EXTRACHROSOMAL FACTOR DETERMINANTS

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In the early days of bacteriology even eminent bacteriologists like Koch, Cohn and Migula believed in the doctrine of monomorphism—the constancy of bacterial species—despite conflicting observations made by Pasteur on attenuated strains of Bacillus anthracis. Any variants that were discovered were dismissed as contaminants or as "Aberrant" or "Degenerate" or "Involutionary" forms. Nowadays they might have called them "L" forms. The concept of variation began to be taken more seriously after the discovery of lactose-fermenting strains of E. coli which appeared in the papilla on the surface of a non-lactose fermenting strain, because such variants could hardly be contaminants or aberrant. There is evidence about another type of variation brought about by small genetic carrying particles in the cytoplasm. This has been shown by their non-random appearance during the growth of culture. These genetic-carrying particles which lie extrachromosomally are known as plamids and episomes.

These are accessory structures and represent addition to the total genetic content of the cell. Despite their size, they play a great part in bringing about variation which is a great nuisance in the action of drugs. This new class of factor-determining bodies was discovered in the past decade so that our knowledge about their action is merely superficial. The word episome was introduced in 1958 by Jacob and Wollman and means "on the body". They can exist in two alternate but often mutual states:

i) the "integrated" state—the element exists on some point on the chromosome and multiplies synchronously with the chromosome;

ii) the "autonomous" state—the episome exists in the cytoplasm and multiplies independently from the chromosome and frequently faster.

GENERAL PROPERTIES OF EPISOMES AND PLASMIDS

i) Under normal conditions the properties they control are non essential.

ii) They can be present in either the integrated or autonomous state—generally the integrated state precedes the autonomous state.

iii) Plasmids exist autonomously but not in the integrated state.

iv) During conjugation, episomes can be transmitted independently of the bacterial chrosomes, either by mere transfer of those lying in the cytoplasm autonomously or elution of those already integrated.

v) Episomes can be eliminated spontaneously. They can also be eliminated by treatment with certain reagents like acridine dyes, divalent salts of Cobalt and Nickel, and certain oxidising agents, particularly periodates. The elimination occurs mainly only when they are autonomous and not when they are integrated.

There are four well established units considered as episomes and several which are still being contemplated.

The forms established are:

i) Genetic material of temperate bacteriophages.

ii) Sex or fertility factors.

iii) Colicinogenic factors.

iv) Factors responsible for the infectious heredity of multiple drug resistance.

Other factors thought to be brought about by episomes are:

i) Factors concerned with lactose fermentation and utilisation.

ii) Fimbriation or piliation factor.

iii) Ability to form spores in Bacillus and probably Clostridia-Sporogenic factors.

iv) Penicillin resistance in Staphylococcus and probably other genera.

A *Temperate Bacteriophage* is one which once in the bacterial cytoplasm, its (phage) nucleic acid provides the genetic information for the synthesis of a series of specific enzymes. which in conjunction with the pre-existing metabolic machinery of the infected cell, catalyze the formation of more phage nucleic acid and phage protein. Only in the late stages of the infectious process are these structural subunits assembled within the infected cell into new virions. The instruction by some phages destroy the nucleus whilst instruction from others do not destroy the nucleus, viz: dependent ones. Immature phage do not appear until there is a pool of precursor materials which are formed in a given order. Amongst the pool are enzymes and phage lysozyme. Although temperate bacteriophage may give the production of active phage and its release by lysis as in lytic cycle, generally it persists in an inactive form indefinitely and it becomes hereditary in the bacterial host cell. A strain of bacteria infected in such a way that active phage is not

produced but persists in an inactive form is said to be lysogenic and the phenomenon is known as lysogeny.

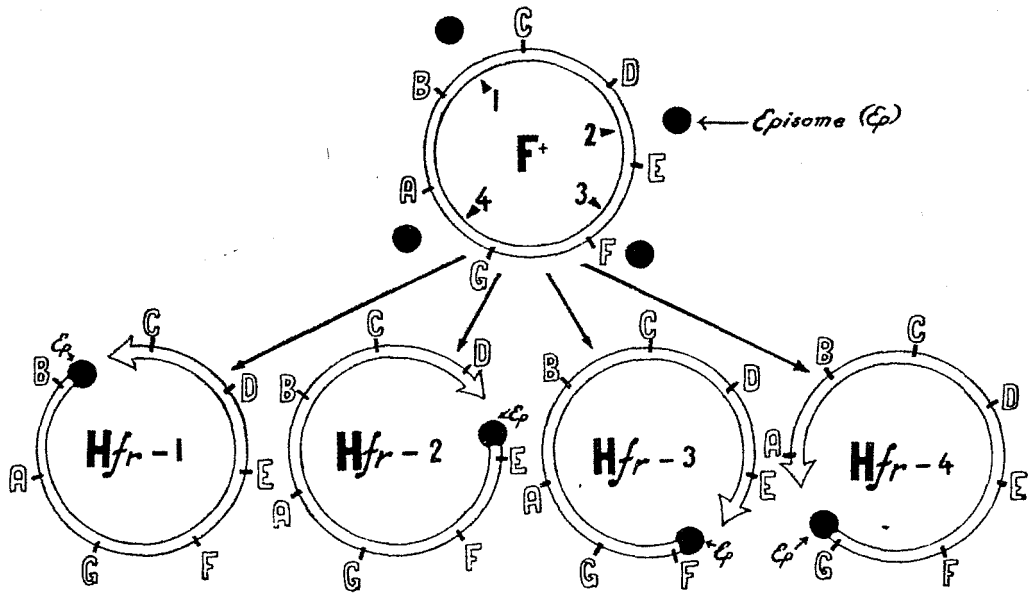
In the lysogenic state the virus is integrated and in the integrated state the genetic material of the phage becomes a constituent of the bacterial cell and behaves as such. Also in this state, the normal viral function is not expressed; the phage persists within the bacterial cells and is transmitted from mother to daughter cell during division and so is not affected by serial culture in antibody containing medium. In bacteria infected by temperate phage—lysogenic bacteria—the entire directive mechanism of the host cell is supplemented by that of the virus unlike the infection of bacteria with virulent phage where the entire directive mechanism appears to be replaced by the virus. In consequence, characteristics of the bacterium, ordinarily regarded as inherent, biologically fundamental properties of the cell, may be attributable to or modified by the presence of phage in the lysogenic state, e.g. the diphtheria bacillus (Corynebacterium diphtheria) is distinguished from its closely similar diphtheroid (Corynebacterium hofmanii) by its production of diphtheria toxin. Toxigenicity, however, has proved to be a characteristic only of lysogenic diphtheria bacilli and may be removed by the abolition of the lysogenic state or conferred on diphtheroid bacilli by making them lysogenic. It appears to be definitely established that all toxigenic diphtheria bacilli are lysogenic, though all lysogenic strains are not necessarily toxigenic and that the induction of toxicity is inseparable from phage. Furthermore, characteristics of a lysogenic bacterium, either seemingly pre-existing, such as biochemical characteristics or content of specific antigens, or acquired, such as drug resistance, may be transferred to another bacterium making it lysogenic with phage derived from the first bacterium.

Virulent phage may act as a transducing agent under appropriate conditions (transduction is the phenomenon by which genetic material is conveyed to the recipient by phage grown on the donor bacteria). The recipient cells (bacterial) acquired a new character and are the transductants. Transductants may be complete or abortive. Complete transductants are recipients in which the donated genetic component is integrated in the recipients genome, e.g. when phage grown on a prototrophic donor strain is allowed to infect a cysteine—requiring recipients—prototrophic transductants are obtained which remain stably prototrophic on subculture. In abortive transductants, on the other hand, the donated fragments are not integrated and when the recipient cell divides, the fragments pass to only one of the two daughter cells and continue to do so on further divisions of each cell containing the fragments—the process of "unlinear transmission". Virulent phage may act as a transducing agent under appropriate conditions, but most often the transduction is by temperate phage.

SEX OR FERTILITY FACTOR

It has been discovered that there are two mating types in E. coli and that during conjugation one partner acts only as a genetic donor or male and the other as the genetic recipient or female. A male retains its fertility even after dying of streptomycin whilst the fertility of the female is destroyed by lethal agents. Thus, the male need not remain viable since its only function is to transfer D.N.A. whilst the female contributes genetic material and a complete viable cell. Both parental strains were equally sensitive to the drug as judged by survival. From this it was deduced that there was a one way transfer of genetic material from a donor (male) to a recipient (female) strain; the donor could be dispensed with once its function had been fulfilled, but the survival of the recipient in which the whole process of recombination and segregation took place was essential. Moreover, the fact that the recombinants inherited most of their characters from the recipient parent further suggested that the genetic contribution of the donor bacteria to the zygotes is fractional. It was then discovered that the donor state is genetically determined not by a chromosomal gene or genes as one might expect but by an infectious agent called the sex factor or "F" (fertility) agent which exists separately in the cytoplasm. This factor promotes conjugation between donor bacteria that harbour it, (termed F+) and recipient bacteria (F−) that lack it, followed by its own efficient transfer to the recipients which are thus themselves converted into F+ donors. When male F+ and female F− conjugate, many of the females are infected and converted to male. "F" agent is transferred only by cell to cell contact, never kills its host, and is never released into the medium as a virulent virus. "F" agent is thus autonomous. In a cross of F+ × F− the sex factor is transferred with up to 100% efficiency. All the progeny are F+. It seems that sex is highly infectious. Actual transfer of the chromosomal marker by F+ is a very rare thing—of the order of 10^{-4} (i.e. $1/10^{4}$). Mutant male strains which transfer their chromosomal genes with very high efficiency are known as "Hfr"—high frequency recombination. They are genetic donors, over 10% transferring chromosomal materials. When recombinants are isolated from

an Hfr × F− cross they are usually found to be F−. (Compare with F+ × F− cross). In other words, Hfr do not transmit infectious F particles but chromosome transfer has increased at least 1000 times and the sex factor is no longer present as an infectious agent. Most of the genetic traits are those of the recipient because it is usually found that most of the genes have been derived from the recipient (F−) parent and only a few characters from the donor parent. This is because the donor cell transfers only a part of its genetic complement during conjugation. The F+ to Hfr mutations are accompanied by the breakage of the chromosome which is often circular; one end of the broken chromosome becoming the leading point in chromosome transfer. There are many types of Hfr and in each type, the break has occurred at a different point so that each transfers its genetic markers in a different order. The order in which the genes are transferred is precisely the order in which the genes are arranged along the chromosome. What matters is where the break occurs.



HYPOTHETICAL FACTORS

A Lactose fermentation
B Methionine +
C Galactose fermentation
D Serine +
E Histidine +
F Proline +
G Coliphage
1, 2, 3, 4, sites of chromosome. One break in each chromosome.

The sex factor is attached to the chromosome at the broken end opposite the end which first penetrates the female F− during the conjugation. Thus if recombinants are selected which have received the very last marker to be transferred, e.g. Methionine in Hfr−1; Histidine in Hfr−2; Proline in Hfr−3 and Coliphage in Hfr−4; then the recombinants also receive sex factor and behave as Hfr males with the same order of gene transfer as their parent. The sex factor is thus transferred only as a chromosome marker being the last to penetrate the female cell. The sex factor is then borne in an integrated state.

METHOD OF STUDY OF SEXUALITY — INTERRUPTED SEX EXPT.

The transfer of genetic material between cells takes place across a bridge and the complete transfer takes about two hours. If the conjugating cells are agitated, as by treatment in a blender, before transfer is complete, partial transfer is found in the progeny. This allows for the determination of a sequence of hereditary units that suplement that deduced from evidence of linkage.

COLICINOGENIC FACTORS

Colicines are proteins or peptides in nature.

They are produced by enterobacteriaceae and are active against other members of the same family. Ability to produce colicine is governed by colicinogenic factor. Colicinogenic factors can be transferred from Col+ to Col− strain.

Evidence of the integrated state is scanty as it is always eliminated by treatment with such reagents like acridine dyes and divalent salts of Cobalt and Nickel. It is thus better described as plasmid rather than episome.

METHOD OF STUDY OF COLICINOGENIC FACTORS

Grow on nutrient agar about fifty colonies and assume that some are Col+. After 24 hours put this over a dish containing chloroform which serves to kill off the bacteria but not the ability to produce colicine. Cover the surface with thin layer of more agar and flood with an indicator which is sensitive to Col. Reincubate and if there is no colicine present there will be confluent growth, but if the original strain is producing colicine, it will diffuse out and produce a number of bare patches hence indentifying the original colonies as being colicinogenic.

SEXUAL HEREDITY OF MULTIPLE DRUG RESISTANCE

Recombination can be selected by means of antibacterial drugs, e.g. streptomycin, with drug resistance as a marker. Ledeberg and Tatum 1946, using E. coli strain K12 isolated prototrophic recombinants(1) of genotype a+ b+ c+ d+ from a mixture of reciprocally marked auxotrophic parent strains(2) a+ b+ c− d− × a− b− c+ d+ by plating on a defined medium lacking the growth factors (a), (b), (c), and (d). The use of doubly auxotrophic parental strains made it almost certain that the prototrophic colonies arose by recombination and not by back mutation because the probability of a double mutation in the same cell is the product of their individual probabilities. When these are 10^{-11} and 10^{-9} respectively ,the probability of both occurring simultaneously is 10^{-20}. Mutation to drug resistance is a serious cause of failure of chemotherapy, when only one drug is used but the risk of drug resistant strains emerging is generally reduced if two drugs are given at the same time, since doubly resistant strains are extremely rare. Occasionally, prototrophic organisms undergo mutation to auxotraphic forms; these can grow on simple medium only if this is supplemented with a particular nutrient compound, e.g. a particular amino-acid or vitamin. The specific nutritional requirements show that the organism has not the power of forming either the enzyme immediately responsible for the synthesis of the required compound or else an enzyme responsible for synthesis of same. Studies in mutations of this kind by Beadle and Tatum have indicated that the formation of each specific enzyme is determined by the action of a different specific gene; this is the "one-enzyme one-gene" theory.

Moreover, since recombinants never arose unless intact bacteria of both parental types were present, it was correctly assumed that the genetic transfer is mediated by cell to cell contact, that is by conjugation.

Sexual heredity of multiple drug resistance is also applicable to the enterobacteriaceae, especially to the pathogenic organisms fed on antibiotics like tetracycline, chloramphenicol, etc. Each factor is a resistance (R) factor and the transfer is controlled by Resistance Transfer Factor (RTF). They exist autonomously and in the integrated state and can be got rid of either physically or by chemicals. It was first discovered in Japan in 1957 in Shigella, and by using volunteers it can be shown that multiple resistant organisms occur in the gut. Resistance Transfer Factors are by definition episomes and like the sex factors of E. coli, to which some seem to be related both genetically and functionally, they mediate conjugation and can spread epidemically through populations of intestinal bacteria which lack them. Some of these factors can also rarely initiate chromosome transfer, but the equivalent of Hfr bacteria have not yet been isolated from strains carrying these factors. Resistance transfer factors are of considerable importance in medicine because they have picked up and incorporated into a single transmissible structure the genetic determinants of bacterial resistance to a wide range of antibiotics in common clinical use such as Kanamycin, Ampicillin, Streptomycin. As many as seven such determinants have been reported to be carried by a single transfer factor. These factors can become extensively disseminated among the normal flora of the intestine in human and animal populations, and thence be transferred by conjugation to a wide range of dangerous intestinal pathogens to initiate epidemics which cannot be treated effectively.

a) Other factors which are very likely to be determined by plasmids and episomes and which are occupying the energies of bacteriologists, biochemists and cell cytologists are—Fimbriation or Piliation factors—these factors allow

(1) Prototrophs: A strain with the nutritional requirements of the wild type strain.

(2) Auxotrophs. A mutant organism unable to synthesize a certain growth factor which can be synthesized by the wild type strain from the more elementary precursor sustances.

certain enterobacteriaceae which initially had no agglutinating ability to develop the power.

b) Ability to form spores in Bacillus and probably clostridium-sporogenic factor. This is transferred either by transduction mediated by phages or by conjugation.

c) Penicillin resistance: More fashionable and better studied is the mechanism by which cells acquire penicillinase activity from scratch by acceptance of extra-chromosomal factors (episomes or plasimds) which can carry the B-lactamase gene complex in a state which is partially autonomous from the chromosome, but they may otherwise function in the chromosomal genes.

In coliforms the penicillinase genetic system may constitute part of an extrachromosomal "R" or resistance factor, which, by linkage with "T" or transfer factor may pass from one cell to another spontaneously (not necessarily within the same species) (Datta and Kontomichalou, 1965).

In Staph aureus, "penicillinase" plasmids can be transferred from one cell to another—not spontaneously, but by transduction through an infecting bacteriophage (Richmond, 1965a: Novick, 1967). It is possible, with Staphylococci, for the cell to possess more than one type of penicillinase plasmid at the same time. In this organism it seems likely that the same or analogous penicillinase genes can, in certain strains, exist fully integrated on the chromosome, though there is yet no clear evidence that they can pass reversibly from the extrachromosomal to the chromosomal state like episomes.

At the present time, our knowledge of these bodies is meagre but it appears that no matter how infinitesimal this may be, it still is invaluable as these small bodies besides being widespread may be implicated in some disease like cancer and might yet be our guiding beacon in striving for the elucidation of the causation of so many diseases which to this very day lie beyond the reaches of the scientist's groping mind.

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