Sigma^{70} of *E. coli* : Many more wonders

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Current trends show studies on regulation of gene expression at various levels form one of the frontier and favourite topics in molecular biology research. A new concept of regulation through intramolecular, interdomain interaction of a protein molecule is fast emerging as one of the most economical and logical modes of regulating the activity of a protein within the cell. Experiments have indicated in the recent past that this may actually be one of commonly used (but surprisingly not recognized until recently) ways of regulation of function. Along this line, recent findings of Gopal *et al.*[1] (CCMB, Hyderabad) in the 9 September issue of *Journal of Molecular Biology* present the required and much sought after proof of a novel mechanism of the regulation of the DNA-binding activity of the transcription factor σ^{70} of *E. coli*. The findings may give an experimental explanation of some of the unusual and even anomalous properties of the σ^{70}.

The transcription factor, essential for the accurate function of *E. coli* RNA polymerase is a protein of 70 kDa molecular weight with several unusual properties. Although the protein is responsible for the promoter recognition by the polymerase, the free protein on its own does not show indication of DNA binding in any *in vitro* experiment. The protein is involved in almost every activity related to promoter-dependent transcription initiation. Thus four major domains[2] of this protein of 613 amino acids are identified as responsible for its binding with core RNA polymerase (made of $\beta\beta'\alpha_2$subunits), recognition of consensus elements at positions −10 and −35 in the promoter by the holoenzyme (core + σ) and DNA melting at the initiation site. In its extremely well organized structure responsible for so many functions, each of which demands high level of accuracy, lies the beauty of the fact that while eukaryotes do not seem to have a protein corresponding to σ^{70} in their cell, diverse functions are carried out by a single protein in the bacterial cell. Rather all the functions of σ^{70} are found distributed over a number of general, basal transcription factors of eukaryotes. In fact, all these factors have segments of sequence similarity to various functional domains of sigma. Prediction of functions of several of these factors could become possible after cloning and sequence comparisons with the sigma sequence; later they were found to match with their actual activities.

As mentioned above, σ^{70} is known to have four major domains, of which several subdomains are conserved[3]. Domain 1 is involved in core binding and domain 3 is thought to be required for stability. Domain 1 is also involved in selectivity while subdomains 2.3, 2.4 and domain 4 at the carboxy-terminal are involved in the recognition of −10 and −35 consensus sequences of the promoter. All four subdomains of the domain 2 are implicated in DNA melting, while subdomains 2.1 and 2.4 are also involved in core binding. Historically, sigma, in spite of its most crucial role in transcription initiation and promoter recognition, proved to be a non-DNA binding protein in all *in vitro* studies aimed to follow the binding of the isolated protein with a specific promoter by a gel mobility shift assay. This observation posed problems in explaining the presence of the DNA-binding domains identified through genetic studies as well as its contacts with DNA when holoenzyme is bound to DNA[4]. However, results could always be explained by telling that binding of sigma with the core results in some conformational change in sigma which makes its DNA binding and promoter recognition domains free to interact in the holoenzyme. In other words, in the sigma without core enzyme, the DNA binding domains are inhibited or masked due to some unknown factors.

Work in the recent past partly revealed some of these factors responsible for the blocking of DNA-binding domains of sigma. Dombroski *et al.*[5] showed that binding of σ^{70} to DNA *in vitro* could be observed if the truncated, carboxy-terminal polypeptide was used. Deletion analysis of the protein showed that amino terminal amino acids in the subdomain 1.1 inhibit the activities of the carboxy-terminal DNA-binding domains. This negative effect was also observed for *E. coli* σ^{32} and *B. subtilis* σ^{K}. Inhibitory effect of N-terminal domain of σ^{70} could also be observed in trans. These results gave strong indication that σ^{70} is subjected to an intramolecular regulation through blocking of its C-terminal domain activity by its own N-terminal domain. Almost at the same time, Juang and Helmann[6] showed that subdomain 2.3 is responsible for both −10 recognition and DNA melting and thus constitutes the most important functional domain of the σ^{70}. Kumar *et al.*[7], based on their C-terminal deletion analysis of σ^{70}, concluded that −35 recognition by σ^{70} is not absolutely essential for its function. This result attributes even more importance to the 2.3 subdomain. These reports also greatly enhanced the significance of the results obtained in the laboratory of Dipankar Chatterji, (Principal investigator) where the work was

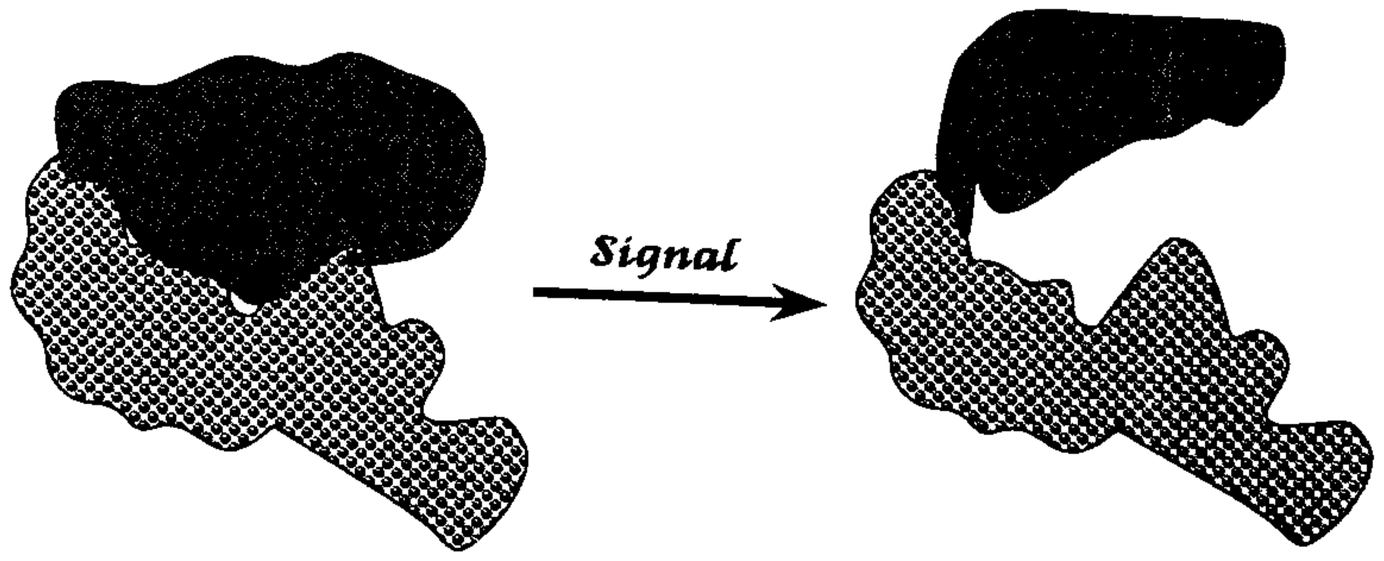

Figure. Intramolecular domain movement of a protein to expose the functional surface in response to a signal which may be thermal energy in case of TBP and binding of the core polymerase in case of sigma.

already in an advanced stage. The work[1] is based on characterization of the σ^{70} mutants generated by PCR-based site directed mutagenesis. He selected two adjacent tryptophans at positions 433 and 434 which lie at the junction of the subdomains 2.3 (amino acids 417 to 434) and 2.4 (amino acids 435 to 453). Point mutations of these tryptophans resulted in mutant σ^{70} with drastically different properties. While changing tryptophan to phenylalanine at position 433 did not affect properties of the protein, change of Trp to Gly at position 434 brought dramatic changes, most striking of which was restoration of normal mobility of protein as 70 kDa on a SDS-polyacrylamide gel. Wild type σ^{70} shows anomalous mobility on this gel, moving as a 90 kDa protein. Proteolytic digestion analysis of the protein showed that the cause of abnormality resides in the fragment harbouring the 434 Trp; rest of the fragments having same mobility in both the proteins. Mutant σ^{70} (named as W434G by the authors[1]) also showed a loss in functions and resulted in partial loss of protection of the −10 box of the promoter by the holoenzyme having the mutant sigma. However, free protein, unlike wild type σ^{70}, showed a transient binding with the promoter DNA which could be detected by the time-resolved fluorescence technique. Thus a direct correlation between abnormal gel mobility and essential function was seen. Based on their results, Gopal *et al.*[1] suggested that 434 Trp is the amino acid which is capable of a stacking interaction with the hydrophobic regions in N-terminal 1.1 subdomain and folding of the protein through this interaction results in exposure of the acidic patch around the middle of the protein and blocking of C-terminal domain by the N-terminal domain. In fact, they already have some additional experimental evidences for this model, wherein mutation of a hydrophobic residue in the subdomain 1.1 to glycine resulted in a mutant having properties similar to W434G (D. Chatterji, pers. commun.). Thus, results of Gopal *et al.*[1] have provided the experimental evidences for the newly suggested mode of intramolecular, interdomain interactions as the regulatory means of a protein's activity.

Several examples are known in eukaryotic gene regulation wherein transcription factors and other essential factors become functional after going through modifications or conformational changes due to their interactions with other effectors. These changes include phosphorylation, acetylation, hydrophobic, protein–protein interactions, etc. A large number of such cases are studied by now. Along with such heteromeric, regulatory interactions, another type of regulation mode is becoming increasingly known. In this new class of regulation mode, part of a molecule regulates the activity of another part of the same molecule, resulting in an almost spontaneous as well as economic way of control. Compared to the classical example of allosteric regulation, aspartate transcarbamoylase, wherein catalytic and regulatory subunits of the molecule are separable entities, in the intramolecularly regulated molecules, both the parts are the inseparable entities of the molecule held together through covalent connections of other parts of the molecule. A recent article in *Biochemistry*[8] puts the phenomenon in right perspective through a large compilation of such molecules and classifies them according to type of domain movements involved in reconforming the protein. The structural basis emphasized therein highlights the enormity with which such domain movements are recognized now.

One of the most important proteins in eukaryotic transcription, the TATA binding protein (TBP) which plays the central role in transcription by all three polymerases of eukaryotes[9] is known to have sequence similarity in its conserved DNA binding C-terminal domain with subdomains 2.3, 2.4 of σ^{70} (ref. 10). TBP also happens to be an unusual protein like σ^{70}, carrying out several important functions. Not surprisingly, even TBP was suggested to change its conformation upon binding to DNA or other components of the transcription machinery. Although TBP can bind to the TATA element of eukaryotic promoters, it requires thermal energy for binding and shows slow on and off rates[11]. Deletion of N-terminal amino acids of TBP[12] resulted in a dramatic rise in its ability to form a stable complex with DNA even at 0°C. Results were used to suggest that thermal energy may be required by the wild type protein to alter the disposition of its N-terminal domain in order to make interaction of its C-terminal domain with DNA efficient. Thus both TBP and σ^{70} seem to have in-built mechanisms to take care of their unwarranted, undesired binding with the promoter which may take place if DNA binding domains are left free. Cell would obviously like this binding to take place only when it is ready to transcribe its DNA using other components of the transcription machinery, supply of which is regulated by other possible ways. This mechanism of intramolecular regulation through interdomain interaction is indispensable, since in course of evolution when distribution of various sigma domains to several factors would have happened, this basic mechanism seems to have remained with TBP which got the DNA-binding function of sigma, though the N-terminal domain of σ^{70} is no more there.

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