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Episomic suppression of phenotype in *Salmonella*

By P. F. SMITH-KEARY and G. W. P. DAWSON

Department of Genetics, Trinity College, Dublin

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In a previous paper we presented evidence in support of the hypothesis that instability at a site within the leucine-suppressor locus (*su-leuA*) of *Salmonella typhimurium* arises as a consequence of the attachment of a controlling episome at that site (Dawson & Smith-Keary, 1963). The only detectable effect of a controlling episome attached at *su-leuA* is to induce mutation in the linear genome at the position of attachment; loss of the controlling episome restores the stable state whether the *su-leuA* allele is mutant or wild-type. In this paper we will discuss a form of instability at a locus involved in proline synthesis that can best be explained by supposing that the function of the gene can be suppressed by an attached controlling episome and that these controlling episomes frequently transpose over short distances of the linear genome.

1. MATERIALS AND METHODS

The media used and the methods for preparing transducing phage and carrying out transduction have been described previously (Smith-Keary, 1960; Dawson & Smith-Keary, 1960 & 1963).

In the account which follows we have used the following abbreviations for the different media:

MM	minimal medium
EMM	enriched minimal medium
MM + P	minimal medium supplemented with 0·002 per cent *l*-proline
NA	nutrient agar

The strains of the proline-requiring auxotroph *pro-401* (Dawson & Smith-Keary, 1963) were *met*$^-$ *try*$^-$ *his*$^-$ and either *leu*$^-$ or *su-leuA*$^-$ *leu*$^-$, and adequate amounts of the corresponding amino acids were added to all the media.

2. RESULTS

(i) *The* pro-401 *mutant*

The proline requiring mutant *pro-401* was isolated in the *leu-151* strain in August 1960 after penicillin screening. It is a relatively stable auxotroph (see 2 (ii)) which grows when proline, but not when hydroxyproline or glutamic acid, is added to the minimal medium.

In January 1961 a subculture was taken from the original stock of *pro-401 leu-151*, and from it a strain that still required proline but had an unstable slow growing phenotype for leucine was selected; this strain has the genotype *pro-401 su-leuA-x(U) leu-151* (Dawson & Smith-Keary, 1963). These two strains, which for brevity will be designated *pro-401* and *su-leu pro-401*, have been independently subcultured and stored for nearly three years.

(ii) *Auxotrophic reversions*

In January 1963, 3 independent broth cultures of *su-leu pro-401* were plated on EMM (about 10^8 bacteria per plate). After 5 days incubation a total of 135 reversions were scored on six plates (22·5 reversions per 10^8 bacteria plated). Ninety-three of these reversions were characterized by shaking in T2 buffer, diluting, and plating between 100 and 200 bacteria on MM and MM + P. Of these, eighty grew as wild-type on MM + P and as the original auxotroph on MM, three grew slowly on both media, and ten were mixtures of both these types. Thus the majority of the clones of these apparent reversions towards prototrophy consist entirely of auxotrophic bacteria, phenotypically identical to the original *su-leu pro-401* auxotroph; these apparent reversions we have termed 'auxotrophic reversions'.

When the original revertant clone of an auxotrophic reversion is streaked out on MM the denser end of each streak shows considerable growth after 48 hours, but the amount of growth declines rapidly as the density of cells along the streak decreases. At the other end of the streak, where individual bacteria are well isolated on the agar, growth can only be detected under the microscope. In addition there are 'reversions' appearing as papillae on the streak, and these also contain only auxotrophic bacteria. On the other hand, when a clone of the original *su-leu pro-401* auxotroph or a clone derived from a single bacterium within an auxotrophic reversion is streaked out the heavy growth at one end of the streak is absent, although occasional papillae, of auxotrophic bacteria, still occur along the streak.

Except for continuing to produce auxotrophic reversions, the bacteria from a clone of an auxotrophic reversion show no evidence of instability; there is no evidence whatsoever of rapid mutation from pro^- to pro^+ and back to pro^- as in the *su-leuA* system (Dawson & Smith-Keary, 1963).

The occurrence of these auxotrophic reversions poses the question: How have they grown on EMM (or on MM) when they contain only auxotrophic cells? The revertant clones are not abnormally small and they eventually grow to at least the size expected of a wild-type reversion.

A further feature of this system became apparent when we attempted to carry out a genetic analysis of two auxotrophic reversions; in homologous transductions involving either the auxotrophic reversions or *pro-401* (*pro-401* or *su-leu pro-401*) considerable numbers of phenotypically wild-type transductants were recovered. These appear to be pro^+. In these experiments *pro-401* did not produce any pro^+ reversions spontaneously.

(iii) *The hypothesis*

To account for these results we propose the following hypothesis:

1. The *pro-401* auxotroph did not arise as the result of a true structural mutation (i.e. due to a change within the linear genome) but arose as a consequence of the attachment of a controlling episome to a site within the *pro* region; the effect of this attachment is to block the functioning of the gene so that the strain is effectively an auxotroph.

2. This controlling episome is not firmly attached to any particular site within the *pro* region, but can transpose over short distances and become attached to other sites within the *pro* gene.

3. Occasionally transposition occurs from one complementation group to another. When this occurs, syntrophism occurs between the cells mutant in different complementation groups and enables the growth of a 'revertant' clone. Such a clone is an auxotrophic reversion.

4. The wild-type transductants arising in homologous transductions arise by recombination between two chromosomes carrying the controlling episome at different sites; this is analogous to recombination between any pair of linked and non-identical mutant sites.

If the auxotrophic reversions arise by transposition of a controlling episome from one complementation group to another, then we can predict that any culture which gives rise to auxotrophic reversions will contain a mixture of bacteria, mutant in different complementation groups, and that when this culture is transduced with homologous phage abortive transductants should be recovered.

We confirmed this prediction by abortive transduction tests, using MM instead of EMM as on this medium the minute colonies characteristic of abortive transduction can be recognized the more easily.

The following transductions were performed:

(*a*) homologous transductions with *pro-401*, i.e. *pro-401* (×) *pro-401*;
(*b*) homologous transductions with each of two different auxotrophic reversions;
(*c*) transductions between two different auxotrophic reversions;
(*d*) transductions between *pro-401* and each of the auxotrophic reversions.

In each transduction small, nearly microscopic, colonies were observed in addition to an approximately equal number of wild-type transductants. The nature of these apparent abortive transductants was confirmed by respreading small areas of the plates, each containing a single isolated minute colony. After further incubation a single minute colony grew in most of the respread areas; in no case was more than one minute colony observed in a respread area. These results are expected only if these colonies were true abortive transductants with unilinear inheritance of the fragment of donor chromosome carrying the *pro* region.

We conclude from these experiments that cultures of *pro-401* and of the auxotrophic reversions contain bacteria mutant at different sites within the *pro* region and further that these mutant sites are distributed between at least two different complementation groups.

(iv) *The distance of transposition*

Two series of experiments provide information on the distance the controlling episome moves by transposition. If the distance is small relative to the length of the proline region the mutant sites among the cells of a clone will be clustered around the site at which the controlling episome was attached in the original cell. In another clone separated from the first by many subculturings the mutant sites will be similarly clustered but probably around a different point; the greater the number of subculturings, the greater the chance of the controlling episome having migrated by successive transpositions to a different part of the proline region. Thus homologous transductions using cultures grown from these two clones should give few prototrophs compared with the number obtained from transductions between the cultures. This result would also be obtained if the distance of transposition is relatively large provided that it occurs with such a frequency that a considerable proportion of the cells of a clone retain the controlling episome at the original site. Alternatively, if the distance of individual transposition is large and its frequency so high that the cells of a clone have the controlling episome distributed almost randomly over the proline region the homologous transductions will produce almost as high a frequency of prototrophs as the transductions between clones.

In the first experiment *pro-401* and *su-leu pro-401* were used. These strains are both derived from the same stock of *pro-401* (see section 2 (i)) and have been separately subcultured since January 1961, so are separated from each other by about ten subculturings. Each strain was streaked out and three clones selected from each streak, clones A, B and C from *pro-401*, and clones D, E and F from *su-leu pro-401*. Two cultures were grown from inocula from each clone; one was used for the preparation of transducing phage, the other as a culture of recipient cells, and transductions effected on EMM in all pairwise combinations. The results (Table 1)

Table 1. *Numbers of transductants from transductions between clones of* pro-401, *between clones of* su-leu pro-401, *and between clones of* pro-401 *and* su-leu pro-401. *Total numbers of transductants on 6 EMM plates (10⁸ bacteria per plate).*

		Donor strain						
		pro-401 clones			*su-leu pro-401* clones			
Recipient strain		A	B	C	D	E	F	Control
pro-401	A	11	113	28	3600	3600	3600	5
	B	9	92	14	2400	1800	3000	1
	C	17	32	20	4800	3000	3000	3
su-leu pro-401	D	362	2000	1500	35	12	10	0
	E	700	3000	2500	32	13	11	0
	F	315	2000	760	23	15	15	0

clearly show that the frequency of prototrophs is very much higher in transductions between a clone of *pro-401* and a clone of *su-leu pro-401* than it is in transductions

between either the *pro-401* or *su-leu pro-401* clones and we conclude that the cells of a clone do not have the controlling episome randomly distributed over the proline region. It remains to decide whether the transpositions are frequent and over short distances or relatively infrequent but over larger distances. These are extremes of a range of possibilities rather than separate and alternative explanations. Three lines of evidence suggest that the transpositions occur frequently and over relatively short distances:

1. If transposition were a relatively rare event clones would vary greatly in the proportion of cells in which transposition had occurred; we would then expect considerable variation in the frequency of prototrophs from homologous transductions of clones of the same strain. The data in Table 1 shows that the variation among these homologous transductions is not conspicuous.

2. The frequency of auxotrophic reversions (2 in 10^7 cells) is a measure of the frequency of transposition of the controlling episome to a different complementation group. Comparing this with the relatively high frequency of prototrophs obtained from homologous transductions suggests that the vast majority of transpositions within a clone are confined to one complementation group.

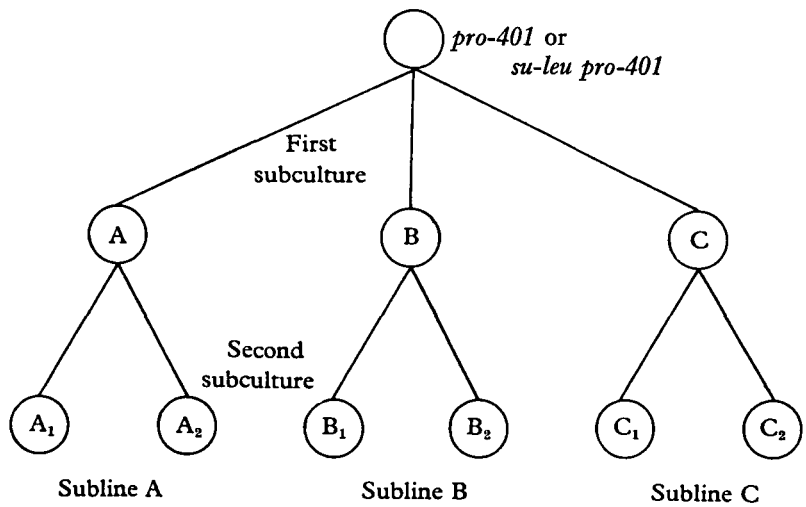

Fig. 1 For description see text.

3. In the present series of experiments no spontaneous wild-type reversions have been observed. If our theory that the attachment of a controlling episome suppresses the expression of a gene is correct then we have never observed its transposition to beyond the limits of the proline complementation groups.

The description that is most consistent with this evidence is that the controlling episome transposes very frequently but over very short distances. Its transposition to a different complementation group is relatively rare and it has never been observed to transpose to beyond the proline complementation groups.

Since the differences in these experiments were so marked, it might be that similar

but smaller differences would be observed when a lesser number of subculturings separates the terminal clones. One of our experiments suggests that this may be so. A single clone of *pro-401* and of *su-leu pro-401* was streaked out on NA, and three of the resulting clones restreaked. Two clones from each streak were selected for further study (Fig. 1).

Thus each pair of terminal clones was separated by one streaking, whereas a clone of one pair is separated from a clone of a different pair by two streakings. Homologous transductions should give the lowest number of transductants, transductions between terminal clones of different sublines (e.g. A_1 ($\times$) B_2, B_2 ($\times$) C_1) the greatest number and transductions between the two clones of the same subline an intermediate number. The results (Table 2) show that this is so although the differences are not as great as in the previous experiment. However, the data are highly heterogeneous and show considerable variation between repeat experiments. This heterogeneity is not unexpected as repeat experiments involve growing different cultures of the recipient strain and transposition may occur at different frequencies or to different sites in the separate cultures. Nevertheless, of the twenty one cultures that were used as recipients, eighteen produced a lower number of transductants when infected with homologous phage than the average number of transductants from the four corresponding transductions between clones in different sublines. Thus these results, while not critically supporting the hypothesis of short-distance transposition, are consistent with it.

During the course of these experiments we observed that when different cultures of the same clone are used in homologous transductions (using the same phage

Table 2. *Numbers of wild-type transductants from transductions between clones of* pro-401 *and between clones of* su-leu pro-401. *Total of 5 EMM plates (10⁸ bacteria per plate).*

(*a*) Transductions between clones of *pro-401*.

Expt.	Recipient clone	Donor clone: subline A A_1	subline A A_2	subline B B_1	subline B B_2	subline C C_1	subline C C_2	Mean for between subline transductions
1	A_1	6	6	4	6	9	6	6·25
	A_2	40	34	55	47	26	24	38·0
	B_1	37	22	8	13	12	14	21·25
	B_2	42	47	45	48	35	37	40·25
	C_1	80	65	55	60	40	58	65·0
	C_2	70	47	39	31	37	16	46·75
2	A_1	620	510	825	562	285	625	574·2
	A_2	605	520	892	485	475	950	700·5
	B_1	700	600	416	510	155	701	539·0
	B_2	780	255	340	330	175	175	346·25
	C_1	815	280	346	480	70	161	480·2
	C_2	875	400	396	700	301	265	592·75

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(*b*) Transductions between clones of *su-leu pro-401*.

Expt.	Recipient clone	Donor clone: subline A A_1	A_2	subline B B_1	B_2	subline C C_1	C_2	Mean for between subline transductions
1	A_1	91	135	109	81	75	80	86·25
	B_1	144	100	95	96	108	138	122·5
	B_2	54	51	25	40	43	41	47·25
	C_1	24	26	23	36	12	30	27·25
	C_2	701	535	361	535	241	190	533·0
2	A_1	78	1113	497	357	575	135	391·5
	A_2	221	327	585	415	315	449	441·0
	B_1	575	578	216	210	380	521	513·5
	B_2	385	456	201	275	341	460	410·5

(*c*) Summary for all transductions. Average numbers of transductants in (*a*) homologous transductions, (*b*) transductions between clones of the same subline and (*c*) transductions between clones of different sublines

	pro-401	*su-leu pro-401*	Combined data
(*a*)	197·8	146·4	175·8
(*b*)	218·8	252·4	233·2
(*c*)	287·6	285·8	286·8

Table 3. *Frequencies of auxotrophic reversions in controls and of wild-type transductants in homologous transductions in two sets of experiments carried out at* 10 *days interval. Numbers of colonies on* 5 *EMM plates* (10^8 *bacteria per plate*).

(*a*) Clones of *pro-401*

	First experiment: auxotropic reversions	First experiment: transductants	Repeat experiment: auxotrophic reversions	Repeat experiment: transductants
A_1	0	6	91	620
A_2	7	34	75	520
B_1	6	8	57	416
B_2	12	48	106	330
C_1	21	40	9	70
C_2	8	16	56	265

(*b*) Clones of *su-leu pro-401*

A_1	35	91	12	78
B_1	150	95	174	216
B_2	39	40	119	275

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preparation) there is a positive correlation between the number of auxotrophic reversions on the control plates and the number of wild-type transductants. In the latter experiment nine of the homologous transductions were repeated about 10 days after the first series of transductions, during which time the clones were stored on NA at room temperature. Table 3 shows the numbers of auxotrophic reversions recovered in the controls and the numbers of wild-type transductants recovered in the homologous transductions. In eight of the nine pairs of transductions this correspondence was observed; in seven of the nine pairs of transductions the culture in the repeat experiment yielded a greater number of auxotrophic reversions than the culture in the first experiment. It appears that transpositions of the controlling episome continue to occur during storage of the clones. If the cultures used in the repeat experiments contain greater numbers of different and more widely separated mutant sites than the original cultures, this would explain the greater number of both auxotrophic reversions and wild-type transductants.

(v) *UV-irradiation experiments*

The following experiment was performed to determine whether or not UV light affects transposition of the controlling episome at the *pro* locus.

A broth culture of *su-leu pro-401* was centrifuged and the cells resuspended in saline (2×10^9 bacteria per ml). One sample of this suspension was irradiated using a Hanovia 'Chromatolite' 2537A UV lamp so as to give about 10% survival, and 0·1 ml. samples spread on EMM (2×10^7 bacteria per plate). A second sample was diluted 10^{-1} in saline and similarly plated (2×10^7 bacteria per plate). After 4 days incubation all the revertant clones were marked and characterized by plating on MM and MM+P. After 5, 6 and 10 days incubation any further clones that appeared on the plates were similarly marked and characterized; after 14 days incubation any further unmarked reversions were counted, but not characterized.

Table 4. *Classification of the reversions of* pro-401. *Appearance of colonies when a suspension of the reversion is plated on MM+P and MM plates. About* 200 *bacteria per plate.*

Symbol for type of reversion	MM+P	MM
SS_1	Colonies of about 3 mm. after 48 hr.	Colonies of about 3 mm. after 48 hr.
SS_2	Colonies 1–2 mm. after 48 hr.	Colonies 1–2 mm. after 48 hr.
SS_3	Colonies 0·5–0·75 mm. after 48 hr.	Colonies 0·5–0·75 mm. after 48 hr.
VVS	Colonies about 3 mm. after 24 hr.	Colonies about 0·5 mm. after 4 days.
A (auxotrophic reversion)	Colonies about 3 mm. after 24 hr.	Microscopic colonies plus occasional larger colonies. The latter consist entirely of auxotrophic bacteria.
M (SS_3+A mixed)	Colonies of 3 mm. after 24 hr. plus colonies of 0·75 mm. after 48 hr.	Colonies of 1 mm. after 48 hr., plus microscopic colonies
WT	Colonies 3–4 mm. after 24 hr.	Colonies 3–4 mm. after 24 hr.

Table 5. *Effects of UV irradiation on the frequency of reversion of* su-leu pro-401. *Total colonies appearing on* 4 *plates, about* 2×10^7 *surviving bacteria per plate.*

	Unirradiated control						Irradiated						
	Number of reversions appearing between days						Number of reversions appearing between days						
					Total						Total		Total induced
Type	0–4	4–5	5–6	6–10	0–10	10–14	0–4	4–5	5–6	6–10	0–10	10–14	0–10
SS_1	0	1	3	2	6		27	0	10	3	40		34
SS_2	1	1	5	7	14		43	8	20	2	73		59
SS_3	1	0	0	5	6		52	0	6	11	69		63
VVS	0	0	0	3	3		60	25	10	12	107		104
A	12	11	28	45	96		124	112	51	35	322		226
M	0	1	13	53	67		0	0	25	44	69		2
TOTAL	14	14	49	115	192	40	306	145	122	107	680	38	448

The types of revertant clones are set out in Table 4 and the frequencies of each type in the control and irradiated samples in Table 5.

Only the type A (auxotrophic) and type VVS reversions responded to exogenous proline, the SS types showing restricted growth whether or not proline was supplied in the medium. The VVS and SS types have not yet been genetically analysed and the site of the mutational change is not known. The mixed (type M) colonies contain both type A and type SS_3 bacteria and serial platings from the two types within these colonies suggest that the SS_3 bacteria are segregating from the auxotrophs. No wild-type reversions were recovered in either the controls or irradiated samples.

With the exception of the mixed-type M reversions, all the types were recovered from among the colonies appearing during the first 4 days of incubation, and all were inducible by treatment with UV light. In contrast, the mixed reversions did not appear until the 5th and 6th days and all are apparently of spontaneous origin. The overall frequency of reversion, in both the control and treated samples, rose steeply between the 4th and 10th days, after which very few further reversions appeared—presumably because all the nutrients in the media were exhausted. The induced reversions continued to appear up to and including the 6th day, after which similar numbers of reversions appeared in both the control and treated samples. Of the 488 induced reversions, 255 were of type A, the majority, if not all, having appeared by the 5th day after plating. We conclude that either the frequency of transposition or the distance of transposition, or both, is increased by UV irradiation. As no wild-type reversions occur it appears that even after UV irradiation the controlling episome does not move beyond the limits of this proline region. This suggests that the UV does not markedly affect the distance of transposition and more probably acts by increasing its frequency.

The results further suggest that the frequency of transposition is not proportional to the frequency of cell division. In the control sample, although only twelve spontaneous transpositions to a different complementation group, recognizable as auxotrophic reversions, occurred between days 0 and 4, 45 similar transpositions occurred between days 6 and 10. The number of cell divisions in the former period is very large indeed compared with the number occurring between days 6 and 10. This result is in good agreement with the observation in section 2 (iv), that after storage clones of *pro-401* yield a greater number of auxotrophic reversions.

3. DISCUSSION

A reasonable hypothesis to explain the experimental results has been discussed in the previous section. Briefly the main experimental results were (*a*) the majority of the revertant clones of *pro-401* contained only auxotrophic bacteria, similar to the original auxotroph, (*b*) when *pro-401* is infected with homologous phage, wild-type and abortive transductants are recovered and (*c*) in transductions between different clones of *pro*-401 more wild-type transductants are recovered than from homologous transductions. To account for these results we have argued that (*a*) the *pro-401* auxotroph arose by the acquisition of a controlling episome by a site within

the proline region of the genome, (*b*) this controlling episome can transpose to other sites in the same or in a different complementation group and (*c*) the controlling episome is recognizable by its suppressing the function of the complementation group to which it is attached.

Two possible alternative explanations deserve to be considered. The first is that the mutant 'sites' in the proline region are structural changes in the linear genome, whether induced by a mutator gene (Miyake, 1960; Treffers *et al.*, 1954) or a controlling episome similar to the one we have described at *su-leuA* (Dawson & Smith-Keary, 1963), rather than sites to which a transposable controlling episome that suppresses phenotypic expression can be attached. Each new mutant site would then have to arise by two events: reversion to wild-type at the original mutant site and a mutation at a new site. If these events are not simultaneous, auxotrophs that have two mutant sites in the proline region and wild-type reversions would appear. While our methods would not have enabled us to detect occasional auxotrophs with two mutant sites, wild-type reversions would have been recovered. No wild-type reversions were found in any of the experiments. The precise simultaneity of the two events which this hypothesis requires but does not explain makes it a less useful explanation than the one we have proposed.

The second possible alternative is that the phenomena we have observed are similar to those recently described by Demerec (Demerec, 1963). He found that eighty-one out of 201 different auxotrophs of *Salmonella typhimurium*, distributed among twenty-two loci, yielded prototrophs when transduced with homologous phage. Prototrophs are also recovered when phage grown on a 'deletion' mutant, which covers the mutant site of the recipient, is used. Demerec concludes that the prototrophs are reversions of the recipient strain that are specifically induced by the transduced fragment. This explanation will not account for three features of the present study. It will not account for the auxotrophic reversions nor for the abortive transductants, and it will not explain the greater frequency of prototrophs that is obtained from transductions between different clones of *pro-401* than from homologous transductions.

The history of the *pro-401* strain that was used in the present study is interesting. The data on the reversions of *pro-401* that were collected early in 1961 (Dawson & Smith-Keary, 1963), soon after its isolation, showed a very different range of types from that in the present experiments. Then, of 246 characterized reversions, 140 were wild-type, fifty-seven were slow growing reversions and forty-nine grew very slowly indeed. There were both stable and unstable reversions among the last two types. Both those that grew slowly and those that grew very slowly grew as wild-type when the medium was supplemented with proline. No auxotrophic reversions were found. By contrast, in the present study two years later there were no wild-type reversions, the slow growing reversions did not respond to exogenous proline and auxotrophic reversions were frequent.

The presence of wild-type reversions, but no auxotrophic reversions in early 1961, and the reverse two years later, seems to show a change in the characteristics of transposition of the controlling episome. Originally it transposed over relatively

long distances to beyond the limits of this proline region: now it transposes over only very short distances within the proline region.

Evidence was presented in a previous paper (Dawson & Smith-Keary, 1963) that the original slow growing and very slow growing reversions arose by transposition of a controlling episome from *su-leuA*, possibly to a suppressor locus. The absence of these reversions, that respond to exogenous proline, in the present study shows that this activity is no longer detectable. The current slow growing reversions have not yet been genetically analysed.

pro-401 was originally isolated in the strain of *leu-151* which was used for the study of instability at the *su-leuA* locus. While this may suggest that the same controlling episome is responsible for the instability at both *pro-401* and *su-leuA* it does not firmly establish this. Our data on the instability at *su-leuA* could best be explained by a controlling episome inducing frequent mutations in the linear genome and transposing infrequently. At *pro-401* the controlling episome suppresses gene expression, does not cause mutations in the linear genome and transposes frequently. Some of McClintock's studies of instability in Maize show that controlling elements can have the property of both inducing mutation and suppressing phenotypic expression. In the *Spm* system the controlling element shows both these activities at the same gene and always shows them together (McClintock, 1956, 1958).

UV irradiation increases the transposition of the controlling episome to different complementation groups; the number of auxotrophic reversions is greatly increased by irradiation. The numbers of SS and VVS reversions are also increased but we can not at present say whether these are likely to have arisen by transposition as they have not yet been genetically analysed. This response of the controlling episome to UV irradiation may be similar to the induction of prophage by UV, which is a transposition of an episome from its site of attachment.

SUMMARY

1. An auxotroph of *Salmonella typhimurium*, *pro-401*, was isolated in a strain that was unstable at the *su-leuA* locus. The auxotrophy of *pro-401* is probably due to the attachment of a controlling episome to the proline region of the genome where it suppresses gene expression.

2. The controlling episome frequently transposes over short distances so that all clones consist of cells, mixed for the site at which the controlling episome is attached; homologous transductions yield prototrophs.

3. The controlling episome can transpose to a different complementation group; homologous transductions yield abortive transductants; syntrophism occurs between cells that are 'mutant' in different complementation groups to give reversions consisting entirely of auxotrophic cells which are called auxotrophic reversions.

4. The controlling episome transposes over very short distances and never to beyond the limits of this proline region of the genome; no wild-type reversions were found.

5. The controlling episome can be located at relatively distant proline sites in different clones; prototrophs from transductions between clones that are separated by many subculturings can be 100 times more frequent than from homologous transductions.

6. The controlling episome has its frequency of transposition to different complementation groups increased by UV; irradiation increases the frequency of auxotrophic reversions.

7. The controlling episome continues to transpose in stored cells.

8. The pattern of reversions of *pro-401* is different in these studies from its pattern two years previously. This is discussed.

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