Developmental switches in a family of temperate phages

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Abstract

P2 is the prototype phage of the non-lambdoid P2 family of temperate phages. A developmental switch determines whether a temperate phage will grow lytically or form lysogeny after infection. P2 related phages have two face-to-face located promoters controlling the lysogenic and the lytic operon respectively, and two repressors. The immunity C repressor of P2 is the first gene of the lysogenic operon and it represses the lytic promoter. The Cox protein, the first gene of the lytic operon, is multifunctional. It represses the lysogenic promoter, acts as a directionality factor in site-specific recombination and activates the P_{LL} promoter of satellite phage P4.

This thesis focuses on comparisons between the developmental switches of P2 and the two heteroimmune family members, P2 Hy *dis* and WΦ. A characterization of the developmental switch region of P2 Hy *dis* identifies a directly repeated sequence which is important for C repression. P2 Hy *dis* Cox can substitute for P2 Cox in repression of the P2 lysogenic promoter, excision of a P2 prophage and activation of P4 P_{LL}. The P4 ε protein can derepress the developmental switch of P2 Hy *dis*.

Functional characterizations of the C repressors and Cox proteins of P2 and WΦ show that both C repressors induce bending of their respective DNA targets. WΦ C, like P2 C, has a strong dimerization activity *in vivo*, but there are no indications of higher oligomeric forms. Despite the high degree of identity in the C-terminus, required for dimerization in P2 C, they seem to be unable to form heterodimers. The two Cox proteins are predicted to have identical secondary structures containing a helix-turn-helix motif believed to be involved in DNA binding. It is, however, not possible to change the DNA specificity of P2 Cox to that of WΦ Cox by swapping the presumed recognition helix. P2 Cox recognizes a sequence repeated at least six times in the different targets, while WΦ Cox seems to recognize a single direct repeat. In contrast to P2 Cox, WΦ Cox binds with a stronger affinity to the switch region than to the attachment site (*attP*). The Cox proteins induce a strong bend in their DNA targets, strengthening the hypothesis that they have a structural role at site-specific recombination. Both proteins show a capacity to oligomerize, but P2 Cox has a higher tendency to form oligomers than WΦ Cox.

The P2 integrase mediates site-specific recombination leading to integration or excision of the P2 genome in or out of the host chromosome. P2 Cox controls the direction by inhibiting integration and promoting excision. In this work it is shown that Cox and Int bind cooperatively to *attP*.

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”Med en gnutta fantasi får man panik”

Jesper Waldensten

”Phages are small packages of genes traveling together in time and space”

Anders Nilsson

List of publications

This thesis is based on the following papers, which will be referred to in the text by Roman numerals.

I. Renberg-Eriksson, S.K., **Ahlgren-Berg, A.**, DeGrooth, J., and Haggård-Ljungquist, E. 2001. Characterization of the developmental switch region of bacteriophage P2 Hy *dis*. Virology 290:199-210.

II **Ahlgren-Berg, A.**, Henriksson-Peltola, P., and Haggård-Ljungquist, E. The oligomeric states of the two immunity repressor proteins of the heteroimmune coliphages P2 and WΦ, and their effects on DNA topology. Manuscript.

III **Ahlgren-Berg, A.**, Eriksson, J.M., and Haggård-Ljungquist, E. A comparative analysis of the multifunctional Cox proteins of the two heteroimmune phages P2 and WΦ. Manuscript.

IV Frumerie, C., Sylwan, L., **Ahlgren-Berg, A.**, Haggård-Ljungquist, E. 2005. Cooperative interactions between bacteriophage P2 integrase and its accessory factors IHF and Cox. Virology 332: 284-294.

Contents

Abbreviations

A	adenine
aa	amino acids
attB	attachment site, bacterium
attP	attachment site, phage
bp	base pairs
C (or CI)	C (or CI) immunity repressor protein
CAT	chlorampenicol acetyltransferase
Cox	control of excision
DNA	deoxyribonucleic acid
EMSA	electromobility shift assay
HTH	helix-turn-helix
IHF	integration host factor
Int	integrase
kDa	kilo Dalton
kb	kilobases
mRNA	messenger RNA
nt	nucleotides
RNA	ribonucleic acid
RNAP	RNA polymerase
T	thymine
wt	wildtype

Introduction

Bacteriophages in a historical context

Bacterial viruses, phages, are the most abundant group of genetic entities on planet earth. They are much more common than their bacterial hosts and if there is an estimated number of 10 million bacterial species there could be as many as 100 million phage species. A majority of the phages are still unknown and therefore there might be several billions of yet unknown open reading frames (potential proteins) (Rohwer, 2003). This represents a gene pool even more over-whelming than the enormous gene pool in the world's rainforests. Phages were recognized as biological entities almost a hundred years ago when they were described by Twort in 1915 and d'Herelle in 1917. d'Herelle immediately showed an interest in the capability of phages to kill pathogenic bacteria and was encouraged by early experiments in which he, for example, treated humans suffering from dysentery caused by *Shigella dysenteriae* using phages. d'Herelle played an important role in establishing a phage institute in Tbilisi, Georgia, which is still active in pursuing phage therapy (Merril *et al.*, 2003). Worldwide, a lot of work was put into phage therapy but when antibiotics were discovered the world thought it now had a cure for most bacterial diseases. This stayed true for many years but their widespread use have resulted in a fast increase of antibiotic-resistant bacterial strains, 70% of hospital acquired bacterial infections in the US are now resistant to one or more of the commonly used antibiotics. Concerns over the increase of antibiotic resistant strains have led to a new interest in phage therapy and there have been many successful attempts (reviewed in Merril *et al*., 2003 and reported on the ASM conference "The New Phage Biology" in Florida in 2004).

The most abundant phage group is the order of *Caudovirales*, tailed phages with a double-stranded DNA, which constitute 96% of the known phages (Ackermann, 1999). There are an estimated 10^{30} (1000000000000000000000000000000) tailed phages in the biosphere and they are believed to play a major role in important environmental processes (Brüssow and Hendrix, 2002). Many of these tailed phages are temperate. Compared to a lytic phage, which will immediately programme the synthesis of viral progeny that is released from the host cell, a temperate phage is capable of establishing a stable coexistence with its host. The viral genome is generally integrated as a prophage in the chromosome of the host by the

activity of a phage encoded integrase, some prophages are however maintained as plasmids, e.g. P1. During the process of prophage induction, the phage DNA can be excised again and enter the lytic cycle. Prophages are very abundant and can constitute 10-20% of a bacterium's genome and they are important factors for horizontal gene transfer between bacteria (Casjens, 2003). Some prophages express "lysogenic conversion" genes with alter the properties of the host. Phage P2, for example, expresses genes rendering the host refractory to infection of phage λ, T5 and T-even phages (Calendar *et al.*, 1998) Some of the known "lysogenic conversion" genes of prophages will result in pathogenic bacterial strains by encoding the structural genes for e. g. diphtheria-, botulinum-, shiga- (pathogenicity factor of EHEC) and cholera toxin (Boyd and Brüssow, 2002).

The P2 family

Phage P2, the focus of this study, belongs to the family *Myoviridae* in the order *Caudovirales*. P2 was discovered when released from the lysogenic Lisbonne and Carrère strain of *Escherichia coli* at the same time as P1 and P3 in the laboratory of Giuseppe Bertani (Bertani, 1951). The P2 family is large with many members that were originally placed in the same group based on serological cross-reactivity with P2, inability to recombine with and serological unrelatedness to the well-studied phage λ (Bertani and Bertani, 1971) (Bertani and Six, 1988) (Nilsson and Haggård-Ljungquist, 2005). With some exceptions, such as 186 and HP1, they can not be induced by UV-light. P2 seems to be common in nature. In a study of the *E. coli* reference library, ECOR (Ochman and Selander, 1984), 25-30% of the strains contained P2-like prophages (Nilsson *et al.*, 2004). The late structural genes show at least 96% identity and could possibly be considered as different isolates of the same phage (Nilsson and Haggård-Ljungquist, 2001) while e.g. 186 is a more distantly related *E. coli* phage (Dodd and Egan, 1996). The P2-like genomes however contain a few genes differing substantially between the different isolates e.g. the *Z/fun* locus which is probably acquired by horizontal gene transfer (Nilsson *et al.*, 2004). P2-like phages may be more of symbionts than parasites on their host since they seem more prone to lysogenize their host than phage λ and carry lysogenic conversion genes beneficial to their host (Nilsson *et al.*, 2004). Since the isolation of P2, P2 related phages have been found in other proteobacteria, such as HP1 in *Haemophilus influenzae* (Esposito *et al.*, 1996), K139 in *Vibrio cholerae* (Nesper *et al.*, 1999), ΦCTX in *Pseudomonas aeruginosa* (Nakayama *et al.*, 1999), SopEΦ and Fels-2 in *Salmonella typhimurium* (McClelland *et al.*, 2001) and PSP3 in *Salmonella potsdam* (Bullas *et al.*, 1991). P2 can grow, not only on *Escherichia coli*, but also on species of *Haemophilus*, *Klebsiella*, *Salmonella*, *Serratia* and *Yersinia*, at least under laboratory conditions.

As a member of the *Myoviridae* family of phages, P2 has an icosahedral head and a long contractile tail which ends with a base plate that carries six tail fibers adhering to the bacteria during infection. The linear, 33.5 kb genome is covalently attached to the tail via the left cohesive end. The cohesive ends of the DNA permit circularization after infection (reviewed in Bertani and Six, 1988; Nilsson and Haggård-Ljungquist, 2005). Like most other temperate phages, the P2 genome is organized in three blocks. One block with genes necessary for lysogenization, another with those genes required for DNA replication (early lytic genes) and a third block encoding genes for making the capsid and lysis of the host (late lytic genes).

Lytic mode

As a temperate phage, P2 has the ability to enter two different life cycles after infection. The lytic cycle results in a fast release of new viral progeny ready for another round of infection. In the lysogenic cycle the P2 genome is integrated in the *E. coli* chromosome and rests as a prophage replicating along with the DNA of the host. A prophage can escape lysogeny by induction and enter the lytic pathway. A developmental, or transcriptional, switch determines which pathway the phage will enter and this will be described in detail in section "Developmental switches of temperate phages". During lytic growth, transcription is initiated immediately after infection and the genes involved in DNA replication (early lytic genes) are switched on. Replication is initiated by the A protein and the DNA is replicated via a modified rolling-circle mechanism (Schnös and Inman, 1971) (Chattoraj, 1978) (Odegrip and Haggård-Ljungquist, 2001). The B protein, believed to be a helicase loader, is also required (Odegrip *et al.*, 2000). Initiation of late gene transcription does not only require ongoing replication but also the transcriptional activator Ogr, containing a zinc finger motif. Ogr is known to interact with RNA polymerase (RNAP) and will activate the four late promoters leading to expression of structural genes and genes needed for lysis of the host cell (Wood *et al.*, 1997) (Ayers *et al.*, 1994). 95% of P2 infections lead to lytic development (Saha *et al.*, 1987b).

Lysogeny

If conditions are such that lysogeny is favoured, the P2 genome will integrate into the host chromosome by site-specific recombination between the phage attachment site, *attP*, and the bacterial attachment site, *attB*, mediated by the P2 encoded integrase. P2 has one preferred integration site but can go to several other sites if location I is occupied or missing. The integration event also requires the host encoded IHF (Integration Host Factor) which serves a structural role (Saha *et al.*, 1990). This system is similar to that of phage λ (Ptashne, 1992). The junctions between the prophage and the bacte-

rial DNA are recognised as *attR* and *attL*. The only phage encoded protein needed for maintenance of lysogenic growth is the C repressor protein repressing the early lytic genes. Excision of the prophage as result of induction is dependent on an additional phage encoded protein, Cox (Control of Excision), and this will be described further in section "Site-specific recombination in P2". C and Cox are the main characters of this thesis. Cox is particularly interesting since it is multifunctional carrying out three different functions; repressing the lysogenic promoter, directing the site-specific recombination and activating a promoter in satellite phage P4.

Transcription

The unique information carried by all organisms in their DNA must be interpreted, first by RNA polymerase (RNAP) during transcription generating messenger RNA (mRNA) and then by ribosomes in the process of translation which results in proteins. Any step can be a target for regulation but the initiation of transcription seems to be the most frequent target. Regulation can be due to DNA structure, by proteins acting as transcription factors or by a combination of these two mechanisms. Phages, in all their simplicity, are excellent for studying transcription regulation and thanks to research on mainly phage λ we have gained a lot of insight into how transcription factors work. *E. coli* has been estimated to encode between 300 and 350 regulatory DNA-binding proteins, 159 regulatory proteins are already characterized. The current numbers of activators, repressors and dual proteins (functioning as both repressors and activators) show a fairly even distribution among them. Considering the diverse environmental conditions where *E. coli* can grow it makes sense with several hundred of transcriptional regulators (Pérez-Rueda and Collado-Vides, 2000). Some transcription factors interact with RNAP while others perform their functions in other ways. Today the exact interaction sites with the *E. coli* core RNAP are identified for only seven of all the transcription factors that are known to directly interact with core RNAP. Three out of these seven are phage encoded and that clearly shows how important the phage systems are to gain further insight into transcription regulation (Severinov, 2000).

RNA polymerase

The *E. coli* RNAP is a complex machinery containing several subunits. Four subunits constitute the core enzyme: two α subunits, one β subunit and one β' subunit ($\alpha_2\beta\beta'$) (Browning and Busby, 2004) (Wagner, 2000). Some researchers include the small ω subunit when describing the core enzyme even though it has no direct role in transcription. The core enzyme is competent for transcription but not for promoter-directed transcription initiation. This

process requires the holoenzyme which is the core enzyme associated with a σ factor. The crystal structure of *Thermus aquaticus* RNAP holoenzyme complexed with promoter DNA has been determined (Murakami *et al.*, 2002). All of the sequence specific RNAP contacts with the core promoter elements are mediated by the σ factor and it facilitates unwinding of the template DNA near the start site for transcription. DNA-binding regions of the σ factor become accessible when it associates with the core enzyme, and a polymerase-promoter complex can be formed (Burgess and Anthony, 2001). σ factors are multi-domain proteins that have three or four domains and domains 2, 3 and 4 are known to be involved in the recognition process (Murakami *et al.*, 2002) (Browning and Busby, 2004). In *E. coli*, there are six σ factors recognizing different promoters types. Depending on the σ factor associated with the core RNAP, different types of promoters will be recognized and used for transcription. The most common σ factor in *E. coli* is the σ^{70} factor responsible for transcription of the "housekeeping" genes, i.e. most of the genes that are being transcribed during exponential cell growth (Browning and Busby, 2004) (Wagner, 2000). The two large β and β' subunits form a crab-claw structure where the cleft contains the active site (Murakami *et al.*, 2002).

DNA elements important for initiation of transcription

Several different sequence elements affect the strength of the promoter and may contribute to a varying degree at different promoters. The two most common elements of a "consensus" σ^{70} promoter are the two structural elements that constitute the core promoter. Both elements are conserved hexameric sequences, 5'-TTGACA-3', known as the -35 region and 5'-TATAAT-3', referred to as the -10 region. These two hexamers will be recognized and bound by the σ^{70} factor (Busby and Ebright, 1994) (Helmann and deHaseth, 1999) (Rivetti *et al.*, 1999) (Gourse *et al.*, 2000). Promoters with several mismatches compared to the consensus sequences can still be recognized and bound by the RNAP; sometimes a -35 region is not recognized at all. There is however a strong correlation between similarity with the consensus sequences and promoter strength (Busby and Ebright, 1994). Transcription starts 5 to 9 nucleotides downstream of the -10 region; the transcription start site is traditionally called +1.

The two hexamers are separated by a stretch of 17 (+/-1) nucleotides, a region known as the spacer. The primary nucleotide sequence of the spacer is generally not of importance for the recognition of the promoter. Instead, it is the number of nucleotides positioning the -35- and -10 regions at an optimal angle and distance from each other that is important. Promoters with non-optimal spacing are sensitive to the twist of the DNA, i.e. the rotations of the two strands around the helix axis, since it will alter their relative orientation (Pérez-Martín *et al.*, 1994) (Wang and Syvanen, 1992). There is also plasticity in the holoenzyme that, together with RNAP induced bending, can

correctly position the -35 and -10 regions relative to each other (Murakami *et al.*, 2002). The -35 and -10 regions are recognized by domain 4 and 2 of the σ factor respectively.

"Extended –10" promoters compensate for a poor -35 region with a TG-motif found immediately upstream of the -10 region and can be found in over 20% of the *E. coli* promoters. This extended motif creates alternative contact points for RNAP via domain 2 of the σ subunit which interacts with bases in the minor groove and with the DNA backbone along the minor groove (Barne *et al.*, 1997) (Burr *et al.*, 2000; Ross *et al.*, 2001). "Extended -10" promoters are frequently found in other genomes but *E. coli*. The *Bacillus subtilis* phage Φ29 has this motif in most of its promoters (Camacho and Salas, 1999).

An upstream (UP) element is found upstream some promoters where it increases the promoter activity by providing a point of contact for the α subunit of RNAP. These are AT-rich sequences, approximately 20 base pairs in length, that are found upstream of the -35 hexamer and that interacts with the C-terminal domain of the α subunit of the RNAP, thereby strengthening the interaction between the promoter and the RNAP (Ross *et al.*, 1993) (Gourse *et al.*, 2000) (Yasuno *et al.*, 2001). Since A-tracts can result in intrinsic DNA bending it was firs suggested that the curvature in itself facilitated transcription but empirical evidence points at the fact that these AT rich regions enhance transcription through the interaction between the DNA and α subunit of RNAP, even if the bending might also influence the outcome (Gourse *et al.*, 2000). It has lately been discovered that sequence–independent upstream DNA interactions with the α subunit of RNAP are major contributors to initiation at many promoters, not only promoters containing a specific UP element (Ross and Gourse, 2005). *U*pstream *a*ctivating sequences (UAS) can sometimes be recognized upstream of the UP elements. These sequences are intrinsically curved; i.e. the DNA is curved because of sequence characteristics within the DNA. This intrinsic curvature upstream of promoters has been shown to affect the activity of the promoter as long as the angular position relative to the promoter is fixed. They can work by increasing the interaction surface between promoter DNA and the RNAP, or by affecting later steps in the initiation of transcription like DNA unwinding and strand opening (Pérez-Martín *et al.*, 1994) (Wagner, 2000).

Initiation of transcription

A schematic description of transcription can be divided into three steps: initiation, elongation and termination. The initiation can further be divided into four subsequent steps, promoter localization and formation of the closed RNAP-promoter complex, isomerization to an open complex, abortive initiation, and promoter clearance (Coulombe and Burton, 1999) (Hsu, 2002) (Browning and Busby, 2004). Since RNAP is known to bind very fast to a

promoter, a one-step binding model where the RNAP localizes the promoter through three-dimensional diffusion is not possible. Instead binding is described in a two-step model where the first step is non-specific binding of RNAP to DNA followed by sliding of RNAP along the DNA molecule until it encounters a promoter to which it will bind specifically forming the closed complex (Guthold *et al.*, 1999). The σ^{70} factor will first bind to the -35 region and subsequently to the -10 region. Binding to these regions induces a bend in the DNA since protein interactions with both hexamers require distortion of the DNA. Formation of the closed complex is reversible and is described by an equilibrium binding constant.

Isomerization from closed to open complex involves melting of about one helical turn of the double stranded promoter at the start site. Untwisting of the spacer region precedes strand opening and will destabilize the -10 region. The untwisting is caused by conformational changes in both DNA and RNAP. This changes are probably the result of bending and extensive wrapping of DNA around the polymerase, the promoter DNA wraps nearly 300^{o} around the polymerase (Rivetti *et al.*, 1999) (Murakami *et al.*, 2002). The interactions between UP elements and the C-terminal domain of the α subunit are thought to participate in this process (Rivetti *et al.*, 1999). The -10 sequence is supposed to be important for opening of the strands because of its relative thermodynamic instability due to the many adenosines and thymines. Intrinsic DNA bends, strategically distributed throughout the promoter region, can be favorable for both wrapping DNA around the RNAP and lowering the energy needed for DNA unwinding (Rivetti *et al.*, 1999) (Murakami *et al.*, 2002).

Once the open complex is formed, nucleotides can be recruited to the complex. Only if the nucleotide in the catalytic site is complementary to the template strand, it will be incorporated into a growing RNA chain. The first transcripts produced will be short and released after incorporation of usually less than 10 nucleotides. This abortive transcription can be repeated for many cycles. The RNAP will not leave the promoter and remains associated with the σ factor. To generate productive transcripts the interaction between the RNAP and the promoter has to be broken. The RNAP-promoter interactions break when the transcript becomes sufficiently long to stabilize the elongation complex. At this point the σ factor is often released and major conformational changes of the polymerase enables stable elongation for thousands of nucleotides. This is known as promoter clearance or promoter escape (von Hippel, 1998) (Hsu, 2002).

Transcription regulation

Every step during transcription can be a target for regulation but for energy conserving reasons it is common to regulate transcription at the initiation stage. Transcription can be regulated by two different mechanisms; the

structure of DNA and the action of transcription factors. During the initiation of transcription, activators and repressors can affect closed complex formation as well as open complex formation and promoter clearance. It is common that the structure of DNA and transcription factors act in concert to influence the expression of the same gene(s).

Regulation by transcription factors

As will be illustrated in the examples of transcription regulation mentioned below, a complex picture often emerges. The action of one transcriptional regulator can often affect two promoters in the same region simultaneously but with different outcome, sometimes even indirectly when transcription from two promoters are mutually exclusive. A consequence of this is that repression of one promoter directly or indirectly often results in activation at another promoter. There are very seldom simple examples! There are also examples, often from the phage world, where it is the concentration of the regulator that decides whether a promoter will be activated or repressed. To further render complexity to the picture, several proteins often work in concert for their final action on a promoter.

Transcription factors form the links between environmental signals and gene expression. For the cell to function efficiently, regulation must be reversible. Some transcription factors work as activators while others repress certain promoters. The members of the LysR family are examples of dual proteins activating several genes and repressing their own expression (Pérez-Rueda and Collado-Vides, 2000). The cAMP receptor protein, CAP or CRP, is another example of a regulator playing a dual role. Certain promoters, such as the *lac* P1 promoter, are activated but the *gal* P2 promoter is repressed by the action of CAP (Wagner, 2000). For several regulators with dual roles, the effect on a certain promoter is often only a matter of the location of the operator relative to the promoter. By moving the operator to a different location relative to the promoter, a repressor might act as an activator, or vice versa, on the very same promoter. Generally it can be said that a transcription factor will act as a repressor when the operator is found overlapping with the core promoter or located downstream of the promoter overlapping with the transcriptional start point. If the transcription factor recognizes an operator located upstream the promoter it will probably function as an activator. There are however many exceptions where the location of the operator does not necessarily tell if it is an activator or a repressor.

Transcription factors generally recognize and bind to symmetrical sequences, in most cases inverted repeats (Wagner, 2000). Some transcriptional regulators have several binding sites where a high affinity binding site stimulates binding to a low affinity site through cooperativity and a stable oligomer is formed. Sometimes these sites can be separated by as much as several kb of DNA and this will require a looping of the DNA to accommodate cooperativity (Révet *et al.*, 1999) (Schleif, 2003). The outcome of regulation

might be different if the occupation of different sites is affected by the concentration of the transcriptional regulator.

Most transcription factors are active as even-numbered multimers, e.g. as dimers, tetramers or even octamers. They often have a modular organization with one domain responsible for DNA binding and one for oligomerization. The DNA binding site is commonly a helix-turn-helix motif (HTH) (Pabo and Sauer, 1992). Of the estimated 314 transcription regulators in *E. coli*, 248 are known or predicted to have a HTH motif (Pérez-Rueda and Collado-Vides, 2000). The oligomerization domain usually has one of the common protein-protein interaction motifs such as an amphipatic helix or a leucine zipper. Some factors that are directly interacting with RNAP can in addition to these domains have an activation domain, often identified by a patch of acidic amino acids or other common protein-protein binding motifs that contact the RNAP (Wagner, 2000).

Repression by transcription factors

Repression can often be explained by the fact that the repressor inhibits RNAP from binding to the promoter due to steric hindrance. This happens if binding of the repressor to its operator cannot take place at the same time as RNAP binds to the promoter and the binding constant is higher for the repressor-operator complex. A repressor that covers the core promoter region often excludes RNAP (Fig. 1). A classical example is binding of the CI repressor protein of bacteriophage λ which represses transcription from promoter P_R by steric hindrance (Ptashne, 1992) and LacI (LacR) repressing the transcription of the *lac* operon (Wagner, 2000).

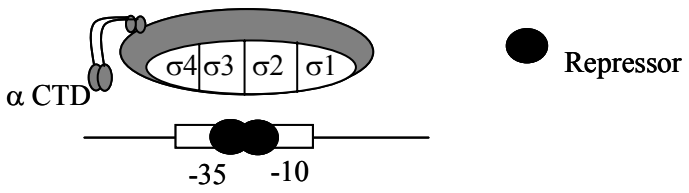


Figure 1. Repression by steric hindrance. Repression is mediated by a direct masking of the core promoter inhibiting RNAP binding. The C-terminal domain of the α subunit (αCTD) and the four domains of the σ^{70} factor (σ1, σ 2, σ 3, σ 4) are shown (modified from Browning and Busby, 2004).

Repression can also be due to inhibition of promoter melting (isomerization from closed to open complex) or inhibition of promoter clearance by direct contact with RNAP or by RNAP collision with a DNA bound repressor. At

these scenarios, the repressor is bound further away or on the opposite side of the DNA helix (Rojo, 2001). Repression can be fine-tuned by an inducer that binds to the repressor and thereby decrease the affinity of the repressor for the operator, or by co-repressors that increase the affinity. These small molecules are often intermediates or end products synthesized by the respective gene(s) to be regulated. LacI for example, is not able to bind to its operator when the inducer allolactose is present and interacts with LacI (Wagner, 2000). The action of repressors can also be altered by anti repressors, e.g. the ε protein expressed by phage P4 that derepresses the early lytic promoter in phage P2 by interacting with the C repressor protein (Giesselsoder *et al.*, 1981) (Liu *et al.*, 1997) (Liu *et al.*, 1998).

Activation by regulators interacting with RNAP

A weak binding of RNAP to a promoter, which can be due to a poor –35 region, can be compensated for by the action of an activator. This activation is often a result of cooperativity in DNA binding of the activator and the RNAP and the activator is commonly said to recruit RNAP (Ptashne and Gann, 2002). Activators binding to promoter proximal sites primarily act by making direct contact with RNAP, i.e. the common definition of activation by recruitment. These interactions increase the affinity of RNAP to the promoter (Busby and Ebright, 1994) (Hochschild and Dove, 1998). Ptashne and Gann (Ptashne and Gann, 1997) however give the definition "activation by recruitment" a broader sense than just increasing the formation of the closed complex. The formation of an open complex is part of the recruitment process if the polymerase is bound stably to DNA only upon isomerization to the open complex. Some activators are therefore said to work by recruitment if they stabilize RNAP binding by increasing open complex formation.

The different mechanisms for activation by recruitment of RNAP are often dependent on the binding site of the activator (Browning and Busby, 2004). Some activators bind to an operator located upstream the core promoter and interact with the C-terminal domain of the α subunit of RNAP (Fig. 2A). This is exemplified by CAP (or CRP) at the *lac* P1 promoter where it stimulates the initial binding of RNAP (Busby and Ebright, 1997). The flexible linker between the N- and C- terminal domains in the α subunit allows an activator to bind at different upstream locations. CAP activates transcription initiation at more than 100 different *E. coli* promoters by different mechanisms. CAP can also, together with the λ CI repressor protein, be used to exemplify a second category of activation where the activator binds adjacent to the -35 region (usually around - 42) (Ptashne and Gann, 1997) (Busby and Ebright, 1997) (Li *et al.*, 1994). CAP activates the *gal* P1 promoter by stimulating both initial binding and isomerization by interacting with both the C- and the N-terminal domains of the α subunit (Busby and Ebright, 1997). λ CI activates the P_{RM} promoter by interacting with domain 4 of the σ factor of RNAP (Fig. 2B) and at the same time represses the P_R promoter (Ptashne, 1992). The N-terminal

domain of λ CI contains a HTH motif and an acidic patch of residues exposed on the first helix mediates activation. Two basic residues in domain 4 of the σ factor of RNAP are critical for the activation by CI, one introduces a bend in the DNA whereas the other interacts directly with CI and it also facilitates the interaction between the σ factor and DNA at this specific promoter (Nickels *et al.*, 2002). The effect of λ CI is primarily on the isomerization step by stabilizing an intermediate between the closed and open complex that is rate limiting (Dove *et al.*, 2000). Whether an activator stimulates formation of the closed or open complex might just be a question of when the interacting surfaces of the activator and RNAP are correctly oriented for interaction (Dove *et al.*, 2000).

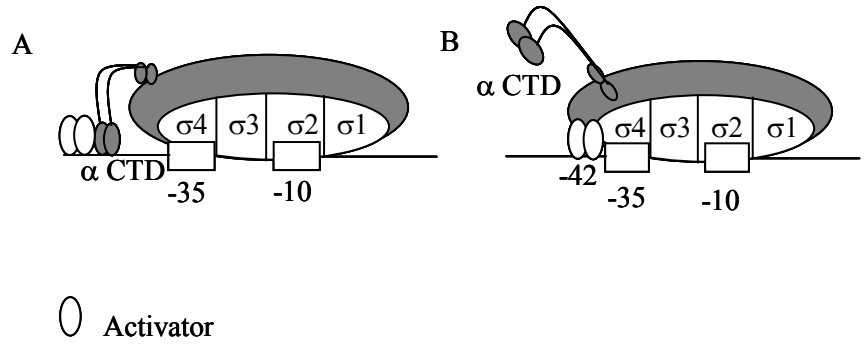


Figure 2. Examples of activation by transcriptional activators. **A**. The activator is bound to an upstream site and contacts the C-terminal domain of the α subunit (αCTD), thereby recruiting RNAP to the promoter. **B**. The activator, e.g. λ CI activating P_{RM}, binds adjacent to the – 35 region (around – 42) and interacts with domain 4 of σ^{70}. The four domains of the σ^{70} factor (σ1, σ 2, σ 3, σ 4) are shown (modified from Browning and Busby, 2004).

Transcription regulation by intrinsic DNA curvature

Considering the importance of wrapping of the promoter DNA around RNAP for formation of a productive transcription complex, it is not hard to see how bending of DNA by different means can have an influence on the regulation of transcription initiation. There is a clear relationship between promoter strength and the presence of upstream regions of intrinsically curved DNA (Pérez-Martín *et al.*, 1994) (Wagner, 2000) (Pérez-Martín and de Lorenzo, 1997). Approximately 50% of the strongly curved sequences in bacterial DNA are found upstream of promoters (Wagner, 2000). The sequence context of the DNA can cause intrinsic curvature, the most common reason being clusters of adenosines and thymines, i.e. A-tracts, at a regular spacing (e.g. UAS). Intrinsically curved DNA is deformed even in the absence of

external factors, such as DNA binding proteins, and has a very rigid structure (Pérez-Martín and de Lorenzo, 1997). These regions can affect transcription in two ways. Curvature can increase the local concentration of interacting groups by bringing different sites closer together or by extending the regions of interacting surfaces between DNA and RNAP, thereby increasing the formation of the closed complex. At other promoters the intrinsic curvature facilitates the melting of the double stranded DNA, i.e. conversion from closed to open complex. Curvature can also provide binding sites for transcription factors.

It is important however to remember that curvature can work in both directions, i.e. both enhance and inhibit transcription. The fact that RNAP can bend promoter DNA to form a transcriptionally active complex predicts that any factor which may bend the same target in the opposite direction should act as a repressor (Pérez-Martín and de Lorenzo, 1997). It has also been shown that the strength of promoters with an UAS region often is rate limited at the step of promoter clearance and that a delay in promoter escape is seen (Strainic *et al.*, 1998).

DNA bending induced by transcriptional regulators

Bends in a flexible region of DNA can be caused by proteins and result in either activation or repression. It seems to be the rule rather than the exception that activators bend their DNA target and there are many examples in the literature (Pérez-Martín *et al.*, 1994). Nucleoid proteins, e.g. IHF, the histone like HU protein, and FIS (Factor for Inversion Stimulation) can influence transcription by introducing bends in the DNA (McLeod and Johnson, 2001) (Xu and Hoover, 2001). These proteins often work in concert with specific transcription factors. IHF was first identified as an essential protein for site-specific recombination in phage λ but has now been recognized to influence transcription from a large number of *E. coli* promoters. IHF creates the sharpest bend of all prokaryotic DNA-binding proteins known and can cause the DNA to form a 180^{o} U-turn. Even though IHF is known to directly repress or activate transcription from certain promoters without other regulators participating (e.g. the early lytic promoter of phage Mu) the most common effect of IHF is to enable other proteins to fulfill their functions by causing the conformational change required. The lytic-lysogenic switch of phage Mu actually represents both scenarios. When the concentration of the CI repressor for lytic growth is low, a bend caused by IHF binding in the region stimulates transcription from the lytic promoter and transcription from the lysogenic promoter is repressed. An increase in the amount of CI repressor protein will result in repressor binding to sites adjacent to and overlapping the lysogenic promoter assisted by the same DNA bend introduced by IHF. This will lead to repression of the lytic promoter (Alazard *et al.*, 1992) (Gama *et al.*, 1992).

Repressors bending the DNA, or causing a loop if they work over large distances, create unfavorable conformation of the promoter region resulting in exclusion of RNAP or reduction in isomerization or promoter clearance (Fig. 3).

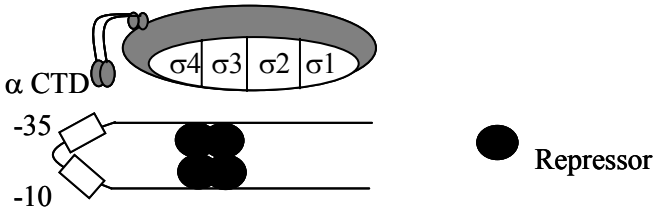


Figure 3. Repression by bending or looping. The intervening promoter is repressed due to unfavorable configuration of the DNA. The C-terminal domain of the α subunit (αCTD) and the four domains of the σ^{70} factor (σ1, σ 2, σ 3, σ 4) are shown (modified from Browning and Busby, 2004.

Two GalR repressor dimers bound to two binding sites in the *galETK* region causes a loop by oligomerizing. This will repress promoter P1, which is found on the inside of the loop, while promoter P2 in the same region is stimulated by its position on the outside of the loop. On the outside of the loop is a CAP binding site which also needs to be occupied for this activation. If nucleotides corresponding to half a helical turn are inserted, the outcome will be the opposite with a stimulated P1 promoter on the outside of the loop (Wagner, 2000). Another example is the negative autoregulation by the CI repressor on the P_{RM} promoter in λ. By causing a loop over a large distance, a conformation of the DNA unfavorable for transcription is generated (Dodd *et al.*, 2004).

In some cases an induced bend is sufficient for activation of a promoter by assisting RNAP binding. At other promoters, a direct contact between the activator and RNAP is necessary for activation. In the *Bacillus subtilis* phage Φ29 the p4 protein, together with the p6 protein, is essential for switching from transcription from the two early promoters, A2c and A2b, to the late promoter A3 (Camacho and Salas, 2001). p4 has two DNA binding regions in the vicinity of these three promoters and upon binding to these sites a bend is generated. It seems like bending is induced whenever p4 is able to bind and a similar final angle is produced no matter if a site contains an intrinsic curvature or not (Pérez-Lago *et al.*, 2005). The p4 induced bend could destabilize the transcription complex at A2c and at the same time p4 is positioned in close proximity to RNAP at A3 favoring the known interaction between p4 and the α subunit of RNAP (Pérez-Lago *et al.*, 2005).

Strand opening is greatly influenced by supercoiling of DNA and superhelical density may control expression of certain genes (Wang and Syvanen, 1992). In general it can be said that negative supercoiling facilitates melting of a promoter. The degree of supercoiling is sensitive to the energy state of the cell because topoisomerase II (or gyrase), responsible for introducing negative coils, requires ATP while topoisomerase I, that relaxes negatively coiled DNA, does not (Drlica, 1990) (Drlica, 1992). This is an ingenious way of the cell to control expression of genes according to environmental conditions.

Developmental switches of temperate phages

Extensive studies of the temperate phage λ gave us the first insights into the molecular mechanisms behind a genetic switch of a temperate phage (Ptashne *et al.*, 1980) (Ptashne, 1992). Today there is a vast number of papers published on the ability of λ to replicate either lysogenically or lytically, and the fairly simple picture that once emerged is today far more complex than originally thought with new modes of transcription regulation being described in recent reviews (Dodd *et al.*, 2005; Friedman and Court, 2001; Hochschild, 2002). A temperate phage has ultimately two pathways to choose between; the lytic or lysogenic. The ability to replicate in either of these two ways is tightly regulated by a developmental or transcriptional genetic switch. To maintain the lysogenic state, the only phage encoded protein that is required is the immunity repressor protein which represses transcription of viral genes involved in lytic growth. During lytic growth the production of the repressor must be shut down so that the viral genes needed for lytic growth can be switched on. These two states are mutually exclusive and the two promoters from which the repressor and genes for lytic growth are transcribed can never be active simultaneously.

In λ, the decision made after infection is effected by the stability of a single phage encoded protein, CII, which is expressed immediately after infection. If CII is rapidly degraded by proteases the phage will enter the lytic pathway (Ptashne, 1992). Lysogeny can be escaped by prophage induction. In λ and phage 186, prophage induction is triggered by the host SOS response which is activated by damage to the host DNA, while P2 can not be induced by the SOS system (Ptashne, 1992) (Lamont *et al.*, 1989) (Bertani, 1968).

The genetic switch of phage λ

Promoters and operators

During lysogenic growth, transcription of the majority of the viral genes must be turned off. These include the genes needed for lytic growth, which

are arranged in a sequential and temporal manner. In the λ prophage, the two early lytic promoters, P_R and P_L, are both repressed by one of the key components of the switch, the CI repressor. These promoters partially overlap with the two CI operator regions, O_R and O_L respectively (for locations of promoters/operators and repressors see Fig. 4A). Each operator region (O_R and O_L) contains three 17 bp operator sites, each containing an inverted repeat, O_R1, O_R2, O_R3 and O_L1, O_L2, O_L3, respectively. Since there are slight variations in the sequences of the operator sites, the affinity of CI varies, with that for $O_R1 > O_R2 \approx O_R3$ and $O_L1 > O_L2 \approx O_L3$. In the lysogen, two CI repressor dimers bind cooperatively to O_R1/O_R2 and O_L1/O_L2. This cooperativity results in occupation of both O_R2 and O_L2 at concentrations normal for a lysogen and is essential for maintaining lysogeny since it results in repression P_R and P_L (Fig. 4B). In addition, the CI dimer bound at O_R2 positively autoregulates its own promoter, P_{RM} (for repressor maintenance), by interacting with RNAP (Li *et al.*, 1994).

Negative regulation of P_{RM} by CI

If the concentration of CI in the cell increases further, the low affinity sites O_L3 and O_R3 will also be occupied in the order mentioned. CI binding to O_R3 will repress the P_{RM}, thus *cI* transcription is both negatively and positively autoregulated. The physiological significance of the negative autoregulation has however been questioned because of the low occupancy of O_R3 seen in lysogens (Hochschild, 2002). The missing level of regulation was recently discovered and it depends on long-range interactions between CI molecules bound at the two operator regions O_R and O_L. The ability of DNA bound CI molecules to interact over very large distances (3.6 kb) was first discovered by Révet et al in 1999 when they showed how CI repressor tetramers preformed on O_R1/O_R2 and O_L1/O_L2 interacted to form octamers.
The CI repressor is a dimer at physiological concentrations (Ptashne, 1992) and for tetramers and octamers to form in solution it requires a hundredfold higher concentration than what is normal in a lysogenic cell where formation of higher order oligomers requires DNA (Dodd *et al.*, 2004).

The CI repressor consists of two domains connected by a linker. The N-terminal domain mediates DNA binding through a HTH motif and is also responsible for contacts with RNAP. The C-terminal domain mediates dimerization as well as cooperative binding resulting in tetramers and octamers on the DNA. Two monomers use identical surfaces of each subunit (head-to-head) for dimerization wherefore further oligomerization using the same surfaces is not possible. The dimer-dimer association, which will only take place when DNA is provided as a scaffold, is however atypical since the two dimers use different interacting surfaces (head-to-tail). Consequently further oligomerization is possible using the same set of interacting surfaces

as used in the dimer-dimer interaction, irrespective of the length of the intervening DNA (Bell *et al.*, 2000).

Tetramers (pairs of dimers) bound to O_R1/O_R2 and O_L1/O_L2 will octamerize, thereby causing a loop of the intervening DNA, and further increase CI repression of P_R and consequently stabilize the lysogenic state (Bell *et al.*, 2000) (Svenningsen *et al.*, 2005) (Fig 4C.). This extra level of cooperativity increases the binding affinity of CI to the two operators thus lowering the concentration of CI needed for a stable lysogen (Svenningsen *et al.*, 2005). The negative autoregulation of P_{RM} has been shown to be dependent on the O_L region found 2.4 kb from the O_R and P_{RM} region. The O_L-CI-O_R loop juxtaposes O_L3 and O_R3 so that a CI dimer bound to O_L3 (higher affinity site than O_R3) can assist a dimer to bind at O_R3 and thereby repressing P_{RM} (Dodd *et al.*, 2004). This seems to be the primary role for O_L3 which previously had no known function in regulation of either P_L, P_R or P_{RM} (Dodd *et al.*, 2004). This additional level of regulation increases the tolerance of the switch to variations in CI repressor concentrations that is known to vary greatly from cell to cell in single-cell studies and the regulatory circuits maintaining a λ lysogen is considered to be extremely stable (Bæk *et al.*, 2003).

The role of λ Cro in the genetic switch

The first gene transcribed from P_R is *cro* which recognises and bind the same operator regions as CI but with the highest affinity for O_R3, the site closest to P_{RM}. The prevalent hypothesis has long been that Cro serves an important role at prophage induction by shutting down P_{RM} upon binding to O_R3, thereby ensuring that no CI is made which could interfere with the switch to lytic growth. This was supported by the findings that *O_R3-* prophages were inefficiently induced (Hochschild, 2002). However, it seems like the DNA loop between O_R and O_L renders the switch insensitive to the presence of Cro as judged by *in vivo* experiments using *cro+* or *cro-* cells to measure transcription from P_R. The switch becomes more unstable and sensitive to the presence of Cro only if the O_L region is missing. The inefficient induction of an *O_R3-* prophage is caused by an over-production of CI since O_R3 is necessary for CI down regulation of P_{RM} (Svenningsen *et al.*, 2005). The role of Cro needs to be re-evaluated. Direct repression of P_{RM} by Cro seems unlikely to be important in the lytic development. However, Cro might be important for weak repression of the early lytic promoters to allow progression into the late lytic phase and also to reduce the expression of CII. The defects in lytic growth in a *cro*$^-$ mutant are suppressed by a *cII* mutation (Svenningsen *et al.*, 2005) (Dodd *et al.*, 2005).

As mentioned above, CII is the protein effecting the decision after infection. It is very sensitive to host cell proteases and therefore very unstable. CII is encoded by the *cII* gene immediately downstream of the *cro* gene. It activates transcription from the P_{RE} promoter (for repressor establishment), which spans the *cro*/*cII* intergenic region upstream of P_{RM}, resulting in ex-

pression of *cI* (Ptashne, 1992). This will result in repression of lytic growth, not only by repression of P_R by CI, but also by interference of P_R transcription through transcription from P_{RE} since the two transcription complexes will run into each other. The transcription from P_{RE} will also result in antisense *cro* RNA that possibly could decrease production of Cro (Spiegelman *et al.*, 1972).

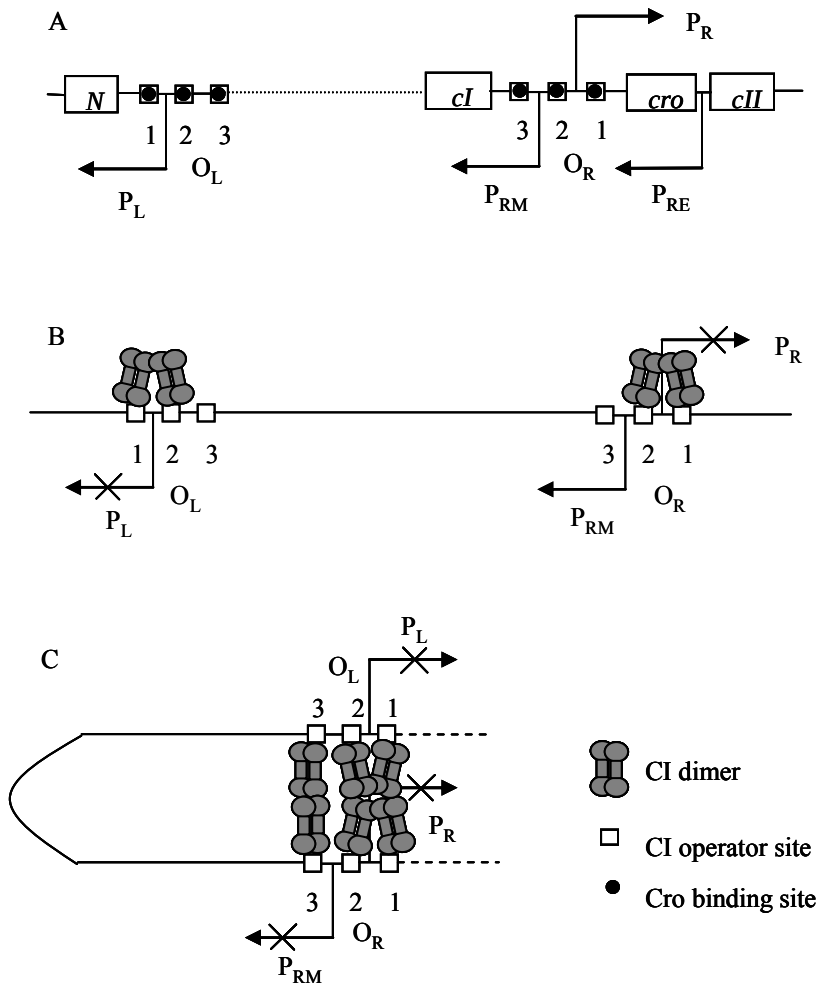


Figure 4. Schematic drawing of the λ immunity region. **A**. Locations of promoters/operators and the repressor genes *cI* and *cro*. **B**. During lysogenic growth, pairs of CI dimers are bound at $O_{L1/L2}$ and $O_{R1/R2}$, thereby repressing the lytic promoters P_R and P_L. **C**. Dimers bound at $O_{L1/L2}$ and $O_{R1/R2}$ octamerize resulting in a DNA loop repressing P_{RM}. See text above for details.

The λ prophage will be induced by DNA damage to the cell. When the SOS system is activated the host encoded RecA is transformed to an active form which will mediate autocleavage of the CI repressor (Ptashne, 1992).

The developmental switch of phage 186

186 is another well-studied temperate phage, unrelated to λ on a sequence level, and their genetic switches illustrate an independently evolved solution to a common need to choose between the alternative lifestyles, lysogeny or lytic growth. 186 belongs to the phages originally classified as a member of the large P2 family and is considered to be a coliphage. However detailed evolutionary studies indicate that 186 could be one of the P2 related phages originating from *Salmonella* and not *E. coli* (Nilsson, 2005). Many 186 CI repressor homologs are found in several *Salmonella* subspecies (Shearwin *et al.*, 2002). 186 is induced by UV damage to the host cell but, unlike λ CI, it is insensitive to activated RecA. 186 CI is instead reversibly inactivated by the SOS-induced 186 encoded protein Tum which is an anti-repressor that binds to CI and prevents it from binding to the CI operator (Shearwin *et al.*, 1998).

The region of 186 that encodes the genetic switch is equivalent to the O_R region of λ with the important difference that the lytic promoter pR and the lysogenic promoter, pL, are arranged face-to-face and not back-to-back as in λ (Dodd *et al.*, 1990) (for location of promoters/operators and repressors, see Fig. 5A). Like in λ, the early lytic promoter, pR, is strong compared to pL and convergent transcription from pR inhibits transcription from pL. This interference is important during lytic growth to ensure that early lytic transcription will occur without transcription from the lysogenic promoter (Dodd *et al.*, 1990) (Reed *et al.*, 1997) (Neufing *et al.*, 2001) (Dodd and Egan, 2002) (Callen *et al.*, 2004). The outcome of regulation by 186 CI is similar to that of λ CI but the mechanisms are different. 186 pR is strongly repressed by the 186 CI repressor and CI activates its own promoter, pL, by "relief-of-interference" while λ CI activates its own promoter by direct contact with RNAP (Dodd and Egan, 2002) (Li *et al.*, 1994). Elongation from pR must pass over pL to generate interference (Callen *et al.*, 2004). 186 pL releases open RNAP complexes much slower than pR, several open complexes can wait as "sitting ducks" to escape pL and this results in head-on collisions between these "sitting ducks" and elongating RNAP complexes transcribing from pR. In addition to the positive autoregulation of its own promoter, pL, 186 CI shows repression of pL at high CI levels in the lysogen. Four DNA regions that bind CI have been localized. The highest affinity site is at pR, where three copies of an inverted repeat that constitute the CI operator are found. On both sides of pR, approximately 300 bp away, two flanking operators are found (FL and FR) and finally a low-affinity site is found at pL. These operator sites are of two different types, A and B (Dodd and Egan, 1996) (Dodd and Egan, 2002). CI binds as an octamer to pR with one dimer free (there are

only three binding sites) to contact FL, FR or pL. Since FL and FR are higher affinity sites compared to pL, a loop will form between one of these sites and pR, and pL will be unrepressed (Fig. 5B). At higher CI levels, FL and FR will be occupied by CI multimers and the free dimer at pR will interact with pL thereby repressing it (Dodd and Egan, 2002) (Fig. 5C). 186 CI has several similarities to λ CI. The N-terminal domain contains the DNA binding HTH motif and by mutational analysis it has been shown that this motif can recognize both A and B type operator. The N-terminal domain dimerizes weakly and the C-terminal domain (connected to the N-terminal domain through a linker region) is responsible for self-association. The native form is a dimer that oligomerizes to octamers via tetramers (Shearwin and Egan, 1996) (Shearwin *et al.*, 2002).

Apl as a repressor in the genetic switch of 186

The analog to λ Cro is Apl, the first gene product transcribed from the early promoter pR. Apl however, in contrast to λ Cro, serves two distinct functions; as a repressor of pL and as an excisionase in site-specific recombination. The recombination events in λ, integration and excision, are controlled by the λ integrase and the excisionase, Xis. The reaction is integrative when only Int is present and excisive in the presence of Xis (Azaro and Landy, 2002). Apl is the functional equivalent to Xis and interacts with DNA in the *attP* region. It is necessary for excision of the prophage and most likely serves an architectural role (Dodd *et al.*, 1993) (Reed *et al.*, 1997). Apl repress transcription from both pR and pL by binding to seven directly repeated sequences between pR and pL. Apl binding overlaps both promoters enough to occlude RNAP but the majority of binding sites are found down stream the promoters. Therefore Apl might repress both by hindering binding of RNAP but also by inhibiting elongation. Repression of pL seems to be important in the initial stages of prophage induction but the protein serves no function in the lysis-lysogeny decision after infection since the lysogenization frequency is not affected by a defective *apl* gene (Dodd *et al.*, 1993) (Reed *et al.*, 1997).

An analog to the λ CII protein is required for establishment of lysogeny in 186. The 186 *cII* gene is found as the second gene in the pR operon, preceded by *apl*. After initial transcription from pR, CII will activate pE, an alternative promoter for CI production, which spans the *apl*/*cII* intergenic region upstream of pL by binding to an inverted repeat. Lysogenization is probably the result of the stability of CII (Neufing *et al.*, 1996) (Neufing *et al.*, 2001).

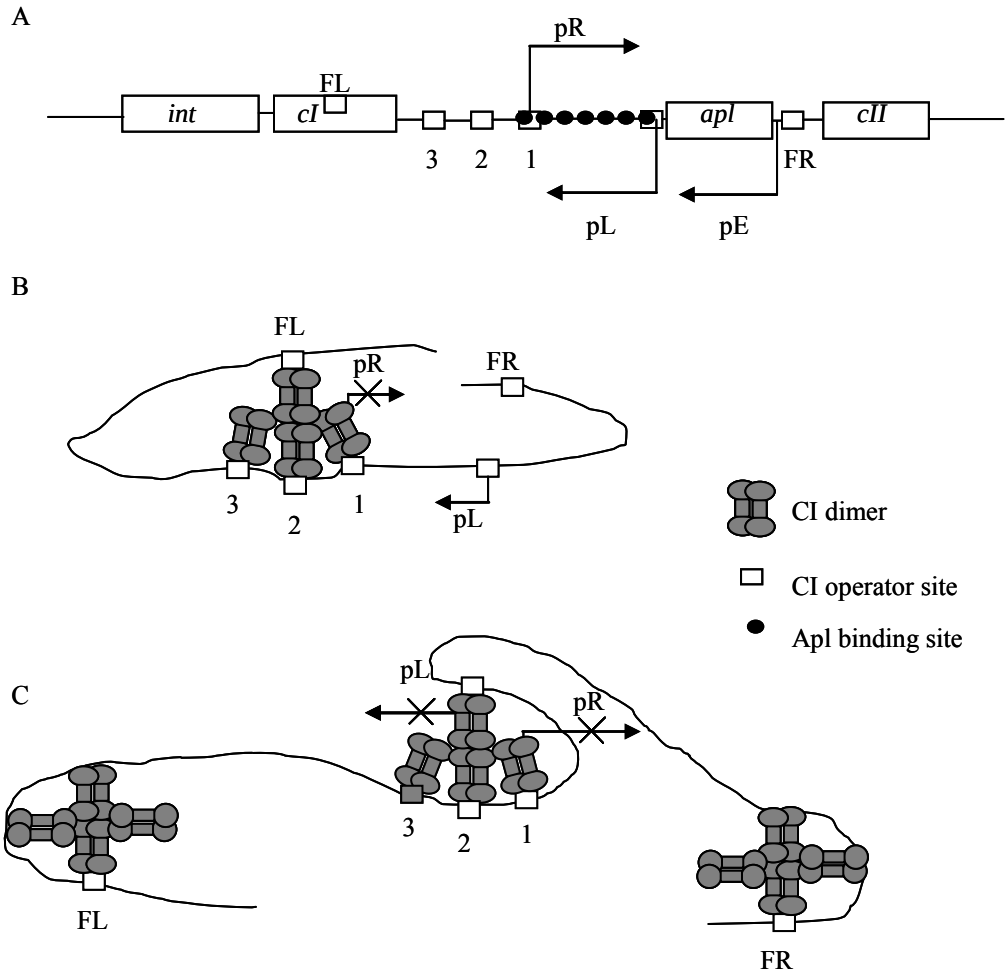


Figure 5. Schematic drawing of the 186 immunity region. **A**. Locations of promoters/operators and the repressor genes *cI* and *apl*. **B**. During lysogenic growth CI will bind cooperatively to CI operators at pR and FL. A loop will form, pR will be repressed and pL will be unrepressed. **C**. At higher CI levels, FL and FR will be occupied by CI multimers and the free dimer at pR will interact with pL and both pR and pL will be repressed (modified from Dodd and Egan, 2002). See text above for details.

The genetic switch of P2

The lytic and lysogenic promoters, Pe and Pc, in P2 are arranged face-to-face (Fig. 6A) as in phage 186 but the overlaps of the two transcripts are different in length in the two phages. The closely related but heteroimmune P2 relative, WΦ, has the same arrangement of the two promoters but the overlap of the two transcripts is longer in WΦ compared to P2 (Liu and Haggård-Ljungquist, 1999). The lytic Pe promoters of P2 and WΦ have higher intrinsic strengths than the lysogenic Pc promoters (Saha *et al.*, 1987a) (Liu and Haggård-Ljungquist, 1999). The interference of lytic transcription on transcription from Pc can be abolished by the C repressor.

With its 99 amino acids the C repressor of P2 is much smaller and shows no sequence similarity to the CI repressor of 186. The N-terminal part contains four α-helices and α helices 2 and 3, as judged from secondary structure predictions, are believed to constitute a HTH motif which might be involved in DNA binding (Fig. 1A in Paper II). One mutation in the N-terminus seems to affect DNA binding without abolishing dimerization (Ljungquist *et al.*, 1984) (Renberg-Eriksson *et al.*, 2000). The native form of P2 C is a dimer and the C-terminus is believed to be responsible for dimerization (Lundqvist and Bertani, 1984) (Liu *et al.*, 1998) (Renberg-Eriksson *et al.*, 2000). The P2 C repressor recognizes a direct repeat flanking the -10 region of Pe (Ljungquist *et al.*, 1984) (Saha *et al.*, 1987a). It is unusual among prokaryotic DNA binding proteins to recognize direct repeats; it is much more common with dimers binding to an inverted repeat as in λ and 186.

P2 C represses Pe and will stimulate transcription from its own promoter (Fig. 6B) by inhibiting convergent transcription from Pe, similar to what is seen in 186. Both for P2 and 186, it is mainly the actual transcription from Pe and pR respectively, and not posttranscriptional control by antisense RNA that inhibits transcription from the lysogenic promoter (Saha *et al.*, 1987a) (Saha *et al.*, 1987b) (Callen *et al.*, 2004). The interference of the strong lytic promoter is however more pronounced for 186 than for P2. 186 pL is more sensitive to interference than P2 Pc which is therefore believed to have a faster promoter clearance than pL even though the rate of open complex formation is slower for Pc than for pL (Callen *et al.*, 2004). Pc separated from Pe is no longer sensitive to activation by the C repressor but still to repression by Cox (Saha *et al.*, 1987b). It is however also seen in DNaseI protection studies that P2 C enhances binding of RNAP to Pc (Saha *et al.*, 1987a) and this has also been assumed to contribute to C activation of Pc (Saha *et al.*, 1987a). An increase of the repressor concentration above what is normally seen in a lysogen, results in a repression of Pc by negative autoregulation (Saha *et al.*, 1987a).

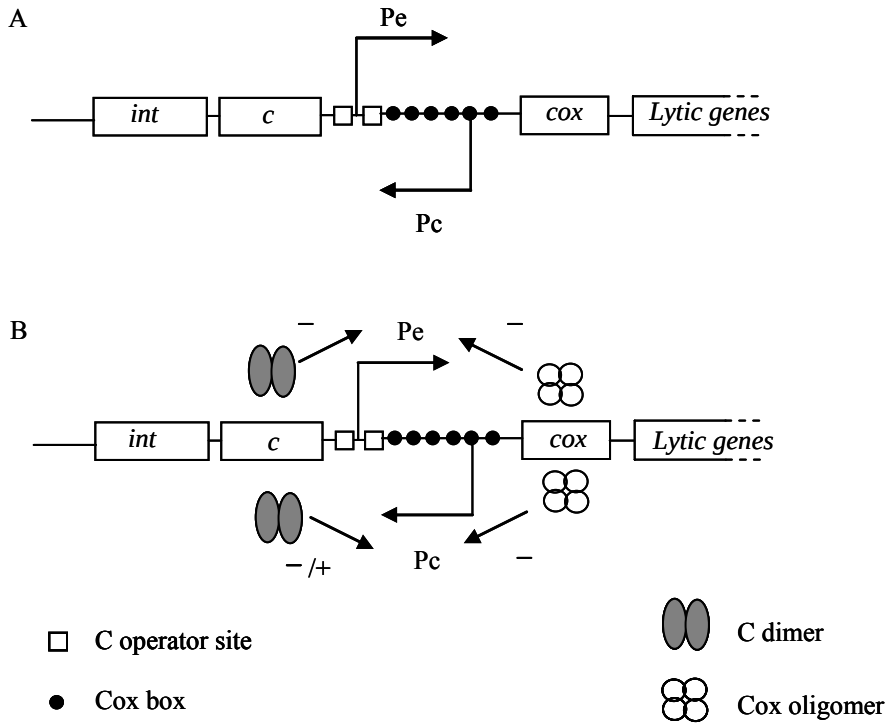


Figure 6. Schematic drawing of the immunity region of P2. **A**. Locations of promoters/operators and repressor genes *c* and *cox*. **B**. P2 C represses the early lytic promoter Pe and also Pc at higher concentrations. Cox down regulates both Pe and Pc.

The role of Cox in the P2 switch

The first gene of the early operon is *cox* which is essential for excisive recombination by serving an architectural role being the equivalent to λ Xis and 186 Apl (Haggård-Ljungquist *et al.*, 1994). P2 Cox will also repress Pc as shown *in vivo* by a frame-shift mutation of the *cox* gene and further proved by adding Cox protein *in trans* (Saha *et al.*, 1987b). Cox also down regulates its own promoter although the inhibition is fairly weak compared to repression of Pe by P2 C (Fig. 6B). This might be due to the distances between the Cox binding sites and Pe (Saha *et al.*, 1987b). As in 186, P2 Cox seems to be important only in prophage induction since P2 *cox* defective mutants lysogenize with a normal frequency (Lindahl and Sunshine, 1972). An overexpression of Cox will however decrease the lysogenization frequency (Saha *et al.*, 1987b). DNaseI protection shows that Cox binds to a region of about 70 nt including the -10 and -35 parts of Pc. In this region there are six potential

Cox boxes (recognition sequences). At higher concentrations it also binds to Pe and this rather weak binding is probably responsible for the moderate autoregulation of Cox (Saha *et al.*, 1987b) (Saha *et al.*, 1989). Cox is known to oligomerize and both tetramers and octamers have been identified in solution (Eriksson and Haggård-Ljungquist, 2000).

The P2 switch is intriguing in two aspects compared to that of λ and 186; the lack of a CII homolog needed for lysogenization and the fact that the P2 prophage can not be induced by UV-damage to the host cell. Despite the fact that CII is needed for lysogenization of 186 but is dispensable in P2, they have similar lysogenization frequencies of 5-15% (Lindahl and Sunshine, 1972) (Dodd *et al.*, 1993). CII promotes lysogenization in 186 by activating production of CI from the pE promoter and simultaneously interfering with transcription from pR by ongoing transcription from pE (Neufing *et al.*, 2001). The spontaneous phage production of P2, approximately 1 out of 50.000 lysogenic bacteria will release phages per cell generation (Bertani, 1951), can not be increased by UV-irridation.

Site-specific recombination

One of the main characteristics of a temperate phage is the ability to integrate into the chromosome of the host cell and to be excised again as a result of prophage induction (Fig. 7). A common pattern for site-specific recombination is seen for many temperate phages, e.g. phage λ, HP1, the and P2 family (Esposito and Scocca, 1994) (Esposito and Scocca, 1997) (Esposito *et al.*, 2001) (Azaro and Landy, 2002) (Yu *et al.*, 1989) (Yu and Haggård-Ljungquist, 1993a) (Yu and Haggård-Ljungquist, 1993b). The catalytic protein is a phage encoded integrase and its binding to DNA is stabilized by a higher-order nucleoprotein complex, the intasome. Integration involves the phage attachment site, *attP*, which contains, in addition to the integrase binding sites, many binding sites for accessory proteins and the fairly simple attachment site on the bacterial chromosome, *attB*.

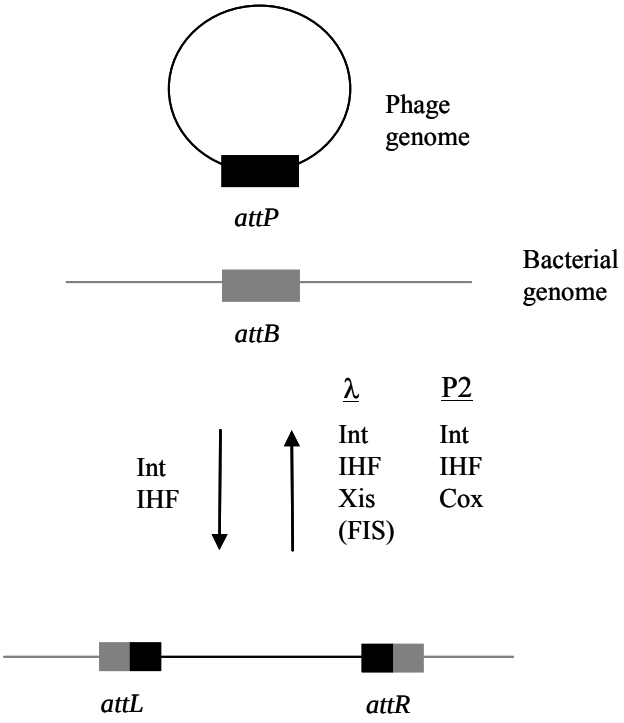


Figure 7. Site-specific recombination in phages λ and P2. In addition to integrase and IHF, excision requires the excisionases λ Xis and P2 Cox respectively.

Site-specific recombination in phage λ

The integrase and the attachment sites

Again, one of the most well studied systems is that of phage λ (reviewed in Azaro and Landy, 2002). The *attP* site (Fig. 8 bottom) contains a core region that is an inverted repeat and an overlap region of seven base-pairs (COC'), and two arm-binding sites, P and P', consisting of an inverted repeat (P1, P2) and three direct repeats (P'1, P'2 and P'3) respectively. *attB* is equivalent to the core of *attP* and these are the regions where the actual strand exchange takes place. The heterobivalent integrase can recognize and bind both core- and arm type sites (Nunes-Düby *et al.*, 1998). The N-terminal part of the integrase has been solved by NMR spectroscopy and is responsible for binding to the arm sites and the DNA binding motif is a three-stranded β-sheet and a stabilizing α-helix (Wojciak *et al.*, 2002). The crystal structure of the C-terminal part of λ Int has been solved and contains the core binding motif together with the catalytic site (Kwon *et al.*, 1997) (Tirumalai *et al.*, 1997) (Tirumalai *et al.*, 1998). The ability to simultaneously bind to different and

well-separated binding sites is a key element in the formation of a functional recombinogenic complex.

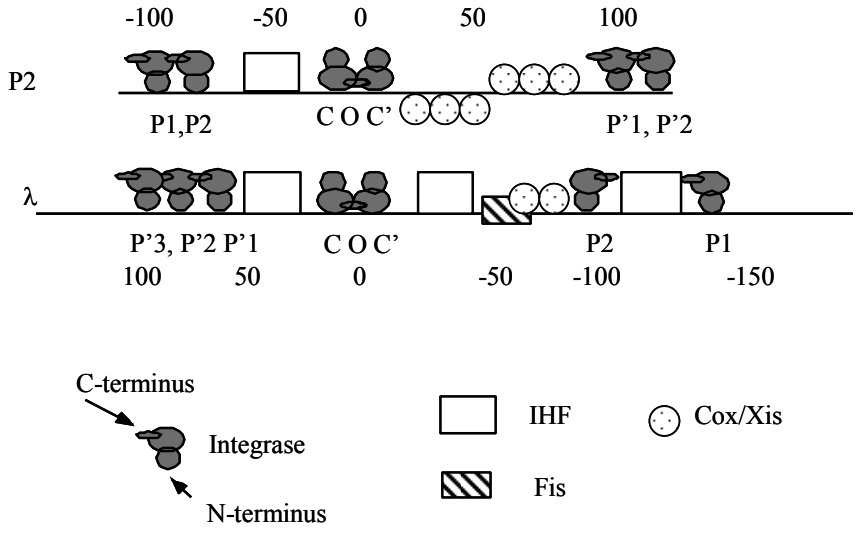


Figure 8. The organization of the *attP* regions of phages P2 and λ. Binding sites for the integrases and accessory proteins are indicated. Note that λ *attP* is inverted compared to how it is usually presented to have the Xis and Cox binding sites at the same side.

Integration requires the accessory host encoded protein IHF that has three recognition sites interspersed between the core and the two arm-binding sites (H, H1 and H2) (Craig and Nash, 1984). IHF stabilizes the formation of the intasome by causing a strong bend in the DNA and the exact position of this bend is vital. The bending will bring integrase binding sites close together, allowing integrase bound at the high-affinity arm-sites to bind the low-affinity core-site (de Vargas *et al.*, 1989). The preformed intasome at *attP* seeks out the naked *attB* and strand exchange can take place (Esposito *et al.*, 2001) catalyzed by the tyrosine residue present in all integrases in the tyrosine family of integrases, e.g. λ, L5, HP1 and P2 (Nunes-Düby *et al.*, 1998). The integration event will result in the two recombinant *attL* and *attR* sites flanking the integrated prophage. These sites can be the substrates for another recombination event as a result of prophage induction.

Excision and the role of Xis

The excision event is tightly regulated by the phage encoded excisionase, Xis. Xis serves a more regulatory function than IHF since it controls the direction of the recombination by stimulating excision and inhibiting integration (Bushman *et al.*, 1984). This control of directionality is important for an efficient growth response of the phage. Xis binding to *attR* enables the formation of a recombinogenic complex containing Xis, Int and IHF. This complex can interact with *attL*, resulting in strand exchange and the regeneration of *attP* and *attB*. Xis binding to *attP* will result in a loop structure unable to participate in recombination.

Excisionases are usually small, basic proteins and little homology is seen between excisionases found in the data bases (Lewis and Hatful, 2001). λ Xis is 72 aa long and recognizes two 13-bp direct repeats between the core and the P-arm (X1 and X2) (Yin *et al.*, 1985). It introduces a sharp bend of approximately 140^{o} which in *attR* promotes the formation of a potent recombinogenic complex but is inhibitory in *attP*. λ Xis has been crystallized and it adopts a "winged"-helix motif that interacts with the major- and minor-grooves of its binding site through a single α-helix and "wing" (loop) structure respectively (Sam *et al.*, 2002) (Sam *et al.*, 2004). The Xis induced bend is stabilized by cooperativity between Int bound to the P2 arm site and Xis bound at the most proximal Xis site, X1, stabilizing a correct *attR* intasome (Numrych *et al.*, 1992) (Swalla *et al.*, 2003) (Warren *et al.*, 2003). The C-terminus of Xis is disordered in the absence of DNA and is presumed to undergo a transition to an ordered state, a solvent exposed α-helix, in the Int-Xis-DNA ternary complex. The interaction surface with Int is believed to be dependent on one or a few positively charged amino acid in the C-terminus. A negatively charged amino acid in the stabilizing α-helix in the N-terminus of Int is important both for interaction with Xis but is also critical for Int dimer formation (Swalla *et al.*, 2003) (Warren *et al.*, 2003).

A fourth protein is active at site-specific recombination, the host encoded FIS, which strongly stimulates excision under conditions where Xis is limiting (Thompson *et al.*, 1987). Binding of Xis to X1 is stabilized either by a second Xis monomer binding to X2 or by FIS binding to the FIS site which partially overlaps X2. Binding of a Xis dimer or a Xis-FIS pair will result in the same bending angle. It has been speculated that the FIS sensitivity is to provide a sensor of host physiology since the FIS concentration varies with the growth phase of the cell (Ball *et al.*, 1992).

Site-specific recombination in P2

P2 *attP* is somewhat different compared to λ *attP* even if the overall organization is similar (Fig. 8 top) (Yu and Haggård-Ljungquist, 1993a) (Yu and Haggård-Ljungquist, 1993b). There are only two integrase binding sites in each

arm, in the form of tandem repeats, the core consists of an inverted repeat and there is only one IHF site between the core and the P-arm. The P2 integrase belongs, together with λ Int, to the large group of tyrosine recombinases and can recognize the two different types of DNA binding sites. P2 Int has been shown to dimerize and the C-terminus as well as central parts are involved in dimer formation (Frumerie *et al.*, 2005). IHF is required for both integration and excision while Cox is the excisionase and also functions as a directionality factor being necessary for excision but inhibiting integration (Fig. 7). Cox recognizes six more or less well conserved repeats (Cox boxes) between the core and the P'-arm (Yu and Haggård-Ljungquist, 1993a). Cox is known to oligomerize and both tetramers and octamers have been identified in solution. The ability to oligomerize seems vital for Cox activity and Cox binds in a cooperative manner to DNA (Eriksson and Haggård-Ljungquist, 2000). The DNA binding domain is believed to be a HTH motif predicted at the N-terminus and a hydrophobic β-strand and/or an amphipatic α-helix in the C-terminus are suggested to make up the oligomerization epitope (Eriksson and Haggård-Ljungquist, 2000).

The fact that the *int* and *cox* genes are found in mutually exclusive transcripts (See Fig. 6A) is a mind twister since both proteins are needed for excision and no integrase can be detected in the lysogen. This might explain why spontaneous phage production is low. Furthermore, induction of the prophage by inactivation of the repressor is abortive, no phages are produced and this can be attributed to the lack of Int (Bertani, 1968) (Ljungquist and Bertani, 1983). There is a weak transcriptional terminator, located between gene *c* and *int*, allowing a 30% read-through and the final transcription termination site is located downstream *attP* which will result in different 3'-ends of the transcripts from the circular phage DNA and from the prophage (Yu *et al.*, 1994) (Yu *et al.*, 1989). In the prophage, the termination site is split from the *int* transcript by the integration event and the *int* transcript proceeds into the host chromosome. Since there are no detectable transcriptional stop signals in this region of the host chromosome, the transcript will probably be uncovered by ribosomes and can therefore be attacked by nucleases. The *C* part of the transcript is presumably protected from degradation by inverted repeats in the spacer between *c* and *int*. This could perhaps explain why there is very little integrase expressed from the prophage. The integrase does also autoregulate its own expression by binding to its own mRNA covering the ribosomal binding site (Yu *et al.*, 1994).

Satellite phage P4

P4 is something as fascinating as a phasmid, an element on the boundary between phages and plasmids. Outside a cell, P4 is a virion with the same looks as P2 but with a smaller head, perfect for the 11.6 kb genome (a third

of the P2 genome). Just like P2, P4 can lysogenize an *E. coli* cell after infection but it can also replicate as a free plasmid after entering the host cell. For lytic growth, P4 is unconditionally dependent on a helper phage from the P2 family that can supply P4 with all the late lytic gene products such as capsid proteins and proteins needed for lysis of the host cell. P4 is therefore said to be a satellite phage parasiting on P2 and it is important to remember that P4 is not a defective P2 phage but a highly specialized genetic element. Several interesting reviews are written on P4 (Dehò and Ghisotti, 2005) (Briani *et al.*, 2001) (Dehò and Ghisotti, 1999) (Lindqvist *et al.*, 1993) (Christie and Calendar, 1990). P4 can take advantage of a helper phage under varying circumstances and P2 and P4 can mutually derepress each other and mutually transactivate late transcription. In the presence of P2, P4 favors the lytic life cycle, whereas, in the absence of a helper, the lysogenic state is preferred. P4 can also establish itself as a multicopy plasmid in the absence of a helper and this occurs in approximately 1% of the P4 infected cells (reviewed in Briani *et al.*, 2001).

P4 immunity

The α operon is transcribed from the two leftwards promoters, P_{LE} and P_{LL} (Fig. 9). It encodes genes needed for both lytic growth, lysogeny and plasmid replication including genes for replication, prophage derepression and immunity which prevents P4 replication. Since the P4 genome is not organized as those of P2 and λ for example, with transcription of genes for lytic versus lysogenic growth being mutually exclusive, it is crucial with the correct expression of the α operon depending on the developmental mode.

The first phase after infection is the uncommitted phase where transcription of the entire α operon from the constitutive P_{LE} promoter results in replication. This happens irrespective of the presence or absence of a helper. Transcripts of different lengths can be seen due to several transcriptional terminators in the region. Soon, the immunity control system will cause premature transcription of all transcripts from P_{LE} at a terminator situated upstream all the genes needed for replication thereby inhibiting all further replication. This premature termination is caused by the 5' untranslated portion of the P_{LE} transcript, rich in direct and inverted repeats allowing the formation of secondary structures. This RNA (CI RNA) is the immunity factor of P4, highly unusual since most repressors/immunity factors identified are proteins (Dehò *et al.*, 1992) (Forti *et al.*, 1995) (Forti *et al.*, 2002). The CI immunity factor is in other words, produced by specific processing of the same transcript as it controls. The uncommitted phase ends when the production of CI RNA is established and a choice of propagation, influenced by the presence or absence of a helper, follows.

P4 choice of propagation

In the presence of a P2 helper P4 favors the lytic cycle. Even if lysogenization is preferred in the absence a helper genome, lysogenization can also occur if a helper is present (Fig. 10). During lysogenic growth only expression of the immunity region, the P_{LE} proximal part of the α operon, is required and genes needed for replication are not expressed. In the plasmid state, where the P4 genome is replicated, the mechanism of premature transcription termination of the α operon is circumvented by transcription from the alternative promoter P_{LL}. The transcript from P_{LL} is insensitive to interactions with CI RNA and genes for replication are transcribed. The plasmid state is further characterized by transcription from the P_{sid} promoter (Dehò *et al.*, 1988). P_{LL} and P_{sid} are both activated by the *δ* gene product which is transcribed from P_{sid} and it is therefore unclear how the transition to plasmid state occur (Dehò and Ghisotti, 2005) It could be a low basal expression from P_{LL} resulting in the *vis* gene product believed to stimulate P_{sid} (Polo *et al.*, 1996) or a very low basal δ-independent transcription from P_{sid}.

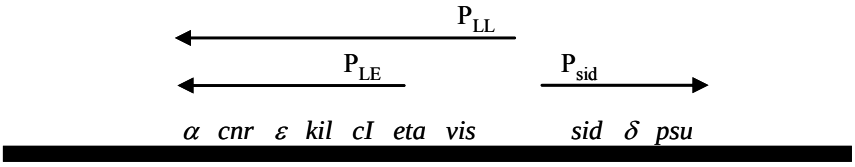


Figure 9. When P4 infects a host cell, transcription is initiated from the constitutive promoter P_{LE} controlling the α operon which includes the immunity factor as well as replication functions. The uncommitted phase is followed by the choice of propagation which appears to be based on whether P_{LL} and P_{sid} are activated or not.

In the presence of a helper the road to lytic growth is open to P4. As in the plasmid state the mechanism of premature transcription termination of the α operon is circumvented by transcription from the alternative promoter P_{LL}. P4 will interfere with the P2 production of phage heads by directing the assembly of smaller capsids suitable for the smaller P4 genome. This is achieved by an interaction between the P4 Sid protein and the P2 N protein (Dokland *et al.*, 2002) (Marvik *et al.*, 1995). P4 Sid is transcribed together with δ from P_{sid} activated by P2 Ogr and/or P4 δ.

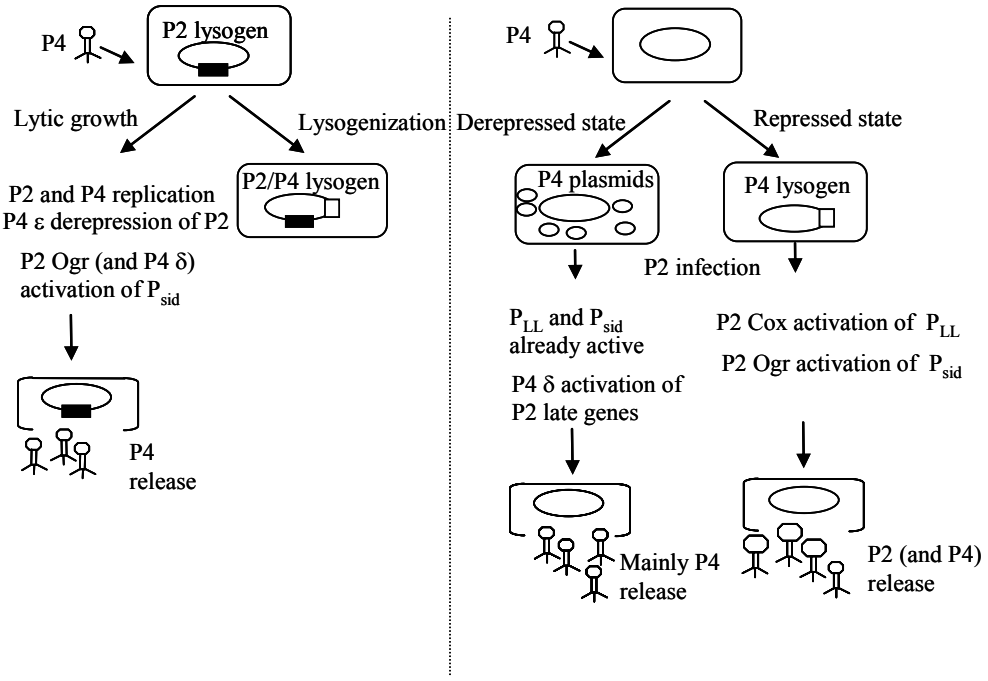


Figure 10. Some of the different outcomes of a P4 infection. When a helper is present at infection (left section) P4 can either lysogenize its host or grow lytically. If there is no helper present at infection (right section) P4 can either lysogenize its host or establish itself as a plasmid. Lytic growth can occur when P4 infects a P2 lysogen, after superinfection of a helper phage or when P2 and P4 simultaneously infects a cell (not shown in the figure).

There are several different scenarios where P4 grows lytically: i) P4 infects a P2 lysogen; ii) a P4 lysogen is being infected by P2; iii) a P4 plasmid containing cell is infected by P2; iv) P4 and P2 simultaneously infect a cell. For an overview see Dehò and Ghisotti, 2005 and Fig 10.

i) When P4 infects a repressed P2 lysogen and the lytic option is set, the P2 prophage immunity is lifted by the P4 ε protein which acts as an anti-repressor by binding the P2 C repressor (Giesselsoder *et al.*, 1981) (Liu *et al.*, 1997) (Liu *et al.*, 1998). This will result in transcription of the early P2 genes and replication of the P2 genome which in turn will result in production of P2 Ogr. Ogr and P4 δ will be responsible for an efficient transcription of the P2 late genes (Halling and Calendar, 1990). Mostly P4 virions are produced and very few P2 particles.

ii) A P4 lysogen can sense an infecting P2 by the P2 Cox protein expressed from the strong Pe promoter. P2 Cox activates P_{LL} thereby allowing

replication of P4 but since Cox does not activate P_{sid} P4 will depend on P2 Ogr for activation of the sid operon. P2 Cox binds upstream the -35 region of P4 P_{LL}, a location common for activators, and a region of approximately 90-95 nt is covered corresponding to nine more or less well-conserved Cox boxes (Saha *et al.*, 1989) (Dehò *et al.*, 1984). In a CAT-assay (see Specific methods) Cox is shown to activate P4*vir1* P_{LL} 15-fold (Saha et al., 1989; in this work the P_{LL} promoter was believed to be wt). The P4 Vis protein has recently been recognized as the excisionase of P4 needed for excision of the P4 prophage and it binds in the *attP* region causing DNA bending (Calì *et al.*, 2004).Very few P4 particles are produced.

iii) In the plasmid state the P_{LL} and P_{sid} promoters are already active and P4 δ will serve the role of P2 Ogr and activate the late P2 genes. P2 growth is severely reduced and the result will mainly be P4 progeny.

iv) In a mixed infection of a non-lysogenic host, progeny of both phages is produced in approximately the same proportions.

Present investigation

Aim of the study

Through this study we have gained further insights into the developmental switches of three P2 family members; P2, P2 Hy *dis* and WΦ. The aim has been to study the switch of P2 Hy *dis* in detail and compare it to the other switches in the family. By also acquiring a deeper molecular understanding of the repressors C and Cox of both P2 and WΦ, it has been possible to do some comparisons revealing interesting differences and similarities. The multifunctional Cox proteins are particularly interesting since they do not only participate in the developmental switches but also function as excisionases in site-specific recombination which has also been addressed in this study. In addition, P2 Cox also functions as an activator of the late promoter P_{LL} in satellite phage P4.

Specific methods

In vivo repressor-operator interaction analysis using a plasmid containing a reporter gene system (Paper I and II)

The wt or operator mutated Pe-Pc region of P2 Hy *dis* together with the P2 Hy *dis C* gene (Paper I) or the Pe-Pc region containing a hybrid early promoter/operator region of P2 and WΦ (Paper II), were cloned in the vector pKK232-8 carrying a promoterless *cat* reporter gene, encoding chloramphenicol acetyltransferase (CAT) (Brosius, 1984). This vector is commonly used in analysis of promoter strength and potential repression/activation by repressors/activators. The P2 Hy *dis* C repressor is transcribed from Pc and its presence will shut down transcription from Pe directing expression of CAT if the operator is recognized by C. In analysis of the hybrid operators, C repressor(s) is (are) supplied *in trans* on a compatible plasmid or from a prophage. By measuring the CAT activity, the repression of Pe by the C repressor(s) can be determined. The CAT activity is calculated as the amount of acetylated [^{14}C]-chloramphenicol divided by the total amount of chloramphenicol.

In vivo dimerization/oligomerization assays using plasmid reporter gene systems (Paper II)

To investigate if WΦ C forms dimers, the λ dimerization system was used (Hu *et al.*, 1990) (Fig.11). It is based on the fact that the N-terminal domain of λ CI mediates DNA binding through a HTH motif but requires dimerization mediated by the C-terminus for efficient DNA binding. If the N-terminus is fused with a protein that dimerizes, the N-terminus will bind DNA and this can be used to examine and quantify the dimerization capacity of a protein of interest. A construct containing the λ high-affinity operator site O_R2 and the promoter P_R controlling the *lacZ* gene is inserted in the chromosome of the assay strain. If the N-terminus is fused to a protein that dimerizes the fusion protein is able to interact with O_R2, thereby repressing P_R seen as a reduction of the β-galactosidase activity.

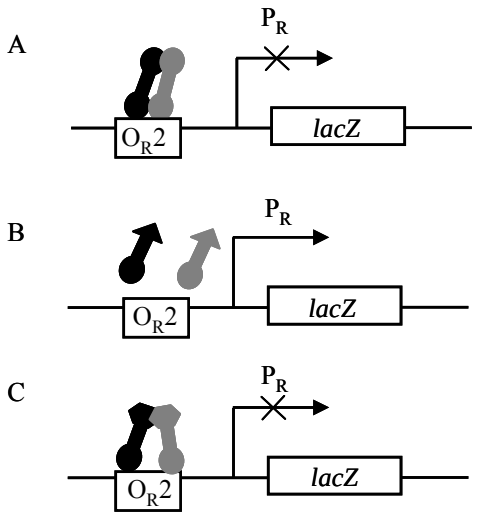


Figure 11. λ CI dimerization assay. **A**. Wildtype λ CI binds to O_R2 thereby repressing P_R and turning off the *lacZ* gene. **B**. A test protein is fused to the N-terminus of CI. The test protein does not dimerize and therefore P_R is not repressed and *lacZ* is transcribed. **C**. A protein that dimerizes is fused to the N-terminus of CI and P_R is therefore repressed resulting in no transcription of *lacZ*.

The system has been further developed to examine oligomerization (Zeng and Hu, 1997) (Fig. 12). Here, the reporter construct contains a weak promoter proximal operator (O_W) and a strong promoter distal operator (O_S). Binding to the weak operator alone is not enough for repression of P_R, occupancy of both operator sites is needed. To occupy both operators the protein must be

able to oligomerize, either in solution or by a dimer at the strong operator recruiting a dimer to the weak operator. A control strain is however used to determine to what extent P_R is repressed by binding to only the weak operator.

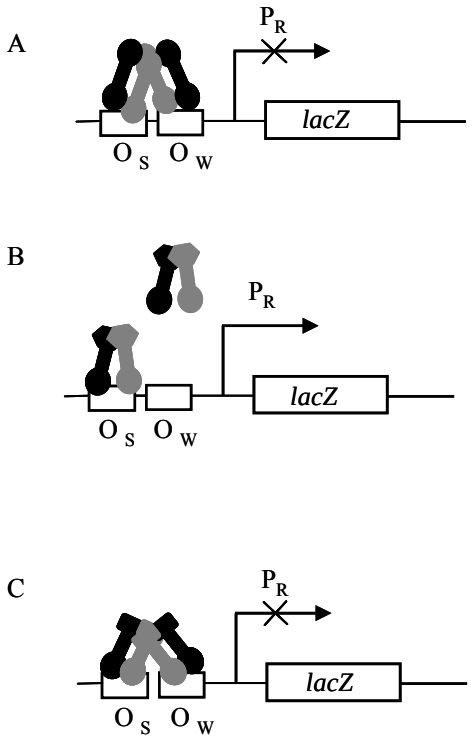


Figure 12. λ CI oligomerization assay. Both the weak (O_W) and the strong (O_S) operators must be occupied for repression of P_R which controls transcription of the *lacZ* gene. Only proteins that oligomerize can stabilize binding to O_W. **A**. Wildtype CI, bound to both operators, turns off *lacZ* transcription. **B**. A protein that can not oligomerize is fused to the N-terminus of CI. This fusion protein will not stabilize binding to O_W and therefore *lacZ* is transcribed. **C**. A protein that oligomerizes is fused to the N-terminus of CI and will stabilize binding to O_W resulting in repression of P_R.

Protein mediated DNA bending analysis using circular permutation assay (Paper II and III)

To investigate the DNA bending capacity of the C and Cox proteins, their respective DNA binding sites were cloned in the pBend2 vector, a pBR322 derivative (Kim *et al.*, 1989). This vector contains a tandemly repeated polylinker sequence with a unique cloning site in between where the C or Cox binding sites were cloned. The region containing the C or Cox binding sites

and the restriction sites from the pBend2 plasmid was amplified by PCR. After cleavage with different restriction enzymes, fragments identical in size but with the protein binding sites at different positions from the ends of the fragments, were generated. The relative mobility of the protein-DNA complexes formed with the circular permutated DNA fragments was determined by electromobility shift analysis (EMSA). If a protein bends the DNA target, the equally long DNA fragments will migrate differently in the polyacrylamide gel depending on the position of the bending center relative to the ends of the fragment. Bending angles were calculated by applying the experimental data from 8% polyacrylamide gel electrophoresis to the formula $\mu_M/\mu_E=\cos \alpha/2$, where μ_M is the complex with the lowest mobility, μ_E is the complex with the highest mobility, and α is the bending angle (Thompson and Landy, 1988).

Quantification of cooperative binding of P2 Int and IHF or P2 Cox (Paper IV)

In paper IV, where we analyzed the cooperative binding of P2 integrase and IHF or P2 Cox, binding patterns where examined with EMSAs and quantifications were made using Image Gauge V 3.45 software for Macintosh using the profile/MW function to define lanes and for automatic peak search. To quantify the cooperativity we compared the **observed** fraction of DNA molecules bound by both proteins, with the **expected** fraction of DNA molecules bound by both proteins, calculated by the product rule assuming independent DNA binding events for each protein. The total fraction of radioactivity in all the complexes formed by one protein alone was determined for each concentration used. This fraction was then multiplied with the total fraction of radioactivity in all complexes formed by the other protein for the respective concentration. The product represents what would be found if the DNA binding events of the two proteins analyzed are independent. This product is then compared to the fraction of the radioactivity obtained in the complexes where both proteins are assumed to be present.

Paper I - Characterization of the developmental switch region of bacteriophage P2 Hy *dis*

Our aim was to study and characterize the genetic switch of a P2 relative; P2 Hy *dis*. P2 Hy *dis* was isolated by Bertani (Bertani, 1957) when it appeared as a result of a P2 infection of an *E. coli* B strain. P2 Hy *dis* was first described as a disimmune (heteroimmune) P2 mutant but after discovering several profound differences such as smaller plaque, more turbid plaque center, heat sensitivity (Cohen, 1959) and 2% more DNA content (Cohen, 1960), P2 Hy *dis* was considered a hybrid between P2 and an unknown de-

fective prophage in *E. coli* B. This hybrid might be a result of three separate replacement events, since heteroduplex mapping between P2 and P2 Hy *dis* shows three loop patterns, i.e. three non-homologous regions (Chattoraj and Inman, 1973). One of the non-homologous regions was located 75.7%-77.4% from the left end that corresponds to parts of the integrase gene, the immunity repressor C and the Pe-Pc region (Cohen, 1960). Differences in this region are consistent with P2 Hy *dis* being heteroimmune to P2. Since then, several intact P2 related coliphages have been shown to have the same immunity as P2 Hy *dis* (J. Karlsson, A, Nilsson and E. Haggård-Ljungquist, personal communication). We were therefore interested in comparing the developmental switch of P2 Hy *dis* to what is known about the prototype switch in P2 and other family members such as WΦ.

Analysis of the Pe and Pc promoters

After sequencing the transcriptional switch region we could conclude that there are two face-to-face located promoters that we located by primer extensions. The two divergent transcripts overlap with approximately 75 base pairs. The Pe promoter has a consensus -35 region and the -10 region has 3 out of 6 base pairs identical to a σ^{70} consensus -10 hexamer. Pc has 4 out of 6 base pairs identical to both the -35 and the -10 regions. In addition, a TG motif can be found one nucleotide upstream the -10 hexamer probably strengthening the initiation of transcription (Barne *et al.*, 1997) (Burr *et al.*, 2000) (Ross *et al.*, 2001). When conducting primer extensions it was obvious that the Pc promoter was considerably weaker than Pe. By including the C repressor in the construct, the Pe promoter was repressed and transcription could be seen from Pc implying that ongoing transcription from Pe might have an inhibitory effect on Pc transcription as in P2 (Saha *et al.*, 1987b).

P2 Cox and P2 Hy *dis* Cox are interchangeable

The P2 Cox and P2 Hy *dis* Cox proteins are very similar, 77 of 91 amino acids are identical in the two proteins. Both have a predicted HTH motif in their N-terminus which is believed to be involved in DNA binding (Eriksson and Haggård-Ljungquist, 2000). Three of the different amino acids are located in the turn of the assumed HTH motif and two in the potential DNA binding helix (α2). Because of the locations of these amino acids it was not obvious from sequence comparison that the two Cox proteins would recognize the same DNA substrate. Our results from EMSAs using the Pe-Pc regions from the respective phage incubated with either P2 Cox or P2 Hy *dis* Cox showed that both proteins, in addition to recognizing their "own" substrate, also bound to that of the other phage. By coupling the respective Pc promoter to the *cat* gene we could further show that P2 Cox and P2 Hy *dis* Cox substitute for each other in repression of either Pc promoter. The Cox protein encoded

by WΦ on the other hand, was unable to repress any Pc promoter but its own and WΦ Pc could not be repressed by P2 Cox or P2 Hy *dis* Cox (this paper and Liu and Haggård-Ljungquist, 1999). The Cox proteins of P2 and P2 Hy *dis* were also functionally interchangeable as excisionases excising a P2 *cox* defective mutant when supplied *in trans*. By infecting a P4 lysogenic strain we could also show that P2 Hy *dis* Cox could activate the P4 P_{LL} promoter resulting in production of free P4 phages.

C repression of the Pe promoter and identification of the operator

It is hypothesized that the N-terminal part of the C protein is responsible for DNA recognition and binding (Liu *et al.*, 1998). As can be seen from a sequence comparison, the N-terminal parts of P2 C and P2 Hy *dis* C show a considerable difference which is consistent with the fact that they are heteroimmune. A direct repeat of 8 base pairs flanking the -10 region are assumed to be the C binding site in the P2 Pe promoter region since these sequences are protected in a DNaseI footprint (Saha *et al.*, 1987a). In WΦ, a direct repeat of 10 base pairs located in a similar position has been shown to affect the repression of WΦ Pe (Liu and Haggård-Ljungquist, 1999). When one mutation is introduced in either half-site in WΦ, repression is not affected. However two mutations, one mutation in each half-site, abolish repression completely (Liu and Haggård-Ljungquist, 1999). In P2 Hy *dis* a direct repeat of 6 base pairs was found flanking the -35 region. Conducting a comparable mutational analysis for P2 Hy *dis* showed consistency with the results for WΦ when mutating one base in the first half-site (O1) but when mutating one base in the other half-site (O2), Pe was no longer repressed by the P2 Hy *dis* C repressor. We then assumed that the repeat in fact included two more nucleotides on the 5' side and that our O2-mutant already carried one "natural" mutation. Therefore this "natural" mutation in the O2-mutant was changed, thereby restoring repression to normal levels. Mutations in both half-sites (O1O2) abolished repression, which was expected. This implies that changing two nucleotides in either half-site might completely abolish repression.

To elucidate whether repression and binding of P2 Hy *dis* C to the Pe promoter are directly correlated, we conducted EMSAs with crude extract containing P2 Hy *dis* C and the Pe-Pc region (wildtype or O1O2 mutant). It was shown that binding is affected by the mutations but only to a minor extent. The C repressor had a slightly lower affinity for the O1O2 mutant substrate compared to the wt and one complex could not be seen at all. Since binding is not abolished this implies that the repressor might act at a different level than inhibiting promoter binding of RNAP by steric hindrance, e.g. formation of the open complex or promoter clearance.

P4 ε derepression of P2 Hy *dis* Pe

An infecting P4 phage can derepress a P2 prophage to get access to the P2 late gene functions. This derepression is mediated by the P4 ε protein which acts as an antirepressor interacting with the P2 C repressor that becomes inactivated (Giesselsoder *et al.*, 1981) (Liu *et al.*, 1997) (Liu *et al.*, 1998). To analyze derepression of a P2 Hy *dis* prophage, the CAT activity of a P2 or P2 Hy *dis C*-Pe-Pc-*cat* construct, where the *cat* gene is under the control of the Pe promoter, was measured. P4 ε was supplied *in trans* from a second compatible plasmid. The CAT activity was derepressed essentially to the same level after ε induction, using the P2 or the P2 Hy *dis* constructs, and we therefore concluded that ε can interact with P2 C and P2 Hy *dis* C with about the same efficiency.

Paper II - The oligomeric states of the two immunity repressor proteins of the heteroimmune coliphages P2 and WΦ, and their effects on DNA topology

Despite the high degree of similarity between the P2 related coliphages, the switch and immunity regions show differences (J. Karlsson, A. Nilsson and E. Haggård-Ljungquist, personal communication). In this work we found it interesting to compare the two C repressors of P2 and WΦ which are only 43% identical at the primary sequence level. This might be reflected in different tendencies to oligomerize, effect DNA topology, repress Pe and autoregulate Pc.

Localization of the P2 Pe and Pc promoters

The initiation sites for transcription from Pe and Pc in WΦ have been determined (Liu and Haggård-Ljungquist, 1999) and we therefore wanted to locate the initiation sites in P2 as well. The lysogenic P2 Pc promoter is considerably weaker than Pe, the very same pattern seen for both P2 Hy *dis* (Paper I) and WΦ (Liu and Haggård-Ljungquist, 1999). In a construct containing the two promoters, the P2 Pe promoter had to be inactivated by deleting the -35 region to achieve a measurable expression from Pc. The two transcripts were shown to overlap by approximately 35 bp, which is much shorter than in WΦ (Liu and Haggård-Ljungquist, 1999) and P2 Hy *dis* (Paper I) . This may have an impact on the relative promoter strengths depending on the mechanism by which ongoing transcription from Pe interfere with transcription from Pc (Saha *et al.*, 1987b).

Dimerization and oligomerization of WΦ C

P2 C is known to form dimers but not higher order oligomers (Liu *et al.*, 1998). In this work the capacity of WΦ C to form dimers and oligomers have been analyzed. We used the same *in vivo* plasmid system as was used for P2 which is based on the interaction between the λ CI repressor and its right operator, O_R (see Specific methods). Just like P2 C, WΦ C dimerizes strongly but is unable to oligomerize in this system. There is however the possibility that the C repressors oligomerize in the presence of operator DNA and that this has an impact on the C regulation of Pe and/or Pc. Since P2 C and WΦ C are quite similar in their C-terminus, which are believed to be involved in dimerization (Lundqvist and Bertani, 1984) (Liu *et al.*, 1998) (Renberg-Eriksson *et al.*, 2000), we wanted to investigate if they could form heterodimers by constructing early promoter/operator hybrids. The picture is however complicated by the fact that P2 and WΦ show different spacing between their operator half-sites corresponding to approximately two and three helical turns, respectively. Since we do not know the importance of this spacing for the proteins ability to repress their respective Pe promoter, four different hybrids were constructed. Our conclusion from this experiment is that P2 C and WΦ C do not form heterodimers, but WΦ C seems to be able to recognize an operator containing only one WΦ half-site in at least one construct. A cryptic WΦ half-site at an acceptable spacing might be the explanation for this.

The two C repressors bend their DNA targets

It seems to be the rule rather than the exception that activators bend their DNA targets and in this work we show that both P2 C and WΦ C bend their DNA targets. This might serve two functions; activation of Pc and repression of Pe. The induced bending could be sufficient for activation of Pc by bending DNA in a way assisting RNAP binding or by stimulating a direct contact between the C repressor and RNAP which might be important for activation. The bending might also cause an unfavorable conformation of the DNA for transcription from Pe. The bending centers are localized to the regions between the half-sites, O1 and O2, of the C operators. When introducing one mutation in each of the P2 C operator half-sites, C repression of Pe is abolished (S. Renberg Eriksson and P. Henriksson Peltola, personal communications) which is consistent with results from P2 Hy *dis* and WΦ (Paper I and (Liu and Haggård-Ljungquist, 1999). P2 C will however still bind the O1O2 mutated target but does not seem to induce any DNA bending (preliminary unpublished results, P. Henriksson Peltola). This indicates that the C induced DNA bending is important for repression of Pe and indicates that this bending causes an unfavorable configuration for transcription from Pe and/or stimulates transcription from Pc.

Spontaneous phage production

An established lysogen is generally stable and the spontaneous phage production is low. A quantification of the spontaneous phage production might reflect the binding affinity of the C repressor to its operator since the repression on Pe by the repressor has to be relieved for the lytic cycle to occur. By determining the titers of free phages in overnight cultures we could conclude that WΦ has a 6.4 fold higher spontaneous phage production than P2 and this might reflect different binding affinities of the two repressors to their operators or different regulation of Pc. There are however other plausible explanations, such as the production and stability of the C repressors.

Paper III- A comparative analysis of the multifunctional Cox proteins of the two heteroimmune phages, P2 and WΦ

The Cox proteins constitute a unique group of directionality factors (Lewis and Hatful, 2001). They link the control of the transcriptional switch, which determines lytic growth versus lysogeny, with control of integration versus excision. The Cox proteins of WΦ and P2 are 90 and 91 amino acids long, respectively, and the predicted secondary structures are identical according to JPRED (Cuff and Barton, 2000) even though they only have 35 amino acids in common. The secondary predictions indicate that the Cox proteins contain a HTH motif in the N-terminus, implicating DNA recognition and binding (Liu and Haggård-Ljungquist, 1999) (Eriksson and Haggård-Ljungquist, 2000). The WΦ Cox protein cannot functionally replace P2 Cox as an excisionase or as a repressor of the P2 Pc promoter, and WΦ Cox is unable to activate the P_{LL} promoter of satellite phage P4, indicating that they recognize different DNA sequences (Liu and Haggård-Ljungquist, 1999). In this work we further characterize and compare the functions of the Cox proteins of P2 and WΦ.

WΦ Cox binding sites in the Pe-Pc region

DNaseI protection studies have shown that P2 Cox covers regions of about 70 nt in the Pe-Pc region and *attP* and both contain six Cox boxes located in different orientations (Saha *et al.*, 1989) (Yu and Haggård-Ljungquist, 1993a). Since WΦ Cox cannot functionally replace P2 Cox, the P2 Cox boxes were not found in the WΦ Pe-Pc or *attP* regions. Instead a direct repeat of 12 nt was found in the WΦ Pe-Pc region with homology to a direct repeat of 9 nt in the *attP* region (Liu and Haggård-Ljungquist, 1999). In this work, the DNaseI protected region in WΦ Pe-Pc was found to include the

direct repeat and probably the Pc transcription start site but not the Pc core promoter region. This is different from P2 where the Cox binding sites covers the Pc core promoter and this difference might reflect a different mechanism behind the repression of Pc even though steric hindrance of RNAP binding is the most likely explanation in both phages. P2 Cox is known to negatively autoregulate the P2 Pe promoter and at higher protein concentrations the Pe promoter is protected probably resulting in the autoregulation (Saha *et al.*, 1987b). WΦ Cox seems to bind in a highly cooperative manner since the whole Pe-Pc region is covered as a result of a slight increase in Cox concentration. This oligomerization on the DNA could be the mechanism behind a possible negative autoregulation of Pe by WΦ Cox.

While P2 Cox seems to have similar affinities to the three different targets; Pe-Pc, *attP* and P4 P_{LL}, WΦ Cox has a substantially lower affinity to *attP* compared to Pe-Pc. In Paper IV we show that P2 Cox and the P2 integrase bind cooperatively to P2 *attP* and it might be that a possible cooperativity between WΦ Cox and WΦ Int is even more important than in P2 for the formation of the recombinogenic complex.

The Cox proteins induce DNA bending

Since the Cox proteins probably have a structural role in the formation of the excisive recombinogenic complex, we wanted to study whether they cause bends in their DNA targets. A possible bending might also have an impact on the abilities of the Cox proteins to repress their respective Pc promoter since an unfavorable bending can cause repression. In circular permutation assays we could show that P2 Cox bends its three DNA targets with the same angle of approximately 125°. In the same assay, WΦ Cox was shown to bend DNA containing the direct repeat in the Pe-Pc region with an estimated angle of 150° and a bending centre between the half-sites of the repeat. P2 Pe-Pc, *attP* and P4 P_{LL} all contain at least six P2 Cox boxes (P_{LL} contains eight) and we wanted to investigate how many boxes are needed for a specific Cox binding. Binding to substrates containing only four boxes is unspecific and in circular permutation assays no bending could be observed using three or four boxes. Thus, at least five boxes are needed for a specific binding and a Cox mediated DNA bending. However, no special feature or arrangement of the boxes seems necessary which is consistent with the different orientations of the P2 Cox boxes in the different targets.

Oligomerization *in vitro*

Both P2 Cox and WΦ Cox form higher order oligomers as shown by *in vitro* cross-linking experiments. P2 Cox has been shown to form higher order complexes in earlier work (Eriksson and Haggård-Ljungquist, 2000) which is confirmed in this work and we can further compare the ability of the two

Cox proteins to form oligomers. Several complexes can be seen and it is obvious that P2 Cox has a higher tendency than WΦ Cox to form higher order complexes. This is consistent with P2 Cox recognizing several Cox boxes while the binding site of WΦ Cox is a direct repeat. However, as mentioned above, WΦ Cox seems to oligomerize strongly in the DNaseI protection studies and the difference seen in these assays could be due to the absence or presence of DNA. WΦ Cox might require DNA for a strong oligomerization.

DNA specificity

In a work by Wharton and Ptashne (Wharton and Ptashne, 1985), the 434 repressor protein was redesigned by exchanging the amino acids occurring in the recognition helix with those of the P22 repressor. This helix swap resulted in a change of DNA specificity for the redesigned 434 repressor from 434 operator DNA to P22 operator DNA. In this work we have tried to conduct the same experiment with P2 Cox and WΦ Cox. Despite the fairly low identity at the amino acid-level, the two Cox proteins are predicted to have the same secondary structure (Fig. 13).

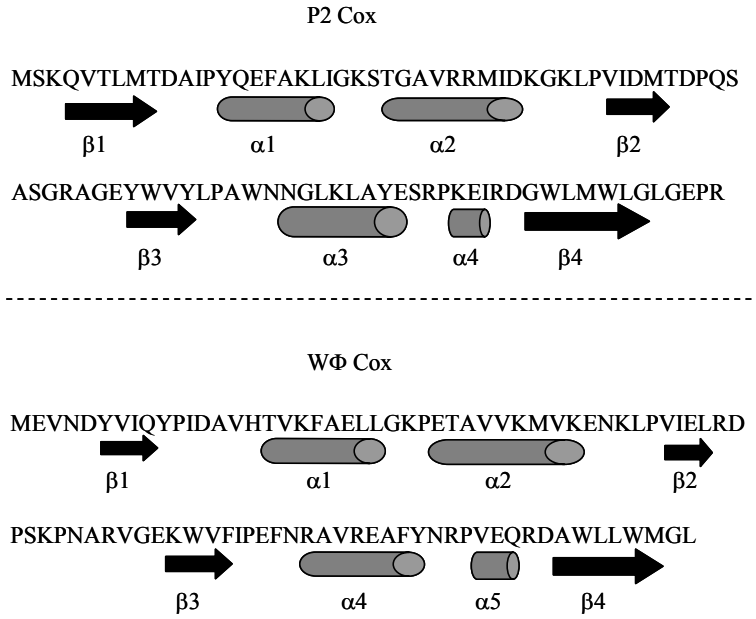


Figure 13. Secondary structure predictions of P2 Cox (top) and WΦ Cox (bottom) according to JPRED (Cuff and Barton, 2000).

The N-terminus contain a HTH motif and in Fig. 14 the proposed DNA-binding surfaces of the predicted recognition helices (α2) are illustrated. In order to determine whether these helices are the DNA binding epitopes, α2 in WΦ Cox was stepwise modified to be identical to α2 in P2 Cox thereby hoping to change the DNA specificity of WΦ Cox to that of P2 Cox. Some of the amino acid changes in WΦ Cox had an impact on DNA binding and K36 seems to play an important role, but the specificity of WΦ Cox was never changed to that of P2. Reasoning that other parts of the protein might be necessary for stabilizing a binding between the α2-helix and the DNA, three P2-WΦ Cox hybrid proteins were made. None of the hybrid proteins resulted in DNA binding, neither to P2 DNA nor to WΦ DNA.

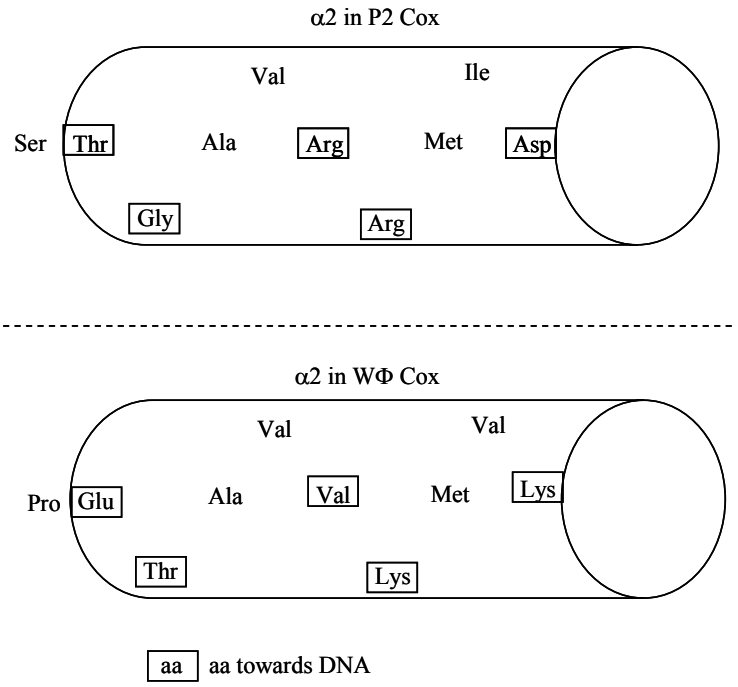


Figure14. The presumed recognition helices (α2) of P2 Cox (top) and WΦ Cox (bottom). Amino acids believed to be oriented towards DNA are boxed.

Paper IV-Cooperative interactions between bacteriophage P2 integrase and its accessory factors IHF and Cox

The intasome formation in λ has been studied extensively and it is known that the arm-binding domain of λ Int is located in the N-terminus (Wojciak *et al.*, 2002). The arm-binding site is a high affinity site and an IHF-induced bend allows binding to the core site (de Vargas *et al.*, 1989). The N-terminal domain is also involved in cooperative interaction with Xis, the λ excisionase (Swalla *et al.*, 2003). We wanted to investigate if the N-terminus of the P2 integrase is associated in arm-binding and potential interactions between P2 Int and its accessory proteins IHF and P2 Cox. Cooperative binding between P2 Int and IHF has been indicated previously (Yu and Haggård-Ljungquist, 1993b).

P2 integrase binds cooperatively with IHF to *attP*

When Int binding to *attP* is analyzed by EMSA, the majority of the DNA is found in a large complex that cannot enter the gel. This complex is presumed to be large intermolecular networks between Int and DNA (Yu and Haggård-Ljungquist, 1993b). In this work it is shown that when IHF is added, these large complexes are solved and several other complexes can be observed. It is further shown that Int and IHF bind cooperatively resulting in more DNA than predicted from independent binding events being found in complexes with Int and IHF. This cooperativity is still seen, but in a reduced form, with an N-terminal truncated integrase where amino acids 2 to 26 have been removed.

The N-terminal truncated P2 integrase is defective in arm-binding

To be able to explain the reduction in cooperativity seen with the truncated Int, we studied the binding to two substrates in addition to the attP substrate, one containing only the P-arm and the IHF site and the other containing the IHF site and the core. We could show that wt Int binds weakly to the P-arm, and this binding is enhanced by the addition of IHF. The N-terminal truncated Int showed no binding at all to the P-arm substrate and binding could not be induced by the presence of IHF. We therefore conclude that the N-terminal truncated Int is defective in arm binding. The cooperative binding of the truncated Int and IHF to the attP substrate should therefore be the result of cooperative binding to the core substrate. This was confirmed by analyzing binding to the core which is strong for both the wt and the truncated proteins and further enhanced by IHF.

The cooperativity is sensitive to the distance between the core- and the IHF binding sites and a large reduction in cooperativity is seen when the distance is enlarged. This is found for both the core- and the attP substrates (data not shown for the attP substrate) and the substantial reduction seen in cooperative binding led us to conclude that the cooperative binding to the core site is the major contributor to the overall cooperativity seen for attP and the cooperative binding to the P-arm should contribute only to a minor extent.

The P2 integrase and Cox bind in a cooperative manner to *attP*

P2 Cox is known to function as an excisionase by promoting excision and inhibit integration, and it recognizes six Cox boxes in the *attP* region (Saha *et al.*, 1987b) (Yu and Haggård-Ljungquist, 1993a). In this work a cooperative binding of Cox and Int to the attP substrate is shown and just like IHF, Cox inhibits the Int formation of large intermolecular networks with attP. The cooperativity seen is reduced but still present when the truncated integrase is used. In the recombinogenic complex, Cox is the equivalent to λ Xis but they seem to be different in their structures. Xis adopts a winged helix motif recognizing a direct repeat while Cox recognizing several Cox boxes arranged as two sets of three direct repeats, positioned in opposite directions, probably by a predicted HTH motif in the N-terminus (Yu and Haggård-Ljungquist, 1993a) (Eriksson and Haggård-Ljungquist, 2000). The C-terminus of Cox is presumed to contain epitopes for oligomerization and interactions with other proteins (Eriksson and Haggård-Ljungquist, 2000). Even though the cooperativity is reduced for the truncated integrase the capacity for cooperative interactions with Cox probably resides in the C-terminus of the P2 integrase.

Concluding remarks and future perspectives

The C repressor proteins

DNA binding

The switch regions of several P2 related coliphages have been sequenced and they constitute at least six distinct immunity groups (J. Karlsson, A. Nilsson and E. Haggård-Ljungquist, personal communication), but more detailed studies of the developmental switches have only been conducted in P2, WΦ and now P2 Hy *dis*. Analyzing the DNA sequences of the switch regions show that there are some differences but also many important similarities to be found; such as a predicted HTH motif in the N-terminus of the Cox and C repressors and that different versions of a direct repeat, known to constitute the C operator in P2, WΦ and P2 Hy *dis* (PaperI, and Liu and Haggård-

Ljungquist, 1999), are found in the vicinity of the presumed Pe promoters in the other immunity groups as well.

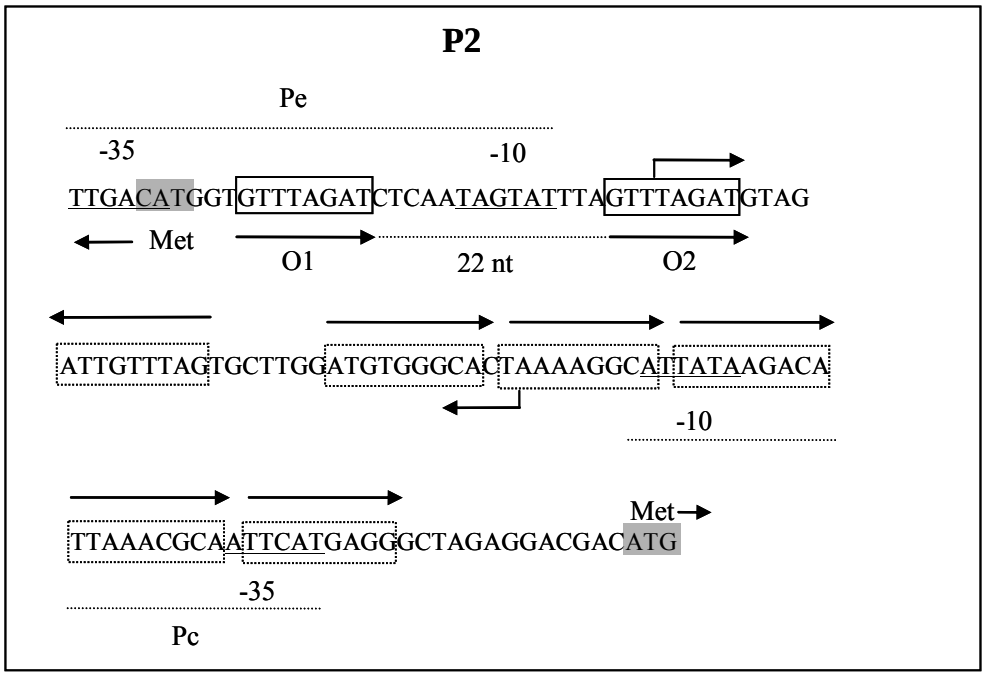
The N-terminus of P2 C has been hypothesized to be involved in DNA binding (Liu *et al.*, 1998). At least one mutation in the N-terminus seems to affect DNA binding. (Ljungquist *et al.*, 1984) (Renberg-Eriksson *et al.*, 2000) and to confirm this it would be valuable to solve the 3D structure of one or several of the immunity repressors of the P2 family, preferably bound to operator DNA.

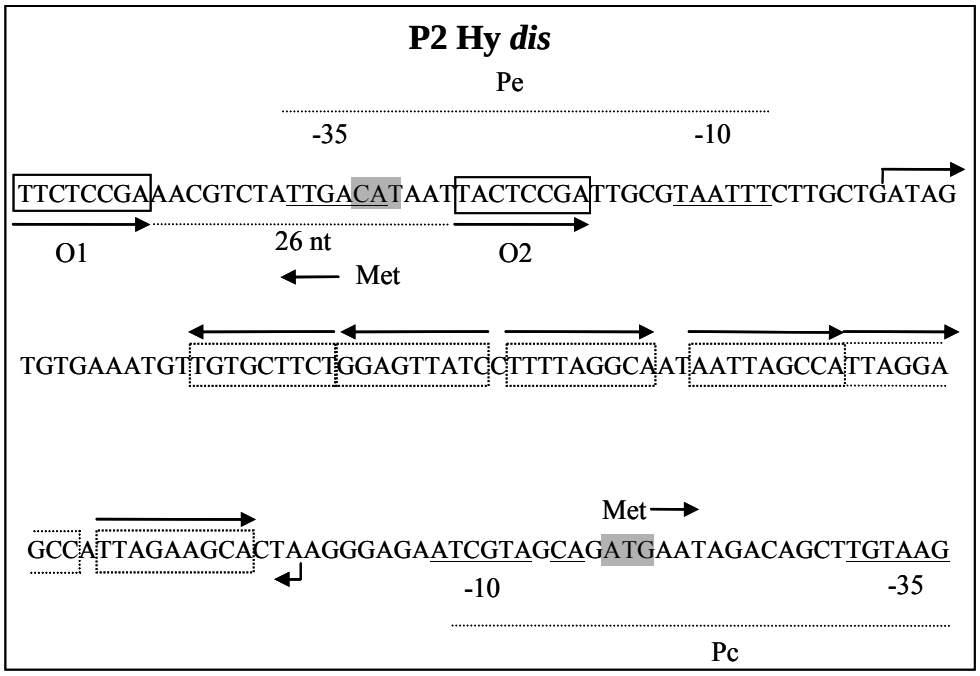
A crystal structure with DNA would be interesting from several perspectives considering that the repressors recognize and bind to a direct repeat. It is not obvious how this is combined with the fact that the active form of the C protein of P2 is a dimer (Lundqvist and Bertani, 1984) (Liu *et al.*, 1998). Binding to a direct repeat is either a result of looping where one interaction surface is enough for dimerization, or a head-to-tail structure where there are two different dimerization epitopes in the protein. The C repressors bend their DNA targets but if symmetrical or asymmetrical interaction surfaces are involved in monomer interactions is not know. AraC, a well studied repressor of the *araBAD* operon, recognizes a direct repeat and causes a DNA loop by bridging distant operator sites together and a single AraC dimer is enough for this loop formation, the half-sites are however separated by a larger distance than in P2 (Martin and Rosner, 2001). λ Xis is another example of a dimer binding to a direct repeat where the half-sites are separated by only seven nt which is more comparable to what is seen for P2 and its relatives where the distances between the half-sites vary from approximately 20 to 35 nt. How Xis binds is not known but the two monomers bound to X1 and X2 are believed to interact and be juxtaposed as a result of the strong Xis induced bending and asymmetric interaction surfaces are then supposed to be exposed to result in a head-to-tail configuration (Sam *et al.*, 2004).

Even if a dimer is the active form of the P2 C repressor in solution (Lundqvist and Bertani, 1984) (Liu *et al.*, 1998), an oligomerization to higher order complexes might take place on the DNA. It could be a dimer that is bound to the direct repeat but it could also be a dimer bound to each half-sites as a result of oligomerization on DNA. Whether the C repressors oligomerize on DNA could be determined by analyzing the complexes generated in DNA-protein binding reactions. By cross-linking the different complexes generated and analyzing their sizes on a protein gel it should be possible to conclude how many C molecules are bound to DNA in the different complexes. The different C repressors might show different binding patterns as indicated in the EMSAs performed to determine the bending capacities of P2 and WΦ C (Paper II). Using WΦ C, three bands are evident, where two bands might constitute binding to one or the other half-site, and the slowest migrating complex should constitute the repressor bound to both half-sites. In the case of P2 C, only one major band is obtained, but a faster migrating weak band is also obtained that might represent binding to one half-site. One

interpretation is that binding of P2 C to one operator half-site leads to a strong cooperative binding to the second half-site, resulting in the fast formation of only one major complex. However, since P2 C bends its DNA target, it is surprising that two weak bands are not seen since the half-sites are located differently relative to the ends. Possible one of the half-sites has a higher affinity for the C protein than the other. In WΦ, as opposed to P2, it seems as if C binds independently to the respective half-sites leading to complexes that migrate differently depending on what half-site is bound and that the cooperative binding to the half-sites is not as strong as the one seen for P2 C. Through mutational analysis it could be possible to determine whether P2 C has different affinities for the half-sites.

The operators of P2, WΦ and P2 Hy *dis* are all slightly different in length as well as in spacing (Fig. 15). In P2 there are the two 8 base pairs long half-sites separated by 22 bp (center to center) and they will probably be presented on the same side of the DNA helix separated by two helical turns. In WΦ, where the repeat is 10 base pairs long, the spacing is even longer, 34 bp. The WΦ half-sites do not seem to be exactly on the same side of the DNA helix since one helical turn corresponds to 10.5 bp and 34 bp therefore corresponds to a bit more than three helical turns. P2 Hy *dis* is intriguing with its imperfect repeat. The location is also interesting with the direct repeat flanking the -35 region instead of the -10 hexamer as in P2 and WΦ. Furthermore the spacing, approximately 26 bp, implies that the half-sites are not to be found on the same side of the DNA helix. These differences might affect the binding of the three C repressors and could be reflected in differences in the structure and/or flexibility of the three repressors even if they are small (99 aa) and/or in the distortion of DNA upon binding. We know that both P2 C and WΦ C bend DNA (Paper II) and it would be interesting to include P2 Hy *dis* C in a circular permutation analysis. One way to examine the importance of an exact distance could be done by varying the distances in the different C operators and then quantify the capacity of the C proteins to repress their respective Pe promoter. It would be interesting to vary the distances so that the half-sites are presented on the same as well as the opposite sides of the DNA helix.


P2
Pe
-35
-10
TTGACATGGTGTTTAGATCTCAATAGTATTTAGTTTAGATGTAG
Met
O1
22 nt
O2
ATTGTTTAGTGCTTGGATGTGGGCACTAAAAGGCATTATAAGACA
-10
Met
TTAAACGCAATTCATGAGGGCTAGAGGACGACATG
-35
Pc


P2 Hy *dis*
Pe
-35
-10
TTCTCCGAAACGTCTATTGACATAATTACTCCGATTGCGTAATTTCTTGCTGATAG
O1
26 nt
O2
Met
TGTGAAATGTTGTGCTTCTGGAGTTATCCTTTTAGGCAATAATTAGCCATTAGGA
Met
GCCATTAGAAGCACTAAGGGAGAATCGTAGCAGATGAATAGACAGCTTGTAAG
-10
-35
Pc

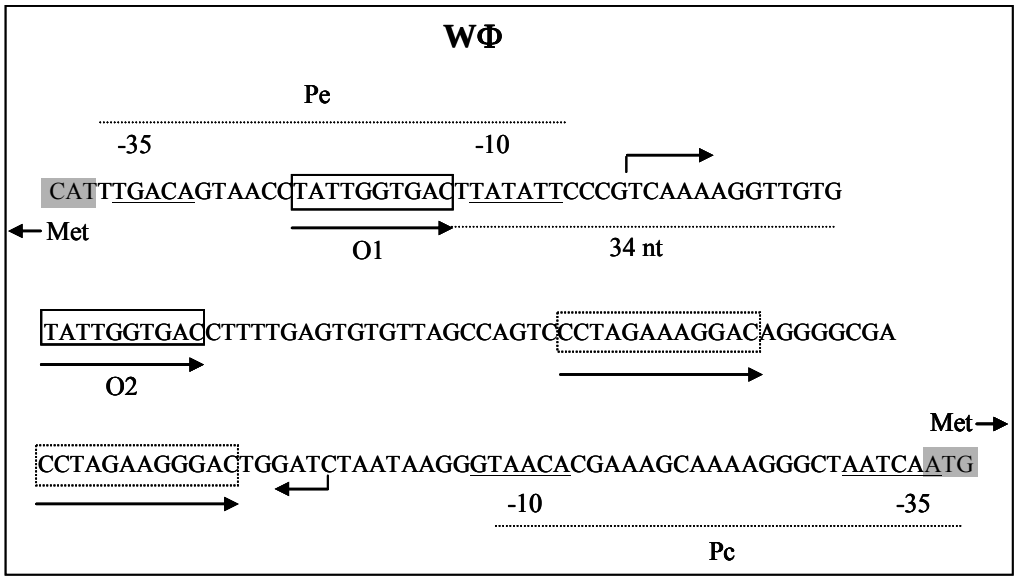


Figure15. The developmental switch regions of P2 (top), P2 Hy *dis* (middle) and WΦ (bottom). Core promoter regions are underlined, transcriptional start sites indicated by bent arrows. C operator half-sites are boxed, Cox boxes are boxed with broken lines and direction of repeats are illustrated by arrows.

Transcription regulation by the C repressors

The effect of C induced bending

Neither the mechanisms behind C induced repression of Pe nor the way P2 C represses and activates P2 Pc are known in detail. It is probably difficult to see repression of Pe and activation of Pc as isolated events. Repression of Pe might automatically lead to activation of Pc since transcription from Pe interferes with transcription from Pc, the same pattern is seen for phage 186 (Saha *et al.*, 1987b) (Callen *et al.*, 2004). It is important to remember that autoregulation of Pc by C has only been confirmed in P2 and it needs to be verified for the other P2 family members.

The pattern seen in EMSAs using P2 Hy *dis* C and the wt or mutated C operator region shows that there is not a direct correlation between repression and binding (Paper I). Even though P2 Hy *dis* C is no longer able to repress the double operator mutant it still binds to the DNA. However, the binding is affected and one band, present with the wt DNA substrate, is missing in reactions with the mutant substrate indicating that this complex can not form. P2 C is also incapable of repressing Pe when two mutations, one mutation in each half-site, have been introduced (S. Renberg Eriksson and P. Henriksson Peltola, personal communication) but C binds to this mutant operator as efficiently as to the wt operator. However, preliminary results indi-

cate that P2 C will not bend this mutated substrate (preliminary unpublished results, P. Henriksson Peltola) which might explain the inability to repress Pe. This might very well be true for P2 Hy *dis* as well. The C induced bend might cause an unfavorable DNA configuration for transcription from Pe. The bending can however work in many ways repressing Pe and activating Pc. DNaseI protection studies show that P2 C enhances the binding of RNAP to Pc and this has been assumed to contribute to C activation of Pc (Saha *et al.*, 1987a). It is not known if this recruitment is dependent on a direct protein-protein interaction between C and RNAP or if DNA bending could be enough. DNA bending by C could create more interaction surfaces between Pc and RNAP. Recruiting RNAP to Pc might not only activate Pc but also repress the stronger Pe promoter by "stealing" RNAP from Pe. C induced bending could bring Pc and Pe closer together and thereby facilitating the possible recruitment of RNAP from Pe to Pc. It can however be other steps in the transcription initiation than the actual recruitment of RNAP that are affected by the C induced bending, e.g. stimulation of the isomerization from the closed to the open DNA-RNAP complex.

The effect of transcriptional interference between Pe and Pc

In P2, the Pe and Pc transcripts overlap with approximately 35 bp, in P2 Hy *dis* with 75 bp, and in WΦ with 82 bp (Paper II, Paper I and Liu and Haggård-Ljungquist, 1999). The Pc promoter of P2 Hy *dis*, as judged by the sequence, seems stronger than our results indicate and also compared to the P2 Pc sequence. This would favor a hypothesis where head-on-collisions between elongating complexes from Pe and Pc is the major cause for interference of transcription from Pc and the longer the overlapping region the more negative is the effect on Pc. For phage 186, on the other hand, it has been shown that elongation from the strong pR promoter passing over pL interfere with transcription from pL since there is a slow release of open complexes ("sitting ducks") formed at pL (Callen *et al.*, 2004). A head-on-collisions could be excluded as a major cause for interference in 186 since increasing the inter-promoter distance had almost no effect on interference. In these studies P2 Pe and Pc were included in the experiments concluding that there is a "sitting duck" mechanism behind the observed interference in P2 as well as in 186, even if P2 Pc has a faster promoter clearance than 186 pL. I think we have a unique opportunity to examine this thoroughly since we can include several of the P2 relatives with known differences in overlaps between the two transcripts in an analysis similar to the one conducted by Callen and co-workers. Whatever mechanism is behind the transcriptional interference, a positive autoregulation will automatically follow as a result of the repression of Pe. A negative autoregulation, on the other hand, probably relies on other mechanisms. The positive autoregulation might be different within the P2 family, different mechanisms might contribute to a varying degree to the overall regulation seen. In P2, RNA polymerase binding to Pc covers 75-80

bp, (from -60 to +20) which should result in P2 C and RNAP being close enough to interact directly which might explain the positive autoregulation of Pc by P2 C, probably in concert with a relief of transcriptional interference when C represses Pe. In WΦ and P2 Hy *dis* however, the promoters are more distal and the C operators are more distant from Pc (Paper I, Paper II and Liu and Haggård-Ljungquist, 1999) and the positive regulation of C on Pc might depend completely on the relief of transcriptional interference when C represses Pe. Additional cryptic C binding sites closer to WΦ and P2 Hy *dis* Pc or the C induced bending (assuming P2 Hy *dis* C also bends DNA) might however allow RNAP and C to come in close contact in WΦ and P2 Hy *dis* as well as P2. It would be interesting to look for direct interactions between RNAP and the different C repressors.

To summarize it can be said that the repression of Pe and activation of Pc by the C repressors are probably two events mutually affecting each other. The C induced DNA bending could be involved in creating an unfavorable DNA configuration for transcription from Pe and simultaneously assist in recruiting RNAP to Pc. The recruitment might be further stimulated by an interaction between RNAP and C. When Pe is repressed there is no transcriptional interference from Pe on Pc, Pc transcription is established and may in part be maintained by an interaction between C and RNAP. I think we should really take advantage of the fact that we have several family members where the switch regions contain interesting differences, such as inter-promoter distances, to investigate to what extent the different mechanisms contribute to the overall picture and study the relative promoter strengths in the family members which might vary and contribute to the complex picture. It is obvious, looking at λ and 186, that there are several solutions to a common need to choose between lifestyles and this might be seen comparing otherwise very similar P2 members as well.

The affinity of the C repressors to their operators

The stability of a lysogen depends on the DNA binding affinity and the production of the C repressor. As presented in Paper II, WΦ has a higher spontaneous phage production compared to P2 and this might reflect a lower binding affinity of WΦ C for its operator than for P2 C to its operator. WΦ C might dissociate with a higher constant allowing higher frequency of prophage induction. This is currently under investigation by surface plasmon resonance analysis (SPR) and EMSA and preliminary data indicate that WΦ C has a lower affinity for its operator compared to both P2 and P2 Hy *dis* to their respective operator, indicating that the WΦ C-DNA complex is less stable (P. Henriksson Peltola, personal communication). This is further supported by the fact that P2 and P2 Hy *dis* show similar frequencies of spontaneous phage production (unpublished results).

The Cox proteins

DNA binding

We believe that the DNA binding motif of the Cox proteins is a predicted HTH motif found in the N-terminus. The helix-turn-helix domain is common in both basal and specific transcription factors in several of the kingdoms of life (Aravind *et al.*, 2005). Still it would be interesting to confirm this by crystallization and analysis of the 3D structure. A 3D structure could also reveal if there are one or two types of oligomerization surfaces in Cox. The P2 Cox boxes are arranged in different orientations in the different targets and it is not obvious if the Cox monomers oligomerize in a head-to-head or head-to-tail manner (or both). In WΦ, Cox binding to a direct repeat is either a result of bending/looping where one interaction surface is enough for dimerization, or a head-to-tail structure where there are two different dimerization epitopes in the protein. Considering the strong Cox induced DNA bending there is a possibility that a loop allows a head-to-head dimerization even though the distance between the half-sites is relatively short. It is also possible that different interaction surfaces are used for dimer formation and the formation of higher order oligomers, as in λ CI (Bell *et al.*, 2000). It would also be of great interest to determine the number of Cox molecules bound to the DNA which might be quite different for P2 and WΦ considering their different types of binding sites.

As implicated previously (Eriksson and Haggård-Ljungquist, 2000), P2 Cox probably lacks independently folded domains due to its small size. This might explain the difficulties we have had to change the DNA specificity of WΦ Cox to that of P2 Cox. Several amino acids, far apart in the primary structure, might come close in the folded protein and stabilize the DNA binding. The difficulties we have had provide support for the idea that there is no simple code for DNA-protein recognition. A residue in the C-terminal half of the CI repressor of the mycobacteriophage L1 plays an important role in binding of CI to its operator although the DNA binding HTH motif is located to the N-terminus (Ganguly *et al.*, 2004). Our results using hybrid proteins indicate that there is something in the C-terminus involved in DNA binding. The structure of the λ Cro protein in complex with its operator DNA has been determined (Albright and Matthews, 1998). It was known from before that the HTH motif, with the recognition helix inserted into the major groove, makes direct contact with the bases in the DNA and that the base-specific contacts that are most important for tight operator binding all occur within the recognition helix (Takeda *et al.*, 1989). This is supported by Albright but a complex picture emerges with many residues making not just one, but several, base-specific contacts and a few residues outside the HTH motif seem to be involved. In a work by van Sinderen and co-workers (submitted for publication) helix swap experiments have been performed involving the CI repressors of the lactococcalphages R1t and Tuc2009. These proteins

are very similar at a primary sequence level and still the helix swap experiment only worked one way (van Sinderen, personal communication) supporting what seems to be a general view that there are several levels of complexity in protein-DNA binding. P2 Cox and WΦ Cox are only similar at a predicted secondary structure level but quite different at a primary sequence level. Their respective way of binding to the DNA and oligomerizing might differ substantially considering the fact that P2 Cox recognizes several Cox boxes while WΦ Cox binds to one direct repeat. This could complicate the construction of a specificity mutant. Maybe oligomerization and an exact bending angle contributes to the specificity for a certain DNA target. If a specificity mutant is to be identified I think the WΦ Cox protein, or possibly one of the hybrid proteins constructed, should be subject to a random mutagenesis and the library of mutants could be screened in a plasmid system where DNA binding would activate P4 P_{LL}.

Cooperativity with the integrase

The data presented in Paper IV, where we show that P2 Int and P2 Cox bind cooperatively to *attP*, raises many new questions. We have weak indications of a direct protein-protein interaction between Int and Cox *in vitro* (A. Ahlgren Berg and C. Frumerie, unpublished results). If this interaction can be confirmed, it would be interesting to identify the amino acids involved in the interaction.

If a "DNA-specificity" modified WΦ Cox protein can be constructed it is important to remember that this modified protein and P2 Int might not be able to bind cooperatively to DNA and therefore a *cox* defective P2 prophage might still not be excised. This could be analyzed by EMSAs using P2 *attP*, P2 Int and the "DNA-specificity" modified WΦ Cox protein. If possible, it would also be interesting to examine if a completely unrelated protein, causing the same bending angle as Cox, could substitute for Cox in the prophage excision event. If the cooperativity seen between Int and Cox is not dependent on protein-protein interactions but only on the strong bending induced by Cox, it might be possible to replace Cox. It is however important to remember that an exact bending angle might be required and that might be difficult to generate.

There are indications that increasing the distance between the Int core binding site and the Cox boxes in *attP* has no impact on the cooperativity, as opposed to Int and IHF, but this needs to be confirmed (A. Ahlgren Berg and C. Frumerie, unpublished results) and different distance should be tried. As mentioned in the description of Paper III, WΦ Cox shows a poor binding to WΦ *attP* compared to what is seen for P2 Cox. Whether this is due to the sequence of the repeat at *attP* or the spacing between the half-sites could be studied by a mutational analysis The cooperativity seen for P2 Int and P2 Cox binding to *attP* might be of greater importance in WΦ than in P2 and it

would be easy to conduct the same study as in Paper IV with WΦ Cox and WΦ Int.

The Cox proteins as repressors and activators

Judging from DNaseI protection studies (Saha *et al.*, 1987b) and Paper III, the Cox proteins might very well function as repressors by excluding RNAP from Pc and, at higher concentrations, from Pe as well. In P2 it is obvious that Pc is protected and the Pe core promoter region is also protected at higher Cox concentrations (Saha *et al.*, 1987b). In WΦ, the footprint seems to cover the transcription start of Pc and the entire Pe-Pc region when the Cox concentration is increased. It is however important to note that a negative autoregulation of Pe has not been examined for WΦ. Even if repression by steric hindrance of RNAP binding is not dependent on bending of the DNA, it might be that the strong Cox induced bending is important for the repression of Pc and/or Pe, especially if subsequent steps in the initiation of transcription is involved in regulation. At P4 P_{LL}, P2 Cox and P2 Hy *dis* Cox function as activators and it would be interesting to determine if activation is caused by Cox induced bending of the DNA or interactions with RNAP. It would therefore be interesting to look for direct interactions between Cox and RNAP. At P_{LL}, the Cox boxes are located upstream the promoter (as opposed to downstream Pc) at a distance where activators can contact RNAP directly.

Evolution of P2-like phages

The P2-like coliphages may be considered as different P2 isolates since they show at least 96% similarity of their structural genes (Nilsson and Haggård-Ljungquist, 2001). However, as shown in this work, the transcriptional switches of the phages characterized so far show a considerable variation. At least six different immunity groups, and three different integration sites, have been identified among the closely related P2 phages (J. Karlsson, C. Cardoso, A. Nilsson, and E. Haggård/Ljungquist, personal communication). P2-like phages are frequent in *E. coli* and most likely compete for a suitable host (Nilsson *et al.*, 2004). A superinfecting phage can either grow lytically or form lysogeny if it belongs to a different immunity group than the residing prophage. If the two phages belong to the same immunity group, the immunity repressor of the resident prophage will block lytic growth of the superinfecting phage. Under these conditions, the superinfecting phage must integrate into the host to be stably maintained. However, the formation of lysogeny is problematic if the two phages share the same integration site, since tandem prophages are unstable due to expression of the integrase from prophages integrated in tandem (Bertani, 1971) (Haggård-Ljungquist *et al.*, 1994). Under these condition the superinfecting phage must either exchange place with the resident prophage or integrate at a secondary site (Bertani and Six, 1988)

(Barreiro and Haggård-Ljungquist, 1992). Thus, the competition between the P2-like coliphages has most likely driven evolution towards different immunities and integration sites. The processes leading to different immunities and integration sites among the P2-like coliphages is impossible to tell from this work, many more P2 related phages from *E. coli* and other enterobacteria must be characterized before a phylogenetic analysis can be undertaken. But recombination with related heteroimmune phages is one possible explanation. An example of such an event is P2 Hy *dis* that was generated by a recombination between P2 and a defective heteroimmune prophage present in *E. coli* B.

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References

Ackermann, H.W. (1999) Tailed bacteriophages: the order of Caudovirales. *Adv. Virus Res.* **51**: 135-201.

Alazard, R., Betemier, M., and Chandler, M. (1992) *Escherichia coli* integration host factor stabilizes bacteriophage Mu repressor interactions with operator DNA *in vitro*. *Mol. Microbiol.* **6**: 1707-1714.

Albright, R.A., and Matthews, B.W. (1998) Crystal structure of λ-Cro bound to a consensus operator at 3.0 Å resolution. *J. Mol. Biol.* **280**: 137-151.

Aravind, L., Anantharaman, V., Balaji, S., Babu, M.M., and Iyer, L.M. (2005) The many faces of the helix-turn-helix domain: Transcription regulation and beyond. *FEMS Microbiol. Rev.* **29**: 231-262.

Ayers, D.J., Sunshine, M.G., Six, E.W., and Christie, G.E. (1994) Mutations affecting two adjacent amino acid residues in the alpha subunit of RNA polymerase block transcriptional activation by the bacteriophage P2 Ogr protein. *J. Bacteriol.* **176**: 7430-7438.

Azaro, M.A., and Landy, A. (2002) λ integrase and the λ Int family. In *Mobile DNA II*. Craig, N.L., Craige, R., Gellert, M. and Lambowitz, A.M. (eds). 1752 N Street NW, Washington, DC: ASM Press, pp. 118-148.

Bæk, K., Svenningsen, S., Eisen, H., Sneppen, K., and Brown, S. (2003) Single-cell analysis of λ immunity. *J. Mol. Biol.* **334**: 363-372.

Ball, C.A., Osuna, R., Ferguson, K.C., and Johnson, R.C. (1992) Dramatic changes in Fis level upon nutrient upshift in *Escherichia coli*. *J. Bacteriol.* **174**: 8043-8056.

Barne, K.A., Bown, J.A., Busby, S.J.W., and Minchin, S.D. (1997) Region 2.5 of the *Escherichia coli* RNA polymerase σ^{70} subunit is responsible for the recognition of the 'extended -10' motif at promoters. *EMBO J.* **16**: 4034-4040.

Barreiro, V., and Haggård-Ljungquist, E. (1992) Attachment sites for bacteriophage P2 on the Escherichia coli chromosome: DNA sequences, localization on the physical map, and detection of a P2-like remnant in E. coli K-12 derivatives. *J. Bacteriol.* **174**: 4086-4093.

Bell, C.E., Frescura, P., Hocschild, A., and Lewis, M. (2000) Crystal structure of the λ repressor C-terminal domain provides a model for cooperative operator binding. *Cell* **101**: 801-811.

Bertani, G. (1951) Studies on lysogenesis I. The mode of phage liberation by lysogenic *Escherichia coli*. *J. Bacteriol.* **62**: 293-300.

Bertani, G. (1957) "A disimmune mutant of temperate phage P2." *Bacteriol. Proc. Soc. Am. Bacteriologists.*

Bertani, L.E. (1968) Abortive induction of bacteriophage P2. *Virol.* **36**: 87-103.

Bertani, L.E. (1971) Stabilization pf P2 tandem double lysogens by *int* mutations in the prophage. *Virol.* **46**: 426-436.

Bertani, L.E., and Bertani, G. (1971) Genetics of P2 and related phages. *Adv. Genet.* **16**: 199-237.

Bertani, L.E., and Six, E.W. (1988) The P2-like phages and their parasite, P4. *The bacteriophages (R. Calendar, Ed.), Volume 2 Plenum Publishing Corporation, New York and London.*

Boyd, E.F., and Brüssow, H. (2002) Common themes among bacteriophage-encoded virulence factors and diversity among the bacteriophages involved. *Trends Microbiol* **10**: 521-529.

Briani, F., Dehò, G., Forti, F., and Ghisotti, D. (2001) The plasmid status of satellite bacteriophage P4. *Plasmid* **45**: 1-17.

Brosius, J. (1984) Plasmid vectors for selection of promoters. *Gene* **27**: 151-160.

Browning, D.F., and Busby, S.J. (2004) The regulation of bacterial transcription initiation. *Nature Rev. Microbiol.* **2**: 57-65.

Brüssow, H., and Hendrix, R.W. (2002) Phage genomics: small is beautiful. *Cell* **108**: 13-16.

Bullas, L.R., Mostaghimi, A.R., Arensdorf, J.J., Rajadas, P.T., and Zuccarelli, A.J. (1991) *Salmonella* phage PSP3, another member of the P2-like phage group. *Virology* **185**.

Burgess, R.R., and Anthony, L. (2001) How sigma docks to RNA polymerase and what sigma does. *Curr. Opin. Microbiol.* **4**: 126-131.

Burr, T., Mitchell, J., Kolb, A., Minchin, S., and Busby, S. (2000) DNA sequence elements located immediately upstream of the -10 hexamer in *Escherichia coli* promoters: a systematic study. *Nucl. Acid. Res.* **28**: 1864-1870.

Busby, S., and Ebright, R.H. (1994) Promoter structure, promoter recognition, and transcription activation in prokaryotes. *Cell* **79**: 743-746.

Busby, S., and Ebright, R.H. (1997) Transcription activation at class II CAP dependent promoters. *Mol. Microbiol.* **23**: 853-859.

Bushman, W., Yin, S., Thio, L.L., and Landy, A. (1984) Determinants of directionality in lambda site-specific recombination. *Cell* **39**: 699-706.

Calendar, R., Yu, S., Myung, H., Barreiro, V., Odegrip, R., Carlson, K., Davenport, L., Mosig, G., Christie, G.E., and Haggård-Ljungquist, E. (1998) The lysogenic conversion genes of coliphages P2 have unusually high AT content. In *Horizontal Gene Transfer*. Syvanen, M. and Kado, C.I. (eds). London: Chapman & Hall.

Calì, S., Spoldi, E., Piazzolla, D., Dodd, I.B., Forti, F., Dehò, G., and Ghisotti, D. (2004) Bacteriophage P4 Vis protein is needed for prophage excision. *Virology* **322**: 82-92.

Callen, B.P., Shearwin, K.E., and Egan, J.B. (2004) Transcriptional interference between convergent promoters caused by elongation over the promoter. *Mol. Cell* **14**: 647-656.

Camacho, A., and Salas, M. (1999) Effect of mutations in the "Extended -10" motif of three *Bacillus subtilis* σ^A -RNA polymerase-dependent promoters. *J. Mol. Biol.* **286**: 683-693.

Camacho, A., and Salas, M. (2001) Mechanism for the switch of Φ29 DNA early to late transcription by regulatory protein p4 and histone-like protein p6. *EMBO J.* **20**: 6060-6070.

Casjens, S. (2003) Prophages and bacterial genomes: what have we learned so far? *Mol. Microbiol.* **49**: 277-300.
Chattoraj, D.K., and Inman, R.B. (1973) Electron microscope heteroduplex mapping of P2 Hy *dis* bacteriophage DNA. *Virology* **55**: 174-182.
Chattoraj, D.K. (1978) Strand-specific break near the origin of bacteriophage P2 DNA replication. *Proc. Nat. Acad. Sci. USA* **75**: 1685-1689.
Christie, G.E., and Calendar, R. (1990) Interactions between satellite bacteriophage P4 and its helpers. *Annu. Rev. Genet.* **24**: 465-490.
Cohen, D. (1959) A variant of phage P2 originating in *Escherichia coli*, strain B. *Virology* **7**: 112-126.
Cohen, D. (1960) Analyse de l'hybridation du phage P2 et d'un prophage défictif d'*Escherichia coli* B par la méthode de centrifugation en gradient de densité. *C. R. Acad. Sci. Paris.* **250**: 946-948.
Coulombe, B., and Burton, Z.F. (1999) DNA bending and wrapping around RNA polymerase: a "revolutionary" model describing transcriptional mechanisms. *Microbiol. Mol. Biol. Rev.* **63**: 457-478.
Craig, N.L., and Nash, H.A. (1984) *E. coli* integration host factor binds to specific sites in DNA. *Cell* **39**: 707-716.
Cuff, J.A., and Barton, G.J. (2000) Application of multiple sequence alignment profiles to improve protein secondary structure prediction. *Proteins* **40**: 502-511.
de Vargas, M., Kim, S., and Landy, A. (1989) DNA looping generated by DNA bending protein IHF and the two domains of lambda integrase. *Science* **244**: 1457-1461.
Dehò, G., Ghisotti, D., Alano, P., Zangrossi, S., Borrello, M.G., and Sironi, G. (1984) Plasmid mode of propagation of the genetic element P4. *J. Mol. Biol.* **178**: 191-207.
Dehò, G., Zangrossi, S., Ghisotti, D., and Sironi, G. (1988) Alternative promoters in the development of bacteriophage plasmid P4. *J. Virol.* **62**: 1697-1704.
Dehò, G., Zangrossi, S., Sabbattini, P., Sironi, G., and Ghisotti, D. (1992) Bacteriophage P4 immunity controlled by small RNAs via transcription termination. *Mol. Microbiol.* **6**: 3415-3425.
Dehò, G., and Ghisotti, D. (1999) P4 phage (satellites). In *Encyclopedia of Virology, 2nd edn*. Granoff, A. and Webster, R.G. (eds). London: Academic Press, pp. 1094-1104.
Dehò, G., and Ghisotti, D. (2005) The satellite phage P4. Calendar, R. (ed): In press.
Dodd, I.B., Kalionis, B., and Egan, J.B. (1990) Control of gene expression in the temperate coliphage 186. VIII. Control of lysis and lysogeny by a transcriptional switch involving face-to-face promoters. *J. Mol. Biol.* **214**: 27-37.
Dodd, I.B., Reed, M.R., and Egan, J.B. (1993) The Cro-like Apl repressor of coliphage 186 is required for prophage excision and binds near the phage attachment site. *Mol. Microbiol.* **10**: 1139-1150.
Dodd, I.B., and Egan, J.B. (1996) DNA binding by the coliphage 186 repressor protein CI. *J. Biol. Chem.* **271**: 11532-11540.
Dodd, I.B., and Egan, J.B. (2002) Action at a distance in CI repressor regulation of the bacteriophage 186 genetic switch. *Mol Microbiol* **45**: 697-710.

Dodd, I.B., Shearwin, K.E., Perkins, A.J., Burr, T., Hochschild, A., and Egan, J.B. (2004) Cooperativity in the long-range gene regulation by the λ CI repressor. *Genes & Dev.* **18**: 344-354.

Dodd, I.B., Shearwin, K.E., and Egan, J.B. (2005) Revisited gene regulation in bacteriophage λ. *Curr. Opin. Genetics & Development* **15**: 145-152.

Dokland, T., Wang, S., and Lindqvist, B.H. (2002) The structure of P4 procapsid produced by coexpression of capsid and external scaffolding proteins. *Virol.* **298**: 224-231.

Dove, S.L., Huang, F.W., and Hochschild, A. (2000) Mechanism for a transcriptional activator that works at the isomerization step. *Proc. Natl. Acad. Sci. USA* **97**: 13215-13220.

Drlica, K. (1990) Bacterial topoisomerases and the control of DNA supercoiling. *TIG* **6**: 433-437.

Drlica, K. (1992) Control of bacterial DNA supercoiling. *Mol. Microbiol.* **6**: 425-433.

Eriksson, J.M., and Haggård-Ljungquist, E. (2000) The multifunctional bacteriophage P2 Cox protein requires oligomerization for biological activity. *J. Bacteriol.* **182**: 6714-6723.

Esposito, D., and Scocca, J.J. (1994) Identification of an HP1 phage protein required for site-specific excision. *Mol. Microbiol.* **13**: 685-695.

Esposito, D., Fitzmaurice, W.P., Benjamin, R.C., Goodman, S.D., Waldman, A.S., and Scocca, J.J. (1996) The complete nucleotide sequence of bacteriophage HP1 DNA. *Nucl. Acids. Res.* **24**: 2360-2368.

Esposito, D., and Scocca, J.J. (1997) Purification and characterization of HP1 Cox and its role in controlling the direction of site-specific recombination. *J. Biol. Chem.* **272**: 8660-8670.

Esposito, D., Thrower, J.S., and Scocca, J.J. (2001) Protein and DNA requirements of the bacteriophage HP1 recombination system: a model for intasome formation. *Nucleic Acids Res.* **29**: 3955-3964.

Forti, F., Sabbattini, P., Sironi, G., Zangrossi, S., Dehò, G., and Ghisotti, D. (1995) Immunity determinant of phage-plasmid P4 is a short processed RNA. *J. Mol. Biol* **249**: 869-878.

Forti, F., Dragoni, I., Briani, F., Dehò, G., and Ghisotti, D. (2002) Characterization of the small antisense CI RNA that regulates bacteriophage P4 immunity. *J. Mol. Biol.* **315**: 541-549.

Friedman, D.I., and Court, D.L. (2001) Bacteriophage λ: alive and well and still doing its thing. *Curr. Opin. Microbiol.* **4**: 201-207.

Frumerie, C., Eriksson, J.M., Dugast, M., and Haggård-Ljungquist, E. (2005) Dimerization of bacteriophage P2 integrase is not required for binding to its DNA target but for its biological activity. *Gene* **344**: 221-231.

Gama, M.J., Toussaint, A., and Higgins, N.P. (1992) Stabilization of bacteriophage Mu repressor-operator complexes by the *Escherichia coli* integration host factor protein. *Mol. Microbiol.* **6**: 1715-1722.

Ganguly, T., Chattoraj, P., Das, M., Chanda, P.K., Mandal, N.C., Lee, C.Y., and Sau, S. (2004) A point mutation at the C-terminal half of the repressor of temperate Mycobacteriophage L1 affects its binding to the operator DNA. *J. Biochem. Mol. Biol.* **37**: 709-714.

Giesselsoder, J., Youderian, P., Dehó, G., Chidambaram, M., Goldstein, R., and Ljungquist, E. (1981) Mutants of satellite virus P4 that cannot derepress their bacteriophage P2 helper. *J. Mol. Biol.* **148**: 1-19.

Gourse, R.L., Ross, W., and Gaal, T. (2000) UPs and downs in bacterial transcription initiation: the role of the alpha subunit of RNA Polymerase in promoter recognition. *Mol. Microbiol.* **37**: 687-695.

Guthold, M., Zhu, X., Rivetti, C., Yang, G., Thomson, N.H., Kasas, S., Hansma, H.G., Smith, B., Hansma, P.K., and Bustamente, C. (1999) Direct observation of one-dimensional diffusion and transcription by *Escherichia coli* RNA polymerase. *Biophys. J.* **77**: 2284-2294.

Haggård-Ljungquist, E., Yu, A., and Bertani, L.E. (1994) Site-specific recombination in bacteriophage P2. *Current Topics in Mol. Genet. (Life Sci. Adv.)* **2**: 75-86.

Halling, C., and Calendar, R. (1990) Bacteriophage P2 *ogr* and P4 *delta* genes act independently and are essential for P4 multiplication. *J. Bacteriol.* **172**: 3549-3558.

Helmann, J.D., and deHaseth, P. (1999) Protein-nucleic acid interactions during open complex formation investigated by systematic alteration of the protein and DNA binding partners. *Biochem.* **38**: 5959-5967.

Hochschild, A., and Dove, S.L. (1998) Protein-protein contacts that activate and repress prokaryotic transcription. *Cell* **92**: 597-600.

Hochschild, A. (2002) The λ switch: cI closes the gap in autoregulation. *Curr. Biol.* **12**: 87-89.

Hsu, L.M. (2002) Promoter clearance and escape in prokaryotes. *Biochimica et Biophysica Acta* **1577**: 191-207.

Hu, J.C., O'Shea, E.K., Kim, P.S., and Sauer, R.T. (1990) Sequence requirements for coiled-coils: analysis with λ repressor-GCN4 leucine zipper fusions. *Science* **250**: 1400-1403.

Kim, J., Zwieb, C., Wu, C., and Adhya, S. (1989) Bending of DNA by gene-regulatory proteins: construction and use of a DNA bending vector. *Gene* **85**: 15-23.

Kwon, H.J., Tirumalai, R., Landy, A., and Ellenberg, T. (1997) Flexibility in DNA recombination: structure of the lambda integrase catalytic core. *Science* **276**: 126-137.

Lamont, I., A, M.B., and Egan, J.B. (1989) UV induction of coliphage 186: Prophage induction as an SOS function. *Proc. Natl. Acad. Sci. USA* **86**: 5492-5496.

Lewis, J.A., and Hatful, G.F. (2001) Control of directionality in integrase-mediated recombination: examination of recombination directionality factors (RDFs) including Xis and Cox proteins. *Nucleic Acids Res.* **20**: 2205-2216.

Li, M., Moyle, H., and Susskind, M.M. (1994) Target of the transcriptional activation function of phage λ cI protein. *Science* **263**: 75-77.

Lindahl, G., and Sunshine, M. (1972) Excision-deficient mutants of bacteriophage P2. *Virol.* **49**: 180-187.

Lindqvist, B.H., Dehó, G., and Calendar, R. (1993) Mechanisms of genome propagation and helper exploitation by satellite phage P4. *Microbiol. Rev.* **57**: 683-702.

Liu, T., Renberg, S.K., and Haggård-Ljungquist, E. (1997) Derepression of prophage P2 by satellite phage P4: Cloning of the P4 ε gene and identification of its product. *J. Virol.* **71**: 4502-4508.

Liu, T., Renberg, S.K., and Haggård-Ljungquist, E. (1998) The E protein of satellite phage P4 acts as an anti-repressor by binding to the C protein of helper phage P2. *Mol. Microb.* **30**: 1041-1050.

Liu, T., and Haggård-Ljungquist, E. (1999) The transcriptional switch of bacteriophage WΦ, a P2-related but heteroimmune coliphage. *J. Virol.* **73**: 9816-9826.

Ljungquist, E., and Bertani, L.E. (1983) Properties and products of the cloned *int* gene of bacteriophage P2. *Mol. Gen. Genet.* **192**: 87-94.

Ljungquist, E., Kockum, K., and Bertani, L.E. (1984) DNA sequences of the repressor gene and operator region of bacteriophage P2. *Proc. Natl. Acad. Sci. USA* **81**: 3988-3992.

Lundqvist, B., and Bertani, G. (1984) Immunity repressor of bacteriophage P2. Identification and DNA binding activity. *J. Mol. Biol.* **178**: 629-651.

Martin, R.G., and Rosner, J.L. (2001) The AraC transcriptional activators. *Curr. Opin. Microbiol.* **4**: 132-137.

Marvik, O.J., Dokland, T., Nokling, R.H., Jacobsen, E., Larsen, T., and Lindqvist, B.H. (1995) The capsid size-determining protein Sid forms an external scaffold on phage P4 procapsids. *J. Mol. Biol.* **251**: 59-75.

McClelland, M., Sanderson, K.E., Spieth, J., and co-authors (2001) Complete genome sequence of *Salmonella enterica* serovar typhimurium LT2. *Nature* **413**: 852-856.

McLeod, S.M., and Johnson, R.C. (2001) Control of transcription by nucleoid proteins. *Curr. Opin. Microbiol.* **4**: 152-159.

Merril, C.R., Scholl, D., and Adhya, S.L. (2003) The prospect for bacteriophage therapy in Western medicine. *Nat rev* **2**: 489-497.

Murakami, K., Masuda, S., Campbell, E.A., Muzzin, O., and Darst, S.A. (2002) Structural basis of transcription initiation: an RNA polymerase holoenzyme-DNA complex. *Science* **296**: 1285-1290.

Nakayama, K., Takashima, K., Ishihara, H., Shinomiya, T., Kageyama, M., Kanaya, S., Ohnishi, M., Murata, T., Mori, H., and Hayashi, T. (1999) The R-type pyocin of *Pseudomonas aeruginosa* is related to P2 phage, and the F-type is related to lambda phage. *Mol. Microbiol.* **38**: 213-231.

Nesper, J., Blass, J., Fountoulakis, M., and Reidl, J. (1999) Characterization of the major control region of *Vibrio cholerae* bacteriophage K139: immunity, exclusion and integration. *J. Bacteriol.* **181**: 2902-2913.

Neufing, P.J., Shearwin, K.E., Camerotto, J., and Egan, J.B. (1996) The CII protein of bacteriophage 186 establishes lysogeny by activating a promoter upstream of the lysogenic promoter. *Mol. Microbiol.* **21**: 751-761.

Neufing, P.J., Shearwin, K., and Egan, J.B. (2001) Establishing lysogenic transcription in the temperate coliphage 186. *J. Bacteriol.* **183**: 2376-2379.

Nickels, B.E., Dove, S.L., Murakami, K.S., Darst, S.A., and Hochschild, A. (2002) Protein-protein and protein-DNA interactions of σ^{70} region 4 involved in transcription activation by λcI. *J. Mol. Biol.* **324**: 17-34.

Nilsson, A.S., and Haggård-Ljungquist, E. (2001) Detecting homologous recombination among bacteriophage P2 relatives. *Mol. Phyl. Evol.* **21**: 259-269.
Nilsson, A.S., Karlsson, J.K., and Haggård-Ljungquist, E. (2004) Site-specific recombination links the evolution of P2-like Coliphages and pathogenic enterobacteria. *Mol. Biol. Evol.* **21**: 1-13.
Nilsson, A.S. (2005) P2-like phages are non-mosaic and evolves vertiacally, tracking the evolution of their γ-proteobacteria hosts. *Manuscript*.
Nilsson, A.S., and Haggård-Ljungquist, E. (2005) The P2-like bacteriophages. In *The bacteriophages*. Calendar, R. (ed): In press.
Numrych, T.E., Gumport, R.I., and Garner, J.F. (1992) Characterization of the bacteriophage lambda excisionase (Xis) protein: the C-terminus is required for Xis-integrase cooperativity but not for DNA binding. *EMBO J.* **11**: 3797-3806.
Nunes-Düby, S.E., Tirumalai, R.S., Kwon, H.J., Ellenberg, T., and Landy, A. (1998) Similarities and differences among 105 members of the Int family of site-specific recombinases. *Nucleic Acids Res.* **26**: 391-406.
Ochman, H., and Selander, R.K. (1984) Standard reference strains of *Escherichia coli* from natural populations. *J. Bacteriol.* **157**: 690-693.
Odegrip, R., Schoen, S., Haggård-Ljungquist, E., Park, K., and Chattoraj, D.K. (2000) The interaction of bacteriophage P2 B protein with *Escherichia coli* DnaB helicase. *J. Virol.* **74**: 4075-4063.
Odegrip, R., and Haggård-Ljungquist, E. (2001) The two active-site tyrosine residues of the A protein play non-equivalent roles during initiation of rolling circle replication of bacteriophage P2. *J. Mol. Biol.* **308**: 147-163.
Pabo, C.O., and Sauer, R.T. (1992) Transcription factors: structural families and principles of DNA recognition. *Annu. Rev. Biochem.* **61**: 1053-1095.
Pérez-Lago, L., Salas, M., and Camacho, A. (2005) A precise DNA bend angle is essential for the function of the phage Φ29 transcriptional regulator. *Nuleic Acids Res.* **33**: 126-134.
Pérez-Martín, J., Rojo, F., and de Lorenzo, V. (1994) Promoters responsive to DNA bending: a common theme in prokaryotic gene expression. *Microbiol. Rev.* **58**: 268-290.
Pérez-Martín, J., and de Lorenzo, V. (1997) Clues and consequences of DNA binding in transcription. *Annu. Rev. Microbiol.* **51**: 593-628.
Pérez-Rueda, E., and Collado-Vides, J. (2000) The repertoire of DNA-binding transcriptional regulators in *Escherichia coli* K-12. *Nucleic Acids Res.* **28**: 1838-1847.
Polo, S., Sturniolo, T., Dehò, G., and Ghisotti, D. (1996) Identification of a phage encoded DNA-binding protein that regulates transcription from late promoters in bacteriophage P4. *J. Mol. Biol.* **257**: 745-755.
Ptashne, M., Jeffrey, A., Johnson, A.D., Maurer, R., Meyer, B.J., Pabo, C.O., Roberts, T.M., and Sauer, R.T. (1980) How the λ repressor and cro works. *Cell* **19**: 1-11.
Ptashne, M. (1992) *A genetic switch. Phage λ and higher organisms. Second edition.*: Cell Press and Blackwell Scientific Publications.
Ptashne, M., and Gann, A. (1997) Transcriptional activation by recruitment. *Nature* **386**: 569-577.

Ptashne, M., and Gann, A. (2002) *Genes & Signals.* Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

Reed, M.R., Shearwin, K.E., Pell, L.M., and Egan, J.B. (1997) The dual role of Apl in prophage induction of coliphage 186. *Mol. Microbiol.* **23**: 669-681.

Renberg-Eriksson, S.K., Liu, T., and Haggård-Ljungquist, E. (2000) Interacting interfaces of the P4 antirepressor E and the P2 immunity repressor C. *Mol. Microbiol.* **36**: 1148-1155.

Révet, B., von Wilcken-Bergmann, B., Bessert, H., Barker, A., and Müller-Hill, B. (1999) Four dimers of λ repressor bound to two suitably spaced pairs of λ operators form octamers and DNA loops over large distances. *Curr. Biol* **9**: 151-154.

Rivetti, C., Guthold, M., and Bustamente, C. (1999) Wrapping of DNA around the E. coli RNA polymerase open promoter complex. *EMBO J.* **18**: 4464-4475.

Rohwer, F. (2003) Global phage diversity. *Cell* **113**: 141.

Rojo, F. (2001) Mechanism of transcriptional repression. *Curr. Opin. Microbiol.* **4**: 145-151.

Ross, W., Gosink, K.K., Salomon, J., Igarashi, K., Zou, C., Ishihama, A., Severinov, K., and Gourse, R.L. (1993) A third recognition element in bacterial promoters: DNA binding by the α subunit of RNA polymerase. *Science* **262**: 1407-1413.

Ross, W., Ernst, A., and Gourse, R.L. (2001) Fine structure of E. coli RNA polymerase-promoter interactions: a subunit binding to the UP element minor groove. *Genes & Dev.* **15**: 491-506.

Ross, W., and Gourse, R.L. (2005) Sequence-independent upstream DNA-αCTD interactions strongly stimulate *Escherichia coli* RNA polymerase-*lacUV5* promoter association. *Proc.. Natl. Acad. Sci. USA* **102**: 291-296.

Saha, S., Lundqvist, B., and Haggård-Ljungquist, E. (1987a) Autoregulation of bacteriophage P2 repressor. *EMBO J.* **6**: 809-814.

Saha, S., Haggård-Ljungquist, E., and Nordström, K. (1987b) The cox protein of bacteriophage P2 inhibits the formation of the repressor protein and autoregulates the early operon. *EMBO J.* **6**: 3191-3199.

Saha, S., Haggård-Ljungquist, E., and Nordström, K. (1989) Activation of prophage P4 by the P2 Cox protein and the sites of action of the Cox protein on the two phage genomes. *Proc. Natl. Acad. Sci. USA* **86**: 3973-3977.

Saha, S., Haggård-Ljungquist, E., and Nordström, K. (1990) Integration host factor is necessary for lysogenization of *Escherichia coli* by bacteriophage P2. *Mol. Microbiol.* **4**: 3-11.

Sam, M.D., Papagiannis, C.V., Connolly, K.M., Corselli, L., Iwahara, J., Lee, J., Phillips, M., Wojciak, J.M., Johnson, R.C., and Clubb, R.T. (2002) Regulation of directionality in bacteriophage λ site-specific recombination: structure of the Xis protein. *J. Mol. Biol.* **324**: 791-805.

Sam, M.D., Cascio, D., Johnson, R.C., and Clubb, R.T. (2004) Crystal structure of the excisionase-DNA complex from bacteriophage lambda. *J. Mol. Biol.* **338**: 229-240.

Schleif, R. (2003) AraC protein: a love-hate relationship. *BioEssays* **25**: 274-282.

Schnös, M., and Inman, R.B. (1971) Starting point and direction of replication in P2 DNA. *J. Mol. Biol.* **55**: 31-38.

Severinov, K. (2000) RNA Polymerase structure-function: insights into points of transcriptional regulation. *Curr. Opin. Microbiol.* **3**: 118-125.

Shearwin, K.E., and Egan, J.B. (1996) Purification and self-association equilibria of the lysis-lysogeny switch proteins of coliphage 186. *J. Biol. Chem.* **271**: 11525-11531.

Shearwin, K.E., Brumby, A.M., and Egan, J.B. (1998) The Tum protein of coliphage 186 is an antirepressor. *J. Biol. Chem.* **273**: 5708-5715.

Shearwin, K.E., Dodd, I.B., and Egan, J.B. (2002) The helix-turn-helix motif of the coliphage 186 immunity repressor binds to two distinct recognition sequences. *J. Biol. Chem.* **277**: 3186-3194.

Spiegelman, W.G., Reichardt, L.F., Yaniv, M., Heinemann, S.F., Kaiser, A.D., and Eisen, H. (1972) Bidirectional transcription and the regulation of phage λ repressor synthesis. *Proc. Natl. Acad. Sci. USA.* **69**: 3156-3160.

Strainic, M.G., Sullivan, J.J., Velevis, A., and DeHaseth, P. (1998) Promoter recognition by *Escherichia coli* RNA polymerase: effects of the UP element on open complex formation and promoter clearance. *Biochem.* **37**: 18074-18080.

Swalla, B.M., Cho, E.H., Gumport, R.I., and Gardner, J.F. (2003) The molecular basis of co-operative DNA binding between lambda integrase and excisionase. *Mol. Micrbiol.*: 89-99.

Svenningsen, S.L., Costantino, N., court, D.L., and Adhya, S. (2005) On the role of Cro in λ prophage induction. *Proc. Natl. Acad. Sci.* **102**: 4465-4469.

Takeda, Y., Sarai, A., and Rivera, V.M. (1989) Analysis of the sequence-specific interactions between Cro repressor and operator DNA by systematic base substitution experiments. *Proc. Natl. Acad. Sci. USA* **86**: 439-443.

Thompson, J.F., de Vargas, L.M., Koch, C., Kahman, R., and Landy, A. (1987) Cellular factors couple recombination with growth phase: characterization of a new component in the λ site-specific recombination pathway. *Cell* **50**: 901-908.

Thompson, J.F., and Landy, A. (1988) Empirical estimation of the protein-induced DNA bending angles: applications to lambda site-specific recombination complexes. *Nucleic Acids Res.* **16**: 9687-9705.

Tirumalai, R.S., Healy, E., and Landy, A. (1997) The catalytic domain of λ site-specific recombinases. *Proc. Natl, Acad. Sci. USA* **94**: 6104-6109.

Tirumalai, R.S., Kwon, H.J., Cardente, E.H., Ellenberg, T., and Landy, A. (1998) Recognition of core-type DNA site by λ integrase. *J. Mol. Biol.* **279**: 513-527.

Wagner, R. (2000) *"Transcription regulation in prokaryotes".* New York: Oxford University Press Inc.

Wang, J.-Y., and Syvanen, M. (1992) DNA twist as a transcriptional sensor for environmental changes. *Mol. Microbiol.* **6**: 1861-1866.

Warren, D., Sam, M.D., Manley, K., Sarkar, D., Lee, S.Y., Abbani, M., Wojciak, J.M., Clubb, R.T., and Landy, A. (2003) Identification of the λ integrase surface that interacts with Xis reveals a residue that is also critical for Int dimer formation. *Proc. Natl. Acad. Sci. USA* **100**: 8176-8181.

Wharton, R.P., and Ptashne, M. (1985) Changing the binding specificity of a repressor by redesigning an alpha-helix. *Nature* **316**: 601-605.

Wojciak, J.M., Sarkar, D., Landy, A., and Clubb, R.T. (2002) Arm-binding by λ-integrase: solution structure and functional characterization of its amino-terminal domain. *Proc. Natl. Acad. Sci. USA.* **99**: 3434-3439.

von Hippel, P. (1998) An integrated model of the transcription complex in elongation, termination, and editing. *Science* **281**: 660-665.

Wood, L.F., Tszine, N.Y., and Christie, G.E. (1997) Activation of P2 late transcription by P2 Ogr protein requires a discrete contact site on the C terminus of the α subunit of *Escherichia coli* RNA polymerase. *J. Mol. Biol.* **274**: 1-7.

Xu, H., and Hoover, T.R. (2001) Transcriptional regulation at a distance in bacteria. *Curr. Opin. Microbiol.* **4**: 138-144.

Yasuno, K., Yamazaki, T., Tanaka, Y., Kodama, T.S., Matsugami, A., Katahira, M., Ishihama, A., and Kyogoku, Y. (2001) Interaction of the C-terminal domain of the *E. coli* RNA polymerase α subunit with the UP element: recognizing the backbone structure in the minor groove surface. *J. Mol. Biol.* **306**: 213-225.

Yin, S., Bushman, W., and Landy, A. (1985) Interaction of λ site-specific recombination protein Xis with attachment site DNA. *Proc. natl. Acad. Sci. USA* **82**: 1040-1044.

Yu, A., Bertani, L.E., and Haggård-Ljungquist, E. (1989) Control of prophage integration and excision in bacteriophage P2: nucleotide sequence of the *int* gene and *att* sites. *Gene* **80**: 1-12.

Yu, A., and Haggård-Ljungquist, E. (1993a) The Cox protein is a modulator of directionality in bacteriophage P2 site-specific recombination. *J. Bacteriol.* **175**: 7848-7855.

Yu, A., and Haggård-Ljungquist, E. (1993b) Characterization of the binding sites of two proteins involved in the bacteriophage P2site-specific recombination system. *J. Bacteriol.* **175**: 1239-1249.

Yu, A., Barreiro, V., and Haggård-Ljungquist, E. (1994) Regulation of *int* gene expression in bacteriophage P2. *J. Virol.* **68**: 4220-4226.

Zeng, X., and Hu, J.C. (1997) Detection of tetramerization domains *in vivo* by cooperative DNA binding to tandem lambda operator sites. *Gene* **185**: 245-249.