# Alteration in template recognition by *Escherichia coli* RNA polymerase lacking the w subunit: A mechanistic analysis through gel retardation and foot-printing studies

Kakoli Mukherjee* and Dipankar Chatterji**,†

*Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India*
**The Swedish Institute for Infectious Disease Control, SE 17182, Sweden*
***Present address: Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India*

†*Corresponding author (Fax, 91-80-334-8535; Email, dipankar@mbu.iisc.ernet.in).*

The *w* subunit of *Escherichia coli* RNA polymerase is a 91 amino acid polypeptide which co-purifies with the enzyme and is thought to help in maturing the rest of the enzyme to its full functionality. Purified *w* when added externally was found to inhibit general transcriptional activity of *w*-less RNA polymerase as well as promoter-specific single-round transcriptional activity at all the promoters tested. In this study we have tried to analyse the observed inhibition of transcription using gel retardation assays and $KMnO_4$ foot-printing. Further, through protein foot-printing we have attempted to identify alterations in the interaction of the *w*-less core enzyme with the $s^{70}$ subunit.

Our results suggest that the *w*-less holoenzyme has lesser affinity towards the DNA template and external addition of *w* destabilizes the open complex for both the wild-type and *w*-less enzyme. The *w*-less core enzyme interacts with the $s^{70}$ subunit to expose the – 35 recognition domain (domain 4·2) unlike that observed in the wild-type interaction. Thus the absence of the *w* subunit leads to the formation of an enzyme which has altered DNA binding and $s^{70}$ binding properties. Circular dichroic measurements also indicate a major conformational alteration of both holo and core RNA polymerase in the presence and absence of the *w* subunit.

## 1. Introduction

The *Escherichia coli* RNA polymerase is a heteromultimer that exists in two enzymatically active forms: the core enzyme ($a_{2}b\,b'\,w$) and the holoenzyme ($a_{2}b\,b'\,w\,s$). Initiation of transcription at defined promoter sites is carried out by RNA polymerase holoenzyme while elongation and termination are carried out by core RNA polymerase (Burgess *et al* 1969; Burgess and Travers 1970). The *w* protein is a 91 amino acid polypeptide, which is strongly associated with purified RNA polymerase and it co-purifies with both the holo and core enzyme and is regarded as a subunit of RNA polymerase. It occurs in a molar ratio of 0·5–2 per enzyme (Gentry and Burgess 1993).

Despite its abundance and ubiquitous presence in all RNA polymerase preparations, *w* is not required for *in vitro* activity. Reconstituted RNA polymerase that lacks *w* has no known alteration in its transcriptional behaviour (Zillig *et al* 1977). This appears also to be true *in vivo*; the *rpoZ* gene encoding *w* can be insertionally inactivated or deleted without a known phenotype (Gentry and Burgess 1989; Sarubbi *et al* 1989), except perhaps a somewhat slow growth. Nevertheless, *w* binds stoichiometrically to RNA polymerase, cross-links specifically to the b ¢ subunit, indicating the proximity of the two subunits in the natural system (Gentry and

Burgess 1993) and is immunologically conserved among bacteria (Gentry 1990). A single *rpoZ* (*spoS*) gene is located at about 82 min on the *E. coli* chromosome, just upstream from and in the same operon as *spoT* (Gentry and Burgess 1986, 1989; Sarubbi *et al* 1989). The proximity of *rpoZ* to *spoT* raised the possibility that w is required for transcriptional regulation by ppGpp (the mediator of the stringent response) directly or indirectly (Igarashi *et al* 1989). However, later this proposition was questioned when strains deleted in *rpoZ* still displayed inhibition of stable RNA synthesis following amino acid starvation (Gentry *et al* 1991). It is thus, likely that w is a factor required for some regulatory function or catalytic activity overlooked by *in vitro* assays and not immediately obvious by the behaviour of mutants *in vivo.*

In order to ascribe a physiological function for *w* we adopted an *in vitro* transcription and reconstitution system involving *w*-less RNA polymerase, purified from a *rpoZ* null mutant and with or without purified *w*-factor added extrinsically. Our previous studies have shown that externally added *w* inhibited promoter specific transcription at all promoters tested. We had also noted that abortive product formation by the *w*-less enzyme was not enhanced corresponding to a shut-off of the run-off transcript. This suggested that w did not increase the flux into the non-productive branch. That *w* might have more of a structural than functional role was indicated when there was maximum renaturation of fully denatured *w*-less RNA polymerase in the presence of *w* (Mukherjee and Chatterji 1997). This was further corroborated by the recent observation that *w*-less RNA polymerase recruits GroEL for its functional activity (Mukherjee *et al* 1999).

In this report, we tried to characterize the cause for inhibition of transcription and also to identify, if any, the difference in conformation of the *w*-less holoenzyme from the wild-type counterpart. The affinity of the two enzymes towards the template DNA has been qualitatively probed by gel retardation assays. We have used $KMnO_4$ as a probe to detect promoter DNA melting during transcription, and to verify whether the affinity or promoter melting is affected on addition of *w*. Efforts have been made to check for differences, if any in core enzyme – s $^{70}$ interaction of the mutant enzyme through protein foot-printing.

## 2. Materials and methods

### 2.1 *Protein purification*

Wild-type RNA polymerase, *w*-less RNA polymerase and w -factor were purified and estimated as described previously (Mukherjee and Chatterji 1997). Purification of s $^{70}$ tagged with a heart muscle kinase (HMK) domain, SARRASVA at the C-terminus is described in a subsequent section.

### 2.2 *End-labelling of DNA template*

A 185 bp *Hin*dIII–*Bam*HI fragment containing the *lacUV5* promoter was amplified by PCR using end labelled 5¢ -CCGCTGGCACGACAGGTTTC-3¢ as the primer for the non-template (top) strand and 5¢ -AGCTTGGCGTAA-TCATGGTC-3¢ as the primer for the template (bottom) strand. The plasmid pLAC12 was used as template (kind gift of Prof. A Ishihama, NIG, Japan). End labelling the 5¢ -ends of both top and bottom strand primers were carried out using T4 polynucleotide kinase (NEB) in the presence of [g -P$^{32}$]ATP. Subsequently the primers were ethanol precipitated using 20 m g/ml glycogen as carrier. The end-labelled primers were then used for polymerase chain reaction (PCR). Following purification of the PCR fragment by 8% polyacrylamide gel electrophoresis (PAGE), it was used for gel retardation assays and foot-printing analysis.

### 2.3 *$KMnO_4$ foot-printing*

$KMnO_4$ analysis was carried out as described (O'Halloran *et al* 1989) with a few minor modifications. Briefly, 5¢-labelled-template DNA-RNA polymerase complexes were formed in 40 m l RNA polymerase binding buffer (50 mM Tris HCl, pH 8·0, 10 mM $MgCl_2$, 100 mM KCl, 1 mM DTT, 10 m g/ml BSA) by incubating at 37°C for 10 min. Typically 200 nM wild-type and 860 nM *w*-less RNA polymerase was complexed along with 30 nM template DNA (~ 20,000 cpm). Purified was added at either 2-fold or 10-fold molar excess where needed (indicated in the figure legend). The complexes were treated with 5·75 m l of 80 mM $KMnO_4$ solution, mixed well and kept at 37°C for 2 min. The reaction was terminated with 15 m l of stop solution containing 3·5 M b -mercaptoethanol and 1·4 M sodium acetate. The solution was extracted with phenol and chloroform twice and ethanol precipitated using 20 m g/ml glycogen as carrier. The precipitated DNA was treated with piperidine (Maxam and Gilbert 1980). Cleavage products were analysed on 8% polyacrylamide (20 : 1), 8 M urea sequencing gels with Maxam and Gilbert (A + G) reaction of template DNA as molecular weight ladders.

### 2.4 *Mobility shift assays*

Binary complexes were formed by combination of a radioactively-labelled 185 bp *Hin*dIII–*Bam*HI fragment containing the *lacUV5* promoter fragment with RNA polymerase (wild-type or *w*-less) in the standard reaction buffer [10 mM Tris HCl (pH 8·0); 0·1 m M KCl; 1 mg/ml BSA; 10 mM $MgCl_2$; 0·1 mM DTT; 0·1 mM EDTA]. Typically, labelled promoter fragment (~ 50,000 cpm/ lane) was used with 0·05 and 0·1 m M wild-type RNA polymerase, 1 and 2 m M *w*-less RNA polymerase. The complex formation was allowed at 37°C for 10 min and then the samples were added to preincubated dye buffer (10 × : 60% sucrose, bromophenol blue, xylene cyanol). The samples were then loaded directly onto a running 4% polyacrylamide (40 : 1 acrylamide/bis) TBE (89 mM Tris borate; 2·5 mM EDTA, pH 8·4) gel at 4°C. Gels were pre-run for at least 1 h (250 V) prior to the application of the sample.

### 2.5 *Protein foot-printing studies*

*s* $^{70}$-tagged with a HMK domain, SARRASVA at the C-terminus was purified from the overproducing strain harboring pNH100 (Nagai and Shimamoto 1998). To label s $^{70}$ using HMK, each reaction mixture (50 m l) contained 20 mM Tris HCl pH 8·0, 100 mM NaCl, 12 mM $MgCl_2$, 1 mM DTT, 0·025% Tween 20, 25 m Ci [g -$P^{32}$]ATP (3000 Ci/mmol; NEN), 5 m g of purified tagged s $^{70}$ and 10 U of reconstituted HMK (sigma P2645). The mixture was incubated for 30 min at 25°C, then passed through a G50 spin column (Pharmacia) pre-equilibrated with TT buffer (50 mM Tris HCl pH 7·9, 100 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 0·025% Tween-20). Labelled s $^{70}$ was used immediately for further experiments. To prepare a marker, labelled s $^{70}$ (final 25 m g/ml) was incubated with 0·5–50 mM CNBr in 70% formic acid for several hours at room temperature. Formic acid and CNBr were removed by evaporation.

For protein foot-printing, reaction mixtures (20 m l) contained 2 pmol of labelled s $^{70}$, 50 mM Tris HCl pH 7·9, 0·1 mM EDTA, 10 mM $MgCl_2$, 1 mM DTT, 0·025% Tween-20, 2 m g of partially hydrolyzed casein, 20 mM Fe-

EDTA complex, 5 mM ascorbic acid and 5 mM hydrogen peroxide. For foot-printing of wild-type holoenzyme, 2 pmol of labelled s $^{70}$ and 10 pmol of RNA polymerase core enzyme were pre-incubated for 5 min. While for *w*-less core enzyme, 2 pmol of labelled s $^{70}$ was incubated with 5–50 pmol of *w*-less core enzyme. The reaction was started by adding Fe-EDTA, ascorbic acid and hydrogen peroxide simultaneously to the mixtures and incubated for 2 min at 37°C, then stopped by adding an equal volume of 2 × sample buffer (125 mM Tris HCl pH 6·8, 4% SDS, 10% b -mercaptoethanol, 10% glycerol, trace amounts of bromophenol blue). In the conditions used here, more than 90% of s $^{70}$ remained uncleaved. The resulting samples were subjected to 10% Tris-glycine SDS-PAGE and 16·5% Tris-tricine SDS-PAGE (Schagger and von Jagow 1987). The gels were dried and analysed with a BAS 2000 image analyser (Fuji Film, Tokyo).

### 2.6 *Circular dichroism measurements*

All circular dichroism (CD) measurements were performed at room temperature (23 ± 1°C) with a JASCO model J715 spectropolarimeter at a scan rate of 50 nm/min and 0·1 nm resolution using a cuvette with a path length of 1 mm (200 m l). Most spectra were recorded as an average of 5 scans. Appropriate buffer base line spectra were subtracted from the protein spectra. The different pro-teins were diluted with a buffer containing 10 mM Tris HCl, pH 8·0, 5% glycerol, 0·1 mM EDTA, 0·1 mM DTT, 0·1 M KCl to a final concentration of 250 m g/ml.

## 3. Results

### 3.1 *The w-less holoenzyme has altered affinity towards DNA template*

During the purification of the *w*-less holoenzyme from the *rpoZ* null mutant, we had observed that binding of the *w*-less holoenzyme to heparin-Sepharose and DNA cellulose was inefficient and negligible indicating an altered DNA binding of the *w*-less holoenzyme. To explore this observation further a mobility shift assay was performed using [$^{32}$P]-labelled promoter fragment. As shown in figure 1, lanes 2 and 3, the *w*-less holoenzyme had markedly less affinity for the DNA template as compared to the wild-type holoenzyme (lanes 4 and 5). Even at 20-fold higher concentration, the mutant enzyme did not saturate the promoter. In addition, the formation of multiple complexes were noticed in the case of *w*-less RNA polymerase. We had also reported earlier that the *w*-less holoenzyme was always around 50% as active as the wild-type holoenzyme. The lesser activity could possibly be explained by the lesser affinity towards the template, which might be due to gross structural changes in the enzyme brought about by the absence of the w subunit.

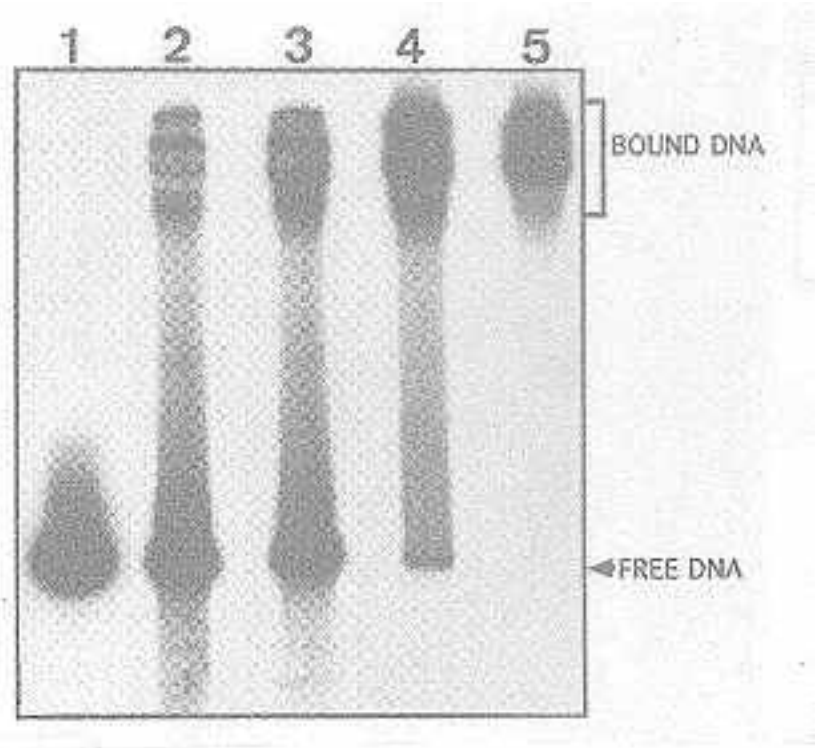


**Figure 1.** Mobility shift of *lacUV5* promoter DNA by *E. coli* RNA polymerase: ω-less and wild-type. Lane 1, Free *lacUV5* promoter DNA; Lanes 2–5, *lac UV5* promoter DNA with 1 μM and 2 μM ω-less (lanes 2 and 3) and 0·05 μM and 0·1 μM wild type (lanes 4 and 5) RNA polymerase.

### 3.2 *$KMnO_4$ foot-printing of RNA polymerase in the presence and absence of w at the lacUV5 promoter*

In our earlier studies we had observed that extrinsically added *w* led to inhibition of both general and promoter-specific transcription (Mukherjee and Chatterji 1997). It was a curious observation in the light of the fact that this inhibition of transcription takes place inspite of w being a subunit of the transcription complex. To determine the cause for inhibition of transcription on addition of w , we carried out $KMnO_4$ foot-printing of RNA polymerase, for both the wild-type and the *w*-less RNA polymerase, in
the presence of different ratio's of RNA polymerase: *w* at the *lacUV5* promoter (a 185 base pair *Hin*dIII–*Bam*H1 fragment, see § 2). Figure 2, panels A and B show the $KMnO_4$ foot-printing on the top and bottom strand respectively. On addition of *w*, the open complex formation is dramatically reduced in case of the *w*-less enzyme (lanes 7 and 8, both panels). The effect is less intense for the wild-type enzyme (lanes 3 and 4, both panels). Addition of *w* alone (lane 5, both panels) where *w* is added in the same concentration as in lane 4 but without RNA polymerase, did not show any sensitivity at – 10/+ 1 region but prominent hypersensitive sites are evident around – 50 and – 90, which were also observed in the presence of RNA polymerase. At higher concentration of w alone (as in lane 9), the presence of higher concentration of glycerol might have lead to a quenching of the $KMnO_4$ reaction. The difference in open complex formation between the wild-type and the *w*-less RNA polymerase was evident from lanes 2 and 6 (both panels).

### 3.3 *Protein foot-printing with HMK-tagged $\sigma^{70}$ and the w-less core enzyme*

Lesser sensitivity to $KMnO_4$ of the *w*-less RNA polymerase-promoter DNA complex prompted us to ask whether there was difference in interaction between the
*w*-less core enzyme and s factor. In a binding assay (data not shown) based on the separation of the core enzyme-s complex (holoenzyme) and free s by native PAGE, we observed difference in affinity between *w*-less core to $s^{70}$ as compared to wild-type core to $s^{70}$. This was further confirmed by protein foot-printing assay of $s^{70}$ as shown below.

The cleavage profile of $s^{70}$ in free form show several segments which are more sensitive to hydroxy radical-mediated cleavage than other portions (Nagai and Shimamoto 1998). Sigma-70 is known to have four functional domains and intra domain interaction is necessary for core RNA polymerase binding and subsequent recognition of promoter (Helman and Chamberlin 1988; Gopal *et al* 1994). Upon binding to core enzyme, certain segments in domain 1·1, domain 2, domain 3 and domain 4 are protected. On comparing the radical-mediated cleavage profile of $s^{70}$ when bound to increasing amounts of the *w*-less core to that of a wild-type core-$s^{70}$ interaction, we found that (figure 3b) a segment which maps near the turn of a putative helix-turn-helix motif in region 4·2 (– 35 recognition domain) of $s^{70}$ (Stragier *et al* 1985; Gribskov and Burgess 1986) became highly sensitive upon binding to the *w*-less core enzyme in a concentration dependent manner. On the other hand, upon comparing lanes F and G in both panels, one can see that the intensity of the clevage of regions 2·2 and 3·1 or 3·2 of $s^{70}$ is more in the case of the *w*-less core -$s^{70}$ interaction in comparision to the wild-type situation. To rule out the possibility of high protein concentration causing ambiguity in interpretation of results, a control reaction was carried out where casein was added to same final concentration as that of the highest concentration of the *w*-less core enzyme.

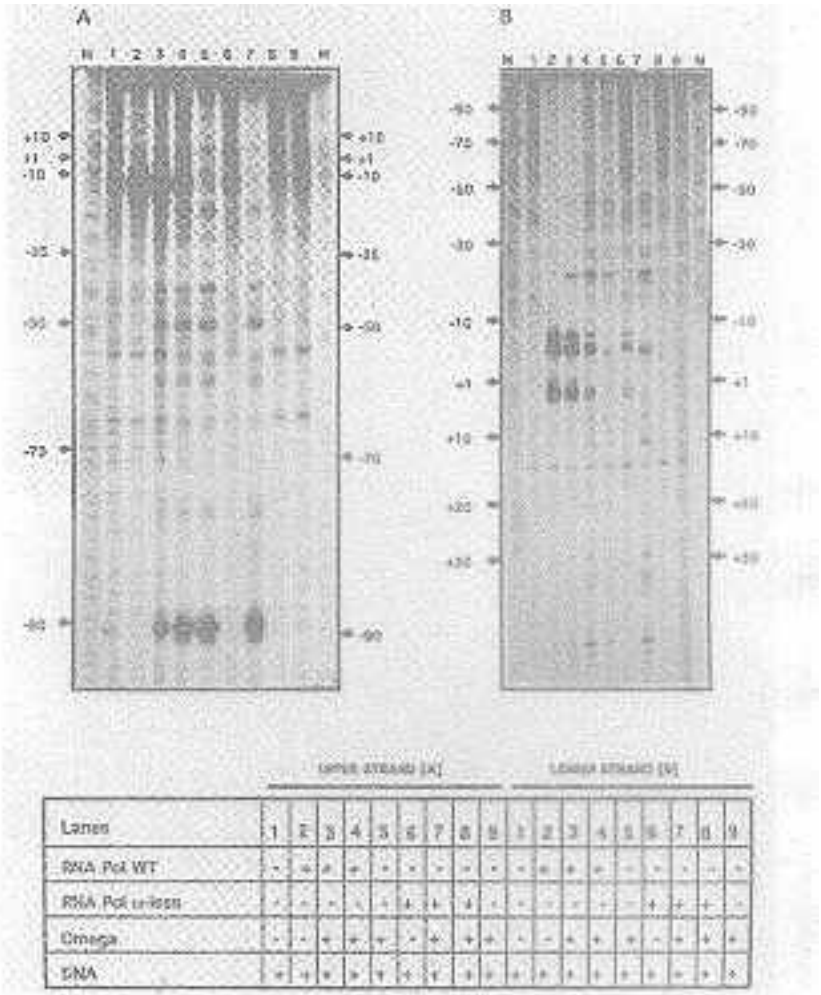


**Figure 2.** *In vitro* $KMnO_4$ foot-printing at the *lacUV5* promoter in the presence of wild-type and ω-less RNA polymerase with or without supplementation of ω. (A) $KMnO_4$ foot-printing of the upper strand. (B) $KMnO_4$ foot-printing of the lower strand. Lane 1, Free DNA; lanes 2 and 6, RNA polymerase alone; lanes 3 and 7, RNA polymerase : ω :: 1 : 2; lanes 4 and 8, RNA polymerase : ω :: 1 : 10; lanes 5 and 9, ω alone (same concentration as in lanes 4 and 8). Lanes 2, 3 and 4 are with wild-type RNA polymerase. Lanes 6, 7 and 8 are with ω-less RNA polymerase. The same order is maintained in both the panels. Lane M, Maxam–Gilbert A + G ladder of respective strands. The concentrations are as given under Experimental Procedure. The ratios are with respect to molar concentrations.

### 3.4 *CD studies of holo and w-less E.coli RNA polymerase in the presence and absence of w subunit*

Probably, the best way to study the gross change in conformation of a protein molecule is through CD measurements. Figure 4 shows CD spectral analysis of the overall conformation of core and holo RNA polymerase purified from both wild-type and *w*-less *E. coli* strains. The profile of the CD spectra were found to be similar to those reported earlier (Ishihama *et al* 1979; Woody *et al* 1987). However, it was reported earlier (Severinova *et al* 1996) that upon addition of $s^{70}$ to core RNA polymerase, noticeable change in conformation of core RNA polymerase takes place as also shown in figure 4. Interestingly, *w*-less core and holo do not show much difference in their CD profile indicating very weak interaction between core RNA polymerase (*w*-less) and $s^{70}$ as also concluded from protein foot-printing experiments.

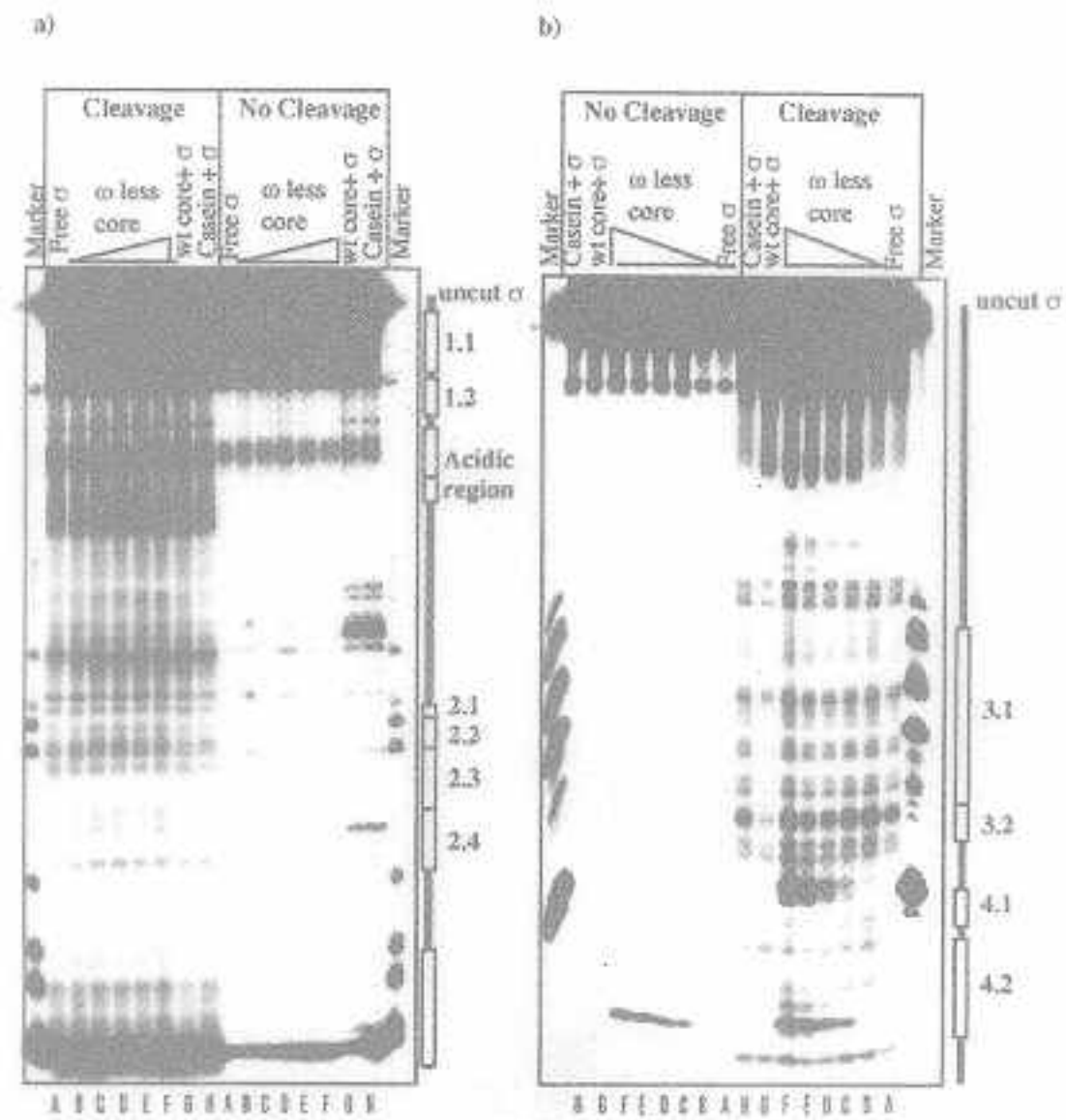


**Figure 3** Hydroxyl radical generated protein foot-print of HMK-tagged $\sigma^{70}$ subunit with ω-less core RNA polymerase. (a) Analysis by 10% Tris-glycine SDS-PAGE. (b) Analysis by 16·5% Tris-tricine SDS-PAGE. Marker lane, $\sigma^{70}$ partially cleaved by CNBr. Lanes A and B, $\sigma^{70}$ alone; Lanes C to F, $\sigma^{70}$ with increasing concentration of ω-less core (see text); lane G, $\sigma^{70}$ with wild-type core; lane H, $\sigma^{70}$ with casein (control). Lanes A–H under "no cleavage" are controls free from radical challenge (both the gels). Same order and composition as in lanes A–H under 'cleavage'.

## 4. Discussion

Despite considerable biochemical and genetic studies on all the constituent subunits of RNA polymerase, the available literature on the *w* subunit as regards to its structure and function is rare. The *w* subunit is not required for the function of the transcriptional apparatus both *in vivo* and *in vitro*. Strain lacking w , that is, a *rpoZ* null mutant is viable, suggesting a non-essential nature of the protein or that there might also be a redundancy in function. The only known phenotype ascribed to the *rpoZ* null mutant is a slower growth time (Mukherjee *et al* 1999).

Recent studies by Dove and Hochschild (1998) pro-vide the first evidence that the *w* protein is associ-ated with RNA polymerase holoenzyme *in vivo* and that
it is accessible at the surface of the enzyme complex. Their experiments further suggest that the amino ter-minal portion of the *w* protein mediate its association with RNA polymerase, since fusion of another protein at the amino terminus abrogates the association with RNA polymerase.

Our previous studies with *w* had given us sufficient reason to believe that *w* might have more of a structural role and it might be necessary for the overall stability of the enzyme. The results presented in this study is an attempt to characterize the changes in RNA polymerase conformation associated with the absence of *w*. Through the gel retardation experiments, we could demonstrate the alteration in interaction between *w*-less holoenzyme and template. $KMnO_4$ foot-printing studies, as well as the time course of reconstitution followed through $KMnO_4$ footprinting indicates that the absence of *w* causes structural alterations which lead to altered promoter recognition and melting. Addition of *w*, destabilizes the open complex both for wild-type and *w*-less enzyme, and *w* protein alone gives rise to hypersensitive sites upstream of the promoter (around – 50 and – 90). When w is present as for the wild-type enzyme, it does not influence any change in the foot-print, indicating that once it finds its proper environment, its inhibitory action is not seen.

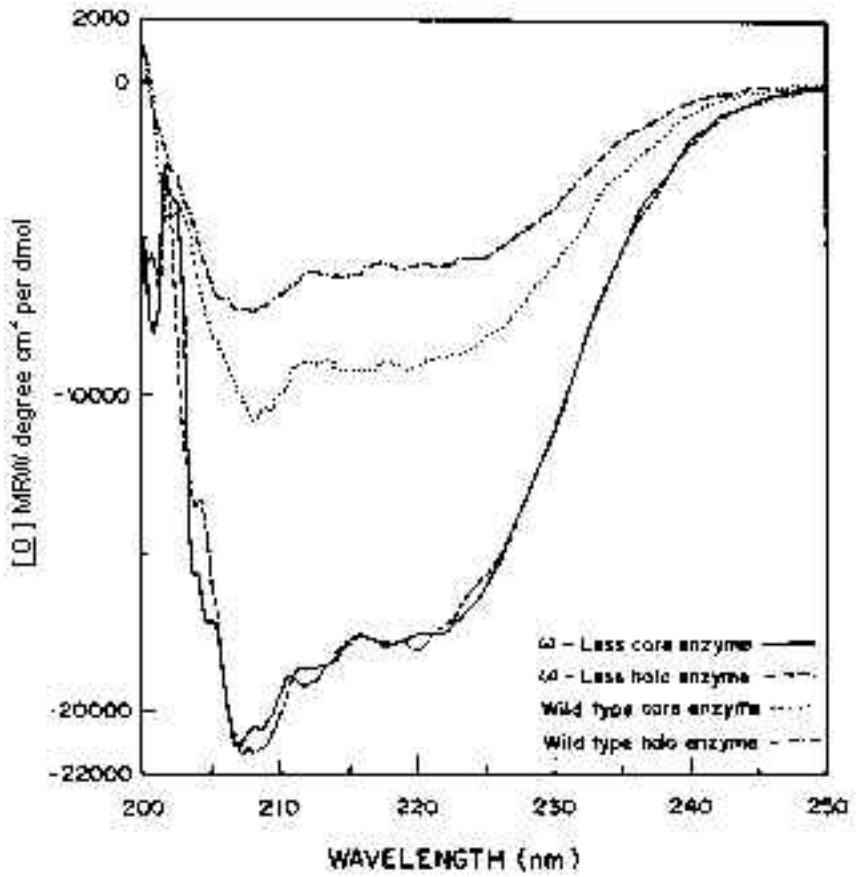


**Figure 4.** CD spectra of core RNAP and holoenzymes from the wild-type and the ω-less strains. CD spectra of the ω-less and the wild-type core RNAP and holoenzymes were measured as described in § 2. MRW, Mean residue weight; dmol, decimol.

*LacUV5* has two promoters P1 and P2, where P2 lies upstream of P1. P2 is much weaker in comparison to P1 and the latter defines the – 10 and – 35 boxes of *LacUV5*. Interestingly, as it is more evident in figure 3b, lanes

3, 4 and 7, addition of *w*, helps in the recognition of P2 promoter of *LacUV5* whose start site is around – 22 (Spassky *et al* 1984) and reactivity at around – 34 reflect weak melting near the – 10 region of the weak P2 promoter which can be active in the absence of cAMP–CRP (Meiklejohn and Gralla 1985). External w might be causing an alteration in recognition of the promoter by the RNA polymerase or decreasing its affinity for P1. We have observed a strong hypersensitive site at – 90 on addition of w . In the *lac* promoter region there are operators O1 and O3 at + 1 to + 21 and – 72 to – 92 respectively. It is known that when *lac* repressor binds to such sequences the region in between shows hypersensitivity at – 29/– 25 region (Borowiec *et al* 1987). We could possibly speculate about the development of a bent repression loop which could prevent the initial stages of recognition by keeping a large section of DNA condensed and distorted.

The protein foot-printing results corroborate our findings of alteration in the structure of the *w*-less RNA polymerase. The decreased and altered interaction with $s^{70}$ could possibly explain the lesser activity of this mutant enzyme and the slow growth phenotype of *rpoZ* deleted strain (Mukherjee *et al* 1999). The hypersensitive site in the – 35 recognition domain indicate an alteration in the conformation of the 4·2 domain of $s^{70}$ when associated with *w*-less core, which can explain the lesser promoter melting in $KMnO_4$ foot-printing studies. However, CD results show only the absence of any major interaction between *w*-less core and $s^{70}$; intricate alteration of 4·2 domain of $s^{70}$ upon association with *w*-less core RNA polymerase cannot be detected with such measurements.

We have recently shown that the *w*-less core RNA polymerase recruits GroEL during purification and any attempt to remove GroEL resulted in irreversible damage to the enzyme (Mukherjee *et al* 1999). Thus, it appears that functionally *w* acts as a chaperonin for the folding of RNA polymerase. Crystal structure analysis of homologous *Thermus aquaticus* core RNA polymerase at 3·3 Å resolution supports this observation (Zhang *et al* 1999). It would be interesting to determine whether the N-terminal domain of *w* controls its GroEL-like function.

## References

Borowiec J A, Zhang L, Sasse-Dwight S and Gralla J D 1987 DNA Supercoiling promotes formation of a Bent Repression Loop; *J. Mol. Biol.* **196** 101–111

Burgess R R and Travers A A 1970 *Escherichia coli* RNA polymerase: purification, subunit structure, and factro requirements; *Fed. Proc.* **29** 1164–1169

Burgess R R, Travers A A, Dunn J J and Bautz E K F 1969 Factor stimulating transcription of RNA polymerase; *Nature (London)* **221** 43–46

Dove S L and Hochschild A 1998 Conversion of the w subunit of *Escherichia coli* RNA polymerase into a transcriptional activator or an activation target; *Genes Dev.* **12** 745–754

Gentry D R 1990 Ph.D. dissertation, University of Wisconsin, Madison, USA

Gentry D R and Burgess R R 1986 The cloning and sequence of the gene encoding the omega subunit of *Escherichia coli* RNA polymerase; *Gene* **48** 33–40

Gentry D R and Burgess R R 1989 *rpoZ*, encoding the omega subunit of *Escherichia coli* RNA polymerase is in the

same operon as *spoT*; *J. Bacteriol.* **171** 1271–1277

Gentry D R and Burgess R R 1993 Cross-linking of *Escherichia coli* RNA polymerase subunits: identification of b ¢ as the binding site of w ; *Biochemistry* **32** 11224–11227

Gentry D R, Xiao H, Burgess R R and Cashel M 1991 The omega subunit of *Escherichia coli* K-12 RNA polymrase is not required for stringent RNA control *in vivo*; *J. Bacteriol.* **173** 3901–3903

Gopal V, Ma H W, Kumaran M K and Chatterji D 1994 A point mutation at the junction of domain 2·3/2·4 of transcription factor sigma-70 abrogates productive transcription and restores it expected mobility on a denaturing gel; *J. Mol. Biol.* **242** 9–22

Gribskov M and Burgess R R 1986 Sigma factors of *E. coli*, *B. subtilis*, phage SP01 and phage T4 are homologous proteins; *Nucleic Acids Res.* **14** 6745–6763

Helmann J D and Chamberlin M 1988 Structure and function
of bacterial sigma factors; *Annu. Rev. Biochem.* **57** 839–872

Igarashi K, Fujita N and Ishihama A 1989 Promoter selectivity of *E coli* RNA polymerase: omega factor is responsible for the ppGpp sensitivity; *Nucleic Acids Res.* **17** 8755–8765

Ishihama A, Aiba H, Saitoh T and Takahashi S 1979 Subunits of RNA polymerase in function and Structure. 7. Structure of Premature core enzyme; *Biochemistry* **18** 972–978

Maxam A M and Gilbert W 1980 Sequencing end-labelled DNA with base-specific chemical cleavages; *Methods Enzymol.* **65** 499–560

Meiklejohn A L and Gralla J D 1987 Entry of RNA polymerase at the *lac* promoter; *Cell* **43** 769–776

Mukherjee K and Chatterji D 1997 Studies on the w -subunit of *E. coli* RNA polymerase: Its role in the recovery of denatured enzyme activity; *Eur. J. Biochem.* **247** 884–889

Mukherjee K, Nagai H, Shimamoto N and Chatterji D 1999 GroEL is involved in activation of *E. coli* RNA polymerase devoid of omega subunit *in vivo*; *Eur. J. Biochem.* **266** 1–9

Nagai H and Shimamoto N 1998 Most conserved regions of the *Escherichia coli* primary sigma factor are involved in the interaction with RNA polymerase core enzyme; *Genes Cells* **2** 725–734

O'Halloran T V, Frantz B, Shin M K, Ralston D M and Wright J G 1989 The MerR heavy metal receptor mediates positive activation in a topologically novel transcription complex; *Cell* **56** 119–129

Sarubbi E, Rudd K, Xiao H, Ikehara K, Kalman M and Cashel M 1989 Characterization of the spoT gene of *E. coli*; *J. Biol. Chem.* **264** 15074–15082

Schagger H and von Jagow G 1987 Tricine sodium dodecyl sulphate polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa; *Anal. Biochem.* **166** 368–379

Severinova E, Severinov K, Kenyo D, Marr M, Brody E N, Roberts J W, Chait B T and Darst S A 1996 Domain Organization of the *E coli* RNA polymerase s $^{70}$ subunit; *J. Mol. Biol.* **263** 637–647

Spassky A, Busby S and Buc H 1984 On the action of the cyclic AMP–cyclic AMP receptor protein complex at the *Escherichia coli* lactose and galactose promoter regions; *EMBO J.* **3** 43–50

Stragier P, Parsot C and Bouvier J 1985 Two functional domains conserved in major and alternate bacterial sigma factors; *FEBS Lett.* **185** 11–15

Woody A Y M, Woody W W and Mlcolm A D B 1987 Effects of ppGpp on transcription by DNA-dependent RNA polymerase from *E coli*: circular dichroism, absorption and specific transcription studies; *Biochim. Biophys. Acta* **909** 115–125

Zhang G, Compbell E A, Minakhin L, Richter C, Severinor K and Darst S A 1999 Crystal structure of *Thermus aquaticus* core RNA polymerase at 3·3 Å resolution; *Cell* **98** 811–824

Zillig W, Palm P and Heil A 1997 Function and reassembly of subunits of DNA-dependent RNA polymerase; in *RNA polymerase* (eds) R Losick and M Chamberlin (New York: Cold Spring Harbor) pp 101–125

*MS received 26 August 1999; accepted 16 November 1999*

Corresponding editor: Deepak Bastia