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A stable duplication of the rII region of bacteriophage T4

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SUMMARY

In the preceding paper (Van de Vate & Symonds, 1974) a deletion, *sd*1, has been reported that stabilizes an rII diploid by penetrating into the region of the duplication from the right-hand side (if we take the rIIB gene located to the right of rIIA). Here we wish to report on a different stabilizing deletion, *sd*2. Evidence will be presented in favour of penetration of the duplication from the left-hand side. The right-hand terminus of the deletion is located within the rIIA gene.

1. INTRODUCTION

The preceding paper describes the identification and properties of a phage particle, *hd*4, containing a genetic duplication with the abnormal characteristic of being completely stable, in the sense that no viable haploid phage particles are derived from it during phage multiplication. This stability was traced to the presence of a particular deletion, *sd*1, which penetrates into the region of the duplication from the right side (if the convention is used that rIIB is to the right of rIIA) and extends through the whole rII region and into the essential genes 39 and 60, which are adjacent to the rIIA cistron. During multiplication haploid segregants are produced, but because of the location of *sd*1 these all obligatorily lack some essential information in their genomes and consequently are non-viable. The *hd*4 strain therefore contains a homozygous duplication, but this does not cover the rII region, which is only present as one copy in the left arm of the duplication. For this reason stable duplications of the *hd*4 type are not compatible with the existence of stable *heterozygous* rII diploids, as these possess two copies of the rII region, one in each arm of the duplication. In this paper we describe the properties of a stable duplication of the heterozygous type in which the absence of haploid segregants is due to the presence of a deletion of a different kind in that it penetrates the region of the duplication from the left side and so removes essential genes from that arm of the duplication. As this new stabilizing

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deletion was also found to remove part of the rIIA cistron it proved possible to locate it by genetic methods.

2. MATERIALS AND METHODS

The techniques and nomenclature used are similar to those described earlier (Van de Vate & Symonds, 1974; Symonds *et al.* 1972). Fig. 1 lists some point and deletion mutants used in addition to those already listed in fig. 2 of the preceding paper.

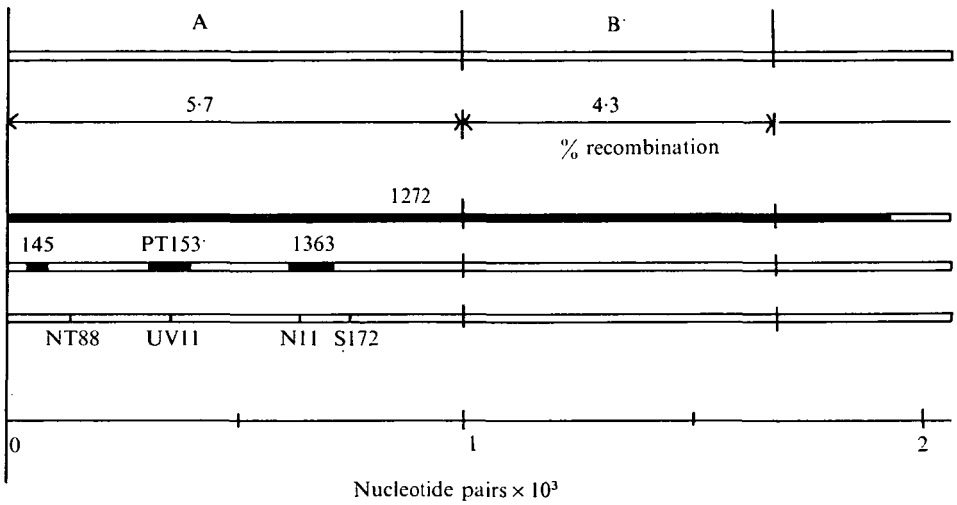

Fig. 1. Map of the T4 rII deletion and point mutants used in addition to those already listed in Fig. 2 of the preceding paper.

3. RESULTS

The new heterozygous diploid, D2, originated as an rII$^+$ recombinant from a cross between the overlapping rII deletions 1589 and 638. Lysates of D2, whether grown in B or K, were always abnormally stable. A few (0·1 %) turbid plaques were found on mixed B + K indicator, but on further testing these were shown by simple spot-crosses to originate from homozygous diploids of the type 1589/1589 and 638/638. These can be formed by double crossing-over between two 1589/638 genomes mated in a 'staggered' position, according to the scheme proposed by Symonds *et al.* (1972).

The stability of the D2 diploid is inherited by the homozygous derivatives. This was shown directly by re-isolation of more than a 1000 progeny plaques from a 1589/1589 (D2) stock, with subsequent spot-crosses against 638. Not one haploid segregant was found. The stability of the homozygous diploids is also implicit in Table 1, where it can be seen that in the cross 1589/1589 (D2) × 638 about 11 % of the cross progeny are rII$^+$ diploids with identical segregation characteristics to D2. On the other hand, the recombination frequency with a segregating

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homozygous diploid like 1589/1589 (M4) (M4 grown in K displays a segregation frequency of 20 %; Symonds *et al.* 1972) is nearly a 1000-times less. This reflects the fact that stocks of homozygous diploids must be grown in B so there is no selection against the segregants. For this reason any stock will contain very few diploid particles, which in turn causes the relatively low recombination frequencies obtained with these strains. The stability of the D2 homozygous diploids is also consistent with the finding that the terminal redundancy of 1589/1589 (D2) is appreciably smaller than that of 1589, and similar to that of 1589/638 (D2) (see Table 2).

Table 1. *Frequencies and segregation characteristics of rII$^+$ progeny diploids from crosses with homozygous diploid derivatives of D2 and M4*

Cross	Recombination frequency*	Segregation frequency (%)†
1589/1589 (D2) × 638	$1{\cdot}1 \times 10^{-1}$	0·1
1589/1589 (M4) × 638	$1{\cdot}9 \times 10^{-4}$	20
1589 × 638	$7{\cdot}0 \times 10^{-7}$	3–60

* Crosses were performed in B under standard conditions. Recombination frequencies were calculated as the ratio of rII$^+$ diploids (assayed on K) to total progeny (assayed on B).

† Twenty rII$^+$ recombinant diploids were isolated from each cross lysate, and plated on a mixed indicator. At least 30 plates containing 500 plaques each were examined for each diploid isolate. Averaged values of turbid plaque frequencies are shown for the first two crosses.

Table 2. *Terminal redundancy measurements*

Phage	h^{2+}/h^{4+} heterozygotes (%)	No. of clear plaques scored
1589/1589 (D2)	0·46	38
D2	0·41	56
1589	0·65	134

Crosses between h^{2+} and h^{4+} derivatives were performed for each phage in B under standard conditions. Progeny phage were plated directly on B/2 + B/4 mixed indicator so as to give about 40 plaques per plate. Clear plaques were picked, and spotted onto B/2/h and B/4/h in order to identify spontaneous *h* mutants, which were disregarded in the above estimates.

As explained in the Introduction, only the models I and II discussed in the previous paper (Fig. 4) can explain the properties of stable heterozygous diploids. In order to discriminate between these two models we crossed D2 with T4 wild-type and isolated 40 independent rII$^+$ diploids. All of these appeared to be identical with the original D2. This result is incompatible with model I because one would expect some segregating recombinant diploids which have lost the *rec*$^-$ mutation. It is, however, compatible with model II if one assumes that the stabilizing deletion is essential for the maintenance of terminal redundancy and hence viability of the D2 diploids (see preceding paper).

We therefore performed genetic tests to locate the stabilizing deletion required in model II. For technical reasons it was not practical to determine whether the

deletion covered the genes 39 and 60 located to the left of the rII region. One possibility that could be tested, however, was that the deletion actually extended into the rII A gene. This is illustrated in Fig. 2(*a*) which is a minor modification of model II as shown in Fig. 4 of the preceding paper. In order to test this possibility

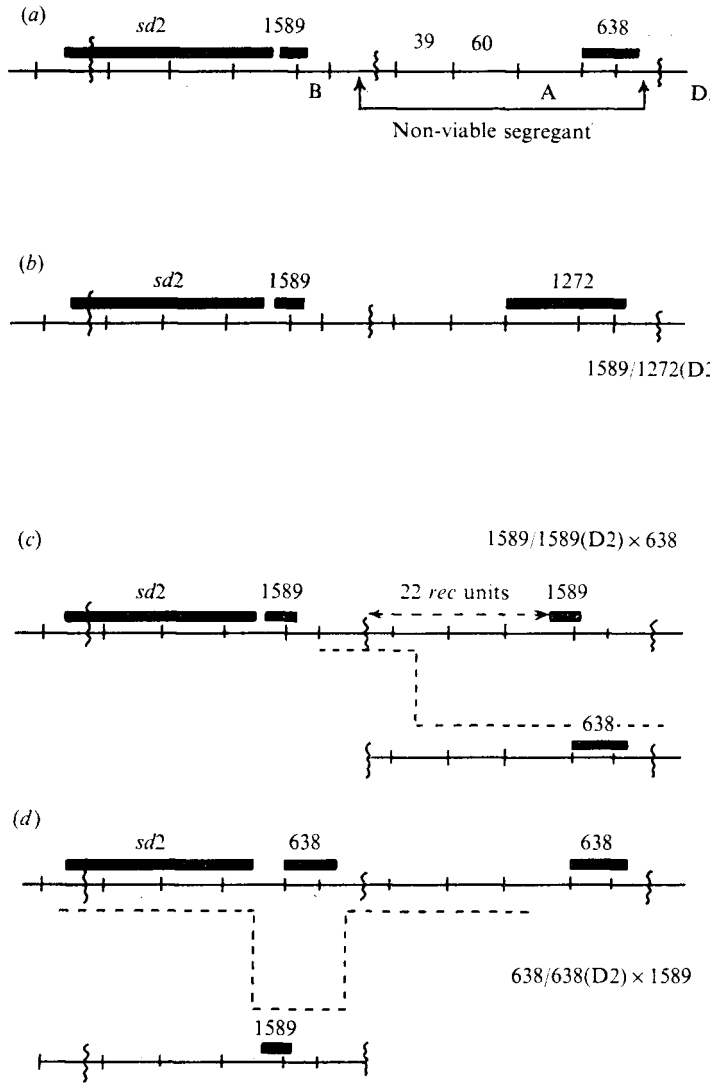

Fig. 2. Proposed structures for D2 and derivatives. The squiggly lines indicate the extent of the duplication. (*a*) D2. The deletion *sd*2 removes the greater part of the homology contained in the duplication, and causes non-viability of the remaining segregants (see double arrow). Capital letters refer to functional genes. (*b*) 1589/1272 (D2). (*c*) 1589/1589 (D2), mated with 638. The single crossover indicated generates an rII$^+$ recombinant diploid similar to D2. (*d*) 638/638 (D2), mated with 1589. The double crossover indicated is necessary for the formation of rII$^+$ progeny because the 1589/638 single cross-over presumably lacks terminal redundancy, and therefore is non-viable.

we first prepared from D2 a 1589/1272 (D2) recombinant diploid which now only has an rII region in one arm of the duplication (see Fig. 2*b*). This newly isolated recombinant was crossed to the gene A mutants 145, PT153, 1368, NT88, N11 and S172 (Fig. 1). Of these strains only S172 recombined to form wild-type phage, at a frequency of 5×10^{-4}. This strongly suggests that *sd*2 does indeed delete the proximal part of gene A. It ends between the point mutants N11 and S172 and is therefore tightly coupled with 1589.

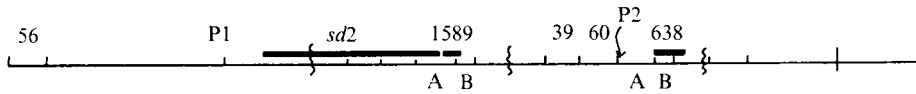

Fig. 3. The diploid D2 shown in its genetic surroundings, with two alternative promoter sites P1 and P2. Neither site can possibly serve both the A and the B gene. The figure is based on the maps of Edgar & Wood (1966) and Sederoff *et al.* (1971*a*).

We have tested the following implications of the model.

(1) In a cross with T4 wild-type, 1589 and 638 should be rescued from D2, but 1589 with a much lower frequency because of its tight coupling with *sd*2. Indeed from 111 rII$^-$ progeny phages from such a cross only 3 were 1589.

(2) rII$^+$ function of D2 is only compatible with the sequence shown, *sd*2–$1589_1/638_2$. The reverse sequence, *sd*2–$638_1/1589_2$, would lack A function. In order to check this we isolated 90 rII$^+$ diploids from the following crosses: 1589/1589(D2) × 638/638(D2)(50 ×), 1589/1589(D2) × 638(20 ×) and 1589 × 638/638 (20 ×), which together should generate both sequences in equal amounts. Each diploid was crossed with T4 wild-type and 50 haploid recombinants were isolated. These contained no 1589, indicating that in any rII$^+$ diploid 1589 remains coupled with *sd*2.

(3) In the cross 1589/1589(D2) × 638 single crossing-over will yield rII$^+$ D2 recombinants, while in the reciprocal cross 638/638(D2) × 1589 double crossing-over is necessary to achieve this (compare Fig. 2(*c*) and 2(*d*); as noted above it is assumed that 1589/638 recombinants without *sd*2 are non-viable because of lack of terminal redundancy). Experimentally the former cross gave 11 % recombinants while the latter gave 0·3 %. The figure of 11 % (which represents 22 recombination units) also indicates that the distance from the repeat-point to 1589 in the right arm of the duplication (see Fig. 2*c*) is about twice the total length of the rII region.

(4) Because the map-positions of *sd*2 and s1231 overlap, no *sd*2–s1231/638 should be formed from the cross 638/638(D2) × s1231. Indeed the recombination frequency in this cross was down to 7×10^{-6}.

DISCUSSION

The diploid D2 has the aberrant property of being stable. This stability is due to a novel deletion in the left arm of the duplication which removes the essential genes 39 and 60 and runs into the rII A gene (Fig. 2*a*). As a consequence all haploid segregants are non-viable. We have obtained direct evidence that the

right-hand end of the deletion is between the map position of N11 and S172 (see Fig. 1). The position of the left-hand end has not been determined directly, but indirect evidence from the cross 1589/1589(D2) × 638 (see Fig. 2*c*) indicates that it is well to the left of gene 39 (according to the map of Sederoff, Bolle & Epstein, 1971 (*a*), the left-hand end of gene 39 is located at about 16 recombination units from 1589; Fig. 2 (*c*) implies that the left-hand end of *sd*2 is at least 22 recombination units away). The left-hand end of *sd*2 is therefore placed in the long region of non-essential DNA separating genes 39 and 56 (Edgar & Wood, 1966). This information about the structure of D2 is incorporated in Fig. 3.

The structure of D2 has some interesting implications with regard to the control of expression of the rII region. Because of the presence of 1589 in D2 the expression of the rIIB gene must be linked to an rIIA promoter (Champe & Benzer, 1962). Two proposals have been considered for the location of the normal rIIA promoter (Schmidt *et al.* 1970):

(1) The function of the region to the left of rIIA is immediate–early (IE), and the RNA polymerase which traverses this IE region continues to transcribe sequentially the delayed–early (DE) rII genes, but only if a phage-determined anti-terminator protein is present. The rII region would then be served by an IE promoter with a wide range of possible locations, for instance the location of P1 in Fig. 3.

Alternatively, (2) transcription of the rIIA gene is under the control of a DE-promoter, located near the left end of the gene, which is recognized by a phage-determined sigma factor. This promoter is indicated by P2 in Fig. 3.

The first proposal has been shown to be true for the majority of the DE regions (Brody *et al.* 1970), but published support in the case of the rIIA gene is still lacking (unpublished evidence is quoted in Sederoff *et al.* (1971*b*)). Therefore the second proposal cannot be disregarded at the moment. In any case Fig. 3 shows that neither promoter location can satisfactorily explain the viability of D2. Promoter site P1 requires an internal promoter in the right arm of the duplication to allow rIIA expression (read-through across the repeat-point is considered to be improbable). Promoter site P2 is deleted in the left arm of the duplication, therefore the expression of the rIIB gene should be under the control of a foreign promoter in that case. Discrimination between these models awaits further research into the location of the rIIA promoter. Some valuable information might result from experiments in which the IE or DE appearance of the rIIA and B genes of D2 is determined.

The origin of *sd*2 is not known. Weil & Terzaghi (1970) have observed a correlated occurrence of duplications and deletions in phage T4. Their explanation is that the deletion is selected because it restores terminal redundancy to an otherwise overlong diploid genome. Because we found *sd*2 in a similar relationship to the D2 duplication, the simplest assumption about the origin of *sd*2 is based on spontaneous selection, and chance penetration into the duplication. We refer, however, to the relevant remarks in the preceding paper about high negative interference, either localized or not, for illegitimate recombination events.

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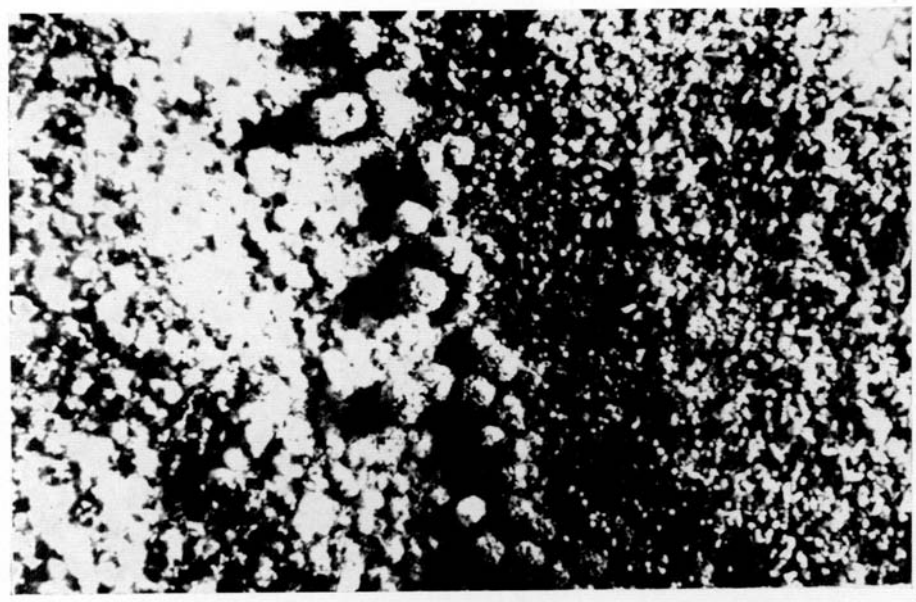

Fig. 1. Photomicrograph of cleistothecia at the junction between strains BWB157 (left) and BWB224 (right) growing on unsupplemented CM. Note the white immature cleistothecia on strain BWB157 (*argB*). × 10.

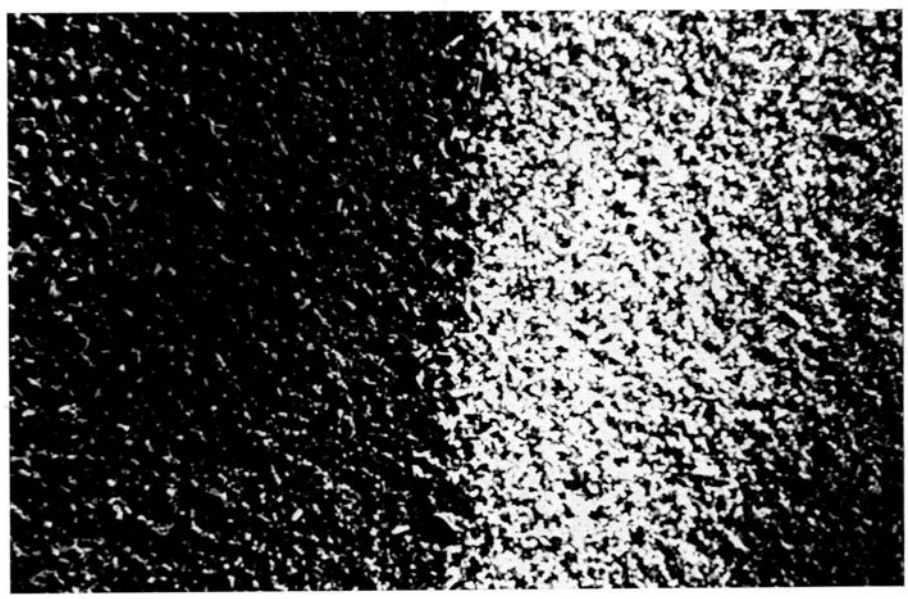

Fig. 2. Photomicrograph of the junction between strains BWB408 (right) and BWB272 (left). Note the complete absence of cleistothecia on either strain. Strain BWB408 carries the *lysB* marker. × 10.