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Location of prophage H90 on the chromosome of *Pseudomonas aeruginosa* strain PAO

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SUMMARY

Prophage H90 has been found to undergo a phenomenon similar to zygotic induction, during conjugal transfer from a lysogenic donor to a non-lysogenic recipient.

It has not been possible to demonstrate that the level of infectious centres increases concomitantly with transfer of the prophage. However, the genetic consequence of zygotic induction was observed with regard to decreased recombinant yield of markers distal to the prophage. This latter fact has been exploited in interrupted mating experiments, to locate the prophage at between 5 and 7 min on the *Pseudomonas aeruginosa* strain PAO map. It was further shown by transduction experiments that the prophage does not appear to be linked to clusters of co-transductional markers at the 5 and 7 min locations.

1. INTRODUCTION

Prophage H90 was shown in a previous communication (Krishnapillai & Carey, 1972) to be located close to a group of *his* markers near 7 min on the strain PAO chromosomal map of *Pseudomonas aeruginosa* (Pemberton & Holloway, 1972) (Fig. 1). This was concluded from the high co-inheritance of the prophage with these markers in unselected marker analysis of plate mating crosses involving lysogenic donors and non-lysogenic recipients, and vice versa. However, placement prior or distal to *his* was not possible from these results. Interrupted mating experiments were undertaken to try and resolve this point. Order of gene transfer in such experiments can be seen from Fig. 1.

Jacob & Wollman (1956) described the phenomenon of zygotic induction in *E. coli*, using phage lambda. This effect is observable in two ways:

(i) A decrease in recombinant recovery for bacterial markers distal to the prophage attachment point on the chromosome transferred from the lysogenic donor to the non-lysogenic recipient.

(ii) A concomitant increase in infectious centres from the time of entry of the prophage into the recipient cell.

Prophage H90 has been shown to be involved in a similar phenomenon, enabling its mapping in *P. aeruginosa* strain PAO.

2. MATERIALS AND METHODS

(i) *Media.* These have been described previously (Krishnapillai, 1971).

(ii) *Bacterial strains.* See Table 1.

(iii) *Bacteriophage strains.* The temperature phage H90 was described in a previous communication (Krishnapillai & Carey, 1972). It has now been assigned to a phage serogroup, designated by the prefix letter H. Phages F116L and E79 have been described previously (Krishnapillai, 1971; Holloway, Egan & Monk, 1960).

Phage and bacterial handling and assay techniques followed essentially those described by Adams (1959).

Table 1

Strain no.	Genotype	Reference
PAO1	prototroph *chl-2* FP2$^-$	Holloway (1955)
PAO381	*leu-38, str-7, FP2*$^+$	Stanisich & Holloway (1969)
PAO824	*pur-66, his-151, arg-163, ese-14*, FP2$^-$	Pemberton & Holloway (1972)
PAO831	*pur-66, his-151, pyr-21, thi-1, pro-71, ese-14*, FP2$^-$	
PAO833	*pur-66, his-151, pyr-21, thi-1, lys-53, ese-14*, FP2$^-$	
PAO850	*pur-66, his-151, pyr-21, thi-1, arg-160, ese-14*, FP2$^-$	
PAO862	*pur-66, his-151, pyr-21, thi-1, arg-166, ese-14*, FP2$^-$	
PAO1225	prototroph *chl-2* (F116L)$^+$ FP2$^-$	This paper
PAO2501	*his-151, pyr-21, pro-71*, FP2$^-$	
PAO2502	*his-151, pyr-21, pro-71*, (H90)$^+$ FP2$^-$	
PAO2504	*leu-38, str-7*, (H9O)$^+$ FP2$^+$	
PAO2510	*pur-66, his-151, pyr-21, thi-1, pro-71, ese-14, nal-1*, FP2$^-$	
PAO2517	prototroph *chl-2, nal-2*, FP2$^-$	

(iv) *Interrupted mating technique.* This was the method described by Stanisich & Holloway (1969) and modified by Pemberton & Holloway (1972). Stationary-phase female cells were mixed with log phase donor cells, at a proportion of 4 to 1. The minimal liquid medium used in matings was described by Pemberton & Holloway (1972) and final cell density was approximately 5×10^8 cells/ml. One ml samples of the mating mixture were vigorously agitated to disrupt mating pairs, and the mixture was then treated with the virulent phage E79 (at a final concentration of 5×10^9 pfu/ml) or nalidixic acid (NA) (at a final concentration of 400 μg/ml) in order to complete interruption of chromosome transfer.

Samples of the mixture were then plated on to minimal medium containing all the growth requirements of the recipient strain, except for the single marker being selected. In experiments using NA, 200 μg/ml NA was present in the plates as well.

Selection of NA-resistant recipient lines utilized concentration-gradient plates as described by Szybalski (1952). The continuous gradient extended up to 1000 μg/ml. Colonies were selected at 500–600 μg/ml.

In experiments to detect possible induction of phage H90 during interrupted

mating crosses, background levels of free phage H90 were reduced: after interruption with NA, 1 ml of the mixture was added to the membrane (0·45 μm pore size) of a Millipore Filtration system, operating at 20 lb/in^2 negative pressure. The cells retained on the membrane were washed with 2 ml of minimal salt solution at 37 °C, and the membrane was then agitated for 1 min in 2 ml of warm minimal salt solution containing NA at 400 μg/ml, to resuspend the cells. Aliquots were then plated as described previously, or diluted in the same medium and plated in soft layer agar seeded with PAO2517 indicator cells to detect phage release.

(v) *Transduction techniques.* This was the standard technique described by Krishnapillai (1971).

To enable identification of transductants acquiring lysogeny for phage H90, as against those becoming lysogenized by the transducing phage F116L, an indicator strain lysogenic for F116L was used (PAO1225).

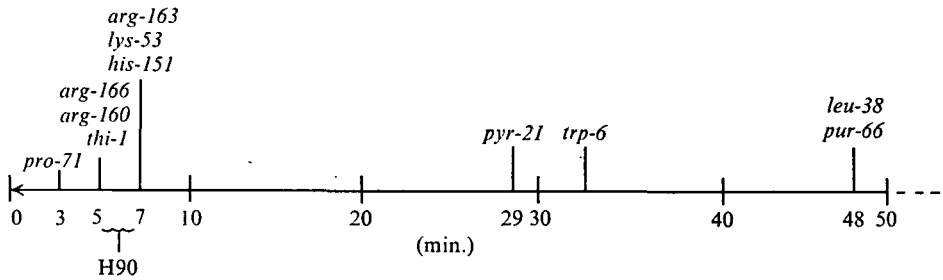

Fig. 1. Relative positions of relevant markers on the *Pseudomonas aeruginosa* PAO linkage map. 'O' denotes the FP2 transfer origin.

3. RESULTS

(i) *Mapping of prophage H90 by depression of recombinant recovery.* Utilizing the minimal salt solution technique of Pemberton & Holloway (1972) interrupted mating experiments were carried out between PAO831 (*pro-71*, *thi-1*, *his-151*, *pyr-21*, *pur-66*, *ese-14*, FP2$^-$) and either lysogenic or non-lysogenic donors of the genotype PAO381 (*leu-38*, FP2$^+$). Fig. 2(*a*) shows the 'non-lysogenic donor × non-lysogenic recipient' cross. Comparison with Fig. 2(*b*) involving 'lysogenic donor × non-lysogenic recipient' shows that a decrease in recombinant recovery is found for His$^+$ and Pyr$^+$, while Pro$^+$ and Thi$^+$ recovery is at a level comparable to that observed in Fig. 2(*a*). At the 60 min point, while Pro$^+$:Thi$^+$ ratio is similar, irrespective of the donor's lysogenic status, it is found that when a lysogenic donor is used the Pro$^+$:His$^+$ ratio increases approximately tenfold, while Thi$^+$:His$^+$ is affected 15-fold. The recovery of Pyr$^+$ recombinants in the latter situation is hardly detectable. The point that the reduction in recovery of His$^+$ and Pyr$^+$ is due to the transfer of a dominant *ese-14*$^+$ (E79 sensitivity) allele located between *thi-1* and *his-151* can be excluded by the fact that the same markers are involved in these two matings, with the single exception of the lysogenic status of the donor. These figures indicate that prophage H90 may be undergoing a zygotic induction-

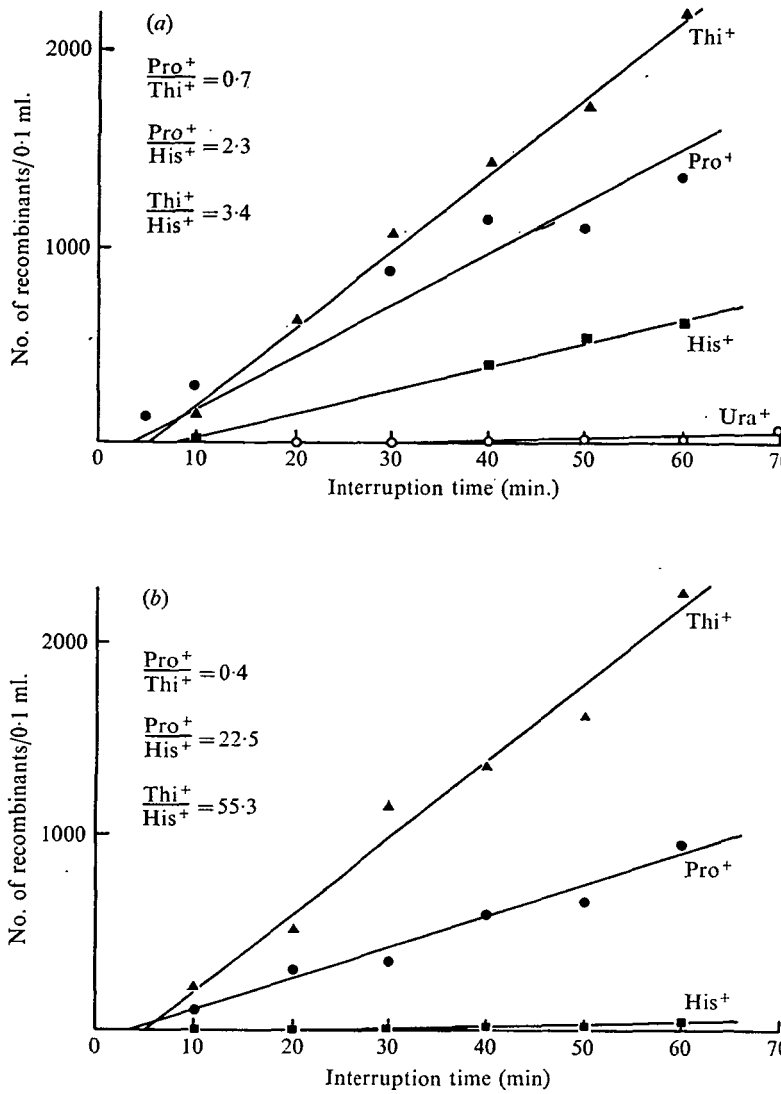

Fig. 2. Time-of-entry curves for donor markers in interrupted mating experiments, using phage E79 to interrupt chromosome transfer. Interrupted mating mixtures were plated on minimal medium supplemented with all the recipient growth requirements except for that being selected. Comparison of recombinant yields using non-lysogenic (2*a*) and lysogenic (2*b*) donor cells. Ratios calculated from the recombinant yields at 60 min. (*a*) PAO381 (*leu38*, *str-7*, FP2+) × PAO831 (*pur-66*, *his-151*, *thi-1*, *pro-71*, *ese-14*, FP2−) (non-lysogenic male × lysogenic female). (*b*) PAO2504 (*leu38*, *str-7*, (H9O+) FP2+) × PAO831 (*pur-66*, *his-151*, *pyr-21*, *thi-1*, *pro-71*, *ese-14*, FP2−) (lysogenic male × non-lysogenic female).

like phenomenon, and that the prophage attachment point is between the bacterial markers *thi-1* (5 min) and *his-151* (7 min) (see Fig. 1).

The strain PAO831 carries a mutation (*ese-14*) conferring resistance to phage E79 which is the interruption agent used in these interrupted mating experiments.

This strain was found to be cross-resistant to infection by phage H90, so that construction of a lysogenic recipient strain to do reciprocal crosses similar to those shown in Fig. 2 has not been possible. However, a recombinant derivative PAO2501 (*pro-71*, *his-151*, *pyr-21*, FP2$^-$) has been obtained, which has lost the *pur-66*, *thi-1* and *ese-14* markers. This has allowed crosses in the four possible combinations of

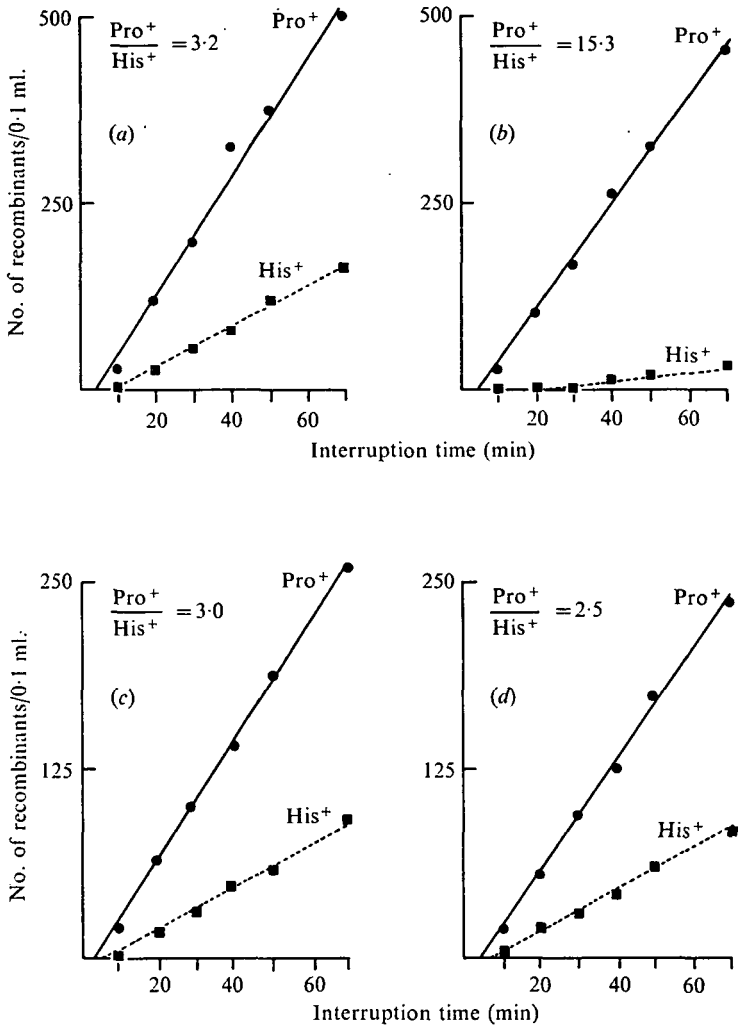

Fig. 3. Time-of-entry curves for donor markers in interrupted mating experiments, using phage E79 to interrupt chromosome transfer. Interrupted mating mixtures were plated on minimal medium supplemented with all the recipient growth requirements except for that being selected. Comparison of recombinant yields using non-lysogenic and lysogenic donor cells. Ratios calculated from the recombinant yields at 60 min. (*a*) PAO381 (*leu-38*, *str-7*, FP2$^+$) × PAO2501 (*his-151*, *pyr-21*, FP2$^-$) (non-lysogenic male × non-lysogenic female). (*b*) PAO2504 (*leu-38*, *str-7* (H9O)$^+$FP2$^+$) × PAO2501 (*his-151*, *pyr-21*, *pro-71*, FP2$^-$) (lysogenic male × non-lysogenic female). (*c*) PAO381 (*leu-38*, *str-7*, FP2$^+$) × PAO2502 (*his-151*, *pyr-21*, *pro-71* (H9O)$^+$FP2$^-$) (non-lysogenic male × lysogenic female). (*d*) PAO2504 (*leu-38*, *str-7*, (H9O)$^+$ FP2$^+$) × PAO2502 (*his-151*, *pyr-21*, *pro-71* (H9O)$^+$FP2$^-$) (lysogenic male × lysogenic female).

donor and recipient, lysogenic and non-lysogenic. Fig. 3(*a–d*) show the gradient of transmission curves for these crosses. It can be seen that the depression of His$^+$ recombinant levels is only observed in the cross 'lysogenic donor × non-lysogenic recipient' (Fig. 3*b*). Of importance is the 'lysogenic donor × lysogenic recipient' cross (Fig. 3*d*) where a normal Pro$^+$:His$^+$ ratio is found. This result argues against the proposition that the decreased recombinant yield may be caused by a mechanical hindrance of some kind due to the attachment of the prophage, and which is manifested during chromosome transfer involving markers distal to this point. Thus, the similarity with the zygotic induction effect is enhanced.

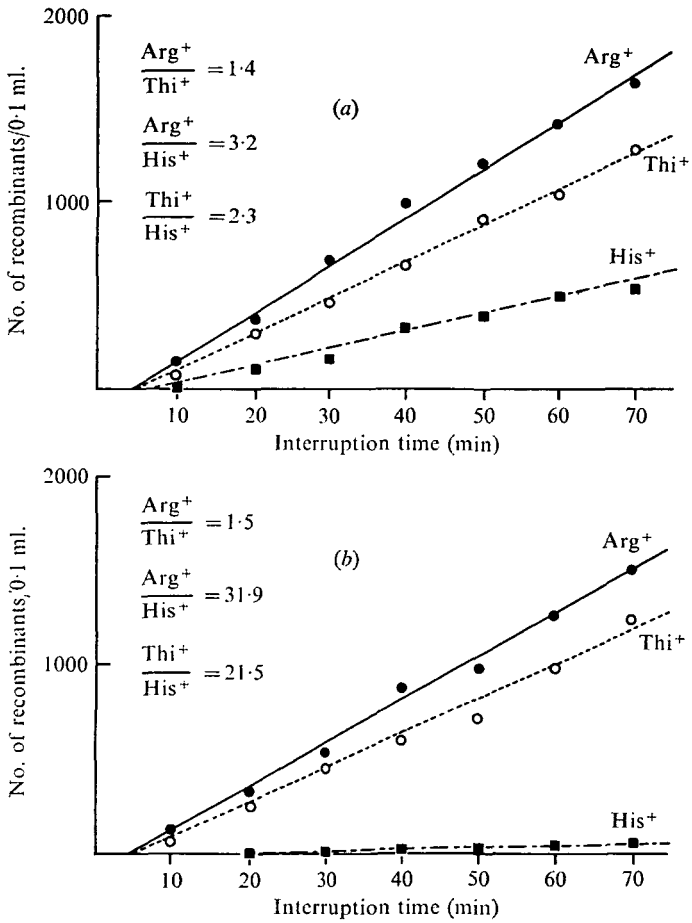

Fig. 4. Time of entry curves for donor markers in interrupted mating experiments using phage E79 to interrupt chromosome transfer. Interrupted mating mixtures were plated on minimal medium supplemented with all the recipient growth requirements except for that being selected. Comparison of recombinant yields using non-lysogenic and lysogenic donor cells. Ratios calculated from the recombinant yields at 60 min. (*a*) PAO381 (*leu-38*, *str-7*, FP2$^+$) × PAO862 (*pur-66*, *his-151*, *pyr-21*, *thi-1*, *arg-166*, *ese-14*, FP2$^-$) (non-lysogenic male × non-lysogenic female). (*b*) PAO2504 (*leu-38*, *str-7*, (H9O)$^+$ FP2$^+$) × PAO862 (*pur-66*, *his-151*, *pyr-21*, *thi-1*, *arg-166*, *ese-14*, FP2$^-$) (lysogenic male × non-lysogenic female).

Further experiments utilizing this effect were undertaken to locate prophage H90 more precisely. Pemberton & Holloway (1972) report that *arg-166* is co-transducible with *thi-1* at 5 min, and also that *arg-166* and *arg-160* appear to be lesions in the same, or closely linked genes, on the basis of prototroph reduction frequencies during transduction tests. However, the relative order of these two markers and *thi-1* is unknown, and the possibility presented itself that prophage H90 may be located between *thi-1* and *arg-160* or *arg-166* with *thi-1* in the most proximal position. The gradient of transmission curves for the cross involving the recipient strain carrying *arg-166*, PAO862 (*arg-166*, *thi-1*, *his-151*, *pyr-21*, *pur-66*, *ese-14*, FP2$^-$) are shown in Fig. 4(*a*) ($\times$ non-lysogenic donor) and Fig. 4(*b*) ($\times$ lysogenic donor). It can be seen that recovery of Arg$^+$ is unaffected by the presence of the prophage H90 in the donor. As expected, His$^+$ recovery is depressed, when the donor is lysogenic. Similar results were obtained with the *arg-160* recipient strain PAO850 (*arg-160*, *thi-1*, *his-151*, *pyr-21*, *pur-66*, *ese-14*, FP2$^-$). It was concluded therefore that prophage H90 is located distally to the three markers *arg-160*, *arg-166* and *thi-1*.

Pemberton & Holloway (1972) have also reported the co-transducibility of *lys-53* and *arg-163* with *his-151* at 7 min. Again, the order is not known, and there was a possibility that prophage attachment was between markers in this group. Examination of recombinant recovery levels in similar crosses to those outlined above showed that the *arg-163* and *lys-53* markers were subject to recombinant depression to an extent comparable with the *his-151* situation. This indicated therefore that the three markers *arg-163*, *lys-53* and *his-151* were all distal to prophage H90 location.

(ii) *Mapping of prophage H90 by infectious centre increase.* Since the reduced yield of certain recombinant classes in crosses between lysogenic donors and non-lysogenic recipients strongly suggested the occurrence of zygotic induction, we next looked directly for such induction by assaying the production of infectious centres as a function of time after mating (Jacob & Wollman, 1956). The virulent phage E79 that is normally used for interruption is unsuitable in this case, as resistance to E79 in the indicator also confers resistance to phage H90, such that assay of H90 infectious centres is not possible. Thus, streptomycin and nalidixic acid were used instead. In addition, specific antiserum for removal of background phage was not available, as phage H90 does not belong to any of the previously known serogroups in this laboratory, and attempts to produce specific antiserum with high activity have been unsuccessful due to the low titre (less than 10^8 pfu/ml) of this phage that is obtained by a variety of procedures. A method was employed, however, which involved removal of free phage from the mating mixture by filtering through membrane filters. Fig. 5 shows the gradient of entry curves obtained in such an experiment using nalidixic acid for interruption. Pro$^+$: His$^+$ ratios are similar to those obtained using the earlier method, and it can be seen that phage H90 levels remain unchanged throughout the time of the study. This indicates that the induction process is not giving rise to an increased number of infectious particles. We have excluded the possibility in this procedure that

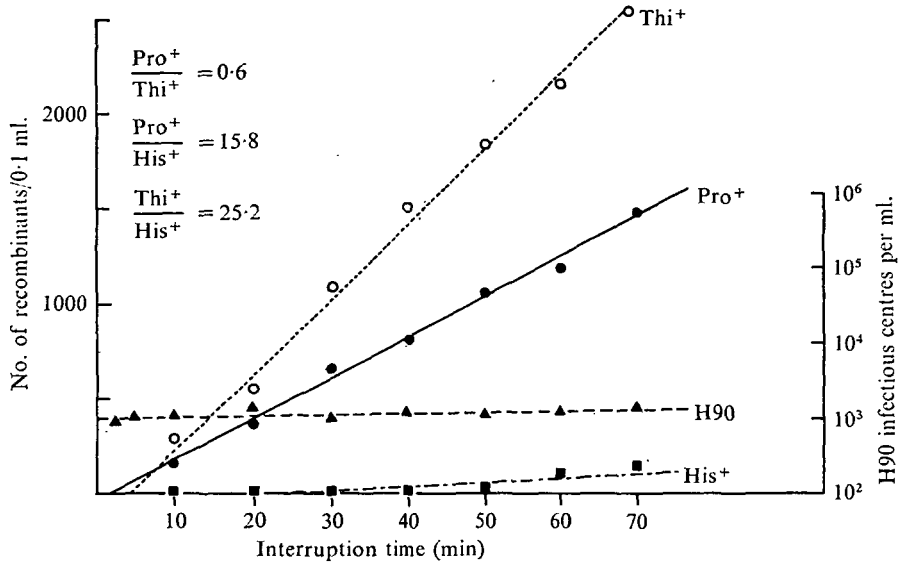

Fig. 5. Time-of-entry curves for donor markers in interrupted mating experiments using nalidixic acid to interrupt chromosome transfer. Interrupted mating mixtures were plated on minimal medium supplemented with all the recipient growth requirements except for that being selected as well as 200 μg/ml NA. Comparison of recombinant yields using non-lysogenic and lysogenic donor cells. Ratios calculated from the recombinant yields at 60 min. PAO2504 (*leu-38*, *str-7* (H9O)$^+$ FP2$^+$) × PAO2510 (*pur-66*, *his-151*, *pyr-21*, *thi-1*, *pro-71*, *ese-14*, *nal-1*, FP2$^-$) (lysogenic male × non-lysogenic female).

reduction of His$^+$ and Pyr$^+$ recovery is due to transfer of a dominant allele for NA-sensitivity, located between *thi-1* and *his-151*.

(iii) *Transductional analysis of prophage H90 location.* From the map positions of the markers we have discussed so far, we are drawn to the conclusion that prophage H90 location is between 5 and 7 min, between two transductional groupings. Efforts have been made, therefore, to demonstrate co-transduction of prophage H90 with markers in these groups, using the transducing phage F116L. It was also felt that transduction frequencies might be altered for markers close to the prophage attachment site due to homology differences leading to recombination inhibition. This might occur when F116L was grown on a donor lysogenic for phage H90 and the recipient was non-lysogenic; alternatively, it might be detected in the case of a preparation grown on a strain non-lysogenic for H90 which is used to transduce a lysogenic recipient strain.

Transductional analysis to the 0·25% level for each of the markers in the two transduction groupings failed to show co-transduction of phage H90 with any of them. Conversely, loss of lysogeny in transductants of lysogenic strains was not found following treatment with transducing preparations grown on non-lysogenic strains. In addition, there was no disturbance of transduction frequency for any of the six markers in either of these two situations.

It was concluded therefore, that prophage H90 is too distant from any of these markers for inclusion in the same transducing particle.

4. DISCUSSION

Our data suggest that prophage H90 is located on the strain PAO map between 5 and 7 min, flanked by the two transductional groupings: *arg-166*, *arg-160*, *thi-1*, around 5 min; and *lys-53*, *arg-163*, *his-151*, around 7 min. Co-transduction of prophage H90 with any of these markers has not been found.

Evidence that prophage H90 is located between these two groups of markers comes from the interrupted mating experiments in which a phenomenon similar to that associated with zygotic induction is found. A depression of recombinant yield is observed for all of the markers in the 7 min group, whereas the 5 min group are unaffected, in crosses of the type 'lysogenic donor × non-lysogenic recipient'. Pro^+ recombinants have been studied, and found to not co-inherit lysogeny for H90 prior to 5 min after initiation of transfer. In addition, a percentage of recombinants for distal markers in crosses showing this effect are lysogenic (Carey, unpublished observations). These co-inheritance values are lower than those reported previously from plate mating experiments, where the zygotic induction effect on recombinant yield of certain classes is not observed (Krishnapillai & Carey, 1972). This indicates a low efficiency of this system, which allows the effect to be concealed, probably due to such factors as multiple mating rounds which may occur in plate matings. Also indicative of an inefficient system is the fact that depression of recombinant yield for the distal markers is only about tenfold.

Another aspect of some interest in the present situation is the absence of an increase in infectious centres in the mating mixture following prophage transfer. Even when the background phage count is reduced to a very low level this remains constant for the 90 min duration of the experiment. This contrasts to the system for λ (Jacob & Wollman, 1956), where an immediate 40-fold rise in infectious centres is observed following transfer of prophage into the non-lysogenic recipient cell, although a 'defective' mutant of λ has been reported (Jacob & Wollman, 1961) that exhibits similar behaviour to prophage H90 in failing to give infectious phage while depressing recombinant recovery. At the present time the reason for the lack of increase in infectious centres is unclear. The recipient cells used in these interrupted mating experiments are normally in stationary phase. It seemed possible that the physiological state of such cells is not conducive to lytic replication of phage while actively growing cells might encourage phage replication over the lysogenic alternative. This did not appear to be the answer to the problem with phage H90 infectious centres, as phage and recombinant levels using recipients in log phase were unaltered from those reported above (Carey, unpublished observations). Certain characteristics of phage H90 lead us to suspect that in fact it may be a partially defective phage, possibly representing an intermediary form between normal bacteriophage, and the defective phage as characterized in *B. subtilis* (Garro & Marmur, 1970) or the R type aeruginocin particles of *Pseudomonas aeruginosa* strain PAO (Ito, Kageyama & Egami, 1970). Further work is in progress to examine the possibility that the burst of phage H90 released from a lysed cell may contain a large proportion of defective particles or unassembled structural materials. With regard to the present report, the situation might exist whereby

induction processes occurring in the recipient cell are giving rise to a very high proportion of non-infectious particles and component parts. This would result in death of the presumptive zygote, without an increase in infectious centres.

Despite the uncertainty with regard to infectious centres, and actual mechanism of recombinant depression for certain classes of transferred bacterial markers, a parallel of the present case with the zygotic induction system in λ is indicated. An important difference exists between the two cases in that λ exists as a prophage that is inducible by various treatments, whereas this has not been shown for phage H90. However, Jacob & Campbell (1959) have isolated a mutant of λ (λ *ind*) which exhibits normal zygotic induction, but which fails to be induced by ultraviolet irradiation. Thus, it appears that the difference between inducible and non-inducible phages may not be as distinct as is thought. Further evidence to support this comes from Woods & Egan (1972), who report that coliphage 186 exhibits essentially the reverse situation to that observed in the present communication; in the case of phage 186, it has been found that zygotic induction is undetectable, while u.v. induction can be demonstrated.

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