Mapping of Trypsin Cleavage and Antibody-binding Sites and Delineation of a Dispensable Domain in the β Subunit of Escherichia coli RNA Polymerase*

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We have mapped principal sites in the Escherichia coli RNA polymerase molecule that are exposed to attack by trypsin under limited proteolysis conditions. The 1342-amino acid-long β subunit is alternatively cleaved at Arg1005 or Lys1006. The cleavage occurs adjacent to a dispensable domain (residues 940–1040) that is absent in the homologous RNA polymerase subunits from chloroplasts, eukaryotes, and archaeabacteria. In E. coli, this region can be disrupted with genetic deletions and insertions without the loss of RNA polymerase function. Insertion of 127 amino acids into this region introduces a new highly labile site for trypsin proteolysis. The dispensable domain carries the epitope for monoclonal antibody PYN-6 (near residue 1000), which can be used for anchoring the catalytically active enzyme on a solid support. We also report the identification of a secondary trypsin cleavage at Arg941 of the β′ subunit within a putative zinc-binding domain that is conserved in prokaryotes and chloroplasts.

DNA-dependent RNA polymerase plays a central role in the expression and regulation of genes. The best characterized RNA polymerase is the enzyme from Escherichia coli, which can carry out in vitro all basic biochemical reactions of the transcription cycle (reviewed in Refs. 1–3). The RNA polymerase molecule also accepts a myriad of regulatory signals that modulate the basic reactions (4). The multiplicity of RNA polymerase function is reflected in its structural complexity. The E. coli holoenzyme (αβ′α′) is composed of the catalytic core component (αβ′α′) and the loosely associated initiation factor σ因子, which is replaceable by other σ factors with different promoter specificities (5). The core subunits are composed of 1407 (β′), 1342 (β), and 329 (α) amino acid residues (6) and carry no function individually. They can be separated from each other only by protein denaturation (7). The localities in the linear sequence of the core subunits that participate in RNA polymerase basic function can be deduced from the mapping of derivatization sites of affinity probes (8–10) and analysis of lethal mutations (11, 12) as well as from homology alignments with evolutionarily distant organisms (see Fig. 1A) (13, 14).

The focus of this report is a region in the β subunit shown in Fig. 1A that is located between two highly conserved sections of the linear sequence. Our interest in this region stems from the presumed functional cooperation between the flanking domains that harbor the sites of lethal mutations E813K (Glu813 → Lys) (15), K1065R (Lys1065 → Arg) (11), and H1237A (His1237 → Ala) (10), which disrupt the catalytic function of RNA polymerase. The sequence region between them that is centered around residue 1000 is present only in eubacteria, where it is highly variable. In other major groups of organisms including closely related chloroplasts, this region is missing, which results in an ~100-residue-long gap in the sequence alignment. On this basis, it can be suggested that the nonconserved region is looped out in the three-dimensional structure of RNA polymerase, whereas the flanking domains interact to perform the enzyme’s catalytic function.

The notion of an exposed loop in this general area seemed to agree with the early observations that, upon limited trypsin proteolysis of RNA polymerase, the β subunit is cleaved once approximately one third along its length (16, 17). In a previous study from one of our laboratories, it was reported that the larger and smaller trypsin fragments of the β subunit represent its amino- and carboxyl-terminal sections, respectively, placing the cleavage site in the general vicinity of the nonconserved region (17). We also found that monoclonal antibodies PYN-2 and PYN-6 selectively reacted with the larger and smaller trypsin fragments of the β subunit, respectively. However, the reported localization of the trypsin cleavage and antibody-binding sites based on indirect data was not precise.

The notion that a significant section of the β subunit in this area may be dispensable for biological function was first put forward by one of us (18, 19) on the basis of the isolation of a viable deletion mutant of rpoB missing ~50 amino acids. However, the location of this deletion based on indirect data was reported in a more distal region of the β subunit (18) that turned out to be highly conserved when sequence comparison became possible. Thus, when this work was initiated, the available data on homology alignment, proteolytic cleavage, and genetic mapping lacked the sequence level resolution and were in disagreement with each other. We report here the
precise localization of the trypsin cleavage and PYN-6 antibody-binding sites on the amino acid sequence level and in relation to the previously reported (18–20) and newly constructed insertions and deletions in this area.

MATERIALS AND METHODS

Bacterial Strains and Plasmids—The map positions of the rpoB deletions and insertions used in this work are indicated in Fig. 1A. The E. coli strains used were C600 (wild type), W31107 rpoBΔ1570-1 (Δ967–1028) (18), and MKDC747 (ΩT888) (20). The principal β subunit expression plasmid was pMKA92 (11), which carries a rifampicin-resistant allele of the rpoB gene under the control of the inducible lac promoter.

rpoB Deletions and Insertions—The sequence of rpoBΔ1570-1 (originally obtained as a chromosomal mutation) was determined by cloning it on a plasmid using the polA(Ts) technique (21), subcloning deletions and insertions used in this work are indicated in Fig. 1B. The deletion is therefore referred to as Δ(967–1028).

ΩT888 is a fortuitous duplication of 127 codons of the β subunit (Asp434–Ile661) inserted in-frame in the TaqI site between codons 988 and 998. It was isolated in the course of in vitro manipulations with cloned rpoB involving TaqI restriction and ligations. The insertion was transferred into the chromosome, where it could function as the sole rpoB allele in the cell (20). The map position of ΩT888 originally inferred from restriction analysis of DNA has now been confirmed by sequencing.

rpoB deletions and insertions originating from TaqI restriction sites were constructed in the pMKA92 plasmid as follows. In the first step, the plasmid DNA was linearized by random cuts at TaqI sites, and its termini were filled in using Klenow DNA polymerase. The DNA was ligated with the EcoRI fragment (with termini filled in using Klenow DNA polymerase) from phage mp17Km carrying the kanamycin resistance gene. Kanamycin-resistant transformants were selected and screened for the appearance of inducible truncated fragments of the β polypeptide that were visualized by gel electrophoresis. The size of these fragments corresponded to the known positions of TaqI restriction sites within the rpoB gene and to the location of kanamycin inserts determined in each case by restriction mapping. In the next step, the kanamycin cassette was excised from individually isolated plasmids with the inserts using BamHI, followed by religation. This procedure was expected to generate insertions of a 22-base pair BamHI linker between the flanked termini of the original TaqI site, thus restoring the rpoB reading frame. The transformants were screened for the restoration of the rpoB reading frame manifested by

![Fig. 1. Genetic context of trypsin cleavage in β (A) and β’ (B) subunits. A, the 1342-amino acid β subunit polypeptide is represented by the heavy bar, with shaded areas corresponding to highly conserved sequence regions. The rifampicin (Rif) region is defined by mutations of rifampicin resistance described in Refs. 45 and 46. The segment between amino acids 984 and 1066 is expanded underneath. The locations of the trypsin cleavage and PYN-6 mAb-binding sites established in this work are indicated over the linear amino acid sequence presented in single-letter code. The top sequence from E. coli (E. c.) (26) is aligned with corresponding segments of homologous RNA polymerase subunits from (top to bottom) P. putida (P. p.) (47); chloroplasts from spinach (S) (38), tobacco (T) (48), and liverwort (L) (49); and Saccharomyces cerevisiae (14), Drosophila melanogaster (50); and Sulfolobus acidocaldarius (51). The dots symbolize identity to the E. coli sequence, and the hyphens represent gaps. The horizontal bars over the sequence represent deletions, and the arrows indicate insertion and substitution mutations. All mutations have viable phenotypes unless indicated otherwise. Note that the large insertion ΩT888 (127 amino acids) is not drawn to scale. B, the alignment is shown of the two bacterial β’ subunit sequences (27, 47) in the area of the putative zinc-binding domain with the corresponding segments from chloroplasts (38, 48, 49).]
the disappearance of the truncated β fragments and the reappearance of inducible normal-sized β fragments visualized as a major overexpressed protein by gel electrophoresis. In the third step, deletions around the introduced linker were generated by opening the plasmids with BamHI, limited digestion with the Bal-31 nuclease, religation, and screening for the maintenance of the reading frame (see above). The complete collection of these mutations will be described elsewhere. Here we present the insertion δA952 and three associated deletions (δA952-964), δA947-967, and δA937-983) (see Fig. 1). Their map location was verified by DNA sequencing.

The three mutations originating from the A11 site at codon 1010 (insertions δA1010-δ1010 and δA1010-δ1010 and deletion δA1010) were obtained previously (20). Their breakpoints have not been verified by DNA sequencing and are therefore tentative. δA1010 removes ~15 amino acids judging by a shift in the electrophoretic mobility of the β subunit (20).

Plasmid-borne mutants were tested for biological function by monitoring their ability to confer isopropyl-1-thio-β-D-galactopyranoside-inducible rifampicin resistance on a rifampicin-sensitive host as described previously (11).

Monoclonal Antibodies —The isolation and initial characterization of the anti-β subunit mAbs δPYN-2 and δPYN-6 and the protocols of Western blots with separated subunits and their fragments have been reported (17). The dot blot activity assay of immobilized polymerase was performed essentially as described (22) using 1 μg of ascites fluid protein/dot. It should be noted that immobilized RNA polymerase apparently used as template the endogenous E. coli DNA present in the extracts since the addition of exogenous DNA to the assay did not have an effect on the activity that was observed in this assay could be completely inhibited by rifampicin, as expected.

Limited Trypsin Proteolysis of RNA Polymerase—RNA polymerase core enzyme was isolated as described (23) with modifications to be reported elsewhere. The wild-type enzyme was obtained from E. coli C600, and the enzyme carrying the elongated βmas subunit from E. coli M2C147 (δT988). The ε subunit was prepared from the overexpression strain as described (24). Type XIII 1:1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin was purchased from Sigma. The 20-μL standard digestion mixture contained 5 μg of RNA polymerase holoenzyme in 40 mM Tris-HCl, pH 7.5, 40 mM KCl, 5% (v/v) glycerol, and either 50 or 5 μg of trypsin in the case of the wild-type or δT988 RNA polymerase, respectively. The samples were incubated for 30 min at 32 °C; and the reactions were terminated by addition of phenylmethylsulfonyl fluoride to a final concentration of 5 mM, followed by electrophoresis on sodium dodecyl sulfate-10% (A) and -5% polyacrylamide gels (25).

N-Terminal Sequencing of Trypsin Cleavage Products —The bands were excised from the gel and electrodialyzed using a Bio-Rad electrodialyzer. The eluted material (~100 μl) was precipitated with 2 volumes of cold acetone; and the pellets were washed with 70% acetone, dissolved in 10 μl of 50% formic acid, and precipitated by adding 100 μl of 500 mM CaCl2. The samples were dried with 70% acetone, and their N-terminal sequences were analyzed in 20 cycles of Edman degradation using an Applied Biosystems Protein Sequencer at the Columbia University Core Protein Facility.

RESULTS

Mapping of Trypsin Cleavage Sites in β and β Subunits—When RNA polymerase is treated with trypsin, a pattern of distinct fragments is generated, which can be visualized by combining the resolution of a 10% (Fig. 2A), and a 5% (Fig. 2B) polyacrylamide gel. Three principal products can be seen after limited trypsin digestion of the wild-type holoenzyme (Fig. 2A, lane 2): bands b1, b2, and s, with apparent M, values of about 105,000, 50,000, and 43,000, respectively. Under these conditions, 30–50% of the β and β' (Fig. 2B, lane 2) and α (Fig. 2A, lane 2) subunits remain intact, whereas the σ subunit is cleaved completely. Of the three major products, band s is likely to be the fragment of the σ subunit that has been observed by others (6) because it is not found among the products of the core enzyme digestion (Fig. 2A, lane 3). The cleavage products b1 and b2 apparently correspond, respectively, to the longer and shorter fragments of the β subunit.

The abbreviation used is: mAbs, monoclonal antibodies.

| A. (10% gel) | B. (5% gel) |
|--------------|-------------|
| β' | | |
| β' | | |
| b1 | b2 | c |

![Fig. 2. Limited proteolysis of wild-type and δT988 RNA polymerases.](image)

Electrophoretic separation of polypeptides in sodium dodecyl sulfate-10% (A) and -5% (B) polyacrylamide gels. Lanes 1–4 present an experiment with the wild-type (WT) RNA polymerase. Lanes 5–8 show the results of treatment of the δT988 RNA polymerase carrying the elongated βmas subunit. Note that the trypsin dose used with the δT988 enzyme was 10-fold lower than that with the wild-type polymerase (see “Materials and Methods”). Lane 8 contains molecular mass markers. Lanes 1 and 5, untreated holoenzymes; lanes 2 and 6, digested holoenzymes; lanes 3 and 7, digested core enzymes; lanes 4 and 7, core enzymes digested in the presence of sonicated denatured calf thymus DNA.
Trypsin, which apparently results from the action by cellular proteases. Judging by its size, band d is generated by cleavage within the inserted amino acid sequence. No C-terminal fragment of the \( \beta_{\text{sub}} \) subunit could be found in this experiment, suggesting that the cleavage within the insertion leads to rapid degradation of the distal sequences. Finally, the exposure of the core RNA polymerase with the \( \beta_{\text{sub}} \) subunit to denatured calf thymus DNA leads to the reappearance of a fragment co-migrating with N-terminal band b, (Fig. 2, A and B, lanes 7). The nature of this fragment has not been investigated.

**Trypsin Cleavage in \( \beta \) Subunit Occurs on Border of Vital and Dispensable Domains**—We report here a corrected sequence position of the large nonlethal deletion mutant originally isolated by Nene and Glass (19). The deletion, \( \Delta(967-1028) \), occurred between codons 966 and 1029 (Fig. 1A), demonstrating that a substantial portion of the nonconserved region is dispensable. To determine how far the dispensable region extends in the upstream direction, we generated a series of deletions expanding bidirectionally from a linker insertion (\( \Omega952 \)) after codon 952 (see Fig. 1). Insertion \( \Omega952 \) and deletions \( \Delta(952-961) \), \( \Delta(947-967) \), and \( \Delta(937-983) \) were engineered in a rifampicin-resistant allele of \( \text{rpoB} \) carried on the inducible expression plasmid pMKA92 under the control of the lacUV5 promoter (11). All of these alleles conferred an isopropyl-1-thio-\( \beta \)-galactopyranoside-inducible rifampicin-resistant phenotype on a rifampicin-sensitive host strain. It should be noted that, in the case of all four mutations, the efficiency of plating and the growth rate in the presence of rifampicin should be noted that, in the case of all four mutations, the efficiency of plating and the growth rate in the presence of rifampicin as well as the maximum concentration of rifampicin tolerated were somewhat lower (by a factor of 2–4) than in the case of the parent plasmid pMKA92. This deficiency probably reflects the lability of the mutant \( \beta \) subunit to cellular proteases and will be analyzed elsewhere. However, the ability of these strains to grow in the presence of rifampicin clearly demonstrated the functionality of the plasmid-encoded \( \beta \) subunit, which extends the upstream boundary of the nonessential region to codon 936, i.e. just beyond the point where the alignment with chloroplasts reveals a gap. We note that a viable insertion of three amino acids, \( \text{Xho-29} \), was recently mapped in this area (12). In conclusion, trypsin cleavage appears to occur immediately upstream from a long nonconserved and dispensable stretch of the \( \beta \) polypeptide and, at the same time, immediately downstream from a highly conserved region that is the site of the dominant lethal mutation \( \text{Xho-19} \) (12).

**Mapping of Epitope for mAb PYN-6**—As was reported previously from one of our laboratories, the shorter trypsin-generated fragment of the \( \beta \) subunit reacted with anti-\( \beta \) subunit mAb PYN-6 (17). The location of the PYN-6 epitope can now be identified using the deletions and insertions shown in Fig. 1. To locate the distal boundary of the epitope, we used the previously described deletion and insertion mutations originated from the \( \text{AluI} \) restriction site at codon 1010 (Fig. 1) (20). Each of these mutants was available on an \( \text{rpoB} \) expression plasmid. As the host strain for the plasmids, \( \text{E. coli} \) MKDC747 specifying elongated \( \beta_{\text{sub}} \) subunit (\( \Omega7898 \)) was used, which permits clear distinction between the plasmid- and chromosome-encoded \( \beta \) subunits by gel electrophoresis. The Western blots of Fig. 3 show the analysis of strains carrying the wild-type \( (\text{lane} \ 4) \) or different mutant \( (\text{lanes} \ 1-3) \) plasmids. It can be seen that, under these conditions, the chromosome-encoded \( \beta_{\text{sub}} \) subunit reacted well with the control PYN-2 mAb (Fig. 3A), but not with the PYN-6 mAb (Fig. 3B). Of the plasmid-encoded \( \beta \) subunits, the one with the \( \sim 15 \)-amino acid deletion, \( \Delta A1010 \) (lane 1), did not react with PYN-6, whereas the 4-amino acid insertion, \( \Omega A1010 \) (lane 2) did. Lane 3 represents the frameshifting insertion \( \Omega A1010-fs \), which leads to the formation of a prematurely terminated fragment of the \( \beta \) subunit. Clearly, this fragment reacts with mAb PYN-6. These results place the distal border of the PYN-6 epitope upstream from residue 1010.

To map the proximal boundary of the PYN-6 epitope, we used deletions \( \Delta(852-964) \), \( \Delta(947-967) \), and \( \Delta(937-983) \) described above. As can be seen from Fig. 4, the \( \beta \) subunits with the two shorter deletions readily reacted with the antibody (lanes 1 and 2, respectively), whereas the longer deletion completely abolished the binding of PYN-6 (lane 3). Thus, the proximal boundary of the PYN-6 epitope maps between residues 967 and 983.

**Solid-state Transcription**—We were interested whether the PYN-6 mAb could be employed to immobilize active RNA polymerase on solid support, a procedure that would have several applications in the transcription field. The experiment of Fig. 5 documents the successful development of such technology. The nitrocellulose strips were each dot-blotted with the PYN-6 mAb as well as the control PYN-5 mAb, which

**FIG. 3. Mapping of distal boundary of PYN-6 epitope.** The two mAbs were used to develop identical Western blots of the gel separation of crude extracts containing the chromosome-encoded elongated \( \beta_{\text{sub}} \) subunit (\( \Omega7898 \)) and the following plasmid-specified variants of the \( \beta \) polypeptide: lane 1, \( \Delta A1010 \); lane 2, \( \Omega A1010-fs \); lane 3, \( \Omega A1010-fs \); lane 4, wild type. Lanes 5 and 6 are the control extracts from haploid strains carrying the wild-type \( \beta \) (\( \text{E. coli} \) C600) and \( \beta_{\text{sub}} \) (\( \text{E. coli} \) MKDC747) subunits.

**FIG. 4. Mapping of proximal boundary of PYN-6 epitope.** Two identical blots were prepared after gel separation of crude extracts expressing the following plasmid-specified variants of the \( \beta \) polypeptide: lane 1, \( \Delta(933-964) \); lane 2, \( \Delta(947-967) \); lane 3, \( \Delta(937-983) \); A was stained with Coomassie Blue, and B was developed with the PYN-6 mAb.

**FIG. 5. Dot blot activity assay of immobilized RNA polymerase.** The two anti-RNA polymerase mAbs were blotted as dots on nitrocellulose strips and exposed to crude RNA polymerase preparations, followed by incubation under RNA polymerase assay conditions, fixation with trichloroacetic acid, and autoradiography (see "Materials and Methods"). Lane 1, wild-type cells (\( \text{E. coli} \) C600); lane 2, cells with shortened \( \beta \) subunits that have lost the PYN-6 epitope due to deletion \( \Delta(967-1028) \) (\( \text{E. coli} \) W3110).
interacts with the α subunit. The strips were incubated with crude extracts of the bacterial strains carrying either the wild-type rpoB gene (Fig. 5, lane 1) or the rpoB1570-1 deletion allele (Δ(967-1028)) (see Fig. 1A) that removes the PYN-6 epitope (lane 2). The strips were washed and then incubated with in vitro transcription mixture as described under "Materials and Methods." After the reaction was completed, the strips were developed with trichloroacetic acid, washed, and autoradiographed to detect the incorporation of [α-32P]UTP into acid-insoluble fractions. Clearly, the PYN-6 dot was able to attract enough wild-type RNA polymerase to generate a massive locus of solid-state transcription (Fig. 5, lane 1), whereas the deletion control displayed no activity (lane 2).

Moreover, the antibody was able to selectively anchor RNA polymerase carrying the epitope from a mixture with an excess of the enzyme that has lost it. Reconstitution experiments (data not shown) demonstrate that as little as 3% of the RNA polymerase with full-sized β subunits can be detected in this way in the presence of an excess of Δ(967-1028) RNA polymerase.

DISCUSSION

Genetic Context of Trypsin Cleavage Site in β Subunit—Since the pioneering paper of Khesin et al. (28), limited trypsin proteolysis has been used by many authors for structural and functional studies of RNA polymerase (16, 17, 29-32), leading in many cases to conflicting conclusions about the location of cleavage sites and identities of the products. Our present results unequivocally identify a single principal site of trypsin attack on the core RNA polymerase that is located in the β subunit. The cleavage occurs immediately downstream from a highly conserved region, the disruption of which by the mutation Xho-19 leads to a dominant lethal phenotype (12). The Xho-19 substitution destroys the phosphate loop part of the bipartite motif Asn-X-X-Asp (29), Gly-X-X-X-Gly-Lys (30), which is found in many GTP-binding proteins (33). Recently, we reported that the dominant lethal substitution Gln→Lys (ES13K), which destroys the upstream part of this motif, affects the catalytic function of RNA polymerase (15). On this basis, it was suggested that the domain located immediately upstream from the trypsin cleavage site participates in the RNA polymerase activity center.

The region on the downstream side of the trypsin cleavage harbors many mutations that do not affect cell viability when the mutant allele is the sole source of the β subunit. These data together with the low level of sequence conservation between E. coli and Pseudomonas putida and the gap in the sequence alignment with chloroplasts, eukaryotes, and archaeabacteria (Fig. 1A) indicate that the trypsin cut in E. coli is located precisely at the border between a large dispensable sequence section and an upstream domain presumed to be involved in the catalytic function. The next highly conserved region downstream in the β subunit sequence includes Lys (31), which has also been shown to participate in catalytic function by genetic and affinity labeling experiments (9-11).

Our data show that, in the wild-type RNA polymerase, the dispensable region is structured enough to make it resistant to proteolysis under conditions where trypsin cleaves the borderline site. This structure must protrude away from the functionally important domains for a distance long enough to accommodate drastic changes caused by deletions and insertions and to permit anchoring to the PYN-6 antibody without loss of RNA polymerase catalytic function. The apparent structural and functional autonomy of the dispensable region justifies categorizing it as a distinct domain in the folded RNA polymerase molecule.

The reason why eubacteria keep an apparently nonessential domain in their RNA polymerase remains obscure. Several eubacteria such as Rickettsia prowazekii (34), Clostridium acetobutylicum (35), Thermus thermophilus (36), and Thermotoga maritima (37) have been shown to possess RNA polymerase β subunits ~10 kDa shorter than the E. coli β subunit. One can anticipate that these species may contain deletions of the nonconserved domain. Our results do not address the possibility that this domain may be important for some fine nonvital aspect of RNA polymerase function. In this connection, we should note the change of in vitro promoter selectivity observed previously with deletions in this area (18).

Cleavage Site in β Subunit—In the wild-type RNA polymerase, the β subunit is relatively resistant to limited proteolysis by trypsin. However, if the core enzyme is exposed to denatured DNA, a site in the β subunit that is located in the middle of the putative zinc-binding domain becomes susceptible to trypsin cleavage. The zinc-binding motif is conserved in all prokaryotic and chloroplast RNA polymerases (38) and may be functionally important. It should be noted that the RNA polymerase molecule is known to contain two Zn²⁺ ions, one in the β subunit and the other in the β' subunit (39-41). Each of them can be individually removed by reconstituting RNA polymerase from subunits obtained under appropriate ionic conditions (42). Thus, it would be interesting to determine whether the removal of the β' subunit Zn²⁺ will affect the kinetics of trypsin cleavage of the β' subunit.

Technical Implications for RNA Polymerase Studies—Our results present several technical advances that will find applications in the field of RNA polymerase structure/function analysis. The characterization of the β subunit with slower electrophoretic mobility (βₘₙ) and of the cleavage products of the two large subunits (bands b₁, b₂, c, and d) (Fig. 2) and the mapping of the PYN-6 epitope will facilitate the analysis of derivatized RNA polymerase labeled with affinity reagents. For example, the use of RNA polymerase with the elongated βₘₙ subunit helped us to map the sites cross-linked to the 5' (10) and 3' (8) substrate derivatives. The elongated β subunit and the PYN-6 mAb can be employed as tags for orienting RNA polymerase image in two-dimensional crystals (43) or neutron scattering analysis (44).

The product of trypsin treatment of RNA polymerase with the βₘₙ subunit (Fig. 2, A and B, lanes 6) presents an interesting model for study: this material seems to have completely lost the C-terminal domains of the β subunit, whereas the N-terminal section as well as the rest of the subunits are still present. Is the σ factor association affected by the loss of the distal domains of the β subunit? Can this preparation interact with promoters? These questions are currently under investigation.

Finally, the anchoring reaction in the format of Fig. 5 constitutes an easy and highly selective way for assessing one type of RNA polymerase (carrying the PYN-6 epitope) in the presence of another (lacking the epitope). Such an assay can be useful for massive biochemical screening of mutations engineered in the cloned rpoB gene, which is the main focus of our current research.

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