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Phosphorylation of eIF2α on Threonine 169 is not required for *Trypanosoma brucei* cell cycle arrest during differentiation

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ABSTRACT

The trypanosome life cycle consists of a series of developmental forms each adapted to an environment in the relevant insect and/or mammalian host. The differentiation process from the mammalian bloodstream form to the insect-midgut procyclic form in *Trypanosoma brucei* occurs in two steps *in vivo*. First proliferating 'slender' bloodstream forms differentiate to non-dividing 'stumpy' forms arrested in G1. Second, in response to environmental cues, stumpy bloodstream forms re-enter the cell cycle and start to proliferate as procyclic forms after a lag during which both cell morphology and gene expression are modified. Nearly all arrested cells have lower rates of protein synthesis when compared to the proliferating equivalent. In eukaryotes, one mechanism used to regulate the overall rate of protein synthesis involves phosphorylation of the alpha subunit of initiation factor eIF2 (eIF2α). The effect of eIF2α phosphorylation is to prevent the action of eIF2B, the guanine nucleotide exchange factor that activates eIF2 for the next rounds of initiation. To investigate the role of the phosphorylation of eIF2α in the life cycle of *T. brucei*, a cell line was made with a single eIF2α gene that contained the phosphorylation site, threonine 169, mutated to alanine. These cells were capable of differentiating from proliferating bloodstream form cells into arrested stumpy forms in mice and into procyclic forms *in vitro* and in tsetse flies. These results indicate that translation attenuation mediated by the phosphorylation of eIF2α on threonine 169 is not necessary for the cell cycle arrest associated with these differentiation processes.

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1. Introduction

Kinetoplastids are protozoa and a large number of species have evolved to infect humans and/or animals. Many of the pathogenic species have complex life cycles and have evolved to proliferate in different niches within one or more host through evolution of a series of developmental forms each adapted to an environment in the relevant invertebrate and, sometimes, vertebrate host. The differences between one developmental form and another include alterations in gene expression and cellular morphology. The transition from one developmental form to another has been used to investigate the regulation of these processes [1]. The best-characterised transition is from the proliferative mammalian bloodstream form to the insect midgut procyclic form in *Trypanosoma brucei*. The process includes two differentiation steps *in vivo*, first proliferating 'slender' bloodstream forms differentiate to non-dividing 'stumpy' forms arrested in G1 [2]. Second, in response to ingestion by a tsetse fly or environmental cues that mimic this event in culture, the cell cycle arrest is ended after a lag of 8–12 h and the trypanosomes re-enter the cell cycle and start to proliferate as procyclic forms [3]. Both steps include alterations in cell morphology and gene expression [4]. The stumpy form retains the ability to differentiate for at least two days and thus the cell cycle arrest can persist from the production of a stumpy form to several hours after the signal to differentiate [5]. Proliferating cells nearly always have higher rates of total protein synthesis than the arrested equivalent, and so the differentiation process is accompanied by a reduction in the overall rate of protein synthesis as the slender to stumpy bloodstream form differentiation occurs, followed by

Abbreviations: RNAPII, RNA polymerase II; eIF2α, alpha subunit of eukaryotic initiation factor 2.

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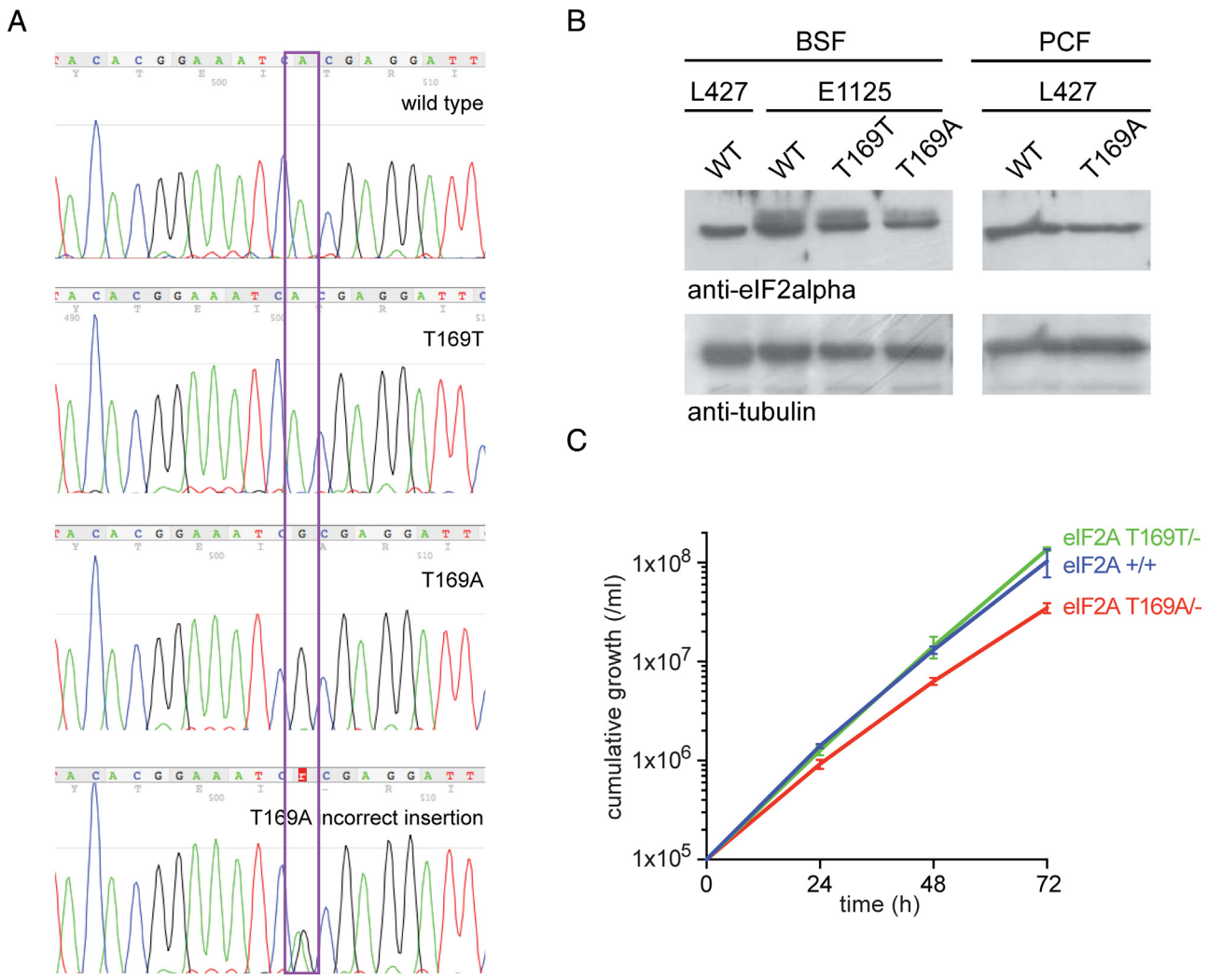

Fig. 1. Characterization of cell lines by direct sequencing of the *eIF2A* locus, western blotting and growth rates. (A) Section of a sequencing trace of the PCR product amplified from genomic DNA corresponding to the part of the *eIF2A* gene encoding T169; the box indicates the mutated nucleotide. (B) Western blot of whole cell extracts of the wild type and mutant cell lines, tubulin is shown as a loading control. BSF, bloodstream forms; PCF, procyclic forms; L427, *T. brucei* Lister 427 cell lines; E1125, *T. brucei* EATRO1125 cell lines. (C) Cumulative growth curve of the indicated cell lines. *T. brucei* bloodstream form EATRO1125 wild type, control T169T and the mutant T169A. Bars indicate standard error (n = 3). Similar results were obtained in three different experiments.

an increase as the cells proliferate as procyclic forms [6]. How is the overall rate of protein synthesis regulated in trypanosomes? The answer is not known for the differentiation associated arrest described above but has been investigated for procyclic cells that arrest due to heat shock [7]. On induction of heat shock, there is a rapid inhibition of initiation, but not elongation, of transcription by RNA polymerase II (RNAPII) and the steady state levels of most mRNAs decrease rapidly, probably via both reduced transcription and a general increase in mRNA turnover. However, cells in heat shock only retain viability for two to six hours; this is significantly shorter than the two or three days that stumpy cells retain viability [8,9].

Selective transcriptional control by RNAPII is unlikely to play a role in the G1 arrest that occurs on differentiation to stumpy forms. In trypanosomes, genes occur in long tandem arrays, usually encoding functionally unrelated proteins, and are transcribed polycistronically from occasional transcription start sites; co-transcriptional processing results in individual monocistronic mRNAs. This lack of transcriptional regulation of individual genes means that the relative levels of individual mRNAs are determined post-transcriptionally. RNAPII transcription must continue in arrested stumpy cells as some mRNAs have increased levels [10]. The overall rate of protein synthesis has been measured in stumpy forms and it is reduced several fold reflected by a reduction in the number of polysomes suggesting that the rate of translation initiation is regulated [11].

In eukaryotes, one mechanism used to regulate the overall rate of protein synthesis involves the phosphorylation of serine 51 (S51) in the eIF2α. eIF2 is a trimeric G-protein that forms a ternary complex with the initiator methionyl tRNA that delivers the Met-tRNA to the pre-initiation complex. At the start codon, release of eIF2 occurs after GTP hydrolysis. The effect of eIF2α phosphorylation is to prevent the action of eIF2B, the guanine nucleotide exchange factor that activates eIF2 for the next rounds of initiation. This is turn reduces the amount of available ternary complex and the rate of translation initiation. The kinases that phophorylate eIF2α contain a catalytic domain attached to different regulatory domains. The best conserved are GCN2, which is activated by amino acid deficiency, and PERK which is activated by an increase in unfolded proteins in the ER. In yeast and mammals, eIF2α phosphorylation also initiates changes in gene expression promoted by proteins whose synthesis is paradoxically increased when there is increased eIF2α-P in cells, such as yeast GCN4 and ATF4 in mammals. Both are transcription factors that regulate the expression of

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