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MORPHOGENESIS AND THE FUNCTION OF THE KINETOPLAST IN "LEISHMANIA"

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The kinetoplast is a structure with mitochondrial properties unique to the parasitic flagellates of the family Trypanosomatidae and to a few free-living species. It has long been known that the kinetoplast is Feulgen-positive and that it also takes up standard mitochondrial stains such as Janus Green B. It does not stain for basic proteins. It is a disc-shaped, slightly concave structure, always found at the base of the flagellum, intimately associated with but not connected to the basal body. Within the matrix of the kinetoplast can be seen a lamellar structure, which in some micrographs seems to be composed of fibrils arranged in a supra-spiral-like configuration. There is good evidence to suggest that this material is, or contains, the stainable DNA.

The unusual nature of this mitochondrion is probably associated with the complex developmental changes occurring during the life cycle of these cells. The members of any one genus can exist in several different morphological forms. The transformation of morphogenetic change from one to the other occurs when the cell moves from one environment to another as, for example, from the vertebrate to the insect host.

For example, *L. donovani* lives in the mammalian host as an intracellular parasite — the so-called LD bodies. The isolated LD bodies can transform to the flagellated leptomonad or culture form in 20-40 hours at 27°C. RUDZINSKA, D'ALESANDRO & TRAGER observed, as early as 5 hrs., a change in the morphology of the kinetoplast-DNA and an apparent biogenesis of mitochondria from the kinetoplast. We thought this system might shed some light on the function of the kinetoplast.

Using the criteria of size and leptomonad form this transformation was found to be dependent on

a source of several amino acids. The addition of further nutrients, such as glucose or blood sped up the process. The extent of transformation was obtained by measuring relative cell size in stained smears at various times. The optical density of the suspension provides another method for measuring increase in cell mass. The cells tend to clump after about 5 hours in culture, but this can be eliminated by mild sonication of formalin-treated samples. Direct cell counts of such preparations demonstrated that no cell division occurs during the first 20-30 hours of the process.

Cyanide-sensitive respiration was found to show a 2-4-fold increase during the transformation, as would be expected from the different QO2's ($\mu MO_2/min/10^7 cells$) of the freshly isolated LD's and the culture leptomonads: The LD's showed a QO2 of 0.29 ($\pm$.001) in saline-buffer-glucose to 0.42 ($\pm$13) in Medium C, while the logphase leptomonads gave values of 1.8-2.2. The final increase is variable because the extent of transformation may vary from 15 to 75% in different preparations. The increase in respiration is inhibited by Actinomycin D, puromycin, mitomycin C, and chloramphenicol. The effect of Actinomycin D is not necessarily due to the selective inhibition of RNA synthesis-which does indeed occur—but may be due to a direct inhibition of respiration — which was demonstrated to occur with the leptomonad.

LD's kept in saline-buffer-glucose for 20 hours at 27°C showed no increase in oxygen uptake and no morphogenesis, but when resuspended in Medium C, their respiration increased 3.2-fold in 28 hours.

It was demonstrated that the initial respiration was due to the LD's and not to splenic mitochondrial contamination as follows: The oxygen uptake of isolated spleen mitochondria exhibited a 50% decrease in 4 hours at 27°C in Medium C, whereas that of LD preparations showed no change. Furthermore, treatment of the spleen mitochondria with a Bactotrypsin preparation at 37°C for 30 mins. brought about an 83% decrease in respiration, while identical treatment of LD preparations had no effect.

We then investigated the leptomonad form, the product of this morphogenetic process. The organism chosen was *Leishmania tarentolae,* which could be grown in a defined medium (Medium C).

Total cell DNA was isolated from *L. tarentolae* by a phenol method. Two bands were evident in CsCl equilibrium centrifugation, the difference in densities being .013 g/cc. Velocity sedimentation show-

ed the DNA to be remarkably homogeneous, as indicated by the sharp boundaries in the tracings. The major CsCl component (nuclear DNA) has an $S_{20,w}$ of about 21.

The respiration of *L. tarentolae* was then studied. The QO2 was found to vary with the age of the culture — being highest in the log phase. Addition of glucose to washed cells stimulated the oxygen uptake two-fold. KCN, amytal, Na azide, and antimycin A all inhibited the respiration, indicating that the normal cytochrome chain was functional here. In the case of the leptomonad of *L. donovani,* there was a four-fold glucose stimulation.

L. tarentolae grown in the presence of acriflavin become *a*- or *dys*kinetoplastic; that is, as shown by MÜLPHORDT and by TRAGER and RUDZINSKA, they exhibit a morphological loss of DNA in the kinetoplast and a general deterioration of the mitochondria. They also lose their ability to grow in axenic culture. The maximum percentage of dyskinetoplastic cells obtained with one strain was over 90%. The "atypical" kinetoplasts in these cells did not stain with Janus Green B as do normal kinetoplasts and functioning mitochondria. DNA was then isolated from normal and dyskinetoplastic cells. Note the complete disappearance of the minor band, confirming that it represents kinetoplast DNA.

Evidence presented indicates that at least one of the initial effects of acriflavin is a specific inhibition of kinetoplast-DNA synthesis, thus leading to a dilution of kinetoplast-DNA upon further cell division. The specificity of effect seems to be due to a specific localization of the dye in the kinetoplast, as seen by fluorescence microscopy. At high dye concentrations, the cells do not divide and rapidly degenerate — and the dye is seen to bind also to the nucleus and cytoplasm. No degenerative effect was noticed at 4ºC, although the dye was found to both organelles. Hence, metabolic activity is necessary for the dye to have an effect. It is interesting to note that acridine orange binds equally at all concentrations to both the nucleus and the kinetoplast and does not have the dyskinetoplastic-producing effect.

We have recently obtained a mutant of *tarentolae* (NW strain) which exhibits an enhanced sensitivity to acriflavin and a decrease in the percentage of dyskinetoplastic cells formed. The mutation seems to be in the permeability of the cell to the dye. The maximum percentage of dyskinetoplastic cells obtained after 6 days of growth was 62%.

It was hoped that selective binding of acriflavin to the kinetoplast would provide a method to selectively inhibit its genetic function by photodynamic action. However, visible irradiation of such cells damage as evidence by a decrease in oxygen uptake and flagellar motility. This does, however, again demonstrate the mitochondrial nature of the kinetoplast.

In summary, the kinetoplast seems to be a highly differentiated mitochondrion uniquely adapted to the unusual life cycles of the hemoflagellates. It contains a DNA of different buoyant density and hence different base ratio than the nuclear DNA and this may represent up to 10% of the total cell DNA. This DNA could not be detected in acriflavin-induced dyskinetoplastic *L. tarentolae*. Finally, a process of cellular differentiation involving the kinetoplast was partially defined in terms of nutritional requirements, nucleic acid metabolism and respiration changes. There was a good correlation between the increase in cell size, the appearance of leptomonad morphology and the increase in respiration. This process may now provide a system with which to investigate the possible role of mitochondrial DNA in mitochondrial biogenesis and in cellular differentiation.

ABSTRACT

The LD-leptomonad transformation of *L. donovani* was found to be dependent on a source of several amino acids, with further nutrients speeding up the process. The LD's have a cytochrome-type respiration which increases several-fold during the transformation. Various inhibitors of RNA and protein synthesis inhibit the morphogenesis and the increase in respiration. The respiration of *L. tarentolae* was shown also to be of the cytochrome type by means of respiratory inhibitors. Acriflavin at low concentrations binds specifically to the kinetoplast and specifically inhibits kinetoplast-DNA replication. Visible irradiation of such acriflavin-treated cells photodynamically inhibits cellular respiration.