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Archaebacteria and eukaryotes possess DNA-dependent RNA polymerases of a common type

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DNA-dependent RNA polymerases of archaebacteria not only resemble the nuclear RNA polymerases of eukaryotes rather than the eubacterial enzymes in their complex component patterns but also show striking immunochemical, i.e., structural, homology with the eukaryotic polymerases at the level of single components. Thus, eukaryotic and archaebacterial RNA polymerases are indeed of the same type, distinct from the eubacterial enzymes, which, however, are also derived from a common ancestral structure.

Key words: archaebacteria/eukaryotes/DNA-dependent RNA polymerases

Introduction

The comparision of catalogues of 16S and 18S rRNA sequences (Woese *et al.*, 1978; Fox *et al.*, 1980; Woese and Gupta, 1981), supported by the recognition of characteristic features of the membrane lipids (De Rosa *et al.*, 1976), the cell envelope (Kandler, 1979) other components of the translation apparatus (Matheson and Yaguchi, 1982; Yaguchi *et al.*, 1982; Schmid *et al.*, 1982) and the DNA-dependent RNA polymerases (Zillig *et al.*, 1982a, 1982b), has led to the division of the prokaryotes into two quite distinct urkingdoms, that of the eubacteria and that of the archaebacteria. A growing body of evidence, however, appears to indicate a specific relationship between several corresponding archaebacterial and eukaryotic proteins as for example the elongation factors 2 (EFs-2) (Kessel and Klink, 1980), ribosomal A protein (Matheson and Yaguchi, 1982; Yaguchi *et al.*, 1982), glycoproteins (Mescher and Strominger, 1975; Yang and Haug, 1979) and DNA-dependent RNA polymerase component patterns (Zillig *et al.*, 1982a, 1982b).

Eubacteria possess single RNA polymerases, with the basic structure $\beta\beta'\alpha_2\sigma$. In some cases, additional binding proteins are known. The specificities depend on the particular factor used (for a review, see Doi, 1982). In contrast, all eukaryotes contain three distinct types of RNA polymerases, A(I), B(II) and C(III), with different transcription specificities (Roeder, 1976), each consisting of >10 components of which some are common to different types (Huet *et al.*, 1982). Like eubacteria, archaebacteria appear to possess single RNA polymerases though enzyme fractions with additional components, possibly representing different forms, have been observed. The archaebacterial polymerases have >10 peptide components, which resemble those of eukaryotic RNA polymerases (Zillig *et al.*, 1982a, 1982b). Furthermore, transcription by archaebacterial RNA polymerases is stimulated by the flavonolignane derivative silybin (Machicao and Sonnenbichler, 1977; Schnabel *et al.*, 1982) as is eukaryotic RNA polymerase A(I). Unlike that by eubacterial RNA polymerases, the transcription is unaffected by rifampicin and streptolydigin.

Polyclonal antibodies are a suitable tool for investigating the phylogenetic relationship between proteins (Prager *et al.*, 1980). For eukaryotic RNA polymerases, a phylogenetic relationship between components of several very distant species could be demonstrated in this way (Huet *et al.*, 1982). We show here that several components of the RNA polymerases of archaebacteria are indeed homologous to components of eukaryotic polymerases.

Results

Spot reactions

We have spotted six archaebacterial and three eubacterial RNA polymerases, two of the former from recently discovered sulfur metabolizing archaebacteria (*Thermoproteales*) (Zillig *et al.*, 1982c, 1982d), on nitrocellulose filters and challenged them with antibodies raised against the native RNA polymerases A(I) and B(II) from yeast and against their single components. The immunological cross-reactions were visualized by autoradiography of iodinated protein A bound to the antigen-linked immunoglobulins.

Antibodies against native yeast polymerase A react with seven of the nine polymerases tested (Figure 1a). Antibodies against native polymerase B react with four of the six archaebacterial enzymes but not with the eubacterial polymerases (Figure 1b). For a more detailed analysis, antibodies against the single components were used. Antibodies against the two large components of polymerase A(I), A_{190} and A_{135}, cross-react with the archaebacterial enzymes, with only one exception. Only that against A_{190} shows a reaction with a eubacterial (*Escherichia coli*) polymerase (Figure 1a). It is surprising that the reaction of both anti-A_{190} and anti-A_{135} with some archaebacterial polymerases is stronger than that with polymerase B of yeast itself. Upon longer exposure of the autoradiogram, antibodies against smaller components were found to react with the polymerases of the following archaebacteria (Figure 1a): anti-A_{49} with *Thermoplasma acidophilum* and *Halobacterium halobium*, anti-A_{43} with *T. acidophilum*, anti-A_{40} with *Sulfolobus acidocaldarius, Desulfurococcus mucosus, H. halobium* and *Methanobacterium thermoautotrophicum,* anti-$A_{34.5}$, anti-A_{27} and anti-$A_{14.5/14}$ with *H. halobium*.

Anti-B_{220} and anti-B_{185} (B_{185} is a proteolytic product of B_{220}, see Huet *et al.*, 1982), react strongly with the enzymes of four archaebacteria, *S. acidocaldarius, D. mucosus, Thermoproteus tenax* and *H. halobium*, and to a much lower extent with the polymerases of the eubacteria *E. coli* and *Lactobacillus curvatus* (Figure 2). Anti-B_{150} only reacts with the enzymes from *T. tenax* and *H. halobium*, but again the reaction is stronger than with polymerase A. On average, the reaction of the antibodies against the large components of both of the yeast RNA polymerases with the archaebacterial enzymes exceeds that with the eubacterial enzymes by a factor of five. A semi-quantitative estimation was made by scanning the autoradiograms (Figure 2).

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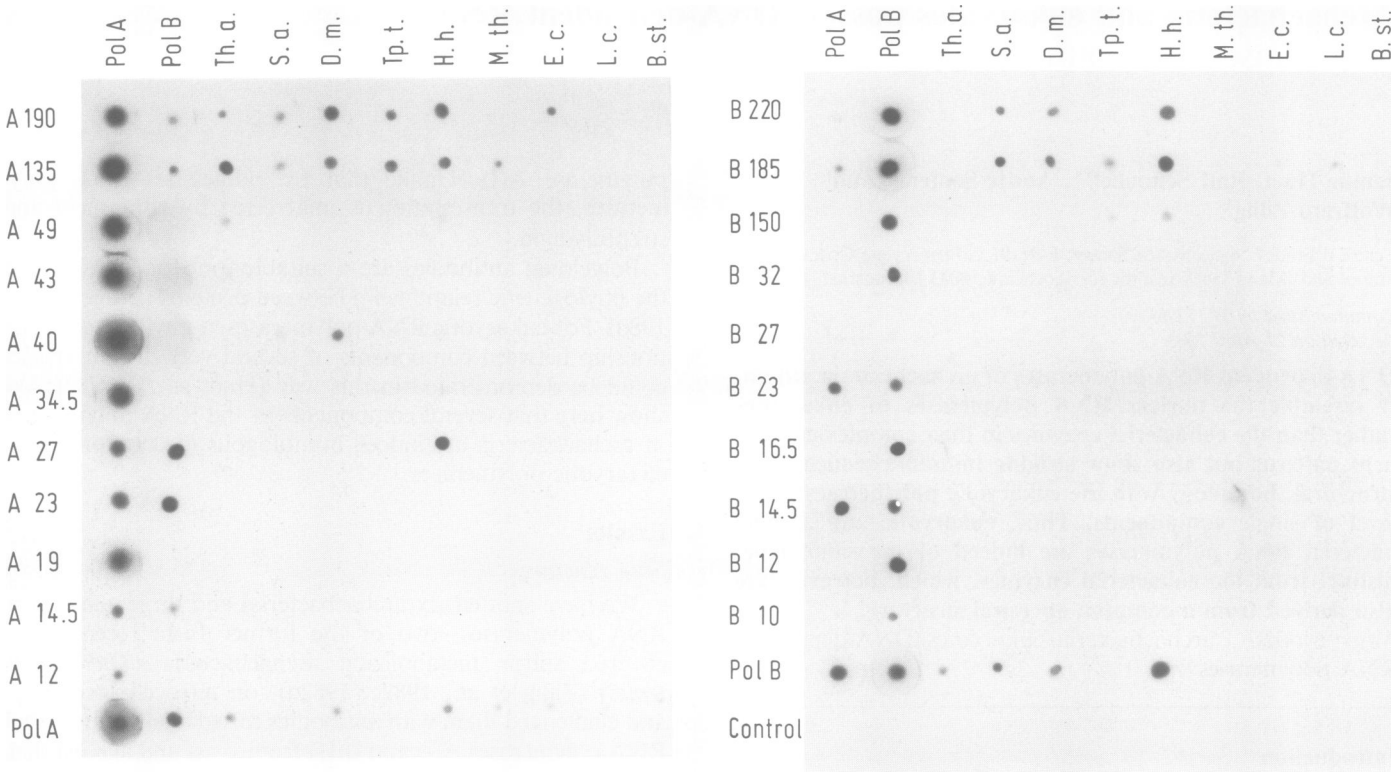

Fig. 1. Immunological cross-reaction of different DNA-dependent RNA polymerases with antibodies raised against native polymerase A (PolA) (**a**) and B (PolB) (**b**) of yeast and single components of these enzymes, as specified by their mol. wts.

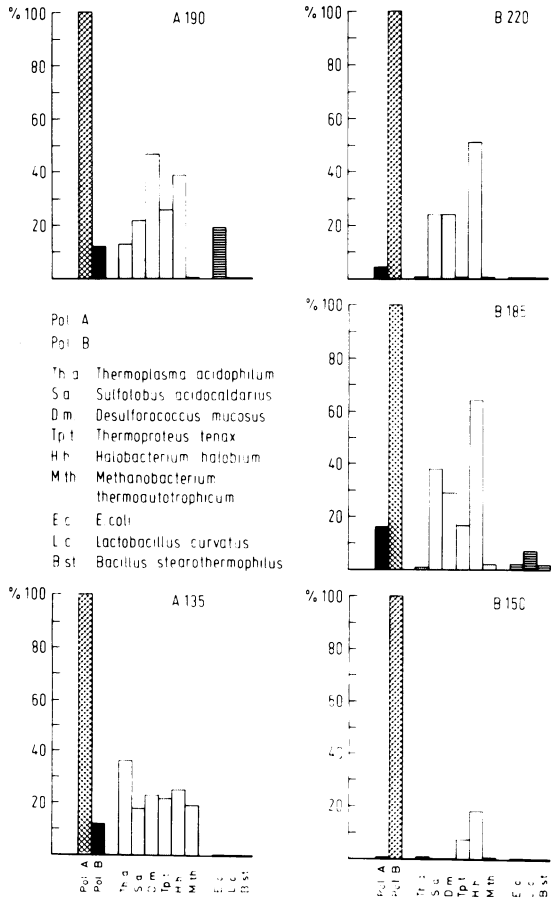

Fig. 2. Quantification of the immunological cross-reaction shown in Figure 1.

Reactions against single components

In 'Western blots' (patterns of RNA polymerase components transferred from SDS-polyacrylamide slab gels to nitrocellulose sheets), antibodies against native polymerase A(I) react with the components B and C of *T. acidophilum* polymerase (for nomenclature of components, see Zillig *et al.*, 1982c), with component A of *S. acidocaldarius* and *D. mucosus* polymerase and components A and B of the *T. tenax* and *H. halobium* enzymes (Figure 3a). Antibodies against the single component A_{190} react with the second largest component, and antibodies against the single component A_{135} with the heaviest component of the polymerases of the thermoacidophilic archaebacteria. This shows that the two large components of the thermoacidophilic archaebacteria appear in reversed order compared with those of the yeast enzyme (Figure 3a). This is the reason for renaming the largest components of the thermoacidophiles B and the second largest A (Zillig *et al.*, 1982c). In contrast, in the pattern of the *H. halobium* polymerase, the two largest components appear in the same order as in yeast polymerase. The weak reaction of the *Thermoproteus* RNA polymerase component A with the antibody against yeast component A_{135} is an exception. The cross-reaction of *Thermoproteus* A with the antibody against yeast A_{190} is much stronger, and both components A and B of *Thermoproteus* react strongly with antibodies against yeast B_{185} and B_{150} in the expected way. The results obtained with antibodies against the components of yeast RNA polymerase B correspond to those obtained with antibodies against component A (Figure 3b).

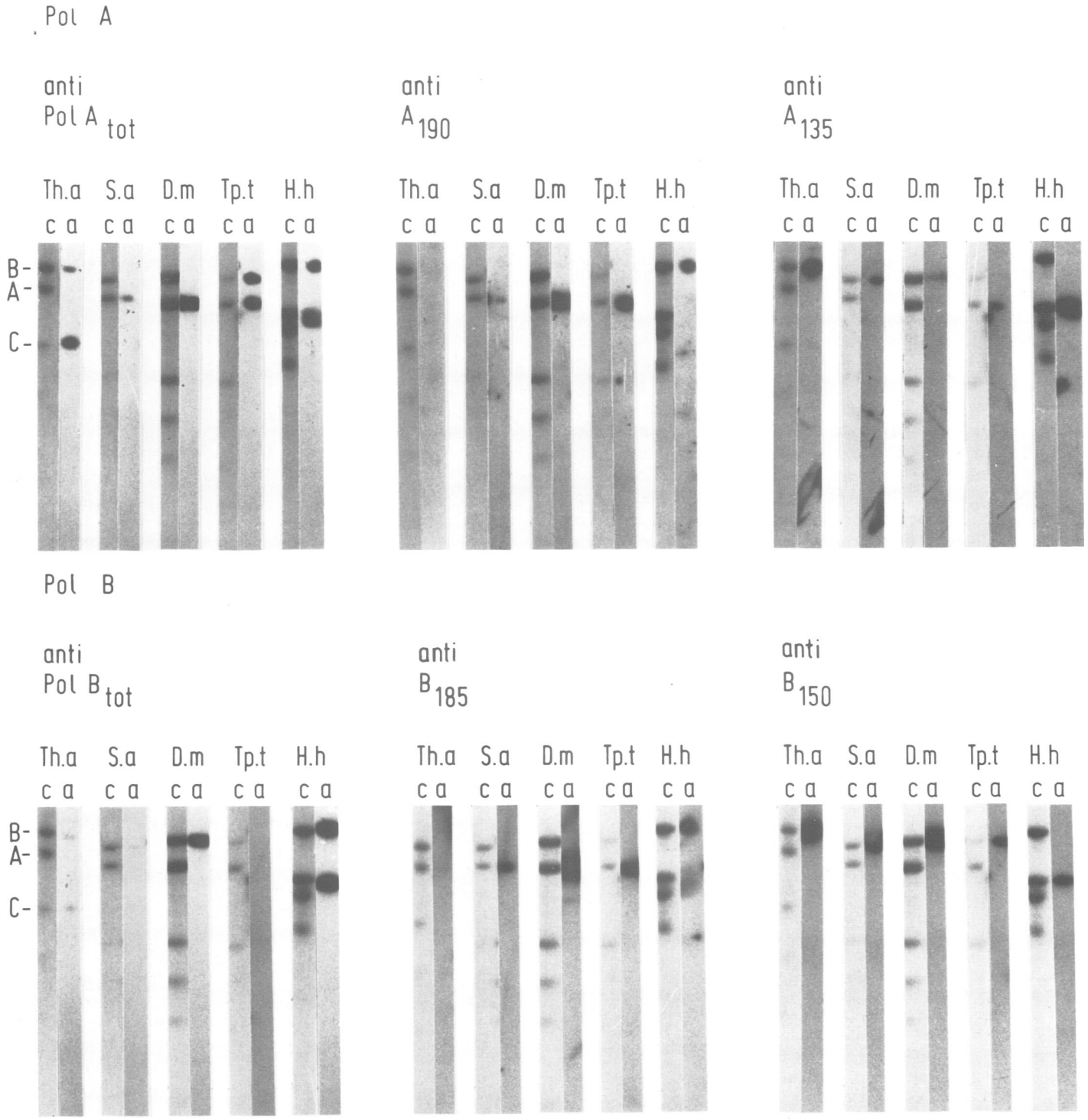

Fig. 3. Immunological cross-reaction of antibodies against the native RNA polymerases and the two largest components of polymerase A (**a**) and B (**b**) of yeast with the components of the archaebacterial RNA polymerases separated on SDS-polyacrylamide gels and transferred to nitrocellulose sheets by diffusion (Western blots). The tracks labelled c show the protein patterns after blotting of the gels as visualized by Coomassie blue staining, the tracks labelled a, the autoradiographs of the corresponding immunoblots.

Discussion

The immunochemical cross-reaction of the RNA polymerases of all three urkingdoms shows that these enzymes have a common root which is probably conserved in the two largest components of the enzymes. The cross-reactions of antibodies against smaller components of yeast with components of archaebacterial enzymes show clearly that both eukaryotic and archaebacterial polymerases must be derived from a common ancestor of a comparably complex pattern. The larger homology of yeast RNA polymerase A with archaebacterial enzymes than that of yeast RNA polymerase B, suggests that polymerase A has a more ancestral (conserved) structure.

The strikingly large homology between archaebacterial RNA polymerases and both RNA polymerases A and B, in contrast to the rather low homology between the large

subunits of the two yeast enzymes themselves, suggests that the archaebacterial enzymes have conserved antigenic groups that have been lost from one or the other yeast polymerase. This implies that the separation of the A and the B types of eukaryotic RNA polymerases occurred after the branching of the eukaryotes from ancestral archaebacteria.

The analysis of the observed cross-reactions with separated components confirms that, in the case of *T. acidophilum*, component C has its counterparts in the polymerases A(I) and B(II) of yeast. The reactions of the antibodies against the two largest components are in agreement with recent results with antibodies against the single components of archaebacterial polymerases (Schnabel *et al.*, 1983). The two largest components of the enzymes of the thermoacidophilic and/or sulfur metabolizing branch are in reverse order compared with those of yeast and those of the methanogenic/halophilic branch of the archaebacteria. We propose to use the order of components in yeast as reference (Schnabel *et al.*, 1983).

In summary, the following conclusions can be drawn from these experiments. (a) Since cross-reactions of antibodies against eukaryotic RNA polymerases with enzymes from archaebacteria as well as from eubacteria have been observed, the DNA-dependent RNA polymerases of all three urkingdoms, are rather conserved molecules. This relationship is not limited to the two large components. (b) Our data show that two of the three urkingdoms, the eukaryotes and the archaebacteria, indeed have a common type of RNA polymerase, as previously suggested by the similarities of their component patterns. This is in accord with other evidence (see Introduction) indicating a closer phylogenetic relationship of both with each other, than with the eubacteria.

Materials and methods

Isolation of RNA polymerases

The RNA polymerases were isolated according to the procedures described earlier (Huet *et al.*, 1982; Sturm *et al.*, 1980; Zillig *et al.*, 1970, 1978, 1979; Prangishvilli *et al.*, 1982; Stetter *et al.*, 1980; Stetter and Zillig, 1974; Rexer, 1976).

Preparation of antibodies

Antibodies were prepared according to the procedure of Buhler *et al.* (1980).

Immunological test of spotted RNA polymerases

The RNA polymerases (0.4 μg) were spotted in 2 μl on nitrocellulose filters and dried. In the case of *S. acidocaldarius* and *M. thermoautotrophicum* 0.2 μg were used. The incubation with antibodies and the detection with ^{125}I-labelled protein A of *Staphylococcus aureus* was carried out as described by Huet *et al.* (1982). As a control, IgGs of a normal rabbit were used. Autoradiograms were exposed for 30 min.

Quantification of the immunological cross-reactions

Spots were scanned with a microdensitometer (Joyce, Loeble and Co., Ltd.). A linear correlation between bound radioactivity and optical density was established by a calibration curve over the density range used. The homologous reactions were taken as 100%. Due to uncertainties in the protein determination and the scanning procedure, the values should be considered as semi-quantitative.

Blotting of component patterns

Components separated by gel electrophoresis were transferred to nitrocellulose (S&S, BA85, 0.45 μm) by diffusion. Gels were equilibrated for 30 min in blotting buffer (50 mM NaCl, 2 mM EDTA, 1 mM β-mercaptoethanol, 10 mM Tris/HCl pH 7.4) and packed between two nitrocellulose sheets. For a gel of 10 x 12 cm, a weight of 1 kg was placed on the sandwich. The transfer was carried out for 24 h. Proteins not completely transferred were visualized by Coomassie blue staining.

Protein determination

Protein concentrations were determined as described by Bradford (1976).

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