A BIOCHEMICAL BASIS FOR APPARENT ABORTIVE TRANSFORMATION IN *BACILLUS SUBTILIS*[1]

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A mutant derivative that was reported to undergo unstable DNA transformation (abortive transformation) was previously isolated from a transformable strain of *Bacillus subtilis* (IYER 1965). In view of the fact that such a system of abortive transformation would be a powerful and much needed tool for analyzing dominance relationships of genes in *B. subtilis*, we report the experimental basis of our conclusion that the behavior of the strain derives from nutritional interactions that depend upon the specific biochemical lesions carried by the mutant. The mutant strains proved to possess a previously undetected biochemical block in the histidine pathway. The latter mutation correlated with the ability of competent cells to interact with transforming DNA from wild-type donors to produce minute colony recombinants (abortive transformants). The biochemical block constitutes a trivial explanation for the apparent abortive transformation that is simulated under a limited set of nutritional conditions.

MATERIALS AND METHODS

Media: Complete medium, both liquid and solid, was TSY (medium A of JENSEN, NASSER and NESTER 1967). Liquid minimal medium was the recipe of SPIZIZEN (1958) and solid minimal medium was modified DAVIS minimal (LEDERBERG 1950). The latter two minimal media were supplemented with a 10^{-3} dilution of the following stock solution of trace elements: $ZnSO_4 \cdot 7H_2O$, 1.0 gram; $FeCl_3 \cdot 6H_2O$, 1.0 gram; $CuSO_4 \cdot 5H_2O$, 0.1 gram; $MnCl_2 \cdot 4H_2O$, 0.1 gram; $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$, 50 mg; $Na_2B_4O_7 \cdot 10\ H_2O$, 75 mg; $CaCl_2$, 1.0 gram; and $CoCl_2 \cdot 6H_2O$, 25 mg. Stock cultures were maintained at room temperature on AK sporulation medium (Baltimore Biological Laboratories).

Bacterial strains: A summary of strain histories and characteristics is detailed in Table 1.

DNA transformation: DNA preparations were made by the procedure of MARMUR (1961). NP 40, a revertant of 168, was used as the source of prototrophic DNA instead of SB 19 used by IYER. SB 19 (NESTER and LEDERBERG 1961) is a genetic hybrid of strains 168 and 23. The latter strains differ strikingly in many respects. For example, levels and sensitivities of regulatory enzymes in the aromatic pathway are different (JENSEN and NESTER 1966; NESTER and JENSEN 1966). Therefore, isogenic strains were used to avoid unnecessary complications due to the possible occurrence of recombinants bearing incompatible or unbalanced gene products. Competent cells were obtained by a regimen involving a shift-up in casein hydrolysate (0.04% to 0.10%) and a shiftdown in temperature (37° to 32°C) (NESTER, personal communication).

Genetic notations, symbols and designations: The cotransfer index, *r*, has been defined by NESTER and LEDERBERG (1961). The shorthand designation of donor and recipient (donor —— ×

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TABLE 1

Strains of Bacillus subtilis

Designation	Genotype	Origin	Reference
168	trp_2^-		BURKHOLDER and GILES 1947
NP 16	aro_2^-	Dr. E. NESTER (SB 167)	JENSEN and NESTER 1965
NP 12	his_2^-	Dr. E. NESTER (SB 32)	NESTER and LEDERBERG 1961
NP 40	prototroph	168, spontaneous revertant	
IF 43	$trp_2^-his_5^-$	Dr. V. IYER (Strain 31)	ELIZUR, SRINIVASAN and ZAMENHOF 1961
IF 44	$trp_2^-his_5^-his_U^-$†	Dr. V. IYER (68c)	IYER 1965
IF 45	his_U^-	NP 40——— × IF 44*	
IF 46	his_5^-	IF 48——— × 168	
IF 47	$trp_2^-his_5^-$	IF 44——— × NP 16	
IF 48	$his_5^-his_U^-$	NP 40——— × IF 44	
IF 49	$his_5^-his_U^-$	IF 44, spontaneous revertant	

* Donor and recipient strains in genetic crosses are designated as proposed by NESTER, SCHAFER and LEDERBERG (1963).

† his_U^- denotes a histidine requirement that is unlinked to the cluster of linked aromatic loci (ELIZUR, SRINIVASAN and ZAMENHOF 1961).

recipient) and the notation for donor genes (111) and recipient genes (000) were described by NESTER, SCHAFER and LEDERBERG (1963). Values of r were determined by plating for transformants acquiring the selective marker followed by replica plating in order to determine the cotransfer of unselected markers. The latter protocol is described by *trp*+ *his*± whereby *trp* is the selective marker and *trp*+ recombinants are obtained on histidine-supplemented media. Replica plating to minimal medium determines the proportion of *his* recombinants, *his* being the unselected marker. In some crosses single transformants carried a nutritional requirement from the donor strain in addition to that of the genetic recipient strain, ruling out a convenient selective medium. In such cases, calculations of r assumed that the unscored class of single transformant was equal in frequency to the remaining class of single transformant. The latter is a rather rough approximation because cotransfer of markers is not necessarily identical in reciprocal crosses (e.g., see Table 4). Such differences have been discussed before by ANAGNOSTOPOULOS and CRAWFORD (1961).

Limiting concentrations of DNA: The determination of accurate linkage relationships of markers by DNA transformation requires non-saturating concentrations of donor DNA in order to avoid co-incidental uptake of unlinked markers into recipient cells. In transformation of competent cells, the most sensitive determination of the DNA concentration range of linear response is by biological assay, following transformation frequency as a function of DNA concentration. This is because the absolute value of the limiting concentration varies with the quality of the DNA and with the concentration and the degree of competence of the recipient cells. Accordingly, appropriate serial dilutions of DNA were assayed against identical aliquots of competent cells. The chemical assay of DNA concentration was by the method of BURTON (1956).

Scoring of transformant classes: Transformants were purified by picking onto the selective medium at least twice before scoring the unselected marker(s) by replica plating. It should be emphasized that the admixture of recombinants of different genotypes within a single clone in routine transformation may occur in 2–5% of the clones. The fact that genotypes which are not selected can nevertheless be recovered from mixed clones at a low frequency is an interesting phenomenon which deserves further attention. Probably, syntrophic cross-feeding is involved. Therefore, in the context of this paper, the observation that the composition of minute colonies may include more than one recombinant type does not particularly distinguish them from ordinary transformant clones.

Ultraviolet (UV) irradiation: The source of irradiation was a Hanovia mercury vapor arc lamp. The output as measured by a Hanovia UV meter was 40 ergs/mm^2/second. An exposure of 50 sec resulted in a survival of 3% of the initial cell population impinged on a membrane filter. Subsequent operations were carried out in subdued light.

Nitrosoguanidine mutagenesis: N-methyl-N'-nitro-N-nitrosoguanidine was prepared in a 0.05 M Tris-maleate buffer, pH 7.5, at a concentration of 1 mg/ml. A 9 ml culture in exponential growth was exposed to the mutagen with aeration at a final concentration of 100 μg/ml for 20 min at 37° in TSY and incubated with shaking at 37°C for three hrs. About 5% of the time the culture underwent mass lysis and was lost in one of the latter steps. Frequently mutagen-treated survivors were preserved at —20°C after quick-freezing in 15% glycerol (v/v).

Chemicals: All biochemicals were obtained as the highest quality products available from either Sigma or Calbiochem. Nitrosoguanidine was purchased from Aldrich Chemical Company.

Chemical and enzyme assays: DNA was estimated by the method of BURTON (1956) using calf thymus DNA as a standard. Anthranilate synthetase was assayed by the protocol of NESTER and JENSEN (1966).

Preservation of competent cells: Cells in the competent state were maintained frozen in 15% glycerol (v/v) for about a month at —80°C or indefinitely in liquid nitrogen (JENSEN and HAAS 1963).

Scoring revertants: Revertants were picked to the medium used for their initial selection and purified once by streaking out on the same medium. This served to eliminate occasional pseudo-revertants which subsequently failed to grow on the selective medium. Only revertants which passed the test of purification were tallied.

RESULTS

The mutant strain of IYER (1965) was initially isolated by selecting for the ability of ultraviolet irradiation-treated survivors of IF 43 (see Table 1) to generate minute-sized transformants in the presence of DNA extracted from the wild-type prototroph. This technique, ingenious in concept, produced mutant 68c (our number, IF 44). A mixture of wild-type DNA and competent cells of IF 44 gave rise to a low yield (about 10^{-4}) of transformants of which up to 40% were "unstable" colonies of minute size on minimal medium, selective for *trp*$^+$ *his*$^+$ transformants.

Unusual recipient characteristics of IF 44: The above results were quite repeatable in our hands. However, a comparison of the IF 44 recipient with the parent strain IF 43 revealed other significant differences: (i) *On minimal agar:* The frequency of *trp*$^+$ *his*$^+$ transformants produced by IF 44 was markedly lower than for IF 43, (ii) *On histidine agar:* The frequency of *trp*$^+$ transformants was similar in both recipients; additionally no minute colonies were found, and (iii) *On tryptophan agar:* Few or no minute colonies were found. The frequency of *his*$^+$ transformants in IF 44 was much lower than in IF 43, as in (i) above.

It was clear that the intrinsic competence of IF 44 was not significantly different from that of IF 43 judging from observation (ii) above. These results implicated some anomalous correlation of small colony formation with the use of histidine as the selective marker and with the marked decrement in the frequency of *his*$^+$ transformants.

New mutation in IF 44. The following results showed that IF 44 carried a *his*$^-$ lesion unlinked to *trp*$_2^-$ *in addition* to the linked *his*$_5^-$ marker already present in parent strain IF 43 (IF 44 was reported (IYER 1965) to be *his*$_2^-$ in genotype

rather than his_5^- (ELIZUR, SRINIVASAN and ZAMENHOF 1961) due to a typographical error): (i) When trp^+ transformants of IF 44 (NP 40 × IF 44) were replica-plated to minimal media to score for the unselected marker, none proved to be his^+ in contradiction to expectations for the joint transfer of these markers which are known to be linked (ELIZUR, SRINIVASAN and ZAMENHOF 1961). Thus the *his* and *trp* markers behave in this strain as though they were unlinked. The latter fact was additionally consistent with the observed low frequency of double transformants and the requirement that saturating concentrations of DNA be used. (ii) When minimal plates that had been imprinted by replica plating with trp^+ transformants from a master plate were allowed to age for four days, numerous revertant papillae developed within about half of the colonies. The remaining colonies never developed papillae regardless of the duration of subsequent incubation. Suggestively, the proportion of trp_2^+ transformants which were revertible on minimal medium resembled the frequency of cotransfer of the linked markers his_5 and trp_2 of IF 43. (iii) When a trp_2^+ transformant of the "revertible" type was picked and purified from the master plate and then used as a genetic recipient, it proved to have a histidine requirement *unlinked* to trp_2 (IF 45, Table 1). (iv) The *trp* and *his* markers of IF 44 behave as though they were linked when the strain is used as a donor, and paradoxically, as if they were unlinked when the strain is used as a recipient.

Genetic crosses. The results which appear in Table 2 permit a comparison of IF 43 and IF 44 as recipients with respect to transformation frequencies. The datum in the first horizontal line indicates no greater difference in recombinant frequency than occurs between different batches of competent cells of the same strain. The classes of single and double transformants obtained with the IF 43 recipient are entirely consistent with the known linkage of these markers. In the IF 44 recipient the fifty-fold lower frequency of his^+ transformants than trp^+ transformants at saturating concentrations of DNA is due to the requirement for the coincidental entry of two *his* loci, unlinked to one another, in order to obtain a his^+ phenotype. The same explanation, of course, applies to the decreased frequency of the trp^+ his^+ class. The number of minute colonies was strikingly similar to the difference between $trp^\pm$ his^+ and trp^+ his^+ classes, a result sug-

TABLE 2

Comparative recipient behavior of IF 44 and its parent, IF 43

NP 40 × IF 44 Number of transformants	Recombinant class	NP 40 × IF 43 Number of transformants
269×10^3	trp+his±	150×10^3
51×10^2	trp±his+	148×10^3
37×10^2	trp+his+	85×10^3
(15×10^2)	trp+his+ (minute colonies)	

Donor DNA was used at a concentration exceeding saturation 5-fold. $trp^+his^\pm$ transformants were scored on minimal + his; $trp^\pm his^+$ transformants were scored on minimal + trp; trp^+his^+ transformants were scored on minimal agar. The viable counts of IF 44 and IF 43 recipients were 4.6×10^8 and 1.4×10^8, respectively, The frequencies cited are based on at least 500 colony counts.

TABLE 3

Comparative recipient behavior of IF 44 and its parent, IF 43

Genetic cross	Number tested	trp+his+ (Class 11)	r	
NP 40 × IF 43				
trp+his±	180	98	0.37	0.44*
trp±his+	177	122	0.53	
NP 40 × IF 44				
trp+his±	269	6	0.011	
trp±his+	204	152	0.59	

* Cotransfer indices on the left of the bracket were calculated by approximating, class 10= class 01. The value of *r* to the right was calculated in the usual manner.
Data were obtained from the experiment of Table 2.

gesting that minute colonies might in fact constitute the trp^- his^+ class. This possibility was verified by nutritional testing of small colony types purified from the background.

Thus the tiny colonies are rarely seen on histidine plates because suitable plating dilutions that yield 100–200 trp^+ transformants contain few or no his^+ transformants (since they must originate by coincidental transfer of two unlinked genes). On the other hand, minute colonies are not seen on tryptophan medium because tryptophan satisfies the nutritional requirement allowing the achievement of normal colony size. The previous data have been reworked in Table 3 in terms of the genetic cotransfer of the two phenotypes. The cotransfer index, *r*, was similar in the IF 43 recipient whether *his* or *trp* was used as the selective marker. In contrast, when IF 44 was used as the recipient, about 75% of the his^+ transformants were also transformed for *trp*; strikingly, very few trp^+ transformants had also acquired the his^+ phenotype. This, of course, is entirely consistent with the discrepancy in the two transformation frequencies (Table 2). Although many trp^+ transformants will have acquired the linked his^+ marker (his_5), few will have acquired the unlinked his^+ marker as well (his_U). Therefore the *phenotype* will nearly always be his^-. On the other hand, when selection is obligatory for recombinants which have acquired the two unlinked *his* markers by coincidence (i.e., on trp medium), the usual linkage of the unselected trp_2 marker will be observed by virtue of its genetic proximity to his_5. The paradoxical linkage results obtained from reciprocal crosses involving IF 44 (Table 4) are explained by the presence of his^-_U in IF 44.

The markers aro_2, trp_2, and his_5 are all known to cluster together within a single linkage group (NESTER, SCHAFER and LEDERBERG 1963). When IF 44 was used as the source of donor DNA, the results showed that aro_2 was indeed closely linked to both trp_2 and his_5. In contrast, when IF 44 served as a recipient, trp_2 was closely linked to aro_2 but appeared not to be linked to the *his* marker. Again this is due to the requirement that a second unlinked *his* marker be integrated together with the linked one in order to achieve the his^+ phenotype.

Relationship of DNA concentration, transformation frequency and r *in IF 48.*

TABLE 4

Paradoxical linkage of IF 44 markers in reciprocal crosses

	IF 44——— × NP 16*					
	$aro_2^+ trp_2^- his^-$——— × $aro_2^- trp_2^+ his^+$					
	Transformant class					
Selective marker	100	101	110	111	r_t^+ ($aro_2 trp_2$)	r_t^+ ($aro_2 his$)
aro_2^+	232	27	73	265	0.39	0.32
	NP 16——— × IF 44					
	$aro_2^- trp_2^+ his^+$——— × $aro_2^+ trp_2^- his^-$					
	Transformant class					
Selective marker	010	011	110	111	r_t^+ ($aro_2 trp_2$)	r_t^+ ($trp_2 his$)
trp_2^+	185	2	387	5	0.51	0.006

The viable counts of NP 16 and IF 44 were 1.1×10^8 and 4.3×10^8, respectively. DNA concentrations were 2-fold and 5-fold greater than saturation, respectively.

* NP 16 maps in the same region as aro_2 and aro_3 (NESTER, SCHAFER and LEDERBERG 1963). Both aro_2 and aro_3 are blocked in dehydroquinate synthetase enzyme activity.

† The cotransfer index was estimated by approximating the frequency of unscored single transformants (classes 010 and 001: IF 44——— × NP 16; and classes 100 and 001: NP 16——— × IF 44) from the frequencies of these classes of single transformants which were scored.

IF 48 (Table 1) arose as a trp_2^+ transformant of IF 44. Table 5 contains data to illustrate that this strain behaves as though the *trp* and *his* markers were linked provided that his^+ is the selective marker (as seen before in Table 3 with IF 44). In Table 5 the value of r is largely independent of DNA concentration as expected if the *trp* and *his* donor markers were linked. However, the transformation frequency (which is low for reasons previously elaborated in the case of IF 44) does *not* show the usual linear dependence upon DNA concentration. At non-saturating concentrations of DNA the transformation frequency falls off precipitously. Thus one would expect that transformant frequencies at DNA concentrations of 0.005 and 0.0005 μg/ml in Table 5 would be 3×10^{-6} and 3×10^{-7}, respectively. However, the latter result does match expectations for the coinci-

TABLE 5

Characteristics of IF 48 as a genetic recipient 168——— × IF 48

DNA concentrations* (μg/ml)	$trp^+ his^+$	$trp^+ his^+$	$trp^- his^+$	r	Transformation frequency
5.0	273	127	146	0.37	5.9×10^{-5}
0.05	150	62	88	0.42	3.4×10^{-5}
0.005	101	44	57	0.39	3.1×10^{-7}
0.0005	0	..	..	..	0

* The DNA concentrations are given as final concentrations in the reaction mixtures of cells and DNA. The saturating concentration of DNA was about 0.04 μg/ml as determined by a saturation curve using strain 168 as a recipient in a separate experiment.

The selective media used in this experiment contained tryptophan; no minute-sized colonies were observed. In other experiments in which minimal agar was the selective medium, colonies did appear.

TABLE 6

Linkage of various his *markers to* trp_2

Genetic cross	trp⁻his⁺ (class 11)*	trp⁺his⁺ (class 01)*	r*
168_____ × NP 12*	354	214	0.45
168_____ × IF 46	327	232	0.41
168_____ × IF 45	6	538	0.006

Donor DNA concentrations were 50-fold below the level required to saturate competent cells. The selective medium was minimal + trp, upon which *his*+ *trp*± recombinants grew. The numbers of double and single transformants were determined by replica plating to minimal medium. See Table 1 for the origins of IF 45 and IF 46.

* The conventions suggested by NESTER, SCHAFER and LEDERBERG (1963) are used. Thus donor and recipient markers are designated 1 and 0, respectively. Cotransfer of donor markers is expressed by *r*, the cotransfer index.

dental transfer of two *unlinked* markers. Thus the rapid decrease in transformation frequency as a function of DNA concentration reflects the obligatory requirement for congression of *his* markers located on different DNA molecules in order that his^+ recombinant phenotypes arise.

Genetic isolation of the two his *markers of IF 44.* The application of appropriate genetic crosses permitted the genetic separation of the *his*⁻ markers carried by IF 44 so that each could be studied independently (see Table 1). It is shown conclusively in Table 6 that the his_5 marker of IF 46 is closely linked to trp_2 as is his^-_2 of NP 12 (NESTER, SCHAFER and LEDERBERG 1963). In contrast, the *his* marker of IF 45 is unlinked to trp_2.

Reversion analysis. It is readily apparent from an inspection of Table 7 that IF 43 and IF 44 differ in stability to reversion of independence of the histidine requirement. IF 43 is revertible; IF 44 is not. This is consistent with the presence of two *his* loci in IF 44 (and IF 48). Reversion to a his^+ phenotype in the latter two strains would require two mutations, and therefore represents an extremely improbable event. It is clear that each of the *his* loci individually present in strains IF 44 and IF 48 is revertible.

TABLE 7

Comparative reversion frequencies of various his⁻ *phenotypes*

Mutagen	IF 43	IF 44	IF 45	IF 46	IF 48	NP 12
Spontaneous	11	0	51	11	0	69
Nitrosoguanidine	14	0	79	9	0	81
Ultraviolet	32	0	401	63	0	399

The above strains were cultured in the logarithmic phase of growth and were then separated into three portions, two of which were mutagenized as described under METHODS. Samples were taken for plating in order to determine viable counts. The cultures were then concentrated 100-fold and frozen in liquid nitrogen following the addition of glycerol to a final concentration of 15% (v/v). The next day the cells were thawed and adjusted to a concentration of 10^9/ml. 10^8 cells were spread on each of 10 minimal + trp plates. The numbers cited above indicate the total number of revertants scored per 10^9 cells after 3 days growth at 37°. See METHODS for further details of scoring for reversions.

TABLE 8

Reconstruction experiment

Plating medium	IF 44	168	IF 44 + 168	IF 43 + 168	IF 43
Minimal	0	0	0	0	0
	(0)*	(0)	(97)	(4)†	(0)
Minimal + trp	0	116	106	121	0
	(0)	(0)	(2)	(2)	(0)

* The upper number denotes the count of normal-sized colonies; the lower number in parentheses designates the count of minute-sized colonies.

† IF 43 does give rise to barely visible clones that can be seen under a dissecting microscope in the presence of cells of 168 but not in the absence of cells of 168. No attempt was made to count the latter clones which were much smaller than the minute colonies arising from the mixture of IF 44 and 168.

Reconstruction of the nutritional phenomenon. If it is indeed true that undersized clones were simply *his*$^+$ *trp*$^-$ transformants under the aforementioned plating conditions, then it should be possible to duplicate these results in a mixing experiment in which the parameter of DNA transformation is removed as a factor. The experiment of Table 8 was carried out by mixing cells of strain 168 and either IF 43 or IF 44 in proportions similar to numbers of minute transformants and numbers of background recipient cells, respectively, on a minimal plate in a transformation experiment. DNAase at a concentration of 10 μg/ml was present at the time of mixing to ensure that genetic exchange could not be a contributing factor. The results show that the number of small colonies which were observed on minimal medium were equal to the input of 168 cells which require tryptophan. The formation of small colonies on minimal medium depends upon a coexisting lawn of IF 44 cells. IF 43 does not support the partial growth of 168. In accord with the latter results IF 48 (or IF 49) cross-feeds 168 much better than does IF 46 on histidine-supplemented medium.

DISCUSSION

Unfortunately, it must be concluded that strain IF 44 (68c, IYER 1965) cannot be utilized to assess dominance relationships of genes in *Bacillus subtilis*. The small colonies which arise on minimal agar in DNA transformation experiments are the consequence of a set of nutritional interactions which probably involve a regulatory relationship between the biosynthesis of tryptophan and histidine.

Detailed genetic analysis of the mutant strain (Tables 2–6) has proven the existence of a second histidine locus in the strain. The two histidine markers were separated genetically by DNA transformation so that they could be examined independently. Histidine loci map in two unlinked clusters in *B. subtilis* (ELIZUR, SRINIVASAN and ZAMENHOF 1961). The locus present in the parental strain IF 43 is closely linked to the trp_2 locus of that strain; the newly induced *his* locus of IF 44 is unlinked to trp_2 and probably resides within the second cluster of *his* loci. Mutational analysis demonstrated that the double *his* auxotroph (IF 44)

did not revert for its histidine requirement at any measurable frequency, a result in distinct contrast to that obtained with the parent strain or with recombinant strains carrying either one of the two histidine loci separately (Table 7).

It was possible to show that the mutant strain differed from the parent strain in its ability to syntrophically supply tryptophan to tryptophan-requiring transformants. The latter elevation of tryptophan is not particularly great. Microbial requirements of tryptophan for protein synthesis are low. Background recipient cells carrying the *his* locus supply enough tryptophan so that *trp*$^-$ transformants form visible colonies, but not enough to allow the achievement of normal colony size. Since small colonies must be *his*$^+$ transformants (i.e., cotransfer of the unlinked his_5 and his_U), they are plated at low dilutions, a condition equivalent to high concentrations of background recipient cells. Fifty-odd small transformants scored at a low dilution are distinctly larger than the five small transformants scored at a 10-fold greater dilution, an observation pointing to the importance of the recipient cells as the source of tryptophan. The reconstruction experiment in which cells of IF 44 and 168 were mixed to mimic the relative concentrations of background recipient cells and transformants, respectively, showed that the phenomenon could indeed occur in the total absence of DNA transformation. The background recipient cells in transformation experiments were his$^-$ trp$^-$ in phenotype. Perhaps IF 44 recipients supply more tryptophan to *trp*$^-$ *his*$^+$ transformants than does the parent strain, either by lysing more readily than the parent strain under plating conditions or by accumulating larger intracellular pools of tryptophan. Exactly why the latter behavior should be associated with the his_U locus is not clear. However, there are reasons to suggest that this may reflect a regulatory relationship between the pathways of synthesis for tryptophan and histidine. Such interrelationships of histidine and tryptophan synthesis characterize microorganisms as different as *Neurospora crassa* (CARSIOTIS and LACY 1965) and *B. subtilis* (NESTER, SCHAFER and LEDERBERG 1963; NESTER and JENSEN 1964). Additionally suggestive of such a regulatory relationship was the observation that revertants of his_U (IF 45) are frequently heavy tryptophan excretors. Also, the level of at least one enzyme in the tryptophan pathway, anthranilate synthetase, is elevated over that of wild type (R. A. JENSEN, unpublished observations). Further enzymological studies in the context of the regulatory interactions that appear to be involved are in progress.

Our findings differ from those previously reported (IYER 1965) on the following points: (i) We could not repeat the results obtained by clonal analyses in support of a unilinear transmission of the exogenote; (ii) transformant classes *trp*$^+$ *his*$^-$ and *trp*$^-$ *his*$^+$ of IF 44 did not yield small colonies when the transformants were used as recipients for a second transformation with wild-type DNA; (iii) transformants of the his$^+$ phenotype (actually requiring the coincidental uptake of two unlinked *his* loci) are not obtained with genuinely limiting concentrations of transforming DNA; (iv) normal cotransfer frequency (NESTER and LEDERBERG 1961) is only obtained when *his*$^+$ is used as the selective marker, not when *trp*$^+$ is used as the selective marker; and (v) although occasionally the two transformant genotypes (*trp*$^+$ *his*$^-$ and *trp*$^+$ *his*$^+$) were found to segre-

gate from small colonies in addition to the his^+ trp^- phenotype, these frequencies were not significantly greater than the frequency of mixed genotypes which can be observed routinely in the purification of transformant clones in general.

The genetic removal of the his_U mutant in genetic recipients correlated with a loss of small colony-type transformants. Additionally, it was obligatory that recipient cells (or donor cells) be trp^- since small colony transformants proved to be trp^- in genotype. Significantly, it was not possible to obtain "abortive transformation" (i) when any of eight other amino acid or nucleic acid markers were substituted for the trp^-_2 locus in the presence of the his_U mutation, or (ii) in recipients bearing the trp^-_2 marker in the *absence* of his_U.

The initial portion of these studies were carried out in the laboratory of Dr. E. NESTER in Seattle, Washington.

SUMMARY

The mutation responsible for the apparent ability of a mutant of *Bacillus subtilis* to undergo abortive DNA transformation was identified in synonymy with a requirement for histidine. This nutritional aspect of the mutation was not obvious originally since it was induced in a strain already possessing another histidine block. Apparent abortive transformants which are expressed as small colonies were found to be tryptophan-requiring double transformants of the two histidine loci. The newly induced histidine block correlated with an increased ability of background recipient cells to accumulate tryptophan in sufficient amounts to partially support the growth of the latter transformant types. The additional biochemical block in the histidine pathway was demonstrated in the mutant by genetic and mutational analysis. A reconstruction experiment demonstrated the nutritional basis of the phenomenon under conditions where DNA transformation was precluded as a variable.

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