MOLECULAR AND CELLULAR BIOLOGY, Oct. 1983, p. 1857–1865
0270-7306/83/101857-09$02.00/0

Vol. 3, No. 10

Cloning of Abundant mRNA Species Present During Conjugation of *Tetrahymena thermophila*: Identification of mRNA Species Present Exclusively During Meiosis

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Received 11 May 1983/Accepted 5 July 1983

A cDNA library was constructed by using as a template the RNA present during the meiotic prophase of *Tetrahymena thermophila*. Clones containing cDNA sequences homologous to moderately abundant to abundant transcripts were detected by colony hybridization and confirmed by hybridizing purified cDNA plasmids on filters with labeled RNA probes. Eighteen clones were isolated, and the sizes of their cDNA inserts were determined. Cross-hybridization of individual cDNA plasmid pairs showed that each of these clones contained cDNA that was homologous to one of eight different RNA transcripts. The sizes of the eight RNA transcripts and the stages of the *T. thermophila* life cycle during which they were present were determined by hybridizing nick-translated cDNA probes against denatured, electrophoresed RNA from various stages. Clones were identified that contained sequences homologous to RNAs present only in early conjugation (meiosis); other clones contained sequences homologous to RNAs which were abundant during conjugation but present at other stages as well. One clone contained a sequence homologous to an RNA that was abundantly present only in nongrowing cells.

Conjugation of the ciliated protozoan *Tetrahymena thermophila* is an easily induced synchronous developmental process (13). When starved cells with different mating types are mixed, pairs form, and the processes of meiosis, cross-fertilization, and nuclear differentiation of somatic and germinal nuclei occur (15).

By pulse-labeling cells with radioactive leucine during various stages of conjugation, we found (D. W. Martindale, C. D. Allis, and P. J. Bruns, manuscript in preparation) a transient increase in the overall rate of protein synthesis during meiotic prophase which declined in postmeiotic cells. This observation of a quantitative change in protein synthesis was extended by monitoring the synthesis of individual proteins during conjugation by two-dimensional gel electrophoresis (Martindale et al., manuscript in preparation). These studies showed that although the majority of proteins synthesized during starvation (a necessary condition for conjugation to occur) continue to be synthesized during conjugation, a number of conjugation-specific proteins are strongly induced, and their relative rates of synthesis generally follow the overall pattern of leucine incorporation, reaching a maximum during meiotic prophase.

The study reported in this paper was initiated to isolate, by recombinant DNA cloning, the genes responsible for the synthesis of conjugation-specific proteins. We describe the construction and analysis of a cDNA library made from RNA isolated during meiotic prophase. We present evidence for clones representing genes showing a remarkable degree of transcriptional control; several of these are transcribed only during early conjugation (meiosis).

MATERIALS AND METHODS

Culture conditions. The *T. thermophila* strains used were CU355 (IV), CU356 (VI), CU399 (VI), and CU401 (VII) (roman numerals following strain designations are the mating types). The cells were grown, starved, and mated at 30°C as described previously (13). Briefly, cells were grown in enriched medium and starved by washing once by low-speed centrifugation into 10 mM Tris, pH 7.4 (starvation buffer). To initiate conjugation, equal numbers of starved cells with different mating types were mixed at 2×10^5 to 3×10^5 cells per ml of starvation buffer.

RNA isolation. (i) Total RNA. All solutions coming into contact with RNA were autoclaved, and all glassware was heat treated at 165°C overnight. Cells (1×10^6 to 2×10^7) were pelleted by low-speed centrifugation and then lysed with 2 to 5 ml of RNA lysis buffer (0.1 M NaCl, 10 mM EDTA, 10 mM Tris-hydrochloride [pH 7.4], 1% sodium dodecyl sulfate, 0.5 mg of heparin per ml). After 5 to 10 min in lysis buffer at room temperature, protein was removed by phenol-chloroform-isoamyl alcohol (25:24:1) extraction fol-

lowed by several chloroform-isoamyl alcohol (24:1) extractions. The RNA was precipitated by adding 0.1 volume of 3 M sodium acetate (pH 7) and 2.5 volumes of 95% ethanol and placing the mixture at −20°C for 2 h or more or at −70°C for 30 min or more. After centrifugation at 12,000 × *g* for 15 min, the pellet was washed with ice-cold 70% ethanol, dried, and dissolved in distilled water or TE8 (10 mM Tris-hydrochloride [pH 8], 1 mM EDTA). An average of 80 or 160 μg of total RNA was obtained from 10^6 conjugating or growing cells, respectively.

(ii) Poly(A)$^+$ RNA. Total RNA was run over an oligodeoxythymidylate-cellulose (Bethesda Research Laboratories) column twice, as described by Aviv and Leder (1). The average yield of polyadenylic acid [poly(A)]-containing [poly(A)$^+$] RNA was 2 to 3% of the total RNA from starved or conjugating cultures and 2.5 to 5% of the total RNA from rapidly growing cultures.

cDNA bank construction. Double-stranded cDNA was synthesized from poly(A)$^+$ RNA that was isolated from conjugating cultures (22). The enzymes used (avian myeloblastosis virus reverse transcriptase, DNA polymerase I, and S1 nuclease) were obtained from Bethesda Research Laboratories. The starting material was 4 μg of poly(A)$^+$ RNA isolated from cells undergoing conjugation (pooled RNA isolated at 2, 2.5, and 3 h after mixing starved cultures of different mating types). After the various reactions and final purification were monitored, ca. 170 ng of double-stranded cDNA was obtained. This DNA was inserted into the *Pst*I site of pBR322 (4) by the polydeoxyguanylic acid-polydeoxycytidylic acid homopolymer extension technique (21). The 3′ DNA Terminus Tailing System from Bethesda Research Laboratories was used for this procedure. The cDNA-containing plasmids were then used to transform *Escherichia coli* HB101 (5). Tetracycline-resistant, ampicillin-sensitive colonies were isolated (480 total).

Colony hybridization. The technique used to detect clones containing cDNA inserts of interest was adapted from techniques previously described (9, 10). Genatran 45 membrane filters (D and L Filter Corp., Woburn, Mass.) were used in place of nitrocellulose filters for this and all other procedures. Colonies were inoculated and grown on LB plates containing 10 μg of tetracycline per ml, transferred by toothpick to duplicate LB plates containing tetracycline, and incubated for ca. 24 h at 37°C. Sterilized filters were then placed on the plates for 5 min and transferred colony side up to LB plates containing 170 μg of chloramphenicol per ml. After 12 to 16 h the filters were removed and air dried on 3MM paper. Cell lysis and DNA denaturation were accomplished by placing the filters in succession, for 5 min each, colony side up on 3MM paper soaked with: (i) 0.5 M NaOH–1.5 M NaCl; (ii) 0.5 M Tris-hydrochloride (pH 7.5)–1.5 M NaCl; (iii) the same solution as in step (ii); (iv) 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The filters were then rinsed in 95% ethanol, air dried, and baked for 2 h at 80°C in vacuo. They were then hybridized with labeled poly(A)$^+$ RNA (ca. 10^6 cpm per filter).

Probe labeling. Poly(A)$^+$ RNA probes were labeled by partially hydrolyzing the RNA (to increase the number of 5′-OH sites) and end-labeling with [γ-^{32}P]ATP, using polynucleotide kinase (P-L Biochemicals, Inc.) (18). The specific activity achieved with [γ-^{32}P]ATP (3,000 Ci/mmol; Amersham Corp.) was typically 1×10^7 to 2×10^7 cpm/μg of RNA. DNA probes were nick translated in the standard way (16) with a nick translation kit from Amersham Corp. The typical specific activity obtained with [α-^{32}P]dCTP (800 Ci/mmol; Amersham Corp.) was 7×10^7 cpm/μg of DNA.

DNA dot blots. To confirm that the colonies selected through colony hybridization contained cDNA of interest, plasmids were rapidly isolated by the boiling method of Holmes and Quigley (11). The plasmids (50 to 100 ng each) were then heated to 90°C for 5 min and spotted (5 μl each) on Genatran 45 filters that had been washed in distilled water and air dried. The DNA was denatured, fixed to the filters, and hybridized with labeled RNA in the same manner as the filters used in colony hybridization.

Plasmid isolation. Plasmids were isolated from sodium dodecyl sulfate-lysed bacteria (7). Centrifugation through cesium chloride purified the plasmids sufficiently so that they could be cut with restriction endonucleases.

Gel electrophoresis. The RNA was denatured with deionized glyoxal and dimethyl sulfoxide (DMSO) (14) and run as described on 0.8 to 1.5% agarose gels in 10 mM NaH_2PO_4 (pH 7.0) at 3 to 4 V/cm for 3 to 4 h. The gels were stained for 45 min with 1 μg of ethidium bromide per ml of distilled water. Glyoxal-DMSO–treated DNA and RNA (lambda DNA digested with *Hin*dIII, φX174 RF DNA digested with *Hae*III, and *E. coli* rRNA) were used as size markers. To visualize the DNA standards, the gel was placed in 50 mM NaOH for 15 min to reverse the glyoxylation, neutralized for 15 min in 0.2 M sodium acetate (pH 4.8), stained for 45 min in 5 μg of ethidium bromide per ml of 1 mM $MgSO_4$, and then destained for 5 to 45 min in 1 mM $MgSO_4$. The gel was rinsed with distilled water between treatments.

DNA was run on 0.8 to 1.5% agarose gels in 8.9 mM Tris-hydrochloride (pH 8.3)–8.9 mM boric acid–2.5 mM EDTA at 3 to 4 V/cm for 3 to 4 h. Both the running buffer and gel contained 0.5 μg of ethidium bromide per ml.

Gel-to-filter transfers of DNA and RNA. Before transfer, DNA gels were denatured in 0.5 M NaOH–1.5 M NaCl for 20 min, rinsed in distilled water, and neutralized in 0.5 M Tris-hydrochloride (pH 7.6)–1.5 M NaCl for 20 min. The DNA was then transferred overnight (17) to a Genatran 45 filter presoaked in 2× SSC for several minutes. Glyoxylated RNA was transferred directly to Genatran 45 filters after the gels were stained and photographed (20).

Filter hybridization. The filters were prehybridized for 18 to 20 h at 42°C in a solution containing 50% formamide (Sigma Chemical Co.), 5× SSC, 1× Denhardt solution (0.02% each of bovine serum albumin, Ficoll, and polyvinylpyrrolidone), 25 μg of yeast soluble RNA (Sigma, type III) per ml, and 0.2% sodium dodecyl sulfate. If the filters were to be hybridized with DNA probes, the prehybridization mixture included 250 μg of denatured herring sperm DNA (Sigma) per ml. Labeled probes (ca. 10^6 cpm per filter) were added directly to the prehybridization mixture, and hybridization was carried out at 42°C for 36 h. Dextran sulfate (Sigma; 10% final concentration) was often added during hybridization; the hybridization time was then decreased to 12 to 20 h at 42°C. The

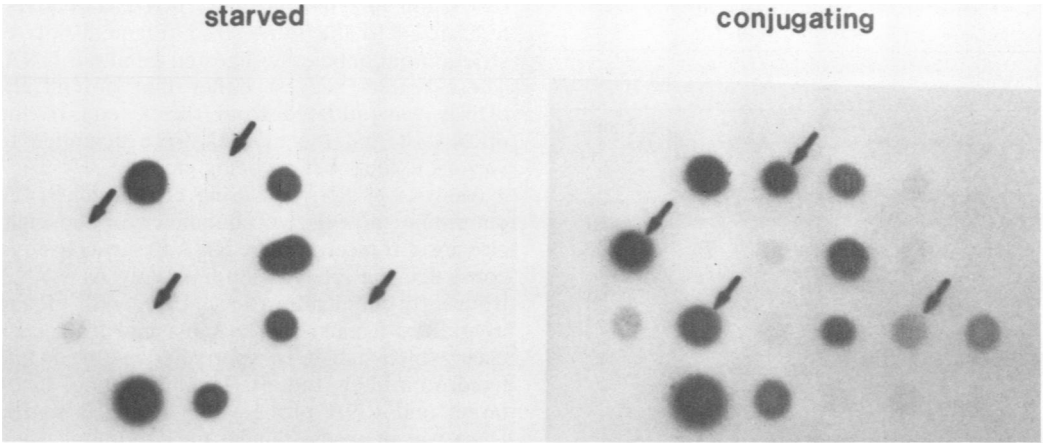

FIG. 1. Identification of clones. Plasmids were dot-blotted onto Genatran 45 filters, denatured, fixed, and hybridized with end-labeled poly(A)$^+$ RNA isolated from either a 24-h starved culture or a culture early in conjugation. Arrows indicate plasmids that appear to contain sequences present only in conjugating RNA.

then washed and exposed to X-ray film (Kodak XS-5) as described by Thomas (20).

Relative abundance. The relative abundances of the cloned transcripts in cells in meiotic prophase (early conjugation) were determined in the following manner. The concentration of each cDNA plasmid was adjusted to 25 ng of pBR322 per μl; this made the number of molecules in a given volume the same for each cDNA plasmid. These cDNA plasmids were then heated to 90°C for 5 min and spotted (4 μl) on dry Genatran 45 filters that had been washed briefly in 2× SSC. The DNA was then denatured by NaOH and fixed to the filters in the same manner as described for the colony hybridizations. The filters were then prehybridized, hybridized with labeled poly(A)$^+$ RNA from meiotic prophase, washed, and exposed to X-ray film in the usual manner.

RESULTS

cDNA library construction. Poly(A)$^+$ RNA was isolated from cells at an early stage of conjugation. Two cultures with different mating types were starved for 22 h and mixed in equal cell numbers to initiate conjugation (3×10^5 cells per ml). Two hours after mixing, 83% of the cells were in pairs. We combined RNA that had been isolated from conjugating cells at 2, 2.5, and 3 h after mixing the two cell cultures. A cytological examination of these cultures (2 to 3 h after mixing) revealed that 95% of the pairs were in early meiotic prophase (stages I through IV of micronuclear elongation) (13, 19). Double-stranded cDNA was synthesized from this RNA. Polydeoxycytidylic acid was added to the 3′ end of these cDNA molecules, polydeoxyguanylic acid was added to the 3′ end of *Pst*I-cut pBR322, and the two types of molecules were annealed. The annealed molecules were then used to transform *E. coli* HB101 (5, 21). Since the cDNA was inserted into the *Pst*I site of pBR322, and this is located within the ampicillin resistance gene, transformants containing cDNA inserts were easily detected (tetracycline resistant, ampicillin sensitive). Furthermore, upon transformation, a pair of *Pst*I sites were generated on each side of the cDNA insert by *E. coli* DNA repair enzymes, permitting the cDNA insert to be cleanly excised from the plasmid by *Pst*I digestion.

cDNA library screen. A total of 480 tetracycline-resistant, ampicillin-sensitive colonies were isolated. Colonies that gave detectable signals upon colony hybridization with labeled poly(A)$^+$ RNA from conjugating cells were isolated. Plasmids from these colonies were rapidly isolated by the boiling method of Holmes and Quigley (11), spotted on duplicate filters, denatured, and then hybridized with labeled poly(A)$^+$ RNA isolated from either a 24-h-starved culture or a culture early in conjugation (2 to 3 h after mixing) (Fig. 1). Plasmids from 18 colonies gave detectable signals on X-ray film after hybridization with labeled RNA from conjugating cells. Several of these plasmids did not give a detectable signal when hybridized to labeled RNA from the starved culture (Fig. 1, arrows). Seven plasmids that did not give detectable signals when hybridized with either labeled RNA in these dot blots were examined further because when they were run on an agarose gel they were found to contain large cDNA inserts. Two of these (pC7 and pC8) had transcripts in sufficient abundance during conjugation to give clear signals when the plasmids were labeled by nick translation and hybridized against total RNA from conjugating cells.

cDNA clone analysis. The sizes of the cDNA inserts (Table 1) were determined by comparing

TABLE 1. cDNA insert and mRNA size determination

Plasmid	cDNA insert (bp)	mRNA (bases)
pC1-1	440	2,800
pC1-2	2,150	
pC1-3	295	
pC1-4	865	
pC2-1	3,410	7,450
pC3-1	300	430
pC3-2	430	
pC4-1	400	560
pC5-1	685	1,450
pC5-2	195	
pC5-3	400	
pC5-4	390	
pC5-5	1,030	
pC5-6	465	
pC5-7	750	
pC6-1	755	1,470
pC7-1	1,270	1,300
pC8-1	1,650	4,200

*Pst*I-digested plasmids with standards after electrophoresis on 1.2% agarose gels.

Cross-hybridization experiments were performed to determine whether any of the selected cDNA plasmids isolated carried inserts with the same (or overlapping) sequence as other cDNA plasmids. *Pst*I-cut cDNA plasmids were run on agarose gels, transferred to filters (17), and then hybridized to various labeled cDNA plasmid probes. From these experiments it was found that several of the plasmids cross-hybridized with other plasmids and were thus independent clones of cDNA from the same mRNA species. Table 1 groups together plasmids which contain homologous sequences. The 18 cDNA plasmids examined represent eight different RNA transcripts.

If all cDNAs were initiated at the poly(A) region at the 3′ end of the mRNAs as expected with oligodeoxythymidylate priming, all undegraded cDNAs made from the same mRNA class should cross-hybridize, since they would all share the sequences at the 3′ end of the RNA. Table 2 presents the results of cross-hybridizations within the pC5 class. Although pC5-3 (cDNA = 400 base pairs [bp]) and pC5-5 (cDNA = 1,030 bp) did not cross-hybridize with each other (note: pC5 mRNA is 1,450 bases long), both cross-hybridized to pC5-4 (cDNA = 390 bp). Figure 2 presents an interpretation of the cross-hybridization and cDNA size data for the pC5 family of clones. Similarly, pC1-3 did not cross-hybridize with pC1-1, pC1-2, or pC1-4; pC1-4, however, did cross-hybridize with pC1-1 and pC1-2. pC1-3 was placed in the pC1 class initially because it hybridized to RNA of the same size, abundance, and transcription pattern as pC1 RNA. This assignment was confirmed by restriction enzyme analysis; pC1-2 and pC1-3 hybridized to the same-sized fragments of restriction endonuclease-digested genomic DNA. These results suggest either that not all the cDNA was initiated from the 3′ end of the mRNA or that the cDNAs were degraded to various extents before being cloned.

Analysis of RNA by using cDNA clones. To determine the relative abundance of the eight classes of transcripts, the RNA that was used to construct the cDNA library [poly(A)$^+$ RNA from early conjugation] was labeled with ^{32}P and hybridized against a cDNA plasmid from each class which had been spotted, denatured, and fixed on a filter (Fig. 3). The amounts of DNA target and RNA probe were arranged so the DNA was in excess. Under the conditions used, the poly(A)$^+$ RNA should have been digested to an average size of 60 bases (12). The amount of labeled RNA hybridizing to a given cDNA plasmid is dependent upon the relative numbers of molecules of a particular mRNA species in the RNA probe (2) and the size of the cDNA insert. The relative amounts of labeled RNA hybridizing to the cDNA plasmids were determined by scanning autoradiographs (Fig. 3) with a laser densitometer (LKB 2202) (Table 3). The exposure of the autoradiograph was chosen for each plasmid so that it was within the linear response range of the X-ray film.

The fraction of total poly(A)$^+$ RNA represented by an RNA species whose sequence is found in more than one clone can be estimated by dividing the number of clones containing that sequence by the total number of clones. This approach assumes that each poly(A)$^+$ RNA molecule has an equal probability of being made into cDNA and cloned; however, since there are many steps in the synthesis and cloning of cDNA at which prejudice for or against a particular RNA may occur, these estimated fractions must be taken as rough approximations at best. The calculated percentages of the total poly(A)$^+$ RNA corresponding to the three cDNA classes for which there are multiple clones are 0.8% (4/480) for pC1, 0.4% (2/480) for pC3, and 1.5% (7/480) for pC5. These values approximate the corrected relative intensities for these three classes (Table 3); at least for these three, the

TABLE 2. Cross-hybridization of pC5 class

Probe	Target						
	pC5-1	pC5-2	pC5-3	pC5-4	pC5-5	pC5-6	pC5-7
pC5-3	−	+/−	++	++	−	−	+
pC5-4	−	+	++	+++	+	+	ND[a]
pC5-5	+++	++	−	+	+++	+++	++

[a] ND, Not done.

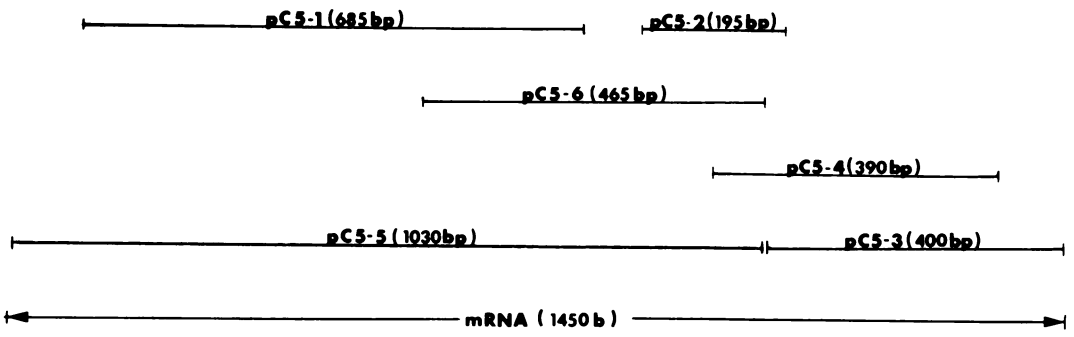

FIG. 2. Possible physical arrangement of the pC5 class of cDNA. With the cross-hybridization data of the pC5 class of cDNA plasmids (Table 2) and the known sizes of the various cDNA inserts, a physical map can be constructed that is consistent with the data. Note that pC5-3 and pC5-5 should define approximately 100% of the mRNA molecule.

number of clones obtained mirrored their relative abundance in the poly(A)$^+$ RNA.

Labeled cDNA plasmid probes were hybridized against total RNAs from various stages of the *T. thermophila* life cycle to determine when mRNAs corresponding to the different classes of cDNA plasmids were present. To conjugate, recently mated cells must become "mature" (3) by undergoing approximately 50 cell divisions. Conjugation is then initiated by mixing, in starvation buffer, mature cells with different mating types that have been starved for at least 70 min (23). There is a brief period of "costimulation" (ca. 30 min; 6), resulting from cell contact between the two mating types, that is required before cell pairing occurs. RNA was isolated from cultures in the following stages: (i) log (mid-logarithmic phase of the vegetative growth cycle, 10^5 cells per ml, with a doubling time of 2.4 h); (ii) stationary (plateau phase of vegetative growth cycle, 6.5×10^5 cells per ml); (iii) starved (a mature [3] culture [CU399] that had reached 2×10^5 cells per ml was washed into starvation buffer to the same cell concentration, and RNA was isolated 2, 4, and 20 h after washing; also a mature culture with a different mating type [CU401] and an immature culture [progeny of a CU399 × CU401 cross] were starved in the same manner, and RNA was isolated 20 h after washing into starvation buffer); (iv) costimulated (6) (after 23 h of starvation, CU399 and CU401 were mixed on a fast shaker that cycled 30 s on, 10 s off, 15 s on, 10 s off repeatedly, thus allowing cell-to-cell contact but preventing pairing; after 6.5 h of this cycle, the total time the shaker was off, allowing costimulation to occur, was 2 h); (v) conjugating (equal numbers of CU399 and CU401 were mixed [0 h] and allowed to mate; RNA was isolated at 2 h [39% pairing], 4 h [73% pairing], 6 h [92% pairing], and 10 h [91% pairing]). The pairing frequencies indicate that cells entered conjugation with less than average synchrony (13).

The RNA was denatured with glyoxal-DMSO (14) and run on agarose gels. After staining with ethidium bromide (Fig. 4A), the RNA was transferred from the gel to Genatran 45 filter paper (20) and hybridized with various labeled cDNA plasmid probes. Glyoxylated DNA and RNA standards were included for the determination of mRNA sizes (Table 1). Also included on the gel shown in Fig. 4 are poly(A)$^+$ RNAs from cultures at late-logarithmic phase (2.5×10^5 cells per ml), 24-h starvation, and early conjugation (same batch of poly(A)$^+$ RNA as was used to make the cDNA bank). Figure 4B shows the autoradiograph of a filter hybridized with pC1-3; no signal was seen with RNA from any stage but conjugation. The stage specificity of pC1 transcription was strikingly demonstrated by examining the amount of label hybridizing to poly(A)$^+$ RNA from conjugating cells as opposed to starved or late-log cultures. Even though the signal from conjugating poly(A)$^+$ RNA was overexposed, no hybridization to the other two poly(A)$^+$ RNAs was seen at all. The transcripts corresponding to pC1 peaked at 4 h after mixing and were at very low levels by 10 h. The culture from which the total RNA was isolated entered conjugation with less than optimum synchrony (seen by pairing kinetics; 13); therefore, the pC1 transcripts were probably present in significant amounts only during meiotic prophase.

The procedure described above was used for all eight classes of plasmids. Figure 5 graphically presents the results obtained by scanning the autoradiographs with a densitometer. The cDNA plasmids were ordered with respect to their transcription patterns. Three of the eight classes of cDNA plasmids hybridized to RNA from conjugating cultures only, although one of

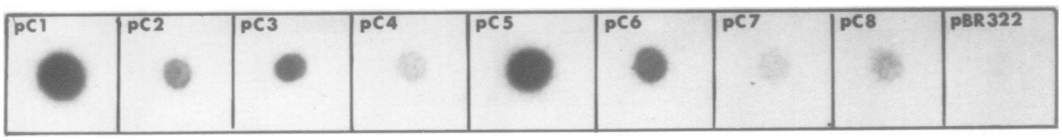

FIG. 3. Relative abundance of transcripts at early conjugation. Each plasmid was spotted on the filter, denatured, fixed, and hybridized with end-labeled poly(A)$^+$ RNA from early conjugation (the same RNA that was used to construct the cDNA bank). Since the plasmids were present in excess, the differences in intensities were due to differences in the abundance of the transcripts corresponding to the plasmids.

them (pC2) hybridized slightly to RNA from a costimulated culture. All classes except pC5 were induced in conjugation above the amount seen after 20 h of starvation. The quantity of all eight classes of transcripts had decreased significantly by 10 h after the induction of conjugation.

DISCUSSION

This paper describes the construction and partial analysis of a cDNA library made from poly(A)$^+$ RNA isolated from *T. thermophila* cells in the early stages of conjugation (meiotic prophase). Colonies containing cDNA inserts made from poly(A)$^+$ RNA species of moderate to high abundance during early conjugation were detected by colony hybridization with end-labeled poly(A)$^+$ RNA and confirmed by dot-blot hybridization, which used the same probe against purified cDNA plasmids. Colonies were detected that contained cDNA made from conjugation-specific RNA and conjugation-induced RNA. The inserted DNA in each of these colonies was sized and used to study *T. thermophila* mRNA. The sizes and relative abundances of the corresponding RNAs were measured in a variety of physiological stages of the *T. thermophila* life cycle.

Most of the mRNA species homologous to the clones analyzed were seen to be present in large amounts for relatively short periods in conjugation. The time when the conjugation-specific mRNAs were synthesized (meiotic prophase) corresponded strikingly to the time of synthesis of conjugation-specific proteins (Martindale et al., manuscript in preparation). These observations show that the genes coding for at least some of the conjugation-specific functions are under transcriptional control.

TABLE 3. Abundance of transcripts at early conjugation

Plasmid	cDNA (bp)	Relative intensity (densitometer)	Relative intensity (corrected)[a]
pC1-2	2,150	1.000	1.00
pC2-1	3,410	0.056	0.04
pC3-2	430	0.084	0.42
pC4-1	400	0.022	0.12
pC5-5	1,030	0.654	1.37
pC6-1	755	0.094	0.27
pC7-1	1,270	0.016	0.03
pC8-1	1,650	0.024	0.03

[a] Correction based upon different sizes of cDNA. Relative intensity is that expected if cDNA insert was 2,150 bp.

Each of the 18 plasmids examined contained a cDNA insert synthesized from one of eight different mRNAs. This was determined by cross-hybridization between the cDNA plasmids and by hybridization of each plasmid to RNA from various physiological states of *T. thermophila*. Since the first strand of cDNA was initiated with oligodeoxythymidylate, we expected all the cDNAs to initiate at the poly(A) region at the 3′ end of the mRNAs. All cDNAs made from the same mRNA class would then cross-hybridize because they all should contain the same 3′ sequences. However, pC5-3 (cDNA = 400 bp) and pC5-5 (cDNA = 1,030 bp) did not cross-hybridize with each other, but both hybridized to pC5-4 (cDNA = 390 bp). This, and other evidence presented above, suggests either that not all of the cDNA was initiated from the 3′ end of the mRNA or that the cDNAs were degraded to various extents before being cloned. An argument in favor of the former suggestion is that the *T. thermophila* genome has an unusually high adenosine-plus-thymidine content (77%) (8), and tracts of adenosine-rich sequences to which oligodeoxythymidylate could bind probably occur within its mRNA. Also, an argument against nonspecific degradation is the fact that we did obtain mRNA-size cDNA inserts (pC3-2 and pC7-1).

F. J. Calzone (Ph.D. thesis, University of Rochester, Rochester, N.Y., 1982) noted an abundant transcript of 1,400 bases when ^{32}P-labeled polyuridylate was hybridized against RNA isolated from starved cells. This may correspond to the pC5 class of mRNA which we determined to consist of 1,450 bases; it was the most abundant transcript during either starvation or early conjugation of any of the clones we have examined. The pC5 transcript was not found in rapidly growing cells but was found in cells that had reached the plateau phase of growth; furthermore, unlike the transcripts from the other seven classes, the pC5 transcript was induced by starvation and remained abundant

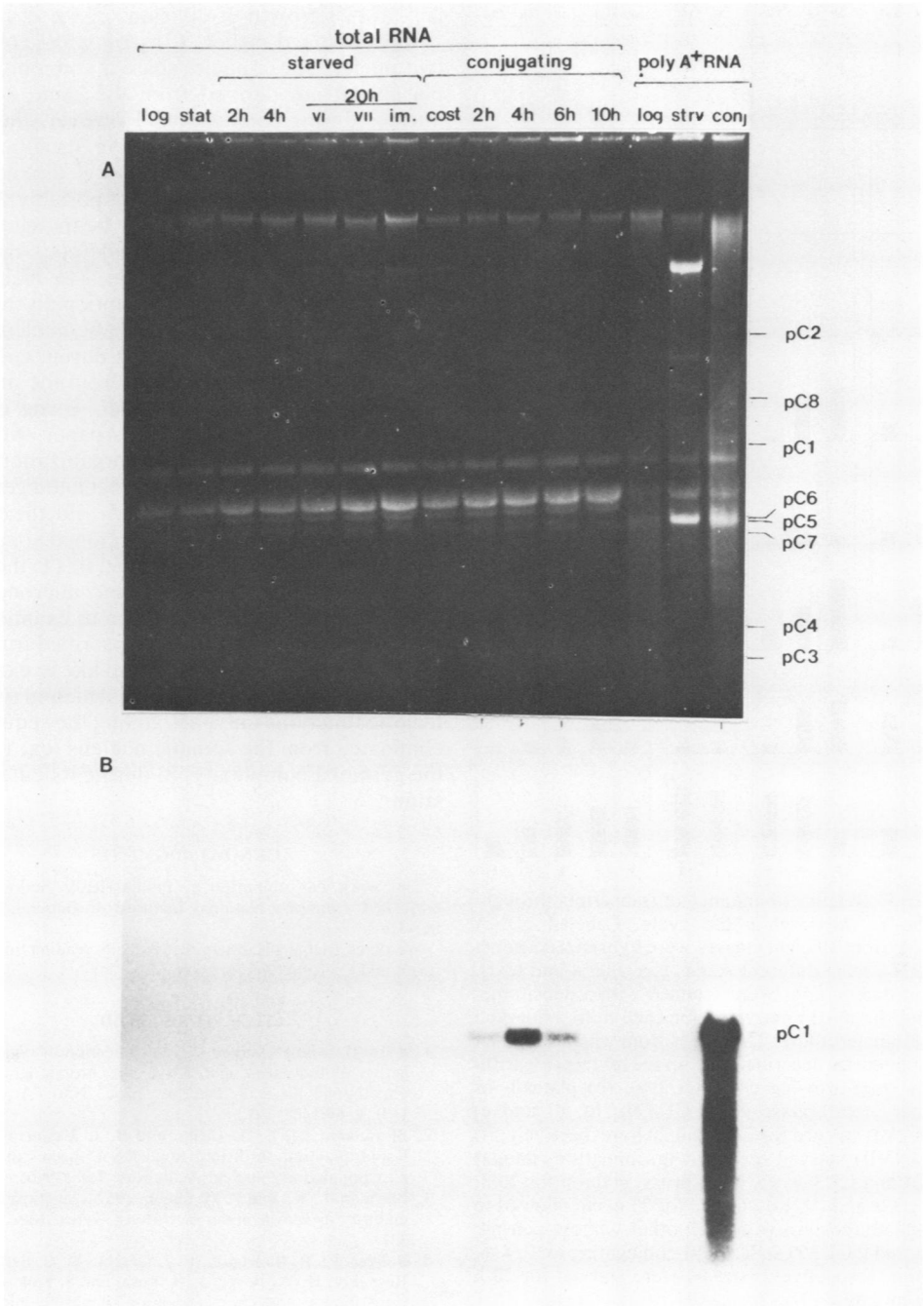

FIG. 4. Hybridization of pC1 against RNA from different stages of the life cycle of *T. thermophila*. RNAs from different life cycle stages (see text) were denatured with glyoxyal-DMSO treatment and run on a 1.2% agarose gel. Both total RNA and poly(A)$^+$ RNA were isolated. (A) Ethidium bromide stain of RNA. The major bands seen with the total RNA are ribosomal. Glyoxal-DMSO–treated DNA and RNA standards had been run in other gels so that the size of the RNAs seen upon staining and those corresponding to the cDNA plasmids could be determined. (B) Autoradiograph of filter (made by RNA blotting with gel shown in A) hybridized with labeled pC1-3 plasmid.

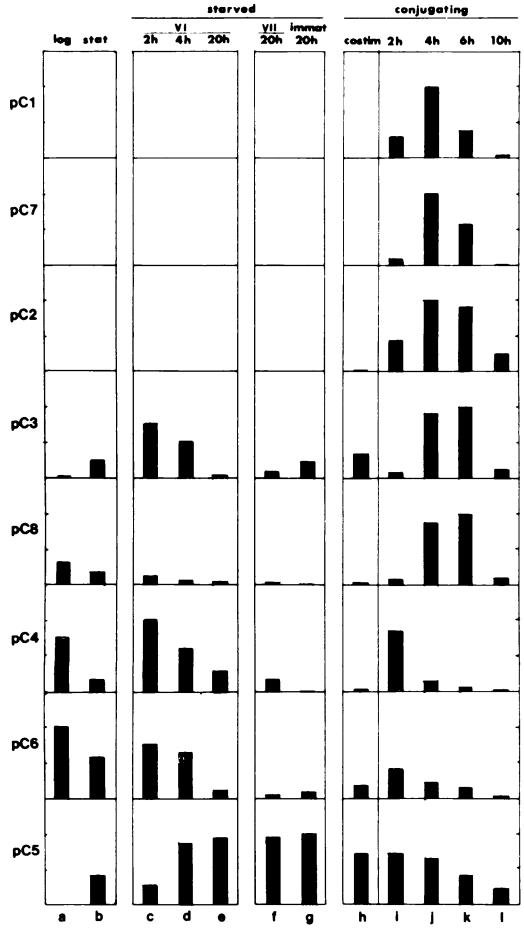

FIG. 5. Relative abundance of transcripts throughout the *T. thermophila* life cycle. Labeled cDNA plasmids from all eight classes were hybridized against total RNA from different stages (see Fig. 4 and text). The autoradiographs were scanned with a densitometer, and the maximum value for each autoradiograph was designated 100%. The stages from which the RNA was isolated are described fully in the text: (a) logarithmically (log) growing cells (CU399); (b) plateau- or stationary (stat)-phase cells (CU399); (c, d, and e) CU399 (VI) starved for 2, 4, and 20 h, respectively; (f) CU401 (VII) starved for 20 h; (g) immature (immat) cells from a CU399 × CU401 cross, starved for 20 h; (h). CU399 and CU401 starved for 23 h and allowed to costimulate (costim) with each other without pairing; (i, j, k, and l) CU399 and CU401 conjugating for 2, 4, 6, and 10 h, respectively (strains were starved for 20 h before mixing).

throughout starvation (more than 20 h). When starved cells are refed and vegetative growth resumes, the pC5 transcript is seen to disappear rapidly; furthermore, this transcript is induced in cells that have been heat shocked (R. L. Hallberg and D. W. Martindale, unpublished data). These results indicate a correlation between the presence of the pC5 transcript and the lack of cell growth or division.

As mentioned earlier, *T. thermophila* contains two nuclei. These nuclei share a common cytoplasm and are derived from the same zygotic nucleus during conjugation. However, they are differentiated from each other in structure and function (8). The micronucleus is diploid and transcriptionally inactive except possibly during meiotic prophase (19), and may be thought of as a germinal nucleus since it undergoes meiosis and gives rise to progeny nuclei. The macronucleus is polyploid and transcriptionally active, and may be thought of as a somatic nucleus; the old macronucleus is eliminated during conjugation after a new one is formed from the meiotic products of the germinal nuclei. Using clones such as those described in this paper, we may now begin to examine the organization and regulation of developmentally regulated genes in the two nuclei of *T. thermophila*. Furthermore, since a number of the genes we cloned are active only during meiotic prophase and this is the only time that there is evidence for micronuclear transcription (19), we would like to examine the possibility that they may be transcribed from the germinal nucleus. We would also like to examine the possibility that such genes, which may have meiotic functions only, are among the sequences eliminated from the somatic nucleus (ca. 15% of the germinal complexity; 8) during its differentiation.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM-27871 from the National Institute of General Medical Services.

We thank Richard Hallberg for critically reading this manuscript and C. Tompkins for typing it.

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