The *Tetrahymena* chaperonin subunit CCTη gene is coexpressed with CCTγ gene during cilia biogenesis and cell sexual reproduction**

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Abstract We report here the cloning and the characterization of the *T. pyriformis* CCTη gene (TpCCTη) and also a partial sequence of the corresponding *T. thermophila* gene (TtCCTη). The TpCCTη gene encodes a protein sharing a 60.3% identity with the mouse CCTη. We have studied the expression of these genes in *Tetrahymena* exponentially growing cells, cells regenerating their cilia for different periods and during different stages of the cell sexual reproduction. These genes have similar patterns of expression to those of the previously identified TpCCTγ gene. Indeed, the *Tetrahymena* CCTη and CCTγ genes are up-regulated at 60–120 min of cilia recovery, and in conjugation when vegetative growth was resumed and cell division took place. Our results seem to indicate that both CCT subunits play an important role in the biogenesis of the newly synthesized cilia of *Tetrahymena* and during its cell division.

Key words: CCTη-chaperonin gene; Ciliated protozoan; Gene structure; Gene expression

1 Introduction

In the cell, as in vitro, the final conformation of a protein is determined by its amino-acid sequence. However, whereas some isolated proteins can be denatured and refolded in vitro in the absence of other macromolecular cellular components, folding in vivo, as well as other aspects of protein assembly, involves interactions with pre-existing proteins that are generally designated as molecular chaperones [1]. One of the particularly well studied families of molecular chaperones is the chaperonins [2,3]. Members of this protein family were first identified in eubacteria (GroEL in *Escherichia coli*) [4] and in certain organelles of eukaryotic cells, such as mitochondria (Hsp60 in mitochondrial matrix) [5] and chloroplasts (Rubisco-subunit-binding protein, RBP) [6]. These proteins assemble into large oligomeric complexes of 60 kDa subunits that are usually arranged as two stacked heptameric rings with a central cavity (for review [2,3]).

Recently a second group of chaperonins in archaebacteria and in the cytosol of eukaryotic cells has been described (for review [7–9]). Indeed, the eukaryotic cytosol contains an abundant ring-shaped chaperonin that seems to be the cytosolic counterpart of the GroEL chaperonin in eubacteria and Hsp60 and RBP in symbiotic organelles. This chaperonin, designated by Chaperonin Containing TCP1 (CCT) [10], is a hetero-oligomeric complex that in mammalian cytosol has a molecular mass of about 850–900 kDa. It is composed of seven to nine distinct polypeptides in the 52–65 kDa size range [10–12]. In mouse, genes coding for eight of the CCT subunit polypeptides have already been isolated and were designated as CCTα (for the original TCP1), CCTβ,... and CCTζ [10,13]. These proteins are ~30% identical to each other in all the pairwise combinations, and weakly related to the traditional chaperonins. Interestingly, a highly significant sequence similarity was detected between the CCT subunits and the archaebacterial proteins TF55 [14] and thermosome [15,16]. The high heteromeric nature of the CCT particle suggests that CCT may function as a more elaborate folding machinery when compared with the other chaperonins as the latter only have one or two subunits species.

It has been shown that in vitro CCT is involved in the folding of actin [17,18], tubulin [12,19] and firefly luciferase [12,20]. In vivo studies indicate that newly synthesized α- and β-tubulin and actin enter in a 900 kDa complex containing TCP1 and that tubulin is released from this complex competent to form heterodimers [21]. In yeast, *S. cerevisiae*, the mutations in CCTβ, CCTδ and CCTγ cause abnormal microtubular structures [22–24] and disruption of actin microfilaments [23].

Tetrahymena is a protozoan ciliate exhibiting highly differentiated microtubule networks in combination with small tubulin gene families [25,26]. In a previous paper, we have described the characterization of the TpCCTγ and its coexpression with tubulin during cilia biogenesis [27]. Now, we report the isolation and characterization of another member of the *Tetrahymena pyriformis* and *Tetrahymena thermophila* CCT subunit gene family CCTη (TpCCTη and TtCCTη, respectively). The expression of these genes was studied during *Tetrahymena* cilia recovery and during the complex process of sexual reproduction (conjugation). Our results show that this CCTη gene follows the same pattern of expression as the CCTγ gene, indicating a possible role of these subunits in the biogenesis of new cilia and during cell division.

2. Material and methods

2.1. Cells and Culture Conditions

T. pyriformis amicronucleated CGL strain was grown axenically in enriched proteose/peptone/yeast extract medium (PPY) at 28°C [28]. Cells were harvested in the stationary phase at a density of 1.5×10^6 cells/ml for DNA extraction. For RNA extraction, cells were collected in the exponential phase at 2×10^5 cells/ml. Cell suspensions were

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deciliated essentially as described by Guttman and Gorovsky [29], except that PPY medium was always used. Cells recovered normal motility after 90-min reciliation and then maintained their normal generation time of about 3 h. Conjugation was performed according to Martindale et al. [30] using the micronucleated *T. thermophila*. Cells were grown, starved and mated at 30°C. Cultures were starved in 10 mM Tris-HCl pH 7.5 for at least 24 h. To begin conjugation equal numbers of cells from the two starved cultures of different mating types (strain B4 and strain SB1918) were gently mixed and then subdivided into samples of 25 ml each (2×10^6 cells). The samples were maintained at 30°C without shaking and at each time point the total cytoplasmic RNA from one sample was prepared. The cytological stages were followed using the Giemsa method described by Burns and Brussard [31].

2.2. *Cloning of* Tetrahymena *CCTη genes*

Degenerated primers (CCTnd — 5′-GCCTCTAGAAAY-GAYGGTGCYACYATY-3′ and CCTcc — 5′-GCCGGATCCRG-CACCACCACCRGSRAC-3′) designed taking into account the conserved regions among all the CCT subunits so far known and *Tetrahymena* codon usage, were used to amplify *T. pyriformis* and *T. thermophila* genomic DNA. The PCR cycling conditions were as follows: 5 cycles of 92°C for 30 s, 45°C for 30 s and 72°C for 2 min, followed by 35 cycles of 92°C for 30 s, 45°C for 30 s and 72°C for 2 min and finally 72°C for 10 min. The reaction products (1.4, 1.55 and 1.7 kb fragments for *T. pyriformis* and 1.55 and 1.2 kb fragments for *T. thermophila*) were cloned into pUC19 plasmid and sequenced. One of the clones, named pTtM1, containing the *T. thermophila* 1.2 kb amplified fragment encodes a protein related to mouse CCTη. This fragment was used to screen a *Sau*3AI genomic library, constructed with macronuclear DNA isolated from *T. pyriformis* and λ Dash II as a vector [27]. Filters were prehybridized and hybridized at 40°C with 6×SSC (1×SSC=0.15 M NaCl, 15 mM trisodium citrate, pH 7.0), 5×Denhardt's solution, 0.1% SDS (mass/vol), 100 μg/ml sonicated salmon sperm DNA and 20 mM sodium phosphate pH 6.5, and washed for 10 min at 50°C with 2×SSC followed by a 10 min with 2×SSC, 0.1% (mass/vol) SDS at 55°C. One of the positive clones, named TpCCTη11.3, was analysed by restriction mapping. Restriction fragments were subcloned in pUC19 plasmid. Sequential deletions were made using the double-stranded Nested Deletion kit (Pharmacia). Sequencing in both strands was performed on denaturated plasmid DNA with the T7 sequencing kit (Pharmacia) according to the supplier's instructions. Nucleotide sequence analysis was carried out using the GCG package (1991) (Genetics Computer Group, Inc., Wisconsin, USA) and DNASIS 5.0 program obtained from Hitachi-LKB.

2.3. *Northern blot hybridization*

Total cytoplasmic RNAs from exponentially growing cells, reciliating cells at different times after deciliation and conjugating cells were extracted from *T. pyriformis* and *T. thermophila* cells essentially as reported by Soares et al. [32]. Total cytoplasmic RNAs were subjected to Northern blot analysis as described by Sambrook et al. [33]. When hybridizations were performed using βTT1 as probe [25], the conditions were the same as described by Cóias et al. [34]. Hybridizations were carried out using probes from *T. pyriformis* TpCCTη and TpCCTγ genes and also *T. thermophila* TtCCTη genes and performed as previously described [27].

2.4. *Radioactive probes*

T. thermophila TtCCTη gene DNA probe was prepared from pTtM1 plasmid DNA hydrolised with *Bam*H1, to generate a 1.2 kb fragment, that contain two introns and 63.3% of the coding region. *T. pyriformis* TpCCTη gene DNA probe was prepared from pTpH2.3 plasmid DNA hydrolysed with *Sfu*I to generate a 0.37 kb fragment containing part of the 5th exon of this gene. The homologous DNA probes encoding *T. pyriformis* TpCCTγ subunit and βTT1 tubulin were prepared from pTpE3 and IB1 plasmids, respectively as described by Soares et al. [27]. Fragments were labelled using the Megaprime DNA labelling system (Amersham) and [α-^{32}P]dATP (3000 Ci/mmol) (Amersham Inter. plc.).

2.5. *Densitometric analysis*

Densitometric analysis of autoradiograms from Northern blots was carried out using the LKB 2222-020 UltraScan XL laser densitometer. The scan data were collected and processed using the LKB 2400 GelScan XL software.

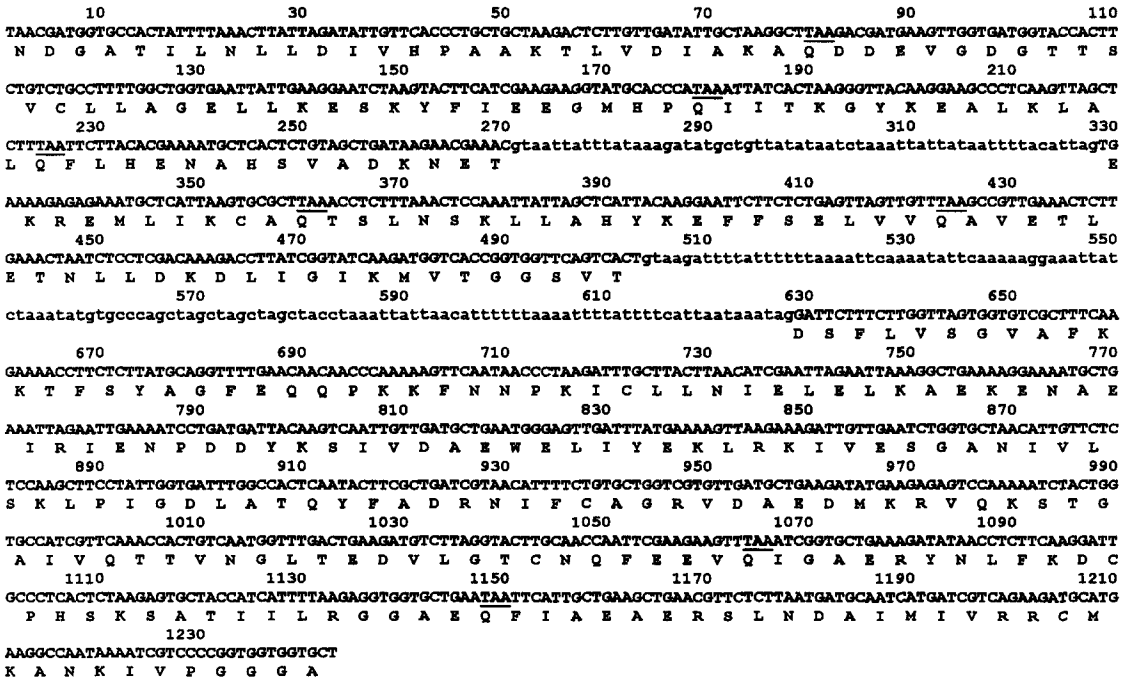

Fig. 1. Nucleotide sequence of the *Tetrahymena* CCTη genes. The complete nucleotide sequence of the TtCCTη (A, left) and the partial nucleotide sequence of the TpCCTη gene (B, right) are shown in uppercase letters whereas introns are indicated in lower case letters. The predicted amino acid sequences of the CCTη gene are shown below the respective nucleotide sequence. The stop codon is indicated by asterisks. Purine-rich sequences with an axis of symetry in the 5′-non-coding-region of the TpCCTη are double underlined. These sequences might be non-canonical TATA boxes in *Tetrahymena* [40]. The codons TAA and TAG coding for Gln are single underlined.

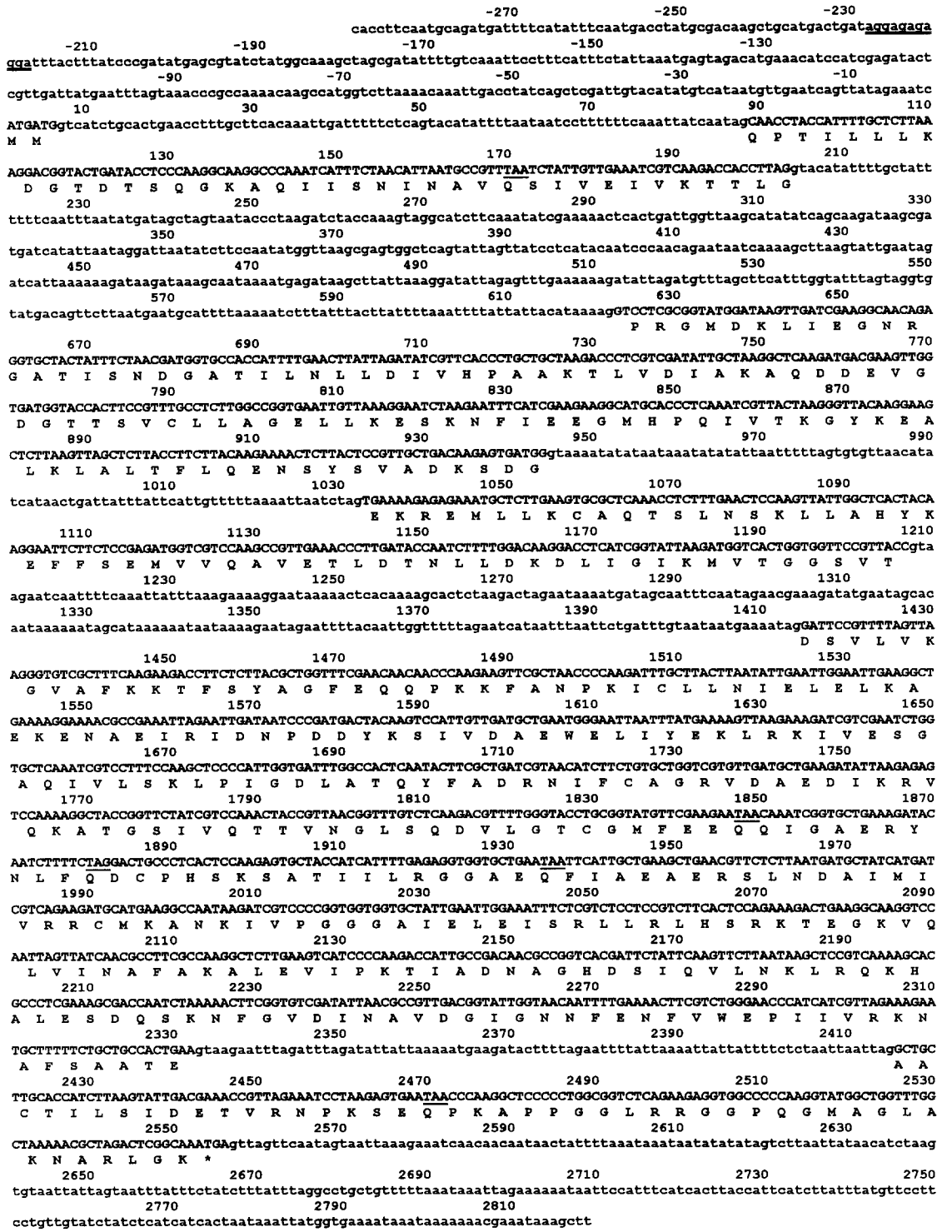

Fig. 1 (continued)

3. Results

3.1. Cloning and structure of the CCTη gene in Tetrahymena

In order to clone the different members of the CCT gene family, we have synthesized several degenerate primers based on conserved regions among CCT subunits. Using these primers and PCR techniques several genomic DNA fragments from *T. thermophila* have been cloned and sequenced. Fig. 1A shows the nucleotide sequence corresponding to the clone pTtM1 containing a DNA insert with 1.244 kb. This DNA insert is part of a gene encoding a protein related to the mouse CCTη subunit. The sequenced coding region is interrupted by two introns with 58 and 125 bp, respectively. Using the 1.244 kb fragment from pTtM1 plasmid as probe we were able to isolate several positive phage plaques from a genomic library of *T. pyriformis*. These clones were analysed by restriction mapping and Southern blotting (results not shown). One of the positive clones, named TpCCTη11.3, contains the com-

plete sequence of the TpCCTη subunit gene coding region as well as 5′- and 3′-non-coding regions. In Fig. 1B the nucleotide sequence of *T. pyriformis* CCTη subunit gene is displayed. The coding region of this gene is interrupted by five introns that range in size from 82 to 419 bp. The intron positions were deduced in both genes as already described for the TpCCTγ [27] and also by comparison of the coding region of mouse CCTη [10]. Interestingly, the two introns of the TtCCTη gene occur in positions corresponding to those of the third and fourth introns of the TpCCTη gene, suggesting that the intron positions are conserved between the two genes of the two species. Thus it might be possible that at least these introns were fixed in the CCTη subunit gene before the split of the two *Tetrahymena* species. However, no sequence similarity was found between introns from the two organisms. The TpCCTη subunit gene encodes a protein consisting of 558 amino acid residues with a calculated molecular mass of 60.9 kDa and a putative pI of 6.44.

3.2. *The TtCCTη and TpCCTη amino acid predicted sequences*

A comparison of the predicted amino acid sequence of the

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                10                  30                  50
TpCCTη  MMQPTILLLKDGTDTSQGKAQIISNINAVQSIVEIVKTTLGPRGMDKLIEGNRG.ATISN
TtCCTη  --------------------------------------------------------
MmCCTη    PTPVI   E   S   IP LV   S C V A A R           VDG  K

                70                  90                  110
TpCCTη  DGATILNLLDIVHPAAKTLVDIAKAQDDEVGDGTTSVCLLAGELLKESKNFIEEGMHPQI
TtCCTη                                                  Y
MmCCTη        K   V             S  A         T   A F  QV PYV   L

                130                 150                 170
TpCCTη  VTKGYKEALKLALTFLQENSYSVADKSDGEKRELLKCAQTSLNSKLLAHYKEFFSEMVV
TtCCTη  I            Q  H  AH      NET      I                    L
MmCCTη  IIRAFRT TQ  VNKIK IAVT KKQDKV Q K  E   M A S   ISQQ V  AK

                190                 210                 230
TpCCTη  QAVETLDTNLLDKDLIGIKMVTGGSVTDSVLVKGVAFKKTFSYAGFEQQPKKFANPKICL
TtCCTη        E                      F  S                    N
MmCCTη  D  MM  .E  QLKM    K Q  ALEE Q  A              M    YK    A

                250                 270                 290
TpCCTη  LNIELELKAEKENAEIRIDNPDDYKSIVDAEWELIYEKLRKIVESGAQIVLSKLPIGDLA
TtCCTη                      E                           N
MmCCTη    V        D      VHTVE  QA       NIL D  E  HQ   KVI         V

                310                 330                 350
TpCCTη  TQYFADRNIFCAGRVDAEDIKRVQKATGSIVQTTVNGLSQDVLGTCGMFEEQQIGAERYN
TtCCTη                      M     S  A          TE       NQ   V
MmCCTη          DM       PE  L  TMM C GSI  S  A VP     H QV   T   G

                370                 390                 410
TpCCTη  LFQDCPHSKSATIILRGGAEQFIAEAERSLNDAIMIVRRCMKANKIVPGGGAIELEISRL
TtCCTη    K                                                 A--------
MmCCTη  F TG  KA TC            ME T    H         AI NDSV A       M L KY

                430                 450                 470
TpCCTη  LRLHSRKTEGKVQLVINAFAKALEVIPKTIADNAGHDSIQVLNKLRQKHALESDQSKNFG
TtCCTη  --------------------------------------------------------
MmCCTη    DY  TIP  Q  L G Y     I  RQLC    F ATNI      AR  ...QGGMWY

                490                 510                 530
TpCCTη  VDINAVDGIGNNFENFVWEPIIVRKNAFSAATEAACTILSIDETVRNPKSE.QPKAPPGG
TtCCTη  --------------------------------------------------------
MmCCTη       N.EN AD  QA      AM  I  LT  S    L V V   IK  R TVD P  SA

                550
TpCCTη  LRRGGPQGMAGLAKNARLGK* (558)
TtCCTη  --------------------
MmCCTη  RG  QARFH* (544)
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Fig. 2. Alignment and comparison of *Tetrahymena* CCTη deduced amino-acid sequence with CCTη from mouse. The deduced amino-acid sequences for the *T. pyriformis* and *T. thermophila* CCTη polypeptides were aligned and compared with mouse CCTη [10], the only CCTη subunit from which the amino acid sequence is available so far. Gaps were inserted when required to maximize the alignment and are represented by points. Dashed line indicates the missing sequence of *T. thermophila* CCTη subunit. Asterisks indicate the end of the open-reading-frames.

TtCCTη gene with the corresponding partial sequence of the TpCCTη gene shows 92.6% identity. When this type of analysis is extended to the distinct CCT subunits from mouse [10,13], an identity of 60.3% with the CCTη subunit was found while values ranging from 27% (CCTθ) to 35.1% (CCTα) were obtained with the other 7 proteins. These results led us to conclude that the TpCCTη and the TtCCTη encode the homologue proteins of mouse CCTη. An identity of 36.4% was also obtained with the TpCCTγ subunit, a value similar to that found when the comparison is performed with mouse CCTγ subunits (32.5% and 33.6%) [10].

The alignment of the predicted amino acid sequences of the *Tetrahymena* TtCCTη, TpCCTη and mouse CCTη subunit protein is shown in Fig. 2. The TpCCTη protein contains 14 amino acid residues more than its mouse counterpart from which 11 are located in the carboxyl terminus. This feature was also revealed for the TpCCTγ. This alignment shows 6 regions (positions 3–37, 98–213, 258–290, 316–371, 415–439 and 474 until the carboxyl terminus) of variable length where the majority of the amino acid substitutions occurs between the TpCCTη and its mouse counterpart. Interestingly, the amino acid substitutions existing between the two *Tetrahymena* proteins, although in a small number, are also clustered in these regions. On the other hand, the conserved regions located between the non-conserved regions show a more similar size than the divergent ones. Among these conserved regions some contain highly conserved motifs that are present in all CCT subunit polypeptides so far described and also in traditional chaperonins (for review [9]). The motif V(P/A)GGG (positions 407–411) has a weak similarity to ATP synthase subunit β and to members of the valosin-containing protein (VCP)/Cdc48p family [9]. Both proteins constitute oligomeric ATPase complexes. Interestingly enough, this motif also occurs in the imperfect repeats present in the C-terminal region of Microtubule Associated Proteins (MAPs) already described as the microtubule binding domain [35]. The last glycine residue in the motif -VP/AGGG- seems to play an important role in CCT function since the change $G^{411} \Rightarrow D$ creates yeast mutants that developed abnormal microtubules after incubation at 37°C [36]. It is therefore possible that the mutation alters affinity of TCP1/CCTα to the tubulins.

3.3. *Expression of the CCTη subunit genes during* Tetrahymena *cilia regeneration and cell conjugation*

We have previously shown that the TpCCTγ gene presents dramatic changes in its expression pattern during cilia regeneration, suggesting that the product of this gene is most probably related to the cilia biogenesis. In order to investigate whether the TpCCTη gene expression is also altered during *Tetrahymena* cilia recovery, we performed Northern blot hybridization. Total cytoplasmic RNAs were obtained from *T. pyriformis* cells under normal physiological conditions and cells recovering their cilia for different periods. As can be seen from Fig. 3A, the TpCCTη gene produces a unique mRNA of about 2.1 kb in exponentially growing cells and cells regenerating their cilia. As for TpCCTγ and tubulin (βTT1), the amount of the steady-state population of TpCCTη mRNAs decreases rapidly until 30 min of cilia recovery as compared to control cells (see Fig. 3B and C). Afterwards one sees that the levels of these three different steady-state populations of mRNAs present a rapid increase.

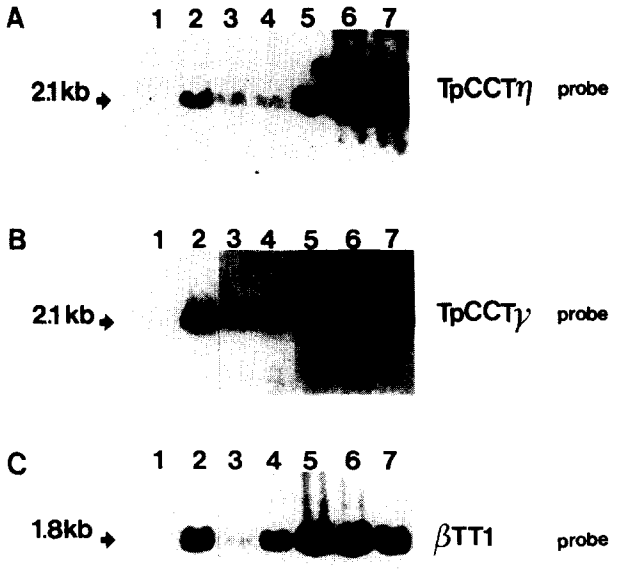

Fig. 3. CCTη, CCTγ and tubulin mRNA levels in *Tetrahymena* cells recovering their cilia. Poly(A)-lacking RNA (lanes 1); total cytoplasmic RNA (30 μg) from exponentially growing cells (lanes 2) and from reciliating cells for 15 min (lanes 3), 30 min (lanes 4), 60 min (lanes 5), 90 min (lanes 6) and 120 min (lanes 7), was analyzed in 1.5% agarose formaldehyde gels, transferred onto nitrocellulose filters, and hybridized with the following probes: (A) a 0.37-kb *Sfu*I DNA fragment from pTpH2.3 plasmid containing part of the 5th exon of the TpCCTη gene; (B) a 0.98-kb *Kpn*I-*Eco*RI DNA fragment from pTpE3 plasmid containing part of the coding region of the TpCCTγ gene; (C) a 3-kb *Hin*dIII fragment from IB1 plasmid containing the β-tubulin gene (βTT1).

TpCCTη mRNAs reach levels higher than those found in exponentially growing cells from 60 min to 120 min of cilia regeneration. The amount of TpCCTγ and tubulin mRNAs reaches the highest levels at about 90 min of reciliation tending toward control levels at 120 min of cilia recovery (see Fig. 3 lanes 6 and 7).

To further understand the role of the CCTη and CCTγ subunits in *Tetrahymena* cells we have studied the expression of the TtCCTη and TtCCTγ genes during *T. thermophila* conjugation. Total cytoplasmic RNAs were extracted from conjugating cells at different periods and immediately after having refed the cells. The vegetative growth resumes under these circumstances (see legend of Fig. 4). Densitometric analysis shows that TtCCTη, TtCCTγ and β-tubulin mRNAs present similar patterns of expression during the conjugation process (Fig. 4). The levels of the three types of transcripts revealed an accentuated decrease until about 300–400 min of conjugation, where the lowest level for each mRNA species is reached. After this period of conjugation the amount of these mRNAs tended to increase slightly to levels that are still lower than those found in control cells. On the contrary, the levels of the 1.8 kb ubiquitin mRNA decrease in the first 135 min to values about 50% of those found in control cells. These levels are maintained during all the studied conjugation stages and may suggest the need for the presence of ubiquitin during the complex process of conjugation. When the starved cells are refed and the vegetative growth is re-initializated, the levels of TtCCTη and TtCCTγ rapidly increase up to about 200%, whereas the tubulin and ubiquitin levels are only 50% of those found in exponentially growing cells.

4. Discussion

The present work reports the structural analysis and expression of the TpCCTη and TtCCTη genes of *T. pyriformis* and *T. thermophila*, respectively. These two genes encode homologue proteins of the mouse CCTη subunit, one of the subunits of the chaperonin-containing TCP1. The identification of these two genes in this ciliate, together with the previously characterized TpCCTγ, strongly support the hypothesis proposed by Kubota et al. [10] that the distinct CCT subunits described in mouse are ubiquitous in all eukaryotes. The predicted amino acid sequence of the TpCCTη gene contains 14 amino acid residues more than its mouse counterpart, a feature also detected in the predicted amino acid sequence of TpCCTγ subunit when compared with the mouse CCTγ. In both *Tetrahymena* CCT subunits, 11 of these extra amino acid residues lie together at the carboxyl terminus. The C-terminus of the CCT subunits is one of the most divergent regions between these proteins and is most likely solvent-exposed parts of the molecule. Indeed monoclonal antibodies against the C-terminus of TCP1/CCTα are able to immunoprecipitate the CCT complex [9–11]. In yeast the CCTα and CCTβ can be C-terminally tagged with additional residues without disordering the CCT complex or affecting viability [22].

We have previously reported that the TpCCTγ subunit gene is co-expressed with tubulin genes and up-regulated during *Tetrahymena* cilia recovery [27]. These results led us to investigate whether the TpCCTη gene is also able to change its pattern of expression in response to the biosynthesis of new

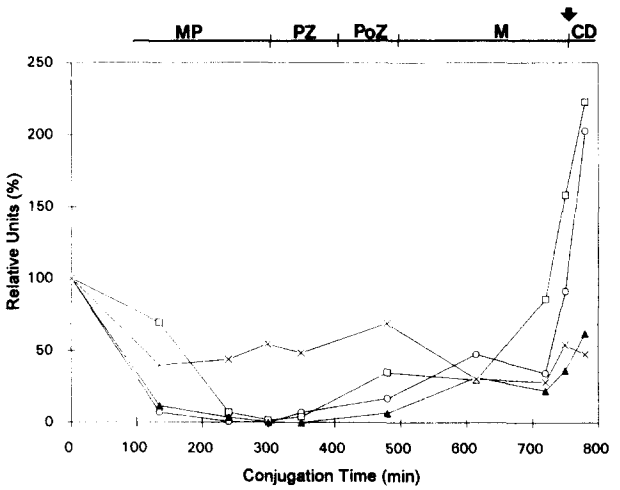

Fig. 4. CCTη, CCTγ and tubulin mRNA levels in *T. thermophila* conjugating cells. CCTη (○), CCTγ (□) tubulin (▲) and 1.8 kb ubiquitin (X) mRNA levels from exponentially growing cells and conjugating cells for different times were determined by Northern blot analysis. Shown is the quantification of linear range autoradiograms performed by densitometric analysis followed by integration. The data represent a standard experiment and the values are expressed in relative units as percentages of the value corresponding to the amounts of these mRNAS in exponentially growing cells. The upper line shows the timing of cytological stages that were followed as described in Material and methods. Each stage is shown to begin at the time by which 50% of the pairs have entered the indicated stages: MP, meiotic prophase; PZ, prezygotic division; PoZ, postzygotic division; M, macronuclear development; and CD, first cellular division. The arrow indicates when the culture was refed.

cilia. Our results show that the TpCCTη gene expression exhibits a similar pattern to that of TpCCTγ gene. In the first 30 min of cilia regeneration we found a decrease of their mRNA levels (see Fig. 3) followed by an increase of their transcripts until 90–120 min. The decline of the TpCCTη and TpCCTγ steady-state mRNA population in the first minutes of reciliation seems to be a general effect to the stress response in this ciliate [32]. The fact that after 30 min of reciliation the levels of TpCCTη and TpCCTγ mRNAs are increased suggests that at least these two CCT subunits might be involved in the biogenesis of the new cilia. This involvement could be related to the folding of newly synthesized tubulin, and/or other ciliary proteins, and/or proteins necessary for cilia assembly. This idea is reinforced by the fact that the abundance of the CCT subunits of the *Tetrahymena* CCT complex already characterized seems to vary under reciliation conditions (unpublished results).

Conjugation of the ciliate protozoan *T. thermophila* is a complex and easily induced synchronous developmental process. When starved cells with different mating types are mixed, then formation of pairs, the process of meiosis, cross-fertilization, and nuclear differentiation of somatic (macronuclei) and germinal nuclei (micronuclei) occur [37]. This also constitutes a unique physiological condition where the transcriptional activity of the micronucleus takes place. As shown in Fig. 4, we observe that the levels of the TtCCTη, TtCCTγ and tubulin transcripts decrease markedly between growing and conjugating cells until 300–400 min. We can assume that this decrease could be the result of the negative transcription regulation of the genes under study. Indeed, Stargell et al. [38] performing run-on transcription assays observed that the apparent rate of transcription of the *T. thermophila* α-tubulin gene is abolished at 240 min after mixing the cells. They also observed that the decrease of transcription levels in nuclei coincides with the absence of messages. Assuming that these genes are not transcribed during this process the assembly/disassembly of microtubules, such as those involved in the exchange of the migratory pronucleus, occurs. In this case it seems that the biosynthesis of the new tubulin and chaperonin subunits is not required for the assembly of these microtubular structures. After the macronucleus differentiation, the marked increase in the amount of TtCCTη and TtCCTγ subunit transcripts could be explained by the entrance of the cells into the phase of vegetative growth and that, in consequence, their active division takes place. Our results give support to the data obtained by Kubota et al. [39] showing that TCP1/CCTα is highly expressed in rapidly growing cells in tissue culture. CCT subunit genes are probably required to be highly expressed during rapid growth in order to maintain enough CCT complex to fold cellular components being newly synthesized in dividing cells [9].

Experiments are in progress in order to establish a functional relationship between CCT complex and the biosynthesis of distinct microtubule structures in protozoa.

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References

[1] Hendrick, J.P. and Hartl, F.-U. (1993) Annu. Rev. Biochem 62, 349–384.
[2] Gatenby, A.A. and Viitanen, P.V. (1994) Annu. Rev. Plant. Mol. Biol. 45, 469–491.
[3] Morimoto, R.I., Tissières, A. and Georgopoulos, C. (1994) In: The Biology of Heat Shock Proteins and Molecular Chaperones. Cold Spring Harbor Laboratory Press, USA, pp. 1–30.
[4] Georgopoulos, C.P., Hendrix, R.W., Casjens, S.R. and Kaiser, A.D. (1973) J. Mol. Biol. 76, 45–60.
[5] Cheng, M.Y., Hartl, F.-U., Martin, J., Pollock, R.A., Kalousek, F., Neupert, W., Hallberg, A.M., Hallberg, M.L. and Horwich, A.L. (1989) Nature 337, 620–625.
[6] Hemmingsen, S.M., Woolford, C., van der Vies, S.M., Tilly, K., Dennis, D.T., Georgopoulos, C.P., Hendrix, R.W. and Ellis, R.J. (1988) Nature 333, 330–334.
[7] Horwich, A.L. and Willison, K.R. (1993) Phil. Trans. R. Soc. Lond. B, 339, 313–326.
[8] Willison, K.R. and Kubota, H. (1994) In: The Biology of Heat Shock Proteins and Molecular Chaperones. Cold Spring Harbor Laboratory Press, USA, pp. 299–312.
[9] Kubota, H., Hynes, G. and Willison, K. (1995) Eur. J. Biochem. 230, 3–16.
[10] Kubota, H., Hynes, G., Carne, A., Ashworth, A. and Willison, K. (1994) Curr. Biol. 4, 89–99.
[11] Lewis, V.A., Hynes, G.M., Zheng, D., Saibil, H. and Willison, K. (1992) Nature 358, 249–252.
[12] Frydmann, J., Nimmesgern, E., Erdjument-Bromage, H., Wall, J.S., Tempst, P. and Hartl, F.-U. (1992) EMBO J. 11, 4767–4778.
[13] Kubota, H., Hynes, G. and Willison, K. (1995) Gene 154, 231–236.
[14] Trent, J.D., Nimmesgern, E., Wall, J.S., Hartl, F.-U. and Horwich, A.L. (1991) Nature 354, 490–493.
[15] Phipps, B.M., Hoffmann, A., Stetter, K.O. and Baumeister, W. (1991) EMBO J. 10, 1711–1722.
[16] Waldmann, T., Nimmesgern, E., Nitsch, M., Peters, J., Pfeifer, G., Müller, S., Kellermann, J., Engel, A., Hartl, F.-U. and Baumeister, W. (1995) Eur. J. Biochem. 227, 848–856.
[17] Gao, Y., Thomas, J.O., Chow, R.L., Lee, G.-H. and Cowan, N.J. (1992) Cell 9, 1043–1050.
[18] Gao, Y., Vainberg, I., Chow, R.L. and Cowan, N.J. (1993) Mol. Cell. Biol. 13, 2478–2485.
[19] Yaffe, M.B., Farr, G.W., Miklos, D., Horwich, A.L., Sternlicht, M.L. and Sternlicht, H. (1992) Nature 358, 245–248.
[20] Frydmann, J., Nimmesgern, E., Ohtsuka, K. and Hartl, F.-U. (1994) Nature 370, 111–117.
[21] Sternlicht, H., Farr, G.W., Sternlicht, M.L., Driscoll, J.K., Willison, K. and Yaffe, M.B. (1993) Proc. Natl. Acad Sci. USA 90, 9422–9426.
[22] Miklos, D., Caplan, S., Mertens, S., Hynes, G., Pitluk, Z., Kashi, Y., Harrison-Lavoie, K., Stevenson, S., Brown, C., Barrel, B., Horwich, A.L. and Willison, K. (1994) Proc. Natl. Acad. Sci. USA 91, 2743–2747.
[23] Chen, X., Sullivan, D.S. and Huffaker, T.C. (1994) Proc. Natl. Acad. Sci. USA 91, 9111–9115.
[24] Vinh, D.B.-N. and Drubin, D.G.(1994) Proc. Natl. Acad. Sci. USA 91, 9116–9120.
[25] Barahona, I., Soares, H., Cyrne, L., Penque, D., Denoulet, P. and Rodrigues-Pousada, C. (1988) J. Mol. Biol. 202, 365–382.
[26] Gaertig, J., Thatcher, T.H., McGrath, K.E., Callahan, R.C. and Gorovsky, M.A. (1993) Cell Motil. Cytoskeleton 25, 243–253.
[27] Soares, H., Penque, D., Mouta, C. and Rodrigues-Pousada, C. (1994) J. Biol. Chem. 269, 29299–29307.
[28] Rodrigues-Pousada, C., Cyrne, M.L. and Hayes, D. (1979) Eur. J. Biochem. 56, 117–122.
[29] Guttman, S.D. and Gorovsky, M.A. (1979) Cell 17, 307–317.
[30] Martindale, D.W., Allis, C.D. and Bruns, P.J. (1982) Exp. Cell Res. 140, 227–236.
[31] Bruns, P.J. and Brussard, T.B. (1974) J. Exp. Zool. 188, 337.
[32] Soares, H., Galego, L., Cóias, R. and Rodrigues-Pousada, C. (1993) J. Biol. Chem. 268, 16623–16630.
[33] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[34] Cóias, R., Galego, L., Barahona, I. and Rodrigues-Pousada, C. (1988) J. Eur. Biochem. 175, 467–474.
[35] Wiche, G., Oberkanins, C. and Himmler, A. (1991) Int. Rev. Cytol. 124, 217–267.

[36] Ursic, D., Sedbrook, J.C., Himmel, K.L. and Culbertson, M.R. (1994) Mol. Biol. Cell 5, 1065–1080.
[37] Bruns, P.J. (1986) in: The Molecular Biology of Ciliated Protozoa (Gall, J.G., Ed.) Academic Press, Inc., Orlando, FL, pp. 27–44.
[38] Stargell, L.A., Karrer, M. and Gorovsky, M.A. (1990) Nucleic Acids Res. 18, 6637–6639.
[39] Kubota, H., Willison, K., Ashworth, A., Nozaki, M., Miyamoto, H., Yamamoto, A., Matsushiro, A. and Morita, T. (1992) Gene 120, 207–215.
[40] Brunk, C.F. and Slader, L.A. (1990) Nucleic Acids Res. 18, 323–329.