

THERMAL AND DEVELOPMENTAL REGULATION OF GENE EXPRESSION
IN Trypanosoma cruzi.

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During development of the complex T. cruzi life cycle, at least three different aspects can be readily observed: a) changes in the cellular morphology, b) variation in the capacity of cell division and c) modifications in the environment and temperature faced by the parasite. Two families of genes are known to be deeply involved with the cellular or environmental alterations described above, the tubulin and the heat-shock gene families (1,2). The tubulin, as a major microtubule protein, is involved with the changes in the cytoskeletal organization, flagellum length and cell division process of T. cruzi. The T. cruzi cells respond to the elevation of temperature with the induction of a group of 4 heat-shock proteins whose precise role in the cells are yet to be fully understood (2,3). For the reasons described above we have chosen those two gene families as a model to study the control of gene expression in T. cruzi.

We have shown that tubulin gene expression is developmentally regulated during T. cruzi life cycle, having its lower level of mRNA accumulation and tubulin synthesis in metacyclic trypomastigotes (4). The tubulin gene arrangement in T. cruzi comprises alpha and beta genes linked in the genome and organized in alternated tandem repeats (5,6). The basic repeat unit is 4.3 kb. However there are indications of other arrangement besides the basic repeat (6).

The mRNA synthesis in Trypanosomatids and other kinetoplastids has been shown to possess peculiar aspects such as the addition of a common leader sequence (mini-exon) to the 5 termini of all mRNA, by a process called trans splicing (7-12). Another equally surprising phenomenon is the so called RNA editing of mitochondrial transcripts. It was shown that the mRNAs from the cytochrome c oxidase subunit II and III genes contain uridines that are not encoded by the respective genes, (13,14). Another new feature in the

RNA metabolism of trypanosomatids is the occurrence of multicistronic transcription units encoding to: variant surface glycoprotein 221 mRNA and seven other stable mRNAs (15,16); alpha and beta tubulins (17), all in T. brucei; alpha tubulin in Leptomas seymouri (18) and the IF-8 multigenic unit in T. cruzi (19). In the majority of the cases the multicistronic RNA precursor was not detected in northern blots, except the RNA from the IF-8 unit of T. cruzi. We present now evidences of occurrence of a stable and common multicistronic transcript encoding alpha and beta tubulin mRNAs in T. cruzi. We also present evidences of accumulation of such transcript under restricted conditions of cell growth, dictated by either developmentally regulated factors (metacyclogenesis), populational density (stationary epimastigotes) or external factors (heat-shocked exponential epimastigotes) possibly due to limitation in a processing mechanism.

RESULTS AND DISCUSSION.

IDENTIFICATION AND COMPARTMENTALIZATION OF TUBULIN RNA TRANSCRIPTS.

The genomic clone lambda Tc3 (4,5) encoding for alpha and beta tubulin was used as a probe to study the expression of tubulin mRNAs in the different stages of development of T. cruzi as well as in heat-treated, and different age epimastigotes. The tubulin transcripts from control epimastigote cells were analysed by northern blot. The result showed the presence of at least seven RNA transcripts hybridizable to the lambda Tc3 clone with molecular weight spanning from 1.6 to approximately 20 kb. The RNA species of 1.6, 2.1 and 2.4 kb were shown to be beta tubulin encoding mRNAs and the 2.1 kb an alpha tubulin mRNA, as evidenced by hybridization with the respective specific cDNA clones isolated from a lambda gt11 cDNA library. The analysis of the cellular compartment distribution of the tubulin RNAs has revealed that the 6.0, 8.0, 13.0 and 20.0 kb species were confined to the nuclear compartment.

SI NUCLEASE PROTECTION ASSAY AND HYBRIDIZATION OF SPECIFIC cDNA CLONES INDICATE A SINGLE MULTIGENIC RNA TRANSCRIPT OF ALPHA AND BETA TUBULIN.

The hybridization of the alpha and beta cDNA clones to northern blots of total RNA revealed that both probes recognize the high molecular weight RNA species. On the other hand only the specific alpha or beta mRNAs were recognized by the respective clone.

SI nuclease protection of the genomic cloned DNA, was used for the detection of contiguous alpha and beta tubulin transcripts in total cell RNA. The protected fragments were analysed in alkaline agarose gel upon hybridization with the specific cDNA clones. Total cell RNA was able to protect lambda Tc3 DNA producing hybridizing bands with molecular weight of that of the mRNAs and above. These results indicate that the alpha and beta tubulin mRNAs are originated from a common high molecular RNA species suggesting thus a multicistronic origin.

CHANGES IN THE EXPRESSION OF TUBULIN GENES IN RESPONSE TO HEAT TREATMENT.

The treatment of exponential growing epimastigotes at 37 and 40 oC induced a gradual, temperature dependent accumulation of a 13.0 kb RNA species. Concomitantly, a decrease in the levels of intermediate species of 8.0 and 6.0 kb. The levels of the mRNA species were not altered. This observation suggest a precursor product relationship of the 13 kb RNA species and the intermediary RNA transcripts, as well as rather stable tubulin mRNAs. On the other hand, the rate of synthesis of tubulin, as measured by in vivo incorporation of ³⁵S-methionine, is markedly reduced in cells incubated at 40 oC. These results suggest a translational control of tubulin synthesis in heat-shocked cells.

The recovery of tubulin synthesis and tubulin RNA processing after heat-shock is shown on fig. 1A and B. After 3h. treatment at 40 oC, the cells were returned to 29 oC and at different time intervals aliquots were taken for pulse labelling with ³⁵S-methionine and RNA extraction. As depicted in fig. 1A, a progressive decrease in the synthesis of tubulin is observed at 40 oC followed by reassumption of synthesis upon return of the cells to 29 oC. After 3h

at 40 oC the cells show the lowest level of tubulin RNA precursors (fig. 1B, lane D) and maintenance of the mRNA levels (lanes A-D). After returning to 29 oC, lanes F-H, the pattern of precursor transcripts is restored with lower level of accumulation of mature mRNAs, possibly due to a higher turnover of these species during the recovering process from the heat treatment.

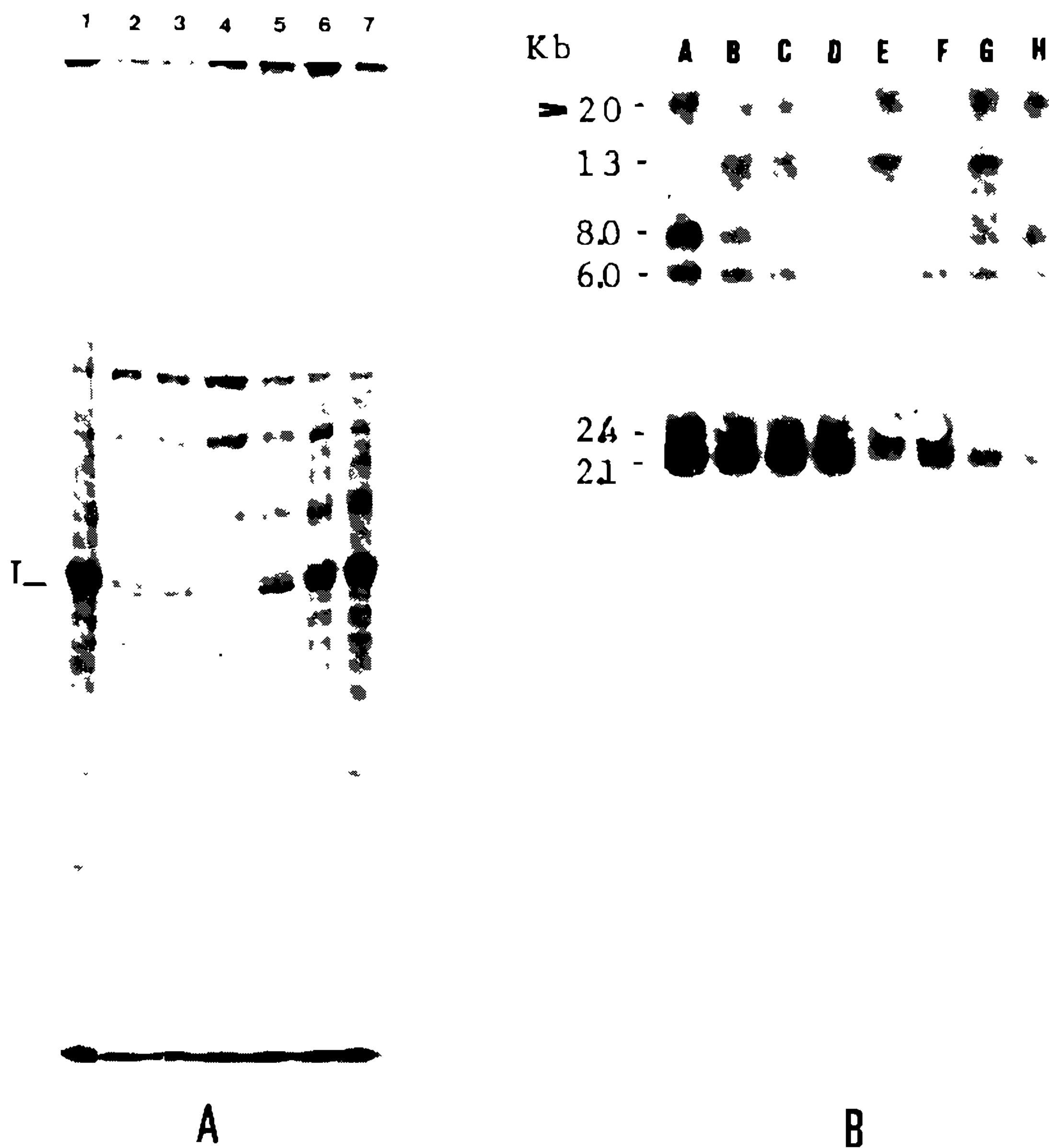


Figure 1: (A) Pulse labeling of epimastigotes cells with ³⁵S-methionine. Control cells (1), cells treated at 40 oC for 2, 3 and 6h (2-4). At the end of 3h at 40 oC the cells were returned to 29 oC; 30 min., 3h and 6h at 29 oC (5-7). (B) Northern blot of total RNA hybridized to nick-translated genomic clone lambda Tc3. Control cells RNA (a), RNA from cells treated at 40 oC for 30 min., 2h, 3h and 6h (b-e), RNA from cells at 30 min, 3h and 6h after returning to 29 oC (f-h).

DEVELOPMENTAL REGULATION TUBULIN GENES DURING
In vitro DIFFERENTIATION OF T. CRUZI.

An vitro cycle of cell differentiation has been developed in our laboratory by using treatment of the cells with 100% serum and incubation in LIT and M16 medium (20,21). The analysis of tubulin RNA transcripts in total RNA from epimastigotes, metacyclic trypomastigotes and amastigotes is shown in fig.2 A,B and C respectively. As it can be noticed the levels of the mature tubulin mRNAs is lowest in metacyclic trypomastigotes. However the comparison of the level of accumulation of the highest molecular weight precursor RNA shows an inverse pattern of accumulation, that is, highest in metacyclics and the lowest in the proliferating forms of T. cruzi. A similar increased accumulation of this RNA species is also observed in stationary epimastigotes. Therefore under restricted conditions of cell proliferation dictated by either developmentally regulated factors (metacyclogenesis), populational density (stationary epimastigotes) or external factors (temperature) there seems to happen a similar limitation in the processing of tubulin RNA precursors.

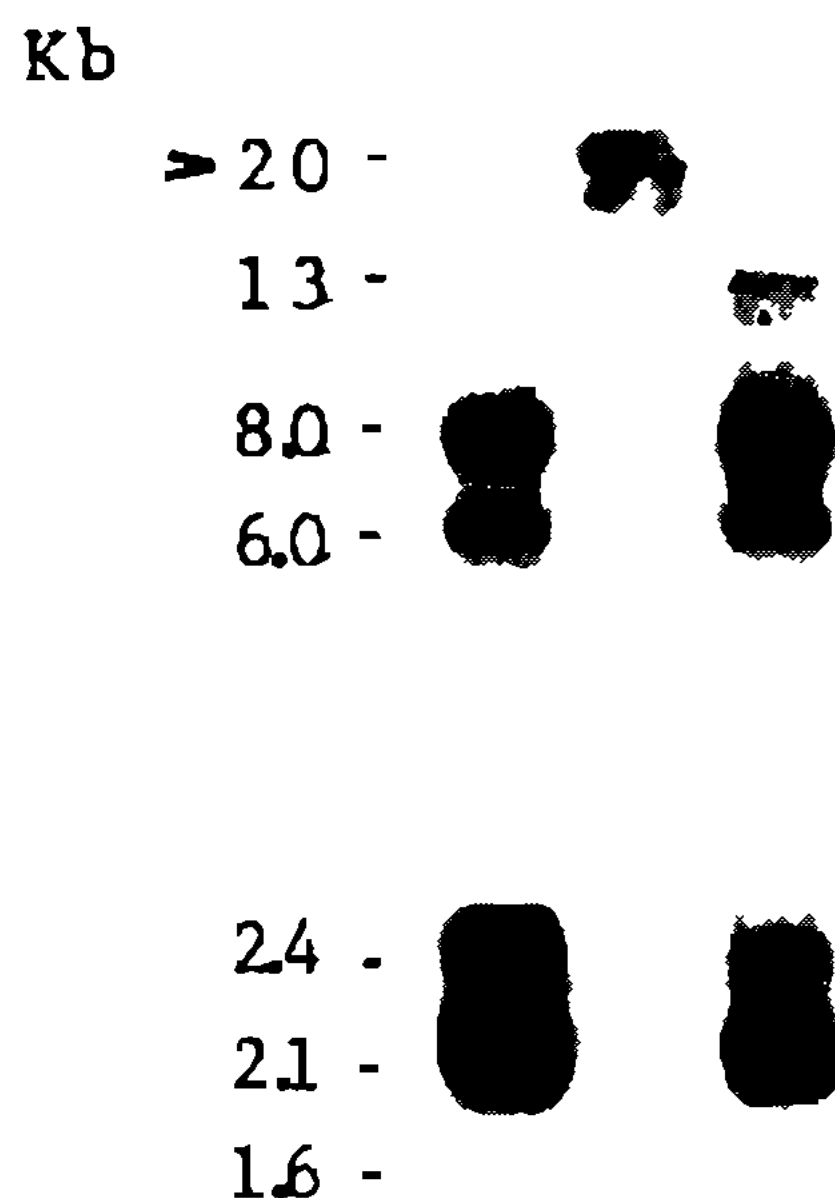


Figure 2: Northern blot of total RNA (5 ug) from epimastigotes (A), metacyclic trypomastigotes (B) and amastigotes (C), hybridized to nick-translated lambda Tc3 genomic clone.

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