The influence of chloramphenicol and cycloheximide on protein synthesis of "Trypanosoma cruzi" : short communication

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The influence of chloramphenicol and cycloheximide on protein synthesis of *Trypanosoma cruzi*

Short communication

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Introduction

Trypanosomes possess the unique feature that up to 25% of their total DNA is extranuclear and found in an organelle, the kinetoplast. The kinetoplast lies within the cell's unique mitochondrion and in the last decade much information has accumulated on the structure and organization of its DNA (K-DNA). It is now known that about 95% of the K-DNA mass consists of minicircles (700–2500 base pairs) which are believed to have non-coding functions and about 5% of the mass consists of maxicircles (20–38 kilobase pairs). The latter are most probably equivalent to the mitochondrial DNA of higher eukaryotes and code for a few mitochondrial proteins (for review see Englund et al., 1982).

Disagreement exists in the literature of the question, to what extent, in trypanosomatids, mitochondrial protein synthesis contributes to overall protein synthesis. It has been reported for instance by Laub-Kuperszejn and Thirion (1974) that in *Crithidia luciliae* up to 50% of the total protein synthesis can be attributed to the mitochondrial system while Kleisen and Borst (1975) working with the same organism under similar conditions attributed less than 1% of total synthesis to the mitochondrial system. As a matter of fact, little is known about the rate of mitochondrial protein synthesis and the coding properties of K-DNA in *Trypanosoma cruzi*.

We, therefore, investigated whether the classical approach for discriminating between pro- und eukaryote-type protein synthesis with the specific inhibitors chloramphenicol and cycloheximide would be of value when applied to *T. cruzi*. Throughout our study we used the *T. cruzi* Y 10 strain which was grown as described by Camargo (1964). The proteins synthesized during exposure to the drugs were analysed by sodium dodecylsulphate (SDS) gel electrophoresis (Laemmli, 1970) and by fluorography (Bonner and Laskey, 1974).

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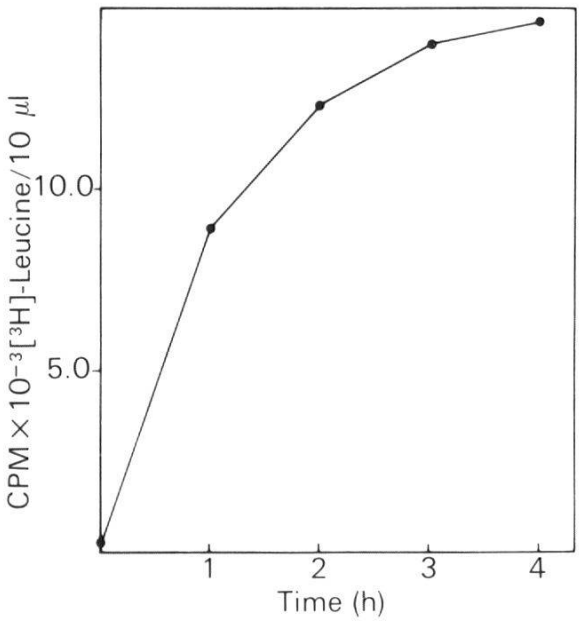

Fig. 1. Incorporation of [^{3}H]-leucine into *T. cruzi*. The system contained: 100 μl of PSG containing 5×10^7 cells, 40 μl PSG 10×, 16 μl of a 2× amino acid mix (Hunt and Jackson, 1974) (without leucine) and 4 μl of [^{3}H]-leucine (56,5 Ci/mM; 1 mCi/ml). The volume was brought up to 500 μl with H_2O, incubation was at 28° C. At the time points indicated 10 μl aliquots were transferred to Whatmann 3 mm filter-paper and the radioactivity incorporated into porteins determined according to standard procedures.

Results and Discussion

The cells were harvested at the beginning of the exponential phase by low-speed centrifugation, washed in PSG (0.6% $NaHPO_4 \times 7\ H_2O$, 0.67% NaCl, 0.9% glucose) and resuspended in PSG to about 5×10^8 cells/ml. In Fig. 1 the incorporation kinetics of [^{3}H]-leucine into cells prepared as described above is shown. The cells seem to incorporate leucine linearly during the first hour and enter the steady-state phase after four hours. Since we were interested in the proteins synthesized during the linear growth phase of the cells, the influence of the antibiotics were tested using a 2-hour incubation time. In Fig. 2 the effects of chloramphenicol and cycloheximide on gross protein synthesis are shown. Cycloheximide (250 ng/ml) inhibits protein synthesis by 97%, results that disagree with those reported by Kleisen and Borst (1975) on the effect of this drug on protein synthesis in *Crithidia luciliae* and with those by Young and Hutner (1979) in *Tetrahymena* sp. Both groups found comparable inhibition rates only with 10-fold higher levels of antibiotic. Chloramphenicol has a much lesser inhibitory effect on overall protein synthesis; 50% inhibition is obtained only when the cells are exposed to 1 mg/ml while exposure to 100 and 500 μg/ml results in about 30% inhibition. With *Tetrahymena* sp. Young and Hutner (1979) obtained results similar to ours while in *Crithidia luciliae* Kleisen and Borst (1975) only found a 2% inhibition. Laub-Kuperszejn and Thirion (1974) report 50% inhibition with chloramphenicol a result comparable with that found in *T. cruzi*.

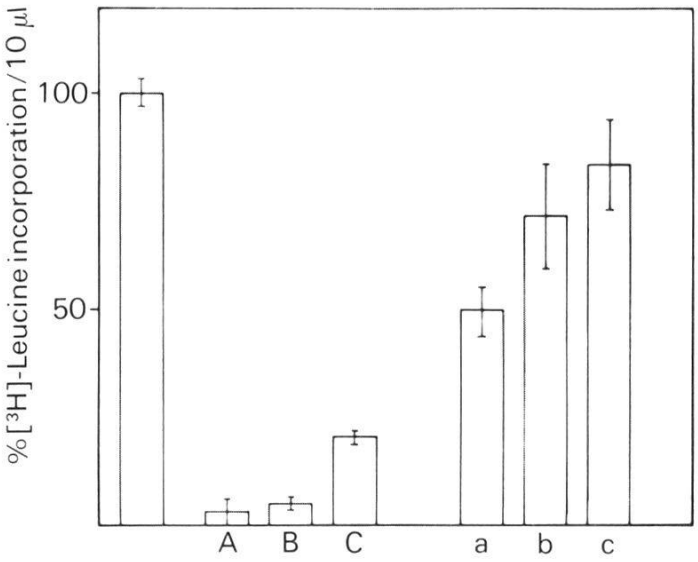

Fig. 2. Effects of cycloheximide and chloramphenicol on gross protein synthesis of *T. cruzi*. A, B and C: cycloheximide 250, 100 and 10 ng/ml; a, b and c: chloramphenicol at 1000, 500 and 100 µg/ml. Prior to addition of the label the cells were preincubated for 15 min in the presence of the drugs and then the incubation was continued for 2 h at 28° C; conditions and determination of radioactivity as described in Fig. 1.

In Fig. 3 an analysis of the proteins synthesized in the presence of the drugs are shown. From Fig. 3 A it is evident that after Coomassie-Blue staining the patterns of the control (slot 4) and of the drug-treated samples (slots 1–3) are identical, an expected result considering the 2-hour exposure time to the drugs. In Fig. 3 B a fluorograph of the same gel is shown: chloramphenicol-treatment (slot 3) results in a significant loss of intensity in seemingly all protein bands and after cycloheximide (slot 2) or both drugs together (slot 1) all but two proteins have disappeared. However, when the exposure time is varied according to the different amounts of gross incorporation, another picture emerges (Fig. 3 C): comparing the control (slot 4) with the chloramphenicol-treated material (slot 3) only two proteins marked with bars (MWs 85,000 and 76,000 daltons) are affected with certainty. All other proteins are still synthesized although in lesser quantities.

In cells treated with cycloheximide (slot 2) or both drugs together (slot 1) a group of proteins in the molecular weight range of 82,000 to 60,000 daltons (marked with a vertical bar) is no longer detectable, all others are still synthesized in minimal quantities.

These results led us to the following conclusions: the classical approach used to discriminate between mitochondrial and cytoplasmic protein synthesis based on the use of "specific" inhibitors (Tsagolof and Macino, 1979) is of limited value when applied to intact *T. cruzi*. A strictly quantitative evaluation of antibiotic-resistant protein synthesis yields little if any information on the differential contribution of the two systems, most probably because of the stringent interdependence of the two systems.

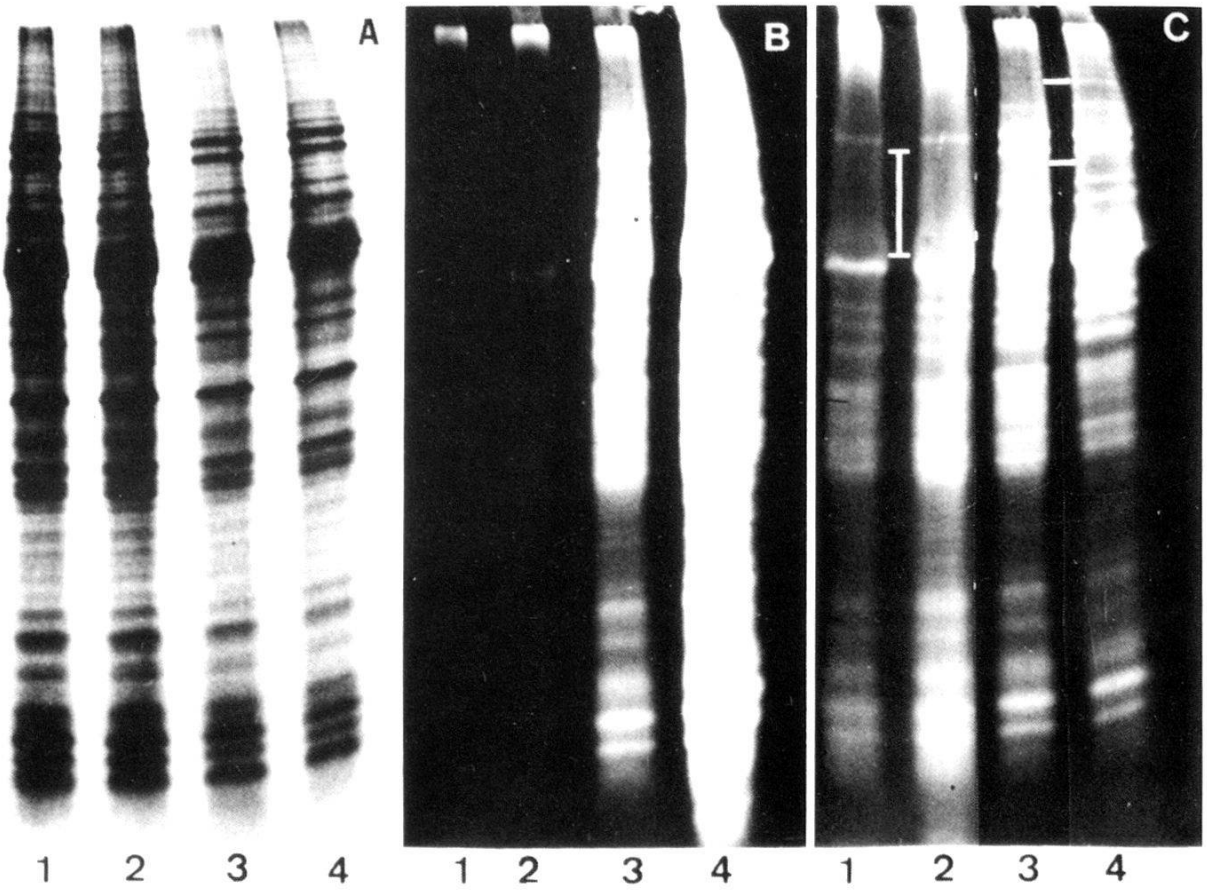

Fig. 3. SDS-gel and fluorographic analysis of the proteins synthesized during exposure to the drugs. Incorporation conditions were as described in Fig. 1 except that the radioactive amino acid was [^{35}S]-methionine (3 μl/500 μl of system; 1220 Ci/mol, 10 mCi/ml). After 2 h of incubation the cells were pelleted, washed and resuspended in sample buffer and about 150 μg of proteins/sample were analysed on 16% gels (Laemmli, 1970). 3 A: Coomassie-Blue staining; 3 B: fluorography; 3 C fluorography with adjusted exposition times. Slot 1: cycloheximide at 250 ng and chloramphenicol at 500 μg/ml; slot 2: cycloheximide at 250 ng/ml; slot 3: chloramphenicol: 500 μg/ml; slot 4: control.

Furthermore, while cycloheximide is a potent inhibitor of overall protein synthesis, only a small group of proteins are knocked-out by the drug, all others are still synthesized. They can hardly be of mitochondrial origin because of the limited coding capacity of the K-DNA. Basically the same arguments hold true for our experiments with chloramphenicol. This drug also seems not to inhibit specific proteins except for two bands found in the SDS gels but rather affects overall protein synthesis.

These findings could be explained by postulating the existence of two protein-synthesizing systems in *T. cruzi* one of which is partially sensitive to the drugs and the other completely insensitive to the antibiotics. On the other hand, one might speculate that in a population of 5×10^7 cells enough antibiotic-resistant mutants are present to account for the remaining incorporation.

One way to discriminate between these possibilities would be the isolation and characterization of pure mitochondrial fractions from *T. cruzi* and the subsequent study of their protein-synthesizing capacity.

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