Control of tubulin and actin gene expression in *Tetrahymena pyriformis* during the cell cycle

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Poly(A)-containing mRNA was isolated from division synchronized populations of the ciliated protozoan, *Tetrahymena pyriformis*. The level of tubulin and actin mRNA at specific cell cycle stages was analyzed by hybridization to tubulin and actin cDNA probes and by gel analysis of their in vitro translation products. The pattern of fluctuation of tubulin mRNA levels was similar to that observed for the in vivo tubulin synthesis previously reported [1]. This suggests that as the cells progress through the cell cycle, tubulin synthesis is controlled at the mRNA level. There was little fluctuation of actin synthesis or actin mRNA levels during the cell cycle, which may be indicative of a different regulatory mechanism for actin than for tubulin.

Tubulin *Actin* *Gene expression* *Cell cycle*

1. INTRODUCTION

Tubulin and actin are cytoskeletal proteins known to be involved in cell morphology and motility; they are thought to play a central role in structure and function of dividing cells [2,3]. Thus, the control of tubulin and actin may be related to specific cell cycle events. The ciliated *Tetrahymena* is readily adapted for cell cycle studies due to the ease of inducing a high degree synchrony [4]. Recent in vivo studies on the regulation of tubulin synthesis in division synchronized *Tetrahymena* cells [1,5] suggest that the induction of tubulin synthesis is dependent on the synthesis of mRNA, however these studies do not differentiate between possible regulation at the mRNA level or at the post transcriptional stages of control of tubulin synthesis. Actin is believed to be fairly universal in all eukaryotic cells [6] yet its identification in *Tetrahymena* is controversial [7–10]. In the present investigation we used cloned cDNA probes for actin and tubulin [11–14] in order to positively identify actin mRNA in *Tetrahymena* and to study the control of tubulin and actin gene expression during the cell cycle.

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2. MATERIALS AND METHODS

2.1. *Cell culture and synchronization*

Tetrahymena pyriformis GL were maintained in axenic culture medium (PPL) consisting of 2% (w/v) proteose peptone (Difco) supplemented with 0.1% (w/v) liver fraction L (Nutritional Biochemical Corp.). Cultures were maintained at 28°C. Cells were synchronized by the one heat shock per generation method in [4]. The experiment was started by inoculation of 3 ml of a 3-day old culture into 250 ml of PPL in a 2.5-l low form culture flask. Cells were given 7 consecutive 30-min heat shocks at 34°C each, separated by 157 min at 28°C. The changes in temperature were

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controlled automatically and cells were shaken gently throughout the treatment. The period of time following the last heat shock is called EH. At the end of 7 shocks, the cell density was 30000–50000 cells/ml.

2.2. *Preparation of poly(A$^+$) mRNA*

Division synchronized *Tetrahymena* were collected at 10, 35, 54, 92, 120, 160 and 190 min EH. Cells were washed with non-nutrient inorganic media (5 mM potassium phosphate buffer, pH 7.0, 1 mM $MgSO_4$ and 4.7 mM NaCl) and in ice cold Tris–Mg buffer (50 mM Tris, pH 7.4, and 5 mM $MgCl_2$). The cells were rapidly frozen in liquid nitrogen and ground to a powder and thawed on ice in lysis buffer (150 mM Tris–acetate, pH 8.5, 200 mM sucrose, 50 mM KCl, 20 mM Mg acetate, 4 mM β-mercaptoethanol, 0.4% Nonidet P-40 and 20 μg/ml polyvinyl sulfate). The polysomes were isolated as in [1]. The polysomal pellet was incubated with predigested proteinase K (200 μl/ml) followed by a phenol extraction. Poly(A$^+$)-containing mRNA fraction was isolated on an oligo(dT) cellulose column [15].

2.3. *Translation of* Tetrahymena *poly(A$^+$) mRNA in vitro*

The reticulocyte cell-free lysate was prepared as in [16]. Poly(A)-containing mRNA was translated in the reticulocyte lysate containing [^{35}S]methionine. The labelled proteins synthesized in the cell-free system were analyzed by 10% SDS–PAGE [17].

2.4. *Fractionation of RNA and hybridization with cDNA probes*

Poly(A$^+$) was isolated at specific cell cycle stages and fractionated on a 1.1% agarose-formamide slab gel. For tubulin and actin gels, 5–10 μg of poly(A$^+$) mRNA was fractionated. The RNA was transferred from the agarose gels and hybridized as in [13]. Hybridization was carried out for 20 h at 42°C using ^{32}P nick-translated tubulin cDNA probe (pT25) and actin cDNA probe (pA72) [11,12]. Quantitative determination of the hybridized mRNA was performed by scanning the fluorograms with a Beckman DU8 spectrophotometer. Similar results were obtained from another preparation of mRNA isolated from another population of synchronized cells.

3. RESULTS

3.1. *Division synchronized cells*

Populations of *Tetrahymena pyriformis* were induced to divide synchronously by the one heat shock per generation method in [4]. Following a series of 7 heat shocks, each spaced one cell generation apart, the cells undergo synchronous divisions at 85 and 200 min after the last heat shock (EH). After release from the last heat shock, the cells progress through G2 phase and more than 80% of the cells display cleavage furrows at 85 min EH. The second division is less synchronous and almost 40–50% of the cells show furrows at 200 min EH. The interval between the first and second synchronous divisions is designated the free running cycle and is considered to be similar to the non-induced log growth cell cycle [19]. The number of cells doubles after each synchronous division indicating that most cells in the population complete their division.

3.2. *Translation of poly(A)-containing mRNA*

Poly(A)-containing mRNA isolated from synchronized *Tetrahymena* at specific cell cycle stages

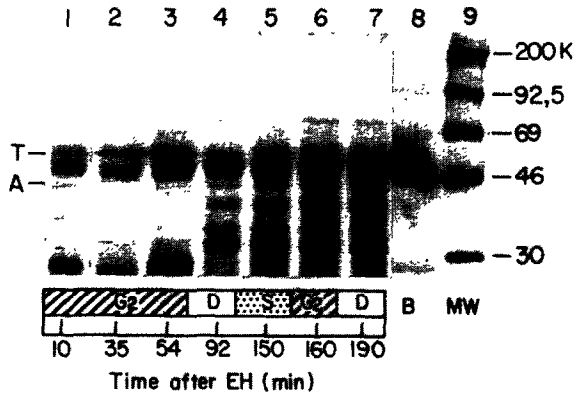

Fig.1. Analysis of translation products in a reticulocyte cell-free translation system directed by poly(A$^+$)-containing mRNA sequences derived from division synchronized *Tetrahymena pyriformis* GL. At specific times after the last heat shock (EH) poly(A$^+$) mRNA was isolated and translated in a cell-free reticulocyte system. In lane 1–7 the RNA was prepared from cells at specific cell cycle stages shown below each lane. Lane 8 cell-free products directed by rat brain (B) poly(A$^+$) mRNA. The M_r-markers are shown in lane 9. Reference bands of actin (A) and tubulin (T) are shown. K, kDa.

was translated in vitro using a rabbit reticulocyte lysate. The cell-free products labelled with [^{35}S]methionine were analyzed by SDS–PAGE. The fluorogram (fig.1) shows polypeptides migrating at M_r 50000 and 45000. These proteins comigrate with tubulin and actin observed in the translation products of rat brain poly(A)-rich mRNA. The same proteins were previously identified [1] among the in vivo labelled proteins of *Tetrahymena* cells. Tubulin present among the cell-free products synthesized with mRNA preparations isolated from cells preceding the first synchronous cell division through the free-running cell cycle. The relative level of tubulin synthesized, seems to be higher before the first synchronous division (during G2) and then falls as the cells progress through division. During the first synchronous division (G2) tubulin is the major product. As the cells enter the second free-running cycle numerous other proteins are synthesized while the tubulin level remains almost constant. In addition to tubulin the mRNA preparations isolated during the free-running cell cycle also directed the synthesis of actin. Densitometric scans performed for quantitation of the gel show that the amount of synthesized actin does not vary to a great extent as the cell progresses through the cell cycle (not shown).

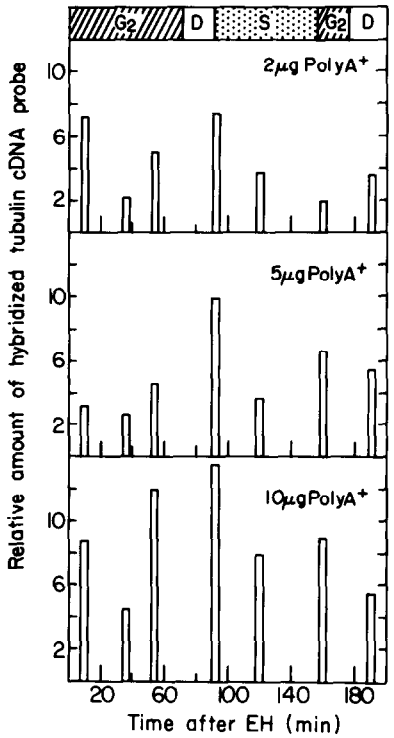

Fig.2. Hybridization of ^{32}P nick-translated tubulin cDNA probe (pT25) to poly(A$^+$)-containing mRNA (2, 5 and 10 μg) from *Tetrahymena* at various times during the cell cycle. After hybridization of the radioactive probe to nitrocellulose discs containing specified amounts of poly(A$^+$) mRNA, the discs were washed and exposed to X-ray film. Quantitative determination of the amount of hybridized mRNA was made scanning the fluorograms.

3.3. *Quantitation of tubulin- and actin-specific sequences*

Using ^{32}P-labelled PT25 tubulin cDNA probe, the level of tubulin-specific sequences at the different cell stages was determined by dot hybridization [20]. Fig.2 shows that maximal level of tubulin

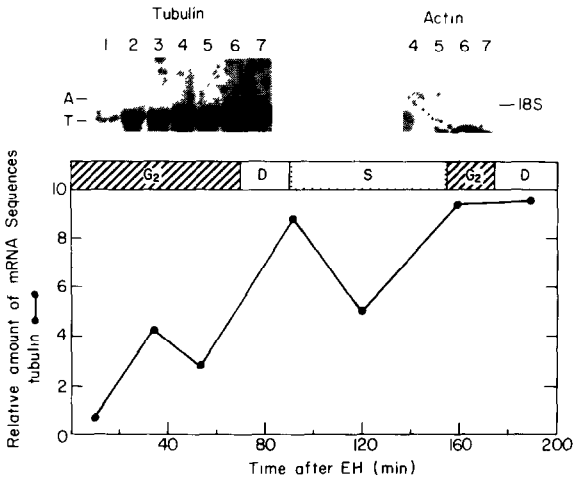

Fig.3. Fractionation of poly(A$^+$)-containing mRNA by agarose-formamide gel electrophoresis and analysis of the hybridization with tubulin and actin cDNA probes. (A) 5–10 μg of poly(A)-containing mRNA isolated from cells at specified times during the cell cycle was fractionated on a 1.1% agarose formamide slab gel, processed and hybridized as described above. Hybridization was carried out using ^{32}P nick-translated tubulin cDNA probe (pT25) and actin cDNA probe (pA72). A fluorogram of the nitrocellulose filter with tubulin and actin cDNA probes is shown. The RNA samples 1–7 were prepared from cells at specific cell stages (see fig.1). Actin (A), tubulin (T) and the 18 S marker are shown. (B) The relative amount of tubulin sequences is plotted at specific cell cycle stages.

mRNA sequences was observed at around 85 min where the first synchronous division occurs and a second lower peak is detected at 160 min just prior to the second division.

In order to further identify tubulin and actin mRNA species, poly(A)-containing mRNA from division-synchronized cells was subjected to electrophoresis on denaturating agarose-formamide gels and hybridized as in [13]. Fig.3 shows that the level of tubulin message was found to rise from a low value immediately after the last heat shock to a peak during G2 prior to the first synchronous division. At this time the amount of tubulin mRNA increases about 13-fold. During the free-running cell cycle the tubulin mRNA level value is reduced at S phase and then rises at G2 phase and is maintained at the second synchronous division.

The actin cDNA probe pA72 hybridizes with *Tetrahymena*-fractionated mRNA at a band position which corresponds to the electrophoretic migration of the identified actin mRNA species [11,13]. It is readily seen during the free-running cell cycle (fig.3) but not clearly identified during the period preceding the first synchronous division. It does not show a clear periodicity and its levels are low and thus precise quantitation is difficult and may be inaccurate.

4. DISCUSSION

We here analyzed the level of tubulin and actin mRNAs sequences and their translatability during the cell cycle of *Tetrahymena*. Our results show a periodicity for tubulin mRNA during the cell cycle. During the free-running cell cycle, tubulin synthesis was viewed by its in vitro translation peaks at G2 phase. In general, levels of actin synthesis and actin mRNA are low and detected only from S phase and show little variability during the same cell cycle stages.

It has been reported [1] that in vivo tubulin synthesis occurs during G2 phase just following formation of the oral apparatus, a process which requires a large quantity of tubulin. It is suggested that tubulin synthesis is regulated by changes in the size of the soluble tubulin pool. Comparison of in vivo tubulin synthesis data [1] with the induction of tubulin mRNA sequences (fig.3) during the free-running cell cycle reveals a close temporal relationship. The absence of a lag phase may provide evidence for transcriptional control of tubulin synthesis. We propose that depletion of the free tubulin pool during the cell cycle initiates transcription of tubulin mRNA sequences. Fluctuations in the size of the unpolymerized tubulin pool have been reported to autoregulate the transcription of tubulin mRNA in several organisms [13,21,22]. The fluctuation of tubulin mRNA levels may differ for the various isotubulin mRNA subspecies perhaps having a functional role in cytokinesis. A cell cycle-dependent induction of tubulin synthesis was reported in vegetative cells in *Chlamydomonas*; induction of tubulin in synchronously dividing cells occurred 1.5–2 h prior to cytokinesis [23]. In addition, newly transcribed tubulin mRNA has been responsible for induction of tubulin synthesis following deflagellation [24,25].

The identification of actin mRNA in division-synchronized *Tetrahymena* by hybridization with cDNA actin probe resolves the question of actin presence in this cell [7–10]. This work supports and extends the electron microscopical and biochemical evidence of actin in log growth cells, namely, recovery of 7 nm wide filaments which decorated with heavy meromyosin, indirect immunofluorescent staining with anti-actin, peptide mapping with *S. aureus* V-8 protease and DNase I chromatography [7,8]. The low level of actin mRNA detected in these experiments which prevent accurate quantitation does not preclude a role for actin in cell division but may be indicative of a different regulatory mechanism from that of tubulin.

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