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MICROBIAN GENETICS: REVERSIBLE INTEGRATION OF THE F' SEX EPISOME IN ESCHERICHIA COLI K 12

[Following is a translation of a report made by Francois Cuzin and Francois Jacob, of the Microbian Genetics Section of the Pasteur Institute, Paris, at the 1 July 1963 Meeting of the Academy of Sciences, Paris, transmitted by Jacques Trefouel and aided by a grant from the National Science Foundation of the United States of America and of the French Fund for Development of Scientific and Technical Research as well as by the Pasteur Institute, in the French-language periodical Comptes Rendus de l'Academie des Sciences (Reports of the Academy of Sciences), Vol CCLVII, No 3, 1963, pages 795-797.]

Some clones, Hfr, were selected, either at high temperature or at low temperature in the presence of acridine on the basis of mutated bacteria whose F' episome does not multiply at high temperature in the autonomous state. The integration mechanisms of the episome and of the chromosomal transfer by I strains may be deduced from the properties of these strains.

The sex episome of Escherichia coli K 12, in the autonomous state, confers on the bacterium that carries it (F^+) the capability of being paired with a bacterium F^- and of transferring episome F to it at high frequency. The integration of the episome and of the chromosome creates a new type of male bacterium (Hfr), which transfers an oriented group of chromosomal genes at high frequency, but does not transfer the autonomous episome F (E. L. Wollman and F. Jacob, La sexualite des bacteries [The Sexuality of Bacteria], Paris: Masson, 1959). A third sexual type, intermediary type (I), has been observed recently (F. Jacob and E. A. Adelberg, Comptes rendus [Reports of the Academy of Sciences], Vol CCXLIX, 1959, p 189; E. A. Adelberg and S. N. Burns, J. Bact., Vol LXXIX, 1960, p 321). The I bacteria transfer the episome by crossing the autonomous state with the same efficacy as the F^+ bacteria. Like the Hfr bacteria, on the other hand, but with a ten times lower frequency, they transfer a characteristic chromosomal sequence. All these characteristics are

acquired simultaneously by the bacteria that receive the episome of an I bacteria (modified episome F') by conjugation.

This modified episome carries a chromosomal segment (including, eventually, genetic markers, for example the Lac genes) which enables it to be integrated in a determined point of the chromosome (F. Jacob and E. A. Adelberg, loc. cit, and E. A. Adelberg and S. N. Burns, loc. cit.). This integration, probably reversible, may result in a transitory Hfr state in a fraction of the I bacteria. We were able to study directly the Hfr variants of I bacteria whose episome, as the result of a mutation, does not multiply at a high temperature (F. Cuzin and F. Jacob, Eleventh Int. Cong. of Genetics, The Hague, 1963, in press).

The mutant episome (F_t-Lac$^+$) is not reproduced at 42° C.; it is diluted during bacterial multiplication. It is regularly observed, after cultivating a Lac$^-$/F_t-Lac$^+$ clone at 42° C., that a fraction (5% to 15%) of the bacteria perpetuate the Lac$^+$ characteristic. One fraction of these thermostable Lac$^+$ variants are F$^-$ haploid recombinations; another fraction is composed of diploid bacteria for the Lac region, which retain the F factor. This last class offers special properties:

1. These bacteria do not transfer the autonomous episome to F$^-$ bacteria at high frequency.

2. They carry a duplication of the Lac chromosomal segment; they are obtained on the basis of a y^-z^+/y^{++} strain, and they segregate haploid clones y^-z^+ and y^+z^+ at a low rate (less than 1%). They synthesize twice the quantity of β-galactosidase produced by a haploid strain. At the time of the crossings they transfer the y^-z^+ and y^+z^+ segments independently.

3. These bacteria transfer, with an increased frequency (2 to 10 times with relation to the I strain), a chromosomal sequence that, according to the clones, is [See Note]:

(a) O Lac(y^+z^+) Pro T L ... Gal Ade Lac(y^-z^+) F

or

(b) O Lac(y^-z^+) Pro T L ... Gal Ade Lac(y^+z^+) F.

([Note:] Lactose (Lac) fermentation genes -- production of galactoside-permease (y), of β-galactosidase (z); fermentation of galactose (Gal), synthesis of proline (Pro), threonine (T), leucine (L), adenine (Ade).)

The y^+z^+ (or y^-z^+) characteristic is the first marker transferred. This is clearly shown by interruption of the crossing at various times

(by mechanical agitation). The Lac recombinations produced in this way are of F^- sex type. A high degree of connection is observed between Lac and Pro. Finally, a fraction of the recombinations selected for the subterminal marker Ade receives the y^-z^+ genes (in the case where the transferred sequence starts as y z). It is possible to ascertain the transfer of one or the other of sequences (a) and (b) by assuming that the integration of the episome takes place by recombination with the chromosome. According to whether the recombination takes place on one side or the other of the Lac marker, we will obtain one or the other of the two Hfr types.

4. The integration of the episome is reversible. A culture of an Hfr diploid at 42° C. is diluted and spread over plates that are then left at 30° C. After growth of the colonies, the plates are replicated in two specimens on an indicator medium (EMB lactose). The two replicas, left for twenty hours at 30° C. and 42 C., respectively, are compared: 0.1% to 0.5% of the colonies are Lac^- at 42° C., although they are Lac^+ at 30° C. These bacteria carry the episome in the autonomous state.

The integration of the F factor, therefore, enables its replication at high temperature under conditions in which it does not occur in the autonomous state. Likewise, we have been able to verify that the acridine compounds, that prevent the reproduction of the autonomous sex factor (V. Hirota, Proc. Nat. Acad. Sc., Vol XLVI, 1960, p 57), are without effect on the thermoresistant Hfr forms of the F_t mutants. Inversely, in the presence of acridine, it is possible to select the two thermoresistant types of Lac^+ bacteria (F^- haploid recombinations and Hfr diploids) from the F_t-Lac^+ cultivated at 30° C.

The episomic mutants with thermosensitive replication, therefore, enable us to select the transitory Hfr forms that appear in the I bacteria. In F-Lac^+ bacteria of the wild type, a similar selection is possible by means of the acridines. We were able to isolate, by using F-Lac^+ bacteria, clones that preserve the Lac^+ characteristic after growth in the presence of acridine. These bacteria display properties similar to those of thermoresistant Hfr variants, but their stability is less: a higher rate of reversion toward the autonomous form (sensitive to acridine) is observed.

Therefore, the mechanism that ensures the transfer of the chromosomal genes in the I strains is similar in form to the one that gives rise to the typical Hfr variants in the F^+ bacteria. It is distinguished from it, nevertheless, because the point of integration is fixed, the high frequency of integration, the frequent return to the autonomous state. This process of reversible integration at high frequency reminds us of the process observed by A. Richter by infecting a bacterium carrying a chromosome modified by an F episome of the wild type (A. Richter, Genet. Res. Camb., Vol II, 1961, p 333).

The characteristics of the Hfr clones studied suggest that the integration of the sex factor is the consequence of a genetic recombination between episome and chromosome. This recombination permits the replication of the episome at high temperatures or in the presence of acridine: the mechanism determined genetically by F which regulates its autonomous multiplication (F. Jacob, S. Brenner and F. Cuzin, Cold Spring Harb. Symp. Quant. Biol., Vol XXVIII, 1963, (in press) does not seem to come into play any longer. The episome and the chromosome, then, form a single replication unit (one single replica).

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