DNA UPTAKE BY BACTERIA AND EUCARYOTIC CELLS

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INTRODUCTION

Although recombination is clearly not essential for the survival of bacteria, yet microbes have evolved at least three different mechanisms for the exchange of genetic material. Several experiments suggest that these processes may actually also occur outside the laboratory, in bacterial populations living in their natural habitats. *Conjugational transfer* of episomal resistance factors has been implied as a major contributor to the quick emergence of antibiotic resistant populations in bacteria (WATANABE, 1966). A sophisticated genetic marking of pneumococcal mutants has allowed detection of spontaneously occurring genetic exchanges (via *genetic transformation*) between pneumococcal mutants living inside an infected mouse (OTTOLENGHI and MACLEOD 1963). The presence of *transducing viruses* in a wide variety of biological specimen suggests that spontaneous transduction-like processes also occur among bacteria in natural habitats.

The unique feature of genetic transformation is, of course, the fact that recipient bacteria can absorb bare DNA molecules. The relative simplicity of the system, the easy access of genetic molecules to experimental manipulation and the availability of a quick test for evaluation of the biological consequences of such manipulations, all would make transformation a method of choice for studies on cellular genetics. Indeed, numerous attempts have been made by a number of investigators to extend the applicability of this system to other microorganisms and--more recently--also to cells of eucaryotes. While some of these experiments did succeed in putting several new species on the list of transformable organisms, most attempts failed because of the difficulties encountered in finding physiological conditions in which the cells were competent to absorb DNA molecules. Even in species, like pneumococci, in which the competent condition appears spontaneously during growth, serious difficulties were encountered with the variability and reproducibility of the competent state. Furthermore, it was recognized that transformation was far from being a "simple" system for studying genetic recombination. While it is true that it is usually a simple matter to prepare pure DNA and to expose it to various physio-chemical

modifications, nevertheless these donor DNA molecules slip out of the control of the experimenter as soon as they have encountered the competent recipient cell with its poorly understood diffusion barrier at the cell surface, studded with adsorption sites, surface-located nucleases, a highly dynamic growth zone and an even more mysterious macromolecular transport mechanism. It seems therefore that for a better understanding of the eventual recombinational process and even for the interpretation of experiments with "modified" DNA molecules a better understanding of the competent state is essential. First, I shall give a brief description of the present status of our knowledge in one of the better developed bacterial transformation systems, that of pneumococci. In the second part of the discussion I shall comment on attempts to introduce nucleic acids into eucaryotic cells. In dealing with both of these topics I shall emphasize the physiology of the recipient cell.

TRANSFORMATION IN BIOLOGICAL CONTEXT

The description of any physiologically relevant recombinational mechanism should include identification of the source of genetic material, the mechanism of exit of donor material from the donor cells, the vectors that participate in the cell to cell transfer and the mechanism of entry into the recipient cell. It may be interesting to briefly recapitulate the important features of conjugation, transduction and transformation, side by side and in the above context, in order to underline analogies and contrasting features.

In sexual conjugation of enteric bacteria the participating cells have a genetically determined polarity: cells carrying the F genetic element are invariably the donors of genetic material. The exit of the genetic material and its transfer to the recipient as well as its entry into the latter: all these stages occur in a concerted fashion during direct physical contact of the conjugating partner. And the whole process is initiated by an interaction between the male-specific surface appendages (F pili) and some sites on the surface of the F^- cells (CURTISS 1969).

In viral transduction, donor and recipient cells are both virus sensitive bacteria; the donor genetic elements originate from a cell that has been host to the transducing virus during a lytic infection. The exit from donor cells as well as the intercellular transfer and entry all occur in a concerted fashion through viral mechanisms. In analogy with conjugation, the entry of donor molecules is preceded by a complex interaction between viral proteins and recipient surface sites. Presumably the interaction is between the phage tail-tips and specific receptors, which are located on the surface of the recipient cell, in close apposition to the anatomical junctions between cell wall and plasma membrane (BAYER 1968).

GENETIC TRANSFORMATION: SOURCE OF DONOR DNA

The recipient condition (called "competent state") in which pneumococci are capable of absorbing DNA molecules from their environment has been known to exist ever since the discovery of genetic transformation. On the other hand a natural, endogenous source of biologically active DNA, which would be available to competent cells without the intervention of the experimenter, has only been identified relatively recently. It was found that sterile filtrates of logarithmically growing pneumococci contained biologically active DNA. Furthermore, by culturing pairs of genetically marked mutants together in a test tube (and actually in living mice!) it was possible to detect a substantial and bilateral exchange of genetic material between the component strains. The emergence of recombinant cells occurred spontaneously (i.e., without the experimenter's intervention) and it could be prevented by adding DNase to the growth medium of the mixed culture (OTTOLENGHI and HOTCHKISS 1962). The most likely interpretation of these findings is that growing pneumococci release DNA molecules into the medium (by some unknown mechanism); these molecules are then absorbed by some cells that happen to be in the competent state resulting in genetic recombination. These experiments clearly indicate that live pneumococci *can* undergo spontaneous genetic recombination through genetic transformation. In this recombination system the competent cells are analogous to the F^- cells of sexual conjugation or to the recipient cells infected with a transducing virus. Unlike in conjugation or transduction, however, the donor materials seem to be bare DNA molecules. This conclusion is based on essentially two observations: (1) that sterile culture filtrates contain transforming DNA, and (2) that DNase can inhibit endogenous transformation in mixed cultures. It should be noted, however, that these findings would also be compatible with the existence in pneumococcal cultures of loose, "conjugal clumps" which might include both donor and recipient cells and in which an imperfect (and thus DNase sensitive) cell contact could be established for the actual transfer of DNA. This specific possibility is mentioned since it was noted that competent cultures have a tendency to clump JAVOR and TOMASZ 1968).

Since the mechanism of exit of donor molecules into the medium is not understood at present, it is not clear whether or not identical cells may some time act as competent recipient and at other times as DNA donors. Although DNA appears in the filtrates of growing pneumococcal cultures in the absence of observable lysis it is still possible that this DNA originates from a small fraction of bacteria that have disintegrated due to some cellular accident (e.g., abnormal triggering of cellular autolysing). Alternatively, living bacteria may "secrete" DNA into the medium (perhaps by a mechanism similar to the excretion of exoenzymes).

The picture of transformation that emerges from its comparison with the other recombinational mechanisms is that of a

less sophisticated mechanism, in which donor and recipient cells exist separated, both in time and in space, and in which the genetic material that escaped from donor cells can be isolated in an extracellular form, unbound to protective vectors and the entry of such molecules into cells would depend on random collisions between DNA and the competent bacteria. It is interesting to note in this connection, that the amount of biologically active DNA in pneumococcal culture filtrates reaches a maximum at about the same time of culture growth when the frequency of competent cells is at a maximum (OTTOLENGHI and HOTCHKISS 1962). Since the probability of transformation is a function of both cell concentration and DNA concentration, the coincidence of these maxima seems to optimize chances for genetic transformation. One might--with some stress of imagination--recognize in this phenomenon an evolutionary "attempt" to better coordinate the donor and recipient states of this primitive recombinational mechanism (TOMASZ 1965).

THE ENTRY STAGE: THE COMPETENT CONDITION

A comparison of the three bacterial recombinational systems reveals further interesting parallels as well as contrasting features. Similarly to the cases of transduction and conjugation, in transformation too, the competent condition (i.e., the ability to absorb DNA molecules) is a *genetically determined property*. There are "genetically incompetent" mutants or clonal isolates known, which are incapable of reacting with DNA (as determined either by the genetic assay or by the binding of radioactive DNA) during the usual growth conditions (Figure 1). Furthermore, it can be shown, that competence is a *mass property* of pneumococcal cultures: in a given culture each cell has an equal chance of developing the competent state. Furthermore, under optimal conditions practically every cell in the population can become "competent" synchronously, within a fraction of a generation time (JAVOR and TOMASZ 1968). Careful experiments also clearly indicate that the competent state is *not a pathological condition:* it does not influence any of the major parameters of cellular physiology and the bacteria that can absorb DNA from the medium are normally growing, "healthy" cells (TOMASZ 1971).

A most striking contrast between the recipient state in transformation as compared to that in conjugation or transduction is evident from Figure 1: the competent state is a *"non-constitutive" property* of pneumococcal cultures (TOMASZ 1966). In bacterial cultures growing from small inocula that competent condition appears abruptly, from low or undetectable levels as the cell concentration enters a critical range; it spreads to most of the cells in a fraction of the average generation time and then it decays, with rates that depend on the growth conditions. There is evidence suggesting that the competent state is also restricted to a fraction of the individual cellular division times (HOTCHKISS 1954). The transient nature, abrupt and explosive "spread" of this physiological

condition in growing cultures has no parallel in conjugation or transduction; in these latter phenomena the capacity of recipient cells to participate in recombination does not show comparable variations within a culture cycle.

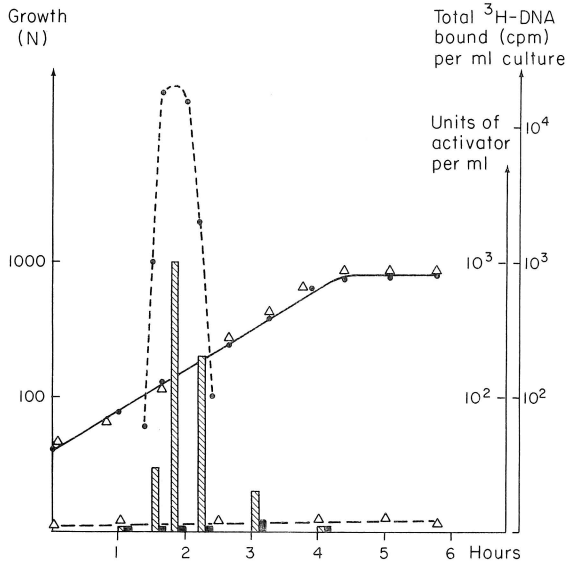

FIGURE 1. Development of the competent state during culture growth of pneumococcus.

Two cultures of pneumococci were grown in synthetic medium: a genetically competent strain (R6) and a genetically incompetent one (RA7). Growth of the cultures was monitored by nephelometry (N) (R6: ●———●; RA7: ▲———▲). Competence was determined by assaying for the binding of radioactive DNA (R6: ●———●; RA7: ▲———▲). The Figure also shows the production of endogenous activator (vertical bars; R6: cross hatched; RA7: solid). Reprinted from Membranes and Walls of Bacteria, Leivy, L., ed. Marcel-Dekker Inc., N. Y., 1973, with permission.

A detailed analysis of the transient expression of competence in pneumococcal cultures has revealed that the recipient condition is under the control of an endogenously produced protein factor, *the activator* (TOMASZ and HOTCHKISS 1964).

Detailed description of the biochemical properties of the activator can be found in the literature (TOMASZ 1966, TOMASZ 1972). For our discussion suffice it to note that the activator is a small protein or possibly glycopeptide, with an approximate molecular weight of about 10,000. It carries a

net positive charge in the physiological pH range, it is sensitive to proteolytic enzymes but not sensitive to lysozyme, DNase or RNase. When added to pneumococci in growth medium and at pH > 7, this factor induces the competent property (measured either as an ability to bind radioactive DNA or by the genetic assay of transformation) in a "reaction" the rate of which is a function of the concentration of both the activator and the incompetent cells (Figure 2). A peculiarity of this reaction

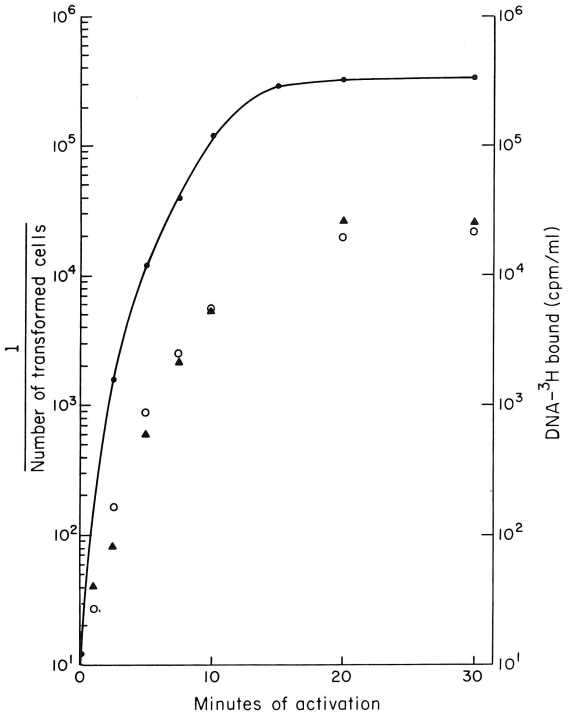

FIGURE 2. Induction of competence by purified activator. Binding of radioactive DNA and the number of genetic transformants (streptomycin resistance) were determined during treatment of cells with purified activator. Solid line: transformants; ▲ : total cell-associated DNA; ● : cell-associated DNA in nuclease resistant form. From Membrane Research, Fox, C. F., ed. Acad. Press, N. Y., 1972, with permission.

is its autocatalytic nature: not only are cells converted to competence but more activator is also produced (TOMASZ 1966). The extreme sensitivity of activator to proteolytic enzymes and the lack of biological activity at pH's below 7 explain the

observations that pneumococci growing in growth media with pH below 7 or in the presence of traces of subtilisin or trypsin, remain incompetent. The activator has extremely high specific activity: purified preparations contain less than 0.1 µg protein per 10^5 activity units (1 unit of activator can induce the competent state in 10^3 bacteria at 30° C and in 10 minutes). Of other transformable species of bacteria only the taxonomically related *Streptococcus viridans* D could be activated by pneumococcal activator. No effect was found on the competent states of *B. subtilis, H. influenzae, strain H streptococci* and *E. coli* spheroplasts. Thus the activator seems to be species specific.

High specificity of action is also evident in two additional senses of the word: (1) no biological activity can be found in physiologically or genetically incompetent cells and (2) the action of activator does not seem to cause any detectable change in general physiological properties of the bacteria such as rates of cellular polymer syntheses and growth and general permeability properties (TOMASZ 1971).

The peculiar kinetics of expression of the competent state in test tube cultures of pneumococci is controlled by the availability of endogenous activator. One can show that physiologically incompetent bacteria taken from low density cultures can be rapidly activated to competence any time during their growth by the addition of activator (TOMASZ 1964). Therefore, the time at which a culture enters the "competent phase" is determined by the endogenous process that produces the initial, threshold concentration of activator. We know very little about this process, except that it requires bacterial growth and that it is this process that makes the competent phase of cultures cell concentration dependent (TOMASZ 1966).

Once the concentration of activator reaches the threshold value, the interaction of activator and cells starts the rapid spread of the competent condition to 80-100% of the cells present in the population.

After a culture has passed through the peak of competence, the bacteria will continue to grow but will lose their transformable condition, sometimes almost as rapidly as they acquired it, sometimes slower. In cases when the decline occurs rapidly one can detect an inhibitory factor in the culture supernatant that can inactivate the activator. This factor is not detectable however under growth conditions when the decline of competence is slow. Yet, one can show that this slow decline of competence can no longer be prevented by adding fresh activator, or transferring the cells to fresh medium. And it is only after a period of growth that the cells again recover their responsiveness to the activator. This latter phenomenon indicates that there exist at least two different kinds of "incompetence" in pneumococci: one correctable by activator, and another that is caused by fluctuations in cellular sensitivity to the activator. One can formally describe this latter phenomenon as a cyclic decay and recovery of cellular "sites" essential for activator uptake or absorption (TOMASZ 1966).

Figure 3 summarizes the set of processes that seems to control the expression and fluctuations of the competent condition in pneumococcal cultures. It should be emphasized again, that no comparable physiological fluctuations in the recipient condition are known in the case of the other bacterial recombination phenomena: conjugation and transduction.

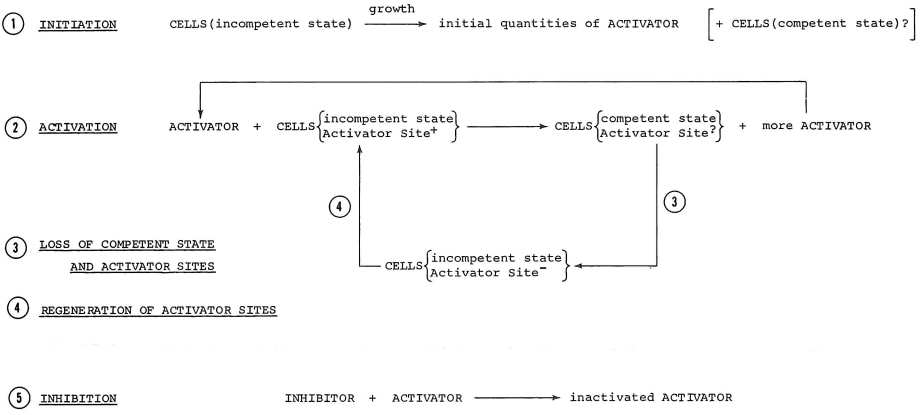

FIGURE 3. A physiological model for the control of competent state. From J. Bacteriol 91:1060 (1966), with permission.

BIOCHEMICAL ANALYSIS OF THE COMPETENT STATE

Since in the usual laboratory transformation experiment competent bacteria are presented with bare DNA molecules (rather than with the DNA-donating cells), it is clear that the entire apparatus for the binding or uptake of these molecules must be present in the competent cell. This situation is in sharp contrast to the case of conjugation or transduction, in which specific structures and activities of the donor cell or the virus dominate the entry phase of recombination and very little is known about recipient activities that might be involved. In contrast, in the pneumococcal system, the competent condition seems to be the result of complex surface alterations in which several specific macromolecular factors take part: the activator; a receptor for the activator (ZIEGLER and TOMASZ 1972); a novel antigenic determinant (NAVA, GALIS and BEISER 1963, TOMASZ and BEISER 1967); an "agglutinin" (TOMASZ and ZANATI 1971). There is also evidence suggesting the importance of the cell wall growth zone for DNA binding and uptake (TOMASZ, ZANATI and ZIEGLER 1971). I shall only briefly summarize here the temporal sequence in which these factors seem to interact during the expression of the competent state.

The process is initiated by the attachment of activator to the initially incompetent bacteria. Upon addition of activator to cells the first observable change is a temporary drop of the activity in the suspension, as if the activator had been destroyed. A little later, parallel with the appearance of competent bacteria, activator also reappears and by the end of the reaction its titer (determined by biological titration) far exceeds the input quantity. This latter phenomenon represents the autocatalytic production of excess activator, already discussed earlier.

A closer analysis of the early (and temporary) loss of activator revealed that this loss in activity represented a physical binding (and reversible inactivation) of activator molecules by a plasma membrane component of the bacteria. Biological activity could be recovered (in soluble form) by brief heating of such membrane fractions at 70° C in mercaptoethanol containing salt solution. The activator-binding component ("receptor" for the activator) has been solubilized and extensively purified. It appears to be a heat-sensitive macromolecule of net negative charge (at physiological pH) with a molecular weight of approximately 80,000 (ZIEGLER and TOMASZ 1972).

By the application of specific metabolic inhibitors a further stage can be recognized in the surface alterations leading to the competent state. It was found that pneumococci treated with activator under conditions that do not permit protein synthesis would bind normal amounts of activator and at the "correct" cellular sites (TOMASZ 1970). However, such bacteria will still not be able to bind radioactive DNA nor will they undergo genetic transformation. The same inhibitory conditions had no effect on the DNA binding (and uptake) of already competent cells. It was shown subsequently, that the binding of activator to cells that were engaged in normal protein synthesis resulted in the deposition of a trypsin-sensitive (protein?) substance (*"agglutinin"*) between the plasma membrane and the cell wall of the bacteria. Such cells show an unusual agglutination phenomenon at low ionic strength and low pH. Cells with blocked protein synthesis or genetically incompetent bacteria did not show agglutination upon treatment with activator (TOMASZ and ZANATI 1971).

The nutritional requirement of pneumococci for choline has allowed the identification of still another factor that seems essential for the appearance of DNA-binding capacity. It was found that bacteria deprived of choline during treatment with the activator would bind nearly normal quantities of these molecules, would also develop the agglutinin property but would not bind radioactive DNA (TOMASZ, ZANATI and ZIEGLER 1971). Upon readdition of choline to the medium, the DNA binding ability developed extremely rapidly, closely paralleling the resumption in the incorporation of choline molecules into the teichoic acid(s) of the cell envelope. Similarly, it was observed that a cessation of the incorporation of choline into teichoic acid causes an immediate, precipitous decline in the

DNA binding capacity of already competent cells also. These close parallels seen between the fluctuation of competence and choline incorporation into the cell surface suggested that the DNA binding capacity of these bacteria is somehow linked to continuous cell wall synthesis. Since the incorporation of new choline containing polymers into the cell surface occurs in a growth zone located at the coccal equator (BRILES and TOMASZ 1970, TOMASZ, ZANATI and ZIEGLER 1971), these observations suggest that DNA uptake depends on this dynamic area of the cell surface.

A more recent set of experiments implicates in a more direct manner this same topographic area of the cell wall as the site of action of activator. In these experiments we utilized a somewhat complex experimental design, which allows one to selectively manipulate the equatorial zone (= wall growth zone) of pneumococci. Pneumocci were grown in a medium in which the normal choline component was replaced by ethanolamine (EA). Such bacteria grow (in long chains) by utilizing EA in place of choline but may remain incompetent even when treated with activator. Upon readdition of choline to such EA-adapted cultures there is an immediate stop in the incorporation of EA into the cell surface and at the same time choline molecules begin to incorporate into the cell wall growth zone (TOMASZ 1968). Within 5-10 minutes after such a shift from EA to choline, the cells recover their responsiveness to activator and can be induced to bind DNA and undergo transformation. In the

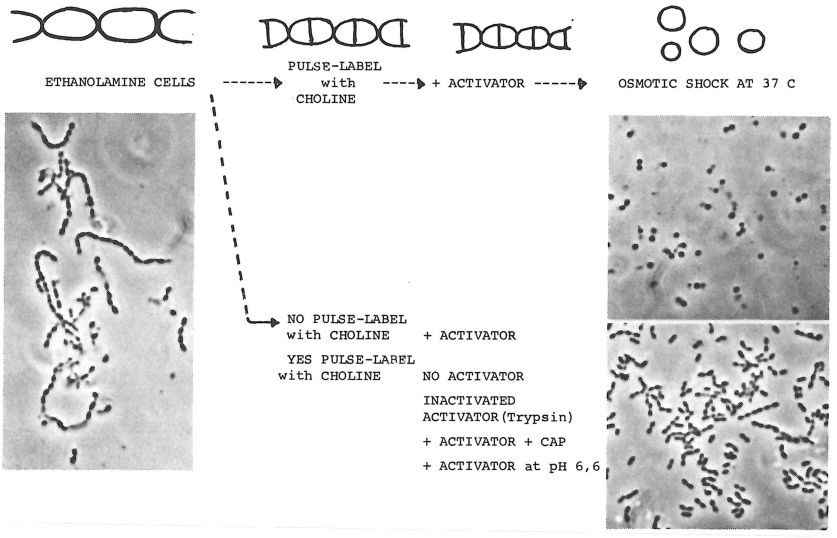

FIGURE 4. Activator-dependent formation of protoplasts. CAP = chloramphenicol.

experiment illustrated in Figure 4, EA-adapted cell were treated simultaneously with choline and activator and, afterwards, the

cells were transferred to a medium containing 20% sucrose. After brief incubation, there was a dramatic change in the appearance of these cells: the long chains of bacteria, characteristic of EA adapted cultures, were quantitatively converted to protoplast! Control experiments--also summarized in Figure 4--indicate that the formation of protoplasts in this complex, multistage experiment was clearly the result of the action of activator. Any condition that is known to prohibit the action of activator (such as inhibitors of protein synthesis, absence of choline, pH below 7, presence of proteolytic enzymes) also prevented protoplast formation. A genetically incompetent strain of pneumococcus did *not* show protoplast formation under these conditions, neither was there any protoplast formation (in a genetically competent strain) if the activator was replaced by extracts made from incompetent cells. Since it is known from separate experiments that the incorporation of choline molecules into EA cells also occurs in the growth zone at the cellular equator (BRILES and TOMASZ 1970), and since choline addition was essential for the activator-induced protoplast formation, this experiment suggests that the activator's action was directed to the same specialized area of the cell surface, namely the equatorial zone. While this experiment does not allow the identification of the mechanism of action of activator in biochemical terms, it represents a substantial step in that direction.

Immunological methods provide still another way of detecting a surface alteration in activator-treated pneumococci. Vaccines prepared from pneumococci in the competent state (but *not* in the incompetent condition) could invoke the formation of specific antibodies in the rabbit (NAVA, GALIS and BEISER 1963). These immunoglobulins could inhibit binding of radioactive DNA to competent pneumococci but not to other, different transformable species. It is not yet clear at which stage of the surface alterations the new, competence-specific antigenic determinants appear on the cell surface. On the other hand, it seems quite certain, that these new determinants are *not* the major pneumococcal surface antigens. This conclusion is based on essentially two findings: (1) in antisera prepared against competent and incompetent pneumococci, over 95% of the antibodies are identical, and (2) antisera prepared against competent pneumococci can also inhibit DNA binding in a transformable streptococcus (TOMASZ 1967), which is taxonomically related to pneumococcus in spite of the fact that the major surface antigens of this bacterium are completely different from those of pneumococci. A summary of the sequence of events leading to the competent state is illustrated in Figure 5.

The foregoing discussion points out two further interesting parallels between genetic transformation and the viral and conjugational recombination mechanisms. As it was pointed out earlier, both in the virus mediated as well as in the conjugational mechanisms there are interactions between structures on the recipient surface (virus receptors and the periodate sensitive sites on the F^- *E. coli* cells) and extracellular non-genetic elements (phage tail tips and F. pili) that precede

entry of DNA molecules. In the genetic transformation of pneumocci the interaction of extracellular activator molecules with cellular receptors seems to be an analogous stage.

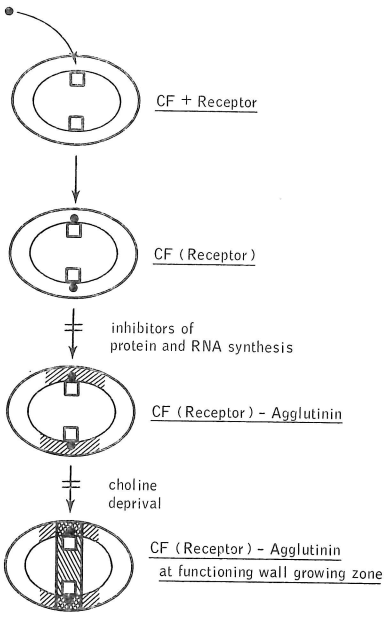

FIGURE 5. Stages in the cell surface alterations leading to competence. CF = competence factor. From Proc. Nat. Acad. Sci. U.S. 68:1851 (1971), with permission.

A second possible analogy is suggested by the fact that both in viral infection and in transformation only a relatively small, and probably specific area of the cell surface participates in the uptake of DNA molecules. These areas are represented by the cell wall-plasma membrane junctions in phage adsorption (BAYER 1968) and the cell wall growth zone in the pneumococcal transformation, respectively. It is already known that the cell wall growth zone is the site for the terminal stage of several outward directed transport processes, in which chemically different macromolecules, such as teichoic acids (BRILES and TOMASZ 1970), peptidoglycan precursor (RYTER, HIROTA and SCHWARZ 1973), surface-located proteins (SWANSON, HSO and GOTSCHLICH 1969), and capsular material (MEYNELL 1965), arrive and incorporate into the "old" cell surface. It is interesting to consider the possibility that import of macromolecules, such as DNA in genetic transformation, might utilize the same area of the cell surface.

NUCLEIC ACID UPTAKE BY EUCARYOTIC CELLS

While a "competent" state analogous to that in bacterial transformation has not as yet been detected in eucaryotic cells, biological phenomena involving nucleic acid transport across membranes do exist. The existence of infectious nucleic acid agents implies nucleic acid uptake by the natural hosts of such viroids (DIENER 1972). Lymphoid cells stimulated by phytohemagglutinins are known to secrete DNA into the medium (ROGERS, BOLDT, KORNFELD, SKINNER and VALERY 1972) and transport of nucleic acids across intracellular membranes (mRNA through nuclear membrane) must be of frequent occurrence. In addition, it has been suggested that transfection-like phenomena, by uncoated viral genomes, may be responsible for some of the unusual symptoms of some types of viral infections and for the vertical transmission of slow viruses (HERRIOTT 1969). Besides these "natural" phenomena there have been numerous. demonstrations of nucleic acid "uptake" by a variety of eucaryotic cells. Reports on the biological activity of such absorbed nucleic acids include initiation of viral infection (CRAWFORD, DULBECCO, FRIED, MONTAGNIER and STOKER 1964), expression (transcription or translation) of parts of a bacterial genome (MERRIL, GEIER and PETRICCIANI 1971), genetic transformation by homologous (SZYBALSKA and SZYBALSKI 1962, FOX and YOON 1970) and even heterologous DNA (LEDOUX, HUART and JACOBS 1971). A sample of the types of systems, methods used and findings claimed may be found in the published proceedings of a recent symposium on this subject (Informative Molecules in Biological Systems).

While many of these uptake phenomena can be quite dramatically stimulated by a variety of agents, such as DEAE-dextran, (VAHERI and PAGANO 1965), poly-L-ornithin (RYSER 1968), ATP (COHN 1971), etc., the mechanism of stimulation is not specific for uptake of nucleic acids; rather, it seems to involve stimulation of endocytosis. In contrast to bacterial transformation, which occurs by a highly specific mechanism, in mammalian cells the uptake of nucleic acids--as well as other macromolecular and even particulate matter--seems to occur by non-specific bulk-transport.

As a rule, macromolecules internalized by endocytosis are rapidly attacked by lysosomal degradative enzymes in the secondary lysosome (COHN 1970). There seem to be only two apparent exceptions from this; one is the case of viruses that enter cells by viropexis (DALES 1965); the other is the pinocytotic transport of ferritin and other macromolecules through capillary walls (BRUNE and PALADE 1968). In the latter case, endocytotic uptake is immediately followed by exocytosis on the tissue side of the cells thus bypassing the secondary lysosomal stage. In virus uptake the escape of intact viral genomes from the endocytotic vacuole may be the result of action of some viral component. For these reasons it is puzzling how DNA molecules fed to mammalian cells can survive intact enough to show subsequent biological activity. It is possible, that at least in some of the cases the molecules entered the recipient

cells by some yet uncharacterized mechanism, which is different from endocytosis. A more likely alternative is that uptake occurs via endocytosis but with a very high casualty rate (due to lysosomal attack) and only rare occasional molecules escape intact. The high DNA concentrations needed in some of the uptake experiments and the all too frequent irreproducibility of findings, may reflect this situation. It should be remembered that the escape of a single viral genome from the lysosomal vacuole is sufficient to initiate a viral infection cycle.

It is clear that for at least some of the kinds of experiments in which nucleic acids are fed to mammalian cells, a better control over the intracellular fate and integrity of the molecules would be essential. It might be, e.g., interesting to examine the role of viral proteins or other factors as possible protective agents or to search for physiological variables that might influence the fate of internalized macromolecules. There seems to be little if any systematic work published on this all-important aspect of the problem.

Although most published experiments with nucleic acid uptake by eucaryotic cells refer to their eventual aim as developing methods for genetic alteration ("reprogramming" or "intervention") of such cells, it may be worth mentioning that the introduction of nucleic acids into mammalian cells may have quite different goals also, not involving genetic recombination of the donor molecules. In the final part of the discussion, I shall briefly outline some of these possible goals.

NUCLEIC ACIDS, AS DRUG-CARRIERS: "ILLICIT TRANSPORT"

Daunomycin, a cytolytic drug capable of strong, non-covalent association with DNA was attached to DNA molecules and was introduced in the form of drug-DNA complex into a variety of mammalian cells via endocytosis (TROUTE, CAMPENEERE and deDUVE 1972). It could be shown that the drug, inactive in the bound form, was reactivated inside the cells through the hydrolysis of the carrier DNA by lysosomal nucleases. In this experiment DNA is simply used as a vector for a DNA-specific drug and the selectivity of drug action is based on the facts that, (a) the drug is inactive in the bound form and, (b) the DNA-bound drug could only be taken up by cells actively engaged in endocytosis. Once inside the endocytotic vacuole, the drug is "reactivated" by its release in free form, due to the hydrolysis of the carrier DNA.

Since several types of malignant cells are known to be unusually active in endocytosis, this elegant strategy could be used to the selective inhibition of the growth of leukemic cells. This method could be extended to the use of several other cytolytic antibiotics that are known to form non-covalent complexes with DNA (GOLDBERG and FRIEDMAN 1971).

In addition, it would seem possible to use some type of "illicit transport" (AMES, AMES, YOUNG, TSUCHIYA and LECOQ 1973) of drugs via endocytosis (instead of the "legitimate" transport processes that are available for the free drug molecules) even by using DNA as covalent "carrier" of nucleic acid base analogs. For instance, polynucleotides containing fluorodeoxyuridine or other base analogs, may be expected to follow the endocytotic path of uptake and once inside the secondary lysosome, the base analog may be liberated in the form of its nucleoside. This methodology may extend the usefulness of the potent carcinostatic fluoropyrimidines (HEIDELBERGER, GRIESBACH, MONTAG, MOOREN, CRUZ, SCHNITZER and GRUNBERG 1958) even for cases in which drug-resistant tumor lines have already developed. The resistance against fluoropyrimidines almost always involves mutational loss of "early" metabolic enzymes (HEIDELBERGER 1965), such as involved in transport mechanism, the PRPP-dependent pyrophosphorylase or ATP-dependent kinase. A successful introduction of fluorodeoxyuridine into such cells through the "illicit transport" outlined above would provide means of feeding the drug into such cells past the resistance-causing metabolic effect.

Unlike this strategy, which is based on the intracellular degradation of DNA, the goals of other types of experiments call for the survival of the internalized DNA intact. Clearly, methodologies of practical effectiveness will have to be developed, so that lysosomal degradation of the nucleic acids could be avoided. Such methods would be essential for the exploration of the feasability of at least two kinds of important investigations.

NUCLEIC ACID UPTAKE, AS GENETIC TOOL

Completely correct translation of a bacteriophage messenger carrying amber mutations have been reported in an *in vitro* protein synthesizing system, in which all components (except the messenger RNA) were derived from mouse L-cells (MORRISON and LODISH 1973). The idea of using genetically characterized microbial nucleic acids for the recognition of suppressor mutations in mammalian cells is an attractive one. In combination with methods by which such molecules could be introduced into mammalian cells it may provide a novel and much needed tool for the genetic characterization of these cells.

NUCLEIC ACID UPTAKE AND VIRUS INFECTION

In an important communication Spiegelman and his colleagues reported the highly selective inhibition of the Qβ replicase by polynucleotides representing incomplete phage genomes, which, in turn, were prepared by the premature termination of replicase-catalyzed *in vitro* synthesis (MILLS, PETERSON and SPIEGELMAN 1967). Preparation of analogous incomplete viral genomes may be possible at least for some viral nucleic acids. Introduction of such polynucleotides (perhaps even structurally

modified) into cells which have already been infected with the same virus might result in selective interference with viral replication. This type of chemotherapy (already suggested by Spiegelman) would be directed at one of the few accessible targets of virus replication that might be selectively inhibited.

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