GroEL is involved in activation of *Escherichia coli* RNA polymerase devoid of the ω subunit *in vivo*

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Highly purified *Escherichia coli* RNA polymerase contains a small subunit termed ω that has a molecular mass of 10 105 Da and is comprised of 91 amino acids. *E. coli* strains lacking ω (ω -less) are viable, but exhibit a slow-growth phenotype. Renaturation of RNA polymerase isolated from an ω -less mutant, in the presence of ω , resulted in maximum recovery of activity. The ω -less RNA polymerase from ω -less strains recruits the chaperonin, GroEL (unlike the wild-type enzyme), suggesting a structural deformity of the mutant enzyme. The GroEL-containing core RNA polymerase interacts efficiently with σ^{70} to generate the fully functional holoenzyme. However, when GroEL was removed, the enzyme was irreversibly nonfunctional and was unable to bind to σ^{70} . The damaged enzyme regained activity after going through a cycle of denaturation and reconstitution in the presence of ω or GroEL. GroES was found to have an inhibitory effect on the core- σ^{70} association unlike the ω subunit. The ω subunit may therefore be needed for stabilization of the structure of RNA polymerase.

Keywords: activation; GroEL; renaturation; RNA polymerase (ω -less); σ^{70} interaction.

Escherichia coli RNA polymerase is a complex enzyme containing multiple subunits. The core enzyme contains four subunits α , β , β' and ω (stoichiometry 2 : 1 : 1 : 1) [1] and is responsible for RNA synthesis. The RNA polymerase core enzyme initiates transcription at specific promoter sites, with the help of a σ factor which binds to the core enzyme [2]. A number of σ factors exist in *E. coli*, each recognizing specific promoter sequences, the major one being σ^{70} (molecular mass 70 kDa). The ω protein (molecular mass 10 105 Da) is an often ignored polypeptide present in highly purified preparations of *E. coli* RNA polymerase [1]. It is a conserved polypeptide in a number of enteric bacteria, including *Serratia marcescens*, *Bacillus subtilis* and *Proteus vulgaris* [3]. Despite its abundance, data on its structure and function are lacking in the literature. It has been difficult to ascribe a function to this factor principally because the ω -less RNA polymerase is active and completely functional *in vitro* at all the promoters tested, and strains with *rpoZ* (gene encoding ω) deleted are viable, which suggests *in vivo* functionality of the ω -less enzyme [4,5]. It is thus likely that ω is required for some unknown or redundant regulatory function or catalytic activity overlooked by *in vitro* assays and not immediately obvious from the behaviour of the mutants.

In order to ascribe a physiological function to ω , we used an *in vitro* transcription and reconstitution system involving the ω -less RNA polymerase, purified from an *rpoZ* null mutant to which purified ω factor was added extrinsically. Our previous studies have shown that externally added ω inhibited promoter-specific transcription at all promoters tested [6]. Although it appears that the ω subunit may have a functional role in inhibiting transcription, we think that its close proximity to the β' subunit [7] perhaps resulted in non-specific blockade of

template binding by the enzyme in the presence of an excess of externally added ω . However, the importance of the ω subunit in *E. coli* RNA polymerase was indicated when maximum renaturation of the fully denatured ω -less enzyme was found in the presence of ω [6].

During purification of the ω -less RNA polymerase holoenzyme from cell lysates, we observed a major protein of molecular mass $\approx$ 60 kDa that copurifies with the enzyme. This protein was also present in significantly lesser amounts in the wild-type RNA polymerase preparations and could be removed by passage through a heparin–Sepharose column [8]. Interestingly, the ω -less RNA polymerase did not bind to heparin–Sepharose during the purification process, and the presence of a large excess of this contaminating 60-kDa protein rendered its removal difficult. However, with the use of more stringent purification conditions, this contaminant was ultimately removed, resulting in a completely inactive RNA polymerase from the ω -less strains. In this work, we have identified this 60-kDa protein through N-terminal sequencing as GroEL.

Chaperonin 60 (Cpn60 or GroEL) and its functional partner cochaperonin 10 (Cpn10 or GroES) co-operate to assist the folding and assembly of other proteins [9–11]. The GroE proteins play a major role in *E. coli* growth at all temperatures, because neither the *groES* nor the *groEL* gene can be deleted from *E. coli* [12]. Genetic and biochemical evidence has demonstrated that the GroES and GroEL proteins interact both *in vivo* [13] and *in vitro* [14].

Association of GroEL with RNA polymerase has been previously reported [15,16], the former constituting 0.4–8% of total cellular protein and the latter 0.5–2%. The copurification of the two proteins raised the possibility that they might be similar with regard to charge and size, at least under the conditions of purification, or might otherwise be associated in a complex. Later work by Ziemienowicz and co-workers [17] showed that the GroEL chaperone protein can protect purified *E. coli* RNA polymerase holoenzyme from heat inactivation.

Table 1. Bacterial strains used.

Strain	Genotype	Source
MG1655	F ⁻	C. Gross ^a
CF2790	W3110, <i>rpoZ::cat</i>	D. R. Gentry ^b
CAG18492	MG1655, <i>zic-4901::Tn10</i>	C. Gross ^a
KM1	MG1655, <i>rpoZ::cat</i>	This work, MG1655XP1 (CF2790) selection for <i>cm^R</i>

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The GroES protein is not essential in this protection reaction, but its presence reduces the amount of GroEL required.

In the present study the enhancement of renaturation of the ω -less enzyme in the presence of ω has been studied in the light of excess association of GroEL with the mutant enzyme. The dependence of the ω -less RNA polymerase on GroEL association for its activity has also been analyzed. Our results indicate that the presence of either ω or GroEL is necessary in core RNA polymerase for efficient reconstitution with the σ^{70} subunit.

EXPERIMENTAL PROCEDURES

Bacterial strains, growth conditions and bacteriological techniques

Table 1 lists the sources, genotypes and derivatives of strains used. P1 transductions were performed as described by Miller [18]. For determination of doubling times, cells were grown in Luria–Bertani medium. Cell density was determined by measuring A_{590} . When appropriate, antibiotics were added to the following concentrations: chloramphenicol, 20 $\mu\text{g}\cdot\text{mL}^{-1}$, and tetracycline, 15 $\mu\text{g}\cdot\text{mL}^{-1}$.

Protein purification

The wild-type holo RNA polymerase, ω -less core holo RNA polymerase, and ω factor were purified as described previously [6]. ω -less core and holo RNA polymerase were purified from an *rpoZ*-deleted *E. coli* strain essentially following the same protocol as for the wild-type enzyme up to Mono Q column (Pharmacia) chromatography. Thereafter, as the mutant enzyme did not bind to heparin–Sephacrose unlike wild-type RNA polymerase, Red–Sephacrose column chromatography was carried out, which separated GroEL from RNA polymerase.

The *E. coli* chaperonins GroEL and GroES were purified from lysates of cells bearing the multicopy plasmid pGroESL [19,20]. The purifications were carried out according to a previously published protocol [17]. The protomer concentrations of GroEL and GroES were measured at 280 nm with absorption coefficients of 2.38×10^4 and $3.44 \times 10^3 \text{ M}^{-1}\cdot\text{cm}^{-1}$, respectively.

Reconstitution of core RNA polymerase

RNA polymerase purified from the *rpoZ* null mutant was reconstituted with various amounts of ω and/or GroEL as described by Igarashi & Ishihama [21]. Briefly, $\approx 1 \text{ mg}$ purified ω -less RNA polymerase was denatured by dialysis against denaturation buffer containing 6 M deionized urea in 50 mM Tris/HCl (pH 8.0 at 4 °C), 1 mM EDTA, 10 mM dithiothreitol, 10 mM MgCl_2 , 0.2 M KCl and 20% glycerol;

an aliquot was removed. The rest of the enzyme was divided into four: in the first portion, there was no supplementation of ω or GroEL; to the second was added a 10-fold molar excess of ω ; to the third was added a 10-fold molar excess of ω and a fivefold molar excess of GroEL; and to the last portion was added a fivefold molar excess of GroEL. After being kept on ice for 30 min, dialysis was carried out at 4 °C against reconstitution buffer containing 50 mM Tris/HCl (pH 8.0 at 4 °C), 0.1 mM EDTA, 1 mM dithiothreitol, 10 mM MgCl_2 , 0.3 M KCl and 20% glycerol [21]. Aliquots removed at different time points were used for multiple-round transcription assays using calf thymus DNA as template as described by Lowe *et al.* [22].

Protein mobility-shift assays

Holoenzymes were allowed to form between the ω -less core RNA polymerase (purified by passage through Red–Sephacrose and devoid of GroEL) and σ^{70} by combining them in a buffer containing 50 mM Tris/HCl, pH 8.0, 0.1 M KCl, 10 mM MgCl_2 , 1 mM dithiothreitol and 0.1 mM EDTA. Typically, 300 nM ω -less core RNA polymerase was used with 350 nM σ^{70} . The wild-type holoenzyme was used as control at 320 nM. Increasing amounts of purified GroEL were added at concentrations varying from 380 nM to 1.9 μM . Where indicated in figure legends, purified ω was added to a final concentration of 1.6 μM ($\approx$ fivefold excess over RNA polymerase concentration). Complex formation was allowed at 37 °C for 30 min. The mixture was then loaded directly on a 4% polyacrylamide gel (40 : 1, acrylamide/bis) and run with 40 mM Tris/borate/1 mM EDTA, pH 8.3, at room temperature for at least 1 h (20 mA). The gel was then silver stained [23].

Similar experiments were carried out with the ω -less core RNA polymerase which was still associated with GroEL, i.e. after the Mono Q column but before passage through Red–Sephacrose. The final concentration of this protein complex was 300 nM. During the retardation assay in the presence of σ^{70} , purified ω and GroEL were added as before. The effect of adding GroES was also followed, with the final concentration of the protein being 750 nM. Denaturation of the ω -less RNA polymerase by dialysis against 6 M urea-containing buffer, followed by reconstitution with ω and removal of the denaturant, was carried out as described above. Thereafter, the interaction of the ω -reconstituted ω -less core RNA polymerase with σ^{70} was also examined in a similar fashion. Single-round *in vitro* transcription at the *lac UV5* promoter was carried out as previously described [24].

RESULTS

Mutation in *rpoZ* reduces the growth rate

Disruption of *rpoZ* by a deletion accompanied by insertion of a chloramphenicol-resistant gene (*cat*) (Table 1) results in a slow-growth phenotype with an increase in generation time. We calculated from the growth curves with exact cell viability counts that the doubling time of the strains carrying the insertion mutation is 15 min longer (45 min) than that of isogenic *rpoZ*⁺ strains (30 min). Gentry & Burgess [4] reported that insertion of a kanamycin-resistant gene at the location of *rpoZ* resulted in a similar slow-growth phenotype, which they thought was due to a polar effect on a downstream *spoT* gene. However, they later observed that the insertion of a *cat* gene instead of kanamycin produced a much weaker polar effect and thus any phenotype associated with ω nulls are at the most due

Fig. 1. Comparison of growth of MG1655, CF2790, KM1, KM2, KM3 and KM4. Cultures of these strains were grown in Luria–Bertani medium and at various time points aliquots were removed and A_{590} was measured. (○) MG1655; (△) KM1; (●) CF2790; (□) KM3 [KM1(*tet^r*, *cm^r*)]; (■) KM4 [MG1655 (*tet^r*)] and KM2 [KM1 (*tet^r*, *cm^r*)].

to a weak secondary polar effect on the downstream genes [25]. They also observed that this growth defect could not be suppressed by supplying a plasmid containing only the *rpoZ*-bearing fragment. In this study, we worked with a strain with a *cat* insertion. In order to rule out the possibility that suppressors in the parent strain (CF2790) cause the mutant phenotype, the *cat* insertion was moved into the background of MG1655 by P1 transduction. The location of the insertion was verified by linkage analysis through P1 transduction. The growth phenotype of a stable transductant (KM1), the wild-type (MG1655) and the parent strain (CF2790) were checked. Figure 1 shows the growth curves of these strains. The growth rates of KM1

and CF2790 were comparable and less than that of the wild-type.

Further, to ensure that the growth defect could be reversed by reintroducing *rpoZ⁺*, P1 lysate of CAG18492 (which has a *tet^r* insertion linked to *rpoZ⁺*) was transduced into both MG1655 and KM1. Subsequently, after selecting for *tet^r* colonies, the *tet^r* colonies were scored for *cm^r*. The growth rates for the strains MG1655, KM2 [KM1 (*tet^r*, *cm^r*)], KM3 [KM1 (*tet^r*, *cm^r*)] and KM4 [MG1655 (*tet^r*)] were checked. As shown in Fig. 1, KM2 and KM4 had identical growth rates.

In the absence of ω , the ω -less RNA polymerase associates with a higher level of GroEL protein

During the purification of RNA polymerase from the ω -less strain, we had reported previously the association of a protein of approximate molecular mass 60 kDa [6]. As shown in lane 1 of Fig. 2A, the contaminant was obtained in the flow through of Red–Sephacel. Lane 2 of Fig. 2A shows the wash and lane 3, the 2 M NaCl eluate. As seen in lane 3, the ω -less RNA polymerase is mostly devoid of this contaminant. Lane 4 depicts the ω -less RNA polymerase obtained after Mono Q chromatography. This protein, which normally copurifies even with the wild-type holoenzyme but in lesser amounts, is separated after passage through heparin–Sephacel [8]. To identify this protein we carried out N-terminal sequencing after transfer to poly(vinylidene difluoride) membrane. The sequence thus obtained for the first 10 amino acids was AAKDVKFGND, which is identical with that of GroEL, a 60-kDa protein.

This led us to ask at which stage during the purification of holoenzyme from the ω -less strain of *E. coli* does the enrichment of GroEL occur. Figure 2B,C shows the purification profile of both wild-type and ω -less RNA polymerase. Upon scanning the gels in Fig. 2B,C and subsequent normalization of the background, we observed that in case of the ω -less enzyme there was about fourfold more GroEL associated with it after passage through the Bio–Gel column (first step of enrichment) (Fig. 2B, lane 5). We purified wild-type holoenzyme as a

Fig. 2. SDS/PAGE analysis of the ω -less RNA polymerase before and after passage through Red–Sephacel. (A) Lane 1, Red–Sephacel flow through; lane 2, Red–Sephacel wash; lane 3, Red–Sephacel eluate; lane 4, the ω -less RNA polymerase after Mono Q chromatography. (B,C) Purification profiles of the ω -less RNA polymerase and wild-type RNA polymerase, respectively. Lanes 1–10 in both cases represent polyethyleneimine P pellet, polymin P supernatant, 0.5 M NaCl wash of polymin P pellet, Bio–Gel A 1.5 m load, Mono Q load, Red–Sephacel load, Red–Sephacel flow through, Red–Sephacel wash, Red–Sephacel eluate and purified holoenzyme, respectively.

Fig. 3. Time course of reconstitution of ω -less RNA polymerase at 4 °C in the presence of ω and/or GroEL. Different sets of points show the reconstitution profile of ω -less RNA polymerase (1 mg·mL⁻¹, 0.4 nmol in 200 μ L) without any other protein addition (control) and in the presence of a 10-fold molar excess of ω (4 nmol), a fivefold molar excess of GroEL (1 nmol), a 10-fold molar excess of ω (4 nmol) + fivefold molar excess of GroEL (1 nmol) after 20, 40, 80, 120 and 300 min, respectively. The assays were multiple-round assays using calf thymus DNA as template. (■) Control; (△) + GroEL; (●) + ω ; (◇) + GroEL + ω . (B) Subunit composition of ω -less RNA polymerase reconstituted with ω after passage through a heparin–Sephacryl column.

marker in Fig. 2B,C, lane 10. However, on Western-blot analysis of whole-cell lysate with anti-GroEL IgG, we observed that the synthesis of GroEL was not affected by *rpoZ* disruption (data not shown).

Denaturation and reconstitution of the *E. coli* ω -less RNA polymerase in the presence of ω and/or GroEL

To investigate whether GroEL had any role to play in renaturation of the enzyme, we reconstituted denatured ω -less

RNA polymerase (2 μ M) in the presence of excess of ω (20 μ M) and/or excess of GroEL (10 μ M). The control reaction was performed in the absence of ω or GroEL addition. Core RNA polymerase from the ω -less strain of *E. coli* after a passage through the Mono Q column, i.e. containing GroEL, was assayed for its activity and this value was taken as 100% activity of the enzyme. We observed that when GroEL was removed from this enzyme by passage through Red–Sepharose, the activity was lost and could not be recovered after a denaturation–reconstitution cycle (Fig. 3A, control). As expected, core RNA polymerase purified from wild-type MG1655 can be eluted from the Red–Sepharose column without any loss of activity. However, on the addition of GroEL or ω during reconstitution of ω -less enzyme, around 40–50% of the original activity could be regained within 90 min. In the presence of both GroEL and ω , maximum reconstitution was observed, which was even more than the original activity of core RNA polymerase containing GroEL, but devoid of ω (Fig. 3A). Subsequently, when the reconstituted enzyme containing both ω and GroEL was eluted through the heparin–Sephacryl column, excess GroEL was removed (Fig. 3B). This is similar to what had been observed during purification of wild-type RNA polymerase; as ω is present with the enzyme, associated GroEL was removed easily when passed through heparin–Sephacryl. The molecular composition of this enzyme, as determined from the gel pattern, is $\alpha_2\beta\beta'\sigma\omega$. If ω protein was not added during reconstitution, GroEL remained tightly bound to the reconstituted RNA polymerase to sustain the regained activity.

The ω -less core RNA polymerase devoid of GroEL cannot associate with σ^{70}

In this study, so far we have reported that the ω -less RNA polymerase recruits more GroEL and this association is favourable for its activity. However, we observed that if GroEL is dissociated after passage through Red–Sepharose, the resulting RNA polymerase retains very little activity. Therefore, we asked whether association between core RNA polymerase and σ^{70} is also affected by the presence of GroEL or ω protein. The concentration of GroEL was varied such that, at maximum molar ratio between GroEL and substrate RNA polymerase, there was a sevenfold excess of chaperonin present in the system. This has to be so as the GroEL chaperonin forms a heptamer over which the substrate folds, with the help of GroES in the presence of ATP [10,11]. Native PAGE (4% gel) was used to record differential mobility between the holoenzyme and the core RNA polymerase. As molecular mass markers cannot be used for native gels, in every case we had run the same sample of native gel in SDS/PAGE to ensure its purity and subunit composition.

It can be seen from lanes 1 and 2 of Fig. 4A that the core RNA polymerase (ω -less) purified through a Red–Sepharose column to remove GroEL moves faster than the holoenzyme. In lane 1, a large excess of GroEL was added along with control wild-type holoenzyme to observe its mobility on native PAGE. It can be seen from Fig. 4A lanes 2–8 that, on incubating the ω -less core RNA polymerase with a fixed concentration of σ^{70} in the presence of various concentrations of GroEL, no trace of holoenzyme was reconstituted, indicating that the ω -less core RNA polymerase is incapable of recognizing σ^{70} . All three components of the protein-folding pathway, namely GroES, ω and ATP, were used in the incubation in various combinations (not shown), but core– σ^{70} association was not achieved.

Two interesting results were obtained from the next set of experiments shown in Fig. 4B. First, the ω -less core RNA

Fig. 4. Mobility-shift assays of *E. coli* core RNA polymerase and its variants on reconstitution with σ^{70} . (A) Lane 1, purified wild-type holoenzyme (6.4 pmol in 20 μ L) and purified GroEL (7.6 pmol) added in the same loading buffer whereas lane 2 represents ω -less core RNA polymerase (6 pmol) alone. Lanes 3–8 show ω -less core RNA polymerase (6 pmol) with σ^{70} (7 pmol) and 0, 7.6, 14.1, 22.8, 30 and 38 pmol GroEL, respectively. In addition, lanes 5–8 have 32 pmol ω -protein. (B) Lane 1, same as lane 1 of (A). Lane 2 represents GroEL-associated ω -less core RNA polymerase (6 pmol in 20 μ L) alone. Lanes 3–8 show GroEL-associated ω -less core RNA polymerase (6 pmol) with σ^{70} (7 pmol) and 0, 7.6, 14.1, 22.8, 30 and 38 pmol GroEL, respectively. In addition, lanes 5–8 have 32 pmol of ω -protein. (C) Lane 1, purified wild-type holoenzyme (6.4 pmol in 20 μ L); lane 2, GroEL-associated ω -less core RNA polymerase (6 pmol); lane 3, GroEL-associated ω -less core RNA polymerase (6 pmol) + σ^{70} (7 pmol); lane 4, GroEL-associated ω -less core RNA polymerase + σ^{70} + GroES (15 pmol); lane 5, GroEL-associated ω -less core RNA polymerase + σ^{70} + ω (15 pmol); lane 6, GroEL + GroES (15 pmol). (D) Mobility-shift assays with reconstituted ω -less core RNA polymerase and σ^{70} . Lane 1, purified wild-type holoenzyme (6.4 pmol); lane 2, ω -less core RNA polymerase (10 pmol); lane 3, GroEL-associated ω -less core RNA polymerase (6 pmol); lane 4, denatured and reconstituted ω -less core RNA polymerase (6 pmol) (in the presence of 10-fold excess ω); lane 5, denatured and reconstituted ω -less core RNA polymerase (6 pmol) (in the presence of a 10-fold excess of ω) + σ^{70} (7 pmol). Incubation volume in all cases was 20 μ L.

polymerase associated with GroEL had different mobility from the core enzyme that was devoid of GroEL. However, 4% PAGE could not differentiate between free GroEL and the core enzyme. Secondly, on incubating with σ^{70} in the presence of various combinations of GroEL and ω , a significant amount of the ω -less core RNA polymerase was converted into holoenzyme (Fig. 4B). In order to find out whether core RNA polymerase was still associated with GroEL after the native gel electrophoresis, the corresponding band was excised and a 13% denaturing gel was run after reconstituting with σ^{70} . It can be seen from Fig. 5A, lane 4 that the gel slice has the full complementation of all the subunits of core RNA polymerase along with GroEL. We will show later that this enzyme was functionally active.

To see whether the association of σ^{70} with the ω -less core RNA polymerase (associated with GroEL), was influenced in any way by the presence of GroES, which is known to assist the release of some proteins complexed with GroEL, we allowed σ^{70} to interact with the core in the presence of GroES and also in the presence of ω . Figure 4C depicts the result. When only σ^{70} was added as in lane 3, to the GroEL-associated ω -less core RNA polymerase, a band corresponding to the holoenzyme was

observed. However, when GroES was added into the mix, the formation of complex appeared to be less (lane 4) than when ω was added (lane 5), when higher amounts of complex were seen.

As we had previously observed that the ω -less RNA polymerase renatured to higher activity when denatured and reconstituted in the presence of ω [6], an experiment was conducted to check if the σ^{70} -core interaction would in any way be affected if the ω -less core RNA polymerase was denatured, reconstituted with ω and then mixed with σ^{70} . We found that in these circumstances the ω -less RNA polymerase indeed regained its ability to bind to σ^{70} in the absence of GroEL. As shown in Fig. 4D, lane 4, the ω -less core RNA polymerase (after reconstitution) regained its correct mobility as against the ω -less core RNA polymerase (lane 2), or the GroEL-associated ω -less core RNA polymerase (lane 3). After addition of σ^{70} the intensity corresponding to the holoenzyme band substantially increased, indicating binding of σ^{70} to the core RNA polymerase (lane 5).

The association between different core RNA polymerases and σ^{70} was further proved by SDS/PAGE analysis of reconstituted enzyme as shown in Fig. 5A. It can be seen

Fig. 5. SDS/PAGE analysis (13% gel) of reconstituted holo RNA polymerase. Lane 1, molecular mass markers; lane 2, purified ω ; lane 3, ω -less core reconstituted with excess ω , passed through heparin–Sepharose and reconstituted with σ^{70} ; lane 4, ω -less, but GroEL-containing core RNA polymerase reconstituted with σ^{70} . (B) Single-round *in vitro* transcription at the *lac UV5* promoter with reconstituted RNA polymerase resulting in a 63-nucleotide long run-off transcript. A 154-nucleotide long recovery marker was used as internal control. Lane 1, wild-type RNA polymerase; lane 2, GroEL-less ω -reconstituted holo RNA polymerase; lane 3, ω -less, GroEL-containing holo RNA polymerase.

here that when ω -less core RNA polymerase reconstituted with excess σ^{70} (equivalent to lane 8, Fig. 4B) was electrophoresed along with the holoenzyme, complementation of σ^{70} was noticed with both ω -containing (lane 3) and GroEL-containing (lane 4) core RNA polymerase. Figure 5B shows a single-round *in vitro* transcription reaction at the *lac UV5* promoter with both these enzymes. Thus, the presence of either GroEL or ω is necessary in RNA polymerase to make it a functionally competent holoenzyme.

DISCUSSION

Despite extensive biochemical and genetic studies on all the constituent subunits of RNA polymerase, literature on the structure and function of the ω subunit is rare. The strain lacking ω , i.e. an *rpoZ* null mutant, is viable, suggesting that the protein is not essential or that there may be some redundancy in its function. The only known phenotype ascribed to an *rpoZ* null mutant is slower generation time.

The *in vitro* association and copurification of this polypeptide with RNA polymerase was reported [1] a long time ago, and later it was found by Berg and others [26] that ω is

selectively lost when the enzyme is subjected to zonal sedimentation in 1 M urea. Igarashi and co-workers [27] were successful in removing ω from the holoenzyme by treating it with 2.5 M urea. However, subsequent controversy on the functional assay [25] leaves some doubt about whether ω can be completely removed from RNA polymerase in this way. Recent studies by Dove and Hochschild [28] provide the first evidence that the ω protein is associated with the RNA polymerase holoenzyme *in vivo*, and that at least part of it is accessible at the surface of the enzyme complex. Their experiments further suggest that the N-terminal portion of the ω protein mediates its association with RNA polymerase, as fusion of another protein at the N-terminus weakens the association with the enzyme.

The P1 transduction experiments reported here and the comparison of growth of the various strains (Fig. 1) demonstrate that the growth defect of an *rpoZ* null mutant is associated only with the *rpoZ* locus and that there is no accumulation of suppressors elsewhere leading to the same phenotype.

The nature of the interaction with σ^{70} , as determined through mobility-shift assays, indicates that, in the absence of ω , the RNA polymerase recruits more GroEL possibly to keep the enzyme in a functional form capable of interacting with σ^{70} . Once the GroEL protein is separated from the enzyme, the core enzyme becomes irreversibly defective and cannot interact with σ^{70} . This could possibly be extended to the *in vivo* situation where only a part of the enzymatic apparatus may be able to access the chaperonin system. In the absence of ω , there would be no release of the RNA polymerase-associated GroEL for recirculation, because, as seen in Fig. 4D, only on reconstitution with ω was the GroEL association with the ω -less RNA polymerase core disrupted. This may explain the lower activity of this mutant enzyme and the slow-growth phenotype of an *rpoZ*-deleted strain.

In an earlier study, Ziemienowicz and co-workers [17] showed that GroEL protein could protect RNA polymerase from heat inactivation and this effect was maximal at a molar ratio of RNA polymerase to GroEL of 1 : 10. In the same study, they showed that more than 60% of the heat-inactivated RNA polymerase could be recovered after a 60-min incubation with GroEL, GroES and ATP. The initial lag of 10–20 min could be overcome when heat-inactivated RNA polymerase was pre-incubated for 10 min at 30 °C with only GroEL, followed by the addition of GroES and ATP at the start of the transcription assay. The gel mobility-shift assays performed here establish that, in the absence of ω , the RNA polymerase has some structural malformation which makes it associate with the GroEL molecular chaperone causing it to remain in the active form. Once this association is disrupted, the functional competence of the enzyme is lost. For the formation of active and structurally correct machinery, the presence of ω is essential, and it also helps in dissociating the enzyme from the chaperone, without loss of activity. The reconstitution data for ω , in the presence of GroEL, suggest a similar protective and scaffold-like activity helping the ω -less RNA polymerase to fold. Interestingly, Fig. 3 shows that the presence of both GroEL and ω are necessary for efficient reconstitution of RNA polymerase. In the presence of ω , an excess of GroEL can be removed; however, the GroEL remains tightly associated with the enzyme in the absence of ω .

The GroEL protein can spontaneously interact with an unstable protein-folding intermediate to form a stable chaperonin–folding protein complex [20]. The step leading to the dissociation of this complex, and the subsequent correct refolding of the assisted protein, requires ATP and is co-ordinated by the cochaperonin

GroES [20,29]. A recent study analysed the quantitative contribution of chaperonin GroEL to protein folding in *E. coli* [30]. In this *in vivo* study, a diverse set of newly synthesized polypeptides, predominantly between 10 and 55 kDa, was found to interact with GroEL, which accounted for 10–15% of all the cytoplasmic protein under normal growth conditions, and for 30% or more on exposure to heat stress. That there might be a size limit to GroEL substrates was therefore indicated.

Recently, it has been reported [31] that T4 bacteriophage does not require *groES* gene product for its assembly. This is because the T4 *gene31* and the *groES* gene encode functionally homologous proteins, making GroES redundant in the folding/assembly of the T4 major capsid protein Gp23 during virus morphogenesis. Taking a clue from this observation, we were tempted to postulate that ω is a GroES-like protein necessary for folding of RNA polymerase; however, neither the sequence homology with GroES and Gp31 nor its functional ATPase assay when complexed with GroEL were conclusive. In addition, the *rpoZ* gene was unable to complement conditional *groES* mutants. It has recently been shown that fragments encompassing the apical domain of GroEL, called minichaperones, facilitate the refolding of several proteins *in vitro* without the presence of GroES or ATP or the cage-like structure of multimeric GroEL [32]. With this background, ω may act as a minichaperone for RNA polymerase folding. We have also noticed that more ω protein accumulates in the late-exponential or stationary phase during the growth of *E. coli* as also has been reported recently [33]. Interestingly, the pattern of ω expression is similar to that of GroEL (not shown).

E. coli RNA polymerase assembly proceeds according to the pathway $\alpha + \alpha \rightarrow \alpha_2 + \beta \rightarrow \alpha_2\beta + \beta' \rightarrow \alpha_2\beta\beta' + \sigma \rightarrow \alpha_2\beta\beta'\sigma$ [34]. As ω protein shows binding affinity towards the β' subunit, we speculate that ω may help the β' association with $\alpha_2\beta$ subassembly. This postulation is currently being tested.

There are a few examples in the literature of diverse proteins performing a similar function, and this redundancy is essential for an organism to survive under stress conditions. The functional commonality between GroEL and ω may be another such example.

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