JOURNAL OF BACTERIOLOGY, June 1990, p. 2819–2824
0021-9193/90/062819-06$02.00/0

Vol. 172, No. 6

Bacteriophage λ DNA: the Beginning of the End

ANDREW BECKER AND HELIOS MURIALDO*

Department of Medical Genetics, Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada M5S 1A8

INTRODUCTION

The mature chromosome of bacteriophage λ is a linear duplex of a unique sequence 48,502 nucleotide pairs long (34). The 5'-terminal ends of this molecule protrude as self-complementary single-stranded chains of 12 nucleotides which can anneal to generate circles. When a λ DNA molecule is injected into the host, it is circularized and closed covalently by DNA ligase action. Early during lytic infection, λ DNA undergoes several rounds of replication in which circles generate circles. At later times of infection, rolling-circle replication takes over and individual rings give rise to linear concatemers (3, 8, 9, 28) which may be 10 chromosomal units long (in the absence of packaging). During DNA packaging, monomeric chromosomal units are cut from these replicative oligomers by the enzyme λ DNA terminase, which regenerates the unique single-stranded ends by introducing two specific nicks staggered 12 nucleotides apart on opposite strands of the duplex, followed by melting of the joint. The DNA site of action of terminase is called *cos*. By its interaction with *cos*, terminase not only catalyzes the formation of ends but, in coupling proheads to DNA, establishes the origin and direction of packaging.

The overview model for packaging and cutting of λ DNA which is currently accepted is as follows: (i) random selection of a *cos* site from among those present on a concatemer for packaging initiation, (ii) polarity of chromosome entry into the prohead from the *Nul* end to the *R* end, (iii) dependence of *cos* cutting on the packaging of a minimal length of DNA, and (iv) processive packaging of two or three chromosomes in series from a concatemer (8, 9, 28).

For previous reviews on this subject, see references 8, 9, and 28. With a few exceptions, references included in these reviews will not be cited specifically; instead, the reader will be directed to one or more of these three reviews, as the case may be.

THE ENZYME

Terminase is a heteroligomer encoded by the two leftmost genes of λ, *Nul* and *A*. The product of *Nul*, gpNu1, is the small subunit (21 kilodaltons) of the enzyme, while gpA is the large subunit (74 kilodaltons).

The enzyme possesses several activities, including DNA binding, *cos* cleavage, and prohead binding. The enzyme also catalyzes DNA-stimulated ATP hydrolysis. In the processive mechanism of λ DNA packaging on concatemers, terminase scans the incoming DNA; upon encountering the terminal *cos* and in response to fullness of the head with DNA, it nicks *cos* and then engages the downstream chromosome to initiate the next round of packaging (5, 8, 9, 28). Terminase function is supported by one or two host factors, integration host factor (IHF) (8, 10, 14, 19, 22, 27, 41) or terminase host factor (13, 38), and by another λ gene product, gpFI (2, 6, 9).

* Corresponding author.

Some of these activities can be correlated with functional domains along the two polypeptide chains of the holoenzyme (Fig. 1). Feiss and colleagues first showed this by making use of phage 21, a phage of the same family as λ (lambdoid phages). This phage has two genes analogous to λ genes *Nul* and *A*, called *1* and *2*, which code for its corresponding terminase. Phage λ and 21 terminases recognize only their homologous DNA and prohead. However, viable 21-λ hybrid phages that code for hybrid terminases can be constructed by crosses. By analysis of such hybrid terminases, the specific DNA-binding domain has been localized to within the first 91 amino-terminal residues of gpNu1, the prohead-binding domain has been localized to within the 38 carboxyl-terminal residues of gpA, and the subunit assembly has been localized to the carboxy and amino termini of gpNu1 and gpA, respectively (8, 40). At the amino terminus of gpNu1 (amino acid residues 5 to 24) there is a putative α2 helix-turn-α3 helix (HTH) motif (31) which likely represents the domain for specific *cos* DNA binding (8, 20) (see below). Biochemical experiments suggest that the nucleolytic (DNA-nicking) center resides in gpA (16), and the existence of mutations in gene *A* that render terminase unable to cleave DNA but do not alter its ability to promote packaging of mature DNA supports this idea (A. Davidson, P. Yau, H. Murialdo, and M. Gold, unpublished observations). The locations of the ATP-binding sites, first inferred from sequence analysis (1), are in both gpNu1 (1, 32) and gpA (15, 16).

THE SUBSTRATE

cos includes all the sequence specificities necessary for terminase to bind, cleave, and start packaging. It comprises about 200 base pairs (bp) and consists of several subsites (Fig. 2). The sequence of the site nicked by the enzyme, *cosN*, has hyphenated twofold rotational symmetry, and the breakpoints are introduced symmetrically within this dyad (Fig. 2A) (28). The rest of *cos*, called *cosB*, comprises a region of about 40 bp to the left of *cosN* and a region of about 170 bp between *cosN* and *Nul* (Fig. 2) (8, 9, 17). Within this region there are three segments, called *R* sites (8, 9), which are known to bind gpNu1 (36). A fourth *R* site, *R4* (Fig. 2A), located to the left of *cosN*, does not bind gpNu1 in vitro (36) but does bind the holoenzyme (R. Higgins and A. Becker, unpublished observations). In addition, *cosB* contains several consensus sequences for IHF binding (*I*) (Fig. 2A) (8). In vitro, however, IHF binds with high affinity only to site *I1* and with only moderate affinity to site *I2* (19, 41). Terminase host factor also binds to *cos* and can stimulate the activities of terminase (13, 38).

Phage 21 contains a *cosN*, with a nucleotide sequence identical to that of phage λ *cosN*, and a *cosB* with three *R* sites and IHF-binding consensus sequences in the same relative positions and orientations as those of phage λ (8). The base sequences of the *R* sites (*R3*, *R2*, and *R1*) of phage 21 are similar but not identical to those of the corresponding *R* sites of phage λ (Fig. 2B), and the differences may underlie

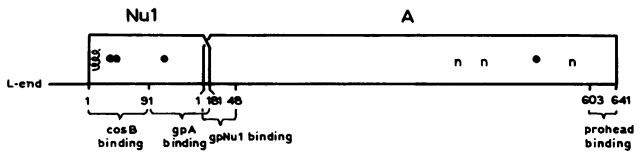

FIG. 1. Genetic map of λ DNA terminase genes and the approximate locations of the functional domains. The numbers refer to the amino acid residues of gpNu1 (1 to 181) and gpA (1 to 641). ●, Domains of ATP-binding sites, as deduced from amino acid sequence comparisons (1, 15). The HTH motif is represented by one helix. n, Approximate locations of three mutations that impair nucleolytic, but not packaging, activity (Davidson et al., unpublished observations).

the DNA recognition specificities of the two terminases. The sequence of *R4* in phage 21 is unknown.

TERMINASE BINDING TO COS

Terminase has separate specificities for *cosN* and for an *R* segment of *cosB*. The enzyme protects specific bases from DNase I cleavage within and near *cosN* (Higgins and Becker, unpublished observations), and a number of *cosN* point mutations can affect terminase function (I. S. Xu and M. Feiss, personal communication). The specificity of terminase for *cosN*, although error prone, is independent of *cosB* binding; specific nicking is seen in substrates from which all four *R* sites have been deleted (16; Higgins and Becker, unpublished observations). For the reasons outlined above, nicking is believed to be a function of the gpA subunit.

Terminase also binds to *cosB* in a phage-specific manner (8, 36). Since the nucleotide sequences of the *R* sites of *cosB* of λ and 21 differ, the small subunit of terminase that binds to the *R* sites must mediate this discrimination.

Alignment of the seven known *R* sites of both phages (Fig. 2B) revealed that several positions (columns) are invariant in all seven *R* segments (positions 8, 9, 11, and 13) or in six of the seven *R* segments (positions 1, 2, and 14). Probably some of these bases provide for the specificity that defines these DNA segments as targets for the small subunits of the terminases. One of these base pairs (at position 8), when mutated by transition (the mutant is called *cos*154), impairs λ *cos* function partially in wild-type cells and completely in IHF mutants and abolishes gpNu1 binding in vitro (8, 36; Xu and Feiss, personal communication).

In contrast, two other positions in *R*, 7 and 10 (Fig. 2B, stars) are distinctive with respect to phage type; that is, the nucleotides are identical among the sites of a particular phage but different between the two phages. We suggest that one or both of these positions play a discriminant role in the binding of the two terminases.

The amino acid residues of the α3 helices of the putative HTH structures in gpNu1 and gp1 can be organized according to the general features proposed for repressors (31). We view four residues—Thr-18, Ile-19, Trp-22, and Gln-23—identical in both λ and 21, as lying on one relatively hydrophobic α-helical face (Fig. 2C), presumably confronting the interior of the protein. Another α-helical residue which is identical in the two phages, Arg-17, falls on the opposite, solvent-exposed face along with three amino acids that differ between the two phages; the latter are Gln-20, Asn-21, and Glu-24 in phage λ or Glu-20, Arg-21, and Ser-24 in phage 21 (Fig. 2C). It would be this hydrophilic face of the α3 helix that could "read" certain bases within a stretch of five or six of the bases of *R* in the major groove. Recent work (summarized in reference 21) suggests that, without much supporting physical data, docking exercises in this realm would appear naive. Nevertheless, in one plausible category of model, we have used the side chain of the common Arg-17 to hydrogen bond in the major groove with an invariant base, such as the critical G(:C) at *R* site position 8, while allowing the side chains of amino acids 20, 21, and/or 24 to provide for the discrimination of λ and 21 through specific hydrogen bonds with bases that include the phage-distinctive (Fig. 2B, stars) bases. Since the *R* sites are 16 bp long and an α helix can contact only five or six bases, it is possible that domains of gpNu1 other than the HTH contact bases in *R*. It is also possible that each *R* site binds two gpNu1 monomers, since some degree of translational symmetry within the *R* sites, more obvious in λ than in 21, does exist.

The terminase protomer (gpNu1 plus gpA) can thus be viewed as a nexus between two different DNA sites: (i) a half-*cosN* symmetry element contacted by gpA for nicking and (ii) an *R* element within *cosB* bound by the gpNu1 subunit. The DNA between would be bent or looped to accommodate this dual interaction (see below).

Poised in this manner, terminase is subject to regulation

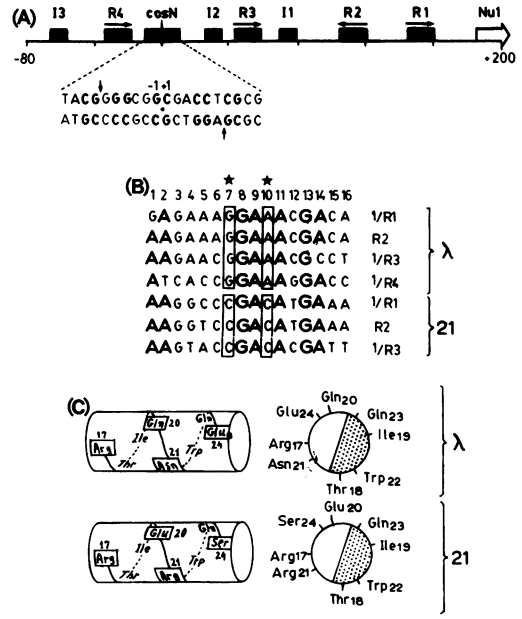

FIG. 2. (A) Map of the protein-binding sites in *cos* DNA and the nucleotide sequence of *cosN*. The binding sites are shown as black boxes and designated *R* for gpNu1-binding site, *I* for IHF-binding site, and *cosN* for the nicking site. The open arrow indicates the start of gene *Nu1* and its transcriptional orientation. Horizontal arrows indicate the relative orientations of the *R* sites. The sequence of *cosN* is shown as an expansion of its block representation in the map. The nucleotide numbering of the map is centered at the twofold rotational axis of symmetry (●) in the sequence of *cosN*. In this sequence, the vertical arrows point to the sites at which terminase nicks the DNA. The bases in boldface type are related by twofold rotational symmetry. (B) The nucleotide sequences of the seven known *R* sites are numbered arbitrarily from 1 to 16. The sequence of some *R* sites corresponds to that of the top (*l*) strand of λ DNA, and that of the *R* sites with the opposite orientation (1/R) corresponds to that of the bottom (*r*) strand of λ DNA. Boldface type indicates nucleotides present in six or seven of the *R* sites. (C) Schematic axial representation and end projections of the α3 helices of gpNu1 (λ) and gp1 (21). Amino acid residue numbering is as deduced from the nucleotide sequences of the genes.

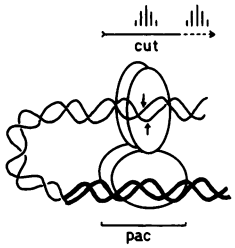

FIG. 3. Schematic representation of a putative DNA-terminase complex for phage Mu. The wavy lines represent the two strands of the DNA, thin for host cell DNA and thick for Mu DNA. The ovals drawn over the Mu DNA represent putative *pac*-binding subunits of Mu terminase, and those drawn over the host DNA represent putative Mu terminase subunits containing the nucleolytic centers indicated by vertical arrows. The horizontal arrow indicates that the site of cutting on DNA can be shifted rightwards. The two Gaussian-like bar diagrams indicate the positions and relative frequencies of the cut sites in block 1 (left) and block 2 (right).

by ATP. ATP serves as an allosteric ligand, markedly enhancing specific contacts of terminase at *cosB* (Higgins and Becker, unpublished observations), stimulating nicking activities at *cosN*, and rectifying the nicking site specificity on the *r* strand of *cosN* (16). Nicking at *cosN* having taken place, the hydrolysis of ATP by terminase results in the melting of the cohesive ends at a physiological temperature (16).

Structurally, the situation appears analogous to that of the site-specific recombination system of λ, in which two autonomous DNA-binding domains of one integrase monomer link two separate sites in *att* DNA, a core-type-binding site (which, like *cosN*, is nicked specifically) and an *arm*-binding site (25, 26). In a biochemical pathway, two or more sequential reactions—nicking and packaging initiation, for example—may be coupled on a multifunctional protein. The DNA targets for these reactions are linked on the DNA thread, and evolution has brought them close to each other. However, the distances between the protein domains and those between the DNA targets need not, and generally will not, coincide in the linear sense. They can be made congruent, however, by DNA bending and/or looping, accompanied in many cases by the formation of a complex nucleoprotein structure (see below).

Up to now, there is no direct evidence that phage terminases (sometimes called *pac*ases) bend or loop DNA near potential ends. However, there is some circumstantial evidence for looping by the terminase of phage Mu. In this phage, the DNA is packaged into the prohead by its terminase directly from an integrated stage in the *Escherichia coli* chromosome (for a review, see reference 4). In mature Mu particles, both ends of the DNA are heterogeneous in length and sequence owing to the presence of host DNA. At the left end, from which packaging starts, the host sequences vary from 56 to 144 bp. Thus, unlike that of the λ terminase, the nuclease action of the Mu terminase is not sequence specific. The host sequences, however, are not continuous from 56 to 144 bp but are distributed in blocks. The first block is 56 to 61 bp, the second is 67 to 72 bp, and so on. Each block represents five or six fragments differing from each other by 1 bp, with a nick-free space of 5 bp between each block. Also noteworthy is that the densities of the bands in each block are Gaussian distributed to a first approximation (12).

One model that would explain the length distribution of host sequences in the particles is shown in Fig. 3. In this model, Mu terminase binds to a *pac* site (*cosB* analog) within the Mu sequence proper, near the left end. The DNA double helix is then bent or looped so that host DNA makes contact with another domain of the terminase. The terminase introduces the nicks into the host DNA only when the DNA is properly positioned within the nuclease active site. Since the rotation of the DNA is restricted by torsional stiffness (39), the host DNA will be properly positioned within the active site only at integral turns of the double helix relative to *pac*. In practice, some rotation of the DNA around its axis between *pac* and the nicking site does take place, so that cutting takes place at the energetically optimal interval, plus or minus a few bases (±3). There now exist many examples of DNA looping in this manner to bring together a pair of interacting sites (25 and references within; 35). As illustrated by the Mu terminase, only one site may be specified by DNA sequence, the other being determined by thermodynamically probable DNA topologies.

ASSEMBLY OF PROTOMERS

Initiation of DNA packaging is a high-order reaction in terminase (9), likely reflecting an initial obligatory occupancy of all or most of the *R* sites of *cosB*. The role of the four *R* sites has been studied with the aid of cosmids. Because of the presence of *cos*, cosmids are packaged upon superinfection with λ. The packageability in vivo and in vitro of a series of *cos* deletion mutants showed that *R1* is not needed for in vivo packaging (23, 24). This result is in agreement with the observation that λ carrying *cos*154 (8), a mutation in *R1* that abolishes gpNu1 binding to *R1* (36), grows well. This mutant, however, is IHF dependent for growth, suggesting that cosmids with *cos* lacking *R1* will not be packaged in vivo in IHF-negative cells. The ability of the various mutants to bind terminase showed that, whereas *R1* is dispensable, *R2* and *R3* are essential for efficient binding.

Cosmids lacking *R3*, *R2*, and *R1* are packaged in vivo with an efficiency of about 10% that of the wild-type cosmid (23, 24). Therefore, terminase can bind, cleave, and initiate packaging at a reduced efficiency in the absence of *cosB*. Interestingly, cosmids missing *R2* and *R1* but with an intact *R3* are packaged much less efficiently than are those lacking all three *R* sites, as if *R3* when present alone were inhibitory. It is possible that binding to *R3* alone results in the formation of a sterile enzyme-DNA complex which can only be overcome by binding of a second terminase or gpNu1 molecule to *R2*.

Terminase binding to DNA containing both *R2* and *R3* is at least 10 times more efficient than is binding to DNA containing only one of the *R* sites, suggesting that the binding is cooperative (11). The cooperativity in binding works better between *R3* and *R2* than between *R2* and *R1*. λ point mutants with mutations in one, two, or three *R* sites grow poorly. The yield of phage is reduced even further if the host cells are IHF negative. The degrees of impairment indicate that the order of importance for the sites is *R3* > *R2* > *R1* (D. Cue and M. Feiss, personal communication). Despite its inability to bind gpNu1 in vitro, the *R4* site binds the holoenzyme (Higgins and Becker, unpublished observations) and is essential for packaging in vivo (Cue and Feiss, personal communication). It is apparent, therefore, that for optimal function, all four *R* sites are needed and that *R1*, although not absolutely essential, does stimulate binding (11).

Of the putative IHF-binding sites, only *I1* appears important. Indeed, deletions and insertions of 1 to 2 bp in *I2* do not significantly alter phage growth. In addition, a mutation in *I2*

that results in four mismatches in the 10 bp that constitute the IHF-binding consensus site has no effect on phage growth (22). These results plus the fact that the concentration of IHF needed to protect *I2* from degradation by DNase I is about 10 times that needed to protect *I1* (41) suggest that IHF, under normal physiological conditions, does not bind to *I2*. Thus, we conclude that, normally only one IHF-binding site, *I1*, is used.

Of particular interest is a 2-bp insertion at the *I2* site. Despite the insertion, the number of mismatches with the IHF-binding consensus sequence is not altered, but the mutant is IHF dependent for growth (22). This result suggests that spacing between *cosN* and *R3* is very critical and, if we accept the notion that *I2* is not normally used to bind IHF, we must conclude that IHF binding to *I1* influences the conformation of DNA in the *cosN-R3* region.

IHF stimulates the binding of gpNu1 to the *R* sites (37) and introduces a very sharp bend in the DNA when bound to *I1* (19). The cooperativity of binding to *R* sites between gpNu1 subunits suggests that these molecules interact among themselves, at least when bound to *cos*. The compensating effect that the presence of IHF has on *R1* mutants and the ability of IHF to stimulate gpNu1 binding could be brought about by a direct IHF-gpNu1 interaction or, more likely, by the ability of IHF to bring into close proximity separate sites because of its DNA-bending action (33). Sequence-induced bending has also been detected in *cos*. The bending is moderate and spans the vicinity of *I1* (A. Yeo and M. Feiss, personal communication; L. D. Kosturko and S. D. Hall, personal communication). It is possible that this intrinsic bending allows interactions between *R* site-bound protomers even in the absence of IHF.

In addition to the *cos*154 mutation in *R1*, other mutations make λ dependent on IHF for growth. One such mutant, λ *cos*59, has a 3-bp deletion in *I2*. It has been shown that a point mutation in *Nu1* which results in a gpNu1 with phenylalanine instead of the normal leucine at amino acid position 40, renders λ *cos*59 IHF independent (10). Position 40 is outside the HTH motif (amino acid positions 5 to 24) believed to mediate the binding of gpNu1 to the *R* sites. Although it is possible that the amino acid change enhances DNA binding through an alteration in protein conformation, it is also possible that the change enhances the interaction between gpNu1 monomers, stabilizing the oligomeric state to the point at which interacting gpNu1 monomers can sustain DNA bending in the absence of IHF.

Along this line of reasoning, it is noteworthy that the same mutation in *Nu1* enables λ *cos*154 to grow in IHF-negative cells (14). This result suggests that under certain conditions of weakened protein-DNA interaction, gpNu1 binding to both *R3* and *R2* is required for efficient DNA cutting and packaging and that binding to these two sites is enhanced either by IHF-assisted DNA bending or by stronger interprotein interactions.

WORKING MODEL

The relative positions and orientations of the *R* and *I* sites are intriguing. The sharp bend that IHF binding at *I1* confers to the DNA (19) brings *R3* and *R2* closer and aligns them in the same orientation (Fig. 4). This observation suggested to us the plausible model for the structure of the *cos*-terminase complex shown in Fig. 4. It should be emphasized that there is no evidence for DNA bending between *R2* and *R1*. Although the sequence in between those sites suggests the presence of a moderate bend, only the interaction between

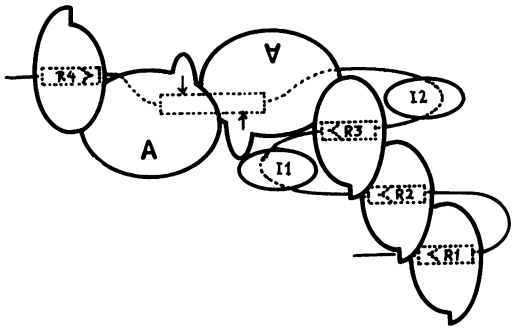

FIG. 4. Schematic model representing the initiation complex. It involves *cos* DNA and its protein-binding sites (broken lines and boxes). Shown are two gpA subunits (A), four gpNu1 subunits (occupying the *R* sites), and two IHF molecules.

the gpNu1 subunits bound at *R2* and *R1* would be able to force the DNA to bend as shown in Fig. 4. The DNA bending between *R2* and *R1* allows the orientation of *R1* to coincide with those of *R3* and *R2* so that the relationship among the gpNu1 molecules bound at the three sites becomes identical. The distance between *cosN* and *R3* is 35 bp longer than the distance between *R4* and *cosN*. This extra length provides room for another loop of DNA, allowing the antiparallel orientation of *R4* and *R3*. This arrangement is in keeping with the twofold rotational symmetry of *cosN* and of the two nicking sites. Two gpA molecules bound to themselves along a twofold rotational axis, to *cosN*, and to the gpNu1 molecules bound to *R4* and *R3* is probably the minimum number of gpA molecules needed to introduce the two nicks. Whether gpA molecules are bound to the gpNu1 molecules at *R2* and *R1* is unknown. The idea that each of the *R3*, *R2*, and *R1* sites is occupied by a holoenzyme protomer is discussed below.

Multiple protein-DNA interactions characterize other transactions of DNA of high specificity, such as the site-specific recombination of λ and the initiation of replication at *ori*λ and *oriC* (7). Echols (7) has argued that the specialized nucleoprotein structures (snups) that assemble at such loci through protein-DNA and protein-protein interactions underly the great precision that characterizes these processes. If the recognition and formation of chromosomal ends in phage proceed through the assembly of a snup (9), two important specificities may be determined by virtue of this assembly—nicking specificity and packaging directionality. These may be the cardinal functions of *cosB*.

As discussed above, in phage Mu there is little cutting specificity; the initial cut sites range with a periodicity over several turns of the duplex at some distance from the *pac* site, the position from which the assembly of a snup might be nucleated. Thus, snups per se need not ensure precision of cutting, if it is not programmed into the terminase enzyme itself, as it is in λ. On the other hand, the nicking sequence specificity of λ terminase is somewhat loose in that various base changes in the canonical *cosN* symmetry elements can be tolerated (Xu and Feiss, personal communication). Furthermore, half-*cosN* sequences or close relatives of these present in λ DNA are readily nicked by terminase (16; Higgins and Becker, personal communication). Thus, in the face of a limiting amount of terminase in vivo, multiple *R* sites in *cosB* would efficiently sequester the enzyme away from pseudosites; this corrective effect would be particularly strong if, by the tethering action of DNA, cooperative protomer-protomer interactions were to take place as shown in Fig. 4.

There is no information on the determinants of packaging directionality. The overall lack of symmetry of the complex shown in Fig. 4 could underly this specificity, and one idea as to how this might be realized is discussed below.

From the genetic experiments of Feiss and co-workers (8, 9) it is inferred that terminase can nick *cosN* and initiate packaging in either of two modes; these may be called (i) the initiation mode and (ii) the termination mode. The initiation of packaging and, presumably, the initial nicking at *cosN* on the first chromosome of a processive run require that terminase bind to *cosB* (initiation mode). Subsequent activities of this terminase, such as scanning and nicking of the terminal *cosN* after headfilling, the transfer of this terminase to the next downstream chromosome, and the initiation of packaging of this chromosome (and of subsequent ones), do not show this requirement for *cosB* (termination mode) or for its trappings, such as IHF (22).

To speculate on the structural basis for these two modes of operation, we suggest that, if holoenzyme protomers could occupy *R1*, *R2*, and *R3* in identical relationships as shown in Fig. 4, they could form a nucleus for the further oligomerization of protomers, resulting in a structure with a cyclic symmetry that surrounds the DNA like a nut does a bolt. Once an end of the chromosome has been formed by cutting, a terminase oligomer in this "nut" configuration could join the prohead connector (which is known to possess cyclic symmetry [18]). Terminase would henceforth be in the packaging, scanning, or termination mode. According to this idea, *cosB* is viewed as the substratum that nucleates the oligomerization of terminase protomers to form an integral part of the packaging machinery. Once this oligomer is formed, it need not be rebuilt during the processive packaging that takes place along the same concatemer, so that *cosB* is no longer needed.

CONTROL OF CUTTING

The cleavage reaction is intimately coupled to DNA packaging. In the absence of competent proheads or of the *D* and *FI* gene products, cutting in vivo is prevented (8, 9, 28). However, under certain conditions, *cos* cutting in the absence of proheads, gpD, and gpFI does take place. A plasmid containing the terminase genes and *cos* is cut very efficiently when, upon derepression, terminase genes are expressed (29). The inhibition of DNA cutting in prohead-deficient bacteriophage infections is, however, dominant. Indeed, when cells harboring the above-described plasmid are infected by a prohead-deficient bacteriophage, DNA maturation is blocked in both the bacteriophage and the plasmid DNAs (30). The mechanism of this inhibition is not presently understood.

ACKNOWLEDGMENTS

We thank our colleagues M. Gold and P. Sadowski and students of the phage group for illuminating discussions and David Cue, Michael Feiss, and Linda Kosturko for helpful comments and suggestions.

This work was supported by grants from the Medical Research Council (Canada) and the National Institutes of Health (United States).

LITERATURE CITED

1. **Becker, A., and M. Gold.** 1988. Prediction of an ATP reactive center in the small subunit, gpNu1, of the phage lambda terminase enzyme. J. Mol. Biol. **199**:219–222.
2. **Becker, A., H. Murialdo, H. Lucko, and J. Morell.** 1988. Bacteriophage lambda DNA packaging the product of the *FI* gene promotes the incorporation of the prohead to the DNA-terminase complex. J. Mol. Biol. **199**:597–607.
3. **Better, M., and D. Freifelder.** 1983. Studies on the replication of *Escherichia coli* phage lambda DNA. Virology **126**:168–182.
4. **Bukhari, A. I.** 1976. Bacteriophage mu as a transposition element. Annu. Rev. Genet. **10**:389–412.
5. **Chow, S., E. Daub, and H. Murialdo.** 1987. The overproduction of DNA terminase of coliphage lambda. Gene **60**:277–289.
6. **Davidson, A., and M. Gold.** 1987. A novel *in vitro* DNA packaging system demonstrating a direct role for the bacteriophage lambda *FI* gene product. Virology **161**:305–314.
7. **Echols, H.** 1986. Multiple DNA-protein interactions governing high-precision DNA transactions. Science **233**:1050–1056.
8. **Feiss, M.** 1986. Terminase and the recognition, cutting and packaging of lambda chromosomes. Trends Genet. **2**:100–104.
9. **Feiss, M., and A. Becker.** 1983. DNA packaging and cutting, p. 305–330. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Feiss, M., S. Fogarty, and S. Christiansen.** 1988. Bacteriophage lambda DNA packaging: a mutant terminase that is independent of integration host factor. Mol. Gen. Genet. **212**:142–148.
11. **Feiss, M., W. Widner, G. Miller, G. Johnson, and S. Christiansen.** 1983. Structure of the bacteriophage lambda cohesive end site: location of the sites of terminase binding (*cosB*) and nicking (*cosN*). Gene **24**:207–218.
12. **George, M., and A. I. Bukhari.** 1981. Heterogeneous host DNA attached to the left end of mature bacteriophage Mu DNA. Nature (London) **292**:175–176.
13. **Gold, M., and W. Parris.** 1986. A bacterial protein requirement for the bacteriophage lambda terminase reaction. Nucleic Acids Res. **14**:9797–9809.
14. **Granston, A. E., D. M. Alessi, L. J. Eades, and D. I. Friedman.** 1988. A point mutation in the *Nu1* gene of bacteriophage lambda facilitates phage growth in *Escherichia coli* with *himA* and *gyrB* mutations. Mol. Gen. Genet. **212**:149–156.
15. **Guo, P., C. Peterson, and D. Anderson.** 1987. Prohead and DNA-gp3-dependent ATPase activity of the DNA packaging protein gp16 of bacteriophage ϕ29. J. Mol. Biol. **197**:229–236.
16. **Higgins, R. R., H. J. Lucko, and A. Becker.** 1988. Mechanism of *cos* DNA cleavage by bacteriophage lambda terminase: multiple roles of ATP. Cell **54**:765–775.
17. **Hohn, B.** 1983. DNA sequences necessary for packaging of bacteriophage lambda DNA. Proc. Natl. Acad. Sci. USA **80**: 7456–7460.
18. **Kochan, J., J. L. Carrascosa, and H. Murialdo.** 1984. Bacteriophage lambda preconnectors. Purification and structure. J. Mol. Biol. **174**:433–447.
19. **Kosturko, L. D., E. Daub, and H. Murialdo.** 1989. The interaction of *E. coli* integration host factor and lambda *cos* DNA: multiple complex formation and protein-induced bending. Nucleic Acids Res. **17**:317–334.
20. **Kypr, J., and J. Mrazek.** 1986. Lambda phage protein Nu1 contains the conserved DNA binding fold of repressors. J. Mol. Biol. **191**:139–140.
21. **Matthews, B. W.** 1988. No code for recognition. Nature (London) **335**:294–295.
22. **Miller, G., and M. Feiss.** 1988. The bacteriophage lambda cohesive end site: isolation of spacing/substitution mutations that result in dependence on *Escherichia coli* integration host factor. Mol. Gen. Genet. **212**:157–165.
23. **Miwa, T., and K. Matsubara.** 1982. Identification of sequences necessary for packaging DNA into lambda phage heads. Gene **20**:267–279.
24. **Miwa, T., and K. Matsubara.** 1983. Lambda phage DNA sequences affecting the packaging process. Gene **24**:199–206.
25. **Moitoso de Vargas, L., S. Kim, and A. Landy.** 1989. DNA looping generated by DNA bending protein IHF and the two domains of lambda integrase. Science **244**:1457–1461.
26. **Moitoso de Vargas, L., C. A. Pargellis, N. M. Hasan, E. W. Bushman, and A. Landy.** 1988. Autonomous DNA binding domains of λ integrase recognize two different sequence fami-

lies. Cell **54**:923–929.
27. **Murialdo, H.** 1988. Lethal effect of lambda DNA terminase in recombination deficient *Escherichia coli*. Mol. Gen. Genet. **213**:42–49.
28. **Murialdo, H., and A. Becker.** 1978. Head morphogenesis of complex double-stranded deoxyribonucleic acid bacteriophages. Microbiol. Rev. **42**:529–576.
29. **Murialdo, H., and W. L. Fife.** 1984. The maturation of coliphage lambda DNA in the absence of its packaging. Gene **30**:183–194.
30. **Murialdo., H., and W. L. Fife.** 1987. Synthesis of a trans-acting inhibitor of DNA maturation by prohead mutants of phage λ. Genetics **115**:3–10.
31. **Pabo, C. O., and R. T. Sauer.** 1984. Protein-DNA recognition. Annu. Rev. Biochem. **53**:293–321.
32. **Parris, W., A. Davidson, C. L. Keeler, Jr., and M. Gold.** 1988. The *Nu*1 subunit of bacteriophage lambda terminase. J. Biol. Chem. **263**:8413–8419.
33. **Prentki, P., M. Chandler, and D. J. Galas.** 1987. *Escherichia coli* integration host factor bends the DNA at the ends of IS1 and in an insertion hotspot with multiple IHF binding sites. EMBO J. **6**:2479–2487.
34. **Sanger, F., A. R. Coulson, G. F. Hong, D. F. Hill, and G. B. Petersen.** 1982. Nucleotide sequence of bacteriophage lambda DNA. J. Mol. Biol. **162**:729–773.
35. **Schleif, R.** 1987. Why should DNA loop? Nature (London) **327**:369–370.
36. **Shinder, G., and M. Gold.** 1988. The Nu1 subunit of bacteriophage lambda terminase binds to specific sites in *cos* DNA. J. Virol. **62**:387–392.
37. **Shinder, G., and M. Gold.** 1989. Integration host factor (IHF) stimulates binding of the gpNu1 subunit of λ terminase to *cos* DNA. Nucleic Acids. Res. **17**:2005–2022.
38. **Shinder, G., W. Parris, and M. Gold.** 1988. Terminase host factor: a histone-like *E. coli* protein which can bind to the *cos* region of bacteriophage lambda DNA. Nucleic Acids Res. **16**:2765–2785.
39. **Shore, D., and R. L. Baldwin.** 1983. Energetics of DNA twisting. I. Relation between twist and cyclization probability. J. Mol. Biol. **170**:957–981.
40. **Wu, W.-F., S. Christiansen, and M. Feiss.** 1988. Domains for protein-protein interactions at the N and C termini of the large subunit of bacteriophage lambda terminase. Genetics **119**:477–484.
41. **Xin, W., and M. Feiss.** 1988. The interaction of *Escherichia coli* integration host factor with the cohesive end sites of phages lambda and 21. Nucleic Acids Res. **16**:2015–2030.