

# Structural homology between different archaebacterial DNA-dependent RNA polymerases analyzed by immunological comparison of their components

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Communicated by W. Zillig

Received on 21 February 1983

The archaebacterial DNA-dependent RNA polymerases have a complex structure containing eight or more components. Immunochemical analysis shows an extensive homology between the components of the enzymes of nine different species. Two enzyme subtypes can be distinguished: that of the thermoacidophilic and/or sulfur-metabolizing archaebacteria with the composition BACDEFGHIJ and that of the methanogenic plus halophilic archaebacteria with the composition ABB'C(D).... Components B and B' of the latter subtype probably evolved by the division of the large component B of the BACD... type enzyme. The existence of the two subtypes corroborates the division of the archaebacteria into two phylogenetic main branches.

**Key words:** archaebacteria/RNA polymerase/components/immunology/evolution

## Introduction

DNA-dependent RNA polymerases have recently been isolated from many species of archaebacteria, representing all phylogenetic orders known at present (Sturm *et al.*, 1980; Zillig *et al.*, 1979, 1983; Prangishvilli *et al.*, 1982; Stetter *et al.*, 1978, 1980; Thomm and Stetter, unpublished data; for review, see Zillig *et al.*, 1982a, 1982b). They are distinct from those of the eubacteria in several ways. The archaebacterial enzymes are not affected by the antibiotics rifampicin and streptolydigin, which are strong inhibitors of eubacterial transcription. They are more complex than their eubacterial counterparts, each containing ~10 different components, normally each present once per monomer, much like the subunits of eukaryotic RNA polymerases (Roeder, 1976). The stimulation of transcription by silybin (Schnabel *et al.*, 1982) and cross-reactions with antibodies raised against yeast RNA polymerases A(I) and B(II) (Huet *et al.*, in preparation) show that the polymerases of archaebacteria and eukaryotes have a close phylogenetic relationship, i.e., they represent one and the same type of enzyme, whereas the eubacterial enzymes with the basic structure  $\beta\beta'\alpha_2\sigma$ , sometimes modified by additional binding proteins, represent another type. The component patterns of the archaebacterial enzymes in SDS-polyacrylamide gels are very similar to each other suggesting homologous components and possibly homologous functions. This paper reports experiments which prove the homology of the component patterns of all known DNA-dependent RNA polymerases of archaebacteria by immunological methods.

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## Results

Homologies between the components of nine archaebacterial DNA-dependent RNA polymerases were visualized by challenging the components separated by SDS-gel electrophoresis and transferred to nitrocellulose filters with antibodies prepared against the single components of the RNA polymerases of *Sulfolobus acidocaldarius* and *Methanobacterium thermoautotrophicum*. Bound antibodies were either visualized with <sup>125</sup>I-labelled protein A or with peroxidase-coupled goat antibodies directed against rabbit  $\gamma$ -globulins.

### Purity of antibodies

As revealed by SDS-gel electrophoresis, the preparatively purified components used for immunization were free of contaminants, with the exception of the two largest components which were slightly contaminated with each other (data not shown).

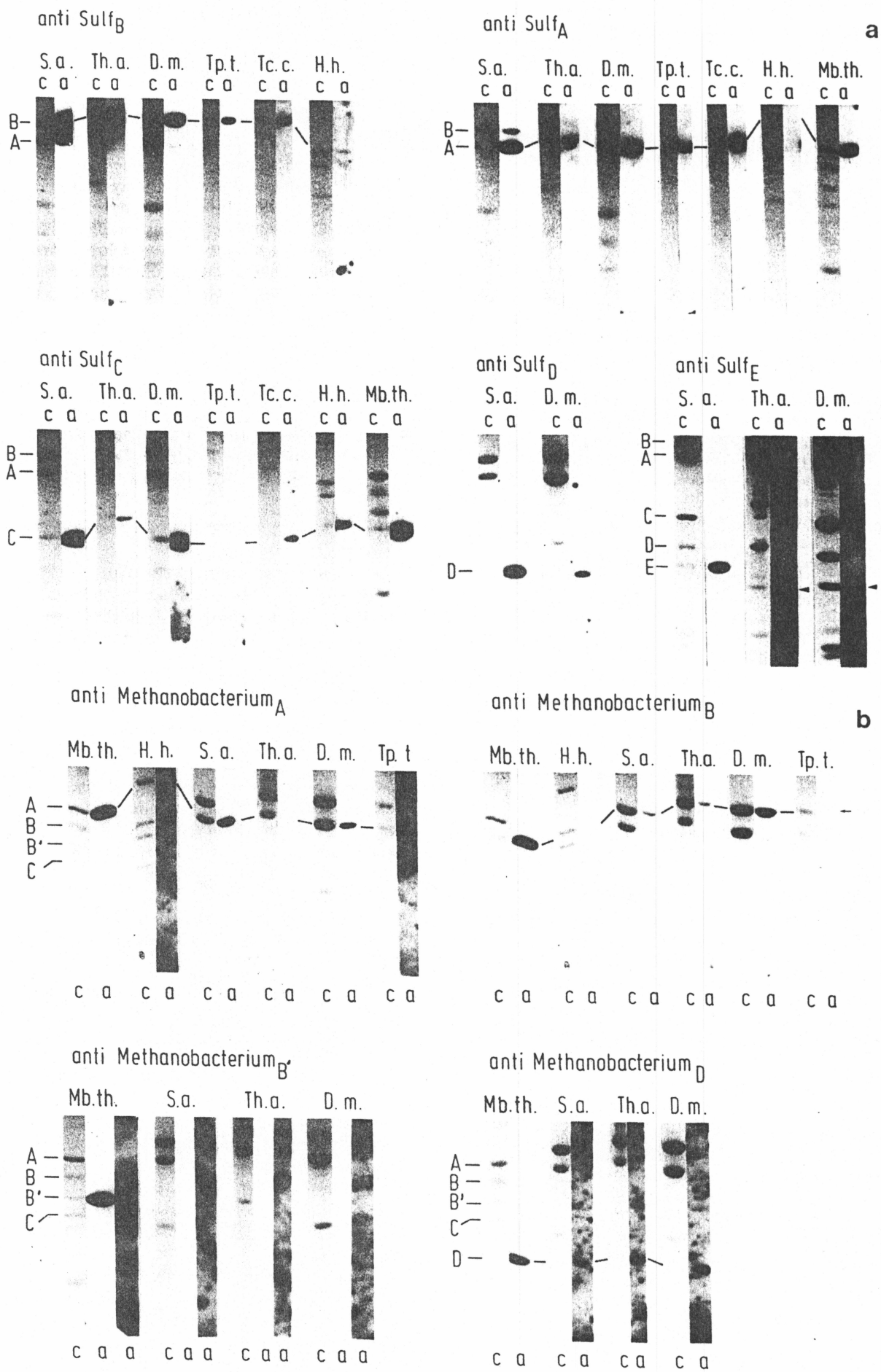
### Reactions with antibodies against *S. acidocaldarius* RNA polymerase

Antibodies raised against the largest component of the RNA polymerase of *S. acidocaldarius* react with the largest components of the enzymes of *Thermoplasma acidophilum*, *Desulfurococcus mucosus*, *Thermoproteus tenax* and *Thermococcus celer*, with the second largest component of *Halobacterium halobium* and with the second and third component of the RNA polymerase of *M. thermoautotrophicum*, *Methanococcus thermolithotrophicus* and *Methanosarcina barkeri* (see Figure 1a and c). Antibodies against the second largest component react with the second largest components of the enzymes of *T. acidophilum*, *D. mucosus*, *T. tenax* and *T. celer* and with the largest component of the enzyme of *M. thermoautotrophicum* (Figure 1a). Antibodies against the third component react with the third components of the enzyme of *T. acidophilum*, *D. mucosus*, *T. celer* and the fourth components of the enzymes of *H. halobium* and *M. thermoautotrophicum*. Antibodies against the fourth component react only with the fourth component of the RNA polymerase of *D. mucosus*. Those against the fifth component react faintly with the fifth component of the enzymes of *T. acidophilum* and *D. mucosus*. Antibodies raised against the further five components only react in the homologous reaction against the RNA polymerase of *Sulfolobus* (data not shown).

### Reactions with antibodies against the RNA polymerase of *M. thermoautotrophicum*

Antibodies against the largest component of the RNA polymerase of *M. thermoautotrophicum* react with the largest component of each of the enzymes of *M. thermolithotrophicus*, *M. barkeri* (Figure 1c) and *H. halobium*, and with the second largest component of each of those of *S. acidocaldarius*, *T. acidophilum*, *D. mucosus* and *T. tenax* (Figure 1b).

Antibodies against the second largest component of the enzyme react with the second largest component of each of the RNA polymerases of *M. thermolithotrophicus* and *M. barkeri* (Figure 1c) and the largest each of *S. acidocaldarius*,



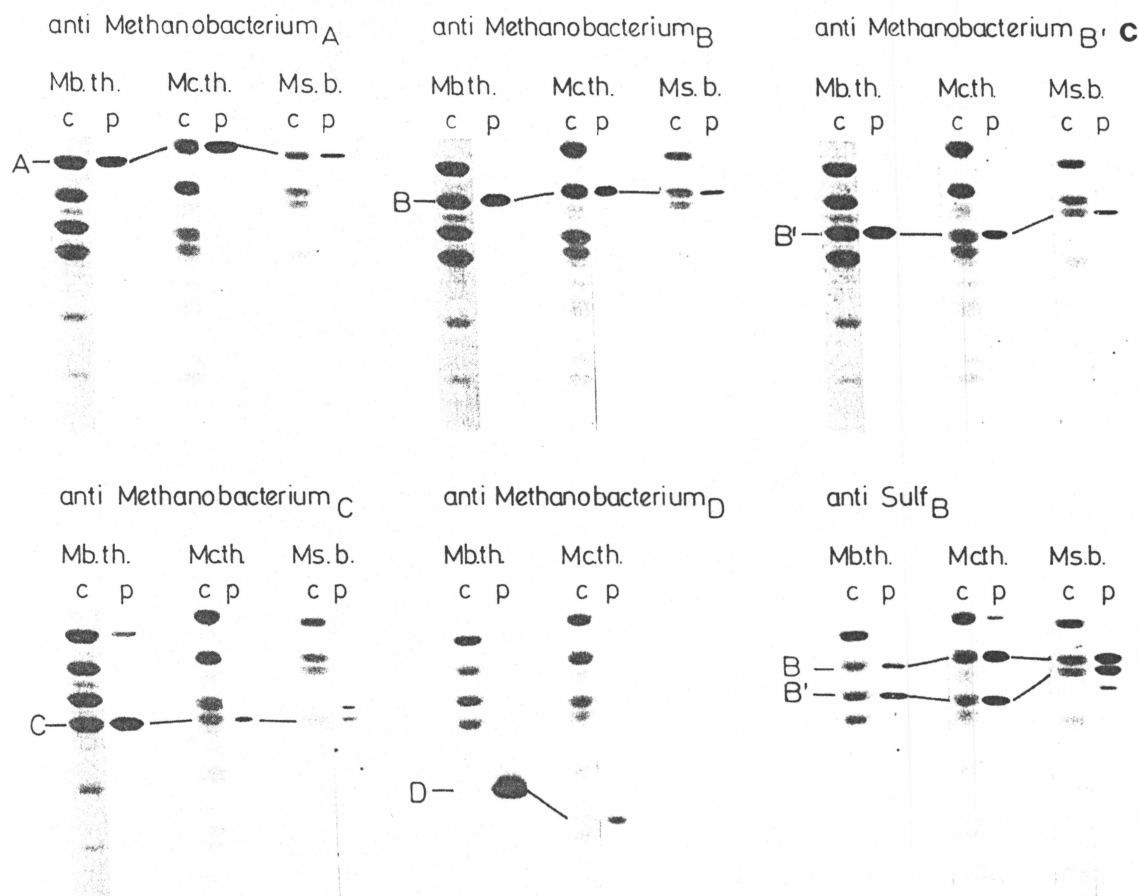


Fig. 1. Immunological cross-reactions of components of archaeobacterial RNA polymerases with antibodies raised against single components of the RNA polymerases of *S. acidocaldarius* (a and c) and *M. thermoautotrophicum* (b and c). 'Western blots' (proteins separated by gel electrophoresis and transferred to nitrocellulose) were challenged with the antibodies. Tracks labelled c show the component patterns visualized by Coomassie blue staining after transfer. Those labelled a show the autoradiographs of the corresponding immune reactions. Tracks labelled p show bound antibodies visualized with peroxidase-coupled antibodies.

*T. acidophilum*, *D. mucosus* and *T. tenax* (Figure 1b). Antibodies against the third component react with the third component each of *M. thermolithotrophicus* and *M. barkeri* but, like the antibodies against the second component, with the largest component of each of the enzymes of *S. acidocaldarius*, *T. acidophilum* and *D. mucosus* (Figure 1b). Those against the fourth component react with the fourth components of the enzymes of *M. thermolithotrophicus* and *M. barkeri* (Figure 1c). Antibodies against the fifth component react with the fourth components of *S. acidocaldarius*, *T. acidophilum* and *D. mucosus* (Figure 1b) and the fifth component of *M. thermolithotrophicus* (Figure 1c). Antisera against even smaller components did not show any reactions.

#### Proteolysis products

Proteolysis products of larger components should react with antibodies against their precursor proteins and *vice versa*. A typical example is the RNA polymerase of *M. thermoautotrophicum* which sometimes contains a polypeptide slightly smaller than the fourth component. Antibodies prepared against the additional protein react with the largest component of the same enzyme (Figure 2), therefore, it is most probably a proteolysis product of this component. This also explains the additional reactions seen with antibodies against the fourth component of the enzyme of *Methanobacterium* in Figure 1c.

In the case of *Sulfolobus* RNA polymerase, only one such

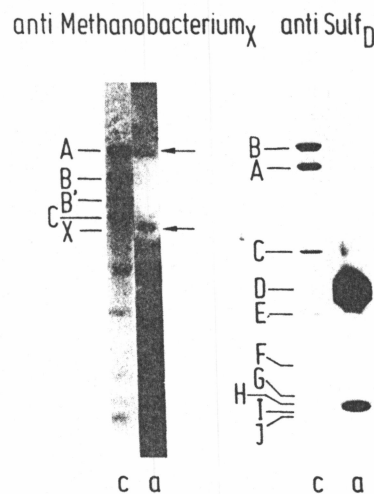


Fig. 2. Cross-reactions of proteolytic proteins in the RNA polymerases of *S. acidocaldarius* and *M. thermoautotrophicum*.

cross-reaction has been detected. The fourth component shows a reaction with a smaller component most probably with the eighth (Figure 2). Antibodies against the largest component of *Sulfolobus* RNA polymerase cross-react not only with the second and third component of the enzyme of *M. barkeri* but also with a proteolytic product of either one.

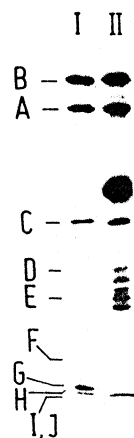


Fig. 3. SDS-polyacrylamide gel of the RNA polymerase of *S. acidocaldarius* isolated by immunoprecipitation with antibodies against the two largest components (II). RNA polymerase isolated by the normal procedure (I). For details see Materials and methods.

### Immune precipitation

SDS-polyacrylamide gel electrophoresis shows that enzyme prepared from a crude extract of cells by immunoprecipitation with antibodies against the two largest components of *Sulfolobus* RNA polymerase lacks component number eight (Figure 3).

### Discussion

Archaeobacterial DNA-dependent RNA polymerases consist of 8–10 components, considering only proteins which co-purify with the enzyme activity through at least three isolation steps. The component patterns in SDS-polyacrylamide gels suggest the existence of two subtypes of RNA polymerase (Zillig et al., 1982a, 1982b). The enzymes of the thermoacidophilic and/or sulfur-metabolizing bacteria contain three large components while those from the halophilic and the methanogenic archaeobacteria contain four large components. The results of the analysis of component homologies prove this hypothesis. In order to analyze homologies on the structural, i.e., the sequence, level antibodies against the single components of the RNA polymerases of two species, *S. acidocaldarius* and *M. thermoautotrophicum*, were used. These two represent the two enzyme subtypes, i.e., the two phylogenetic branches of the archaeobacteria defined by 16S rRNA data (Fox et al., 1980; Tu et al., 1982). Immunological cross-reactions not only indicate structural similarities between enzyme components but also imply functional analogy.

### Nomenclature

On the basis of the immunologically established homologies of the constituents of archaeobacterial polymerases, we propose to name the components by capital Roman letters, generally, but not strictly, following the order of their mol. wts. The order of the large components of the RNA polymerases A (I) and B (II) of yeast which cross-react with the large components of the archaeobacterial enzymes was used for reference, of course arbitrarily. Compared with the yeast RNA polymerases, the two largest components of the enzymes of the thermoacidophilic and/or sulfur-metabolizing branch of archaeobacteria appear in reverse order. Therefore, the largest components of these were named B and the second largest A. As presented in the Results,

the order of mol. wts. of the components is conserved in all RNA polymerases from the thermoacidophilic and/or sulfur-metabolizing archaeobacteria. Therefore, the components are named B A C D E F G H I J in order of decreasing mol. wt. For the smallest components (from F onward), the nomenclature remains tentative since cross-reactions are lacking. The largest component of the RNA polymerases from the methanogenic/halophilic branch of the archaeobacteria is homologous to component A of the thermoacidophilic and/or sulfur-metabolizing bacteria and is therefore also called A. Both the second and third components of the methanogenic/halophilic RNA polymerase type are homologous to different parts of component B of the thermoacidophilic/sulfur-metabolizing type and are therefore named B and B'. The fourth component is called C because it is homologous to the third component of the thermoacidophilic/sulfur-metabolizing polymerase type. The fifth components of the enzymes of *M. thermoautotrophicum* and *M. thermolithotrophicus* are homologous to component D of the polymerases from thermoacidophilic/sulfur-metabolizing archaeobacteria. Hence, the general formula for the methanogenic/halophilic enzyme type can be written ABB'C(D)...

### Evolution of component B

As already mentioned, the large component B of the thermoacidophilic/sulfur-metabolizing enzyme type corresponds to two separate smaller components in the other type. This could be explained either by gene fusion or division depending on the direction of evolution, or by post-translational processing of a precursor. We favour the division hypothesis since *Thermoplasma* has the BAC... type of enzyme and 16S rRNA catalogues (Fox et al., 1980) and cross-hybridisation experiments (Tu et al., 1982) show that the lineage leading to *Thermoplasma* branched off from the sulfur-metabolizing archaeobacteria before the methanogenic/halophilic branch separated from *Thermoplasma*. Hence the BAC... type of enzyme seems to be the elder one. From our experiments, we cannot conclude if, in the halophiles and methanogens, one gene was split into two, or if post-translational processing takes place. However, we can exclude artefactual proteolysis since the content of both components is invariably one each per enzyme monomer in all enzymes of this branch.

### What is a component?

Proteolysis of components yielding new peptides which may appear similar to additional components can be a problem during enzyme preparation. With the exception of the described cross-reaction between a small component, probably H, and component D, no cross-reactions between components of the RNA polymerase of *Sulfolobus* have been observed. It is, therefore, most unlikely that any of these components are proteolytic products. Minor cross-reactions between components A and B are due to slight cross-contaminations of the antigens used. Component H appears to be present in four copies in *Sulfolobus* RNA polymerase. In fact, this component is missing from an enzyme isolated by immunoprecipitation. It is, however, always present in the enzyme isolated by the normal procedure. Therefore, this protein could be a binding protein rather than a true component. It is possible that band H is a mixture of different polypeptides of which one is a proteolysis product of component D which often appears as two bands. The smaller of these may arise by proteolysis.

### Phylogenetic implications

Besides the 16S rRNA data (Fox *et al.*, 1980; Tu *et al.*, 1982), the existence of two types of RNA polymerases is a further strong argument for the division of the archaeobacteria into two main branches, that of the methanogens and halophiles and that of the thermoacidophiles and/or sulfur-metabolizing archaeobacteria.

### Materials and methods

#### Isolation of RNA polymerases

The RNA polymerases were isolated according to procedures published earlier (Sturm *et al.*, 1980; Zillig *et al.*, 1979, 1983; Prangishvilli *et al.*, 1982; Stetter *et al.*, 1978, 1980; Thomm and Stetter, unpublished data).

#### Preparation of the components

In one step, 0.8 mg of purified RNA polymerase was separated preparatively on a 1.5 mm SDS-polyacrylamide gel. Proteins were visualized by equilibration of the gel with 0.1 M KCl solution at 4°C for at least 30 min. The components were collected by cutting the gel into strips. The proteins were extracted from the gel by homogenizing the strips with 3 ml of a 0.175 M NaCl, 0.1% SDS solution. The suspension was kept at 4°C overnight and centrifuged at 100 000 g for 30 min. The protein concentration of the supernatant was estimated by comparing 20 µl aliquots with a dilution series of the same RNA polymerase with known concentration on a SDS-polyacrylamide gel stained with Coomassie blue.

#### Preparation of antibodies

Rabbits were immunized according to the schedule described in Stetter (1977). In each immunization step the amount of component used corresponded to an equivalent of 0.1 mg total RNA polymerase.

#### Gel electrophoresis

Polyacrylamide gel electrophoresis was carried out as described previously (Stetter *et al.*, 1978).

#### 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.

#### Immunological reactions

The immunological reactions and the visualization with protein A was carried out according to Huet *et al.* (1982). For the results presented in Figure 1c, peroxidase-coupled antibodies were used as described by Towbin *et al.* (1979).

#### Immunoprecipitation

The immunoprecipitation with antibodies against the largest component was carried out as published earlier (Zillig *et al.*, 1980).

### Acknowledgements

We thank Petra Frischeisen and Volker Schwass for excellent technical assistance. The work was supported by a grant from the Deutsche Forschungsgemeinschaft to K.O. Stetter.

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