TRANSFORMATION OF *BACILLUS SUBTILIS* TO MOTILITY AND PROTOTROPHY: MICROMANIPULATIVE ISOLATION OF BACTERIA OF TRANSFORMED PHENOTYPE

B. A. D. STOCKER[1]

Department of Genetics, Stanford Medical Center, Palo Alto, California

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ABSTRACT

STOCKER, B. A. D. (Stanford Medical Center, Palo Alto, Calif.). Transformation of *Bacillus subtilis* to motility and prototrophy: micromanipulative isolation of bacteria of transformed phenotype. J. Bacteriol. **86**:797–804. 1963.—A nonmotile (nonflagellated, *fla*$^-$) *try*$^-$ strain of *Bacillus subtilis* was transformed to *fla*$^+$ and to *try*$^+$ by wild-type deoxyribonucleic acid (DNA) at comparable rates. Bacteria of *fla*$^+$ phenotype were recognized by their motility approximately 3 hr after uptake of DNA, and bacteria of *try*$^+$ phenotype at about the same time by their elongation into filaments in a medium lacking tryptophan. Of phenotypically transformed bacteria of each sort isolated by micromanipulation, the majority produced only transformed progeny, a mixture of transformed and untransformed, or a mixture of two kinds of transformant. Some produced only untransformed progeny, or progeny transformed only at a locus linked to that concerned in their phenotypic transformation. In a few clones, some partial heterozygotes were present even ten generations after DNA uptake. In nonmotile clones derived from motile isolates, the unilinear transmission of motility to one to four descendants was detected; it is attributed to persistence of a corresponding number of units of some product of an unincorporated *fla*$^+$ gene, probably flagella or cell walls each carrying several flagella. No pedigrees indicating unilinear transmission of an unincorporated *fla*$^+$ gene were observed.

Motility is a bacterial character which can be scored by inspection of the live individual. Study of the phage-mediated transduction of motility to nonmotile strains of *Salmonella typhimurium* led to the discovery of abortive transduction, i.e., the transmission of a nonreplicated transduced gene down a single line of descent for many generations. A motile species susceptible to deoxyribonucleic acid (DNA)-mediated transformation being now available in *Bacillus subtilis* (Spizizen, 1958), it was of interest to seek a nonmotile mutant and to study its transformation to motility by the methods used in work on transduction of motility. Because of various experimental difficulties in the *B. subtilis* system, the results here described are less extensive than those obtained in the transduction system with *S. typhimurium* (Stocker, 1956*a*; Lederberg, 1956). However, they suffice to establish (i) that the transformant phenotype, in respect of both motility (*fla*$^+$) and tryptophan independence (*try*$^+$), is not expressed until 3 or more hr after uptake of DNA; (ii) that some bacteria which develop the transformant phenotype produce only untransformed progeny; and (iii) that others produce both transformed and untransformed progeny (or more than one type of transformant) or progeny some of which are still heterozygous for the transformed character several generations after DNA uptake.

MATERIALS AND METHODS

Most of the strains used, all derivatives of the transformable (Spizizen, 1958) tryptophan-exacting (*try*$_2^-$) mutant 168 of Burkholder and Giles (1947), were listed by Nester and Lederberg (1961). The media, methods of cultivation, and routine for obtaining competence were those of Nester and Lederberg (1961), and DNA was extracted and purified as described by them. Competent cultures were incubated with excess of DNA for 30 to 60 min, usually followed by incubation with deoxyribonuclease. Samples were then plated on defined media for estimation of the number of *try*$^+$ transformants, and standard loop-

[1] Present address: Guinness-Lister Research Unit, Lister Institute of Preventive Medicine, London, England.

ful amounts (ca. 0.003 ml) of tenfold dilutions were seeded onto semisolid medium (Stocker, Zinder, and Lederberg, 1953) in 5-cm petri dishes or in 1-ml volumes in cups in a plastic sheet (Disposo-Tray; Linbro Chemical Co., Inc., New Haven, Conn.) sterilized by ultraviolet irradiation. Growth spreading across and through the medium showed the presence of one or more motile transformants in the inoculum.

Competent cultures, after treatment with DNA, were incubated under various conditions as described in the text, and were examined at intervals for the detection by microscopy of the *fla*$^+$ or *try*$^+$ transformant phenotype. For this purpose, samples were set up as hanging drops in oil-filled micromanipulation chambers, generally with a "trapping-drop" of fresh medium into which motile bacteria could migrate; for description of this technique, and that of micromanipulative isolation of selected bacteria, see Stocker (1956*a*) and Lederberg (1956). The chambers were incubated at bench temperature. It was necessary to determine the motility of the progeny of bacteria isolated in microdrops before these became crowded, since bacterial death and lysis then followed, presumably because of the inadequate supply of oxygen or nutrients. In some experiments, incubation of DNA-treated competent cultures was interrupted by overnight storage at 4 C. This resulted in death of up to half the bacteria but did not seem to affect the outcome otherwise. To determine the nutritional character of the progeny of a bacterium isolated in a microdrop, the entire contents of the droplet containing some hundreds or thousands of bacteria were taken up in a micropipette, and were transferred with washing to the surface of a nutrient agar plate. The nutritional character of the colonies obtained was determined by replication to test media; this technique, though it easily detects even a single less-exacting colony, may fail to detect a small proportion, perhaps $<0.5\%$, of colonies more exacting than the rest.

RESULTS

Transformation to motility of nonmotile strains. Among many stock sublines of *B. subtilis* strain 168 (*try*$_2^-$) examined, a glycine-exacting mutant obtained by ultraviolet irradiation, strain SB 53, was found to be nonmotile in broth and nonspreading in semisolid medium. This strain gave only a poor yield of *try*$^+$ transformants; its nonmotile character was therefore transferred by transformation reaction to strain SB 1 (*try*$_2^-$, *his*$_1^-$; Nester and Lederberg, 1961). Of 128 *his*$^+$ clones obtained from SB 1 in two experiments by treatment with an excess of DNA from SB 53, 4 were nonmotile as a result of coincidental transformation to *fla*$^-$. One of these clones, SB 108, was used in the present work. No motile bacteria have been seen in cultures of strain SB 108, and it rarely mutates to motility, as judged by selection in semisolid medium. Its nonmotility results from absence of flagella, for it is not agglutinated by an antiflagellar serum and no flagella are seen after staining by the method of Leifson (1951).

When a competent culture of strain SB 108 was treated with an excess of DNA from a *try*$^+$ *fla*$^+$ strain, generally about 0.2% of the cells gave *try*$^+$ colonies. The proportion of standard loopful inocula of a 1:100 dilution giving spreading growth in semisolid medium was generally about 0.5; this indicates that about 0.1% of the bacteria were transformed to *fla*$^+$. In many experiments, inocula from the 1:10 and 1:1 dilutions did not give spreading growth, even though some inocula from more dilute suspensions did so. No such negative "pro-zone" was seen when artificial mixtures of *fla*$^-$ and *fla*$^+$ cells were tested. A possible explanation is discussed below. No "trails," such as result from abortive transduction of motility in *S. typhimurium* (Stocker et al., 1953), were seen. The proportion of *fla*$^+$ transductants which were also *try*$^+$, or also *str*^{r}, when the DNA used came from a streptomycin-resistant strain, was not higher than expected for unlinked characters.

Appearance of motile bacteria and filaments. Cells of strain SB 108, after treatment with *fla*$^+$ DNA, were incubated with aeration at 30 or 37 C in the tryptophan-supplemented acid-hydrolyzed casein medium [CHT-10 medium of Nester and Lederberg (1961)] used to induce competence. No motile bacteria were seen in samples taken up to 3 hr after the end of DNA treatment, even though about 0.1% of the DNA-treated bacteria produced motile progeny in semisolid agar, and though a much lower proportion than 0.1% of motile bacteria can easily be detected by microscopy. The DNA-treated suspensions were therefore diluted two- to eightfold in fresh medium, to allow a longer period before growth ceased. Small numbers of motile bacteria then appeared, generally between 3 and 4 hr after the end of DNA treatment; on longer incubation, they became

numerous. Many early-appearing motile bacteria were isolated by micropipette and transferred to droplets of fresh CHT-10 medium. For reasons discussed in the accompanying paper (Nester and Stocker, 1963), it was thought that exposure of the DNA-treated bacteria to penicillin would increase the proportion of transformants in the surviving fraction and so facilitate isolation of the first-appearing motile bacteria. The DNA-treated cells were therefore sometimes incubated in CHT-10 medium with penicillin (2000 units/ml); after 90 min, penicillinase was added and incubation was continued. Motile bacteria appeared at about the same time as when penicillin was not used. The progenies of motile bacteria isolated by micromanipulation from suspensions, some of which had been treated with penicillin, are described below.

In some experiments, bacteria of strain SB 108 (try_2^- *fla*$^-$) after treatment with *try*$^+$ *fla*$^+$ DNA were washed, resuspended in casein hydrolysate medium (as CHT-10 but omitting tryptophan), and incubated for various periods before addition of tryptophan; the object was to prevent multiplication of the majority of untransformed *try*$^-$ *fla*$^-$ bacteria during most of the long period of incubation before the appearance of motile bacteria. It was then observed that after about 3 hr of incubation in medium without tryptophan some filaments were present, though no very long bacteria had been seen in earlier samples. On further incubation in the micromanipulation chamber at room temperature, these filaments grew into long tangled skeins. No filaments appeared in the following controls: without DNA, with DNA from a try_2^- strain, when deoxyribonuclease was added with the *try*$^+$ DNA, and on incubation in tryptophan-supplemented medium. The filaments must therefore arise from bacteria which can grow in length in medium lacking tryptophan because they have become *try*$^+$, at least in phenotype, as a result of the uptake of *try*$^+$ DNA. Several filaments were isolated by micromanipulation, with some difficulty because they stuck to the oil-medium interface, were too rigid to enter the micropipette except endways, and usually broke up during pipetting. The progenies resulting from their growth in droplets of tryptophan-supplemented medium are described below. In one experiment, a try_2^- *fla*$^+$ (motile) strain, instead of the try_2^- *fla*$^-$ strain SB 108, was treated with *try*$^+$ DNA, and then incubated in medium without tryptophan. Motile filaments, shorter than the nonmotile filaments produced by the *fla*$^-$ strain, appeared. Nine of these motile filaments were isolated in droplets of medium without tryptophan. Four multiplied normally, which shows that the progeny of these four filaments, or at least a fraction of them, were *try*$^+$. The other five filaments produced only 3 to 30 descendants during overnight incubation at room temperature. Two bacteria from each of these five small clones were transferred to droplets of tryptophan-supplemented medium; six of the ten then multiplied, which suggests that the cessation of growth in the original droplets resulted from the absence of tryptophan, not from accidental causes. The five filaments which grew for only a few generations in droplets of medium without tryptophan were presumably *try*$^+$ in phenotype as a result of uptake of *try*$^+$ DNA, but were untransformed, i.e., *try*$^-$, in genotype, so that all their progeny rapidly reverted to the *try*$^-$ phenotype.

When the *try*$^-$ *fla*$^-$ strain SB 108 treated with *try*$^+$ *fla*$^+$ DNA was incubated in medium without tryptophan, small numbers of motile bacteria, of normal length, appeared soon after the development of filaments, i.e., at about the same time as motile bacteria appeared in similar suspensions incubated in tryptophan-supplemented medium. Such motile, i.e., phenotypically *fla*$^+$, bacteria did not appear when try_2^- *fla*$^+$ DNA was used instead of *try*$^+$ *fla*$^+$ DNA. It is inferred that they are bacteria which have become, at least in phenotype, both *try*$^+$ and *fla*$^+$, by the coincidental uptake of two DNA molecules, each bearing one of these unlinked loci. As these motile bacteria were much easier to take up by micropipette than filaments, many were isolated in droplets of tryptophan-supplemented medium for investigation of the try_2 and linked his_2 character of their progeny.

It has been observed in this laboratory and elsewhere that DNA extracted from some independently isolated strains of *B. subtilis* evokes few or no *try*$^+$ transformants from the *try*$^-$ Spizizen strain. When an excess of DNA from strain SB 45, a thymineless mutant of a prototrophic strain unrelated to the Spizizen strain (Brabander and Romig, 1960), was added to a competent culture of strain SB 108, the yield of *try*$^+$ transformants was only about 3/ml, whereas DNA from a *try*$^+$ derivative of the Spizizen strain evoked about 2.3 $\times$ 10^5/ml. No filaments or motile bacteria were seen in the SB 108 suspension treated with the

heterologous *try*$^+$ DNA and incubated for 4.5 hr in CH medium, and no motile bacteria appeared in samples of the same suspension further incubated in tryptophan - supplemented medium. Thus, DNA from a "foreign" strain entirely or almost entirely fails to cause the appearance of the *try*$^+$, *fla*$^+$, and *try*$^+$ *fla*$^+$ phenotypes, as well as failing to effect genotypic transformation to *try*$^+$.

Progeny of bacteria of transformant phenotype. Droplets seeded with single motile bacteria isolated from DNA-treated suspensions were examined at intervals, and the motility of the progeny was recorded. In some experiments, most of the motile bacteria isolated, termed *initials* (Stocker, 1956*a*; Lederberg, 1956), failed to grow; in others, they grew as filaments, which obscured motility. The results of all technically satisfactory experiments are recorded in Table 1. A large majority of motile initials, both from suspensions incubated in tryptophan-supplemented medium, with or without penicillin exposure, and from medium without tryptophan, produced progeny most of which were motile when they numbered hundreds or thousands. The minority of nonmotile bacteria observed in these clones were probably genotypically *fla*$^+$ and nonmotile through accidental causes only. The immediate progeny, numbering from two to eight, of 47 motile initials were separated; in 41 of the 47 pedigrees, all the resulting subclones were motile; in 6 pedigrees, both motile and nonmotile subclones were observed.

TABLE 1. *Motility of progeny of motile initials isolated from DNA-treated suspensions of strain SB 108*

Post-DNA treatment	No. of motile initials with progeny of indicated kind				
	Motile	Non-motile	Non-motile except 1-4	Non-viable	Total
Incubated in CHT	76*	3	7	3	89
Incubated in CH	64†	0	1	2	67
Incubated in CHT with penicillin (2000 units/ml) for 60 min	34‡	0	0	1	35
Total	174	3	8	6	191

* All of 20 initials whose immediate progeny were separated produced only motile subclones.

† All of five initials whose immediate progeny were separated produced only motile subclones.

‡ Of 22 initials whose immediate progeny were separated, 16 produced only motile and 6 produced both motile and nonmotile subclones.

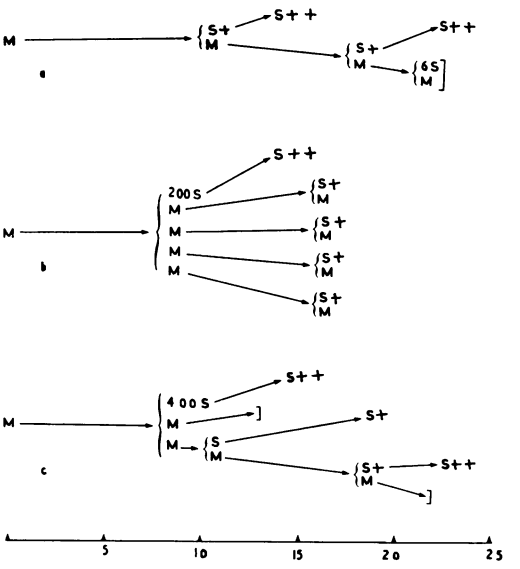

FIG. 1. *Pedigrees of three motile initials isolated from DNA-treated suspensions of strain SB 108 incubated in tryptophan-containing medium.* M = *a motile bacterium;* S = *a nonmotile bacterium;* S+ = *hundreds of nonmotile bacteria;* S++ = *thousands of nonmotile bacteria; arrows run from cells to their progeny;*] = *cells did not multiply or were lost; scale shows number of generations from initial, calculated from estimated population sizes.*

Of the 185 viable motile initials, 11 produced only nonmotile clones and 6 produced nonmotile subclones as well as motile subclones; inspection of these 17 nonmotile progenies when they numbered 100 to 1000 resulted in the detection of one to four motile bacteria in 8 of them. From five of these eight clones, all the motile bacteria (numbering four, two, two, one, and one) were isolated and transferred to separate droplets. Three of these ten failed to grow; the remaining seven all produced nonmotile clones, in six of which a single motile descendant was detected at the 100- to 1000-cell stage. The single motile descendant was isolated from two of these six progenies; only one grew, producing six nonmotile and one motile descendant, at which point the clone was accidentally lost. This pedigree and those of two initials with, respectively, four and two motile descendants are shown in Fig. 1. In summary, a few of the bacteria which acquired the *fla*$^+$ phenotype as a result of uptake of DNA produced *fla*$^-$ clones,

with a very few motile (i.e., phenotypically *fla*$^+$) members, each of which, so far as they were tested, transmitted motility to only a single descendant, i.e., unilinearly (Stocker, 1956*a*; Lederberg, 1956). No motile bacteria at all were detected at the 100- to 1000-cell stage in the nonmotile subclones produced by immediate progeny of the six motile initials which also produced motile clones, i.e., among the descendants of sibs of cells transformed to *fla*$^+$. In three of these six pedigrees, all the immediate progeny bacteria which gave nonmotile clones were themselves nonmotile at the time they were transferred to separate droplets; in the other three, the progenitors of the nonmotile subclones showed only rotatory or twitching movements, instead of the translational movement typical of *fla*$^+$ *B. subtilis*.

In some experiments, strain SB 108, *fla*$^-$ *try*$_2^-$, was treated with DNA from a *fla*$^+$ donor, which was *try*$^+$ and histidine-exacting through mutation at the *his*$_2$ locus, closely linked to *try*$_2$. For the reasons given above, motile bacteria appearing in medium without tryptophan are believed to be phenotypically *try*$^+$ as well as *fla*$^+$. In one experiment, all of 16 motile initials after transfer into droplets of tryptophan-supplemented medium produced motile clones. Ten of these clones were removed from the chamber and streaked *in toto* on nutrient agar; the resulting colonies were later scored for their nutritional character (Table 2). Among eight apparently homogeneous clones, all four possible combinations of the *try* and *his* characters were represented, including one clone with the *try*$^-$ character of the recipient strain and the linked *his*$^-$ of the donor. One of the two mixed clones contained two kinds of transformant, *try*$^+$ and *try*$^+$ *his*$^-$. The other produced some hundreds of *try*$^+$ *his*$^-$ colonies (i.e., transformed for both *try* and *his*) and a single colony containing both *try*$^+$ *his*$^-$ (doubly transformed) and *try*$^-$ *his*$^+$ (untransformed) components.

Filaments (14) produced in medium without tryptophan after exposure to *try*$^+$ *his*$^-$ DNA were isolated and transferred to droplets of tryptophan-supplemented medium. Most filaments broke up during this operation, and it was impossible to be sure that all the fragments of a single filament had been recovered and delivered into the droplets. After incubation at room temperature, the resulting clones, of some hundreds of rods, were removed, plated, and later scored for nutritional character (Table 2). Like the motile initials inferred to have been *try*$^+$ in phenotype, some filaments produced only *try*$^-$ *his*$^+$, i.e., untransformed, progeny, some produced only singly transformed, *try*$^+$, progeny, and others produced only doubly transformed, *try*$^+$ *his*$^-$, progeny. Two clones yielded some hundreds of *try*$^+$ *his*$^-$ colonies and, respectively, three and eight *try*$^+$ *his*$^+$ colonies. Several of these few *try*$^+$ *his*$^+$ colonies on the original nutrient agar plates were streaked on nutrient agar, and 20 colonies from each were tested. Each of the two original clones gave the same results, *viz.*, most of the few original colonies which scored as *try*$^+$ *his*$^+$ gave 20/20 *try*$^+$ *his*$^+$ daughter colonies but some (one of three and two of five tested) gave a majority of *try*$^+$ *his*$^+$ daughter colonies and a few

TABLE 2. *Character of progeny of filamentous (phenotypically try*$^+$*) and motile (phenotypically try*$^+$ *and fla*$^+$*) initials isolated from strain SB 108 (try*$^-$ *his*$^+$*, fla*$^-$*) treated with try*$^+$ *his*$^-$*, fla*$^+$ *DNA and incubated in medium without tryptophan*

Kind of progeny* (and ratio of types)	No. of clones of indicated sort derived from		
	Filaments†	Motile† initials	Total
Homogeneous clones			
try$^+$ **his**$^-$	3	2	5
try$^+$ *his*$^+$	5	4	9
try$^-$ *his*$^+$	2	1	3
try$^-$ **his**$^-$	0	1	1
Mixed clones			
try$^+$ **his**$^-$ (0.3) / *try*$^-$ *his*$^+$ (0.7)	1	0	1
try$^+$ **his**$^-$ (1) / *try*$^-$ *his*$^+$ (0.001)	0	1	1
try$^+$ **his**$^-$ (0.7) / **try**$^+$ *his*$^+$ (0.3)	0	1	1
try$^+$ **his**$^-$ (1) / **try**$^+$ *his*$^+$ (0.001)	2‡	0	2
Sum	13	10	23

* Gene symbols in bold-face indicate the exogenous alleles resulting from incorporation of donor DNA.

† All progenies from filaments were nonmotile, and all from motile initials were motile.

‡ In each of these two clones, some bacteria of the minority type bred true, and others segregated **try**$^+$ *his*$^+$ and **try**$^+$ **his**$^-$.

colonies made up mainly of *try*$^+$ *his*$^-$ bacteria with a few *try*$^+$ *his*$^+$. Thus, in the progeny of these two filaments heterozygosity for *his* had persisted for many generations in a small fraction of the descendants.

DISCUSSION

The detection of a nonmotile, because nonflagellated (*fla*$^-$), mutant of the transformable *try*$^-$ Spizizen stock of *B. subtilis* has permitted observation that transformation to motility (or to nonmotility, by treatment of *fla*$^+$ lines with DNA from the *fla*$^-$ mutant) can be effected by DNA at about the same rate as transformation of other characters. Nasser and Koffler (1962) briefly reported similar transformation of another nonmotile mutant, obtained by irradiation.

Motile bacteria resulting from uptake of DNA did not appear until at least 3 hr after the end of DNA exposure, and at about the same time regardless of whether the DNA-treated *try*$^-$ bacteria were incubated in tryptophan-supplemented medium supporting their growth, or in medium without tryptophan, in which only bacteria of *try*$^+$ (transformed) phenotype could grow. There was about the same delay before bacteria which had acquired the *try*$^+$ phenotype began to elongate by growth during incubation in medium without tryptophan. Assays for tryptophan synthetase activity of *try*$^-$ bacteria treated with *try*$^+$ DNA likewise demonstrate a lag of 240 min before the enzyme activity acquired by transformation is first expressed (Nester, 1963; Nester and Stocker, 1963). As the wild-type *try*$^+$ and *fla*$^+$ characters would presumably be dominant, it is unlikely that the delay in expression results from the time needed for segregation of a transformed nucleus, or DNA strand, from an unaltered one. The delay in expression of *try*$^+$ and *fla*$^+$ in *B. subtilis* transformation contrasts with the immediate appearance of amylomaltase activity in competent maltose-negative pneumococcus cultures exposed to wild-type DNA (Lacks and Hotchkiss, 1960). A hypothesis proposed in the accompanying paper (Nester and Stocker, 1963) attributes the phenotypic delay in the *B. subtilis* transformation system, and an equal or greater delay before new transformants begin to multiply, to biosynthetic inactivity or "latency" of competent bacteria, persisting for 2 hr or more after they have taken up DNA. Delay in expression, and probably also in multiplication, of *fla*$^+$ transformants suffices to explain the paradoxical failure of large inocula, e.g., 10^5 cells, of DNA-treated *fla*$^-$ cultures to give spreading growth in semisolid medium when smaller inocula did so; with a large inoculum, the bacterial concentration at the inoculated area might reach a level preventing further growth before the *fla*$^+$ transformants came out of their state of latency.

A minority of the motile bacteria (initials) resulting from treatment of *fla*$^-$ bacteria with *fla*$^+$ DNA produced both *fla*$^+$ and *fla*$^-$ subclones (Table 1), which might result from segregation of a transformed and an untransformed nucleus present in the initial or from segregation of an unchanged from a recombinant strand of DNA. The 12 motile initials which produced *fla*$^-$ clones may have been untransformed sister cells of transformed bacteria giving *fla*$^+$ clones, and motile only through the inheritance of some product of the *fla*$^+$ gene from a parent bacterium with both a transformed and an untransformed nucleus, or with an as yet unintegrated *fla*$^+$ gene. Or they may have been bacteria in which a DNA molecule carrying the *fla*$^+$ gene was present but failed to be integrated, or was integrated in part by crossing-over in such a way that the *fla*$^+$ gene was lost. Though the data are scanty, the unilinear transmission of motility in these pedigrees (Fig. 1) is exactly like that observed in *S. typhimurium* when a motile, i.e., phenotypically flagellate, bacterium produces progeny none of which can manufacture new flagella, a phenomenon observed both in phage-mediated transduction of motility to *fla*$^-$ strains (Stocker 1956*a*; Lederberg, 1956) and in other circumstances (Stocker, 1956*b*; Quadling and Stocker, 1957; Quadling, 1958). It therefore seems likely that, as in *S. typhimurium*, some nonreplicated motility-conferring product of the *fla*$^+$ gene is being transmitted to only one daughter cell at each division, and so unilinearly to a single motile descendant. Of various hypotheses as to the nature of the postulated gene product, discussed by Lederberg (1956), the most plausible are: (i) that it is a flagellum, or a granule which synthesizes a flagellum; and (ii) that it is a cell wall carrying several flagella. In *Salmonella*, on division of a cell with several flagella, the parental flagella are shared about equally between the daughters (Quadling and Stocker, 1962); this and other evidence (Quadling and Stocker, 1957; Quadling, 1958) indicate that in *Salmonella* the motility-conferring particle is a

flagellum. It may be that the unilinear transmission of motility in *B. subtilis* reported above likewise results from unilinear transmission of single flagella, and that, for instance, the motile initial whose progeny are recorded in Fig. 1b had four flagella, whose distribution among its descendants resulted in only four of them being motile. But in the absence of evidence of how the parental flagella are distributed at cell division in *B. subtilis*, it is possible that a whole cell wall, probably bearing several flagella, is unilinearly transmitted, and that in the pedigree recorded the exogenous *fla*$^+$ gene (or its products) persisted for a time such that all the four second-generation descendants had flagella on their cell walls and such that they budded off daughter cells with new cell walls, and unable to make new flagella. No pedigrees of the kind ascribed in *S. typhimurium* to abortive transduction of motility, i.e., the unilinear transmission of a nonintegrated but functional *fla*$^+$ gene (Stocker, 1956*a*; Lederberg, 1956), were encountered in the transformation experiments, nor were the trails which result from this phenomenon seen on semisolid medium. This indicates that *fla*$^+$ genes taken up as exogenous DNA if not soon incorporated are soon destroyed, or at least cease to determine the *fla*$^+$ phenotype. The absence on selective defined media of the minute colonies ascribed to abortive transduction of nutritional characters (Ozeki, 1956) leads to the same inference in respect to transformation at *try* and other loci.

Only 13 clones derived from filaments, that is, from bacteria of *try*$^+$ (transformed) phenotype, and 10 from bacteria selected because they became motile in medium lacking tryptophan, i.e., for their *fla*$^+$ *try*$^+$ phenotype, were analyzed in respect to the try_2 and linked his_2 characters. In each set, the most common sort of clone was homogeneous, transformed to *try*$^+$ but still *his*$^+$; the next most common were homogeneous *try*$^+$ *his*$^-$ clones, i.e., transformed in respect to both the linked characters. The absence of a *try*$^-$ *his*$^+$, i.e., untransformed, component from these clones was surprising, since it would be expected from segregation of the untransformed nucleus of a binucleate rod whatever the mechanism of recombination in transformation. However, a sister cell untransformed in respect to *try* may have separated from a motile initial before it was caught, and a short, because nongrowing, *try*$^-$ element in a filament may sometimes have been lost when a filament broke up during isolation. Of the 18 apparently homogeneous clones, 4 yielded only *try*$^-$ colonies; thus, for a nutritional character as for *fla* some bacteria of transformant phenotype produce only untransformed progeny. The *try*$^-$ *his*$^-$ clone shows that the progeny of a phenotypically *try*$^+$ bacterium may be transformed at the linked *his* locus though not at the *try*; presumably, the *try*$^+$ gene accompanying the *his*$^-$ gene in a DNA molecule conferred the *try*$^+$ phenotype on the bacterium which adsorbed this molecule, but was lost (or was integrated in a sister cell) when the *his*$^-$ gene was integrated. The clone made up of about 70% of a double-transformant component, *try*$^+$ *his*$^-$, and 30% of a single transformant component, *try*$^+$ *his*$^+$, suggests that more than one recombinant type may result from uptake of a single DNA molecule, perhaps by delayed crossing-over in a persistent heterozygote. The significance of the three clones containing a majority of one genotype and a very small minority of another is not clear. One initial of *try*$^+$ *fla*$^+$ phenotype produced some hundreds of *try*$^+$ *his*$^-$, i.e., double transformant, progeny and one descendant which gave a colony containing some bacteria of this genotype and some which were *try*$^-$ *his*$^+$, i.e., untransformed. The minor component may have resulted from reincorporation by crossing-over of an endogenous chromosomal fragment which had been transmitted unilinearly for some eight generations after it had been displaced by the exogenous *try*$^+$ *his*$^-$ fragment, or perhaps from one untransformed nucleus of a competent multinucleate bacterium remaining "latent" for some eight generations longer than its transformed sister. In the other two instances, the major component was *try*$^+$ *his*$^-$, i.e., doubly transformed, and the minor component was *try*$^+$ *his*$^+$, i.e., apparently transformed only in respect to *try*. But the segregation of *try*$^+$ *his*$^-$, i.e., double transformant, progeny by some of the *try*$^+$ *his*$^+$ bacteria shows that some of them were still heterozygous for *his* about ten generations after uptake of DNA.

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