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The bacteriophage λ CI protein finds an asymmetric solution

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Abstract

The CI protein of bacteriophage λ (λ CI) is both a repressor and activator of transcription that has served as a model for understanding how gene regulatory proteins work. A dimeric DNA-binding protein, λ CI also forms higher-order oligomers that allow it to bind cooperatively to both adjacent and nonadjacent operator sites within the phage genome. The ability of phage λ to transition efficiently from one program of gene expression to another depends upon the formation of these higher-order protein-DNA complexes. A recently determined crystal structure of a DNA-bound λ CI dimer reveals that the two subunits of the dimer adopt different conformations. This unexpected asymmetry helps explain how these higher-order complexes are assembled.

Introduction

Bacteriophage λ provided one of the earliest paradigms for achieving a mechanistic understanding of gene regulation [1–3]. Its CI protein and the bacterial Lac repressor were the first transcription repressors to be isolated [4,5]. Since that time, λ CI function has been probed by a combination of genetic, biochemical and structural approaches, revealing a sophisticated regulatory circuit that allows for the efficient transition from one pattern of gene expression to another [1,2].

A lysogenic phage, λ can access two alternative developmental pathways upon infection of a bacterial host cell: the lytic pathway, which results in host cell lysis and the release of a crop of progeny phage, or the lysogenic pathway, which results in the stable integration of the phage chromosome into the bacterial chromosome and a general shut down of phage gene expression. Once established, the lysogenic state is highly stable [6,7]; nevertheless, specific environmental conditions (those that elicit the cellular SOS response) trigger a highly efficient switch from lysogeny to lytic development (referred to as prophage induction) [1,2].

The off position: λ CI and the maintenance of lysogeny

Both the maintenance of lysogeny and prophage induction depend critically on the CI protein, which functions as both a repressor and an activator of transcription [1]. A key aspect of λ CI function is its ability to form higher-order oligomers, permitting it to bind cooperatively to both adjacent [8] and widely separated operator sites [9,10]. The phage genome contains six

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individual operators sites, three in each of two control regions, O_R and O_L , which are separated by ~2.4 kilo base pairs (kb) (Fig. 1) [1]. The O_R region consists of operator sites O_{R1} , O_{R2} , and O_{R3} ; these are flanked by promoters P_R and P_{RM} , which direct transcription of early lytic genes and the *cI* gene, respectively. The simpler O_L region consists of operator sites O_{L1} , O_{L2} , and O_{L3} and a single promoter, P_L , which, like P_R , directs transcription of early lytic genes. During lysogenic growth, λ CI represses transcription of the early lytic genes by occupying sites O_{R1} and O_{R2} in O_R [11] and sites O_{L1} and O_{L2} in O_L [1]. In addition, λ CI bound at O_{R2} activates transcription of the *cI* gene from P_{RM} [12], an autoregulatory function that also contributes to the maintenance of lysogeny [1,13]. Finally, λ CI has the potential to repress transcription of its own gene from P_{RM} (negative autoregulation), which occurs when λ CI binds to O_{R3} [14].

λ CI, which binds as a dimer to individual operator sites, contains two structurally distinct domains that are connected by a protease-sensitive linker region. The N-terminal domain (NTD) contains a helix-turn-helix DNA-binding domain [15] and also a small surface-exposed patch that contacts RNA polymerase to effect transcription activation [16]. The C-terminal domain (CTD) mediates dimer formation and also the higher-order dimer-dimer interactions that enable λ CI to bind cooperatively to two (or more) operator sites [17,18].

Two parameters dictate occupancy of the correct sites during lysogeny and the proper functioning of the molecular switch that mediates the process of prophage induction: (i) the intrinsic binding affinities of the individual operator sites for λ CI dimers and (ii) the ability of pairs of λ CI dimers to bind cooperatively to adjacent operator sites [1,8]. O_{R1} and O_{L1} are both high-affinity λ CI binding sites, whereas the remaining sites bind λ CI with intrinsically lower affinities. Thus, a λ CI dimer bound at O_{R1} or O_{L1} can stabilize the binding of a second dimer to the adjacent site (O_{R2} or O_{L2}), increasing the apparent binding affinity of the weaker site. The resulting cooperatively bound λ CI dimer pair is, however, unable to interact with a third adjacently bound λ CI dimer. As a result, higher λ CI concentrations are required for the occupancy of O_{R3} and O_{L3} , which ensures that lysogenic concentrations of λ CI do not repress transcription from P_{RM} . This inability of more than two adjacently bound λ CI dimers to interact is known as “alternate pairwise cooperativity”, because a λ CI dimer bound at O_{R2} normally interacts with a λ CI dimer bound at the higher-affinity O_{R1} site, but can alternatively interact with a λ CI dimer bound at O_{R3} provided that O_{R1} has been inactivated by mutation (Fig. 1b) [8].

As expected, a genetically altered λ phage bearing a mutation that specifically disrupts λ CI cooperativity was found to be incapable of forming stable lysogens [19**]. Strikingly, however, Babic and Little [19**] were able to restore qualitatively wild-type behavior to the mutant phage by the introduction of two suppressor mutations (one that increased the strength of lysogenic promoter P_{RM} and the other that strengthened O_{R2} as a λ CI binding site). These findings suggest that λ CI cooperativity may have evolved in the context of a cruder, but functional form of the regulatory circuit that governs lysogenic development. The authors note, however, that CI cooperativity likely evolved early on because, with one possible exception, all lambdoid phages examined encode a CI protein that exhibits cooperativity.

From off to on: λ CI and prophage induction

During prophage induction, which is triggered under conditions that elicit the cellular SOS (DNA damage) response, an activated form of the bacterial RecA protein binds to the λ CI CTD and induces a self-cleavage reaction that separates the λ CI CTD from the λ CI NTD [20]. As a consequence, the operator sites are vacated and transcription from P_R and P_L commences. The first gene that is transcribed under the control of P_R encodes a second repressor (Cro) [21] that binds to the same six operator sites as does λ CI [1,22]. However, Cro, which binds

noncooperatively to the individual operator sites, exhibits a different order of preference for these operators, binding with the highest affinity to O_R3 [23]. When bound at O_R3 , Cro (like λCI) represses transcription from P_{RM} [23]. Early on, the suggestion was made that the antagonistic effect of Cro on cI transcription likely plays a critical role during prophage induction, by preventing a recovery of λCI synthesis [24]. Although this proposal has been challenged [25,26], a more recent study provides definitive evidence in support of the original proposal [27**].

Cro is also required for lytic development after infection, but in this case its essential role is to turn down early lytic transcription by binding to sites O_R1 and O_R2 and sites O_L1 and O_L2 [28]. Cro's affinity for these sites is lower than its affinity for O_R3 [23], ensuring that there is a delay before Cro accumulates sufficiently to occupy these sites [29]. Presumably, Cro-mediated repression of early lytic transcription is also required for completion of the lytic program after prophage induction, but this has not been demonstrated directly [27**].

Tuning the switch: higher-order looped complex facilitates negative autoregulation

The molecular interactions depicted in Fig. 1a summarize the understanding of λCI -mediated regulation as it stood until about 10 years ago. Although this picture provided a fairly satisfactory explanation for the regulation of lysogeny, certain anomalies remained unexplained. For example, no obvious regulatory role could be ascribed to O_L3 . Furthermore, experiments carried out with single-copy P_{RM} - $lacZ$ fusions suggested that lysogenic concentrations of λCI were insufficient to mediate a significant amount of O_R3 -mediated repression of P_{RM} transcription [14], suggesting that negative autoregulation might be relatively insignificant. In the meanwhile, various lines of biochemical, structural and genetic evidence pointed to the possibility of another level of regulation. In particular, biochemical studies suggested that λCI can assemble into an octamer in solution [30], and crystallographic studies of the λCI CTD revealed the structural basis for octamer formation [18,31]. Furthermore, the striking observation that pairs of cooperatively bound λCI dimers could interact with one another over distances of several kb, forming looped protein-DNA complexes, suggested a physiological role for the λCI octamer -- namely, to link events occurring at O_R with those occurring at O_L [9].

A breakthrough in understanding was achieved by Dodd *et al.* [10,32], who uncovered a critical function for O_L3 in helping to mediate negative autoregulation. They found, unexpectedly, that mutation of O_R3 in context of an otherwise wild type λ lysogen resulted in a substantial defect in prophage induction that was due specifically to the disruption of negative autoregulation by λCI . (Importantly, they used an O_R3 mutation that disrupted λCI binding to O_R3 , but had no effect on Cro binding.) Their findings thus indicated that the fractional occupancy of O_R3 in a lysogen is higher than previous experiments (performed with O_R -based reporter constructs lacking the O_L region) suggested. Additional experiments revealed that in a lysogen, interaction between the cooperatively bound λCI dimer pair at O_R1 and O_R2 and another cooperatively bound λCI dimer pair at O_L1 and O_L2 results in the formation of a higher-order octameric complex (Fig. 1c). The resulting 2.4 kb DNA loop juxtaposes O_L3 and O_R3 such that a pair of dimers can now bind cooperatively to these distant sites, bringing about the occupancy of O_R3 at lower concentrations of λCI than would be predicted simply by its intrinsic λCI binding affinity. In fact, Dodd *et al.* [32] found that mutation of O_L3 , in the context of an otherwise wild-type λ phage, resulted in an ~3-fold increase in the lysogenic λCI concentration and a substantial defect in prophage induction due to the loss of negative autoregulation.

Structure-based modeling

The formation of both λ CI tetramers and λ CI octamers, postulated by the model depicted in Fig. 1c, is consistent with a wealth of biochemical, structural and genetic data. The crystal structure of the λ CI CTD (the oligomerization domain) [18], which was determined almost 20 years after the structure of the λ CI NTD (DNA-binding domain) was first reported [15], permitted direct visualization of the biologically relevant λ CI tetramer (as established based on genetic data [33,34]) and suggested how two tetramers might associate to form an octamer (Fig. 2). Subsequent crystallographic studies permitted direct visualization of the λ CI CTD octamer [31]. Nevertheless, the lack of a structure for the intact λ CI dimer left unexplained the structural basis for pairwise cooperativity (the ability of adjacently bound dimers to interact, forming a CTD tetramer). In addition, it was unclear why no more than two adjacently bound dimers can interact, even though up to four dimers can interact provided the two dimer pairs are bound at a distance from one another on the DNA.

The recent crystallographic analysis of a DNA-bound λ CI dimer provides a plausible explanation for the rules governing higher-order complex formation, based on a striking lack of global symmetry exhibited by the dimeric complex [35**]. Rather than a single axis of symmetry as might have been expected, the dimeric complex is described by two nearly orthogonal symmetry axes, one that relates the two NTDs and another that relates the two CTDs (Fig. 3). As a result of this asymmetry, the NTD and CTD of one subunit of the dimer, but not the other, are in contact, creating an interface that is comparable in terms of buried surface area to the CTD-dimer interface. What makes this asymmetry possible is the flexibility of the short linkers that connect the N-terminal and C-terminal domains; these linker segments adopt markedly different conformations in the two subunits of the dimer. Previously reported biochemical evidence, based on the analysis of a mutant λ CI protein in solution, also suggested that the intact dimer might adopt an asymmetric conformation [36].

Although crystal-packing forces could, in principle, be responsible for the observed asymmetry, the asymmetric dimer assembly neatly accounts for the rules that govern the cooperative binding of λ CI to both adjacent and nonadjacent operator sites [35**]. To model the higher-order interactions of DNA-bound λ CI dimers, the CTDs of the intact operator-bound λ CI dimer were superimposed onto the structures of the λ CI CTD tetramer and octamer. Consider first the superimposition of the CTDs of two intact operator-bound λ CI dimers on the structure of the λ CI CTD tetramer [18]. Because of the inherent asymmetry of the intact operator-bound dimer, the superimposition can be performed in two different ways, one of which allows for cooperative binding to adjacent operator sites. Specifically, when the superimposition is performed as shown in Fig. 4a, the DNA of one λ CI-operator complex comes into rough alignment with that of the second λ CI-operator complex, so that the two λ CI dimers are adjacently bound on what is essentially a continuous DNA helix. Thus, this superimposition provides a model for the interaction of adjacently bound λ CI dimers at either O_R1 and O_R2 or O_L1 and O_L2 . When the superimposition is done in the other way (Fig. 4b), the DNA-binding domains of the two interacting dimers point in opposite directions, a configuration that allows for binding to nonadjacent operator sites, such as O_R3 and O_L3 (see Fig. 1c). λ CI dimers have also been found to bind cooperatively to nonadjacent operator sites that are separated by several (e.g. five or six) turns of the DNA helix on artificial constructs [37]; the superimposition shown in Fig. 4b also accounts for these cooperativity complexes, in which the intervening DNA is bent.

To model the higher-order octameric complex that forms when the pair of cooperatively bound λ CI dimers at O_R1 and O_R2 interacts with another pair of cooperatively bound λ CI dimers at O_L1 and O_L2 , the CTDs of four intact operator-bound λ CI dimers were superimposed onto the structure of the λ CI CTD octamer [31]. When two tetrameric complexes configured as shown

in Fig. 4a are brought together, the physiologically relevant octameric complex is formed (Fig. 4c). Two alternative superimpositions are also possible (involving either one or two tetrameric complexes configured as shown in Fig. 4b), as depicted in Fig. 4d. Notably, no matter how the superimposition is done, no more than two λ CI dimers can be positioned at adjacent DNA sites, providing a structural explanation for alternate pairwise cooperativity [35**].

Future directions

Genetic analysis may permit definitive confirmation that the asymmetric configuration of the DNA-bound dimer seen in the crystal structure of the λ CI-operator complex is the biologically relevant configuration. A possible approach would be to use the crystal structure to identify amino acid substitutions predicted to disrupt the observed interaction between the λ CI NTD and CTD in the compact subunit of the DNA-bound dimer (Fig. 3). Such a mutant, which might or might not manifest a defect in binding to a single operator site, would presumably be unable to bind cooperatively to adjacent operator sites (or in any case no longer exhibit alternate pairwise cooperativity). However, cooperative binding should be restored in the context of a wild-type/mutant heterodimer (Fig. 4e). Moreover, such heterodimers should bind in a defined orientation when the pairwise cooperativity complex is formed, with the mutant subunits bound to the outer half-sites (see Fig. 4a, e). The requirement for oriented binding could, in principle, be tested by altering the DNA-binding specificity of one or the other subunit so that it can be targeted to a specifically mutated operator half-site [38] (Fig. 4e).

Further structural work could also provide additional support for the proposed structural models. It may be possible to crystallize the pairwise cooperativity complex by using a DNA template containing a pair of appropriately spaced operator sites. The direct visualization of a pair of λ CI dimers interacting as predicted by the model would provide strong confirmation for the proposed significance of the asymmetric DNA-bound λ CI dimer.

Many transcription regulators bind as dimers and participate in higher order oligomeric interactions. It will be interesting to learn whether asymmetry also plays a role in other cases to facilitate formation of the biologically relevant higher-order complexes, while preventing the formation of competing (unwanted) higher-order complexes.

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Fig 1. The interactions of λ CI at the right and left operator regions in a λ lysogen

(a) λ CI dimers bind cooperatively to adjacent operator sites in O_R and O_L . The λ CI dimers are shown in blue. Each subunit of the dimer consists of an N-terminal DNA-binding domain (N), a C-terminal oligomerization domain (C), and a linker region (black) connecting the two. The dimer pair bound cooperatively at O_R1 and O_R2 represses transcription from P_R and the dimer bound at O_R2 also activates transcription from P_{RM} . The dimer pair bound cooperatively at O_L1 and O_L2 represses transcription from P_L .

(b) Alternate pairwise cooperativity. No more than two adjacently bound λ CI dimers can interact. Ordinarily, a dimer bound at high-affinity site O_R1 stabilizes the binding of a second dimer to O_R2 . But if O_R1 is inactivated by mutation, λ CI dimers can bind cooperatively to O_R2 and O_R3 .

(c) Higher-order looped complex that facilitates negative autoregulation. The cooperatively bound λ CI dimer pair at O_R1 and O_R2 interacts with the cooperatively bound λ CI dimer at O_L1 and O_L2 , forming an octameric complex that loops 2.4 kb of intervening DNA. Formation of the looped complex juxtaposes O_L3 and O_R3 , allowing another pair of λ CI dimers (shown in pale blue) to bind cooperatively to these sites. When bound at O_R3 , λ CI represses transcription from P_{RM} (negative autoregulation); however, in a lysogen O_L3 and O_R3 are only partially occupied.

Fig 2. Formation of the λ CI CTD octamer

(a) A pair of λ CI CTD dimers aligned for interaction. The structure of the λ CI CTD dimer is shown in surface representation (left side), with one subunit colored salmon and the other subunit colored blue. The two dimers are related by a 180° rotation about an axis perpendicular to the plan of the page. The amino acid residues implicated in cooperativity [33,34] form two patches on the surface of each CTD monomer, which are highlighted in red and dark blue (note that the blue patch actually consists of two neighboring but noncontiguous regions). The two dimers are also shown in cartoon representation as wedges of a cylinder (right side). The blue (B) and red (R) cooperativity patches are labeled and the dotted lines indicate how the patches pair for tetramer formation.

(b) The λ CI CTD tetramer. The two dimers shown in (a) are docked to form the λ CI CTD tetramer, depicted in surface representation on the left side. Notice that four cooperativity patches (two red and two blue) are still exposed on the surface of the tetramer. Shown on the right side are two tetramers in cartoon representation, each of which resembles a half-cylinder with staggered ends. Pairing of the indicated cooperativity patches results in octamer formation, as shown in (c).

(c) The λ CI CTD octamer. Shown on the left is the octamer that is formed when two tetramers associate; the bottom tetramer is depicted in surface representation and the top tetramer depicted in ribbon representation so that the cooperativity patches underneath can still be seen. To form the octamer, a second tetramer is rotated approximately 180° about a horizontal axis in the plane of the page, forming a sandwich-like complex. Shown on the right is the octamer in cartoon representation (depicted as a cylinder with staggered ends). The octamer shown (left and right) has three identical dimer-dimer interfaces, those contained within each tetramer and another that is formed when the upper dimer of the bottom tetramer pairs with the upper dimer of the top tetramer. The structural details of octamer formation preclude interaction between the unpaired cooperativity patches exposed on the lower dimer of each tetramer (see, for example, the exposed blue patch at the bottom left).

Fig 3. Views of the dimeric λ CI-operator complex

(a) Cartoon representation of the asymmetric DNA-bound λ CI dimer. One subunit of the dimer (salmon colored) adopts an extended conformation and the other adopts a compact conformation (blue). The radii of the spheres were determined based on the actual size of the NTD and CTD. The NTD and CTD of the compact subunit associate through the cleavage site region (CSR) (highlighted in dark blue, but only partially visible), a pair of antiparallel β -strands and a connecting loop that contains the site of RecA-mediated autocleavage [20, 35**]. The NTD and CTD of the extended subunit do not interact. The DNA is shown in pink.

(b) Crystal structure of the DNA-bound λ CI dimer in ribbon representation. The color scheme is as in (a), with the compact subunit in blue and the extended subunit in salmon. The CSR of the extended subunit is highlighted in red and that of the compact subunit is highlighted in dark blue. The symmetry axes of the individual domains are shown as green rods, and are nearly perpendicular to one another.

(c) Crystal structure of the DNA-bound λ CI dimer in space-filling representation. The color scheme is as in (a) and (b). This representation emphasizes that the CSR of the blue subunit (highlighted in dark blue) forms the interface between the NTD and CTD.

Fig 4. Structural modeling of higher-order λCI complexes

(a) Model depicting interaction of adjacently bound λCI dimers. The pairwise cooperativity complex was modeled by superimposing the CTDs of two intact DNA-bound dimers onto the structure of the CTD tetramer. The color scheme is as in Fig. 3. This superimposition brings the DNA of one λCI-operator complex into rough alignment with that of the second complex. Also shown are the C-terminal α-helices of the NTDs (visible only on the left-hand dimer), which mediate formation of a weak NTD dimer.

(b) Model depicting interaction of nonadjacently bound λCI dimers. Alternative superimposition shows how two λCI dimers might look when bound cooperatively to O_L3 and O_R3 in the context of the looped complex depicted in Fig. 1c.

(c) Model depicting the interaction of two adjacently bound λ CI dimer pairs. The octameric complex that links O_R with O_L was modeled by superimposing the CTDs of four DNA-bound dimers onto the structure of the CTD octamer. In this case, the superimposition was performed by first constructing two pairwise cooperativity complexes, as shown in (a).

(d) Alternative superimpositions illustrating that no more than two adjacently bound λ CI dimers can interact. The CTDs of four DNA-bound dimers can be superimposed onto the structure of the CTD octamer in three different ways, the one shown in (c) and the two alternatives shown here. One of the alternatives (top) is generated by docking a pairwise cooperativity complex with the alternative tetrameric complex shown in (b). The other alternative (bottom) is generated by docking two tetrameric complexes configured as shown in (b). Regardless of how these superimpositions are performed, no more than two DNA operators come into alignment.

(e) Cartoon illustrating the design of oriented heterodimer experiment to detect λ CI dimer asymmetry. The crystal structure of the asymmetric DNA-bound λ CI dimer is used to design a mutant (purple subunit) that is unable to adopt the compact conformation (due to one or more amino acid substitutions predicted to disrupt the NTD-CTD interface). In addition, the DNA-binding specificity of the mutant subunit is altered by introduction of a suitable amino acid substitution on the DNA-binding surface of the NTD. Wild-type/mutant heterodimers are assembled in vitro. Only the wild-type subunit (blue) can adopt the compact conformation. These heterodimers should bind in an oriented fashion to an asymmetric operator bearing a half-site (purple) that has been mutated so that it is specifically recognized by the mutant subunit only. Furthermore, the wild-type/mutant heterodimers should bind cooperatively to a pair of asymmetric operators, configured as shown, with the mutant half-sites on the outside. Alternative configurations of the adjacent operator sites (not shown) are predicted to preclude cooperative binding by the wild-type/mutant heterodimers.