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Recombination in phage λ: one geneticist's historical perspective[1]

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Abstract

Several features of bacteriophage λ suit it for the study of genetic recombination. Central among them are those that make it possible to correlate inheritance of DNA with the inheritance of information encoded by DNA through density-label equilibrium centrifugation. Such studies have revealed relationships between DNA replication and recombination, have identified roles for double-strand breaks in the initiation of recombination, and have elucidated the role of the recombination-stimulating sequence, χ.

Keywords: Genetic recombination; RecA; RecBCD; Chi; Red; DNA replication; Phage lambda; Density gradient centrifugation

1. Introduction

Soon after the discovery of phage λ, mutants were isolated, making possible the demonstration of genetic recombination (Jacob and Wollman, 1954; Kaiser, 1955). The data from lytic cycle crosses were coherent when analyzed within the population mating theory of Visconti and Delbrück (1953; see also Kaiser, 1955). However, the data differed from those obtained with phages T2 and T4, which had provoked the theory, in that λ recombined at a lower rate than did those phages. Later analyses demonstrated that the λ linkage map obtained in lytic cycle crosses was linear, unlike the circular map of the T-even phages, and congruent with the virion chromosome (Hershey, 1958; Foss and Stahl, 1963; Streisinger et al., 1964). These properties of λ recombination underlay the success of density transfer crosses with this phage.

In their pioneering density-transfer crosses, Meselson and Weigle (1961) were blessed by additional serendipitous features of λ infections:

(1) Some chromosomes appearing in progeny virions have failed to replicate as indicated by conservation of their density label.
(2) These 'free-loaders' are not excluded from other activities – in particular, they indulge in genetic recombination with each other as well as with chromosomes that have replicated.
(3) Non-replicated chromosomes are packaged into progeny particles only if they have enjoyed an exchange (which need not be genetically detectable) (Stahl et al., 1972a; but see Thomason et al., 1997). This ensures that recombination frequencies among the 'free-loaders' are conveniently high, even when the rate of recombination among intracellular phage is low.
(4) In crosses that are wild-type for recombination functions, most of the recombination involving non-replicated chromosomes occurs near the ends of the chromosome, preserving the discrete fully conserved density peak that identifies free-loaders (Stahl et al., 1974).

Meselson and Weigle (1961) addressed the hypothesis that recombination in λ occurs by a cooperative replication process, in which parental chromosomes act as templates but contribute no atoms to the jointly created progeny chromosome ('Copy-Choice'). The hypothesis was a popular one in phage circles at that time (see, e.g., Levinthal, 1954; Hershey, 1958) for several reasons:

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Abbreviations: Chi (χ), DNA sequence 5′-GCTGGTGG, which stimulates *E. coli* recombination; DSB, double-strand break; Exo, exonuclease; H, heavy due to isotope content; L, light (not H).

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(1) The apparent non-reciprocality of recombination in T-even phage seemed to rule out exchanges like those resulting in meiotic crossing over.
(2) There was a reluctance to break, transversly, the beautiful double helices so recently conceived by Watson and Crick (1953).
(3) Uncertainties about chromosome structure permitted one to think of eukaryotic chromosomes as strings of phage-like DNA molecules. In that context, the non-reciprocal nature of meiotic gene conversion suggested a replicative process homologous with phage recombination.

The lytic cycle crosses of Meselson and Weigle (1961) involved one density-labeled ('Heavy') and one ordinary ('Light') parental type λ. The two parents differed at a pair of loci near the right end of the map, which corresponds with the right end of the virion chromosome. The results obtained demonstrated that recombination in λ was not exclusively by Copy-Choice (or by the Fragmenting Copy-Choice scheme of Delbrück and Stent, 1957) since recombinant chromosomes were found near the fully conserved (H/H) peak of freeloaders. However, the crosses left open the possibility that the recombinants observed had arisen by Break–Copy, in which the heavy parent donated all but the right end of its chromosome and the light parent provided the right-end marker by allowing itself to be used as a template for DNA synthesis. This interpretation was addressed by Meselson (1964), who made both parents heavy and employed markers defining a centrally located interval. Fully heavy recombinants were recovered from these crosses, arguing that recombinants in lambda arise by a Break–Join process.

Soon thereafter it was demonstrated that generalized (homology-dependent) recombination in λ can occur by more than one ensemble of enzymes ('pathway'). For instance, λ recombines well in hosts lacking *Escherichia coli*'s RecA protein (Takano, 1966; Van de Putte et al., 1966; Brooks and Clark, 1967). This recombination proved to be dependent on λ's *red* genes (Signer and Weil, 1968). Conversely, λ missing its *red* genes recombines in wild-type *E. coli*, revealing the action of *E. coli* pathways on λ. Especially relevant to Meselson's (1964) analysis was the demonstration that the site-specific *att*/Int system of λ operated in the very interval for which Meselson had demonstrated Break–Join recombination (Weil and Signer, 1968; Echols et al., 1968). Thus, it remained possible that the conclusions drawn by Meselson were applicable to only some of the systems that operated in that interval and might not characterize generalized, homology-dependent recombination at all. This possibility provoked Mary Stahl and me to re-investigate λ recombination using modifications of Meselson's (1964) density-label methods.

We employed three modifications:

(1) We compared results from crosses in which different pathways were operating individually as a consequence of mutational elimination of recombination functions of the host and/or the phage.
(2) Following Weil and Signer (1968), we marked a central and a terminal interval in the same cross, so that one interval could be subjected to site-specific recombination while the other was not. This sometimes allowed comparisons between generalized and site-specific pathways to be made within the same data set.
(3) We reduced or eliminated chromosome replication (Stahl and Stahl, 1971a,b).

Reduction of replication served three purposes:

(i) By reducing the numbers of progeny particles carrying chromosomes that had replicated, the peak of particles carrying unreplicated chromosomes was better resolved by the equilibrium density gradient.
(ii) By reducing replication that was occurring independently of recombination, we might reveal any replication that was associated with recombination.
(iii) When replication was fully eliminated (McMilin and Russo, 1972), density gradient centrifugation of the progeny from crosses between phages marked near their chromosome ends, one parent heavy and one light, displayed the full distribution of exchanges along the length of the unreplicated λ chromosome (Stahl et al., 1974; McMilin et al., 1974; Lam et al., 1974).

2. The Red pathway, with and without RecA

The results of the various crosses (Stahl et al., 1974) revealed that the pathways did differ from each other with respect to the involvement of DNA replication. The site-specific *att*/Int system did, in fact, catalyze Break–Join recombination in the central interval. Furthermore, in the absence of DNA replication, λ's generalized system, Red, operating in a *recA*$^+$ host, generated few recombinants in that interval even though recombination near the termini was robust. Thus, Int could have been responsible for the Break–Join recombinants Meselson saw in the central interval. On the other hand, when replication was allowed to the extent of giving three density peaks (H/H, H/L and L/L) of comparable height, recombinants in the central interval were well represented in the L/L peak, while recombinants in the terminal interval were a constant fraction of the phage in all three peaks. A second way of reporting this result is to say that, when modest amounts of chromosome replication were allowed, the average density of recombinants for the central interval was less than that for terminal recombinants (and for total phage progeny), implying that DNA replication enhances recombination, but does so differentially in the two intervals. Stahl et al. (1972b) posed the question: does

replication stimulate completion (as in Break–Copy) or initiation of recombination? Within each of those classes of explanation, we had to account for the differential behavior of the generalized recombination system (Red$^+$ RecA$^+$ Int$^-$) in the central and terminal intervals.

For some time, Mary and I aimed our experiments at the following Break–Copy explanation: recombination far from the ends of the chromosome is sensitive to the replication block because a lot of DNA must be replicated to complete the recombination act. (Perhaps a full replication fork was required unless the event was close to the end of the chromosome, in which case chain extension by DNA polymerase might be sufficient.) The demonstration that λ's Red recombination system stimulated DNA synthesis (Skalka, 1974) argued for such an interpretation, since shown to be valid for T-even phage (for review, see Mosig, 1994). However, we could never obtain convincing evidence for Break–Copy with our methods. That may be because, as described below, DNA replication does appear to stimulate the initiation of recombination, and this may have confounded our efforts to show Break–Copy.

Support for replication-dependent initiation came most directly from crosses in which one parent (or the other) was cut in vivo with a Type-II restriction enzyme acting at a unique site in the central interval (Thaler et al., 1987a). In a cross between a heavy and a light parent, recombinants arose whose density and genotype indicated that they received duplex DNA to one side of the restriction site from one parent and duplex DNA from the other side from the other parent (Stahl et al., 1990a). Thus, recombination proceeded by Break–Join and was evidently initiated by the restriction-catalyzed double-strand break (DSB). This evoked the proposal (i) that the reason recombination at the termini is replication-independent is that the termini act like DSBs in their ability to *initiate* recombination and (ii) that the dependence on replication for centrally located recombination is because replication is the major creator of DSBs located there (Thaler et al., 1987b; Stahl et al., 1990a, 1997, q.v. for earlier references), presumably during λ's transition from theta to sigma replication.

Lambda's DSB-induced recombination in RecA$^+$ hosts is like that postulated for yeast (for review, see Stahl, 1996): the DSB is followed by Red-dependent formation of a 3′ overhang (Hill et al., 1997), which, with the help of RecA protein, invades an intact homologue. The resulting intermediate is cut by a 'resolvase' to give recombinants in which the two segments are spliced together by 3′ overhangs (White and Fox, 1974). Features of the λ experiments suggest that it is only luck when both ends invade the same homologue, as must happen routinely in yeast meiosis. When only one end invades, the reaction continues, but only one recombinant chromosome results, at least directly.

The focusing of the recombination event near a DSB implies factors that limit the extent of Red-mediated resection and other possible degradations of DNA at the DSB. T. Tarkowski, L. Thomason, D. Mooney and F.W.S. (in preparation) showed that functions in λ's *ninR* region are required for those focused events. Their work suggests that Orf (Sawitzke and Stahl, 1992) assists RecA protein and that Rap (Hollifield et al., 1987; G. Sharples, personal communication) is a resolvase. Neither of these functions is essential for the event, but Tarkowski et al. suggest that they limit degradation by getting the event over with quickly.

The dependence of recombination on RecA asserted above is demonstrated by the results of replication-blocked crosses in RecA$^-$ cells. Recombination does occur, but at a 100- or 1000-fold reduced rate compared to that in RecA$^+$ cells (Stahl et al., 1974). The small amount of recombination that occurs is focused at λ's right end. This result is apparently in conflict with the observation cited above that λ *red* genes mediate robust recombination in RecA$^-$ hosts. The resolution is that the Red system does do so, but that the recombination depends on replication-induced breaks throughout the chromosome. When RecA protein is present, a DSB in one parent is sufficient to stimulate recombination, which proceeds by invasion, as described above. In the absence of RecA, invasion occurs at only a low rate. Nevertheless, the Red system alone can catalyze near wild-type levels of recombination as long as both parents are broken and the breaks are non-allelic. The resulting recombinants are typically heteroduplex from one break to the other. In RecA$^-$ replication-blocked density-labeled crosses, these recombinants have the density expected for two segments of DNA spliced together from one break site to the other (Stahl et al., 1997). This is exactly the result predicted by Cassuto and Radding's refinement (Cassuto and Radding, 1971) of the models of Szybalski (1964) and of Thomas (1966). The in vitro properties of the exonuclease encoded by the *redα* gene provoked the refined model, in which resection in a 5′–3′ direction occurs hand-in-hand with annealing of complementary chains and is terminated when the annealing is complete. The annealing is presumably catalyzed by Red*β*, which has such activity in vitro (Kmiec and Holloman, 1981).

3. χ and the RecBCD pathway

Phage λ stripped of its recombination genes *redα*, *redβ* and *redγ* (or *gam*) recombines in *E. coli* by the RecA-dependent RecBCD pathway of the host. However, it does so poorly. Recombination is improved by λ mutations (Lam et al., 1974; Stahl et al., 1975; Henderson and Weil, 1975) that create the χ (Chi) sequence 5′-GCTGGTGG-3′ (Smith et al., 1981). This sequence

is present about once per 5 kb (Malone et al., 1978) or 1000 times in *E. coli* DNA (Blattner et al., 1997), but is lacking in wild-type λ. In the absence of χ, density-labeled crosses with blocked DNA replication revealed that recombination by Break–Join was approximately uniform across the length of the chromosome – there was no hint that the ends were playing a special role (Stahl et al., 1974). A single χ mutation gave a density peak of recombinants signifying a Break–Join recombination event focused near the χ (Lam et al., 1974; Stahl et al., 1975).

A role for λ's ends in the RecBCD pathway became apparent following the demonstration that χ was functional only when in one of its two possible orientations in the λ chromosome (Faulds et al., 1979; Yagil et al., 1980). The basis for this orientation-dependence is an asymmetry in the two ends of the linearized λ chromosome – if *cos*, the sequence that is cut to create the ends of the packaged chromosome, is inverted, χ in its standard orientation becomes inactive, but an inverted χ becomes active (Kobayashi et al., 1982). An inverted χ can be activated as well by a restriction cut delivered to circular λ in vivo (Stahl et al., 1983). This observation, combined with the observations by Rosamond et al. (1979) that RecBCD (=ExoV, the product of the *recB*, *C*, and *D* genes) can travel through duplex DNA from a DSB entry site, led to the proposal that when λ is cut at *cos*, preparatory to packaging, the right end becomes an entry site for RecBCD, while the left end does not (Kobayashi et al., 1984b). This view was supported by the demonstration that RecBCD does, in fact, interact with χ (Ponticelli et al., 1985).

At this point, the field appeared to be paralyzed by denial. Everyone knew that linear duplex DNA in *E. coli* was rapidly degraded by ExoV (Simon and Lederberg, 1972; Oliver and Goldberg, 1977), the principal activity of the RecBCD protein, but this knowledge was in apparent contradiction to the notion that RecBCD was required for recombination, and that it facilitated recombination by interacting with χ. How could an enzyme that demolished its substrate be a recombinase? The possibility that the enzyme digested as far as χ and then stopped may have escaped consideration because of early demonstrations that *E. coli* recombination, presumably effected by RecBCD, was often apparently reciprocal (Herman, 1965; Meselson, 1968), even when known to be χ-stimulated (Kobayashi et al., 1984a).

Shumo Liu (cited in Stahl et al., 1990b) proposed that RecBCD *does* degrade DNA from its point of entry until it encounters χ and that the apparent reciprocality is the result of three-body interactions. Liu supported his proposal with experiments, the data from which were buried in my desk drawer (I was still in denial). However, when Andrei Kuzminov (cited in Stahl et al., 1990b) weighed in with the same proposal, we tested its predictions (Stahl et al., 1990b). Our results confirmed those of Liu: when χ is present in only one parent and the two parents are present equally in the cross, the recombinant that inherits χ appears in the progeny in lower frequency than does the χ^0 recombinant. However, if the χ^+ parent is present in numerical excess, the complementary recombinants are recovered about equally. This supported the view that the elementary event is non-reciprocal, but that involvement of a third participant can produce the recombinant that could not appear in the primary interaction because of DNA destruction from λ's right end up to the χ site. This view of χ-induced events was supported by the triparental nature of exchanges resulting in formation of a χ-stimulated cointegrate between λ and a plasmid carrying a segment of λ DNA (Stahl et al., 1990b, 1995).

Evidence from genetic crosses, combined with that from in vitro biochemistry, established the view that, following entry into the right end of λ's chromosome, ExoV moves leftward, chopping up the DNA until it encounters χ so oriented as to be recognizable to the moving enzyme. The enzyme then has a chance of undergoing a change of state, losing nuclease activity but gaining recombinagenic activity. This view, which is supported by in vitro analyses (Dixon and Kowalczykowski, 1993; Dixon et al., 1994), accounts for the observations that χ^+ is dominant to χ^0, stimulates recombination to its left, and results in the formation of the χ^0 recombinant more frequently than the χ^+-containing one (Stahl et al., 1980). It also accounts for the interactions between χ sites seen in vivo when a chromosome contains two such sites – the χ site to the right is about 50% epistatic to the one on the left (Stahl et al., 1990b).

The picture presented above is widely accepted (although you would not know it from looking at recent textbooks of Genetics). Within its framework, a number of issues have been raised: What is the nature of the change of state of RecBCD? Why does the rate of recombination fall with distance to the left of χ? By what means does the changed enzyme provoke recombination? What are the 'rules' of that recombination? i.e., which chains do what? λ has made its contributions not only to the posing of these questions but to answering them, as well.

3.1. What is the nature of the change that RecBCD undergoes when it meets χ?

The χ site is inactive in λ crosses conducted in a $recD^-$ mutant host, and recombination between freely replicating λ chromosomes occurs in each marked interval at about the rate expected if χ were in that interval (Chaudhury and Smith, 1984; Amundsen et al., 1986). These properties of $recD^-$ mutants led Thaler et al. (1988) to propose that when RecBCD encounters

χ the enzyme becomes functionally equivalent to the enzyme present in a *recD*$^-$ mutant. This model made the strong prediction that recombination in *recD*$^-$ mutants would be focused at DSBs – immediately upon entering a duplex at a DSB the mutant enzyme would be recombinagenic, acting like wild-type enzyme that had just seen χ! That result was obtained (Thaler et al., 1989), supporting the notion that the role of χ is to alter the enzyme and that the nature of the alteration is equivalent to the loss of the RecD subunit from the enzyme. Since *recD* mutants had been selected on the basis of being exonuclease-deficient (Chaudhury and Smith, 1984; Amundsen et al., 1986), it was economical to suppose that the role of χ is to eject or alter RecD, suppressing the exonuclease activity of the RecBCD enzyme and allowing it to express its recombinase activity as it continues to travel (leftward in ordinary λ) (Stahl et al., 1990b).

This model for χ stimulation of recombination was further supported by 'in vivo biochemistry'. Kuzminov et al. (1994) showed that a multicopy χ-free cosmid linearized in vivo by terminase was destroyed by RecBCD. However, more of the linearized DNA survived if the cosmid contained χ. This protection by χ acted in *trans* (and presumably also in *cis*) – χ-free cosmids simultaneously linearized in the same cells were protected by the χ sites. This suggested that the cells were rendered RecD$^-$ by exposure of the RecBCD enzyme to multiple χ sites.

Myers et al. (1995a) conducted λ crosses in such cells. They found that the λ indeed recombined as if the cross had been conducted in a *recD* mutant cell – χ activity was decreased and recombination was focused near the enzyme's entry site, at the right end of the λ chromosome. The RecD$^+$ phenotype was restored by overproduction of RecD subunits, further supporting the view that the χ-induced change involves a loss of RecD subunits.

3.2. *Why does χ activity fall off with distance to the left of χ?*

The notion that χ effects a change of state in the leftward-traveling RecBCD enzyme accounts for the precipitous rise in recombination rate on the right side of χ, but it does not explain why the rate falls off with distance to the left. Myers et al. (1995b) distinguished among three possibilities: (i) the χ-induced alteration (presumed loss of RecD subunit) reduces the enzyme's processivity, (ii) the RecD subunit reassociates with the leftward traveling enzyme, (iii) the altered enzyme can effect only one recombination act and it does so with high probability per unit distance traveled leftward. Myers et al. (1995b) showed that the latter was the correct interpretation by showing that the fall off to the left depends on the presence of homologous DNA in the same cell.

3.3. *By what means does the changed enzyme provoke recombination?*

In vivo studies (Rinken et al., 1992) imply that the enzyme lacking the RecD subunit by mutation retains helicase activity. That conclusion justifies the hypothesis that the enzyme altered by interaction with χ retains helicase, but loses nuclease, functions. Retention of helicase activity could acount for its recombinase activity. In vitro data (Anderson and Kowalczykowski, 1997) suggest that upon encountering χ the enzyme not only loses its predominant nuclease activity on the chain ending 3′ to the right, but gains an activity on the opposite chain. Helicase activity plus this new nuclease activity would generate a 3′ overhang to the left of χ. Conventional wisdom has it that these structures are the world's best for RecA-catalyzed strand invasion and exchange with an intact duplex. That view is supported by the demonstration that λ recombinants produced in all recombination pathways, including RecBCD and RecBC(D$^-$), comprise duplex segments spliced together predominantly by 3′ overhangs (White and Fox, 1974; Stahl and Stahl, 1974; Siddiqi et al., 1990). However, markers patched into λ by χ-stimulated recombination and recovered while still in a heteroduplex state are found predominantly on the chain that ends 5′ at the right end (Hagemann and Rosenberg, 1991). The somewhat paradoxical observations on strand polarity were speculatively reconciled as follows: the overhanging 3′ end is responsible for most of the splices that lead to the replacement of the digested right arm, and the 5′ ended chain, unwound (and partially digested?) by the helicase action of the altered enzyme, is primarily responsible for the interactions that lead to the patching of markers from one replicon to another (Stahl et al., 1995).

3.4. *Break–Copy still to be found in λ?*

Many of the observations cited above were made with density-labeled λ blocked for DNA replication. These data demonstrate that the RecBCD-mediated exchanges can be completed without a requirement for DNA replication of any appreciable extent (relative to the length of the 48.5 kb λ chromosome). That conclusion is potentially at odds with the Break–Copy view of RecBCD-mediated recombination in *E. coli* (for review, see Kuzminov, 1996). The conflict could be resolved by the demonstration of a λ-encoded nuclease, like the resolvase of T4 (Kemper et al., 1981), active on the intermediate arising by RecA-mediated strand-invasion.

4. Conclusions

Some of the omissions in this review are justified within the spirit of the title. Others are excused by my wish to keep the paper accessible to a wide audience. Armed with these two shields, I have presented a condensed chronology of efforts to understand the formal genetics of phage λ in terms of the structure and behavior of its chromosome. Aspects of formal genetics biased early workers toward Copy-Choice schemes for recombination. The pioneering work of Meselson and his colleagues in the 1960s, using density-transfer combined with conventional linkage analysis, swung heads around 180°, so that Break–Join, independent of chromosome replication, became the favored view. Subsequent studies have revealed an interdependence of replication and (generalized) recombination – each appears to stimulate the other in all the generalized recombination pathways described herein. I suspect that the denouement, which cannot be far away, will have much in common with the scheme for Red-mediated recombination of λ offered in 1974 by Ann Skalka.

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