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SHORT REVIEW

Energetics of the First Steps of Phage Infection

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Introduction

Infection of a bacterium by a virulent phage is a complex process which may be described as a cascade of sequential events. The first event is the *adsorption:* after random collision between a phage and a bacterium, one or several reversible steps lead to an irreversible attachment of the phage to a specific receptor present on the surface of the host cell envelope. A succession of interactions between different phage organelles and membrane components will allow the phage DNA to be released from the capsid (*DNA ejection*) and then to be *injected* into the cytoplasm. Once inside, the viral genome will monopolize the host cell biosynthetic machinery for its own replication. Finally, the self-assembly of new virions (morphogenesis) will lead to the lysis of the infected bacterium, liberating several hundreds of new phages into the external medium.

This review will be devoted to different energetic aspects of the infection of *E. coli* by phages. We will focus our discussion on the following questions: What are the energetic requirements for the first steps of adsorption, ejection, and DNA injection? What are the initial effects of adsorbed phages on the host cell metabolic energy?

Energetics of the Infectious Cycle

The Adsorption Step

Every component present on the surface of *E. coli* outer membrane has been found to be used as a specific receptor by a phage (for a recent review, see

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Randall and Philipson, 1980). Some phages (e.g., T4, T7) adsorb to different sites of the lipopolysaccharide hydrophilic chain. Others (e.g., T5, λ, etc.) attach to different outer membrane proteins. In this last case, one of the most interesting aspects of these protein receptors is their multifunctionality, as summarized in Table I.

For most phages, adsorption occurs whatever the temperature (between 0 and 37°C) and in the presence of various metabolic inhibitors (uncouplers, arsenate, cyanide) (Goldberg, 1980). Furthermore, when the isolated receptor is available, the irreversible attachment step, which will prevent the phage from adsorbing to entire bacteria further (i.e., phage inactivation), is equally possible *in vitro*. However, there is at least one well-studied example where irreversible adsorption is both temperature and energy dependent. In contrast to T5, T1 and ∅ 80 are not inactivated *in vitro* by outer membrane vesicles bearing Ton A protein, and cannot irreversibly attach to entire bacteria in the presence of uncouplers (Hancock and Braun, 1976). Since the irreversible attachment of these two phages also requires the presence of the Ton B protein (Gratia, 1964), Hancock and Braun (1976) proposed that the functional state of the Ton A receptor protein might be directed by the energized state of the cytoplasmic membrane, the Ton B protein serving as a coupling device between the two membranes. Since the protein Ton B has been recently characterized as an inner membrane protein (Postle and Reznikoff, 1979; Plastow and Holland, 1979), such a Ton A–Ton B direct interaction would be possible only at the adhesion zones between the two membranes that have been described by Bayer (1979). On the other hand, Reynolds *et al.* (1980), working on another Ton B-dependent system, the transport of vitamin B 12, proposed that a "diffusible messenger" X would be generated in the periplasmic space by the combined action of the protonmotive force and the Ton B protein (possibly acting as an antiport X/H^+). This "messenger" X would activate the outer membrane receptor Btu B. Such a hypothesis could also hold for the Ton A–Ton B interaction.

Whatever the exact mechanism, this was the first example of a system channeling the information between the two membranes of *E. coli* in an energy-dependent manner.

Table I. Multifunctionality of the Proteins of the *E. coli* Outer Membrane

Receptor	Substrate	Phages	Colicins
LamB	Maltose	λ, K10	—
	Maltodextrins	TP1	
Tsx	Nucleosides	T6	col. K
Ton A	Ferrichrome-Fe^{3+}	T1, T5	col. M
		ϕ80, UC-1	
Btu B	Vitamin B12	BF23	col. E

The DNA Ejection Step

Immediately after irreversible attachment of the phage to its outer membrane receptor, a succession of conformational changes is transmitted along the capsid, signaling the opening of the head–tail junction and allowing the DNA to exit (Goldberg, 1980). *In vitro,* the DNA can be released from the capsid after the phage has interacted with its isolated receptor (Goldberg, 1980). This suggests that the energy which has been built into the condensed DNA during the morphogenesis is sufficient to release the DNA (for a detailed discussion, see Zarybnicky, 1969, and Hendrix, 1978). However, *in vivo,* the ejection process may be blocked as soon as the tip of the DNA emerges outside the capsid (Labedan and Goldberg, 1982).

In the case of the well-studied T4 phage, successive interactions of the long and short fibers with different sites of the lipopolysaccharide allow both the irreversible adsorption and the contraction of the tail (Goldberg, 1980). This contraction, which requires ATP carried by the phage itself (Kozloff and Lute, 1959), triggers the penetration of the internal tube through the outer membrane. Benz and Goldberg (1973), using naturally contracted phage mutants, showed that the mere interaction of these isolated phages with phosphatidylglycerol was sufficient *in vitro* to open the tube and allow the exit of DNA. This was confirmed more recently by Furukawa and Mizushima (1982). They showed that T4 wt phages, adsorbed to reconstituted OmpC protein + lipopolysacharide + peptidoglycan complex, could contract their tail but eject their DNA only when the tip tube was in close contact with liposomes made of phosphatidylglycerol but not phosphatidylethanolamine. Furthermore, they showed that DNA was only ejected outside the liposome. From these experiments it can be concluded that metabolic energy is not necessary for the T4 DNA ejection step.

In the case of phages with noncontractile tails (e.g., λ, T5), the initial interaction between the distal end of the tail and the outer membrane receptor protein triggers a conformational change of this distal end which is transmitted all along the tail to eventually induce the opening of the head–tail junction. In the case of T5, this reaction occurs both *in vivo* and *in vitro,* at any temperature and in the absence of metabolic energy (Zarybnicky *et al.,* 1973; Labedan 1976; Labedan 1978; Filali Maltouf and Labedan, 1983). In the case of λ, an energy requirement might be indirectly involved. Indeed, Scandella and Arber (1974) isolated a bacterial mutant on which λ could normally adsorb but not inject its DNA. This mutation has been tentatively mapped in the *ptsM* gene (Elliott and Arber, 1978), which codes for the enzyme II^{Man}, the cytoplasmic membrane component of the phosphotransferase system (PTS) specific for the entry of glucose, mannose, and glucosamine (Postma and Roseman, 1976). This mutation can be complemented by specific mutations in two proteins of the phage tail, V and H (Scandella and Arber,

1976). Thus, it seems that an interaction between these tail proteins and cytoplasmic membrane protein involved in a specific ATP-dependent transport system is necessary to open the capsid to deliver the phage DNA. How an integral inner membrane protein can influence the conformational change of the capsid occurring at the outer membrane level deserves further studies.

The Injection Step

After ejection, the DNA must cross the inner membrane to penetrate into the cytoplasm. How a long (e.g., 50 μm for T4 DNA) polyanionic molecule can traverse the hydrophobic cytoplasmic membrane in less than half a minute is not yet understood. Different models have beeen proposed through recent studies with phages T4 and T5.

Injection of T5 DNA is very unusual two-step process (Lanni, 1968). First, there is the entry of the so-called first-step-transfer (FST) DNA, a fragment which amounts to 8% of the total T5 chromosome. Secondly, there is an obligatory pause during which FST-encoded proteins are synthesized to allow the injection of the rest of the DNA [second-step-transfer (SST) DNA]. Moreover, a pre-FST state has been described where the DNA has emerged but stayed attached to the outer membrane, just by incubating phage-cell complexes at 0°C (Labedan, 1976). In both pre-FST and FST stages, it has been shown possible to decapsidate the noninjected DNA without breaking it away (Labedan and Legault-Demare, 1973; Labedan, 1976). Furthermore, it was demonstrated that this naked uncoiled DNA, attached by one extremity to the bacterium and freely floating into the external medium, can enter the cell after either a temperature transfer (Labedan, 1976) or a transfer to a rich medium allowing protein synthesis (Labedan and Legault-Demare, 1973). These two results demonstrate that the energy necessary for DNA penetration cannot come from the release of some tension introduced at the time of DNA packaging inside the head during the morphogenesis, thus excluding the theoretical model of DNA injection proposed by Zarybnicky (1969) and Hendrix (1978). This work was actually the first demonstration that the energy required for phage DNA injection must come from the cell and not from the phage capsid.

Several years ago, Grinius (1976) proposed that the energy required for injection would be of chemiosmotic nature, more precisely that DNA traversal would occur in symport with protons. Results obtained by two different groups (Kalasauskaite and Grinius, 1979; Labedan and Goldberg, 1979; Kalasauskaite *et al.*, 1980) concerning the energetics of T4 injection seemed at first to agree with this hypothesis. Indeed, it was found that protonmotive force and not ATP was required for T4 DNA injection. Labedan and Goldberg (1979) further demonstrated that only one of the components of the protonmotive

force, the membrane potential ($\Delta\Psi$), was absolutely required for DNA injection. Furthermore, Labedan *et al.* (1980) found that T4 DNA injection only occurs above a threshold of $\Delta\Psi$, independently of the ΔpH. This last result has recently been contested by Kalasauskaite *et al.* (1983) who suggested that ΔpH may actually be involved. However, their data based on indirect measurements of T4 DNA injection do not seem sufficiently convincing compared to the previous demonstration of Labedan *et al.* (1980) that a large ΔpH (90 mV at pH 6) could not be used to promote DNA injection even when $\Delta\Psi$ was collapsed.

More recently, Filali Maltouf and Labedan (1983) demonstrated that in contrast to T4, phage T5 is able to inject efficiently its entire DNA in bacteria deprived of all known metabolic energy sources (protonmotive force and ATP). The same indifference to metabolic energy was observed if DNA was decapsidated and uncoiled before injection. These results strongly suggested that T5 phage DNA (a molecule 34 μm long) can enter the host cytoplasm by simple diffusion. To account for the irreversibility and fastness of T5 DNA entry (especially when it is naked), Filali Maltouf and Labedan proposed that this diffusion process would be facilitated by interaction of the penetrating DNA with the cytoplasmic DNA-binding (histone-like?) proteins. Indeed, these proteins are known to condense DNA independently of the metabolic energy (Geider and Hoffmann-Berling, 1981), and the coiled-DNA protein complex has been shown to have a free energy level lower than that of the uncoiled free DNA (Camerini-Otero and Felsenfeld, 1977; Rouviere-Yaniv *et al.*, 1979).

The results obtained with T4 and T5 phages seem to be contradictory. Two schemes can be considered. First, it might be that the process of DNA penetration is not unique and that T4 DNA traversal, in contrast to T5 DNA, would require the protonmotive force. However, the hypothesis of a DNA-proton symport (Grinius, 1976; Wagner *et al.*, 1980) still remains to be tested. The second scheme, which seems to us the more plausible, would be that there is a unique mechanism for DNA traversal. In this case, phage DNA injection would occur via a facilitated diffusion process through specific pores or channels. Because of the existence of a threshold for T4 DNA injection, it has been postulated (Labedan and Golberg, 1982) that the configuration of the DNA channels which determines their interaction with the tip of the DNA or their opening would be voltage-dependent. On the other hand, the entry of T5 DNA is not accompanied by any ion leakage even when the DNA is blocked at the FST stage, the SST DNA being kept traversing the membrane. This suggests a very special configuration of these DNA channels implying either that the physical presence of DNA is sufficient to plug the channel, or that the process of blocking injection includes a specific closing device (Filali Maltouf and Labedan, 1983). In all cases, DNA condensation would be mediated by

cytoplasmic proteins. More experimental data are necessary in order to be able to choose between these two models.

Phage-Induced Effects on the Host Metabolic Energy

Evidence has accumulated that many host membrane functions are dramatically, but transiently, affected during the first minutes of the infection process.

The increasing permeability of the host membrane toward potassium, which was first observed in the case of T4 (Silver *et al.*, 1968), has been shown for many other phages, e.g., T7 (Ponta *et al.*, 1976), T5 (Oldmixon and Braun, 1978; Glenn and Duckworth, 1980a), and T1 (Wagner *et al.*, 1980; Bakker, personal communication). The amplitude of this potassium release is dependent on the multiplicity of infection. This efflux is independent of the genome expression and, in the specific case of T7, has been shown to require the presence of the specific phage protein M (Ponta *et al.*, 1976). In the cases of T5 (Glenn and Duckworth, 1980a) and T1 (Bakker, personal communication), this efflux has been shown to be followed, after about 5 min, by a reentry of potassium. This recovery was shown to be independent of protein synthesis; moreover, in the case of T1 and T4, potassium leakage was prevented by the addition of high (10 to 25 mM) concentrations of Mg^{++} (Silver *et al.*, 1968; Bakker, personal communication).

This permeability for potassium is also accompanied by a partial inhibition of the accumulation of several protonmotive force-driven substrates (proline, TMG). PTS-mediated uptake of α MG is stimulated, suggesting that these phages can directly affect the protonmotive force, (Winkler and Duckworth, 1971; Glenn and Duckworth, 1979; Wagner *et al.*, 1980; Wilson and Okabe, 1982), since it has been shown that a decrease of protonmotive force stimulates the PTS system (Reider *et al.*, 1979; Robillard and Konings, 1981). ATP-dependent glutamine uptake is also partially increased after infection by T5 (Glenn and Duckworth, 1979), T1 (Wagner *et al.*, 1980), and λ (Wilson and Okabe, 1982). A parallel reduction of the ATP pool has been observed in these cases (Wagner *et al.*, 1980; Hulen, personal communication; Wilson and Okabe, 1982). This drop in ATP could be the consequence of its utilization by the ATPase in order to restore the initial level of the protonmotive force.

In the cases of T5 (Glenn and Duckworth, 1979) and λ (Wilson and Okabe, 1982), it has been shown that the inhibition of these active transports is reversed 3–5 min after the onset of infection. In the case of λ, this recovery was shown to be independent of protein synthesis but strictly dependent on the presence of the specific phage protein S (Wilson and Okabe, 1982). Although

no other data are available at the present time, it is tempting to suggest that this repair mechanism carried by the phage could be a general process for all productive infection.

On the other hand, in the case of T4 ghosts (phage particles lacking their DNA and internal proteins), a more dramatic and irreversible scheme occurs (Duckworth, 1970). There is a permanent inhibition of all transport systems, depletion of cytoplasmic potassium, and exhaustion of internal ATP. Furthermore, ghosts induce a release of phosphorylated sugars (αMGP, TMGP). However, Duckworth and Winkler (1972) demonstrated that ghosts do not create "holes" or a generalized breakdown of the permeability barrier and, even then, in certain conditions, it is possible to reverse their effects.

Some of these effects can be correlated with the results obtained by Hantke and Braun (1974) and later by Glenn and Duckworth (1980b), using ANS (8-anilino-1-naphthalenesulfonate) and NPN (*N*-phenyl-1-naphthylamine) probes. Hantke and Braun (1974) showed that phages T2 to T7, which have different outer membrane receptors, induce the same conformational changes in the cell envelope, i.e., an increase of the fluorescence intensity and a shift to a more hydrophobic environment. The same increase was found with T4 ghosts, showing that it is independent of the DNA injection process. Although the fluorescence changes of these two probes may not correlate directly with the state of membrane energization in all cases (Cramer *et al.*, 1976), this provides additional evidence of the membrane modifications following infection.

Fluorescent cyanine dyes, which allow a continuous monitoring of the transmembrane potential (Letellier and Shechter, 1979), have provided new information about the very early effects of phages T4 and T5 on the cell membrane. Labedan and Letellier (1981) showed that the irreversible adsorption of these two phages trigger the same partial depolarization of the cytoplasmic membrane. This is followed (e.g., after 20 sec at 37°C) by a repolarization to a new steady state $\Delta\Psi$, slightly lower than the preinfectious value. Moreover, the level of depolarization reflects the number of added phages. This depolarization–repolarization process was shown to be independent of the presence of the phage DNA and internal proteins, and of DNA injection and expression as it occurs with T4 ghosts as well. These results were demonstrated independently using the radioactive membrane-potential cationic probes $^{86}Rb^+$ and tetraphenylphosphonium (TPP^+) (Labedan and Letellier, unpublished results). Further results have shown that the T4-induced $\Delta\Psi$ changes may be suppressed by addition of EGTA, a chelator of calcium. Since it is known that calcium is only present in the bacterial envelope, this implies that part of this calcium would become accessible to EGTA only as a consequence of phage adsorption. Remarkably, this cellular calcium may be replaced by externally added calcium to reverse the inhibitory

effect of EGTA. Furthermore, the repolarization is probably a response to the depolarization, as suggested by the fact that addition of EGTA before the phage does not induce a hyperpolarization (Letellier and Labedan, 1983).

We proposed that this depolarization process may be the result of a conformational change transmitted from the outer membrane receptor to an inner membrane component. In the case of T4 this conformational change would trigger the unmasking of part of the envelope-bound calcium which would be necessary to activate the cytoplasmic membrane component. This activation would be necessary to induce the depolarization of the cytoplasmic membrane.

As the kinetics of this early transient depolarization and that of the inhibition of the different transport processes do not overlap, it is not clear whether there is a direct relationship between these two kinds of events. However, it must be underlined that even a slight decrease of $\Delta\Psi$ may have a pronounced effect on active transport of proline (Therisod *et al.*, 1982).

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