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PHYLOGENY OF THE ARCHAEBACTERIA AND EUKARYOTES: HOMOLOGY OF THE DNA-DEPENDENT RNA POLYMERASES

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Introduction

The effort to establish a systematics of bacteria and eukaryotes by comparison of the 16/18S rRNA sequences lead Carl Woese in the late seventies (Fox et al., 1980) to the discovery of a second prokaryotic urkingdom which was not more related to the true bacteria (eubacteria) than both prokaryotic urkingdoms to the eukaryotes. This finding was based only on the analysis of few species all living in extreme habitats which resembled the common notion of the early environment in the development of the earth. Therefore Carl Woese called them archaebacteria. Meanwhile the number

of species has been greatly enlarged and many features we discovered which put a new light on the archaic nature these prokaryotes.

Systematics

As shown by 16S rRNA catalogues (Fox et al., 1980) an crosshybridisation experiments (Tu et al., 1982) th archaebacteria consist of two main branches, the methanogen and halophiles and the thermoacidophilic and/or sulfu metabolizing archaebacteria; Thermoplasma acidophilu appears to represent a link between the two branches. Sinc 1980 the orders Sulfolobales and Thermoproteales (Zillig et al., 1980, 1981, 1983) and a new genus, Thermococcus (Zillig et al., 1983 b), were established in the thermoacidophilic and/or sulfur metabolizing branch. The methanogens were expanded by five new members (Huber et al., 1982, Konig & Stetter, 1982, Stetter et al., 1981, Wildgruber et al., 1982, Zehnder et al., 1980) and the square bacteria represent a new halophile (Javor et al, 1982). A dendrogram summarizing the phylogenetic relations is shown in Figure 1.

Environments and metabolism

The methanogens live all in strictly anaerobic environments gaining their energy from the reduction of CO_2 and other components to CH_4. The halophiles live in

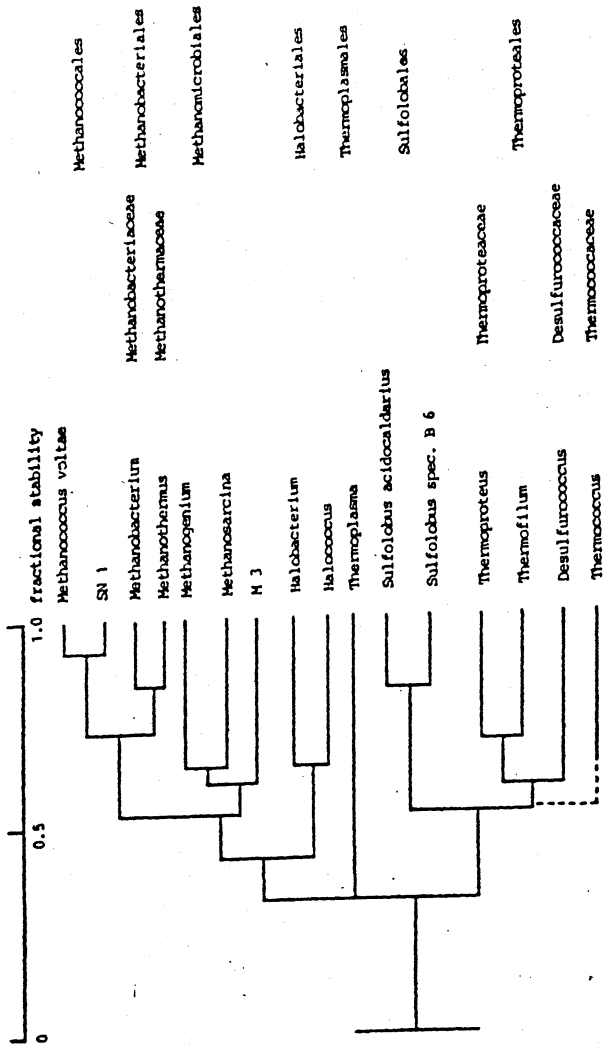

Figure 1: Dendrogram derived from crosshybridisation of 16s-rRNAs and DNAs showing the phylogeny of the archaebacteria (Tu et al., 1982).

saturated salt solutions. Some of them have the capacity produce ATP with light energy under low oxygen tension wi the help of the membrane bound bacteriorhodopsin containir the eye pigment retinal as chromophor (Oesterhelt Stockenius, 1973).

Thermoplasma was isolated from burning coal refuse piles It grows heterotrophically between pH 0.5 and 3 around 60°C

The members of the second branch are sulfur metabolizin organisms. The Sulfolobales isolated from hot acidi volcanic (solfataric) springs are growing either auto- o heterotrophically oxidizing H_2S and/or elementary sulfur t sulfuric acid. The strictly anaerobic Thermoproteales isolated from the anaerobic depths of hot solfataric springs with temperatures of more than 70°C up to the boiling point also grow either auto- or heterotrophically but reducing elementary sulfur to H_2S.

The RNA polymerases

The archaebacterial RNA polymerases are very complex molecules consisting of about nine components (Fig. 2) all present once per enzyme monomer. In complexity and spacing the patterns resemble those of eukaryotic RNA polymerases (Fig. 2). They are very different from the composition $\beta\beta'\alpha_2\sigma$ of the eubacterial polymerases. The archaebacterial

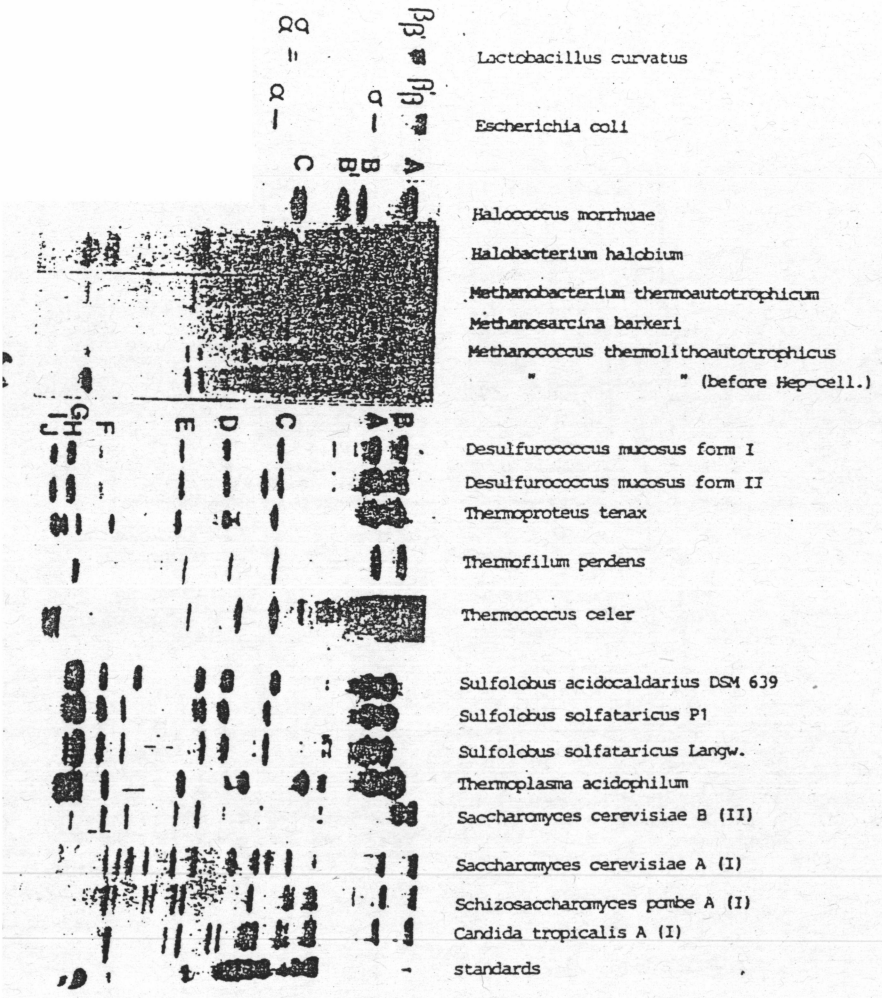

Figure 2: Component patterns of the DNA-dependent RNA polymerases of eukaryotes (yeast), archaebacteria and eubacteria separated by SDS-gel electrophoresis.

enzymes are not inhibited by the antibiotics rifampicin an streptolydigin which are typical inhibitors of eubacteria transcription (Zillig et al., 1982 a, 1982 b)

The components

Judged by the component patterns in SDS-polyacrylamide gels (Fig. 2) the RNA polymerases of the different species are very similar suggesting a homology of their components. Two subtypes can be distinguished corresponding to the two main phylogenetic branches. One contains only three large components and is found in the thermoacidophilic and/or sulfur metabolizing branch, the other contains four large components and is common to the halophilic/methanogenic branch.

To establish the homology of components of the polymerases of nine archaebacteria within and between the branches antibodies were raised against the single components of the RNA polymerases of Sulfolobus acidocaldarius and Methanobacterium thermoautotrophicum (Schnabel et al., 1983). "Western blots" (components separated by SDS-polyacrylamide gelelectrophoresis and transferred to nitrocellulose filters) were challenged with these antibodies and bound antibodies were visualized either with ^{125}J-labelled protein A of Staphylococcus aureus or by peroxidase coupled antibodies. The homology for the five

(six) largest components as analyzed by this method is summed up in Table 1. According to the crossreactions two subtypes of enzyme are identified. The subtype of the thermoacidophilic and/or sulfur metabolizing branch is characterized by the general formula BACDEFGHIJ and the subtype of the methanogenic/halophilic branch by the general formula ABB'CD... Component B' probably arose by division of the largest component of the BACD... type of enzyme. Antibodies against the largest component B of the thermoacidophilic and/or sulfur metabolizing branch react with the second and third component of the methanogenic/-halophilic polymerases (which do not crossreact with each other) and vice versa. This also shows that the two largest components of the RNA polymerases are in reversed order. A division of component B is very likely since Thermoplasma which has the BACD... type of enzyme branched off very early from the methanogenic/halophilic branch so that the development of the ABB'CD... type enzyme probably occurred later.

What are components ?

Polypeptides which copurify through at least 3 isolation steps with the enzyme activity (for a review see Zillig et al., 1982a) are considered components. Sometimes, in the case of the Sulfolobus and Thermoplasma enzymes forms

HIJ	FG	E	D		C				A		B		Components
	yes	e	d	p		c			a	a	bb'	b	Sulfolobus acidocaldarius
		e	d			c			a	a	bb'	b	Thermoplasma acidophilum
		e	d	p		c			a	a	bb'	b	Desulfurococcus mucosus
									a	a	b	b	Thermoproteus tenax
						c				a		b	Thermococcus celer
						c				b	a		Halobacterium halobium
			p		c	c	b'	b	b	b	a	a	Methanobacterium thermoautotrophicum
			p		c		b'	b	b	b	a		Methanococcus thermolithotrophicus
					c		b'	b	b	b	a		Methanosarcina barkeri
			D		C		B'		B		A		Components

Table 1: Crossreactions of antibodies against the single components (small letters) of the RNA polymerase of Sulfolobus (upper lines) and Methanobacterium (lower lines) with the components of the enzymes of the other archaebacteria (Schnabel et al., 1983).

missing components F and H can be isolated which are not affected in their basic activity. Component H which is present in the enzyme of Sulfolobus in 4 copies crossreacts with antibodies raised against the fourth component D and therefore probably arises by proteolysis of this component.

No other crossreactions are observed between different components of the polymerase of Sulfolobus, confirming that no "false" components due to proteolysis are present in the enzyme.

The archaebacterial RNA polymerases are enzymes of the eukaryotic type

Judged by their component patterns the archaebacterial RNA polymerases resemble eukaryotic polymerases (Roeder, 1976), and one could assume that they may be of a common type as opposed to the eubacterial type. This hypothesis was tested by two experiments. It was known that the flavonolignane derivative silybin activates the RNA polymerase A of rat liver (Machicao & Sonnenbichler, 1977). The archaebacterial polymerases from the thermoacidophilic and/or sulfur metabolizing branch share this feature with RNA polymerase A(I) (Schnabel et al., 1982). The enzymes from two eubacteria, E. coli and Lactobacillus curvatus are not affected at lower but inhibited at higher concentrations (Schnabel et al., 1982). This was the first indication that eukaryotic and archaebacterial RNA polymerases are phylogenetically related. This is confirmed by the use of antibodies raised against native RNA polymerases A(I) and B(II) of yeast and their single components (Huet et al., submitted for publication).

The RNA polymerases of six archaebacteria, thr eubacteria and, as control, the polymerases A(I) and B(I of yeast were spotted on nitrocellulose filters an challenged with the antibodies against the nativ polymerases and the single components of the two eukaryoti enzymes. Antibodies against the native polymerase A(I) reac with all enzymes tested (Table 2) which leads to th conclusion that the polymerases of all three urkingdoms hav a common ancestor. Antibodies against the two largest components A_{190} and A_{135} react with all archaebacterial enzymes except one, and A_{190} shows a reaction with E. coli polymerase. The reaction with the archaebacterial enzymes is stronger than that with another polymerase, B, of yeast itself. Antibodies against several smaller components react with some polymerases of the archaebacteria (Table 2).

Antibodies against native polymerase B(II) react with four of the six arachaebacterial polymerases (Table 2) but not with the eubacterial enzymes. Anti B_{220} and anti B_{185}, where B_{185} is a proteolysis product of B_{220} (Huet et al., 1982) react strongly with the RNA polymerases of Sulfolobus acidocaldarius, Desulfurococcus mucosus, Thermoproteus tenax and Halobacterium halobium and to a much lower extent with those of the eubacteria E. coli and Lactobacillus curvatus. Anti B_{150} only reacts with the enzymes of Thermoproteus tenax and Halobacterium halobium but again stronger than

Antibodies against	Pol A	Pol B	Thermoplasma acidophilum	Sulfolobus acidocaldarius	Desulfurococcus mucosus	Thermoproteus tenax	Halobacterium halobium	Methanobacterium thermoautotrophicum	E. coli	Lactobacillus curvatus	Bacillus stearothermophilus	Antibodies against
A190	++	+	+	+	++	++	++		+			
				++	++	++	++		+	+		B185
A135	++	+	++	+	++	++	++	+				
						+	+					B150
A 49	++		+				++					
A 43	++		+									
A 40	++			+	++		+					
A345	++						+					
A 27	++						++					
A 23	++											
A 19	++											
A145	++											
A 12	++											
PolA	++	++	+	+	+	+	+	+	+	+	+	
	++	++	++	++	++		++					PolB

Table 2: Crossreactions of antibodies against the RNA polymerases A (I) and B (II) of yeast and their single components, with the polymerases of some archaebacteria and eubacteria (+ weak, ++ strong reactions), (Huet *et al.* submitted for publication).

with one of the other polymerases, A(I), of yeast itself.

The observed crossreactions were further analyzed by incubation of the antibodies with the separated components transferred to nitrocellulose filters. Those against the native polymerase A(I) and B(II) react with the components B and C of the Thermoplasma acidophilum enyzme, showing that the third largest component C has counterparts in both classes of eukaryotic RNA polymerases. A_{190} and B_{185} are homologous to the second largest components of the enzymes of the thermoacidophilic and/or sulfur metabolizing branch and to the largest of the methanogenic and halophilic branch. A_{135} and B_{150} are homologous to the largest components A of the enzymes of the thermoacidophilic and/or sulfur metabolizing branch and to the second largest components of the polymerases of the other branch.

The following major conclusions can be drawn:

- All RNA polymerases have a common ancestor since polymerase A has homologies with the enzymes of all three urkingdoms which are preserved in the two large components of the enzymes.

- The similar, complex component arrangement, the crossreactions of antibodies against the smaller components of RNA polymerase A(I) with the archaebacterial enzymes and the activation of the transcription of

both polymerase A and the archaebacterial enzymes by silybin show that these are the same type of enzymes as opposed to the type present in eubacteria.

- In the archaebacteria two subtypes of RNA polymerases with the compositions BACDEFGHIJ and ABB'C(D)... exist which justifies in addition to the 16S rRNA data (Fox et al., 1980, Tu et al., 1982) the division of the archaebacteria into two main branches.

Impact on the understanding of early evolution

Catalogues of 16S rRNA fragments are suitable to distinguish clearly between three urkingdoms of organisms but are not sensitive enough to resolve the very early path of evolution, i.e., the branching of the urkingdoms (Hori et al., 1981). The suspicion of the archaic nature of the archaebacteria appeared soon placed in question by many findings which demonstrated that the archaebacteria have a close phylogenetic relationship to the eukaryotic cytoplasm.

The DNA-dependent RNA polymerases are of the same type as those of eukaryotes and many features of the translational apparatus resemble that of the eukaryotes. The sequences of the ribosomal A proteins are related to those of wheat germ and yeast (Matheson et al., 1981). The elongation factor EF2

can be ADP-ribosylated by diphtheria toxin (Kessel & Kli 1981). The translation is insensitive to the antibiot chloramphenicol but sensitive to anisomycin (Schmid et al 1981). The initiator t RNAs are not formylated. Two furth eukaryotic features are the existence of glycoprotei (Mescher and Strominger, 1975, Yang & Haug, 1979) and t retinal containing bacteriorhodopsin in Halobacteri (Oesterhelt & Stockenius, 1973).

The sequence of 16S rRNAs seems so far to be unique a judged by the catalogues. However, the sequences an structures given by the base pairing of the 5S rRNAs show continuous flow from more eubacterial structures in th methanogenic/halophilic branch to almost perfect eukaryotic structures in the thermoacidophilic and/or sulfur metabolizing branch. (Fox et al., 1982, Hori et al., 1982). Similarly, though not as significantly, the sequence of the initiator tRNA of Sulfolobus appears closer to that of yeast, that of Halococcus closer to that of E. coli whereas that of Thermoplasma is again intermediate (Kuchino et al.,1982). A model which considers the known characteristics is presented in Figure 3. It is proposed that the archaebacteria are survivors of an intermediate stage in the evolution from prokaryotic precursors to the eukaryotic cytoplasm: Features which are common to the archaebacteria and eukaryotes should have developed after branching off

from the eubacteria, e.g. RNA polymerases, ribosomes, glycoproteins, etc. The phylogenetic depth between the two

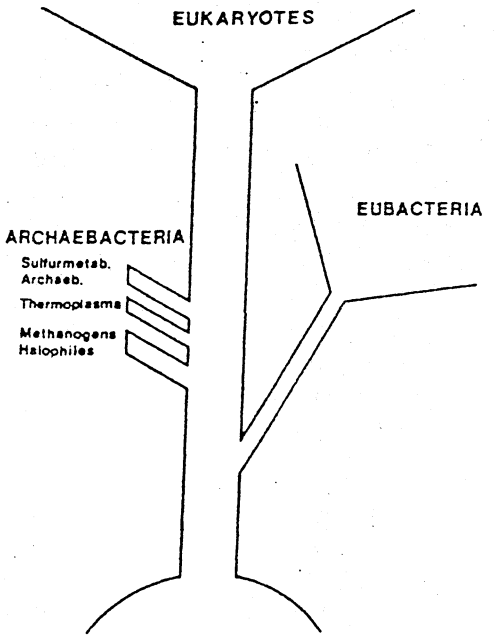

Figure 3: Model for the early evolution and the branching of the eubacteria, archaebacteria and eukaryotes.

archaebacterial branches, which is almost as deep as that between the three urkingdoms itself suggests, in accordance with the two subtypes of RNA polymerases and the differences in 5S rRNA structure, two different separation points for

the two archaebacterial branches out of the main lir leading to the eukaryotes.

A difficulty of this model is given by a unique featur of the archaebacteria, the existence of isopranyl ethe lipids (Langworthy et al., 1981). It is, however, possible that ether lipids were widespread in early organisms as ar adaptation to a hot environment. Even in recent eubacteria living in hot environments alkyl ether lipids have been found (Langworthy et al., 1983). Up to 2.5 billion years old organic sediments contain large amounts of typical archaebacterial isoprenoids suggesting much higher populations of these bacteria than today (Hahn, 1981). The invention of ester lipids could then have been a secondary adaptation to milder environments. Fatty acids have indeed been shown to occur in the lipids of Thermoplasma and the sulfur metabolizing archaebacteria (Thermoproteales) (Zillig et al. 1981).

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