Edited by Prof. Dr. Otto Kandler, München

Archaebacteria

Proceedings of the First International Workshop on Archaebacteria, Munich June 27th – July 1st, 1981

1982. approx. 360 pp, 152 fig. and 50 tab., cloth, approx. DM 98,–

Enlarged and completed reprint from "Zentralblatt für Bakteriologie, Mikrobiologie and Hygiene, Series C: General, Applied and Ecological Microbiology" Vol. 3 (1982) Nr. 1/2 (Gustav Fischer Verlag Stuttgart, New York)

Table of contents: General Aspects · RNA Structures · Genom Organisation and Phages · Proteinsynthesis · Cell Wall Structures · Ribosomal Proteins · RNA Polymerases · Lipids and Membranes · Coenymes · Metabolism

This is the first book dealing with all aspects of the "concept of the archaebacteria".
The concept of "archaebacteria" based on the sequence similarity of ribosomal 16S (18S) RNA, has provided us with startling unexpected new insights into genealogical relationship between organisms and must be considered a break-through in the search for a natural system of bacteria, the final goal of bacterial systematics. In addition, the concept of the archaebacteria is exerting a marked stimulatory effect on molecular genetics, comparative biochemistry and physiology and has far-reaching implications for ecological and applied microbiology.
The main emphasis of these contributions is in the area of the molecular biological and biochemical aspects of archaebacteria. There are also papers on geochemistry, paleontology and systematics which have been included to give a complete picture.
This book provides the most complete and compact account of this new fast rising multidisciplinary research area. It is essential for any scientist, who wants to have profound and extensive information on this field.

SEMPER BONIS ARTIBUS

Gustav Fischer Verlag Stuttgart · New York

Zbl. Bakt. Hyg., I. Abt. Orig. C 3, 128–139 (1982)

Lehrstuhl für Mikrobiologie der Universität Regensburg, Regensburg, Federal Republic of Germany

Histone-like Proteins in Eu- and Archaebacteria*

MICHAEL THOMM, KARL O. STETTER, and WOLFRAM ZILLIG [1]

Received July 27, 1981

Summary

The DNA of 5 species of eubacteria and 5 species of archaebacteria was isolated by isopyknic centrifugation in metrizamide density gradients. It is associated with high amounts (protein: DNA $\approx$ 0.25 w/w) of small, acid-soluble proteins with molecular weights ranging from 5,500 to 14,300. Electrophoreses of these proteins according to charge density showed that nearly all of them are very basic, some of the archaebacterial proteins even as basic as calf thymus histones.

Antibodies against the histone-like protein of *Escherichia coli* formed precipitates with extracts of 13 species of eubacteria, of which some were phylogenetically quite distant from *Escherichia coli,* but not with extracts from archaebacteria. Dodecylsulfate polyacrylamide gel electrophoresis of the precipitates yielded bands of identical molecular weights as those obtained after metrizamide centrifugation. No precipitation could be detected with extracts from archaebacteria or with calf thymus histones.

Antibodies against the histone-like protein of *Thermoplasma* reacted only with the corresponding extract, but not with those from other archaebacteria e. g. *Sulfolobus, Thermoproteus, Methanobacterium, Methanococcus* and *Methanosarcina.* They also did not yield precipitates with extracts of eubacteria and with calf thymus histones.

Key words: Archaebacteria – DNA – Eubacteria – Histone-like proteins – Nucleoprotein

Introduction

The nucleus of eukaryotes contains large amounts of basic proteins, the histones (*Kossel,* 1884). Since significant amounts of proteins were previously not found in association with prokaryotic DNA, it had been assumed that histone-like proteins do not exist in bacteria (*Leaver* and *Cruft,* 1966; *Raaf* and *Bonner,* 1968; *Bonner* et al., 1968; *Makino* and *Tsusuki,* 1971; *Hsiang* and *Cole,* 1973).

---

* Paper given at the First International Workshop on Archaebacteria, München, June 27 to July 1, 1981.

[1] Max-Planck-Institut für Biochemie, D-8033 Martinsried, Federal Republic of Germany.

A prokaryotic small basic DNA-bound protein was first detected in large amounts in *Thermoplasma acidophilum* by *Searcy (1975)*. As he could not find a similar protein in *Escherichia coli*, he suggested that the histone-like protein of *Thermoplasma* is an adaption of this thermophilic bacterium helping to stabilize the DNA in its extreme environment.

However, *Varshavsky* et al. (1977) found two histone-like proteins associated with the isolated DNA of *Escherichia coli*. The localization of one of them, the HU-protein, on the DNA of *Escherichia coli* could be confirmed by Rouvière-Yaniv (1978). A serologically cross-reacting protein was detected by *Haselkorn* and *Rouvière-Yaniv* (1976) in *cyanobacteria*.

In this paper we report on the distribution of histone-like proteins in various members of eu- and archaebacteria of different habitats. These proteins were identified by antibody precipitation and by isopyknic centrifugation in metrizamide density gradients, a method already applied to the isolation of eukaryotic chromatin (*Birnie* et al., 1973).

Materials and Methods

1. Bacterial strains and growth conditions

The origin, the source of the bacteria, and the culture conditions are summarized in Table 1.

All eubacteria with the exception of *Thermus sp.* were grown in HD-medium (pepton 5 g/l, yeast extract 2.5 g/l, glucose 1 g/l; pH 7.0) at 37 °C.

Thermus sp., Abano Terme, isolated by *W. Zillig* and *K. O. Stetter* was grown aerobically at 78 °C and at pH 7.2 (adjusted with 1 n NaOH) in a medium containing per liter: 1.46 g NaCl, 0.21 g $NaHCO_3$, 0.20 g $KHSO_4$, 0.37 g $MgSO_4 \cdot 7\,H_2O$, 0.51 g $KCl \cdot 2\,H_2O$, 0.0132 g $(NH_4)_2SO_4$, 0.028 g $FeSO_4 \cdot 7\,H_2O$ and 2 g yeast extract (Difco). The cells were harvested by continuous centrifugation in a Padberg centrifuge type 61 G and stored at −80 °C.

2. Isolation of the nucleoproteins

Procedure A: The nucleoprotein of *Thermoplasma acidophilum* was isolated by the procedure of *Searcy* (1975) with the following slight modifications: the DNA was sheared with an Omni-mixer (Sorvall) for 2–3 min at 10,000 rev./min and the concentrated ribosome-free supernatant was applied to a chromatography column (97 × 3 cm) containing Biogel A 15 m, 50–100 mesh (Bio Rad).

Procedure B: This method was generally used for the isolation of the nucleoproteins: 4 g of frozen cells were thawed in 20 ml buffer A (0.01 mol/l Tris-HCl, pH 7.4, 0.2 mol/l NaCl, 0.01 mol/l $MgCl_2$; *Searcy*, (1975)), and suspended employing a potter (Elvehjem) homogenizer with a loosely fitting pestle. The cells were lysed in a French Pressure Cell (34 ml, Aminco) at 88,600 kPa and the crude extract was centrifuged at 50,000 rev./min for 85 min at 5 °C in a Beckman L 5–50 ultracentrifuge, rotor 50 Ti.

To 2–3 ml of the clear supernatant a metrizamide stock-solution (800 g/l in buffer A) was added to a final metrizamide concentration of 520 g/l. Then a solution of ethidium bromide (10 g/l) was added to a final concentration of 7 mg/l and the mixture was centrifuged for 96–104 h in polyallomer-tubes in the rotor 50 Ti (Beckman L 5–50), at 45,000 rev./min and 5 ° C. After the run a sharp band was visible and could be isolated by suction with a syringe.

For a further purification, 1 ml of DNA-fraction from the metrizamide gradient was mixed with 5 ml metrizamide stock-solution, 7.5 ml buffer A and 10 μl ethidium bromide (10 g/l) and centrifuged under the same conditions as described above.

Table 1. Strains and culture conditions

Strain	Designation	Origin	Culture conditions and references
Escherichia coli K 12	M 1132	Merck	HD-medium ae*)
Enterobacter aerogenes	ATCC 13047	ATCC	HD-medium ae
Citrobacter sp.	ATCC 6750	ATCC	HD-medium ae
Proteus mirabilis	WS 100399	WS	HD-medium ae
Pseudomonas acidovorans	DSM 39	DSM	HD-medium ae
Pseudomonas putida	DSM 291	DSM	HD-medium ae
Thermus sp.	-	*W. Zillig*	*Thermus* medium
Staphylococcus epidermidis	ATCC 12600	ATCC	HD-medium ae
Eubacterium limosum	DSM 20402	*N. Weiß*	HD-medium an**)
Bacillus stearothermophilus	DSM 22	*B. Rexer*	HD-medium ae
Bacillus subtilis	DSM 10	DSM	HD-medium ae
Micrococcus luteus	DSM 20030	DSM	HD-medium ae
Lactobacillus bulgaricus	WS 6	WS	*De Man* et al., 1960
Lactobacillus bavaricus	ATCC 31063	*K. O. Stetter*	*De Man* et al., 1960
Methanobacterium thermoautotrophicum, strain ΔH	DSM 1053	*R. Wolfe*	*Balch* et al., 1979
Methanococcus vannielii, strain SB	DSM 1224	*J. Winter*	*Balch* et al., 1979
Methanosarcina barkeri	DSM 804	*H. Hippe*	*Balch* et al., 1979
Thermoplasma acidophilum	ATCC 25905	*E. A. Freundt*	*Sturm* et al., 1980
Sulfolobus solfataricus	DSM 1616	*W. Zillig*	*Zillig* et al., 1980
Sulfolobus acidocaldarius	DSM 639	DSM	*Zillig* et al., 1980
Sulfolobus brierleyi	DSM 1651	*C. L.* and *J. A. Brierley*	*Zillig* et al., 1980
Thermoproteus tenax	DSM 2078	*W. Zillig*	*Zillig* et al., 1981

M = Merck, Darmstadt
ATCC = American Type Culture Collection, Rockville, USA
DSM = Deutsche Sammlung von Mikroorganismen, Göttingen
WS = Weihenstephan
*) ae = aerobically grown
**) an = anaerobically grown

In the case of *Methanobacterium thermoautotrophicum* all solutions were gassed for one h with N_2 and contained 0.001 mol/l 2-mercaptoethanol.

For preparation in large scale, portions (38.5 ml) were centrifuged in polyallomer-tubes in the rotor 60 Ti at 55,000 rev./min for 72 h at 5 °C.

3. Extraction of acid-soluble protein

The nucleoprotein fraction was dialysed against distilled water (2–14 h) and diluted with 3 volumes of water. Then, the acid-soluble protein was extracted with 0.2 M sulfuric acid (*Searcy,* 1975) and precipitated with ethanol at −20 °C. The precipitate was dried in vacuo and dissolved in the required electrophoresis sample buffer (*Laemmli,* 1970 or *Machicao* and *Sonnenbichler,* 1971).

4. Analytical procedures

Electrophoretic procedures: Dodecylsulfate polyacrylamide gel electrophoresis was performed according to *Laemmli* (1970), but in 5–25 % exponential polyacrylamide gradient gels (*Mirault* and *Scherrer,* 1971).

Cellogel-electrophoresis was performed as described by *Machicao* and *Sonnenbichler* (1971).

Determination of DNA, RNA, and protein: 50–250 µl nucleoprotein fraction, isolated by metrizamide centrifugation, were diluted with water to a final volume of 1 ml. The ethidium bromide was removed with n-butanol at 0 °C. The nucleic acids were then precipitated with perchloric acid according to *Rickwood* et al. (1973). From the precipitate RNA and DNA were separated (*Hutchinson* and *Munro,* 1961) and then assayed by the orcinol and diphenylamine reaction (*Burton,* 1956), respectively.

Protein was determined by the micromethod of *Heil* and *Zillig* (1970).

5. Preparation of antibodies

A rabbit was immunized with a total of 250 µg purified histone-like protein of *Thermoplasma,* another rabbit with 1 mg of the histone-like protein of *Escherichia coli* using a micromethod described previously (*Stetter,* 1977). The γ-globulins were enriched from the serum by the method of *Linn* et al. (1973).

6. Enrichment of heat-stable proteins for antibody precipitation

The antibodies showed only precipitation with crude extracts of the corresponding bacterial strains, while in heterologous systems the histone-like proteins had to be enriched before by the following procedure:

1 g cells were suspended in 1 ml TAG buffer consisting of 0.05 M Tris-HCl buffer pH 7.3 containing NH_4Cl 0.02 mol/l, $MgCl_2$ 0.02 mol/l, and glycerol 100 g/l.

The cells were disrupted in an French pressure cell (Aminco) at 100,000 kPa. Pancreatic DNAase (Boehringer) was added at a final concentration of 20 mg/l. After 1 h at 37 °C the suspension was diluted with 1 ml buffer and then centrifuged at 45,000 rev./min at 2 °C in the rotor 50 Ti. The clear supernatant was heated in a boiling water bath for 10 min. The precipitate was removed by centrifugation (10 min 21,000 rev./min, 2 °C, rotor JA 21, J2–21 Beckman centrifuge) and the supernatant concentrated by precipitation with 4 vol. of ethanol (1 h at −20 °C). The precipitated protein was collected by centrifugation (20 min, 20,000 rev./min, rotor JA 20, at 0 °C), dried in vacuo and redissolved in 20–50 µl TAG buffer. For the investigation of thermophilic bacteria, the boiling-step was omitted, as no or very little precipitation occurred.

7. Antibody precipitation

For immunoprecipitation in solution, 20 µg heat-stable protein fraction were combined with 10 µl purified antiserum. This mixture was incubated for 16 h at 4 °C.

8. Preparation of immunoprecipitates for SDS polyacrylamide gel electrophoresis

After the incubation the immunoprecipitate was collected by centrifugation (15 min, 21,000 rev./min, JA 21, 2 °C), washed 2 times with 200 µl TAG buffer containing 3 moles/l NH_4Cl, 2 times with 50 µl TAG, heated for 2 min at 95 °C in 25 µl sample buffer (*Laemmli,* 1970) and then loaded onto the polyacrylamide gel.

Results

Purification and composition of the bacterial nucleoprotein

Isopyknic centrifugation of extracts containing bacterial DNAs in the presence of metrizamide yields sharp bands as visualised by ethidium bromide. These were further purified by repetition of the centrifugation. They contained in all cases, besides the DNA, large amounts of protein, which could not be removed from the DNA by repeated centrifugation. The protein/DNA ratios in the nucleoprotein complexes of *Thermoplasma acidophilum, Sulfolobus acidocaldarius* and *Sulfolobus brierleyi* (Table 3) are around 0.25 (w/w). For the nucleoproteins of other bacteria similar ratios were roughly estimated from relating the intensities of the stained protein bands in the polyacrylamide gels with the amounts of DNA, from which the proteins had been extracted.

No significant amounts of RNA could be detected in the purified nucleoprotein.

The DNA-binding proteins of *Escherichia coli, Bacillus stearothermophilus, Lactobacillus bavaricus, Thermus sp., Thermoplasma acidophilum, Sulfolobus acidocaldarius, Sulfolobus solfataricus* and *Sulfolobus brierleyi* are soluble in 0.2 M sulfuric acid, while that of *Methanobacterium thermoautotrophicum* is not acid-soluble.

Table 2. Molecular weights of DNA-associated proteins of different eu- and archaebacteria determined from mobilities in SDS polyacrylamide gel electrophoresis*) (Fig. 1 and 2)

Organism	M.W. (daltons)
Thermoplasma acidophilum	9300
Sulfolobus acidocaldarius	
component A	9300
component B	6200
Sulfolobus solfataricus	
component A	9300
component B	5800
Sulfolobus brierleyi	
component A	12200
component B	10600
component C	9300
component D	7100
component E	5500
Methanobacterium thermoautotrophicum	14300
Thermus sp.	9300
Bacillus stearothermophilus	8100
Lactobacillus bavaricus	
component A	12000
component B	7400
Escherichia coli K 12	7000**)

* The calf thymus histones H3, H2A, H4 (*Sonnenbichler* and *Zetl*, 1975) and the histone-like protein of *Escherichia coli* (*Rouvière-Yaniv* and *Gros*, 1975) were used as standards.

** calculated by *Rouvière-Yaniv* and *Gros* (1975) from the amino acid composition.

Table 3. Ratio of acid-soluble protein/DNA in the nucleoprotein of different organisms

Organism	acid-soluble protein/DNA*
Prokaryotes	
Sulfolobus acidocaldarius	0.26
Sulfolobus brierleyi	0.25
Thermoplasma acidophilum	0.25 (*Searcy*, 1975)
	0.36
Escherichia coli	0.12 (*Rouvière-Yaniv*, 1978)
Anabaena sp.	0.14 (*Haselkorn* and *Rouvière-Yaniv*, 1976)
Eukaryotes	
Neurospora crassa	0.25 (*Hsiang* and *Cole*, 1973)
Rat liver	1.0 (*Marushige* and *Bonner*, 1966)

* w/w

Characterization of the bacterial DNA-associated proteins by SDS polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis of the isolated nucleoprotein complexes yielded 1 to 5 bands with molecular weights ranging from 5500 to 14 300 (Fig. 1 and 2, Table 2).

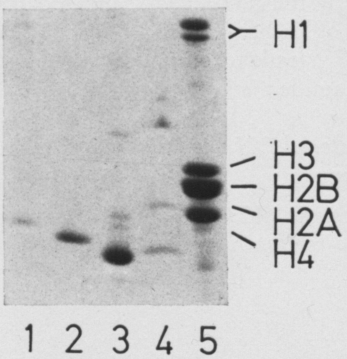

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the DNA-associated proteins of different eubacteria. 1 = *Thermus sp.*; 2 = *Bacillus stearothermophilus*; 3 = *Escherichia coli*; 4 = *Lactobacillus bavaricus*; 5 = calf thymus histones.

In the case of *Bacillus stearothermophilus* the nucleoprotein was isolated with and without the proteinase-inhibitor phenyl-methylsulphonylfluoride (Sigma). No difference in the molecular weight of the polypeptide chain could be observed, however (not shown).

The nucleoproteins of *Lactobacillus bavaricus* (Fig. 1, lane 4) *Sulfolobus acidocaldarius* (Fig. 2, lane 3) and *Sulfolobus solfataricus* (Fig. 2, lane 4) contain 2 protein components, while that of *Sulfolobus brierleyi* (Fig. 2, lane 5) contains 5. The DNA of *Escherichia coli* (Fig. 1, lane 3), *Bacillus stearothermophilus* (Fig. 1, lane 2), *Thermus sp.* (Fig. 1, lane 1) and *Thermoplasma acidophilum* (Fig. 2, lane 2), however, is associated with only one protein component.

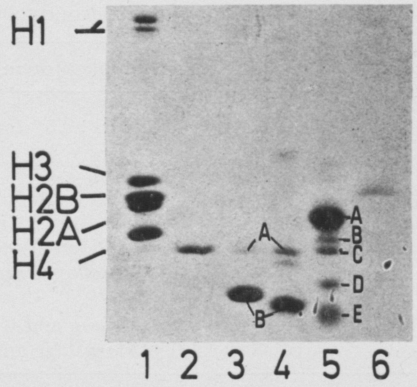

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the histone-like proteins of archaebacteria. 1 = calf thymus histones; 2 = *Thermoplasma acidophilum;* 3 = *Sulfolobus acidocaldarius;* 4 = *Sulfolobus solfataricus;* 5 = *Sulfolobus brierleyi;* 6 = *Methanobacterium thermoautotrophicum.*

The isolation of the "histone-like" protein of *Thermoplasma* by the method of *Searcy* (1975) yielded a polypeptide (Fig. 3, lane 5) with the same electrophoretic mobility as that isolated by the metrizamide method (Fig. 3, lane 2–4). The isolation of the HU-protein of Escherichia coli by a method similar to that of *Rouvière-Yaniv* and *Gros* (1975) leads to a single protein comigrating in SDS gels with that isolated by the metrizamide method (not shown).

Characterization of bacterial DNA-associated proteins according to charge densities (cellogel electrophoresis)

In cellogel electrophoresis at pH 10, the DNA-associated proteins of *Sulfolobus acidocaldarius, Sulfolobus solfataricus, Sulfolobus brierleyi* and *Thermoplasma*

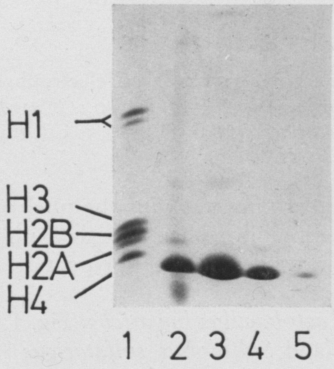

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of DNA-binding proteins of *Thermoplasma acidophilum.* 1 = calf thymus histones; 2, 3, 4 = protein extracted from nucleoprotein fractions purified by metrizamide centrifugation; 5 = acid soluble protein extracted from the nucleoprotein purified by column-chromatography.

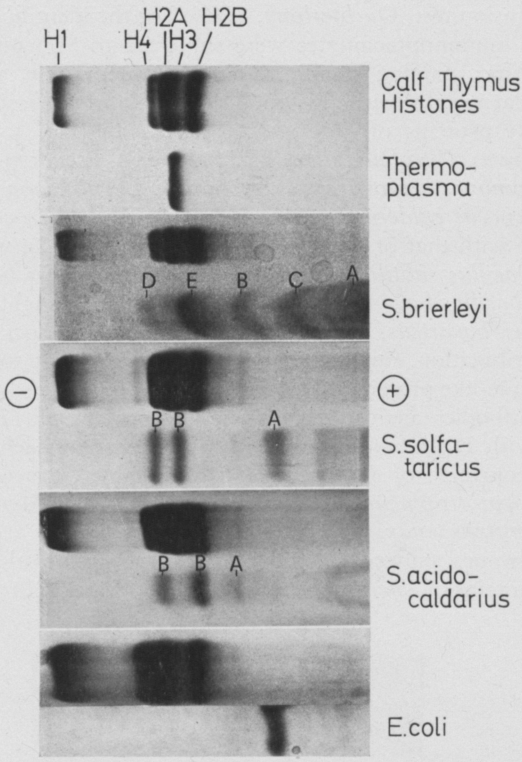

Fig. 4. Cellogel electrophoresis of calf thymus histones and bacterial histone-like proteins of different origin. The numbering of the bands refers to the corresponding positions in the SDS polyacrylamide gel (Fig. 2).

acidophilum migrate to the cathode similar to calf thymus histones (*Machiaco* and *Sonnenbichler*, 1971), (Fig. 4), indicating a similar basicity.

In the preparations of *Escherichia coli* and *Thermoplasma*, only one band could be detected (Fig. 4). The protein of *Escherichia coli* is less basic than that of *Thermoplasma* and than calf thymus histones. Each of the different *Sulfolobus* species, however, yields several bands in the cellogel electrophoresis (Fig. 4), differing significantly in charge densities, 5 in *Sulfolobus brierleyi* and 3 each in *S. acidocaldarius* and *S. solfataricus*. After cellogel electrophoresis, the bands were identified and eluted as described by *Heil* and *Zillig* (1970) and the molecular weights were determined by SDS polyacrylamide gel electrophoresis. The two most basic bands of *Sulfolobus acidocaldarius* (Fig. 4) and *Sulfolobus solfataricus* (Fig. 4), respectively, migrate to the same position in polyacrylamide gels (Fig. 2, lanes 3 and 4), indicating an identical molecular weight.

Serology

The heat-stable protein fractions of 11 eubacteria yielded precipitation lines (not shown) with an antibody against the histone-like protein of *Escherichia coli*

in the immunodiffusion test (*Ouchterlony*, 1960). For the identification of the cross reacting proteins, immunoprecipitates were subjected to SDS polyacrylamide gel electrophoresis. After staining small proteins became visible, which were very similar in molecular weight to the histone-like protein of *Escherichia coli* (Fig. 5).

The histone-like proteins of *Enterobacter aerogenes* (Fig. 5, lane 4), *Proteus mirabilis* (not shown), *Citrobacter sp.* (Fig. 5, lane 3), *Pseudomonas putida* (Fig. 5, lane 5), *Pseudomonas acidovorans* (not shown), *Eubacterium limosum* (Fig. 5, lane 7), *Staphylococcus epidermidis* (Fig. 5, lane 8), and *Micrococcus luteus* (not shown) comigrate with that of *Escherichia coli* (Fig. 5, lane 2), while the histone-like proteins of *Bacillus subtilis* (Fig. 5, lane 6), *Lactobacillus bulgaricus* (Fig. 5, lane 9) and *Lactobacillus bavaricus* (Fig. 5, lane 10) migrate slightly slower.

In *Lactobacillus bavaricus*, which yielded two DNA-associated proteins by metrizamide centrifugation, only the smaller one turned out to be serologically related to the histone-like protein of *Escherichia coli*.

In contrast, antibodies against the histone-like protein of *Thermoplasma* did not cross react with DNA-binding proteins from other archaebacteria including the purified nucleoprotein of *Sulfolobus* and heat-stable extracts from *Methanobacterium thermoautotrophicum*, *Methanococcus vannielii*, *Methanosarcina barkeri* and *Thermoproteus tenax*.

Calf thymus histones showed no precipitation line both with the sera against the histone-like proteins of *Thermoplasma* and *Escherichia coli*.

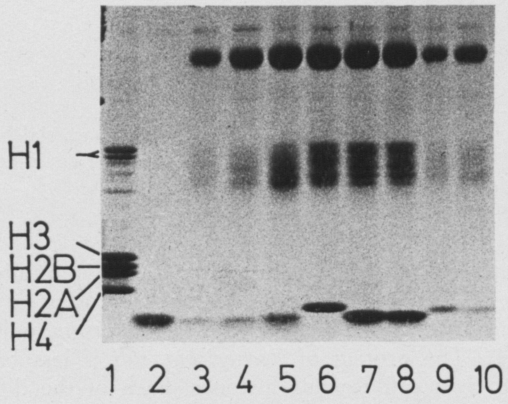

Fig. 5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of immunoprecipitates formed by antibody precipitation with an antiserum against the histone-like protein of *Escherichia coli* and extracts of different eubacteria. 1 = calf thymus histones; 2 = histone-like protein of *Escherichia coli;* 3–10 = immunoprecipitates of: (3) *Citrobacter sp.;* (4) *Enterobacter aerogenes;* (5) *Pseudomonas acidovorans;* (6) *Bacillus subtilis;* (7) *Eubacterium limosum;* (8) *Staphylococcus epidermidis;* (9) *Lactobacillus bulgaricus;* (10) *Lactobacillus bavaricus.*

Discussion

The existence of the histone-like protein bound to the DNA of *Thermoplasma* (*Searcy*, 1975) could be confirmed by metrizamide density gradient centrifuga-

tion. In *Escherichia coli,* only one of the two DNA-binding proteins described by *Varshavsky* et al. (1977) was found by the metrizamide method. This protein migrates to the same position in SDS polyacrylamide gels as the *Escherichia coli* DNA-binding protein HU described by *Rouvière-Yaniv* and *Gros* (1975) and therefore appears to be identical with it. The second protein found in *Escherichia coli* by *Varshavsky* et al. (1977) possibly binds only to the DNA at the very low ionic strength used by these authors.

Histone-like proteins are thus not restricted to thermophiles, as previously supposed by *Searcy* (1975). On the contrary, they appear to be bound to the DNAs of all members of both prokaryotic kingdoms, the eubacteria and the archaebacteria.

In eubacteria, the histone-like proteins appear to be very conservative in structure as already known for eukaryotic histones: Even proteins of bacteria phylogenetically far distant from *Escherichia coli,* e. g. *Micrococcus, Lactobacillus* and *Eubacterium* show precipitation with the antibodies against the HU-protein of *Escherichia coli.* Besides their serological relationship, these proteins are also very similar in their molecular weights. In analogy to histones they are heat-stable and soluble in dilute mineral acids, indicating a strong basicity. Although these features fit also to eukaryotic histones, no serological cross reaction between the latter and the histone-like protein of *Escherichia coli* was observed.

In archaebacteria, the histone-like proteins are usually as basic as eukaryotic histones (exception: *Methanobacterium thermoautotrophicum*). Although they were found in all species inspected, no serological cross reaction with antibodies against the *Thermoplasma*-protein could be observed, possibly because of a higher phylogenetic diversity of the archaebacteria or a less conservative structure of these proteins.

The function of the histone-like proteins remains still unclear. As they are present on the DNA in as high amounts as histones in lower eukaryotes (Table 3), they may play a similar role, possibly in the formation of a quarternary structure (*Rouvière-Yaniv* et al., 1979) of the "chromatin" in prokaryotes.

Acknowledgements

We wish to thank Dr. *I. Grummt* for stimulating discussions and Dr. *J. Sonnenbichler* for the generous gift of calf thymus histones. Thanks are also due to *P. Frischeisen* for excellent assistance. This work was supported by a grant of the Deutsche Forschungsgemeinschaft to *K. O. Stetter.*

References

Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R., Wolfe, R. S.: Methanogens: Reevaluation of a unique biological group. Microbiol. Rev. *43,* 260–296 (1979)

Birnie, G. D., Rickwood, D., Hell, A.: Buoyant densities and hydration of nucleic acids, proteins and nucleoprotein complexes in metrizamide. Biochim. Biophys. Acta *331,* 283–294 (1973)

Bonner, J., Dahmus, M. E., Fambrough, D., Huang, R. C., Marushige, K., Tuan, D.: The biology of isolated chromatin. Science *159,* 47–56 (1968)

Burton, K.: A study of the conditions and mechanism of diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. *62*, 315–322 (1956)

Haselkorn, R., Rouvière-Yaniv, J.: Cyanobacterial DNA-binding protein related to *Escherichia coli* HU. Proc. nat. Acad. Sci. (Wash.) *73*, 1917–1920 (1976)

Heil, A., Zillig, W.: Reconstitution of bacterial DNA-dependent RNA polymerase from isolated subunits as a tool for the elucidation of the role of the subunits in transcription. FEBS Lett. *11*, 165–168 (1970)

Hsiang, M. W., Cole, R. D.: The isolation of histones from *Neurospora crassa*. J. Biol. Chem. *248*, 2007–2013 (1973)

Hutchinson, W. C., Munro, H. N.: The determination of nucleic acids in biological materials. Analyst *86*, 768–813 (1961)

Kossel, A.: Über einen peptonartigen Bestandtheil des Zellkerns. Z. physiol. Chem. *8*, 511–515 (1884)

Laemmli, U. K.: Cleavage of structural proteins during the assembly of the head of the bacteriophage *T 4*. Nature (Lond.) *227*, 680–685 (1970)

Leaver, J. L., Cruft, H. J.: Investigation into the distribution and properties of histones and other basic proteins in bacteria. Biochem. J. *101*, 665–673 (1966)

Linn, T. G., Greenleaf, A. L., Shorenstein, R. G., Losick, R.: Loss of the sigma activity of RNA polymerase of *Bacillus subtilis* during sporulation. Proc. nat. Acad. Sci. (Wash.) *70*, 1865–1869 (1973)

Machicao, F., Sonnenbichler, J.: Cellogel electrophoresis of histones. Biochim. Biophys. Acta *236*, 360–361 (1971)

Makino, F., Tsusuki, J.: Absence of histone in the blue-green alga *Anabaena cylindrica*. Nature (Lond.) *231*, 446–447 (1971)

Man, J. C. de, Rogosa, M., Sharpe, M. E.: A medium for the cultivation of *Lactobacilli*. J. appl. Bact. *23*, 130–135 (1960)

Marushige, K., Bonner, J.: Template properties of liver chromatin. J. molec. Biol. *15*, 160–174 (1966)

Mirault, M. E., Scherrer, K.: Isolation of preribosomes from Hela cells and their characterisation by electrophoresis on uniform and exponential-gradient-polyacrylamide gels. Europ. J. Biochem. *23*, 372–386 (1971)

Ouchterlony, O.: Diffusion-in-gel methods for immunological analysis II. In: Progress in allergy, Vol.. VT, pp. 30–154, (*P. Kallod* and *B. H. Waksman*, eds.). Basel, Karger 1962

Raaf, J., Bonner, J.: Properties of *Escherichia coli* deoxyribonucleoprotein as compared with the deoxyribonucleoprotein of higher organisms. Arch. Biochem. Biophys. *125*, 567–579 (1968)

Rickwood, D., Hell, A., Birnie, G. D.: Isopycnic centrifugation of sheared chromatin in metrizamide gradients. FEBS Lett. *33*, 221–224 (1973)

Rouvière-Yaniv, J., Gros, F.: Characterisation of a novel, low-molecular-weight DNA-binding protein from *Escherichia coli*. Proc. nat. Acad. Sci. (Wash.) *72*, 3428–3432 (1975)

Rouvière-Yaniv, J.: Localisation of the HU-protein in the *Escherichia coli* nucleoid. Cold Spring Harbor Symp. Quant. Biol. *42*, 439–447 (1978)

Rouvière-Yaniv, J., Yaniv, M., Germond, J.: E. coli DNA binding protein HU forms nucleosomelike structure with circular double-stranded DNA. Cell *17*, 265–274 (1979)

Searcy, D. G.: Histone-like protein in the prokaryote *Thermoplasma acidophilum*. Biochim. Biophys. Acta *395*, 535–547 (1975)

Sonnenbichler, J., Zetl, I.: The quantitative protein composition of calf thymus chromatin. Hoppe-Seyler's Z. physiol. Chem. *356*, 599–603 (1975)

Stetter, K. O.: Transcription in *Lactobacillaceae:* DNA-dependent RNA polymerase from *Lactobacillus casei*. Isolation of transcription factor γ. Hoppe-Seyler's Z. physiol. Chem. *358*, 1093–1104 (1977)

Sturm, S., Schönefeld, U., Zillig, W., Janécovic, D., Stetter, K. O.: Structure and function

of the DNA dependent RNA polymerase of the archaebacterium *Thermoplasma acidophilum*. Zbl. Bakt. Hyg., I. Abt. Orig. C *1*, 12–25 (1980)

Varshavsky, A. J., Nedopasov, S. A., Bakayev, V. V., Bakayeva, T. G., Georgiev, G. P.: Histone-like protein in the purified *E. coli* deoxyribonucleoprotein. Nucleic Acid Res. *4*, 2725–2745 (1977)

Zillig, W., Stetter, K. O., Wunderl, S., Schulz, W., Priess, H., Scholz, J.: The *Sulfolobus*-"Caldariella" group: Taxonomy on the basis of the structure of DNA-dependent RNA polymerase. Arch. Microbiol. *125*, 259–269 (1980)

Zillig, W., Stetter, K. O., Schäfer, W., Janécovic, D., Wunderl, S., Holz, I., Palm, P.: Thermoproteus: Two types of extremely thermoacidophilic anaerobians isolated from icelandic solfataras represent a novel group of Archaebacteria. Zbl. Bakt. Hyg., I. Abt. Orig. C *2*, 205–227 (1981)

Prof. Dr. *K. O. Stetter*, Lehrstuhl für Mikrobiologie der Universität Regensburg, Universitätsstr. 31, D-8400 Regensburg, Fed. Rep. of Germany