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Control of Proliferation and Differentiation of *Trypanosoma brucei* Bloodstream Forms

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Trypanosoma brucei proceeds through a complex life cycle in the mammalian host and tsetes insect vector. During the ascending parasitemia in the mammalian blood, replicating slender bloodstream forms differentiate to cell cycle arrested stumpy forms. This transition is induced by a density sensing mechanism operating via the cAMP signal transduction pathway (Vassella et al. J. Cell Science, 110, 2661 ff). Parasite density control is important for host survival and thus for persistence of the infection. To uncover the intracellular signalling mechanisms inducing cell cycle arrest and differentiation, 3 isoforms of the catalytic subunit of protein kinase A were cloned and biochemically characterized. The cAMP pathway and its role for the life cycle is being dissected by a combination of reverse genetic tools: 1) gene disruption of individual PKA-C subunits, 2) inducible expression of a PKA-C subunit, of a specific protein kinase inhibitor peptide (PKI) and of a phosphodiesterase. To this end, gene targeting technology and the tetracycline inducible expression system have been established for the first time in a differentiating (pleomorphic) *T. brucei* strain.

A second signalling pathway was identified which functionally antagonizes the cAMP pathway and stimulates cell cycle progression of slender bloodstream forms in culture. This was concluded from the phenotype of a homozygous disruption of an atypical protein kinase C of *T. brucei* in a differentiating (pleomorphic) strain but the absence of this phenotype in a targeted non differentiating (monomorphic) strain.

Thus, we are at the beginning to decipher the signalling mechanisms which control part of the life cycle of trypanosomes. Understanding the control of proliferation in the mammalian stages will provide new targets to fight this important tropical pathogen.

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