On the association between DNA and membrane in bacteria

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[Plates 13 to 16]

Work in genetics, as in most other branches of biology, has so far been essentially analytical. A century of dissection of heredity has led to a reasonable picture of what the ‘factor’ disclosed by Mendel’s work is made of and how it carries specificity. The gap created between the factor and the character is progressively filled by well-defined molecules. It seems justified to hope that, in some not too distant future, the structure and properties of such molecules will be sufficiently understood.

The main programme for the Mendel bicentenary will probably consist essentially in determining how molecular structures recognize each other to form cellular superstructures and how they interact in vivo to coordinate the extraordinarily complex chemistry of the cell. In genetics, this problem is merely that of the chromosomal structure, of its regulation and its reproduction in strict coordination with cellular growth and division. It seems fair to say that, if some analytical information concerning the components of a chromosome is now available, little is known about the molecular arrangement of these components. As for the reproduction of chromosomes, this is obviously a very complicated process since it represents a crucial event in the chemistry of the cell and must therefore be closely connected with a great many other processes. The complexity of this problem is exemplified by the following argument: there exists good experimental evidence indicating that DNA does replicate according to the mechanism that Watson & Crick (1953) predicted from their model; furthermore, from the work of Kornberg (1961) and his colleagues, an enzyme is known which, in vitro, is able to order and polymerize deoxynucleotides according to the sequence imposed by a DNA template; yet if a fragment of bacterial DNA is transferred to a recipient bacterium, by transformation or incomplete conjugation, it appears to be unable to be replicated as such. It is replicated only when it has been integrated, as a result of genetic recombination with one of the DNA structures present in the bacterial cells.

Bacterial replicons

In bacteria, DNA is arranged in structures which are much simpler than those found in cells of higher forms. The basic information necessary to the growth and division of a bacterium is carried by one of these structures, which is known as the ‘bacterial chromosome’. Other, dispensable structures, such as the so-called episomes, can be added to the bacterial cell. They control non-vital functions which
can be superimposed on to the bacterial economy. From what is known, each of
these structures behaves as an integral unit. The most studied among these units
is the bacterial chromosome (see contributions to this symposium by Hayes and
by Pritchard): genetically it constitutes a single linkage group which appears as
a closed (circular) unit; structurally, it seems to contain a single DNA duplex,
about 1.2 to 1.4 mm long, which is actually closed; biochemically it forms also a
single unit, since replication appears to start at one fixed point and to proceed
along the structure until it is completed; finally, the regulation of this unit appears
also to be an integral process since, under normal conditions of growth, a new cycle
of replication cannot be initiated until the previous one is completed. Although
much less is known of other bacterial units capable of autonomous reproduction,
such as the sex factor, yet the available evidence points to a similar behaviour.

The genetic equipment of a bacterium may therefore be visualized as made up
of independent structures, each of which contains a single DNA molecule of
different length and replicates in strict coordination with growth. As already
stated, the capacity for autonomous replication does not belong to any fragment
of bacterial DNA introduced into the bacterial cell. It is the property of the
integral units, which have been called replicons in a model we proposed with
Dr S. Brenner (Jacob & Brenner 1963; Jacob, Brenner & Cuzin 1963). The replicon
model has two main features.

(1) Since every replicon is a structure able to replicate itself independently of
the others, it should control genetically its own replication. In other words, every
replicon must carry genetic determinants which control specifically the regulation
of its own replication. This predicts that mutants of every replicon should be
obtained in which the replication of this very replicon, but not of others, is
altered.

This has proved to be the case in the three units studied so far. By use of
thermosensitive, conditional mutants, it has been possible—in the temperate
bacteriophage (Jacob, Fuerst & Wollman 1957), in the sex factor F of E. coli
(Jacob et al. 1963), or in the chromosome of E. coli (Kohiyama et al. 1963)—to
obtain mutations which prevent specifically the replication of the mutated unit,
but not of others. It is not possible to describe in detail here the properties of these
mutations. Suffice it to say that in contrast to regulation of gene expression
which is negative—i.e. repressors inhibit messenger production—the properties
of these mutations indicate that the regulation of DNA replication must in some
way be positive—i.e. every replicon must produce some compound which plays
an active role in initiating its own replication. The most economical hypothesis is
to assume that a structural gene of the replicon controls the synthesis of a cyto-
plasmic protein, or initiator, which is susceptible to cytoplasmic signals and
recognizes a particular segment of the replicon DNA, or replicator, where it allows
replication to begin. More information concerning the chemistry of replication
should be obtained before biochemical analysis of such a system becomes possible.

(2) The second point of the replicon model deals with a possible association of
cellular genetic units, such as the chromosome or the sex factor, with the bacterial
membrane. This hypothesis comes from a study of bacterial conjugation. On the
Association between DNA and membrane in bacteria

The efficiency of genetic transfer (close to 100% of the formed pairs) compared with the small number of sex factors (one per chromosome in all male types) suggests that the sexual episome is attached to the cell membrane in the immediate vicinity of the very region where conjugation with F⁻ females, and transfer of genetic material from male to female, occur. On the other hand, all the features of bacterial conjugation can best be interpreted by assuming that the pairing with a female triggers in the male a replication cycle which begins at a precise point of the sex factor and from there proceeds along the DNA attached to it. Of the two newly formed DNA replicons, one would remain in the male while the other would be driven into the female by the very forces which allow replication to proceed (see figure 41).

Figure 41. Schematic representation of genetic transfer by replication during mating. In the donor (Hfr or F⁺) the F complex of replication (F rep) is assumed to be a part of the bacterial membrane, at the vicinity of the point of contact with an F⁻ female. The DNA is assumed to revolve for replication through the replication complex, one of the daughter replicons remaining in the male while the other is driven through the bridge into the female. (From Jacob et al. 1963.)

This model of genetic transfer through replication during conjugation has recently been supported by several lines of evidence. First, male bacteria were used which carry such a conditional mutation that bacteria grow at 30 °C; but, after transfer of the culture to 40 °C, DNA synthesis is inhibited (99% within 5 min). When mated with F⁻ females, these male bacteria transfer their genetic material at 30 °C. When the mating mixture, first incubated at 30 °C, is shifted to 40 °C, genetic transfer is inhibited (99%) within 5 min (Kohiyama & Jacob, unpublished). The second piece of evidence comes from the work of Caro & Gross (1965). Using radioautographs, they were able to show that DNA transferred by conjugation is composed half of old material formed before conjugation, and half of new material synthesized in the male during conjugation. The third piece of evidence comes from a physiochemical study, by Ptashne (1965), of the DNA transferred by conjugation. All the material transferred to the female appears to have been replicated in the male before transfer. These various results indicate that genetic transfer during conjugation is a particular effect of a replication which
occurs in the male, probably close to the region of contact with the female and triggered by a surface reaction.

If extended to other cellular replicons, such as the bacterial chromosome, this idea of an association with the membrane can account for some properties of DNA replication (figure 42).

(a) If cellular replicons are attached to the membrane where their replication occurs and is regulated, one may conceive how reproduction of DNA structures can be correlated with cellular growth through signals transmitted by the membrane to the enzyme system of replication.

(b) Since bacteria are deprived of a complex mitotic apparatus, the separation of the newly formed chromosomes and their distribution to the two daughter cells at every division might result from the growth of the membrane in the area located between the points of attachment of the chromosomes to the membrane.

![Figure 42. Model for the regulation of DNA synthesis and an equal distribution of DNA units among daughter bacteria. The bacterium (F+) is assumed to carry two replicons, the chromosome and an independent sex factor, each of which are attached to the bacterial membrane. At a certain stage of the bacterial cycle, the membrane transmits to each replicon a signal initiating their replication which proceeds linearly, each of the DNA structures revolving slowly through the membrane where the replication systems are located. For each region, two daughter structures are thus formed and are attached side by side to the membrane. The membrane is then assumed to be synthesized between the points of attachment of the daughter replicons, which are thus progressively separated, and the septum is formed in the middle part. No new replication is permitted until the membrane, having reached the original state as a result of division and separation of the daughter bacteria, transmits a new signal. The process is oversimplified in the sense that (1) bacteria have generally 2 to 4 (and not 1 to 2) DNA complements per cell, DNA replication being one cycle ahead of cellular division, and (2) every step is assumed to be completed before the following one is initiated. (From Jacob et al. 1963.]

This model leads to some very precise experimental predictions:

(i) The bacterial chromosome should be found connected in some way with the membrane.

(ii) The synthesis of the membrane during the division cycle should not occur randomly but at well-defined regions.

(iii) Old fragments of the membrane should segregate with old chains of DNA.

(iv) Under all conditions the replicating fork of DNA should be found associated with the membrane since, because of the conjugational replication, the model implies that it is not the replication enzyme which progresses along the DNA chain, but the whole DNA structure which revolves slowly, during replication, through a complex enzyme system incorporated into the membrane.
Figure 43. Sections of growing *B. subtilis*, showing mesosomal structures (M) and nuclear bodies (N). The nuclear bodies are connected to the membrane by the intermediacy of mesosomes. (From Ryter & Jacob 1964.)

(Facing p. 270)
Figure 44. Sections of growing *B. subtilis* after 30 min in 0.5 M saccharose. Mesosomes have been expelled from cytoplasm (one (M) can be seen as a vesicle at the left pole of the top picture). Nuclear bodies then appear directly connected with the membrane at region C. (From Ryter & Jacob 1964.)
Consequently the enzyme system responsible for replication should be found in the membrane.

**Replicons and bacterial membrane**

Some of these predictions have already received direct experimental support. The first demonstration of a direct association between the chromosome and the membrane was obtained by direct observation of *Bacillus subtilis* with electron microscopy (Ryter & Jacob 1963, 1964). *B. subtilis* contains a small number of structures called *mesosomes*, which represent invaginated segments of the membrane. When serial sections of *B. subtilis* are examined, every nucleus is found in close contact with one, or sometimes two, mesosomes (see figure 43, plate 13). This contact persists during the whole cycle of *B. subtilis*, except in dormant spores where the mesosomes disappear and the nucleus then is seen in direct contact with the membrane of the spore. When the spore is placed under conditions of germination, the first event to be seen is the reappearance of a big mesosome which ties the nucleus to the membrane. Actually, the attachment of the nucleus to the mesosome is so strong that, if bacteria are placed in 0-5 m saccharose, the mesosome is progressively expelled by osmotic forces but during this process it pulls with it the nucleus, which then appears directly attached to the membrane (figure 44, plate 14).

If bacteria observed at random during the division cycle are ordered with respect to nuclear size (see figures 45 and 46), it is possible to obtain a representation of the division cycle. First the nucleus appears as a small structure attached to a single mesosome. Then the nucleus increases in size. At a certain stage, it appears attached to two mesosomes, close to one another, which seem to result from the doubling of the initial mesosome. Then the two mesosomes appear farther and farther apart from each other and the pictures indicate a separation of the nucleus into two nuclei, each of which is associated with one of the mesosomes. This strongly suggests that the two mesosomes become progressively separated, each one pulling apart its attached chromosome. Obviously one cannot tell from pictures whether the separation of mesosomes, and therefore of nuclei, results from membrane synthesis occurring between the mesosomes. There is, however, some indication that this might be the case: the only place in the cell where one can be sure, from electronmicrographs, that membrane synthesis occurs is the region of septum formation; indeed this region, whether during the vegetative phase or during sporulation, is always found to contain one and sometimes two mesosomes, a result which suggests that these structures are in fact directly involved in membrane synthesis.

It can be shown more directly in *B. subtilis* that membrane synthesis does not occur uniformly throughout the whole membrane but at some, well-defined points (Ryter & Jacob, unpublished). When growing cultures are exposed to potassium tellurite, tellurium needles precipitate all along the membrane and are easy to see in the electronmicroscope. If the exposure is short enough, bacterial growth resumes upon removal of tellurite. If bacterial samples taken at various times of growth are observed in the microscope, it is found that the needles are not randomly diluted but remain at well-defined regions, namely at the two poles of the cell and
often as a strip in the middle region of the cell, these areas being progressively separated by growth (see figures 47, 48, plates 15 and 16; figure 49). When bacteria grow in chains, after several generations, only the two extreme poles are labelled by tellurium plus a narrow strip in the central region of the chain. This result obtained with the membrane is very similar to those found by Cole & Hahn (1962) by labelling the cell wall of streptococci with fluorescent antibodies.

It should become possible therefore to label both the membrane with tellurium and the DNA strands with tritium and to determine whether or not the two structures segregate together during growth.

**Figure 45.** Two-dimensional representation of growing *B. subtilis* reconstituted from serial sections. Growing bacteria were fixed, included in Vestopal and serially cut so that about seven to eight sections were obtained per bacterium. Every section was examined in the electron microscope. For each of them, the position of mesosomes and nuclear bodies was drawn on a transparent film. For every bacterium, the superposition of the films allows a three-dimensional reconstitution of the cell from which the relation between nuclear bodies (hatched regions), the mesosomes (regions with small circles) and the membrane can be inferred. (From Ryter & Jacob 1963.)

**Description of plates 15 and 16**

**Figures 47 and 48.** Labelling of membrane with tellurite in *B. subtilis*. *B. subtilis* growing exponentially in broth were exposed to 0.05% of potassium tellurite without aeration. After 20 min, the bacteria were washed from tellurite, resuspended in broth and aerated so that growth resumed. At various times, samples were removed and examined in the electron microscope.

**Figure 47A.** Bacteria at time 0 after treatment. Tellurium needles are homogeneously distributed all along the membrane.

**Figure 47B.** Bacteria after one generation. Most bacteria still carry tellurium needles all over the membrane except in the central region of the cell.
**Figure 48C.** Bacteria after two generations. Most bacteria carry tellurium needles only at one pole.

**Figure 48D and E.** Bacteria after three generations. Only the extreme cells carry tellurium needles at their exterior pole. The cells at the centre do not carry needles.
The second experimental approach to the problem of the membrane-DNA association is a biochemical one. The most direct evidence would obviously consist in demonstrating a chemical linkage, which is hardly possible in the state of our knowledge concerning the chemistry of the membrane. Several authors have reported that, upon careful lysis of protoplasts, a DNA fraction sediments with membrane pieces. The value of these observations, however, can hardly be estimated without the demonstration, for instance by means of genetic markers, that this is the result of a specific association between DNA and membrane.

**Figure 46.** Diagrammatic representation of the division cycle in *B. subtilis*. This diagram is obtained by ordering the representations of bacteria, as obtained in figure 45, according to the size of the nucleus. (From Ryter & Jacob 1964.)

**Figure 49.** Diagrammatic representation of the most frequent distribution of tellurium needles during growth of *B. subtilis* treated for 20 min with potassium tellurite.

More easily obtainable appears the information concerning the site of DNA replication, which, in the replicon model, should always be associated with the membrane, since the DNA is assumed to revolve through the membrane structure, ensuring replication. Several experiments have been reported which suggest that both in *B. subtilis* and in *E. coli* the segment of DNA which is engaged in replication sediments in a heavy particulate fraction (Goldstein & Brown 1961). In studying the ‘replicating fork’ of the *E. coli* chromosome, Hanawalt & Ray (1964) found that it was difficult to extract unless treated with proteolytic and lipolytic enzymes. Recently it was observed by Ganesan & Lederberg (1965) that replicating DNA, labelled by very short pulses of isotopes, sediments in the heavy
fraction where membrane pieces are found. Chase experiments rapidly displace the label from the heavy fraction. This result obtained with non-synchronized bacteria supports the prediction drawn from the replicon model that the site of DNA synthesis must lie in the membrane. The results of biochemical experiments can be understood only if DNA revolves through an enzyme complex which is part of the membrane, but not if the enzyme moves along the DNA chain. The exact role of the enzyme which in vitro catalyses DNA synthesis has already been questioned, because of the peculiar properties of the product and of the difficulty in obtaining net synthesis endowed with transforming activity (Schildkraut, Richardson & Kornberg 1964). In the view that DNA replication occurs in the membrane, one has to assume that this enzyme must represent a part of a complex structure organized in the membrane or that it is not involved in the in vivo process of replication.

**The unit of segregation in bacteria**

The present experimental evidence supports the picture of the genetic material in bacteria as composed of integral units or replicons, each of which is associated with the membrane where replication occurs and is controlled. If the regular segregation and distribution of replicons among daughter cells result from localized synthesis of the membrane, the different replicons of the cell might be attached to a same membrane element. This can be experimentally investigated by determining whether two replicons, such as the chromosome and the sex factor, segregate together or randomly in the course of bacterial divisions. Two types of experiments have been performed to investigate this point (Cuzin & Jacob, unpublished).

In the first type of experiment, the killing effect of $^{32}$P incorporated in the chromosome was used to label DNA. The bacteria carry a mutant sex factor $F_{r}$-Lac which replicates autonomously at 30 °C, but not at 40 °C. These bacteria were first grown at 30 °C in a medium containing radiophosphorus of high specific activity. After several generations, the isotope was removed and the bacteria grown in $^{31}$P at 42 °C to dilute the $F_{r}$-Lac. After 0, 1 and 4 generations, samples were removed, thawed and the killing effect of $^{32}$P decay was measured, on both the whole population and that fraction of the population which still harbours the sexual episome. As can be seen in figure 50, at 0 generation both populations decay at the same rate. After 0.8 generation, they still decay at a similar rate which is a little more than half the rate observed at 0 generation. After 3.6 generations, however, while the whole population decays at a very low rate as expected, the population harbouring a sex factor, although diluted more than 8 times, still decays at the same rate as after one generation. It is known that, because of size difference, the decay of the sex factor is negligible as compared with that of the chromosome. The results of this experiment indicate that the cells still containing the sex factor, which did not multiply at high temperature, also contain about half of the radioactivity, i.e. in all likelihood one of the original DNA chains of the chromosome which did multiply.

The other type of experiment aims at answering the same question using a slightly different method. Bacteria carrying a mutation in the gene for galactose
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Primerase lyse when fed with galactose. Bacteria which can make β-galactosidase and are fed with a β-galactoside also lyse because the enzyme releases galactose. If bacteria have a deletion of the lactose region on the chromosome and carry a thermosensitive F$_r$-Lac, the only gene for galactosidase is in the sex factor. When grown at 40 °C the F$_r$-Lac episome is diluted out. As shown by reconstruction experiments, one can at any time lyse those cells which still carry the sex factor by feeding a β-galactoside to the cultures. If the DNA has been labelled with a radioactive compound before growth at 40 °C, one can thus determine at various times the fraction of the previously labelled DNA which remains in those cells still carrying a sex factor. The results of such an experiment give a similar answer as the previous one. After several generations at high temperature, when the sex factor has been diluted by a factor of 10 or more, the radioactivity of DNA

**Figure 50.** $^{32}$P decay in bacteria carrying an F$_r$-Lac. Bacteria E. coli K12 200 PS/F$_r$-Lac carrying a y$^{-}$ mutation on the chromosome and a thermosensitive F$_r$-Lac (which multiplies autonomously at 30 °C but not at 40 °C) were grown at 25 °C for about seven generations in a radioactive medium containing 100 mc/mg phosphorus. Bacteria were then washed, resuspended in a medium containing $10^{-1}$m $^{32}$P and aerated at 42 °C (time 0). After 0, 0.8 and 3.6 generations, samples were removed and frozen in liquid nitrogen. After 3.6 generations, the F$_r$-Lac factor was diluted by a factor of more than 8. From day to day, samples were thawed and assayed for viability of the total population (○) and of bacteria still carrying an autonomous F$_r$-Lac factor (●). Abscissa: time, or per cent of disintegrated $^{32}$P atoms. Ordinate: survival of the two populations on a logarithmic scale.
released by the cells still harbouring the sex factor is only diluted by a factor of about 2. In all likelihood one of the DNA strands of the chromosome has remained associated with the sex factor.

One has therefore to conclude that bacterial replicons, such as the chromosome or the sexual episome, do not segregate randomly, but together, in the course of bacterial multiplication. From the previously described evidence, the most likely explanation is that they are attached to a common cellular component, probably an element of the membrane, which remains intact during bacterial growth and division.

Concluding remarks

The picture resulting from this analysis is as follows: the genetic equipment of bacteria is made of independent self-replicating units, or replicons, which are associated with a common segment of membrane controlling their replication in coordination with cellular division. In bacteria, it is the membrane, or fragments of the membrane, which represents the unit of segregation and, in this respect, is the most akin to chromosomes of higher forms. The basic genetic information is contained in the largest replicon, the so-called bacterial chromosome, but more information can be added to the bacterial cell by attachment of other replicons to the membrane.

It is indeed remarkable that this picture of replicons attached to a backbone, the membrane, found in bacteria has much in common with that reached in the study of chromosomes of higher forms (see in this Symposium, the contributions by Ris and by Pelling). The labelling of mammalian chromosomes during replication by Taylor (1964), for instance, shows that DNA synthesis is initiated at several, well-defined points along the chromosome. These points differ from one type of chromosome to another, but appear to be always the same for a particular type. The conclusion drawn by Taylor is that a chromosome is made of several, probably a large number, units of replications which, in all likelihood, are attached to a non-DNA backbone. A similar conclusion can be drawn from the study of giant chromosomes in Diptera, by Pavan (1963), who finds that, under certain circumstances, the puffing of chromosomes involves DNA synthesis at certain regions of the chromosomes, but not at others.

Although there is of course a wide difference between the organization of genetic material in bacteria and in cells of higher forms, the remarkable similarity between the pictures obtained in both cases—i.e. DNA units attached to a backbone—is probably not fortuitous. It should be pointed out that in bacteria the membrane is also known to contain the energy-generating enzymes system. The bacterial membrane plays the role of at least one class of organites described in higher cells, the mitochondria (see Stanier 1964). The analysis reported in this paper suggests that the bacterial membrane also plays the role of a chromosome and it would not be surprising if other functions differentiated in some cellular organites were found in the bacterial membrane. It seems that an important step of the evolution from prokaryotic to eukaryotic cellular organization involves an invagination of the membrane and a structural differentiation of specialized organites, whose functions originally belonged to the membrane of bacteria.
In fact, between the very simple bacterial system and the highly sophisticated mitotic apparatus as observed in cells of higher organisms, a great many intermediate types can be found, especially among Protozoa. For instance, in some of the 'endonuclear mitoses' observed in certain Protozoa where the nuclear membrane never disappears, the chromosomes are attached to the nuclear membrane by their centromeres. At division, the two twin centromeres, while still attached to the nuclear membrane, become progressively separated from each other, each centromere pulling one of the two newly formed chromosomes, until a constriction of the nuclear membrane in the central region results in the division of the whole structure into two equal nuclei. Such a picture is obviously similar to what is found with the bacterial membrane. It is not known, however, whether or not chromosomal separation in such Protozoa results from the growth of the nuclear membrane between the two newly formed centromeres. In other Protozoa, it has long been known (see Chatton 1931) that the kinetosome, or basal body, while closely connected with the cellular membrane where it controls cilia formation, attracts the nucleus at mitosis and plays the role of a centrosome. Many of such particular types of mitoses suggest the existence of some relation between organelles in the membrane and the reproduction of DNA followed by the distribution of chromosomes at cellular division.

The continuity reported in cells of higher organisms between such structures as the cellular membrane, the endoplasmic reticulum and the nuclear membrane might well ensure some relation between cell surface and genetic material. This may have an important value in view of the point which emerges from the study of bacteria, namely that regulation of DNA replication in coordination with cellular growth and division is likely to occur in the membrane of bacteria. This is best exemplified by conjugation where a very surface reaction due to the pairing of the male with a female appears to trigger a DNA cycle of replication in the male at the level of the sex factor. It is clear that in higher forms the cell surface must play an important role in cellular division, signals from the surface being in some way transmitted to the nucleus as exemplified by morphogenetic processes or in cell contact phenomena, where transmission and reception of signals have recently been individualized (Stocker 1964). The situation in such cells is obviously much more complicated than in bacteria. However the relation observed between the membrane constituents via the endoplasmic reticulum, while reflecting in some way an evolutionary differentiation of internal organelles, might keep a functional value for a necessary transmission of signals from cell surface to DNA.

The work done in the authors' laboratory has been supported by grants from the National Science Foundation, the Délégation Générale à la Recherche Scientifique et Technique and the Commissariat à l'Energie Atomique.
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