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Probing yeast RNA polymerase A subunits with monospecific antibodies

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Monoclonal antibodies were raised in mouse against native RNA polymerase A from *Saccharomyces cerevisiae*. After screening with the spot-immunodetection technique, 14 hybridomas were selected and the antibodies produced in mice. Their specificity, analyzed by blot-immunodetection, was found to be markedly biased towards a few RNA polymerase subunits: A_{135}, A_{49}, A_{43}, and $A_{14\cdot5}$. A different monoclonal antibody directed against the largest subunit, A_{190}, was obtained by immunizing a mouse with RNA polymerase A dissociated into its subunits with SDS. Two antibodies, which probably recognized the same antigenic determinant on subunit A_{135}, inhibited *in vitro* RNA synthesis. Inhibition was prevented by preincubation of the enzyme with DNA, suggesting a role for the A_{135} subunit in template binding. The antibody directed against $A_{14\cdot5}$ interacted with the $A_{14\cdot5}$ kd subunit present in all three forms of the yeast nuclear RNA polymerases but did not interfere with RNA polymerase activity. These antibody probes will be useful to study subunit function in reconstituted transcription systems.

Key words: RNA polymerase A/monoclonal antibodies

Introduction

In yeast, as in all eukaryotic cells, there are three nuclear DNA-dependent RNA polymerases, each with a different specificity (Roeder, 1976). The yeast enzymes have been extensively studied at the molecular level (Sentenac *et al.*, 1976; Hager *et al.*, 1976). They can be obtained in relatively high yield and are good immunogens, compared with the animal enzymes. The immunological approach has contributed greatly to unravelling their complex molecular structure. Antibodies to native RNA polymerases A or B revealed the core of common subunits of low mol. wt., present in enzymes A, B, and C, as well as two additional polypeptides shared by enzymes A and C (Buhler *et al.*, 1980). These results were confirmed with antibodies to isolated individual subunits (Huet *et al.*, 1982). The two large subunits present in each enzyme are clearly distinct gene products (Buhler *et al.*, 1976; Ruet *et al.*, 1980). However, a small level of cross-reaction was found among the three forms of RNA polymerase with antibodies directed against the large polypeptides (Buhler *et al.*, 1980; Huet *et al.*, 1982). There is also a marked conservation of antigenic determinants in RNA polymerase B from a variety of eukaryotic organisms, especially on the large subunits (Huet *et al.*, 1982; Kramer and Bautz, 1981).

At this point, it would be most interesting to confirm and extend these observations using monospecific antibodies which would be directed at antigenic sites conserved during evolution. These sites are likely to be part of essential domains of RNA polymerases. Studies with polyspecific antibodies also showed that *in vitro* transcription can be blocked by antibodies against several subunits (Buhler *et al.*, 1980). This suggested that it might be possible to obtain monoclonal antibodies binding to vital parts of the enzymes. The availability of such tools, as well as the development of specific *in vitro* transcription systems, would provide a way to investigate subunit function. With this in mind, we have explored the feasibility of constructing a library of monoclonal antibodies against yeast RNA polymerases. Monospecific antibodies were obtained which are directed at five different subunits of enzyme A.

Results

Screening of hybridomas

Antibodies raised in rabbits against native RNA polymerases are directed at all the subunits of the enzyme (Buhler *et al.*, 1980). In our first attempts to produce monoclonal antibodies, we therefore used native RNA polymerase A as the antigen. Mouse spleen cells were fused with the myeloma cells and hybridomas growing in the hypoxanthine-azaserine selection medium (Buttin *et al.*, 1978) were assayed for antibody production by the spot-immunodetection technique previously described (Huet *et al.*, 1982). RNA polymerase was spotted on nitrocellulose filters and incubated with an aliquot of the culture medium. After washing, the immune complexes were directly revealed by ^{125}I-labelled Protein A. Out of 280 wells, 160 showed growing hybridomas of which 17 gave a positive response. The intensity of the response, as visualized on the autoradiogram, varied considerably. This could be attributed to various causes: a difference in the affinity of the antibodies for the antigen, a difference in cell density, or a difference in the efficiency of production and excretion of the antibody. All the hybridoma cultures were cloned by limit dilution into microtiter plates with a feeder layer of thymocytes. Upon cloning and subculturing, some strong positives became much weaker. The positive clones, including the weak ones, were cultured on a larger scale for storage in liquid nitrogen and production of antibody in mice. The monoclonal antibodies were purified from the ascitic fluid by ammonium sulfate precipitation and DEAE-cellulose chromatography, as described under Materials and methods. Figure 1 summarizes the final screening process where each antibody was assayed by the spot-immunodetection technique. Both RNA polymerases A and B were spotted on each filter to reveal antibodies reacting with common determinants (Huet *et al.*, 1982). The assay was performed using a constant concentration of immunoglobulins purified from the different ascitic fluids and therefore reflected the relative affinity of the antibodies for RNA polymerases. A large range of interactions were found from very weak to very strong (see Figure 1). Interestingly, in two cases, both enzymes A and B were recognized by the antibody. These two antibodies also reacted with spots of enzyme C (result not shown). For further characterization, we selected five antibodies giving the

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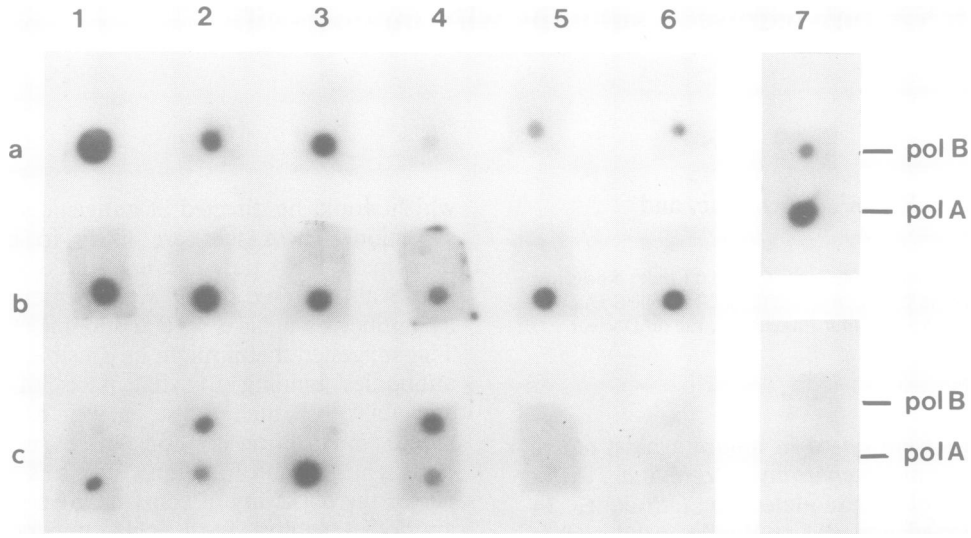

Fig. 1. Screening hybridomas by spot-immunodetection; a summary. Hybridomas from the 17 original positive cultures were cloned by limit dilution, grown as ascites tumors and assayed by spot-immunodetection using both RNA polymerases A and B on the same filter. The filters were incubated with immunoglobulins purified from the different ascitic fluids and processed as described under Materials and methods. The above experiment shows the antibody binding signal obtained on the autoradiogram. Hybridomas are named after the original cultures: filter $\mathbf{a_1}$, 2; $\mathbf{a_2}$, 5; $\mathbf{a_3}$, 16; $\mathbf{a_4}$, 29; $\mathbf{a_5}$, 34; $\mathbf{a_6}$, 35; $\mathbf{b_1}$, 39-1 (clone 1); $\mathbf{b_2}$, 39-6 (clone 6); $\mathbf{b_3}$, 86; $\mathbf{b_4}$, 96; $\mathbf{b_5}$, 150; $\mathbf{b_6}$, 151; $\mathbf{c_1}$, 130; $\mathbf{c_2}$, 131; $\mathbf{c_3}$, 146; $\mathbf{c_4}$, 128; $\mathbf{c_5}$, 129; $\mathbf{c_6}$, 107. **Lane 7** corresponds to a control experiment with rabbit antibodies against yeast RNA polymerase A (top) or with control rabbit immunoglobulin (bottom).

strongest binding signals and one giving a dual response on enzymes A and B.

Characterization of the monoclonal antibodies and their subunit specificity

The purity of the antibodies was determined in two cases (clones 131-17 and 39-6), by electrophoresis in a polyacrylamide slab gel, in the presence of SDS. The immunoglobulins, upon reduction and heating with 1% 2-mercaptoethanol, exhibited two sharp protein bands of mol. wt. 56 000 and 23 000 (clone 39-6) or 56 000 and 21 000 (clone 131-17). After staining the proteins in the gel with Coomassie blue, the purity of the γ-globulins was estimated to be in the range of 90%. In all cases, the antibodies were characterized immunologically as being IgG_{2a} immunoglobulins (C.Leguern, unpublished data). This was in keeping with the fact that the antibodies were not retained on DEAE-cellulose at pH 7.4 at low ionic strength (Dissanayake and Hay, 1975). A selection of only IgG_{2a} antibodies can result from the mice antibodies being directly revealed by Protein A. Although mice IgG_1 immunoglobulins are retained on Protein A-Sepharose (Ey *et al.*, 1978) it appears that they are not visualized directly by the spot-immunodetection technique (C.Leguern and J.Huet, unpublished data). To detect mouse IgG_1 antibodies, one has to include an incubation step with rabbit anti-mouse immunoglobulins before adding Protein A.

RNA polymerase subunits bearing the antigenic determinants recognized by the different antibodies were identified by protein blotting, using the same approach as that originally used to characterize rabbit polyspecific antibodies (Buhler *et al.*, 1980). The blot-immunodetection was carried out both with the culture supernatants obtained after cloning and with the ascitic fluid, with identical results. After electrophoresis the subunits of RNA polymerase A were transferred to a nitrocellulose filter and reacted with the different antibodies (Figure 2). All subunits reacted with control rabbit antibodies directed against native RNA polymerase A (Figure 2, lane 1). In sharp contrast, the six monoclonal antibodies recognized

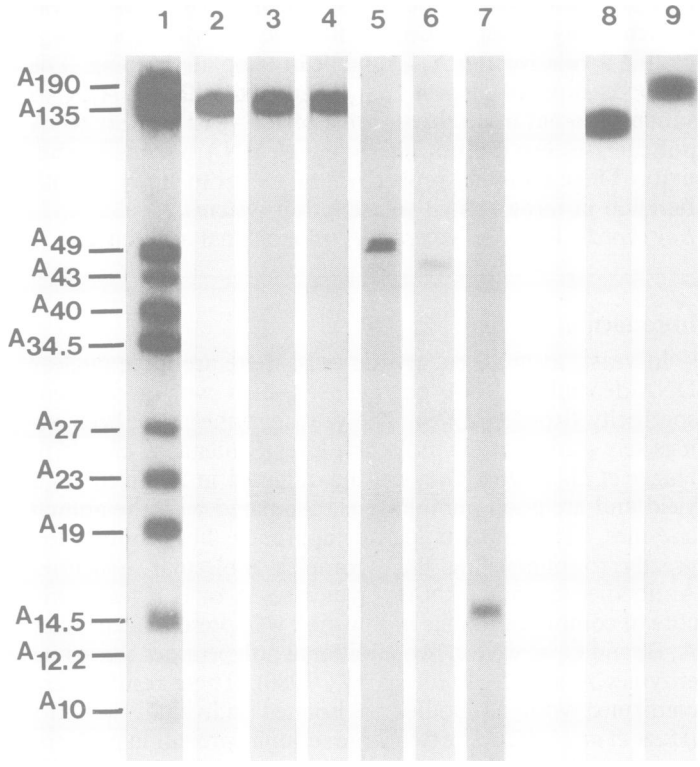

Fig. 2. Specificity of antibodies towards RNA polymerase A subunits. RNA polymerase A subunits were separated by electrophoresis, transferred to a sheet of nitrocellulose and probed with the different antibodies as described under Materials and methods. In **lane 1**, the blotting was controlled using rabbit antibodies against RNA polymerase A. The subunits are identified on the left. **Lanes 2–7**, antibodies purified from the ascitic fluid of hybridomas 2, 39-6, 86, 151, 146, or 131, respectively, shown in Figure 1. In **lanes 8** and **9**, the two hybridomas were derived from a different fusion with spleen cells from a mouse immunized with RNA polymerase A dissociated with sodium dodecylsulfate. From this experiment, the monospecific antibodies are named after the subunit they recognize, i.e., **lanes 2–9**, $A_{135\text{-}1}$, $A_{135\text{-}2}$, $A_{135\text{-}3}$, $A_{49\text{-}1}$, $A_{43\text{-}1}$, $A_{14\cdot5\text{-}1}$, $A_{135\text{-}4}$, and $A_{190\text{-}1}$.

only one polypeptide component, either A_{135}, A_{49}, A_{43}, or $A_{14.5}$ (Figure 2). The intensity of the response varied greatly (compare, in Figure 2, lane 4 and lanes 5 or 6). Since the antibodies were purified and used at the same concentration, the variation in their binding efficiency may reflect a variable affinity for the antigenic sites or the partial denaturation of the antigenic determinant during electrophoresis or the transfer process. Three out of six antibodies bound to subunit A_{135}, which therefore must contain a very immunodominant site. Two additional fusions were made, under the same conditions, to isolate antibodies with different specificities. Several positive hybridomas were obtained but the antibodies were again directed at subunits A_{135} or $A_{14.5}$. No antibodies were directed at subunits A_{190}, A_{27}, A_{23}, or A_{19}. To extend our collection of monoclonal antibodies, another fusion was made using, as antigen, RNA polymerase A that had been dissociated into subunits by SDS (instead of using the native enzyme). In the spot-immunodetection screening, for that experiment, an incubation step with rabbit anti-mouse immunoglobulins was added before adding Protein A. A large number of positive hybridomas were detected. Antibodies were prepared as above from two strong positive clones and analyzed by blot-immunodetection: one recognized subunit A_{190} while the other bound to subunit A_{135} (Figure 2, lanes 8 and 9).

A monospecific antibody against a subunit common to the three forms of RNA polymerases

The monoclonal antibody $A_{14.5\text{-}1}$, binding to $A_{14.5}$, was shown by spot-immunodetection to bind to both enzymes A and B (see Figures 1–2C). This subunit was previously shown by fingerprinting (Buhler *et al.*, 1976) and with polyspecific antibodies (Buhler *et al.*, 1980; Huet *et al.*, 1982) to be present in the three forms of RNA polymerases. It was interesting to demonstrate the identity of one specific antigenic site within the same subunit of the three enzymes. In the experiment shown in Figure 3, the three purified RNA polymerases, were electrophoresed under denaturing conditions and analyzed either with the monospecific antibody or with antibodies raised in rabbits against native RNA polymerase A. The two blots are directly comparable as the protein transfer was carried out by direct contact with two nitrocellulose sheets, one on each side of the gel. On one sheet, the polyspecific antibodies revealed the subunits common to the three enzymes: AC_{40}, ABC_{23}, AC_{19}, and $ABC_{14.5}$. Subunit ABC_{27} was only weakly detected (Buhler *et al.*, 1980). On the other blot, the monospecific antibody revealed exclusively $A_{14.5}$ in the three enzymes. The intensity of the band corresponded to the respective amount of protein as estimated by staining the gel after blotting (result not shown). The signal was not much weaker than with the polyspecific antibodies, suggesting that the latter reacted with very few determinants. The second monoclonal antibody that reacted with both RNA polymerases A and B (see Figures 1–4C) was also found by immuno-blotting to recognize subunit $A_{14.5}$ and was not analyzed further.

Effect of monospecific antibodies on transcription

Monospecific antibodies that interfere with the interaction of polymerase with template or factors could be a powerful tool to investigate subunit function during transcription. In the absence of a specific assay for RNA polymerase A, we measured the effect of antibody binding on the basic steps of RNA synthesis using a non-specific template. The purified monospecific antibodies were incubated with RNA

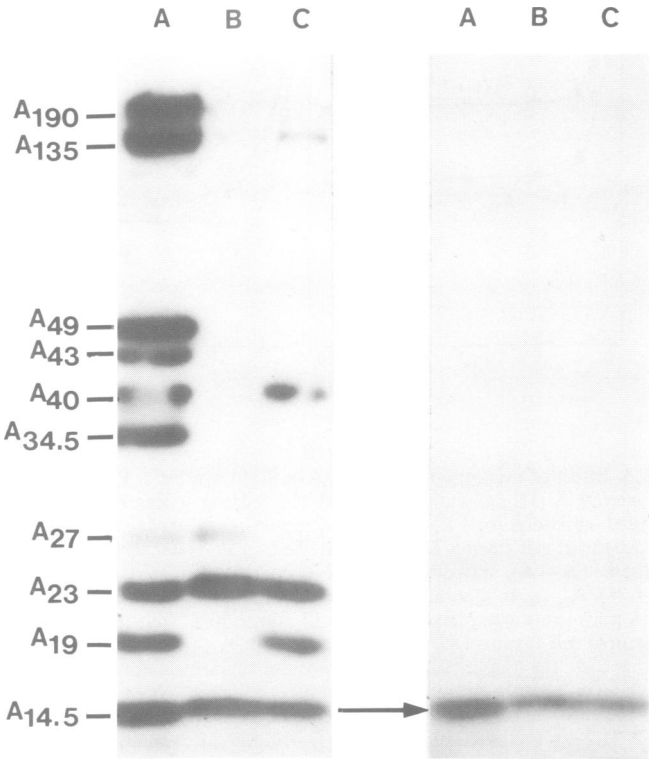

Fig. 3. Antibody $A_{14.5\text{-}1}$ recognizes the same polypeptide in RNA polymerases A, B, and C. RNA polymerases A, B, and C were subjected to electrophoresis under dissociating conditions, blotted to nitrocellulose and probed with purified antibodies to native RNA polymerase A **(left panel)**, or with the monospecific antibody $A_{14.5\text{-}1}$ **(right panel)**, as described under Materials and methods. RNA polymerases are identified by the letters A, B, or C. Subunits of RNA polymerase A are identified on the left. The arrows show the reaction of the monospecific antibody with subunit $A_{14.5}$ from the three enzymes.

polymerase A prior to RNA synthesis. Among the monospecific antibodies shown in Figure 2, only two were inhibitory (antibodies $A_{135\text{-}1}$ and $A_{135\text{-}2}$) (Figure 4A). However, in both cases, maximal inhibition was only 50% and could not be increased by adding a large molar excess of antibody over the enzyme. The inhibitory effect of the two antibodies was not additive, and enzyme A* (lacking subunits A_{49} and $A_{34.5}$) was similarly inhibited 50% (results not shown). Two explanations could account for this partial inhibition. The antibody could simply slow down, by a factor of two, one step of RNA synthesis, (e.g., the elongation step), without blocking a vital function of the enzyme. Alternatively, it could bind to only a fraction of the enzyme molecules while inactivating them totally. To explore the latter possibility, the immune complexes formed at varying antibody concentrations were removed by Protein A-Sepharose and the supernatant was assayed for RNA polymerase activity. With antibodies $A_{135\text{-}1}$ or $A_{135\text{-}2}$, ~40% of the input activity was recovered in the supernatant (Figure 4B). In contrast, using a different antibody, $A_{135\text{-}3}$, also specific for subunit A_{135} but non-inhibitory, all of the RNA polymerase activity could be immunoprecipitated. Therefore, antibodies $A_{135\text{-}1}$ or $A_{135\text{-}2}$ seem to distinguish between two populations of RNA polymerase molecules, and once bound, totally inhibit the enzyme. The inhibition can be partly suppressed by preincubating the enzyme with DNA (100 μg/ml) prior to antibody addition (Figure 4A). This result suggests that the antibody

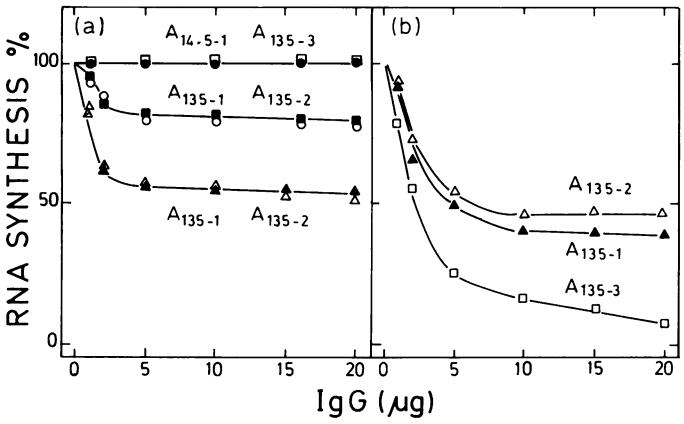

Fig. 4. Effect of monospecific antibodies on RNA synthesis. **Panel a**, RNA polymerase A (1.2 μg) was preincubated with varying concentrations of purified antibodies for 1 h at 37°C, and transcription activity was assayed on denatured calf thymus DNA, as described under Materials and methods. (▲—▲), antibody $A_{135\text{-}1}$; (△—△), $A_{135\text{-}2}$; (□—□), $A_{135\text{-}3}$; (●—●), $A_{14\cdot5\text{-}1}$. In a parallel experiment, before addition of the antibody, RNA polymerase was preincubated for 10 min at 30°C with 10 μg/ml of denatured calf thymus DNA. (■—■), antibody $A_{135\text{-}1}$; (○—○), $A_{135\text{-}2}$. In that case, the transcription mixture was not supplemented with DNA. The activity of RNA polymerase in control experiment (100% value) corresponded to the incorportion of 420 pmol UMP. **Panel b**, RNA polymerase A (1.2 μg) was preincubated similarly with the different antibodies. The immune complexes were adsorbed on Protein A-Sepharose and the residual enzyme activity was assayed in the supernatant (see Materials and methods). Symbols are the same as in **panel a**.

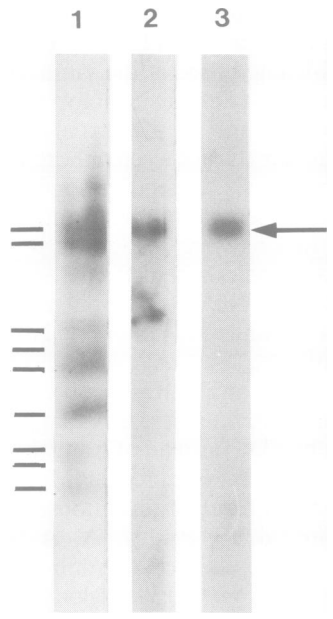

Fig. 5. Binding of antibodies $A_{135\text{-}1}$ and $A_{135\text{-}2}$ to the same peptide derived from A_{135} subunit. A_{135} subunit was isolated by electrophoresis, subjected to partial proteolysis, the peptides were transferred to nitrocellulose and probed with rabbit antibodies against A_{135} or with the monospecific antibodies $A_{135\text{-}1}$ or $A_{135\text{-}2}$. The Figure shows the autoradiogram of the filters after incubation with ^{125}I-labelled Protein A. **Lane 1**, rabbit antibodies to A_{135}; **lane 2**, antibody $A_{135\text{-}1}$; **lane 3**, antibody $A_{135\text{-}2}$. The spots in **lane 2**, under the band, are artefactual and are not seen in other experiments.

interferes with DNA binding.

It was possible that antibodies $A_{135\text{-}1}$ and $A_{135\text{-}2}$ recognized the same antigenic determinant since they both bound subunits A_{135} and inhibited enzyme activity. To investigate this, subunit A_{135} was isolated by electrophoresis and subjected to partial proteolysis according to Cleveland *et al.* (1977). The peptides were analyzed by protein blotting and immunodetected with the two monoclonal antibodies as well as with rabbit antibodies directed against the denatured subunit. Figure 5 shows that the same peptide was recognized by the two monoclonal antibodies which suggests that they recognize the same antigenic site. On the other hand, antibody $A_{135\text{-}3}$ did not react with any peptide in the same experiment (results not shown) and therefore recognized a different antigenic site.

Discussion

We have undertaken the production of a collection of monospecific antibodies directed against yeast RNA polymerases. The native multimeric enzyme A was first used as antigen to preserve the possibility of hitting functionally important sites. The immunological response obtained with the yeast enzyme as antigen appeared much higher than with insect (Krämer *et al.*, 1980) or mammal (Christmann and Dahmus, 1981) RNA polymerase B. However, the collection of antibodies produced was markedly biased with respect to specificity since they were directed at only a few RNA polymerase components (A_{135}, A_{49}, A_{43}, or $A_{14\cdot5}$). There were four antibodies to A_{135}, a very immunodominant subunit. Two of these probably recognize the same antigenic site as they bind to the same peptide and have the same inhibitory properties. Two additional antibodies recognized the small polypeptide $A_{14\cdot5}$. In an attempt to isolate a different population of monospecific antibodies, a mouse was immunized against RNA polymerase A which had been dissociated by SDS. One antibody obtained from this fusion recognized the largest subunit A_{190}. It is possible that, by changing the form of the antigen, one also changed the pattern of immunodominant sites. As this antibody binds to the native enzyme bound to nitrocellulose, it could prove useful to study subunit function.

All of the antibodies presented in this study recognized specifically one subunit of RNA polymerase A and did not cross-react with some other component. This is in agreement with our previous conclusion that the various components of this multimeric enzyme are different gene products (Buhler *et al.*, 1976, 1980). Krämer *et al.* (1980) isolated a monoclonal antibody which interacted with the two large subunits of *Drosophila* RNA polymerase B, while Weeks *et al.* (1982) found no cross-reactions between the two large subunits of this enzyme. One possibility for this discrepancy, evoked by Krämer and Bautz (1981), was that a portion of the largest subunit was proteolytically converted to a product co-migrating with the second subunit. Christmann and Dahmus (1981) described a monoclonal antibody directed against the largest subunit of calf thymus RNA polymerase B (subunit IIa). They noticed a slight interaction with two small polypeptides of mol. wt. 20 500 and 16 500. The significance of this cross-reactivity was not investigated.

Using polyspecific antibodies, a core of common subunits was shown to be present in the three forms of yeast RNA polymerases ABC_{27}, ABC_{23}, and $ABC_{14\cdot5}$ (Buhler *et al.*, 1980; Huet *et al.*, 1982). We obtained a monospecific antibody directed against the common polypeptide $A_{14\cdot5}$ which allowed a clear demonstration of the presence of the same determinant in this component of the three enzymes.

Aside from the information which can be gained at the structural level, monospecific antibodies can be a tool to investigate subunit function. An example of this is the inhibitory effect of antibodies A_{135-1} and A_{135-2} on transcription. The enzyme engaged in a binary complex with DNA is protected from inhibition. These results suggest that A_{135} is an essential subunit involved in the basic steps of RNA synthesis and participates in template binding, probably together with other subunits. The reason antibodies A_{135-1} or A_{135-2}, which recognize the same determinant, binds only 50% of the enzyme molecules is unclear. This demonstrates the heterogeneity of the enzyme preparation, distinct from the $A \rightarrow A^*$ transition (Huet *et al.*, 1975). There is the possibility that a fraction of RNA polymerase molecules is already complexed with a small nucleic acid and is therefore protected from the antibody. The mechanism of inhibition by this specific antibody is being further investigated.

The other monoclonal antibodies described here, or by others (Krämer *et al.*, 1981; Christmann and Dahmus, 1981), were not inhibitory in a standard non-specific transcription assay. However, these probes will be important to investigate the function of their target polypeptide in a reconstituted system where the RNA polymerase is likely to interact with specificity factors.

Materials and methods

RNA polymerase and other materials

RNA polymerase A from *Saccharomyces cerevisiae* (4094 B) was purified according to Huet *et al.* (1975), including the phosphocellulose step. RNA polymerase B was purified according to Dezélée *et al.* (1976). RNA polymerase C was obtained by a modification of the procedure described by Wandzilak and Benson (1978). Antibodies raised in rabbits against native RNA polymerase A or its isolated subunits were previously described (Buhler *et al.*, 1980). Monoclonal antibodies were purified by ammonium sulfate precipitation and DEAE-cellulose chromatography as for conventional antibodies (Buhler *et al.*, 1980). Protein A, purchased from Pharmacia, was labelled with ^{125}I to a specific activity of ~35 μCi/μg (Buhler *et al.*, 1980). Protein A-Sepharose was obtained from Pharmacia and Proteinase K from Merck; nitrocellulose membranes were from Schleicher and Schüll (AE 99) or Sartorius (SM 11306).

Cells

The 8-azaguanine resistant mouse myeloma cell line SP2/O-Ag was kindly provided by Dr.Shulman (Shulman *et al.*, 1978). The cell hybrids were grown in Eagle medium reinforced by doubling the amino acids, vitamins, and glucose in the presence of 10 μM azaserine and 50 μM hypoxanthine. This was supplemented with 1 mM pyruvate, 2 mM glutamine, and 10% (v/v) heat inactivated horse serum. Cells were grown in Petri dishes incubated at 37°C in a 10% CO_2 atmosphere.

Immunization and cell fusion

Three-month-old BALB/c mice were injected in the hind footpads with 250 μg RNA polymerase A (in 0.1 ml 50 mM Tris-HCl, pH 8, 50 mM ammonium sulfate, 10% glycerol) emulsified with complete Freund's adjuvant. Three weeks later, a booster injection of 250 μg RNA polymerase A emulsified with incomplete Freund's adjuvant was given. Ten days later, each mouse received a booster injection of 250 μg RNA polymerase A, in saline. Three days after the last immunization, the mouse was killed by neck dislocation, and the spleen removed. The spleen cell suspension was washed three times and resuspended in serum-free medium for fusion. Fusion of spleen cells and the SP2/O-Ag cells was performed on membrane filters, as previously described by Buttin *et al.* (1978), or by the mass fusion technique (Claflin and Williams, 1978). In the two experiments, a 45% (w/v) solution of PEG 1000 (Merck, 9729) was used. Hybrid cells secreting anti-polymerase antibody were cloned by limiting dilution in flat bottom microtiter plates (Falcon, 3040F), in the presence of 1 x 2 10^6 BALB/c fresh thymocytes as a feeder layer. The single clones selected by microscopic examination were reassayed for antibody secretion.

Screening for hybridomas by spot-immunodetection

Positive hybridomas were screened by incubating an aliquot of culture medium with RNA polymerase A (0.4 μg) spotted on a nitrocellulose filter. Bound antibodies were revealed with ^{125}I-labelled Protein A. The filters, prepared as previously described (Huet *et al.*, 1982), were incubated with 0.4 ml culture medium, diluted with 1 volume of phosphate-buffered saline (PBS) x 2 (PBS x 1 is 10 mM phosphate, 140 mM NaCl, 2.7 mM KCl, 0.2% (w/v) Triton X-100, 0.2% (w/v) SDS and 0.5% bovine serum albumin), for ~2 h at 37°C with gentle shaking. Subsequent steps of washing, incubation with ^{125}I-labelled Protein A and autoradiography have been previously described (Huet *et al.*, 1982).

For production of monoclonal antibodies directed against the dissociated enzyme, the same general protocol was followed, with two modifications. The mouse was immunized with RNA polymerase A (250 μg for each injection) dissociated with 0.2% SDS (w/v) at room temperature. In the spot-immunodetection screening, prior to the incubation step with ^{125}I-labelled Protein A, the filters were incubated with rabbit anti-mouse immunoglobulin serum (a gift from C.Leguern) at a 1000-fold dilution in the PBS buffer described above for 2 h at 37°C or 16 h at 0°C. The filters were then washed and incubated with radioactive Protein A as previously described (Huet *et al.*, 1982).

Antibody specificity

RNA polymerase subunits bearing the antigenic site recognized by the antibodies were identified by protein blotting. RNA polymerase A (100 μg) was subjected to electrophoresis under dissociating conditions in the buffer system of Laemmli (1970)in a polyacrylamide slab gel (10 x 9 x 0.1 cm). The subunits were transferred by diffusion for 24 h onto two nitrocellulose sheets placed on each side of the gel (Bowen *et al.*, 1980). After transfer, the membrane was treated as in the spot-immunodetection technique (Huet *et al.*, 1982), first with serum albumin then with the antibody and ^{125}I-labelled Protein A followed by autoradiography. Incubations were carried out in sealed plastic bags, using strips of membrane (0.4 x 10 cm), and 10 μg/ml of purified immunoglobulins or the culture supernatant directly as for the spot-immunodetection.

Mapping antigenic determinants by partial proteolysis

The antigenic site on subunit A_{135} was located after limited proteolysis by an extension of the method of Cleveland *et al.* (1977). Subunit A_{135} was isolated by electrophoresis in polyacrylamide gel in the presence of SDS and subjected to a second SDS-gel electrophoresis in the presence of 10 ng of proteinase K as previously described (Cleveland *et al.*, 1977; Ruet *et al.*, 1980). The peptides obtained were transferred to nitrocellulose and probed with different antibodies (10 μg/ml) as described above.

Effect of antibodies on in vitro transcription

RNA polymerase A (1.2 μg) was preincubated with varying concentrations of purified immunoglobulins in 50 μl of buffer containing 10 mM Tris-HCl (pH 7.4), 2 mM NaCl, and 0.1 mM EDTA, for 1 h at 30°C. RNA synthesis was initiated by addition of 50 μl of a mixture containing 150 mM Tris-HCl, pH 8, 2 mM dithiothreitol, 10 mM $MgCl_2$, 10 μg of calf thymus denatured DNA, 2 mM each of ATP, GTP, CTP, and 1 mM [α^{32}P]UTP (38 c.p.m./pmol) and stopped after 20 min incubation at 30°C. RNA was estimated by the conventional acid precipitation technique on nitrocellulose filters (HAWP 025). A control experiment showed that enzyme activity was not affected by the preincubation step, up to 2.5 h at 30°C.

Binding of the antibodies to RNA polymerase was estimated in a parallel experiment. After the preincubation step, the RNA polymerase-antibodies mixture was supplemented with a 50 μl suspension in the same buffer of Protein A-Sepharose (100 μg of Protein A) and further incubated for 30 min at 0°C with gentle shaking. After centrifugation of the Sepharose, enzymatic activity was assayed on 50 μl of the supernatant supplemented as above with 50 μl of the transcription mixture.

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References

Bowen,B., Steinberg,J., Laemmli,U.K., and Weintraub,H. (1980) *Nucleic Acids Res.*, **8**, 1-20.

Buhler,J.M., Huet,J., Davies,K.E., Sentenac,A., and Fromageot,P. (1980) *J. Biol. Chem.*, **255**, 9949-9954.

Buhler,J.M., Iborra,F., Sentenac,A., and Fromageot,P. (1976) *J. Biol. Chem.*, **251**, 1712-1717.

Buttin,G., Leguern,C., Phalente,L., Lin,E.C.C., Medrano,L., and Cazenave,P.A. (1978) in Melchers,F., Potter,M., and Warner,B. (eds.), *Current Topics in Microbiology and Immunology*, vol. **81**, Springer Verlag, Berlin/Heidelberg, pp. 27-36.

Christmann,J.L., and Dahmus,M.E. (1981) *J. Biol. Chem.*, **256**, 11798-11803.

Claflin,L. and Williams,K. (1978) in Melchers,F., Potter,M., and Warner,B. (eds.), *Current Topics in Microbiology and Immunology,* vol. **81**, Springer Verlag, Berlin/Heidelberg, pp. 107-109.
Cleveland,D.W., Fisher,S.G., Kirschner,M.W., and Laemmli,U.K. (1977) *J. Biol. Chem.,* **252**, 1102-1106.
Dezélée,S., Wyers,F., Sentenac,A., and Fromageot,P. (1976) *Eur. J. Biochem.,* **65**, 543-552.
Dinassayake,S., and Hay,F.C. (1975) *Immunochemistry,* **12**, 101.
Ey,P.L., Prowse,S.J., and Jenkin,C.R. (1978) *Immunochemistry,* **15**, 429-436.
Hager,G., Holland,M., Valenzuela,P., Weinberg,F., and Rutter,W.J. (1976) in Losick,R., and Chamberlin,M. (eds), *RNA Polymerase,* Cold Spring Harbor Laboratory Press, NY, pp. 745-761.
Huet,J., Buhler,J.M., Sentenac,A., and Fromageot,P. (1975) *Proc. Natl. Acad. Sci. USA,* **72**, 3034-3038.
Huet,J., Sentenac,A., and Fromageot,P. (1982) *J. Biol. Chem.,* **257**, 2613-2618.
Krämer,A., and Bautz,E.K.F. (1981) *Eur. J. Biochem.,* **117**, 449-455.
Krämer,A., Haars,R., Kabisch,R., Will,H., Bautz,F., and Bautz,E.K.F. (1980) *Mol. Gen. Genet.,* **180**, 193-199.
Laemmli,U.K. (1970) *Nature,* **227**, 680-685.
Roeder,R.G. (1976) in Losick,R., and Chamberlin,M. (eds.), *RNA Polymerase,* Cold Spring Harbor Laboratory Press, NY, pp. 285-330.
Ruet,A., Sentenac,A., Fromageot,P., Winsor,B., and Lacroute,F. (1980) *J. Biol. Chem.,* **255**, 6450-6455.
Sentenac,A., Dezélée,S., Iborra,F., Buhler,J.M., Huet,J., Wyers,F., Ruet, A., and Fromageot,P. (1976) in Losick,R., and Chamberlin,M. (eds.), *RNA Polymerase,* Cold Spring Harbor Laboratory Press, NY, pp. 763-778.
Shulman,M., Wilde,C.D., and Köhler,G. (1978) *Nature,* **276**, 269.
Wandzilak,T.M., and Benson,R.W. (1978) *Biochemistry (Wash.),* **17**, 426-431.
Weeks,J.R., Coulter,D.E., and Greenleaf,A.L. (1982) *J. Biol. Chem.,* **257**, 5884-5891.