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Trypanosoma brucei: Differentiation of in Vitro-Grown Bloodstream Trypomastigotes into Procyclic Forms[1]

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ABSTRACT. *Trypanosoma brucei* strain 366D trypomastigotes grown at 37°C in the presence of a human fibroblast cell line formed foci underneath the feeder cells whereas trypanosomes grown in the presence of a human epithelial cell line grew only in the culture supernatant. A culture system was developed to study the differentiation of bloodstream trypomastigotes grown in the epithelial cell system into procyclic trypomastigotes at 27°C. The morphological differentiation into the procyclic form was complete by 48 h. Cell division did not occur until 30–40 h after transfer to 27°C. Various characteristics of this system were examined, including the effect of the feeder layer, the type of medium, the presence of the metabolites cis-aconitate and citrate, the preadaptation period, and the trypanosome cell concentration. The respiration of the recently differentiated procyclic cells was less sensitive to inhibition by CN^- than that of established procyclic forms, implying a delayed appearance of complete mitochondrial oxidative pathways. This trypanosome differentiation system has the advantage that the animal host is not needed and the entire process is carried out in in vitro culture.

THE differentiation of the bloodstream trypomastigote form of *Trypanosoma brucei* into the procyclic culture form, which survives in the tsetse midgut, involves some external morphological change combined with several dramatic internal ultrastructural and physiological changes. The long slender bloodstream trypomastigote has a glycoprotein surface coat and contains a single mitochondrion that lacks well-developed cristae and the associated mitochondrial enzymes. Within the bloodstream, the initial event in the differentiation process is the appearance of the 'stumpy' trypomastigote form, which is a non-dividing cell fixed in G_0 that contains an active mitochondrial lipoyl dehydrogenase enzyme activity but does not contain mitochondrial cytochromes. The next step in the process, the differentiation of the stumpy form to the procyclic form, occurs in nature in the insect midgut. It involves a loss of the surface coat and the establishment of a functional mitochondrion containing cristae.

Cultivation of animal-infective monomorphic and pleomorphic bloodstream forms of African trypanosomes at 37°C in the presence of mammalian feeder cells has been described (7, 11, 12, 15, 17) as has the in vitro differentiation of bloodstream forms into procyclic culture forms. Most work has involved the use of bloodstream forms from the animal although Brun et al. (7) noted, without providing data, that a small portion of cultured polymorphic bloodstream forms transformed within three days into procyclic forms in SDM-79 medium. Brun & Schonenberger (6) observed that the addition of the Krebs cycle metabolites, citrate and cis-aconitate, markedly stimulated this process. Simpson et al. (15) confirmed the stimulatory effect of citrate and cis-aconitate on the differentiation process and also showed that continued exposure of the bloodstream trypomastigotes to these metabolites in culture at 37°C was toxic. The mechanism of action of citrate and cis-aconitate is unknown.

Overath et al. (13) examined the repression of surface glycoprotein synthesis and the loss of the surface coat in *T. brucei* stock 427 during the procyclic differentiation process. Bienen et al. (2–4) found that *T. brucei* LUMP 1026 bloodstream trypomastigotes from the rat underwent a reproducible differentiation into the procyclic form without cell death in modified Cunningham's medium in approximately 72 h at 27°C. They also examined antimycin A, CN^-, and SHAM-sensitive respiration as well as the appearance of cytochromes by spectral analysis. They concluded that the morphological differentiation to the procyclic form occurs independently of the biochemical differentiation that progresses through the initial appearance of mitochondrial dehydrogenases by day 5 to the establishment of a complete cytochrome complement by day 20. Ultrastructural correlates of the biochemical changes occurring during this life cycle change have also been presented (10). The single mitochondrion undergoes a gradual change from the "narrow tube with only few short cristae" (10), characteristic of the long slender bloodstream form, to the extended and branched structure containing abundant cristae found in the established procyclic form. The finding that these morphological changes are incomplete even after 11 days of culture at 27°C (10) is in agreement with the biochemical studies.

In this paper we examine various factors affecting the in vitro differentiation of cultured bloodstream trypomastigotes of *T. brucei* strain 366D into procyclic forms. A preliminary account of this work was reported previously (15).

MATERIALS AND METHODS

The human fetal lung fibroblast cell line (F2000) was obtained from Flow Labs. The human skin epithelium cell line (NCTC 3075) was obtained from the American Type Culture Collection (ATCC CCL 19.1). A human epithelial tumor cell line (SCaBER) was obtained from Dr. Ahmed of the UCLA Dermatology Department (1); this line was originally derived from a human bladder cancer. All cell lines were maintained in T25 flasks at 37°C in RPMI 1640 medium with 10% (v/v) fetal calf serum (heat-inactivated). Gamma irradiation of the F2000 cell monolayer was performed using a cobalt source. Gamma irradiation of the CCL 19.1 cell line often led to extensive cell sloughing and therefore was not generally employed.

The *T. brucei* strain 366D cells were obtained from Dr. Leo Jenni of the Swiss Tropical Institute and were routinely maintained in culture at 37°C in RPMI medium with 10% (v/v) fetal calf serum in the presence of the F2000 fibroblast cell line. Trypanosomes were transferred to the CCL 19.1 culture at least one week prior to use for differentiation. Established procyclic cells were maintained at 27°C in modified REI medium (16), also known as REIA medium (14).

Measurement of percentage of procyclic forms was performed by mixing 100 μl of culture with 100 μl of 10% (v/v) formalin in 0.15 M NaCl–0.015 M Na citrate (pH 7) and scoring cell types in a hemocytometer under phase contrast microscopy at 400× magnification. Cell concentration was also measured in a hemocytometer. At least 200–600 cells were counted for each point. The data in Figs. 4–9 are representative results from experiments that were repeated several times with similar results.

Respiration studies were performed using a Clark oxygen electrode with a 2-ml water-jacketed chamber. Cells were resuspended in REIA medium for respiration measurements at 37°C

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