

RNA Polymerase and its Accessory Factors in *Escherichia coli*

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RNA polymerase is one of the most important enzymes in living organisms as it controls the synthesis of the key intermediate RNA from DNA message. Regulation of gene expression is primarily governed by the interplay among various protein factors and this enzyme. In this review we tried to emphasize and catalogue all the factors that are known till-date in prokaryotes, taking *E. coli* as a model. Structural requirements for intricate protein-protein and protein-DNA interaction are also discussed.

Key words: *E. coli*, RNA polymerase; Subunits, Factors, Domains, Interactions, Regulations
Protein factors

Introduction

Gene expression requires the transfer of genetic information from DNA into RNA molecules, the direct templates for protein synthesis. Transcription entails copying of a defined portion of DNA into RNA in response to the needs of the organism. The transcription machinery must, therefore, be responsive to environmental signals, and also be able to recognize "start" and "stop" signals on the DNA. These intricate events are precisely performed by an enzyme called DNA-dependent RNA polymerase (EC 2.7.7.6) or RNAP, and the enzyme function is regulated by various interacting factors.

The modern era of regulatory biology began with Jacob and Monod's proposal for gene

regulation by repression, induction and feedback inhibition of enzyme activity (Monod & Jacob 1962). On the basis of the enormous body of regulatory information available in prokaryotes, it has been technically straightforward to determine the sites at which regulatory proteins act, analyze the nature of the regulatory response and reconstruct each regulatory system using isolated, well characterized components. However, an obvious deficiency in our knowledge of transcription regulatory mechanisms in prokaryotes and eukaryotes, is the detailed information on the structures of the various prokaryotic and eukaryotic RNAPs, the roles of polymerase accessory factors on one hand, and how protein-protein interactions influence polymerase function on the other, are

the arena where there is not much information available.

In eubacteria, the synthesis of mRNA, rRNA and tRNA is catalyzed by a single type of RNAP. Although the enzyme has been purified from a number of sources, much of our knowledge on the structure and mechanism of action of this vital enzyme comes from the enzyme purified from *Escherichia coli*. Earlier biochemical studies, followed by studies on the structure, function, synthesis and assembly of the polymerase subunits were made possible through the discovery and analysis of the subunit structure of RNAP (Burgess 1976) as well as the introduction of genetic means to dissect the genes involved in biosynthesis of the polymerase subunits in *Escherichia coli* (Scaife 1976).

The *E. coli* RNAP is a hetero-multimer of a molecular weight of approximately 460 kDa (Chamberlin & Berg 1962). RNAP exists in two enzymatically active forms: the RNAP holoenzyme [$\alpha_2\beta\beta'\sigma(\omega)$] and the core enzyme [$\alpha_2\beta\beta'\sigma(\omega)$] which is devoid of the σ - subunit (Chamberlin 1976). In addition to the essential subunits (α, β, β' , and σ) a number of proteins/polypeptides which are strongly associated with purified RNA polymerase have been reported. In a recent report (Sukhodolets & Jin 1998), a novel ATPase named RapA (RNAP-associated protein) has been identified which copurifies with RNAP holoenzyme as a 1:1 complex and has homology with SWI/SNF family of eukaryotic proteins. Amongst others is the polypeptide designated as omega (ω), occurring at a molar ratio of 0.5 to 2 per enzyme molecule, it copurifies with both the holo and core enzyme, and is regarded as a subunit of RNAP (Burgess 1969). The core RNAP is capable of polymerization of ribonucleotides, while the holoenzyme containing the σ -subunit, can also initiate transcription at specific promoter sites in a catalytic manner (Burgess & Travers 1970).

The genes coding for the holoenzyme subunits *rpoA* (α -subunit), *rpoB* (β -subunit), *rpoC* (β' -

subunit), *rpoD* (σ -subunit) and *rpoZ* (ω -subunit) have been located on the *E. coli* genome (Yura & Ishihama 1979, Gentry & Burgess 1986). Table 1 summarizes the subunit name, map position on the *E. coli* genetic map and some properties of each of the *E. coli* RNAP subunits (Burgess et al. 1987) and its associated factors.

The Subunits of RNA Polymerase

The Alpha (α) Subunit

The α -subunit, present as a dimer in *E. coli* RNAP, consists of two independently folded domains: an amino terminal domain (α NTD, residues 8-235) and a carboxy terminal domain (α CTD, residues 219-329) connected through an unstructured and/or flexible linker of 14 amino acids (Blatter et al. 1994, Igarashi & Ishihama 1991, Negishi et al. 1995). The alpha subunit performs at least three critical functions: it serves as the initiator for RNAP assembly both *in vivo* (Hayward et al. 1991), and *in vitro* (Igarashi et al. 1991, Kimura et al. 1994, Kimura & Ishihama 1996); it participates in promoter recognition by sequence-specific protein-DNA interaction (Ross et al. 1993, Rao et al. 1994, Blatter et al. 1994); and it is the target for transcriptional regulation by a large set of transcriptional activator proteins (Ishihama 1992, 1993, Busby & Ebright 1997).

The principal determinants for dimerization of α and almost all determinants for interaction of α with other RNAP subunits, are located within α NTD (Kimura et al. 1994, Kimura & Ishihama, 1995a, b, Heyduk et al. 1996). In a recent study, the X-ray crystal structure of α NTD was determined at 2.5 \AA resolution (Zhang & Darst 1998) revealing the basis for the tight dimerization of the α -subunits (α NTD dimer forms an elongated, flat structure) providing insight into the organization of core RNAP subunits. The RNAP α subunit serves as the initiator for RNAP assembly subsequent to dimerization, which proceeds according to the pathway $\alpha + \alpha \rightarrow \alpha_2 + \beta \rightarrow \alpha_2\beta + \beta' \alpha_2\beta\beta' + \sigma \rightarrow \alpha_2\beta\beta'\sigma$ (Ishihama 1981).

Table 1 Properties of *E. coli* RNA polymerase subunits and its associated factors

| Subunit factor | Gene | Map position (min) | Total amino acids | M.W (dal tons) | Size in SDS gels | Charge (pH 7) | Function |
|----------------|-------------|--------------------|-------------------|----------------|------------------|---------------|---|
| α | <i>rpoA</i> | 73 | 329 | 36,512 | 39 | -14 | Serves as initiator for RNAP assembly, recognizes UP-element, α -CTD interacts with activators of class I system |
| β | <i>rpoB</i> | 90 | 1342 | 150,615 | 155 | -34 | Active site for RNA synthesis, binds inhibitors like rifampicin, streptolydigin and ppGpp |
| β' | <i>rpoC</i> | 90 | 1407 | 155,159 | 165 | +6 | DNA binding, termination/antitermination |
| σ^{70} | <i>rpoD</i> | 67 | 613 | 70,263 | 87-90 | -40 | Major initiation specificity factor. Contact site for class II activators |
| ω | <i>rpoZ</i> | 82 | 91 | 10,105 | 10-11 | -4 | (This study) |
| σ^{32} | <i>rpoH</i> | 76 | 284 | 32,363 | 33-36 | -3 | Initiation specificity factor (Heat shock) |
| σ^{54} | <i>rpoN</i> | 70 | 477 | 53,978 | 55-60 | -25 | Initiation specificity factor (under N-starvation) |
| NusA | <i>nusA</i> | 69 | 494 | 54,570 | 64-69 | -36 | Termination/antitermination |
| ρ (rho) | <i>rho</i> | 85 | 49 | 46,094 | 48 | +1 | Termination |
| GreA | <i>greA</i> | - | 158 | 19,000 | 19 | nk | In elongation, release of di/tri-ribonucleotides |
| GreB | <i>greB</i> | - | 158 | 19,000 | 19 | nk | In elongation, release of longer oligo-ribonucleotides |
| RapA | <i>rapA</i> | 1.3 | ~100 | 110,000 | 110 | nk | Has an ATPase activity, no known role |
| Rsd | <i>rsd</i> | 90 | | 21,000 | 21 | nk | Stationary phase specific anti- σ^{70} factor |

nk: not known

Deletion analysis of α has indicated that the residues between positions 21 and 235 are involved in assembly (Kirmura et al. 1994); while insertion analysis has been used for fine mapping of the sites within this region required for subunit-subunit contacts. The region around residue 80 is involved in binding both β and β' ; the region between residues 180 and 200 plays a role in β' binding; while α dimerization involves multiple contact sites (Kimura & Ishihama 1995b).

The α CTD is proposed to serve as a flexibly tethered DNA-binding activator-binding "adaptor" module. It is proposed to occupy different locations at different promoters: that is, it makes no specific contact at a simple promoter (like *lacUV5*); makes specific DNA protein contacts at promoters containing the UP element (eg. *rrnBPI* promoter); and makes specific protein-protein interactions with the activator at a promoter having an appropriate activator (eg. *lac* promoter and CAP) (Ishihama 1992, 1993, Busby & Ebright 1994). It is possible to remove as much as 99 amino acid from the carboxyterminus without preventing formation of a functional holoenzyme *in vivo* and the region between residues 220-230 contains the C-terminal boundary of the assembly domain (Zou et al. 1997). However, the extreme thirteen C-terminal residues are crucial for stabilising the α -polypeptide against natural proteolysis occurring approximately at residue 271. Further work has shown that the extreme C-terminal region of α is exposed in the holoenzyme and substituted deletions in this region are not commonly functional at class I promoters (Thomas et al. 1997). A schematic representation of the functional map of α subunit is represented in figure 1.

The Beta (β) Subunit

This is the second largest subunit in *E. coli* and is intimately involved in most of the known activities of prokaryotic RNAP. Binding sites for substrates, and inhibitors like the antibiotics, rifampicin, streptovaricin and streptolydigin, which inhibit initiation and elongation are located in this subunit

(Zillig et al. 1976, Krakow et al. 1976). The rifampicin binding site has been well characterized and falls exclusively on the β subunit (Frischauf & Scheit 1973, Armstrong et al. 1976). The active site geometry has been mapped, through fluorescence spectroscopy by using TbGTP and rifampicin (Kumar 1990, Kumar et al. 1992, Chatterji & Gopal 1996). Analysis of *rpoB* mutants implicated β subunit in σ binding (Travers 1975); ppGpp (the stringent response effector) binding (Nene & Glass 1983); promoter interactions (Glass et al. 1986a) and core assembly (Ishihama et al. 1990). The binding site of ppGpp has been mapped on the β subunit and is estimated to be 27 Å from the rifampicin binding site (Reddy et al. 1995).

It is possible to develop a composite model towards a functional map at the β subunit on the basis of the existing biochemical and genetic data (Lisityn et al. 1998, Nene & Glass 1984).

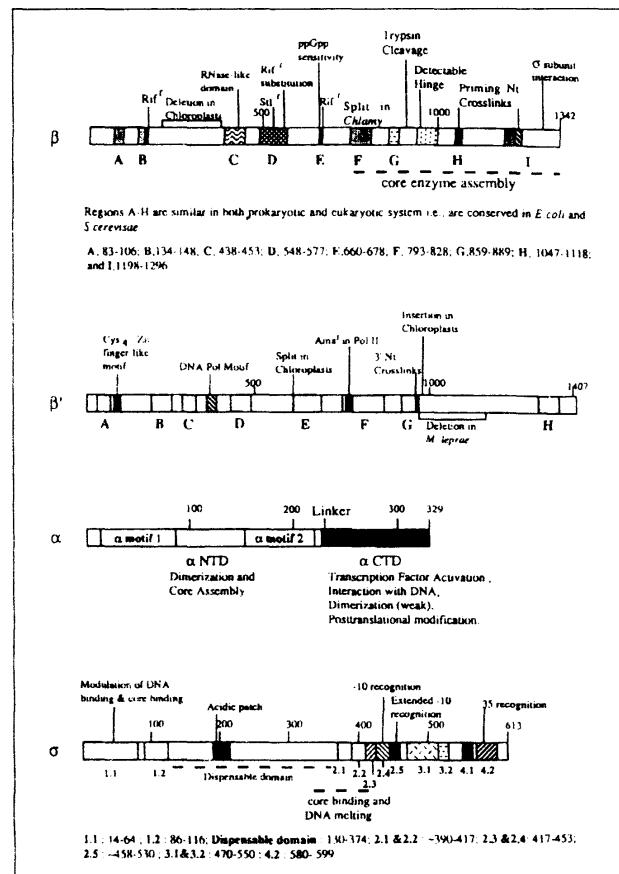


Figure 1 Different functional domains of *E. coli* RNA polymerase subunits

Crosslinking experiments have implicated segments 515-660 and 1091-1107 in β subunit to be facing the 3' terminus of RNA in the catalytic pocket (Markovtsov et al. 1996). Lys¹⁰⁶⁵ and His¹²³⁷ of the β subunit are located on the 5' side facing the priming nucleoside triphosphate (Grabcev et al. 1989, Mustaev et al. 1991). The rifampicin binding site is located upstream of the 5' face along the exit path of the transcript (Severinov et al. 1995). The region from residue 800 to 1200 is responsible for ppGpp binding (Reddy et al. 1995, Chatterji et al. 1998). The C-terminal portion of the β subunit (from 1200-1342) is required for binding of σ to core (Glass et al. 1986b). Several alterations in the N-terminal third of the β subunit do not eliminate catalytic activity (Landick et al. 1990) but are important for proper folding and assembly of the β subunit.

Through insertion analysis it was shown that the region between 965 to 1080 was dispensable (Glass et al. 1986a) and the β subunit can be tryptically digested at two major sites, Arg⁹⁰³ or Lys⁹⁰⁹, which is adjacent to the dispensable domain (Borukhov et al. 1991). Identification of intragenic suppressor mutations has provided evidence for interactions between the conserved regions B (residue 134-148) and D (548-577), and regions E (660-678) and H (1047-1118) (Tavormina et al. 1996). Thus, residues quite distant in the primary structure of β may interact in the tertiary structure, raising the possibility that many of the conserved regions of the β subunit may interact to form a framework for the activities of the polypeptide but may help determine promoter selectivity. Evidence also suggests that the β subunit may serve as an activation target at least in one case (Lee & Hoover 1995). A schematic representation of the functional map of β subunit is represented in figure 1.

The Beta-prime (β') Subunit

The β' subunit is the most basic among the subunits of RNAP. Template binding is attributed to the β' subunit because of its high affinity to DNA and other polyanions as well as the inability of α , β complex to bind to DNA (Zillig et al. 1971). It

is the only subunit that can bind to DNA by itself (Zillig et al. 1970, 1971), and this property presumably contributes to the non-specific DNA binding exhibited by the core enzyme. That the β' -subunit is responsible for template binding was shown by means of crosslinking of poly-4-thiothymidylic acid to holoenzyme (Frischauf & Scheit 1973). Several bacterial mutants whose RNAPs have impaired DNA-binding properties, contain altered β' subunits (Panny et al. 1974, Gross et al. 1976, Sugiura et al. 1977). Using photocrosslinkable nucleotide analogs and genetic mapping, it has been possible to identify a conserved stretch of amino acids in the β' subunit (330 to 366 and 932 to 1020) that interact with the nucleotides in the 'i+1' site (elongation site) (Borukhov et al. 1991). A conserved motif of NADFDGD lying between residues 400-500 in the β' subunit has been implicated in the binding of Mg²⁺ ion (binds to 'i+1' site) at the catalytic center of RNAP (Zaychikov et al. 1996), and is proposed to form the base of the cleft which forms the crucial element of the active centre. Other sites identified through crosslinking experiments in both β and β' , probably line the surface of the cleft where the active center is located.

E. coli RNAP is a Zn-metallocenzyme and binds to 2 moles of Zn per mole of the enzyme (Wu & Wu 1981). One of the Zn²⁺ is present in the β subunit and the other in the β' subunit (Wu & Tyagi 1987). Amino acid sequence analysis predicted the presence of a DNA binding Zn-finger motif in the N-terminal part of the β' subunit (residue 68-88) (Chatterji & Guruprasad 1988), this was later corroborated with experimental verification (Clerget et al. 1995). Further, it was shown that the ω -subunit crosslinks predominantly with β' subunit, indicating their proximity in the natural system (Gentry & Burgess 1993). Recently, the β' subunit has been identified as the target of action of N4SSB, an activator, that functions without binding to DNA (Miller et al. 1997). A schematic representation of the functional map of β' subunit is represented in figure 1.

The Sigma (σ) subunit

Sigma factor confers sequence specific initiation capability to the bacterial core RNAP, and therefore has two functions; it binds to the core RNAP, and thereafter, binds to DNA in a sequence specific manner. There are two major families of sigmas, those related in their protein sequence to σ^{70} , the primary vegetative sigma factor of *E. coli*, and those related to σ^{54} , an alternative sigma factor of *E. coli*, involved in transcribing nitrogen-regulated genes (Gribskov & Burgess 1986, Helmann & Chamberlin 1988, Lonetton et al. 1992).

A comparison of sequences of σ factors belonging to the σ^{70} family in *E. coli* and other bacteria, have led to the identification of four homologous conserved domains which can be divided into at least 10 subdomains in terms of function and sequence homology (Gribskov & Burgess 1986, Helmann & Chamberlin 1988, Lonetton et al. 1992). Domain 1 is involved in core RNAP 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 C-terminus are involved in the recognition of -10 and -35 consensus sequences of the promoter. All four subdomains of domain 2 are implicated in DNA melting, while subdomains 2.1 and 2.4 are also involved in core RNAP binding (Gross et al. 1992). Some primary σ factors carry a large non-conserved segment between domains 1 and 2. On the other hand, the σ^{54} family of proteins have two strongly conserved domains (Sasse-Dwight & Gralla 1990).

In addition to their structural distinction, the process of transcription initiation by holoenzymes containing these two types of sigma factor differs. Promoters recognized by σ^{70} -containing holoenzyme consist of two blocks of conserved sequences located at about -10 and -35 bp, relative to the transcription start site at + 1 and are separated by 15-20 bp of non-conserved sequence (Reznikoff et al. 1985). In contrast, σ^{54} dependent promoters are characterized by highly conserved dinucleotides positioned near -12 and -24 bp

relative to the +1 site and separated by only 5 bp (Gussin et al. 1986). Moreoever holoenzymes containing σ^{54} type sigmas require an auxiliary protein to form "open complexes" whereas those containing σ^{70} type sigmas are competent in this aspect independent of the presence of any auxiliary factor.

The affinity of σ^{70} for free core RNAP is between $2 \times 10^9 \text{ M}^{-1}$ to $5 \times 10^9 \text{ M}^{-1}$ (Wu et al. 1975, Gill et al. 1991). The interaction of σ with core RNAP is tighter in the open complex (that is, following the binding and melting of DNA), than in free holoenzyme (Gill et al. 1991) indicating additional sigma-core RNAP interactions in the open complex. Interactions between σ and DNA may also stabilize the complex. In contrast, the interaction of σ^{70} with core RNAP is weaker in elongating complex, (affinity is $\leq 5 \times 10^5 \text{ M}^{-1}$) (Gill et al. 1991) than in the free holoenzyme.

Although σ subunit is implicated in the primary recognition of promoter DNA sequences it cannot bind to promoter DNA in the absence of the core RNAP (Zillig et al. 1970, 1971). Mutational analysis, by amino acid substitution, has implicated conserved domain 2.4 of σ^{70} in recognition of the -10 hexamer and conserved domain 4.2 of σ^{70} in recognition of the -35 hexamer of the promoter, respectively (Gardella et al. 1989, Siegele et al. 1989, Zuber et al. 1989, Daniels et al. 1990, Waldburger et al. 1990, Tatti et al. 1991). Recent studies have suggested that the residues 437, 440 and 441 interact with the non-template strand of promoter DNA (Roberts & Roberts 1996, Severinova et al. 1996, Marr & Roberts 1997). Barne et al. (1997) have reported that an amino acid substitution of the residue 458, which lies downstream of domain 2.4, alters the extended -10 recognition.

Removal of an N-terminal segment upto 100 amino acids from σ^{70} , rendered the truncated σ^{70} subunit capable of binding to -35 promoter sequences even in free form (Dombroski et al. 1992, 1993). The inhibitory effect of N-terminal domin could also be observed in *trans*, partial

polypeptides of σ^{70} , containing residues 1-100 could inhibit the binding of a fragment consisting of residues 507-613 (terminal part of domain 3.1 to the C-terminus), to DNA with a -35 promoter sequence (Dombroski et al. 1993).

The work of Gopal and coworkers (1994) have shown that a tryptophan to glycine mutation (W434G) at the junction of the 2.3/2.4 subdomain of σ^{70} resulted in its transient DNA binding ability. On the other hand, a mutation in the 1.1 subdomain of σ^{70} led to disruption in its overall structure, and also resulted in its transient DNA binding ability like W434G (Gopal & Chatterji 1997). This indicated that the 1.1 subdomain might be interacting hydrophobically with the 2.3/2.4 subdomain, thereby resulting in the exposure of the acidic patch around the middle of the protein and blocking of the C-terminal domain by N-terminal domain.

A small segment overlapping domain 2.1 (residues 361-390) was shown by deletion analysis to be sufficient for core RNAP binding (Lesley & Burgess 1989). Several amino acid substitution mutations in the domains 2.1 and 2.2 of other related σ factors have shown defects in core RNAP binding (Shuler et al. 1995, Joo et al. 1997). Recently, protein footprinting techniques have been used to study the subunit-subunit interaction of *E. coli* RNAP. Various domains in σ^{70} , including segments in domain 1.1, 2, 3 and 4 get substantially protected from radical attack upon binding the core RNAP (Nagai & Shimamoto 1997) indicating that besides the conserved domain 2, other conserved domains may also be involved in core RNAP binding. C-terminal truncations of σ^{70} retaining residues 574, 556, 540 or 529 show fourfold reduced affinity for the core RNAP. One of these C-truncated σ^{70} retaining residue 529, can promote the initiation of transcription from the extended -10 promoter but not from -35 dependent promoters (Kumar et al. 1994).

The crystal structure of a tryptic fragment of σ^{70} spanning residues 114-448, carrying a part of the conserved domain 1.2 and all but the C-

terminal 8 residues of conserved domain 2, reveals an entirely helical, 'v' shaped protein with all the domains of conserved primary sequence closely associated with one another (Malhotra et al. 1996). This fragment can bind to the core RNAP albeit with 30-fold less affinity than intact σ^{70} , and the resulting complex is capable of binding to the non-template strand and promoter DNA (Severinova et al. 1996). All these findings again suggest that domains 1.1, 3 and 4 also contribute to core binding. A schematic representation of the functional map of σ subunit is represented in figure 1. Recent reports also suggest that the σ subunit is represented in figure 1. Recent reports also suggest that the σ subunit can function as the contact surface for activators (Hochschild 1994, Kuldell & Hochschild 1994, Li et al. 1994, Artsimovitch et al. 1996, Gerber & Hinton 1996).

This mechanism of intramolecular regulation through interdomain interactions are indispensable to regulate the activity of σ^{70} where unwarranted DNA interactions may be inhibited in the absence of RNAP, wherein DNA binding domains are revealed only on binding to the core RNAP.

The Omega (ω) Subunit

Omega (ω) is a small protein (Mol. wt. 10,105 Da) found associated with both core and holo *E. coli* RNAP (Burgess 1969). The ω protein has been called a subunit of RNAP on the basis of its copurification with RNAP core and holoenzyme. When antibodies raised against ω were used to probe fractions of an *E. coli* lysate fractionated by gel filtration, it was found that the majority of ω was bound to RNAP indicating that it was not merely an abundant contaminant (Gentry 1990).

The function of ω is unknown and unlike the other subunits, ω is not required for transcription either *in vitro* (Heil & Zillig 1970) or *in vivo* (Gentry & Burgess 1989, Gentry et al. 1991). Cells deleted for the gene encoding ω (*rpoZ*) have been reported to have no discernible mutant phenotypes (Gentry & Burgess 1989, Gentry et al. 1991). The *rpoZ*

deleted strain, however, exhibits a defect in the degradation of ppGpp (Gentry & Burgess 1989, Sarubbi et al. 1989), which might be due to polarity effect on the expression of the *spoT* gene, which is in the same operon and immediately downstream of *rpoZ* (Gentry & Burgess 1989).

A recent study (Dove & Hochschild 1998) demonstrated that ω can function as a transcriptional activator when linked covalently to a DNA-binding protein, implying that the ω protein is a component of the RNAP holoenzyme *in vivo*, and that it is accessible at the surface of the enzyme complex. Further their work has also suggested that the amino-terminal portion of the ω protein mediates its association with the RNAP. Interestingly, despite its not having an obvious function, ω is evolutionarily conserved among bacteria (Gentry 1990), and crosslinks specifically to the β' -subunit (Gentry & Burgess 1993).

Accessory Regulatory Factors

In addition to the primary subunits and σ -factors, several accessory factors regulate the functioning of RNAP either negatively or positively, and their interplay has important bearings on transcriptional control. There are three general ways by which proteins interacting with the transcription machinery exert regulatory effects: (i) The binding of proteins (IHF, Hu, H-NS etc.) at regions upstream of *E. coli* promoters result in the bending of DNA and enhancement of specific steps of initiation (Schmid 1990), (ii) Activator binding to specific sites proximal to, or overlapping with, promoter regions facilitates the recruitment of RNAP to the promoter (Ptashne & Gann 1997), on account of protein-protein contacts between the activator and $\alpha/\sigma/\beta/\beta'$ subunits of RNAP. A large number of regulatory proteins in *E. coli* adopt this mechanism

Table 2 Activator proteins and factors that interact with RNA polymerase subunits

| Subunit | Effector | Region of contact | Operon/Gene | Reference |
|---------------|----------------------|--------------------|------------------------------|---------------------------|
| α | Fnr | C-terminal domain | <i>narX, ndh</i> | Ishihama 1993 |
| | OxyR | " | <i>katG, oxyX, ahpC</i> | " |
| | OmpR | " | <i>ompC</i> | " |
| | CAP | " | <i>lac, uxuAB</i> | " |
| | IHF | " | <i>1P_L</i> | " |
| | MelR | " | <i>mel</i> | " |
| σ^{70} | AraC | " | <i>araBAD</i> | " |
| | Mu Mor | " | <i>Mu mor</i> | Artsimovitch et al. 1996 |
| σ^{54} | CAP | Domain 4 | <i>gal P1</i> | Kumar et al. 1992 |
| | PhoB | Domain 4 | <i>pst</i> | Makino et al. 1993 |
| | λ -repressor | Domain 4 | <i>cI</i> | Kuldell & Hochschild 1994 |
| | T4 MotA | Domain 4 | <i>T4 motA</i> | Li et al. 1994 |
| β | T4 MotA | Domain 4 | | Gerber & Hinton 1996 |
| | NtrC | -35 binding region | <i>glnA P2</i> | Lee et al. 1993 |
| β' | C_4D^* | " | <i>C_4D^*</i> | Lee & Hoover 1995 |
| | ppGpp | C-terminal domain | <i>rrn, tufB, rplJ, tyrT</i> | Chatterji et al. 1998 |
| C_4D^* | | " | <i>C_4D^*</i> | Lee & Hoover 1995 |
| | N4SSB | C-terminal domain | <i>phage N4 genes</i> | Miller et al. 1997 |

* C_4D : C_4 -dicarboxylic acid transport protein D

and are detailed in table 2 (Ishihama 1993, Busby & Ebright 1994, 1997, Ebright & Busby 1995), (iii) Direct activator-RNAP contacts without the former binding to the DNA template, can regulate transcription as reported for the N4SSB protein interacting with the RNAP β' subunit, leading to activation of bacteriophage N4 promoters (Miller et al. 1997). Another class of these factors are the repressors, examples include the Trp repressor (Ashimizu et al. 1973, Rose et al. 1973) and the lac repressor (DeCrombrugghe et al. 1971).

Among the other important protein factors which interact with RNAP are rho (ρ) factor, NusA, NusG, S10, GreA and GreB, while effector molecules include ppGpp and pppGpp. These factors function to increase the initiation rate, augment the elongation rate, decrease the frequency of chain termination, release terminated enzymes from the DNA for restarts, or rescue RNAP from abortive termination (Greenblatt 1992, Jones & Peterlin 1994).

The *E. coli* rho (ρ) factor is an essential homohexameric protein composed of 47 kDa (419 amino acid) subunits that is required to mediate transcription termination at various genomic and bacteriophage sites (Platt & Richardson 1992, Das 1993, Nowatze et al. 1996). It serves an important and essential function in *E. coli* by causing RNAP to terminate transcription at the ends of genes and operons, and at regulatory sites at the beginning of operons (Platt & Richardson 1992). It also aids in transcription termination within genes when the cell is stressed by starvation for amino acids (Richardson 1991). Rho-dependent terminators have an upstream RNA component that slows RNA chain elongation when the polymerase is near the 3' endpoints. Rho also exhibits various partial activities such as ATP binding, RNA binding, RNA-dependent ATP hydrolysis and ATP-dependent unwinding of RNA-DNA hybrids. The RNA-DNA hybrid helicase activity of Rho presumably facilitates termination of transcription by dissociating the RNA from its complex with DNA template and RNAP. Genetic evidence

suggests a direct interaction between the Rho factor and RNAP in transcription termination, but the P factor neither co-purifies nor co-precipitates with RNAP by specific antibodies (Das et al. 1978, Guarente 1979).

During the development of bacteriophages such as T4, λ , and others, the host polymerase is altered, either by phage encoded "protein-modifying enzymes" or by phage encoded polypeptides which become associated with the host enzyme and change its properties. In case of T4 phage infection, an anti-sigma factor called AsiA, synthesized from early RNA, binds very tightly to *E. coli* σ^{70} to allow gp55, a T4 bacteriophage sigma factor, to bind to and redirect *E. coli* core RNAP to transcribe from T4 middle promoters (Ouhammough et al. 1995). However, AsiA is a coactivator of MotA-dependent middle transcription, which also requires the σ^{70} subunit (Ouhammough et al. 1995). Although several roles have been proposed for the AsiA (Brody et al. 1995), in a recent report it has been shown that AsiA interacts in a 1:1 stoichiometry with σ^{70} (in the region encompassing residues 506-613, i.e in the -35 recognition domain) both in solution, and as a part of the holoenzyme. Further it has been shown that AsiA acts at a step prior to open complex formation i.e., at the initial binding step, it decreases the affinity for host promoters but enhances affinity of the holoenzyme at T4 middle promoters alongwith MotA (Pahari & Chatterji 1997, Adelman et al. 1997).

During the development of λ phage, the N polypeptide (11.6 kD) becomes associated with the host polymerase and transforms it into a highly processive, termination-resistant transcription apparatus (Barik et al. 1987, Das et al. 1996). This protein can be co-precipitated with *E. coli* RNAP by anti RNAP serum (Barik et al. 1987). The N protein can also suppress multiple ρ -dependent and intrinsic terminators that are located kilobases away from a cloned *nut* site (Whaler & Das 1990, Lazinski et al. 1989). The amino-terminus of N protein, rich in arginine, binds to the stem-loop

structure (*boxB*) in the *nut* mRNA and contacts RNAP through RNA looping to convert it to the termination-resistant form (Lazinski et al. 1989, Chattopadhyay et al. 1995). These factors are NusA, NusB, NusG, and the ribosomal protein S10 (NusE). In addition to the N-*boxB* complex, each of these factors also bind to the RNAP elongation complex (Mason & Greenblatt 1991). NusA, NusG, and the ribosomal protein S10 each interact with RNAP directly as shown by affinity chromatography (Gottesmand & Weisberg 1995). NusB is thought to gain access to the complex through its ability to bind both to S10 and to *boxA*, which lies upstream of *boxB* (Patterson et al. 1994). The Nus factors influence cellular gene transcription as well (Henkin 1994). Using affinity columns containing immobilized core RNAP three abundant *E. coli* RNAP binding proteins were identified; σ^{70} , NusA and NusG (table 3) (Greenblatt 1992, Das et al. 1996).

NusA was originally identified genetically as a protein involved in transcriptional antitermination by the N protein of bacteriophage λ (Friedman & Baron 1974) and further biochemical studies indicated that NusA is an adapter that couples the N protein to RNAP

(Greenblatt 1981). The identification of NusA led to studies showing that NusA induces pausing by RNAP at certain sites (Landick & Yanofsky 1987) and is a transcription termination factor at some terminators (Schmidt & Chamberlin 1987, Barik et al. 1987). Thus, NusA is both a termination factor and a confactor required for antitermination by N.

The ribosomal S10 protein or NusE, was isolated as a component of transcription initiation or elongation complexes in the absence of any elongation factor, but immobilized S10 was unable to bind RNAP (Mason & Greenblatt 1991). The NusB protein, however, bound selectively to S10 and S10 probably therefore, interacts with DNA-bound RNAP and couples NusB to RNAP.

NusG is an *E. coli* elongation factor which was identified through its requirement for antitermination by N *in vitro* (Li et al. 1992). Like NusA and S10, NusG also binds to RNAP (Li et al. 1992), and is essential for Rho to function *in vivo* (Sullivan & Gottesman 1992). NusG also becomes important for termination by Rho *in vitro* under conditions of elevated ionic strength where Rho does not work well on its own.

Table 3 Some protein-protein interactions in transcription detected by protein affinity chromatogr (Greenblatt 1992)

| Ligand | Interacting protein | Apparent Kd(M) | Actual Kd (M) | References |
|--------------------------------------|----------------------------------|--------------------|---------------------|-------------------------------|
| Bacteriophage λ N protein | <i>E. coli</i> NusA | 5×10^{-7} | 3×10^{-7} | Greenblatt and Li (1981) |
| <i>E. coli</i> NusA | <i>E. coli</i> RNA polymerase | 10^{-7} | 3×10^{-8} | Greenblatt and Li (1981) |
| <i>E. coli</i> rRNA polymerase | <i>E. coli</i> σ^{70} | - | 3×10^{-10} | Li et al. (1992) |
| | <i>E. coli</i> NusA | - | 3×10^{-8} | Li et al. (1992) |
| | <i>E. coli</i> NusG | - | - | Li et al. (1992) |
| <i>E. coli</i> NusG | <i>E. coli</i> rho factor | 10^{-5} | - | J. Li (unpubl.) |
| <i>E. coli</i> ribosomal protein S10 | <i>E. coli</i> NusB | 3×10^{-6} | 10^{-7} | Mason et al. (1992) |
| <i>E. coli</i> NusB | <i>E. coli</i> ribosomal protein | S10 | - | 10^{-7} Mason et al. (1992) |

Pausing sites function as regulatory elements in both eukaryotes and *E. coli*. The RNAP can escape these sites if the nascent transcript in the elongation complex is cleaved and repolymerized (Das 1993, Eick et al. 1994). Although the cleavage reaction itself is probably mediated by the core polymerase, protein factors that promote the cleavage reaction have been identified. In *E. coli*, the GreA and GreB proteins are associated with the RNAP and promote the release of di-ribonucleotides and tri-ribonucleotides and of longer oligoribonucleotides (upto 18 nucleotides long), respectively, depending on the type of ternary elongation complex (tEC) (Borukhov et al. 1992, 1993, Nudler et al. 1994). In addition to the antiarrest activity, GreA is believed to have a proof reading role in transcription because it facilitates the removal of misincorporated nucleotides (Erie et al. 1993). Both Gre factors act independently to suppress elongation arrest. The two factors also differ in that GreA can rescue the elongation complex only if added before the arrest took place, whereas GreB can act both before and after the arrest (Borukhov et al. 1992, 1993). GreA and GreB factors are proteins of low abundance whose content in the total soluble fraction of *E. coli* is less than 0.01% (w/w) and 0.002% (w/w) respectively, and its purification involves multiple steps (Borukhov & Goldfarb 1996). The susceptibility of different TEC to hydrolytic reactions induced by GreA and GreB varies substantially depending on the length of the transcript, its sequence and the condition of RNAP in ternary complexes (Borukhov & Goldfarb 1996).

Among the other proteins which are copurified with RNAP or co-precipitated alongwith RNAP by treatment with anti-RNAP antibodies, are the "tau" (τ) proteins (Burgess 1976). Molecular weights of this class of proteins are \sim 100 kD. Most RNAP preparations purified under mild conditions contain three major species of analogous proteins. The middle τ protein can be separated from RNAP by glycerol gradient centrifugation or by gel filtration in the presence

of high salt concentrations. The large τ protein separates from polymerase after phosphocellulose chromatography (obtained in the flowthrough fraction). Enzymes eluted at higher salt concentrations contain increasing amounts of small τ proteins. The small τ proteins interfere with and decrease core enzyme activity (Ishihama et al. 1980). Recently, it has been shown that the τ protein is identical to RapA, a novel RNAP associated protein which is a homolog of the SWI/SNF family of eukaryotic proteins whose members are involved in transcription activation, nucleosome remodelling and DNA repair (Sukhodolets & Jin 1998). Though the exact function of RapA is yet to be determined, this 110 kD protein interacts with RNAP both physically (binds to RNAP) and functionally (the ATPase activity of RapA is stimulated by RNAP).

The σ subunit required for transcription of most of the genes expressed in the exponential growth phase is σ^{70} , the product of the *rpoD* gene. Upon entry into stationary phase, the σ^{70} subunit is replaced with σ^{38} or σ^s (encoded by *rpoS*). The concentration of σ^{70} , which is around 700 molecules per cell, in exponential phase remains constant even though the frequency of transcription of genes under the control of σ^{70} decreases by more than 10-fold (Ishihama 1991). In a recent report, a fraction of the σ^{70} subunit in stationary phase cell extracts was found to exist as a complex with a novel protein designated Rsd (Jishage & Ishihama 1998). The authors have shown that intracellular levels of this protein increase during transition from exponential to stationary phase. Purified Rsd could interact with only σ^{70} amongst different σ -factors *in vitro*, at or downstream of region 4 of σ^{70} . It could also, inhibit σ^{70} -driven *in vitro* promoter-specific transcription to various extents depending on the promoters used. Rsd is possibly an anti-factor for σ^{70} or σ^s .

A novel ATPase was found in most partially purified RNAP by different methods (Ishihama et al. 1976). Isolated ATPase did not rebind to

RNAP under the conditions tested nor to DNA. However, the morphology of the ATPase as well as the ATP hydrolyzing activity indicated that it was the *groE* protein which is involved in the morphogenesis of several bacteriophages (Hendrix 1979). The synthesis of this ATPase was transiently stimulated if the culture temperature was shifted up (Yamamori et al. 1978). Copurification of the two proteins, RNAP and ATPase probably simply indicates a similarity of physical and chemical properties between the two proteins. Later work by Ziemienowicz and co-workers (1993) showed that the GroEL chaperone protein can protect purified *E. coli* RNAP holoenzyme from heat inactivation. In this protection reaction, the GroES protein is not essential, but its presence reduces the amount of GroEL required.

The stringent-starvation protein (SSP) of molecular weight 22.5 kD synthesized predominantly under extreme amino acid starvation (Reeh et al. 1976) was found to be stably associated with purified RNAP (Ishihama & Saitoh 1979). This protein binds to the holoenzyme but not to the core enzyme, and inhibits its activity with some template DNA and under certain salt conditions. Induction of the stringent response, causes a dramatic increase in the intracellular levels of two unusual guanosine nucleotides, initially termed MSI and MSII (Cashel 1969). These were later identified as 3'-pyrophosphate derivatives of GDP, guanosine-5'-diphosphate-3'-diphosphate (ppGpp) and GTP, guanosine-5'-triphosphate-3'-diphosphate (pppGpp), respectively.

Thus, from the above discussion it becomes apparent that a clear understanding of the reversible and dynamic interplay between RNAP and a variety of regulatory factors and effectors is essential, not only in determining the functional interconversion and structural alteration of the RNAP and in the control of transcription *per se*, but also perhaps in the coordinate control of other processes such as DNA replication and cell division.

Regulation of Synthesis and Assembly of RNA Polymerase

(a) Regulation of RNA Polymerase Synthesis

The level of RNAP limits transcription, and thus the control of RNAP formation is a major facet in the control of cell growth (Ishihama et al. 1976). The number of core enzyme molecules in an *E. coli* cell is approximately 2,000 (Ishihama et al. 1976, Ishihama 1991), whereas there are more than 4,000 genes on the *E. coli* chromosome to be transcribed at widely varying rates (Ishihama 1991, Blattner et al. 1997). The concentration of σ^{70} during the exponential stage is about 700 molecules per cell, as measured by immunoprecipitation of radiolabelled cell extracts (Iwakura & Ishihama 1974) or Western blot analysis with specific antibodies (Jishage & Ishihama 1995, Jishage et al. 1996). In growing cells, at any given moment, about two-thirds (about 1,300-1,400 molecules) of the core enzyme are actively involved in elongation of transcripts, and are therefore, devoid of σ and are associated with the chromosome. Therefore, most of the remaining core enzyme (around 600-700 molecules) is likely to be associated with σ^{70} . When *E. coli* cells are exposed to a nutritional or temperature shift, a specific regulatory mechanism seems to operate immediately to adjust the core RNAP enzyme concentration to the level characteristic of the post shift rate of growth (Nakamura & Yura 1975). However, the kinetic patterns of response vary with nature of change in growth conditions.

The lack of gene dosage effect, stimulation of RNAP synthesis when exposed to rifampicin, and mutations that affected the level of active RNAP but stimulated the rate of subunit synthesis, particularly the β and β' subunits implicated an autogenous regulation of RNAP synthesis (Yura & Ishihama 1979, Lang-Yang & Zubay 1981). A direct demonstration of the autogenous regulation model was provided when the *in vitro* synthesis of β and β' subunits in a coupled transcription-translation system, was specifically repressed by

the addition of either holoenzyme or the $\alpha_2\beta$ complex, while none of the individual subunits or the core RNAP was found to have significant repressor activity (Taketo et al. 1978, Fukuda et al. 1978). It was later shown that the feedback regulation was at the level of translation (Kajitani et al. 1980, Peacock et al. 1982). A limitation of $\beta\beta'$ synthesis results in a 2-3 fold increase in the translational efficiency of $\beta\beta'$ synthesis (Dennis et al. 1985). While the induction of RNAP overproduction is characterized by an initial large burst of $\beta\beta'$ synthesis, followed by a gradual decrease as the concentration of RNAP increases, indicating that RNAP feedback regulation controls $\beta\beta'$ synthesis *in vitro* (Bedwell & Nomura 1986).

(b) Assembly of RNA Polymerase Subunits

Despite its large molecular size and complex subunit composition the *E. coli* RNAP holoenzyme can be reversibly dissociated into σ subunit and core polymerase ($\alpha_2\beta\beta'\omega$) and can be separated by passage through phosphocellulose or the cationic resin Biorex 70 (Lowe et al. 1979). The first successful attempts to reconstitute RNAP from isolated individual subunits were made in early 70s, all of them involved denaturation through dialysis of RNAP against concentrated (6-7M) urea or guanidinium hydrochloride solution, resulting in complete loss of activity (Heil & Zillig 1970, Ishihama 1972, Lowe & Malcolm 1976). When such a denatured enzyme was diluted 100-200 fold into a buffer, enzyme activity was partially restored indicating renaturation of the dissociated enzyme. Presence of a thiol compound and glycerol during denaturation protects the enzyme from irreversible inactivation (Lill & Hartmann 1970). Dialysis of denatured core enzyme at 4°C yields an inactive complex, generally called "premature core enzyme", which is converted in a time dependent process upon incubation at 37°C and in the presence of either DNA or σ subunit, to the native RNAP. The rate limiting step in *in vitro* reconstitution is the activation ("maturation") of the premature core enzyme (Harding & Beychok 1974, Palm et al. 1975, Levine et al. 1980).

Another breakthrough was brought about after two decades. Following sequencing, cloning and establishment of the transient overexpression systems for individual subunits of RNAP, another approach of obtaining highly purified wild-type and mutant RNAP became possible using reconstitution and assembly of recombinant RNAP from individually overexpressed and purified subunits (Zalenskaya et al. 1990, Igarashi & Ishihama 1991, Fujita & Ishihama 1996). Subsequent refinement has suggested that the presence of both σ factor and $ZnCl_2$ (10 μM) led to higher yields with an optimal molar ratio of the subunits $\alpha : \beta : \beta' : \sigma$ being 2 : 8 : 4 : 1, rather than the stoichiometric 2 : 1 : 1 : 1 (Borukhov & Goldfarb 1993). Use of a hexahistidine-tagged α derivative has been utilized to perform an efficient recombinant reconstitution of RNAP, where crude recombinant β , β' and σ^{70} subunits from inclusion bodies were incubated with tagged- α and the resulting, reconstituted, recombinant RNAP was purified by batch-mode, metal-ion-affinity chromatography (Tang et al. 1995, Kashlev et al. 1996). The histidine tag technology also allows the study of mechanisms of transcription, by allowing one to trap defined intermediates of initiation, elongation, and termination (Kashlev et al. 1996).

Reconstitution from individual recombinant subunits produced by cloning and high level overexpression of individual subunit genes, permits construction of mutant RNAP derivatives (Zalenskaya et al. 1990, Kashlev et al. 1990, Igarashi & Ishihama 1991, Mustaev et al. 1991, Lee et al. 1991, Martin et al. 1992, Zou et al. 1992, Severinov et al. 1993, Tang et al. 1994) including lethal mutant derivatives (Kashlev et al. 1992, Igarashi & Ishihama 1991, Mustaev et al. 1991, Lee et al. 1991, Martin et al. 1992). Recombinant reconstitution is an extremely powerful tool for genetic dissection of RNAP structure and function.

The concept of reconstitution of subunits of RNAP proceeding by a process of self-association was proved by *in vitro* experiments. Mixtures of α , β ; α , β' ; and β , β' subunits dialyzed against

reconstitution buffer and analyzed by glycerol gradient centrifugation, showed that aggregates of subunits α and β (2:1), as well as β and β' (1), but not α and β' are formed. Addition of β' subunit to preformed $\alpha_2\beta$ aggregate followed by dialysis against reconstitution buffer and incubation at 37°C, results in an enzymatically active complex with sedimentation coefficient similar to that of the native core enzyme. In contrast, the preformed aggregate of $\beta\beta'$ subunits does not form an enzymatically active complex, indicating that isolated subunits assemble in a sequential process (figure 2) (Ishihama & Ito 1972, Palm et al. 1975, Kumar 1981).

Since then, several lines of evidence suggested that subunit assembly *in vivo* proceeds in the same sequence as that found *in vitro*. The assembly intermediates involved in *in vitro* reconstitution are present not only in assembly-defective strains of *E. coli* (Taketo & Ishihama 1976, 1977) but also in wild-type strains, pulse-labelled enzyme subunits are integrated into the polymerase structure in the order expected from the assembly sequence (Ito et al. 1975). Moreover, most temperature-sensitive β and β' mutants were

found to accumulate one or more assembly intermediates at high temperature (Taketo & Ishihama 1976, 1977).

The concept that the assembly of RNAP is controlled, was strengthened by the finding that newly synthesized β and β' subunits in stationary phase cells of wild-type *E. coli* are degraded without being assembled into the enzyme structure, though the pre-existing polymerase formed in log phase cells remains undegraded during the stationary phase (Kawakami et al. 1979). There is also a decrease in the synthesis rate of the α subunit as *E. coli* cells cease to grow exponentially. Thus, the abortive subunit assembly in the stationary phase cells can be attributed at least in part, to the lack of an initiating α dimer. The amount of functional RNAP (core enzyme) which is nucleoid bound varies depending on growth phase; 60-70% in log phase cells to 30-40% during the linear phase or early stationary phase (Kawakami et al. 1979). The increase in the cytoplasmic pool of unused RNAP and the concomitant decrease in the rate of subunit synthesis in early stationary phase is again consistent with autogenous regulation of RNAP synthesis.

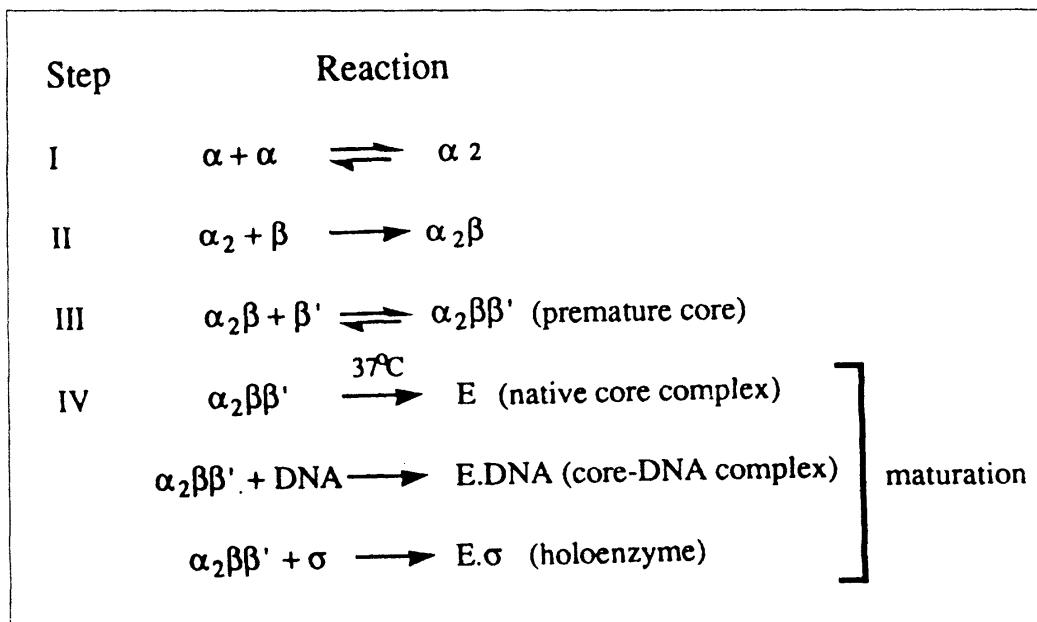


Figure 2 Maturation of *E. coli* RNA polymerase from individual subunits

Eukaryotic RNA Polymerase

Unlike bacterial polymerase, eukaryotic RNA polymerase is a complex enzyme made up of at least 11 subunits, producing a total mass greater than 500 kDa (Woychik et al. 1990). The two large subunits are conserved in amino acid sequence across species from bacteria to man and are believed to play fundamental roles in RNA synthesis (Allison et al. 1985). Structure of RNA polymerase enzyme from any species is not very well characterized, apart from T7 bacteriophage RNA polymerase, which is a single polypeptide chain and its X-ray structure is known (Chung et al. 1990). However, two dimensional crystal structure over positively charged lipid bilayer for *E. coli* and yeast RNA polymerase II are reported in literature (Darst et al. 1988, Darst et al. 1991). Interestingly, both the structures show an arm-like projection surrounding a cleft about 25 Å in

diameter, and appropriate size for binding double helical DNA. It is definitely worthwhile to wait for single crystal structure of this enzyme for more surprises.

Concluding Remarks

One of the most important aspects of the transcription assembly is the interplay among various subunits with different transcription factors. We tried to emphasize this point throughout the manuscript. It is now clear that conventional X-ray crystallography for RNA polymerase molecule is not an easy task to achieve. New technological breakthrough is necessary. However, in the next century efforts will be mainly on to define minimal catalytic surface that is necessary to carry on gene expression. Today we somewhat understand the control of gene expression, but the structure still remains elusive.

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