

University of St Andrews

Exploring the activity and essentiality of the Δ-6 desaturase in *Trypanosoma brucei*

Michela Cerone^a and Terry K Smith^a



^a School of Chemistry, BSRC, University of St Andrews, North Haugh, St Andrews, Fife, Scotland, UK





Fig 1. Schematic representation of Δ -6-desaturase trans-membrane enzyme and its reaction mechanism. Green indicates the ω -3 PUFAs biosynthetic pathway and orange the ω -6 PUFAs biosynthetic pathway.

3. Looking for a phenotype: polyunsaturated fatty acids production and growth curve



Fig 2. Immunofluorescence microscopy pictures of BSF *T. brucei* OE- Δ 6. The HAtag (green) and mitotracker (red) show that $\Delta 6$ -desaturase (white arrows) is a mitochondrial associated (white arrows) enzyme. The same result was obtained in PCF *T. brucei* OE- Δ 6 (not shown).





Fig 3. Panel A and B show bar charts (top and middle) of the different polyunsaturated fatty acids (X axis) and the relative abundance (Y axis) found in T. brucei wild type (WT), overexpression (OE) and knockdown (KD) of \Delta6-desaturase cultured in high fat (10% FBS) media (top) and low fat (5% FBS) media (middle) in presence of tetracycline. Panel A and B also show the conversion rate of the substrate (22:4) into product (22:5) for T. brucei PCF (panel A, bottom), and into product (22:6) for T. brucei BSF (panel B, bottom) by Δ6-desaturase. Panel C represents the growth rate of T. brucei wild type (WT), overexpression (OE) and knockdown (KD) of Δ6-desaturase cultured in low fat (5% FBS) media with (solid line) or without (dotted line) supplementation of 10 μ M of the product 22:6, respectively for PCF (top) and BSF (bottom).

5. Looking for a phenotype: inositolphosphoryl-ceramide (IPC) production in OE-Δ6 in BSF

Α



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m/z (Da

Fig 4. Panel A shows ESI-MS/MS spectra in negative mode for precursor of m/z 241 to detect IPC species (CE 60 eV) respectively in BSF T. brucei wild type (WT) (left) and overexpression (OE) (right) of Δ6-desaturase cultured in low fat media (5% FBS) for 48 h in presence of tetracycline. The red arrows highlight the surprisingly formation of IPC species when Δ6-desaturase is overexpressed, which is only normally observed in PCF or in the *T. brucei* stumpy form. Panel B shows immunofluorescence microscopy images of samples of the same cells, revealing the possible presence of the surface transporter for the differentiation signal, PAD1 (red, white arrows), in cells overexpressing Δ6-desaturase.

Reference Nat. Rev. Mol. Cel. Bio., 1, 31-39 (2000); *Nat.*,459, 213-217(2009).

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