Comprehensive investigation of the Trypanosoma brucei kinetoplast and the discovery of a slew of new protein constituents.

M Hammond[1]; J Pyrih[2]; L Cadena[3]; M Svobodova[3]; V Raskova[3]; A Alves[4]; S Dean[5]; JD Sunter[6]; R Wheeler[7]; K Gull[8]; C Benz[9]; I Durante[3]; J Lukeš[10];

[1] Biology Centre, Institute of Parasitology, Czech Republic; [2] Department of Biochemistry, University of Cambridge, UK; [3] Institute of Parasitology, BioCenter, České Budějovice, Czech Republic; [4] Oxford Brookes University, UK; [5] Division of Biomedical Sciences, Warwick Medical School, UK; [6] University of Oxford, UK; [7] Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford, UK; [8] University of Oxford, Sir William Dunn School of Pathology, UK; [9] Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic, Czech Republic; [10] Institute of Parasitology, Biology Centre, ASCR, Czech Republic

The TrypTag mitochondrial investigation of *Trypanosoma brucei* (MitoTag) revealed a fluorescent tagged association with the concentrated mitochondrial DNA structure known as the kinetoplast, documented in several hundred mitochondrial proteins for the first time. Combined with transmembrane domain prediction, this enabled the sub-localisation of 1,053 mitochondrial proteins within this organelle, circumventing the need for electron microscopy or other intensive localisation methods. Furthermore, we demonstrate a method to distinguish genuine kinetoplast proteins from artificial tagging-induced associations among mitochondrial proteins, and accordingly demonstrate over a dozen novel kinetoplast components. From this, we expand the functions of the kinetoplast to metabolic pathways of dUMP synthesis and One Carbon metabolism. This constitutes the largest single expansion of the kinetoplast repertoire to date and represents an exciting development for a complex long considered an attractive drug target due to its clade-specific presence.