On the Physical Structure of \( \lambda \) Recombinant DNA

V. E. A. Russo *
Institut für Biologie III der Universität Freiburg

Received February 19, 1973

Summary. Crosses of \( \text{sus O29} \times cJ26 \text{ sus O125} \) with \( ^{15}\text{N}^{13}\text{C} \) labelled phages have been performed in \( ^{14}\text{N}^{12}\text{C} \) medium with conditions designed to prevent the duplication of the infecting chromosomes (less than 4% duplication among the mature phages) to determine how much of the recombinant molecule is inherited from each parent, and how much is newly synthesized.

Both the \( \text{rec} \) and the \( \text{red} \) mediated recombinants have 5% newly synthesized DNA, while at least 10% of the DNA is inherited from one parent and at most 85% from the other. The \( O \) gene is at 18.5% (or more) from the right end of \( \lambda \) DNA (Table 2).

The genetic structure of the recombinants in these types of crosses was analyzed:

I. \( \text{sus O29 tsR2} \times cJ26 \text{ sus O125} \)
II. \( \text{sus O29} \times cJ26 \text{ sus O125 tsR2} \).

The double heterozygotes in \( cJ26 \) and \( tsR2 \) regions were (almost) always ++ in cross I and \( cJ26 \text{ tsR2} \) ++ in cross II (Table 3). The \( \text{rec} \) and \( \text{red} \) system seem different in some aspects of the regulation of the recombination process.

These results suggest the following Partial Hybridization model for both \( \text{rec} \) and \( \text{red} \) mediated recombination:

a) The first step in recombination is a hybridization of the single strand of one parent molecule to the other parent DNA. The hybridization starts from one end of the molecule.

b) The mismatched base pairs formed in this hybrid can be repaired through a mechanism of excision and resynthesis.

The model can explain qualitatively the high negative interference phenomenon. A critical analysis of the published results in \( \lambda \), T1, and T4 shows that this model can explain the experimental results much better than does the Break and Join model.

Introduction

The experiments of Meselson and Weigle (1961) and G. Kellenberger et al. (1961) have shown conclusively that there is parental DNA in recombinant phages. However, from those experiments it is impossible to decide how the structure of recombinant DNA is.

Tentatively Meselson and Weigle suggested a Break and Join model and/or a Break and Copy model.

Meselson (1964) has ruled out the Break and Copy model as the only model of recombination.

Fox and Allen (1964) have shown in the transformation of \( \text{Diplococcus pneumoniae} \) that there is a physical insertion of a single strand of transforming DNA into the genetic material of the recipient bacterium.

Crosses of \( \text{sus O29} \times cJ26 \text{ sus O125} \) have been performed in order to distinguish between a Break and Join model and a Single Strand Assimilation model. The \( \text{sus} \) markers were both in gene O that is 18.5% from the right end (Kumar and Szymbalski, 1970).

* Recipient of an EMBO fellowship.
The experiments were designed to measure:

(i) how much of the recombinant DNA is inherited from one parent, how much from the other and how much is newly synthesized,

(ii) how long is the hybrid overlap and possibly heterozygous region.

The \textit{Break and Join model} would predict that 18.5\% of recombinant DNA is inherited from one parent and the hybrid region is relatively small.

In contrast the \textit{Single Strand Assimilation model} would predict that as low as few percent is inherited from one parent and the hybrid region is as long as the assimilated strand.

To do this type of experiments it is essential to be able to recover all the recombinant DNA before any duplication. For that reason the system used was the one described by McMilin and Russo (1972) where there is less than 4\% duplication, the analysis was carried out for both \textit{rec}– and \textit{rec}+ crosses separately. In view of the data obtained, a model of \( \lambda \) recombination DNA invoking partial hybridization of one parent DNA strand to the other parent DNA and subsequent repair of heterozygous regions shall be discussed.

\textbf{Materials and Methods}

\textit{Media and Plates:} see Russo \textit{et al.} (1970); Stahl and Stahl (1971).

\textit{Bacteria and Phages:} see Table 1.

\textit{Phage Crosses:} see McMilin and Russo (1972).

The cross performed in the absence of replication in FA-77 between density labelled phages was always of the same genetic type \( O29 \times cJ260125 \), where \( O29 \) and \( O125 \) are two \textit{am} mutants (\textit{sus}) in the \( O \) gene. For the analysis of heterozygotes two types of crosses were used:

\( O29 \times cJ260125 \, t e R2 \) and \( O29 t e R2 \times cJ260125 \).

\textbf{Heavy Phage Stocks}

\( K12SH28 \) was diluted from an overnight in M9 1:100 in M9 heavy and shaken at 37\(^{\circ}\) for 4 to 6 hours to a titer of \( 10^8 \) ml. The bacteria were centrifuged 5 min at 5000 rpm, the supernatant was saved and the pellet was resuspended in 1/10 of the original volume using the supernatant. The bacteria were infected with a m.o.i. = 3 of the desired phage genotype and after 10 min adsorption at 0\(^{\circ}\)C the rest of the supernatant, prewarmed at 30\(^{\circ}\)C was added. The culture was shaken at 30\(^{\circ}\)C for 4 to 6 hours, until lysis was visible. The advantage of growing the infected bacteria at 30\(^{\circ}\)C instead of 37\(^{\circ}\)C is:

a) The reversion is low so that any stock did not contain more than \( 10^{-5} \) revertants.

b) The titer is higher: from \( 1 \) to \( 4 \times 10^{10} \).

The heavy phage stocks were not purified for density because the phages with \( L/L \) and \( H/L \) DNA represent less than 2\% of the total phages.

\textbf{Density Analysis}

Cross lysates were banded in Cs formate gradients as described in McMilin and Russo (1972). To measure the amount of shift of the different peaks, a semilogarithmic paper with the recombinant gradient curves was superimposed on a semilogarithmic paper with the parents gradient curves. The two papers were shifted, one with respect to the other, until the fitting curve of one parent peak was lying over the experimental points of one of the recombinant peaks. It was easy to measure the shift, in millimeters, of the two peaks and the distance, in millimeters, of the HH and LL parent peaks. The ratio gives the percentage of recombinant DNA that is light, or heavy, depending if the parents curves were in the heavy position, or in the light one.

\textbf{Analysis of Heterozygote}

A cross was made (in FA-77 or FA-773 \textit{rec}–) between \( O29 \times cJ260125 \, t e R2 \) and \( O29 t e R2 \times cJ260125 \) according to the procedure explained above.