; being a circular permutation of the order of markers on the lytic map.

When a genetic map was constructed from analysis of lytic crosses it had a most peculiar appearance. At one end was a group of genes responsible for late functions and at the other end was a group of early functions. The two groups were apparently separated by a region constituting about 80% of the genetic map but containing no identifiable genes.

Int mutants, (mutants which did not produce the integration enzyme and therefore could not integrate unless complemented by int^+ P2 helpers and once integrated could not excise) were isolated. It was shown that the int function promoted site specific recombination and that this was responsible for the distortion of the genetic map described earlier. When crosses were made between two P2 phages both carrying int^- mutations the apparent genetically blank region no longer appeared.

The reason why the site specific recombination system could so distort the P2 genetic map is that the general level of recombination is very low. (It can, however, be increased 100-fold by ultra violet irradiation of the parental particles.) This low level is uninfluenced by the recombination system of the bacterium and it is very interesting to consider the reasons why the rec system fails to act upon P2. Dr. Lindahl noted that recombination was low in phage φx174 and speculated that there may be similarities in the replication patterns of the two phages and that the low level of recombination might be a reflection of these patterns.

A particularly interesting notion is that the int function, though not expressed in the prophage may not be repressed by the immunity system. Thus, when a P2 particle superinfects a bacterium lysogenic for P2 it seems to be capable of making the int product. It may be that the int gene is part of an operon which is physically broken by the recombination step in the integration of the prophage. Thus, the prophage cannot express the int gene but cannot prevent its expression in the superinfecting genome.

When integrated at a well defined site in *E. coli* K12 between the histidine operon and the shikimate A gene P2 prophage shows a peculiar propensity to undergo excision not by an interaction between the ends of the prophage but by recombination between one end of the prophage and a point on the bacterial chromosome. This type of excision eliminates not only the prophage but also a segment of the bacterial chromosome including the histidine operon. This process is termed eduction and requires an active int^+ gene.

Escherichia coli

Dr. R. Devoret (Gif-sur-Yvette) described his experiments on the effects of ultraviolet light on the transfer of F′ episomes between strains of *E. coli*. The transfer of Flac, recognised by the formation of lac^+ recipients was extremely resistant so long as the recipient was capable of rescuing the lac^+ marker by recombination with the chromosome, regardless of whether the recipient had an active repair (hcr) system. If, however, the recipients were incapable of this recombination (e.g. because it was rec^- A) the transfer was much more susceptible to U.V. and Dr. Devoret calculated that a single lethal hit (pyrimidine dimer) was enough to prevent the formation of a lac^+ recipient.

Thus it seems that the genetic markers of the incoming Flac are susceptible to rescue by recombination with homologous genetic material of the bacterial chromosome but not to repair by stripping and resynthesis, which suggests (but does not prove) that the genetic material may be transfered as a single strand.

Confirmation of these conclusions was provided

by studies with an F′ carrying the attachment site of bacteriophage λ at which a phage was integrated. Transfer of such an episome to a non-lysogenic recipient leads to induction of the prophage (zygotic induction). The entry of the phage's genetic material by this pathway may be directly compared with normal infection. A number of differences are noted. For example, U.V. irradiated bacteriophage populations show a higher proportion of plaque formers on hcr^+ bacteria than on hcr^- (bacteria incapable of stripping). On the other hand, mating with U.V. irradiated F′ donors leads to the same efficiency of zygotic induction in hcr^+ or hcr^- recipients.

These observations can be explained by the hypothesis that in mating only a single strand of the genetic material is transfered while in normal infection the λ genome is certainly injected as a double stranded molecule. Thus repair mechanisms can operate on double but not on single stranded molecules.

Dr. Stacey (Sussex) described the known mutants affecting the recombination pathway. The best known are the rec A, B and C genes. Mutants at all of these genes increase the susceptibility of the bacteria to X-rays, U.V. and various chemicals. All reduce the level of recombination.

The mutants with the most dramatic effects are the rec^- A mutants. These are also distinguished by a tendency to break down their DNA much more activy than wild type, while res^- B and C mutants on the other hand, are notable in that even after U.V. irradiation they strip their DNA less than do wild type cells. It has recently been shown that these mutants lack an ATP-dependent nuclease found in normal (rec^+) cells. Abnormally high activity of the enzyme is found in rec^- A cells.

Other mutants known as lex^- and exr^- mutants known to increase X-ray sensitivity have also been shown to decrease the level of recombination though less dramatically than the rec^- mutants.

Recently attempts have been made to isolate mutants which reduce the frequency of recombination without affecting radiation sensitivity. A strain was synthesised carrying a small deletion in the z gene of the lactose operon. Into this was introduced an Flac with a point mutant also in the z gene. The two genes could recombine to produce lac^+ recombinants and such recombinants could be observed as papillate colonies on differential medium. Colonies without papillae were isolated and some had reduced ability to form recombinants. Some of these mutants had a resistance to U.V. irradiation not distinguishable from normal, but most on further examination appear rec^- for duly trivial reasons.

A similar system was used to isolate mutants with an abnormally high level of recombination (i.e. producing very many papillae). Two such mutants have been isolated. These appear no more sensitive to U.V. irradiation than do normal cells.

The recombination enzymes of *E. coli*

Dr. A. Rörsch (Rijswijk) described the various classes of radiation sensitive mutants. Notable among these was a class of ror mutants which are sensitive to X-rays but not to U.V. These mutants which map at, at least two regions of the chromosome, do not abolish the ability to undergo recombination.

He then outlined the general nature of the steps involved in recombination and compared them with the steps in repair of damaged DNA. It seems that several steps may be common to both processes but that other steps may be specific. Many points are, however, still obscure, for example, a mutant with much reduced activity of the Kornberg DNA polymerase is U.V. sensitive but still capable of undergoing recombination.

One of the steps which could be imagined as important in both repair and recombination involves an exonuclease capable of hydrolysing DNA starting from a single strand break and so producing an extensive single stranded region. The lack of rec^- B and C mutants of an ATP dependant nuclease present in wild type, apparently explaining the low level of stripping of DNA after U.V. irradiation of these mutants, their U.V. sensitivity and the low level of recombination.

Extracts of wild type bacteria contain an inhibitor of the ATP dependant nuclease but extracts of rec^- A mutants seem to lack this inhibitor. Some at least of the properties of rec^- A mutants (e.g. the excessive breakdown of the DNA) might be explained in terms of a loss of regulation of the nuclease.

Another enzyme which may be involved in repair and recombination is the DNA ligase. This could be the enzyme that completes the rejoining of DNA

strands after recombination. Crellin Pauling has isolated a mutant in which both ability to grow and ligase activity are temperature sensitive. At high temperatures, U.V. sensitivity is increased but there are no reports so far on recombination ability.

A whole set of temperature sensitive mutants of phage T4 affecting genes known to produce enzymes involved in DNA metabolism are known and it may be hoped that the study of such mutants will throw light on our understanding of both repair and recombination.

Fungi

Dr. R. Holliday (London) discussed the mechanisms of recombination found in eukaryotes (especially fungi). In some fungi, it is possible to observe that DNA duplication precedes sexual (nuclear) fusion and that recombination occurring at the subsequent meiosis must involve breakage and reunion of preformed DNA molecules.

He emphasised the role of heteroduplex DNA molecules as intermediates in the formation of recombinants. These heteroduplexes are presumed to be subject to repair where marker mutations prevent normal base pairing. Phenomena such as gene conversion and the 5:3 segregation can be explained in terms of the repair of mismatched bases in heteroduplex molecules.

A particularly interesting suggestion was that the well-known fact that in certain crosses a particular allele may predominate over its homologue in conversion tetrads (so that $6a_1:2a_2$ tetrads are much more frequent than tetrads containing $2a_1:6a_2$) could be explained by specificity of the stripping enzymes and evidence of Trautner and Spatz was quoted to show that such specificity could be demonstrated for the stripping enzymes of *Bacillus subtilis*.

One marked peculiarity of fine structure mapping in fungi is that in a series of 2 point crosses the frequency of recombination between two markers several intervals apart, may be greater than the sum of the recombination frequencies for the adjacent intervals between them. This phenomenon is called map expansion and must be due to the effect of the markers themselves on genetic recombination.

In the discussion Dr. Leupold (Berne) emphasised that transversion mutants (producing purine:purine and pyrimidine:pyrimidine mismatched base-pairs) showed marked differences from transition mutants (which produce guanine:thymine and adenine:cytosine mismatches) in their influence on recombination.

Bacteriophage f1

Dr. T. Boon (Belgium) spoke on some results on recombination in phage f1. The DNA of this phage is a single stranded circle. After injection the DNA is converted to a double stranded form (probably by a bacterial enzyme). Further development requires the activity of the bacteriophage gene II. If a cell is injected with two bacteriophage particles carrying separable mutants abolishing the gene II function there will be no replication of the primary double stranded circle unless recombination between the two mutants produces a phage genome with an intact gene II.

In cells in which such a recombinant is formed phage development can occur and not only can the recombinant genome multiply but it can provide the enzyme allowing the other genome produced by recombination to multiply also.

Thus by analysing the total progeny of the individual phage producing cells (single burst analysis) it is possible to detect the product(s) of individual recombination events.

In the large majority of cases the bursts were shown to contain (beside the wild type recombinant) one or other, but not both, of the parental types.

Such a result is not compatible with either copy choice or breakage reunion and a model was proposed based on breakage of single strands, exchange of partners and replication beginning at the fork produced by this exchange (Boon and Zinder, Proc. Natl. Acad. Sci. U.S. 64 (1969) 573–577).

Bacteriophage λ

Dr. F. Stahl (Edinburgh) described some of his recent experiments on the involvement of DNA replication in recombination. Experiments by Meselson had shown that recombinants between genomes of phage λ could be produced in the absence of detec-

table DNA synthesis but they did not establish whether this dissociation was typical of recombination in this phage. Dr. Stahl's experiments indicate that crossover events near the middle of the phage genome are associated with clearly significant amounts of DNA synthesis but that crossovers near the 'right-hand' end of the chromosome are not. Intermediate results were observed for crossovers near the 'left-hand' end.

These results can be interpreted in terms of the Boon and Zinder model for replication if one assumes that replication is polarized in such a way that when the recombination event occurs near the right-hand end, the region replicated is the short segment from the point of crossover to the end. If the crossover occurs near the middle then there will be extensive replication ending either at a chromosome end or at the next point of recombination.

Since the Boon and Zinder model proposes that the replicating fork running from the point of the switch will be active up to the point of a switch back the amount of DNA synthesis associated with a double crossover might be expected to be correlated with the distance between the two crossover sites. This prediction was confirmed by Stahl in a further series of crosses.

It was most exciting that a recombination model, proposed to account for results obtained in phage f1, should have such value in explaining and predicting results of recombination in so different a phage as λ and from the subsequent discussion it seems that the model may well have significance for understanding recombination mechanisms in a very wide range of systems.

Dr. Stahl also described results of recombination experiments with red$^-$ phages. He found evidence for extensive degradation of the bacteriophage DNA molecules but that those phages which had undergone recombination seemed to have been spared this stripping. He postulates that single strand breaks can lead either to stripping or recombination and that if recombination is prevented excessive stripping will occur. This very closely resembles the fate of the chromosomes of rec$^-$ A mutant bacteria.

The meeting was remarkable for the discussion following each paper. A theme which arose at many points was the possibilities of interaction between the different recombination mechanisms. For example the genome of phage λ is subject to recombination mechanisms catalysed by the enzymes of the rec, red, int, and ter systems.

There is no evidence that recombination events catalysed by the various systems are identical processes and a good deal of evidence that the int and ter systems, with their extremely high substrate-specificity, may be rather different from the generalized recombination mechanisms. For example, it seems that recombination promoted by the int system may be reciprocal whilst that promoted by the red system may, typically, be non-reciprocal. The interactions between these systems and between them and other systems (e.g. the relationship of the xis effect and the int mechanism) are the focus of much interest and are likely to prove the subject of investigations aimed at elucidating the integration and regulation of recombination mechanisms.