RECOMBINATION IN BACT.COLI K 12: UNI-DIRECTIONAL
TRANSFER OF GENETIC MATERIAL.

Five years ago Lederberg and Tatum[1] demonstrated that when
two mutant strains of Bact.coli K 12 having double and complementary
growth factor requirements were plated together in a basal synthetic
medium, colonies appeared in the ratio of one to every 10^6 or 10^7
cells seeded, although colonies did not arise by back mutation when
the mutants were plated separately.   These colonies were
prototrophic and similar to the wild type in their independence of
added growth factors.   Not only did they breed true, but it was
also shown that if each mutant was further denoted by complementary
differences in characters not selected by the basal medium (such as
fermentative capacity for various carbohydrates and sensitivity or
resistance to specific virus infection), the pattern of these
characters in prototroph cultures was usually different from that
in either mutant.   It was clear, therefore, that prototroph
colonies arose from the transfer of genetic material between the
mutants and not from complementary exchange of growth factors.
Unlike type transformation in Pneumococcus or the "directed mutation"
of similar mechanism in Bact.coli, growth of one K 12 mutant in
culture filtrates of the other failed to stimulate prototroph
formation.   This, in conjunction with recent evidence for the
occasional occurrence of diploid heterozygous prototrophs[2], strongly
support the view that genetic recombination is mediated by the

classical mechanism of sexual conjugation.   Attempts to
reproduce the phenomenon by similar techniques in other strains
of Bact. coli and in Salm. typhimurium have failed, though Cavalli
and Healot[3] succeeded in out-crossing K 12 mutants with a naturally
nutritionally exacting strain of Bact. acidi lactici.

In the following experiments, Bact. coli K 12 mutants 58-161,
requiring biotin and methionine, and W 677, requiring leucine,
threonine and aneurin, were employed.   The aneurin requirement
of W 677 was neutralised by the addition of this substance to the
basal medium.   An attempt was made to investigate the dynamics
of recombination by spreading a mixture of 58-161 and a streptomycin-
resistant mutant of W 677 (W 677/S^r) on a series of plates of
basal medium, and then adding streptomycin to each plate in turn
at different intervals after seeding.   It was anticipated that
the streptomycin would rapidly block the recombination mechanism
by inactivating 58-161, while allowing resistant prototroph cells
formed prior to its addition to develop into colonies.   It was
found in practice that the number of prototroph colonies did not
differ greatly whether streptomycin was incorporated in the basal
medium before plating, or was added up to four hours later.
Since similar results were obtained when the mutants were mixed
for the first time during plating, the occurrence of recombination
in mixtures before contact with streptomycin was excluded.   Either
prototrophs arose before the action of streptomycin on the

sensitive mutant became effective, or else those functions of the
cell affected by streptomycin were not involved in recombination.

Logarithmic phase broth cultures of 58-161 were treated with
either 1000 or 2000 µg./ml. streptomycin, under conditions
optimal for bactericidal effect, for from 4 to 18 hours. After
washing and concentration in saline, volumes of suspension
turbimetrically equivalent to from 1.5×10^8 to 4.0×10^8 cells
(i.e. double the inoculum used in recombination experiments)
frequently proved sterile and rarely yielded more than 100 colonies
on nutrient agar after 72 hours at 37°C. Nevertheless such
suspensions ($58-161/S^t$) invariably stimulated prototroph formation
when mixed with W $677/S^r$ on basal medium containing 200 µg./ml.
streptomycin. The recombination rate, however, was usually
appreciably less, and showed much more marked variation between
experiments than when viable, untreated 58-161 suspensions were
used. Whenever streptomycin treatment failed to produce sterility,
control reconstruction experiments showed that about 1000 times as
many untreated 58-161 cells as those which had survived treatment
failed to yield prototroph colonies when mixed with W $677/S^r$
suspension under similar conditions.

Mixtures of streptomycin-treated W 677 (W $677/S^t$) and $58-161/S^r$,
on the other hand, invariably failed to produce prototrophs although
comparable recombination rates were given by the mixtures
(W $677/S_s^r$ + 58-161) and ($58-161/S^t_s$ + W677). In these experiments

streptomycin was not incorporated in the basal medium since previous analysis of proven prototrophs had shown that about 95 per cent carried the S^r or S^S character of W 677. The clearcut distinction between 58-161/S^t and W 677/S^t in ability to participate in recombination was shown to be independent both of the presence of the S^r character in the complementary mutant and of the basal medium environment. Thus (58-161/S^t + W 677) produced prototroph colonies on every occasion whether cultured directly oh basal medium or initially on nutrient agar. The mixture (W 677/S^t + 58-161), however, failed to do so repeatedly on basal medium and, in a single experiment, when seeded on nutrient agar.

These findings require a revision of the current concept of recombination. It is unlikely that sensitive cells which have been acted upon for 18 hours by very high concentrations of streptomycin are still able to participate in conjugation in the continued presence of the drug. Moreover, if conjugation under these conditions was possible for 58-161/S^t it might also reasonably be assumed for W 677/S^t which is equally sensitive to streptomycin. Yet suspensions of the latter are inactive to recombination. It is simpler to suppose that recombination is mediated by genetic elements which are extruded by the viable cell but remain adherent to the cell wall and which, like viruses, are unaffected by streptomycin. Thus the cell could be regarded

merely as a passive carrier for its genetic elements after their expulsion, a function which could be performed equally well by dead or by living cells. The incompetence of $W\ 677/S^t$ becomes intelligible only if we suppose that the role of $W\ 677$ is the vital one of accepting genes and incorporating them into its genetic structure. It is possible that $W\ 677$ is also a gene donator but, if this is so, then 58-161 is inherently incapable of accepting them.

It has recently been shown that symbiotic bacterial viruses can transfer hereditary characters to heterologous cells which they infect[4]. It is also known that K 12 carries a symbiotic virus which is liberated from the cells in appreciable numbers only after UV irradiation. This virus can infect and lyse mature cultures of the K 12 mutant "S" although young cultures of "S" display the peculiar property of resisting lysis while still absorbing the virus[5]. The existence of a latent virus in 58-161 has been unmasked by X-radiation (personal observation). It seem possible that such a virus might be the agent of genetic transfer. The apparent restriction of the recombination phenomenon to one strain of Bact.coli, its relatively low rate and the inability of culture filtrates to induce it, the stimulation of recombination by doses of UV light too low to increase the mutation rate[6], the competence of streptomycin-"sterilised" cultures of 58-161 and the one-way transfer of the

genetic agent are all in conformity with such a concept. Work is now in progress to determine whether filtrates of 58-161, from which latent virus has been released by UV irradiation, can induce prototroph formation in W 677.

Department of Bacteriology,   W. Hayes.
Postgraduate Medical School of London.

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