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Control of transcription initiation*

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Abstract Mechanism of control of transcription initiation have expanded far beyond the classical operon concept. Control elements are multipartite and well separated from each other. The *trans*-factors bound to these sites make contacts with RNA polymerase: promoter complexes by DNA bending or looping to influence the initiation event. Activators and repressors are like two faces of the same coin and their function depends on the site of action, mode of interaction with DNA and also the nutritional status of the cell.

Keywords. RNA polymerase; promoter; activator; repressor; transcription.

1. Introduction

Regulation of gene expression is a fundamental event in every cell and organism. The regulatory events occur during each step of macromolecular synthesis. The control of transcription initiation is by far the major regulatory event determining whether a gene is turned 'on' or 'off'. The enzyme RNA polymerase occupies center stage during this process. A precise, productive initiation is possible only by the direct and specific interaction of the enzyme with functional promoter sequences (Mishra and Chatterji 1993). RNA polymerase is one of the conserved proteins in prokaryotes in terms of its structural organization and function. The size, composition and function of different subunits of core polymerase does not vary much in different organisms. On the other hand, promoter structures vary significantly from species to species and even within species depending on the kind of sigma factor (protein that binds to core enzyme to direct correct initiation) bound to the polymerase. Further, different *trans*-factors influence promoter recognition by the holoenzyme. No doubt promoter: polymerase interaction plays a major role, but that alone is not sufficient for proper initiation with a large number of promoters. Based on extensive *in vitro* experiments (Hoopes and McClure 1987) the promoter: polymerase interaction leading to transcription initiation can be divided into several steps as depicted in figure 1.

The first step, wherein RNA polymerase binds to promoter to form a relatively weak closed complex, is represented by the binding constant K_B. In the next step, the closed complex gets converted to a more stable open complex and K_f represents the rate constant of this irreversible reaction. During this slow isomerization process, the DNA sequence around –10 region of the promoter opens up along with a conformational change in RNA polymerase. In the next step, the enzyme synthesizes oligoribonucleotides of less than 10 nucleotides in length.

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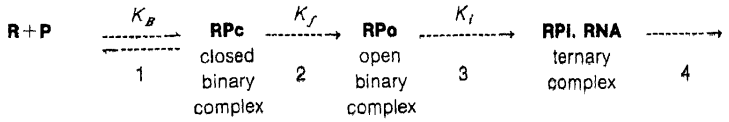

1. DNA binding
2. DNA melting (isomerization step)
3. Phosphodiester bond formation
4. Promoter clearance (polymerase in elongation mode)

Figure 1.

This initial phosphodiester bond formation event may have several intermediate stages and occurs while polymerase is still bound to the promoter. Once cleared from the promoter by the dissociation of the sigma subunit, RNA polymerase starts elongation of RNA chain. The control of initiation could be exerted at any one of these steps. This article attempts to highlight the importance of *trans*-acting factors (either activators or repressors) and also the role of methylation and phosphorylation in influencing transcription initiation. It is not intended to review the wealth of information available; for these readers should refer to other excellent reviews (Raibaud and Schwartz 1984; Reznikoff *et al* 1985; Hoopes and McClure 1987; Adhya 1989), Only some selected examples are chosen that reflect on our current understanding of the field, Also, we focus on emerging future trends.

Activation or repression of transcription is brought about by different mechanisms. Activator proteins are involved in facilitating productive transcription initiation while repressor proteins prevent it. The action of different sigma factors in the phage development pathway or heat shock response is not included here but has been dealt elsewhere in this issue (Mishra and Chatterji 1993).

2. Transcription activation

RNA polymerase fails to interact in a specific fashion with promoters that have a poor "consensus" sequence. The factors which assist the polymerase to develop a productive interaction are called transcription activators, By implication, therefore, positively controlled promoters are not fully functional in the presence of RNA polymerase alone. They are also known as weak promoters with poor —10 and — 35 sequences. Figure 2 represents a compilation of such promoter sequences, Some of these promoters lack the entire –35 sequence. *Trans*-activating proteins could directly bind to DNA around these promoter regions or form a complex with RNA polymerase and then bind to the promoter to stimulate transcription. It was believed that activator proteins bind at or near the –35 sequence of the weak promoters and thereby increase the rate of open complex formation (Raibaud and Schwartz 1984). However, it is more likely that various proteins act differently at different steps (figure 1) of the promoter: polymerase interaction. For example, catabolite activator protein (CAP) increases the binding constant (K_B) to the *lac* promoter 20-fold (Malan *et al* 1984). cI protein of phage lambda binds to promoter P_{RM} to increase the isomerization rate 11-fold (Hawley and McClure

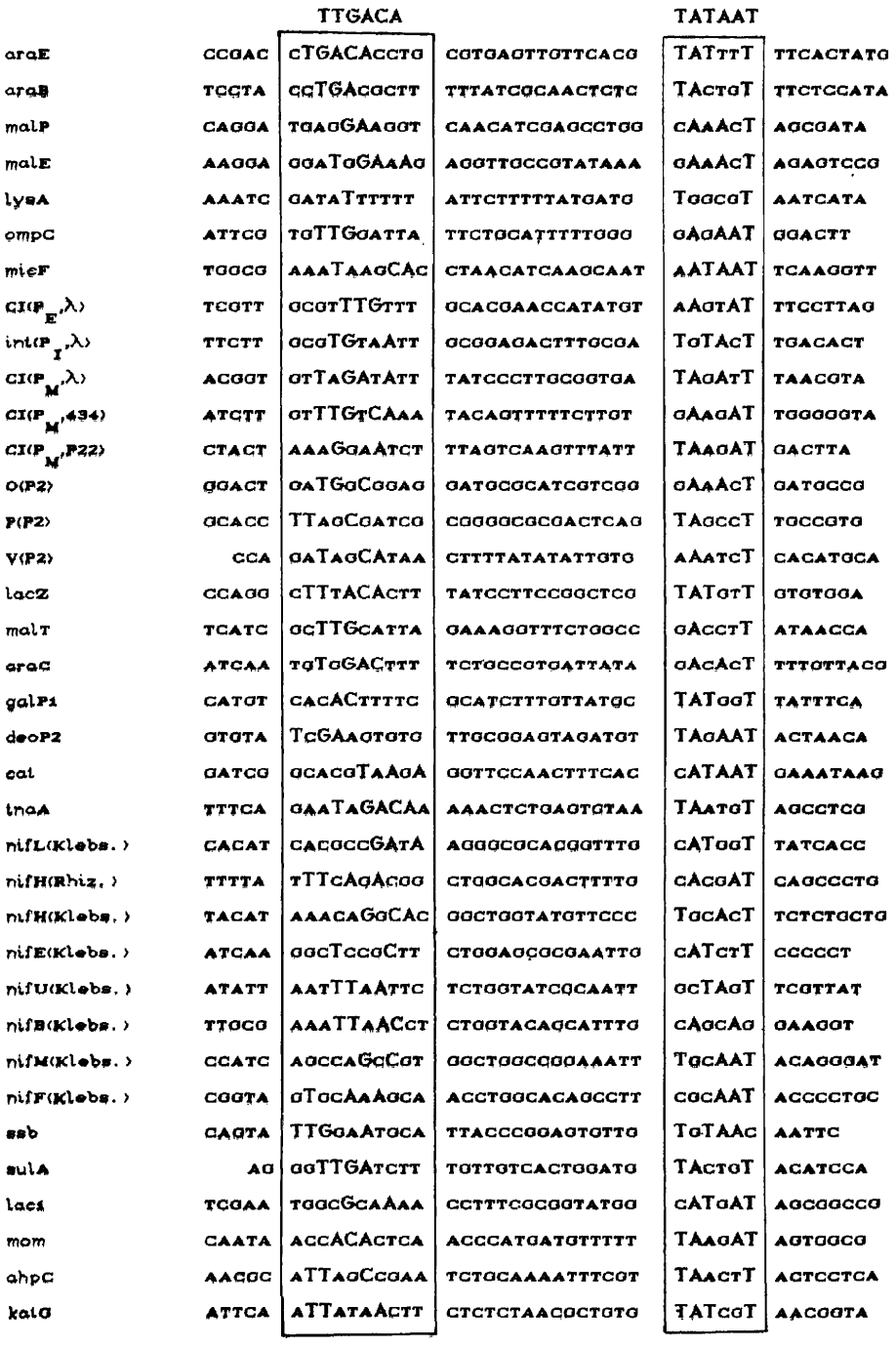

Figure 2. Activation dependent weak promoters. Most of the information is taken from Raibaud and Schwartz (1984). Rest of the promoter sequences are from Balke *et al* (1992), Hoopes and McClure (1987) and Tartaaglia *et al* (1989). The sequences are aligned to consensus –10 and –35 sequences (top) and are boxed. Letters in bold face indicate nucleotides identical to the consensus nucleotide at the particular position.

1982) while cII influences both binding and isomerization (Shih and Gussin 1984). It should be noted here that the sequence to which CAP binds is located adjacent to the - 35 sequence, while the cII protein binds to the spacer region between the –10 and –35 sequences on opposite phase of the RNA polymerase contact. In this respect, the bacteriophage Mu C protein function could be analogous to that of lambda cII. C protein binds to two sites on DNA, one adjacent to - 35 and the other at the spacer region but closer to –10 (V Nagaraja, T Gindlesperger and S Hattman, unpublished observations).

Many proteins influence rates of initiation by binding to DNA farther away from the promoter. In such cases, the protein makes direct contacts with RNA polymerase by looping over intervening DNA sequences. When the DNA sequence is not that distant, the important consideration is the bending and stereospecific positioning of the c*is* sequence (activator binding site) relative to that of the promoter. In other words, both protein recognition sequences should be located on the same phase of the helix to facilitate protein: protein contacts. These aspects are discussed in § 4.

3. Repression

Unlike transcription activation, in which activator binds to DNA and RNA polymerase to activate transcription, repressors were thought to act by preventing the RNA polymerase binding to promoter through steric hindrance (Beckwith 1987). This dogmatic view has its roots in the classical operon concept. A large body of literature has been accumulated on Operons (Miller and Reznikoff 1978) mainly from the elaborate analysis of the *lac* system. The basic premise was that operator-bound repressor impeded RNA polymerase binding because the operator site was located adjacent or overlapping to the promoter sequence. But the situation is different in many cases including, as it happens, the *lac* operon itself. A middle operator which is crucial for repressor binding is located downstream of the *lac Z* mRNA start point and not upstream as originally proposed. The *lac* operator, like many other regulatory sequences, is palindromic. However, the inverted repeats in the sequence are not identical. More operator mutants, denoted O^c due to their dominant constitutive phenotype, are found on the left half of the operator site. Mutations on the right half of the operator which make the sequence completely symmetric increase the affinity of the repressor to the site 10-fold. The repressor protein is a tetramer of identical subunits and thus symmetry in DNA sequence reflects a symmetry in the protein. The imperfect two-fold symmetry of the operator could be the result of evolutionary optimization of repressor :DNA interactions for the precise tuning of regulatory circuits. With the detailed analysis of several Operons the following additional features have emerged.

(i) Multiple operator elements have been found in *lac, gal, ara* and other Operons (Adhya 1989).

(ii) Operators are located some distance away from promoter. The two operators in the *gal* system are separated by 114 base pairs, one lying next to overlapping promoters (see §6) and the other within the first structural gene (Adhya 1987). The regulatory region of the *ara*BAD genes in the arabinose operon consists of three operator sites distributed over a length of 220 base pairs (Schleif 1987).

(iii) Both RNA polymerase and repressor can bind to their respective sites and make a ternary complex (Straney and Crothers 1987).

From these observations, it is clear that the mechanism of negative control is not as simple as believed previously. In theory, repressors can interfere with the productive initiation of RNA synthesis by acting in any one of the steps outlined in figure 1 and the experimental observations support this view.

3.1 *Steric hindrance for RNA polymerase binding*

If the operator and – 35 sequence overlap, repressor could prevent the formation of a closed complex by preventing polymerase binding. It is also possible that repressor binding can prevent the activator's interaction with polymerase and/or DNA (Bolker and Kahmann 1989).

3.2 *Inhibition of open complex formation*

Isomerization is a step in which RNA polymerase conformation is changed and a more stable complex is formed spanning the region of + 5 to beyond – 35 sequence. The exact boundary of nucleotides protected by RNA polymerase in footprinting experiments varies depending on the promoter used in an experiment (V Nagaraja, unpublished observations). The repressor when bound at or near the – 10 region of the promoter (the site of complex formation) could distort the DNA and as a consequence, formation of stable open complexes may be prevented. The Arc repressor involved in bacteriophage P22 lysogeny is believed to repress the P_{ant} promoter in this fashion (Vershon *et al* 1987).

3.3 *Inhibition of initiation complex formation*

RNA polymerase initiates RNA synthesis with the formation of nascent RNA of 3-8 nucleotides. During this stage, polymerase is still bound to the promoter. The repressor which is bound to the operator may make direct contacts with the enzyme present in open complex state, thus preventing the subsequent step, *i.e.* oligoribonucleotide formation. Studies with the *gal* repressor (Adhya 1989) support this mechanism. The *gal* repressor does not block open complex formation at the *gal* operon promoter and hence is believed to interfere at the stage of oligoribonucleotide formation (Adhya 1989).

3.4 *Increased abortive initiation*

Once an initiation complex is formed, RNA polymerase moves out of the promoter and gets locked into the elongation mode. The repressor could make contacts with RNA polymerase and block promoter clearance. More recently, experimental evidence has been provided for the mechanism of *lac* repressor action at the *lac* UV5 promoter (Lee and Goldfarb 1991), The authors of this paper contend that the repressor modifies the initiation complex resulting in continued abortive synthesis of RNA oligomers. It should be pointed out that these *in vitro* experiments were

carried out with isolated DNA. fragments. Control may be exerted during the release of the sigma subunit from the RNA polymerase. Dissociation of sigma subunit from rest of the polymerase is an important step for the movement of the transcription bubble, and it is possible that sigma dissociation is blocked by the repressor resulting in continued synthesis of short oligomers. There is no experimental observation as yet to support this model.

A repressor bound to an operator located downstream in the coding region of the gene can create a "road block" in the path of the transcription bubble. This could result in premature termination of transcripts. Infact *lac*I gene which codes for the repressor protein is autoregulated in this fashion. The operator 03 of the *lac* operon is located at the end of *lac*I gene leading to a reduction in *lac*I gene expression (Sellitti *et al* 1987). Other *in vitro* experiments also support this mechanism (Deuschle *et al* 1986). Although this mechanism operates at the post-initiation level, I have included it here because the nature of the interaction makes it relevant.

4. The role of stereospecific positioning, DNA bending and looping in positive and negative control

When the *trans*-activator binding site is located right next to the promoter, the interaction between the activator protein and RNA polymerase can be brought about by protein-induced DNA bending. It is known that the CAP protein can bend the DNA by 90 thus facilitating its direct contact with polymerase bound to the adjacent promoter (Wu and Crothers 1984). But when distances between the *cis* sites are large, other mechanisms must be operative to make protein: protein contacts. To account for the action of regulatory proteins bound at many distant sites in influencing the promoter: RNA polymerase function the model of DNA looping has been developed (Schlief 1988). Involvement of DNA looping in gene regulation was first suggested by Schliefs group working with the arabinose operon (Dunn *et al* 1984). Addition of integral and half integral turns of DNA helices between the promoter for *ara*BAD and the upstream operator O_2 resulted in different levels of *ara*BAD expression Repression was nearly normal when integral helical turns of DNA were introduced; repression was impaired when half integral turns of DNA were introduced. These initial observations were later extended by Ptashne and co-workers. By using the lambda el repressor as a model system, they provided electron microscopic evidence for DNA looping (Griffith *et al* 1986; Hochschild and Ptashne 1986). Importance of DNA looping for positive and negative regulation is depicted in figure 3. The two protein molecules bound to far-apart sites in DNA make direct contact with tine another resulting in looping of intervening regions of DNA. Formation of the loop by protein: protein contacts should be a periodic function of the distance between the two protein binding sites located on the same side of the helix If the distance between the two sites is altered by inserting or deleting 5 base pairs the contact between the two proteins is lost, releasing the DNA from looped structure with decisive regulatory consequences (figure 3). Requirement for stereospecific positioning of *cis* sites for gene activation has been demonstrated by Maeda *et al* (1988). *Omp*C gene transcription in *E. coli* is regulated by the transactivator protein *Omp*R *Omp*C gene *trans*-activation is dependent on *Omp*R even when the *Omp*R binding site is separated from –35 and

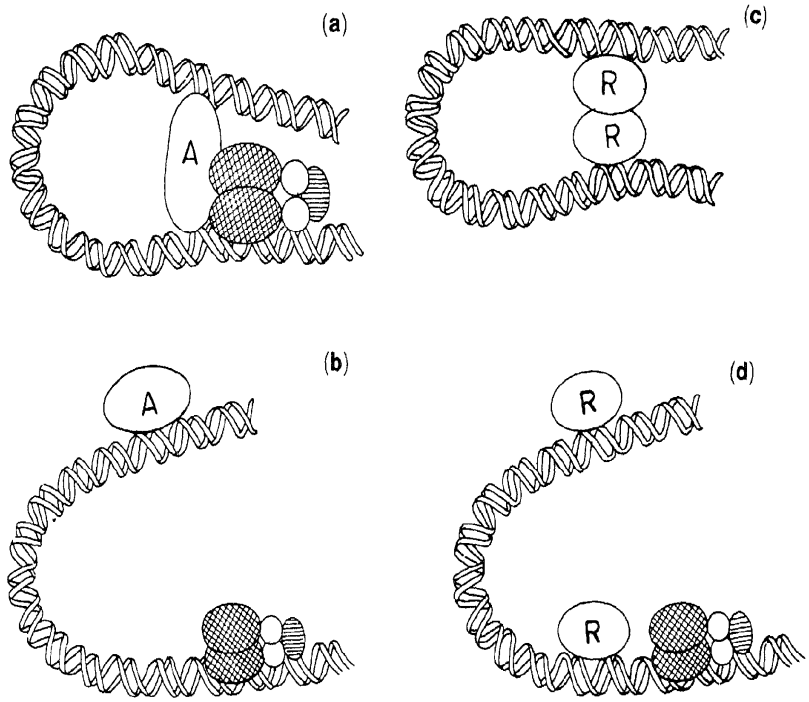

Figure 3. DNA looping in transcription activation and repression. Importance of protein: protein interaction for transcription activation **(a, b)** and repression **(c,d)** are represented. Activator protein (A) bound upstream makes contact with RNA polymerase located at promoter to initate transcription. When the distance between the two binding sites is changed by half integral of DNA helix, the activator lies on the opposite side and hence cannot make contact with RNA polymerase. The two repressor molecules (R) bound at two distant operator sites make contact with each other by formation of the DNA loop and thus prevent RNA polymerase binding to promoter (c). When the distance between the two operators is altered by half integral turns of the helix, DNA looping is abolished. Now RNA polymerase can bind to the nearby promoter to initiate transcription.

–10 sequences by several turns of the double helix, but the distance between them is an integral multiple of one turn of the double helix. Importance of such stereospecific positioning is now well documented (Adhya 1987; Schleif 1987).

The transcription activator protein NTRC *(gln*G gene product) activates transcription from the glutamine synthetase *(gln*A) promoter of various enteric bacteria. NTRC binds at two sites distant to the promoter, one at –108 and the other further upstream at –140 (Reitzer and Magasanik 1986). Two different sets of experiments establish the looping of intervening sequences between the promoter and NTRC binding sites. The NTRC sites can be positioned either downstream or upstream several thousand nucleotides away from the promoter without affecting the transcription initiation. Further, deletion analysis of the region revealed the requirement for a minimum 70 base pair spacer between the promoter and the NTRC binding site for *trans*-activation (Reitzer and Magasanik 1986). The relative inflexibility and torsional stiffness of short DNA fragments (having reduced spacer

length of less than 70 bp) could account for the absence of DNA looping and the resultant inability to *trans*-activate *gln*A transcription in such deletion mutants. These properties qualify the NTRC binding sites to be called prokaryotic enhancers (Kustu *et al* 1991). NTRC protein by itself has interesting properties (discussed in the next section). Intrinsic DNA bending as well as protein-induced bends result in DNA looping and the formation of a multiprotein complex at the transcription start site. Not all activators can bend the DNA to the same extent and some of them may not induce site-specific bends at all. In such cases, loop formation is assisted by the integration host factor, IHF, which is known to introduce sharp bends to DNA when bound to its site. The IHF binding site is located between the promoter and activator binding sites and it facilitates loop formation after protein binding (Kustu *et al* 1991).

5. Role of covalent modification of *cis* elements and transfactors

DNA methylation and protein phosphorylation are frequently observed phenomena influencing gene expression. The adenine residue in the sequence GATC is modified to 6 methyl adenine by the Dam methylase of *E. coli* and some coliphages. Methylation status of DNA can influence promoter activity in different ways when methylable sequences are located in the promoter region (Sternberg 1985). DNA methylation also serves as a link between regulation of transcription (positive or negative) and replication since DNA is transiently hemimethylated just after the passage of a replication fork. Generally, Dam methylation has a negative role (Roberts *et al* 1985; Sternberg 1985): IS10 and Tnl0 serve as good examples. In these cases, the *dam* sites located within the –10 and –35 regions, when methylated are presumed to influence the interaction of RNA polymerase with the promoter. A burst of RNA synthesis is observed after the passage of the replication fork before the hemimethylated DNA gets remethylated. Here, DNA replication serves as an indirect activation event. Interestingly, the remethylation process which is immediate and very rapid at all these sites, is delayed at *dam* sites present at the *ori*C and *dna*A promoters. It is important to recall here that hemimethylated *ori*C cannot undergo replication reinitiation *in vivo;* the *dna*A gene product is critical for replication initiation. Once remethylated, the *dna*A promoter is transcriptionally active and the *dna*A protein is available for a new round of replication initiation at *ori*C sites. These results imply that each round of replication initiation serves for transient down regulation at *ori*C and *dna*A while methylation has the opposite effect (Campbell and Kleckner 1990). A requirement for Dam methylation in gene activation is unusual though observed for the *mom* gene of bacteriophage Mu (Bolker and Kahmann 1989). The positive role of methylation on *mom* gene expression is discussed in the next section.

A particularly well characterised example for the role of phosphorylation in *trans*-activation is the NTRC protein described earlier. The phosphorylated form of NTRC has been shown to bind its two binding sites and then loop out to catalyze the isomerization reaction in order to convert the closed complex of sigma-52 RNA polymerase and *gln*A promoter into an open complex. However ATP hydrolysis is an essential prerequisite for generating the open complex. The phosphorylation of NTRC activates its ATPase activity 500-fold (Weiss *et al* 1991). Requirement for

ATP hydrolysis during open complex formation seems to be a general phenomena for sigma-52 RNA polymerse reactions (Kustu *et al* 1991).

6. A repressor can be an activator and *vice versa*

Sequence comparisons and structural studies indicate that both activators and repressors have similiar structures and hence interact with DNA in a similar fashion (Harrison and Aggarwal 1990). The first examples is that of bacteriophage cI repressor which turns of transcription at P_{RM} while stimulating its own synthesis at P_R (Ptashne 1978; Guarente *et al* 1982). Similarly, CAP protein, the first activator to be purified, acts in both ways in the *gal* system (Adhya 1987) as well as in the *lac* operon (Malan *et al* 1984). In both cases these proteins exert their activation and repression functions simultaneously while bound to one site. CAP protein stimulates transcription initiation at the P1 promoter while repressing it at P2. Positive control mutants of both CAP and cI have been isolated which do not alter DNA binding properties but fail in *trans*-activation. Such mutants of cI repress the promoter P_R but have a minimal influence at P_{RM} (Guarente *et al* 1982; Hochschild *et al* 1983). Unlike the CAP or lambda cI protein, several other regulatory proteins bind to more than one site and carry out different functions. *Ara*C protein is one of the best examples. It binds to three different sites in the arabinose operon and responds differently to different concentrations of L-arabinose (Schlief 1987). In absence of L-arabinose, it represses the expression of the arabinose operon. This is achieved by the direct contact of *Ara*C protein bound to two operator sites separated by approximately 200 base pairs and the looping out of intervening DNA sequences. In the presence of arabinose, repression is relieved and *Ara*C acts as an activator of *ara*BAD. However, its own gene expression is autoregulated by the protein in a concentration-dependent manner. At low concentrations of the sugar, *Ara*C transcription is favoured while in the absence or high concentration of arabinose, synthesis is abolished.

It is noteworthy that the *lac* repressor functions as a transient gene activator in the presence of the gratuitous inducer IPTG (Straney and Crothers 1987). The initial binding of RNA polymerase to the promoter is increased more than 100-fold in presence of the repressor protein. Addition of the inducer results in an enhanced rate of productive transcription on the first round. These observations are contrary to the classical view and suggest a complex mechanism of action for the repressor.

Regulation of phage Mu *mom* gene expression by *Oxy*R protein serves as a unique example of a regulatory protein recognising different sequences in totally distinct systems. Further, Dam methylation requirement for *mom* transcription makes it quite unusual. A cluster of three *dam* (GATC) sites located upstream of the promoter region have to be methylated for transcription initiation. Only in the absence of methylation, *Oxy*R protein protects all the three sites by serving as a repressor (Bolker and Kahmann 1989). However, the OxyR protein has been discovered and characterized as a general activator of oxidative stress response genes binding to different sequences in the promoter regions of a set of genes (Tartaglia *et al* 1989). It autoregulates its own synthesis as well.

The presence of overlapping or tandemly arranged promoters leads to interesting regulatory consequences due to competition for the binding of RNA polymerase

(figure 4). *Lac* operon (Malan *et al* 1984; Goodrich and McClure 1991), galactose operon (Adhya 1987) and *mom* (Balke *et al* 1992; V Nagaraja, V Balke and S Hattman, unpublished) systems are examples of competing promoters oriented in tandem that produce transcripts encoding the same protein. Here RNA polymerase bound at one promoter may serve to repress at the other. The *ant/mnt* system of bacteriophage P22 and bacterial *mer*R/*mer*T systems have competing promoters in divergent orientation (Goodrich and McClure 1991; Vershon *et al* 1987).

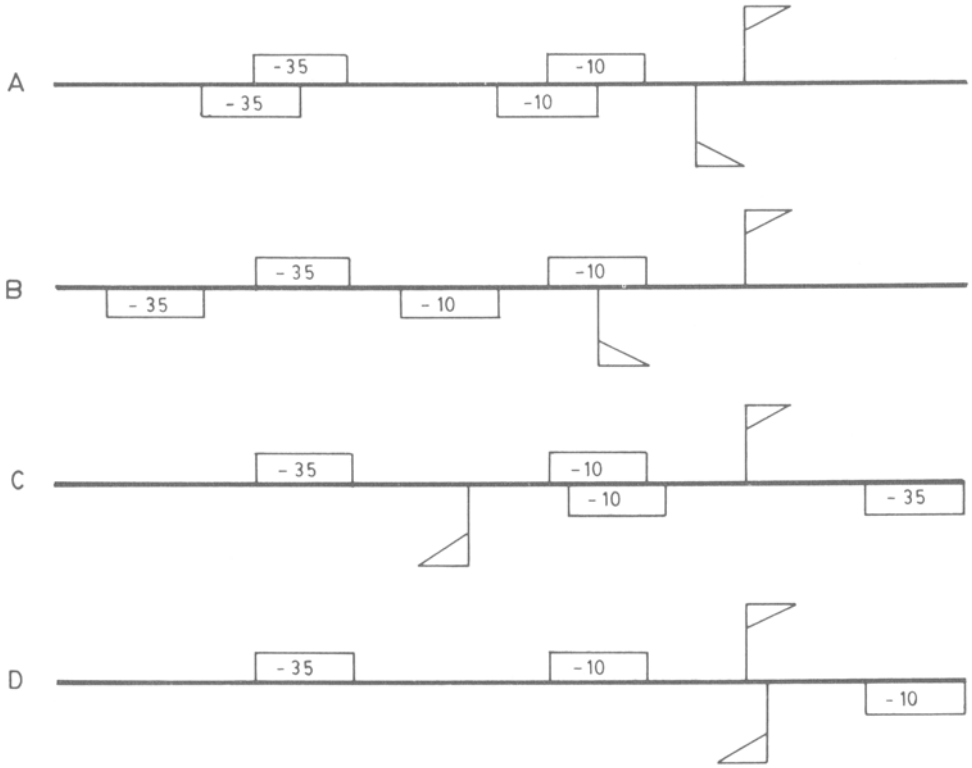

Figure 4. Competing promoters in gene regulation. **(A)** and **(B)** represent two overlapping competing promoters oriented in tandem to produce transcripts for the same gene products wherein the spacing of the two promoters with respect to each other is different. *E. coli gal* and *lac* operons are the examples for **(A)** and phage Mu mom operon for **(B)** Two divergently oriented promoters (transcribing different strands of DNA) located at different distances from each other are depicted in **(C)** and **(D).** The – 35 box is not shown in **(D).** The flags represent mRNA start sites.

7. Summary

It is clear that the subject of regulation of transcription initiation has expanded far beyond the imagination and boundaries of the classical operon concept. In the light of these developments, one could summarise the control of transcription initiation as follows: The rate of transcription initiation by RNA polymerase from a promoter

is determined by regulatory protein(s) that affect the activity of RNA polymerase by interacting with specific control sites in DNA and/or direct contact with RNA polymerase. Regulatory proteins could be either repressors bringing about negative regulation or activators of RNA polymerase activity resulting in positive regulation. These regulatory proteins are present in two states — active or inactive — as determined by the environmental state. The change from one state to the other takes place by enzymatic covalent modification, ligand-induced allosteric transition or protein: protein interaction. Regulatory elements in DNA are often multipartite and positioned over a long distance from the promoter. DNA looping helps in the formation of multiprotein complexes involving multiple *cis* sites and promotes a molecular communication network. Some regulatory proteins are activators in one state and repressors in other. A few of them can exert both functions simultaneously when bound to a single site.

8. Future outlook

The identification of many elements far upstream and downstream of genes/operons and their decisive role as part of control circuits *in vivo* has lead to a variety of regulatory consequences. Recently multiple regulatory elements have been discovered in several new systems and in classical ones such as the *lac, gal* and *ara* operons (Adhya 1989). The emerging picture is complex and not totally predictable. For example, *lac* repressor itself serves as a transient activator of the system (Straney and Crothers 1987). In a dual promoter system, RNA polymerase along with activator protein could activate one promoter resulting in the repression of the other (Goodrich and McClure 1991). Detailed analysis of open complex formation *in vivo* under different environmental conditions and genetic backgrounds would provide greater insight into regulatory mechanisms.

Regulatory proteins have very similar structural motifs (Harrison and Aggarwal 1990). Although this parameter takes care of general principles of protein-DNA contact, it is likely that specific residues determine the specificity of interaction. Regulation of gene expression is influenced by both DNA-protein and protein-protein interactions. The structure and conformation of DNA at or near promoters seems to have a decisive effect. The cell probably contains factors which can interact with such structures to facilitate transcription initation. The intracellular concentrations of the factors and small molecules influence the initation event. Proteins like IHF play a major role in bending DNA for loop formation and the development of a proper transcription initiation complex.

Regulatory events occurring during initation of RNA synthesis in prokaryotes is discussed in this review and references to more complex eukaryotic systems have been left out. However, it is noteworthy that positive control mechanism is a rule in eukaryotic gene regulation and repressors in the classical sense have not been characterized. Sequestration of activator proteins by heterodimerization event could be considered as an aspect of negative control. On the other hand, it could be argued that eukaryotic DNA is rendered inaccessible to RNA polymerases by its organization into higher order structures thus relieving the need for specific control. As yet there is no clear answer in this regard and it remains an open question.

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