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Molecular analysis of rearrangements in human ribosomal RNA gene clones

(Recombinant DNA; deletion; recombination; promoter; operator; bacteriophage λCharon 16A; electron microscopy)

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SUMMARY

Human placental DNA, enriched for ribosomal sequences, was cloned in the phage vector λCharon 16A. Recombinants containing 28S rDNA sequences were isolated, and all were found to have deletions in the insert and/or vector DNA. Electron microscopic analysis was used to map the deletions and provide evidence that unstable forms of the recombinants can revert to the original vector or undergo further rearrangements. Specific deletions are manifested as previously unreported plaque phenotypes.

Genes coding for human ribosomal RNA (rRNA) have been cloned in the phage vector λCharon 16A and subjected to structural analysis (Wilson et al., 1978; Erickson et al., 1981). In an initial study (Wilson et al., 1978) it was found that eleven of 978 recombinant clones generated in λCharon 16A contained an *Eco*RI fragment encoding most of the 18S gene (*Eco* RI-B fragment; Wellauer and Dawid, 1979). No recombinant, however, was found to contain the known 7.3-kb *Eco* RI-A fragment containing most of the 28S gene. We expected to find equal numbers of A and B fragment clones, since the fragments occur in equal amounts and the lengths are within the cloning capacity of the vector. Subsequent efforts have resulted in the cloning of the *Eco* RI-A fragment in λCharon 16A, but in all cases it was observed that each recombinant contained one or more deletions. Further analysis of several recombinants was undertaken to clarify the nature of the deletions.

Human placental DNA was extracted and digested with *Eco*RI as described (Wilson et al., 1978; Schmickel and Knoller, 1977) and ligated into the *Eco*RI site of λCharon 16A. The recombinant phage DNA was transferred into the Lac$^+$ *E. coli* strain K802, and the bacteria were plated in the presence of the chromogenic substrate 4-bromo-5-chloro-3-indolyl-D-galactoside (X-gal) as described

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Abbreviations: kb, kilobases or kilobase pairs; rDNA, DNA coding for ribosomal RNA.

by Williams and Blattner (1979). Under these plating conditions λCharon 16A are dark blue with blue halos resulting from expression of the phage *lacZ* gene. Recombinant plaques are pale blue. An insert at the *Eco*RI site interrupts the *lacZ* gene of the phage, but the adjacent *lac* operator binds the host's *lacI* product allowing derepression of the host *lacZ* gene (Blattner et al., 1977).

Out of 576 recombinants 13 hybridized to a 28S [^{125}I]rRNA probe. These included an unexpected colorless plaque designated λA_6. Plaque purification of λA_6 showed that it had a stable phenotype. Analysis of λA_6 by gel electrophoresis, R-loop analysis, and heteroduplex analysis (see Fig. 1 for references) revealed that the recombinant contained a 7.3-kb insert that hybridized to the 28S [^{125}I]rRNA and was identical in size to the 7.3-kb *Eco*RI-A fragment of rDNA. λA_6 also had a deletion of approx. 6 kb which included the promotor-operator portion of the *lacZ* gene (Fig. 1). Deletion of the *lac* promoter from the phage would account for the colorless plaque.

A second recombinant λA_{64}, was identified as a pale blue plaque with a distinct dark blue ring (ring phenotype). Banding of plaque-purified λA_{64} in CsCl resulted in two phage bands labeled λA_{64A} (the less dense band) and λA_{64B} (the more dense band). Analysis of λA_{64A} showed that it was a stable revertant to the parental λCharon 16A and gave dark blue plaques with blue haloes. Southern analysis (Southern, 1975) of λA_{64B} showed a 4.1-kb insert that hybridized to the 28S [^{125}I]rRNA. Electron microscopic analysis confirmed that the insert had an internal deletion of approx. 3.2 kb (Fig. 1). Plating of plaque-purified λA_{64B} produced four types of plaques among a total of 12402 plaques observed: ring (99.63%), colorless (.25%), light blue (.08%), and parental (.04%).

Several derivatives of λA_{64B} (each cloned from a single plaque), including one light blue plaque (λA_{64D}) and seven colorless (λA_{64C}, λA_{64E}–λA_{64H}), were analyzed by electron microscopy as shown in Fig. 1. In addition to the 3.2-kb deletion of the parent phage, all have other deletions either in the right vector arm or in the insert. Four of the isolates appear to be identical to each other. Subsequent plating of phage from light blue and colorless plaques gave stable plaque phenotypes.

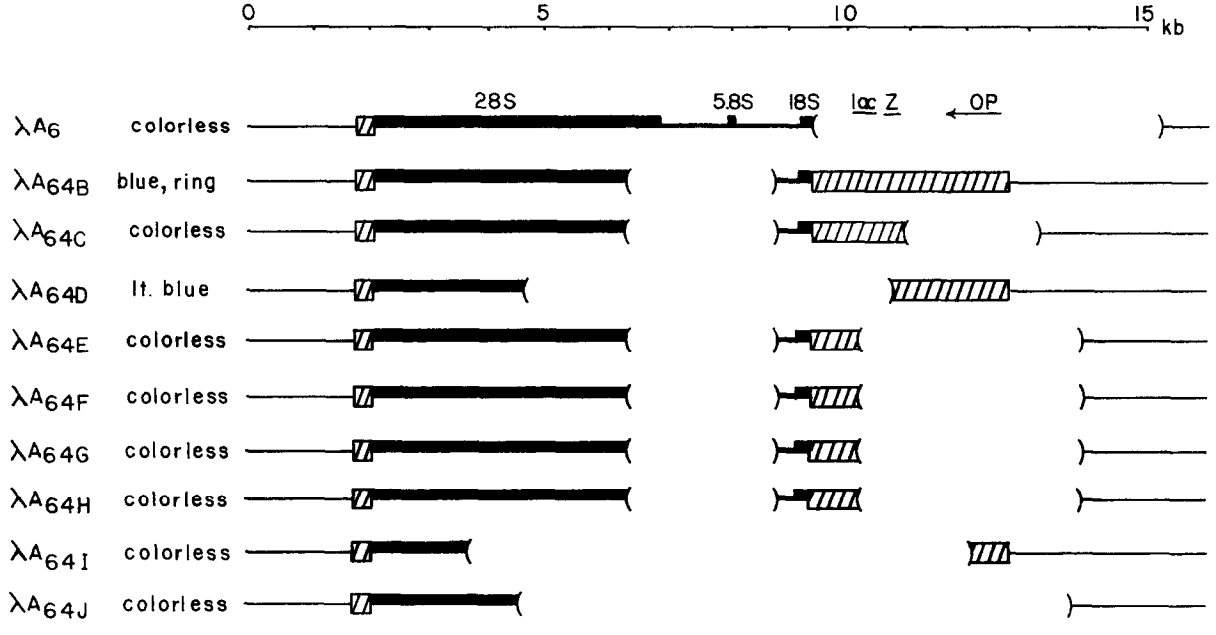

Fig. 1. λCharon 16A recombinants containing human 28S rDNA, as analyzed by gel electrophoresis and Southern blotting (Helling et al., 1974; Southern, 1975), heteroduplex analysis formamide technique of Westmoreland et al. (1969) as modified by Davis et al. (1971), and R-loop analysis (Thomas et al., 1976). Plaque phenotypes are indicated after each clone designation, and only the central portions of the recombinant DNAs are depicted. Thin lines indicate λ sequences, heavy lines with black boxes: fragment A sequences, and shaded boxes: *lac*5 sequences from λCharon 16A. The positions of the 18S, 5.8S (tentative) and 28S genes, the *lac* promoter (P), operator (O), and *lacZ* gene are shown. The arrow indicates the direction of transcription.

The expected recombinant of λCharon 16A containing the 7.3-kb *Eco*RI-A fragment has not been isolated. Fig. 2 summarizes the evolution of the recombinants starting with a putative recombinant containing the full length A fragment. The instability of the initial isolates suggests that deletions are the result of cloning and do not represent naturally occurring variants of the 28S rRNA gene. The source of instability may be a deleterious sequence in the A fragment that is transcribed from the *lac* promoter. It is noted that the A fragment contains the complete 5.8S human rRNA gene and most of the 28S gene, both of which have considerable homology with the *E. coli* 23S rRNA gene. Deletion of either the *lac* promoter or a portion of the A fragment sequence leads to a stable phage. Colorless plaques result from destruction of the *lac* promoter while light blue plaques generated from λA_{64} result from deletion within the insert. Ring phenotypes are the result of metastable genotypes. As the plaque develops, revertants to parental phenotype and also to deleted progeny are being produced, giving a mixture of recombinant (pale blue), *lac* deleted (colorless), and revertant (dark blue, halo) phenotypes.

Regeneration of parental phage (e.g., λA_{64A}) from plaque purified recombinants must entail the reconstitution of the *lacZ* gene. It is probable, given the significant number of revertants observed (4×10^{-4}), that precise deletion of the A fragment insert occurs by the mechanism of recombination. That mechanism would not require the precise excision of the insert but rather a double crossover event involving the homologous sequences of the phage *lacZ* gene and that of the host.

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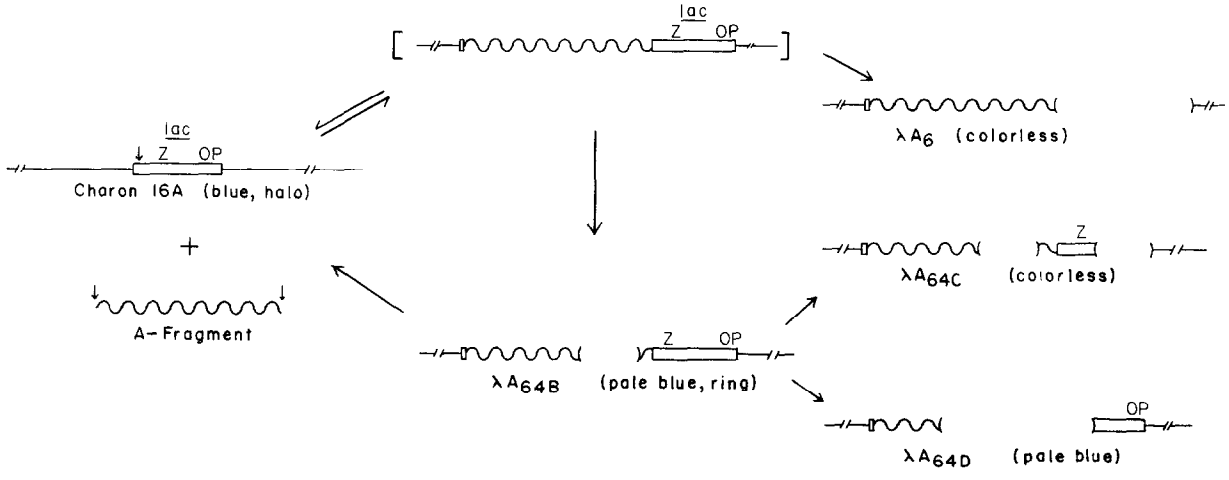

Fig. 2. Possible evolutionary relationship between metastable λCharon 16A-28S rDNA recombinants. This diagram shows examples of the evolution of recombinants starting with the insertion of the rDNA A fragment into the *Eco*RI site (small vertical arrows) of λCharon 16A. Boxes indicate *lac*5 sequences, and wavy lines indicate inserted sequences. Deletions are indicated by parentheses. The recombinant in brackets is putative and was never isolated. Examples are labeled with λA isolate designations and with plaque phenotype. The relative location of the *lac* promoter (P), operator (O), and *lacZ* gene are shown.

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