Comparative Chromatin Analysis of *Trypanosoma congolense*

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The chromatin of Trypanosoma congolense *was analyzed by electron microscopy. The chromatin is organized as nucleosome filaments but does not form a 30 nm fiber. There are five groups of histones, including a histone H1-like protein, which has a molecular weight within the range of the core histones, and is extremely hydrophilic. Weak histone-histone interaction, a typical feature of trypanosome chromatin, was found. These results are similar to those for* T. cruzi *and* T. b. brucei, *but differ significantly from those for higher eukaryotes. The results confirm the model of trypanosome chromatin, and support the theory of their early separation from the other eukaryotes during the evolution.* T. congolensis *is an excellent model for chromatin research on trypanosomes, because it is easy to cultivate and its chromatin has, a relatively high stability, compared to that of other trypanosomes.*

Key words: *Trypanosoma congolense* - chromatin structure - histones - lower eukaryotes - procyclic culture forms

Trypanosomes, protozoan parasites of man and animals, have various nuclear features different from those of higher eukaryotes. No condensed chromosomes can be seen during nuclear division (Vickerman & Preston 1970), and the compaction of the chromatin in the nucleus is less pronounced as compared to the chromatin of rat liver nuclei (Hecker & Gander 1985). The histones of the trypanosomes differ significantly from those of higher eukaryotes in amino acid composition and sequence (Bender et al. 1992b, Toro et al. 1992, Hecker 1993). There are weaker interactions, histone-histone and histone-DNA, and this leads to a reduction in chromatin stability (Hecker et al. 1989, Burri et al. 1993).

The chromatin of *Trypanosoma b. brucei* procyclic and of *T. cruzi* epimastigote culture forms was shown to be organized in a nucleosome filament-like form, but did not form a 30 nm fiber. Histone H1 was thought to be absent in trypanosomes for a long time, mainly because of the poor condensation behaviour of the chromatin, and also because no protein with the characteristics of histone H1 of higher eukaryotes was found (Hecker et al. 1994). Toro and Galanti (1988) showed for *T. cruzi* and Schlimme et al. (1993) for *T. b. brucei* that histone H1-like proteins do exist, but that these proteins are different in several ways from histone H1 of higher eukaryotes. They are very hydrophilic and have a molecular weight of about 13'000 D instead of the 21'000 D of H1 of higher eukaryotes (Van Holde 1989), and the globular part appears to be quite small (Schlimme et al. 1993).

Most investigations on the chromatin of trypanosomes have been carried out on *T. cruzi* and *T. b. brucei* (Hecker et al. 1994). In the study described here, the chromatin of *T. congolense*, the casual agent of African bovine trypanosomiasis, an important disease of cattle and domestic animals, was investigated.

MATERIALS AND METHODS

T. congolense STIB 802 procyclic culture forms were used. They were grown in SM medium containing 20% heat-inactivated fetal bovine serum, 10 µg/ml hemin, 10 µg/ml gentamicin, 5% CO_2 at 27°C (Cunningham 1977). *T. cruzi* Y strain epimastigotes were cultured in modified Mattei medium (Mattei et al. 1977) containing 10% heat-inactivated fetal bovine serum, 10 µg/ml hemin, 10 µg/ml gentamicin, 30 mM leucine, at 27°C.

T. b. brucei STIB 345 AB strain procyclic culture forms were cultured as described by Brun and Schönenberger (1979).

Exponentially growing procyclic trypanosomes (2 to 3×10^{10}) were harvested and nuclei were prepared as described for procyclic culture forms of *T. b. brucei* (Schlimme et al. 1993).

Preparation of soluble chromatin - Nuclei of *T. congolense* were digested with 0.2 units micrococcal nuclease (Sigma, N-3755) per 20 A_{260} at 30°C for 50s. Nuclei of rat liver were digested with 0.4 units micrococcal nuclease per 20 A_{260} at 30°C for 50s. The nuclei were pel-

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leted, and the chromatin solubilized by nuclear lysis in a hypotonic buffer containing 1 mM triethanolaminehydrochloride (TEACI) and 0.2 mM Na_2EDTA, pH 7.4. Insoluble material was removed by centrifugation (Thoma et al. 1979, Hecker & Gander 1985).

Gradient analysis of the chromatin digest - Gradient analysis was done in 5.5 - 28.5% (w/v) 17 ml isokinetic sucrose gradients containing 5 mM TEACI pH 7.4, 0.2 mM Na_2EDTA and 10 mM NaCl (Noll 1969). Centrifugation was performed for 14 hr at 25'000 g in a Kontron TST 28/17 swing out rotor. The gradients were monitored at 254 nm and the bottom fractions containing the larger fragments of soluble chromatin were used for analysis by electron microscopy (Thoma & Koller 1981).

Electron microscopy - The fractions with the large chromatin fragments were divided into four aliquots and dialyzed against 5 mM TEACI (pH 7.4), 0.2 mM Na_2EDTA containing 0, 10, 40 or 100 mM NaCl respectively for 4 hr. Then the dialysis buffer was made 0.1% (v/v) with glutaraldehyde and samples were fixed at least for 15 hr and prepared for EM observation (Thoma et al. 1979).

Histone extraction from purified nuclei - Nuclei were resuspended in 0.25 NHCl (Elpidina et al. 1979) or in 5% (v/v) perchloric acid (PCA) (Sanders 1977) and proteins extracted for 60 min under constant agitation. Insoluble material was pelleted at 4000 g for 5 min. The supernatants, containing the histones, were removed and dialyzed against 1 mM TEACI, 0.2 mM Na_2EDTA pH 7.4 and lyophilized. All the preparations were carried out at 0-4°C if not otherwise stated.

SDS Tricine PAGE (Schägger & von Jagow 1987) - Lyophilized histones were solubilized in sample buffer, and separated in a linear 17.6% SDS Tricine polyacrylamide gel as described (Schlimme et al. 1993).

Triton acid urea polyacrylamide gel electrophoresis (Alfageme et al. 1974) - Lyophilized histones were dissolved in sample buffer, and electrophoresis in a linear 15% triton DF-16 acid urea polyacrylamide gel was carried out as described (Schlimme et al. 1993).

RESULTS

Structure and compaction pattern of soluble chromatin - Soluble chormatin of *T. congolense*, centrifuged through a sucrose gradient containing 10 mM NaCl, dialyzed against concentrations of 0, 10, 40, or 100 mM NaCl, and prepared for EM observation, is organized in the form of nucleosome filaments. The soluble chromatin shows some condensation at increasing ionic strength (Figs 1-4). However, solenoids (30 nm fibers) which are typical for rat liver chromatin (Figs 5-8), are not formed. Free linker DNA can barely be seen in rat liver chromatin (Figs 5-7), but is clearly visible in trypanosome chromatin at low salt concentrations (Figs 1-3).

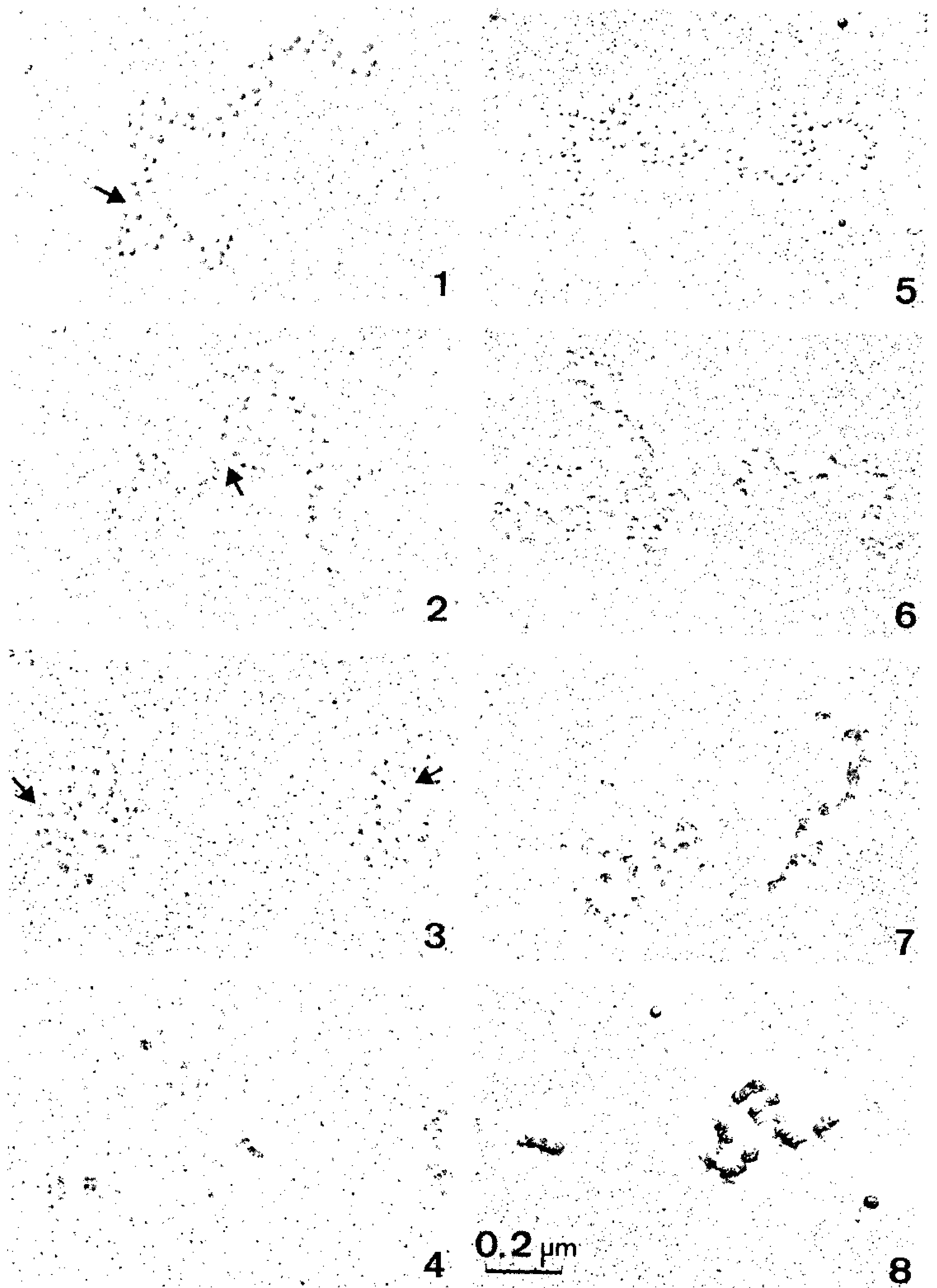
SDS Tricine PAGE - Histones of higher eukaryotes separate into four core histones and into two H1 variants in SDS Tricine PAGE (Fig. 9, lane A). Core histones of trypanosomes and higer eukaryotes differ in their electrophoretic mobility and number of variants and/or modifications. The trypanosome histones H3 and H4 have a very similar migration behaviour, while histones H2A and H2B are quite heterogeneous (Fig. 9, lanes B-E). Histone 2 of *T. congolense* has three variations, and histone 3 has two (Fig. 9, lane D, E). Neither *T. cruzi, T. congolense* nor *T. b. brucei* has any protein migrating in the region of histone H1 of higher eukaryotes (Fig. 9, lanes B-E). Proteins extracted with 5% PCA migrate in the lower region of the core histones and are metachromatically stained with Coomassie brilliant blue (Fig. 9, lane F). These PCA-extractable proteins of *T. congolense* migrate in only one band.

Triton acid urea PAGE - In Triton acid urea gels, which separate proteins according to their hydrophobicity, the core histones of *T. congolense* migrate in four main complexes 1, 2, 3 and 4 (Fig. 10, lane A). Note that the numerical sequences of the histones in the SDS and Triton gel systems do not correspond. A fast migrating, very hydrophilic complex of H1-like proteins (Nr. 5), (Fig. 10, lanes A-F) can be seen. No proteins of higher eukaryotes can be seen in the region of H1-like proteins (lane G). Although the protein complexes 1-4 do run in the same region as the core histones of higher eukaryotes (Fig. 10, lane G), all the histones of the trypanosomes differ from those of higher eukaryotes.

Differences exist between the histones of various trypanosomes species in the number of bands, their position in the gel and their relative amount. This is specially true for the H1-like proteins (Fig. 10, lanes A-F, complex Nr. 5). All the proteins in this area can be selectively extracted with 5% perchloric acid. These proteins are metachromatically stained with Coomassie brilliant blue. The H1-like proteins can also selectively be removed by 500 mM NaCl (not shown).

DISCUSSION

The banding pattern of the core histones of *T. congolense* in Triton acid urea gels differs significantly from that of histones from higher eukaryotes and also from that of other trypanosomes. This indicates that the core histones have different amino acid compositions and sequences. In SDS Tricine gels the core histones of trypanosomes migrate in the same region as those of higher eukaryotes, which means that they have a similar molecular weight. Histones



Soluble chromatin from a sucrose gradient containing 10 mM NaCl. Nucleosome filaments of *Trypanosoma congolense* procyclic culture forms do condense at increasing salt concentrations (Figs 1-4), but do not form compact 30 nm fibers at 100 mM salt like rat liver chromatin (Figs 5-8). Free linker DNA can barely be seen in rat liver chromatin (Figs 5-7), but is clearly visible in trypanosome chromatin at low salt concentrations (Figs 1-3, arrows). Figs 1 and 5: 0 mM NaCl. Figs 2 and 6: 10 mM NaCl. Figs 3 and 7: 40 mM NaCl. Figs 4 and 8: 100 mM NaCl.

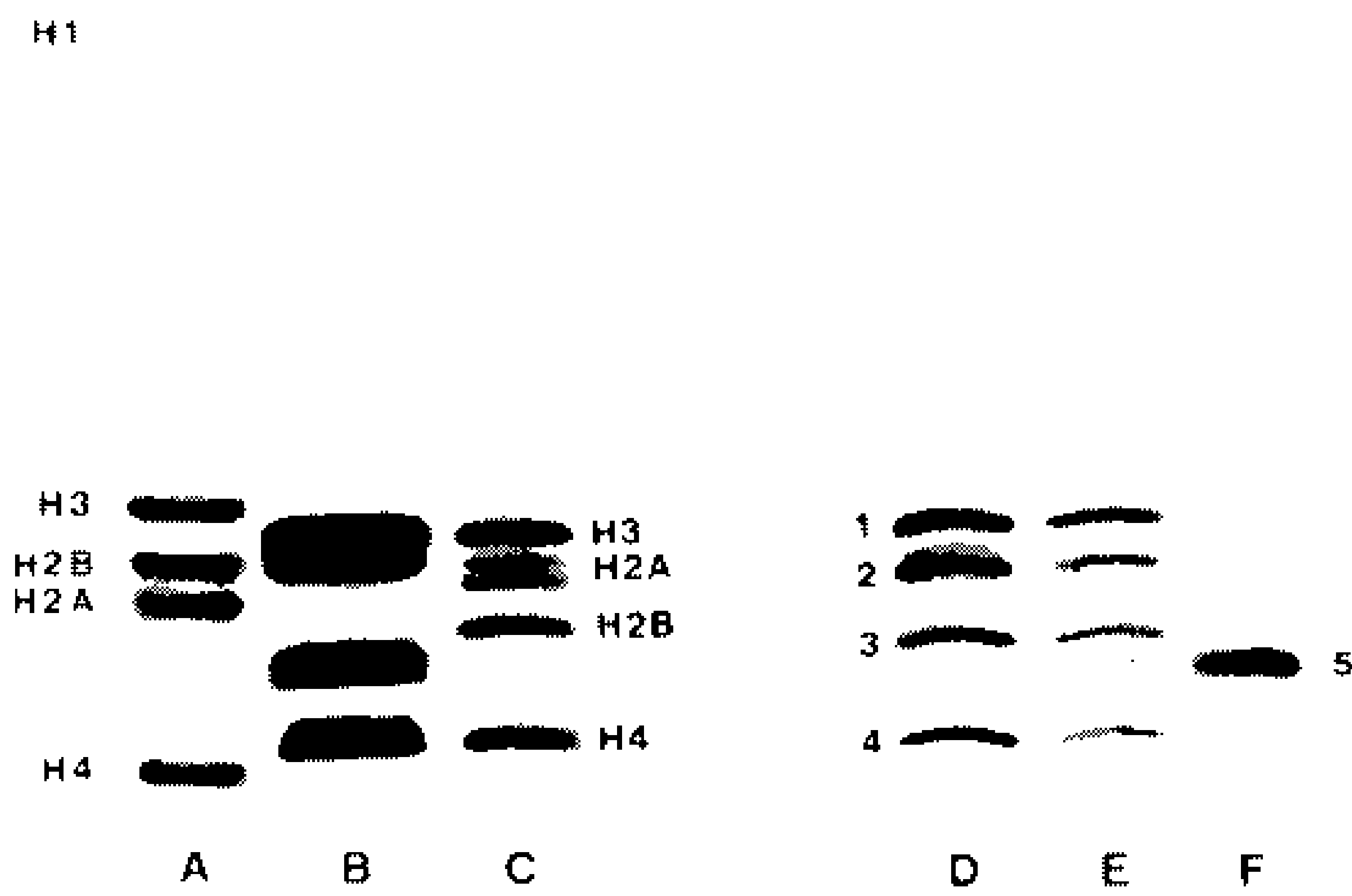


Fig. 9: histone analysis in SDS Tricine PAGE. Lane A: calf thymus histones. Histones extracted with 0.25 N HCl of *Trypanosoma cruzi* (lane B), of *T. b. brucei* (lane C) and of *T. congolense* (lane D). Histones of *T. congolense* extracted with 0.4 N H_2SO_4 (lane E) and histone H1-like proteins of *T. congolense* extracted with 5% perchloric acid (lane F). None of the trypanosomes has a protein migrating in the region of histone H1 of higher eukaryotes (lanes B-F). Proteins of *T. congolense* extractable with 5% perchloric acid migrate in the region of the core histones of the trypanosomes and are present in just one band (lane F). No significant differences can be seen between proteins extractable in 0.25 N HCl and 0.4 N H_2SO_4 (lanes D, E).

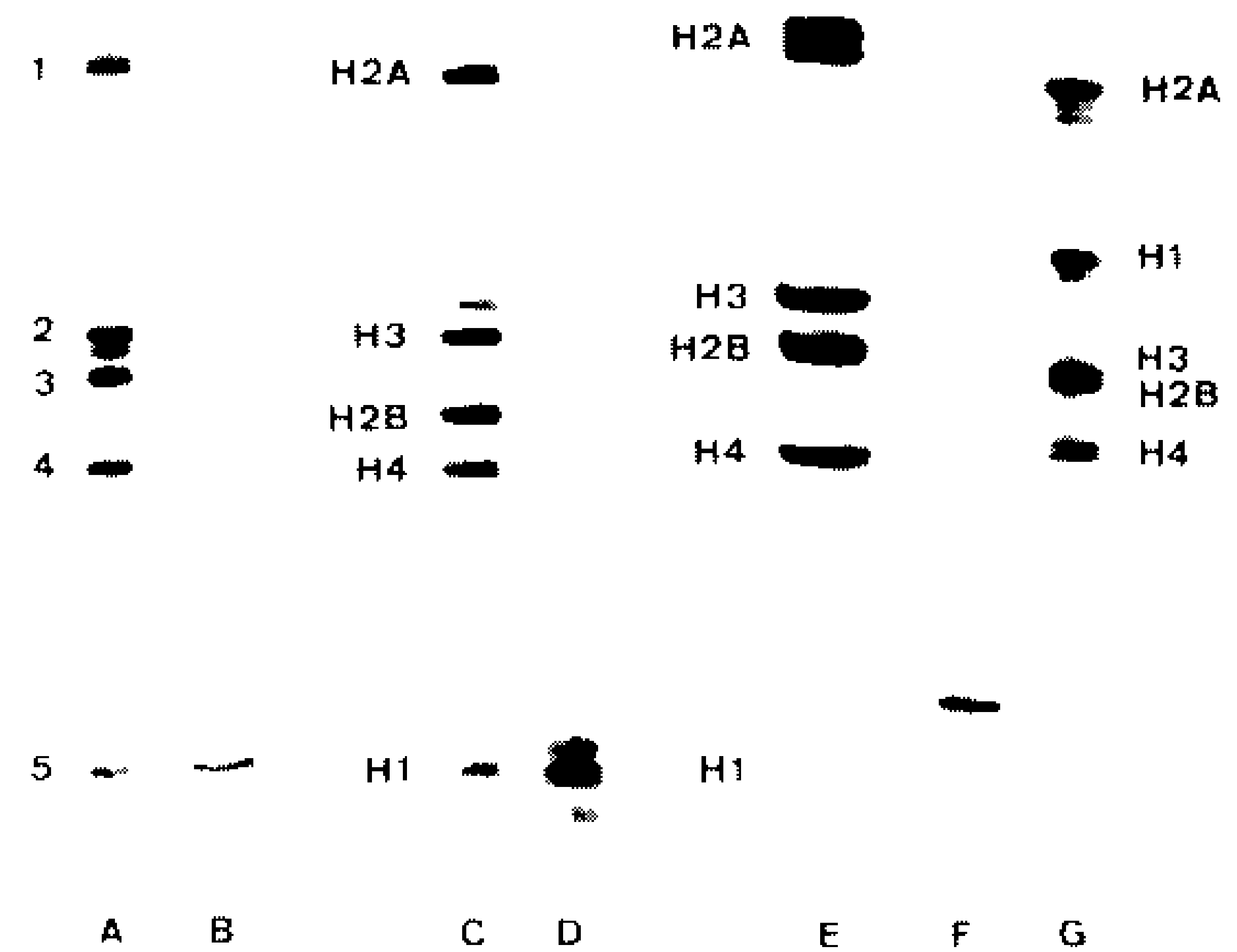


Fig. 10: histone analysis in Triton acid urea PAGE. HCl extracted histones of nuclei of *Trypanosoma congolense* (lane A), of *T. cruzi* (lane C) and *T. b. brucei* (lane E). 5% perchloric acid extracted histones of *T. congolense* (lane B), *T. cruzi* (lane D) and *T. b. brucei* (lane F). Calf thymus histones (lane G). All trypanosome species show different fast-migrating H1-like proteins (Nr 5), none of which occur in higher eukaryotes. These proteins are extracted with 5% perchloric acid, a procedure which selectively extracts histone H1 and nonhistone proteins in higher eukaryotes.

are known to be highly conserved proteins. Among them histone H4 is the most conserved, followed by H3, H2A, H2B and H1 (Van Holde 1989). Bander et al. (1992b) and Toro et al. (1992) showed for *T. b. brucei* and *T. cruzi* a sequence divergence in trypanosomes of about 35% in the N- and C-terminal regions even for histone H4, suggesting a very early separation of the trypanosomes from other eukaryotes during evolution (Hecker 1993). The observation that the band corresponding to H4 in *T. congolense* ran in the same position in both gel systems as H4 of the other trypanosomes further supports this theory. The positions in the SDS gel of the proteins 2 and 3, the counterparts of histone H2A and H2B (Bender et al. 1992a, b) are quite variable between the different trypanosome subgroups, and the considerable differences between the histones H2A and H2B reflect this fact.

In *T. congolense*, a histone H1-like protein is present, but does not have as many variants or modifications as its counterparts in *T. cruzi* or *T. b. brucei*. Like H1 of the other trypanosomes, it is extractable with 5% PCA and shows metachromasia, which is a sign of a high content of lysine (Duhamel et al. 1980). Furthermore it can be selectively removed by 500 mM NaCl. The similarity of the migration behaviour of this protein as compared to other trypanosome H1-like proteins Duschak and Cazzulo (1990), and all the other properties mentioned above, allow the conclusion that the PCA-extractable proteins of *T. congolense* correspond to the H1-like proteins of other trypanosomes. However, the question how such a small histone H1 can execute its role in chromatin condensation is not yet answered. The poor condensation of the chromatin of *T. congolense* might be due to the small size of this H1-like protein. Genetically inactive chromatin of higher eukaryotes is organized as nucleosome filaments and forms 30 nm fibers (solenoids) at 100 mM NaCl (Thoma et al. 1979). The chromatin of *T. congolense* is also organized in the form of nucleosome filaments, but does not form 30 nm fibers. The compaction pattern of the chromatin of *T. congolense* procyclic culture forms is comparable to that of *T. b. brucei* blood stream forms (Schlimme et al. 1993) and to that of *T. cruzi* epimastigote culture forms (Hecker et al. 1994), but is more pronounced than that of *T. b. brucei* procyclic culture forms (Hecker & Gander 1985, Schlimme et al. 1993). Trypanosomes do not form chromosomes *sensu stricto*, and we do not know, whether a high degree of condensation is necessary for the function of the genetic system, as it is in higher eukaryotes. One of the main questions that still remains is what type of condensation in trypanosome chromatin is achieved. The stability of chromatin depends on the nature and strength of the protein-protein and the protein-DNA interactions (Yager et al. 1989). The stability of the soluble chromatin of *T.*

congolense is reduced as compared to that of higher eukaryotes. The interactions of histones and DNA are weak and easily destabilized by experimental conditions, as described for other trypanosomes (Hecker et al. 1989, Bender et al. 1992c, Schlimme et al. 1993, Burri et al. 1993).

Differences in the stability of the chromatin among the trypanosomes exist not only between the different species (Hecker et al. 1994), but also between different stages of the life cycles (Schlimme et al. 1993).

The chromatin of *T. congolense* is a very good model for chromatin research in trypanosomes, since it has some major advantages as compared to the other trypanosomes investigated so far. First, *T. congolense* procyclic culture forms are easy to cultivate *in vitro* in large numbers. Second, the instability of the soluble chromatin is not as pronounced as in *T. b. brucei* procyclic culture forms. Therefore reconstitution of soluble chromatin and other experiments to investigate the condensation behaviour can be better carried out with this parasite. Last but not least, makes the fact, that *T. congolense* is not pathogenic for humans, it is very convenient to work with.

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