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. (CCMB, Hyderabad) in the 9th 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 $\sigma^{70}$ of *E. coli*. The findings may give an experimental explanation of some of the unusual and even anomalous properties of the $\sigma^{70}$.

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 of this protein of 613 amino acids are identified as responsible for its binding with core RNA polymerase (made of $\beta\beta'$ $\alpha_{sub}$ units), recognition of consensus elements at positions -10 and -35 in the promoter by the holoenzyme (core + $\sigma$) 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 $\sigma^{70}$ in their cell, diverse functions are carried out by a single protein in the bacterial cell. Rather all the functions of $\sigma^{70}$ 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, $\sigma^{70}$ is known to have four major domains, of which several subdomains are conserved. 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. 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. showed that binding of $\sigma^{70}$ 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* $\sigma^{70}$ and *B. subtilis* $\sigma^{5}$. Inhibitory effect of N-terminal domain of $\sigma^{70}$ could also be observed in trans. These results gave strong indication that $\sigma^{70}$ 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 showed that subdomain 2.3 is responsible for both -10 recognition and DNA melting and thus constitutes the most important functional domain of the $\sigma^{70}$. Kumar et al., based on their C-terminal deletion analysis of $\sigma^{70}$, concluded that -35 recognition by $\sigma^{70}$ 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](current_science/10.1007/s10071-004-4904-9)

**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.

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already in an advanced stage. The work1 is based on characterization of the 70\nmutants 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 70\nwith 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
bramatic changes, mostly striking of which
was restoration of normal mobility of
protein as 70 kDa on a SDS-polyacryl-
lamide gel. Wild type 70\nshows
abnormal 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 frag-
ment harbouring the 434 Trp; rest of the
fragments having same mobility in both the
proteins. Mutant 70 (named as
W434G by the authors) also showed a
loss in function and resulted in partial
loss of protection of the -10 box of the
promoter by the holoenzyme having the
mutant sigma. However, free protein, un-
like wild type 70\n, 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 intra-
molecular, interdomain interactions as the
regulatory means of a protein’s activity.

Several examples are known in eukar-
yotic gene regulation wherein transcrip-
tion 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, hydrophob-
exchange reaction, etc. A large
number of such cases are studied by now.
Along with such heteromorphic, regulatory
interactions, another type of regulation
mode is becoming increasingly known.
In this new class of regulation mode,
pair 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. Com-
pared 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 pairs are the inseparable entities
of the molecule held together through
covalent connections of other parts of the
molecule. A recent article in Bio-
chemistry\2
putts 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 high-
lights 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 polyn-
merases of eukaryotes\3
is known to have
sequence similarity in its conserved DNA
binding C-terminal domain with sub-
domains 2.3, 2.4 of 70 (ref. 10). TBP
also happens to be an unusual protein
like 70,\4
running out several important
functions. Not surprisingly, even TBP
was suggested to change its conformation
upon binding to DNA or other com-
ponents of the transcription machinery.
Although TBP can bind to the TATA
element of eukaryotic promoters, it re-
quires thermal energy for binding and
shows slow on and off rates.\5
Deletion
of N-terminal amino acids of TBP\6
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 70
seem to have in-built mechanisms to take
care of their unwarranted, undesired bind-
ing with the promoter which may take
place if DNA binding domains are left
free. Cell would obviously like this bind-
ing to take place only when it is ready
to transcribe its DNA using other compo-
ments of the transcription machinery,
supply of which is regulated by other
possible ways. This mechanism of
intramolecular regulation through inter-
domain interaction is indispensable, since
in course of evolution when distribution
of various sigma domains to several fac-
tors 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
70 is no more there.

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