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Differentiation of *Trypanosoma brucei* Bloodstream Forms to the Procyclic Vector Stage: Signals and Mechanisms

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The differentiation of bloodstream forms of *Trypanosoma brucei* to the procyclic stage takes place in the midgut of the tsetse insect vector and is associated with a temperature shift from 37℃ to 27℃. The induction mechanism operative in the tsetse midgut environment is unknown. In culture, differentiation is very efficiently induced by addition of *cis*-aconitate in combination with the temperature shift. Here we show that *cis*-aconitate is alone sufficient to trigger differentiation. A careful single cell analysis with cell cycle progression and differentiation markers has shown that bloodstream forms are only responsive to *cis*-aconitate in the Go phase of the cell cycle. This is consistent with the fact that only nondividing stumpy forms differentiate synchronously and with fast kinetics. Thus, stumpy forms are an obligatory intermediate stage in the life cycle.

To investigate the mechanism of induction of differentiation by *cis*-aconitate, we have cloned the aconitase gene of *T. brucei*. The enzyme which has strong sequence similarity with iron response proteins is localized in the cytoplasm as well as in the mitochondrion and is stage-regulated at the level of translation. Though iron response proteins in the mammalian system are cytoplasmic aconitases with a regulatory RNA-binding activity, a homozygous genetic disruption of the *T. brucei* aconitase shows that this gene is not involved in the induction of differentiation by *cis*-aconitate. However, the mutant strain documents that aconitase activity and thus citric acid cycle activity is not essential in procyclic trypanosomes in culture. For further investigation of the role of citrate and *cis*-aconitate for induction of differentiation, strains with inducible expression of a heterologous citrate synthase have been generated. The phenotype of these strains will be discussed.

The hallmark of differentiation to the procyclic stage is a fast and complete exchange of surface proteins. The variant surface glycoprotein of bloodstream forms (VSG) is replaced by procyclin/PARP within a few hours upon induction, and the entire membrane protein trafficking occurs through a tiny surface area called the 'flagella pocket'. To analyse the kinetics of surface protein exchange and surface protein dynamics during differentiation, we have constructed fusions of both surface proteins with the green fluorescent protein. Using three-dimensional fluorescence microscopy and digital deconvolution software, we follow the fate of the fusion proteins in living organisms in real time and three dimensions. To refine this analysis, markers for the endocytic pathway of trypanosomes are required. Therefore, a small GTP-binding protein which is homologous to rab7 of other organisms has been cloned and shown to be specifically associated with late endosomes. A combination of gene targeting methods and advanced fluorescence microscopy and image analysis tools with the excellent inducibility of differentiation provides a unique system to study the dynamics and sorting of GPI-anchored surface proteins.

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