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Laurie A. Stargell
University of Rochester

Kathleen M. Karrer
Marquette University, kathleen.karrer@marquette.edu

Martin A. Gorovsky
University of Rochester

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Transcriptional regulation of gene expression in *Tetrahymena thermophila*

Laurie A.Stargell, Kathleen M.Karrer[1] and Martin A.Gorovsky
University of Rochester, Department of Biology, Rochester, NY 14627 and [1]Marquette University, Department of Biology, Milwaukee, WI 53233, USA

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ABSTRACT

The only well-characterized study of gene expression in *Tetrahymena thermophila* (1) demonstrates that the temperature dependent expression of the Ser H3 gene is regulated at the level of mRNA stability. A run-on transcription assay was developed to determine if regulation of RNA stability was a major mechanism regulating gene expression in *Tetrahymena* or if transcriptional regulation dominates. The relative transcriptional activities of 14 *Tetrahymena* genes were determined in different physiological/developmental states (growing, starved and conjugating) in which many of the genes showed striking differences in RNA abundance. In every case except Ser H3, changes in transcription accompanied changes in RNA abundance. Thus differential transcription, not differential RNA degradation, is the major mechanism regulating RNA abundance in *Tetrahymena*.

INTRODUCTION

Regulation of mRNA abundance in higher eukaryotes can occur at the level of transcription, nuclear processing and mRNA stability. Nevertheless, transcriptional control is indicated to be the most frequently employed mechanism (reviewed in [2]). In lower eukaryotes, such as yeast, transcriptional regulation also appears to dominate. Comparatively little is known about the regulation of gene expression in *Tetrahymena*, a ciliated protozoan which is evolutionarily distant from both yeast and higher eukaryotes. The regulation of the serotype H3 surface antigen (Ser H3) gene is one case which has been well-characterized in this organism (1). Ser H3 message accumulation and protein synthesis are limited to incubation temperatures of 20 to 30°C, and the increase in mRNA abundance is the consequence of a dramatic temperature-dependent increase in Ser H3 message stability. We wished to determine if the regulation of mRNA stability is a major mechanism regulating expression of genes in *Tetrahymena* or if transcriptional regulation dominates. Hence, a run-on transcription assay was developed and optimized using isolated macronuclei. Analysis of nascent transcripts via run-on transcription assays allows one to examine the process of transcription without the complications of RNA processing or turnover and has proven useful in other systems for studies involving transcription initiation rates (3,4) and termination (5). With this assay the transcriptional activity of 14 *Tetrahymena* genes was determined in growing, starved and conjugating cells and compared to the abundance of RNA in these different physiological states. These studies demonstrate that differential transcription is in fact, the major mechanism regulating RNA abundance in *Tetrahymena*.

MATERIALS AND METHODS

Cells and Culture Conditions

Tetrahymena thermophila (strains CU428, SB1969 and SB210) were grown axenically in enriched proteose peptone at 28°C as described (6). Cells were starved at a density of $2-3\times10^5$ cells/ml in 10 mM Tris, pH 7.4 for 18–22 hours, without shaking. Conjugation was induced by mixing equal numbers of cells of strains SB1969 and SB210.

The RNA abundance data compiled in this study was obtained from the literature. Hence, cell culturing conditions, growth densities, starvation conditions and incubation lengths, as well as conjugation time points for the nuclei isolations were matched as closely as possible with the conditions reported for the message abundance data.

Nuclei Isolation

Macronuclei from *Tetrahymena thermophila* were isolated as described (7), with the following modifications. All operations were carried out on ice. One liter of cells, at a density of about 250,000 cells/ml, was harvested at 250× g in a Sorvall HL-8 rotor for 2 minutes and resuspended in 40 ml per packed cell volume in 1× nucleus isolation medium containing 0.3% or 1% octanol for starved and conjugating, or log cells respectively. Blending was as described. Nuclei were collected by centrifugation at 2250× g in the HL-8 rotor for 10 minutes. Supernatant and 'skin' were reblended once. Nuclei were washed once in nucleus isolation medium with octanol and once without octanol, resuspended in 50% glycerol/50% nucleus isolation medium at 4×10^7 nuclei/ml and stored at −20°C.

Run-on Transcription

Transcription reactions were carried out essentially as described (1) with several modifications. Frozen nuclei were pelleted by centrifugation, washed once in Transcription Buffer I (50 mM Tris, pH 8, 50 mM KCl, 5 mM $MgCl_2$, 1 mM spermidine, 1 mM spermine, 1 mM $CaCl_2$, 2 mM β-mercaptoethanol, 0.1 M sucrose, 25% glycerol) and resuspended at 2×10^8 nuclei/ml in

Transcription Buffer I. Transcriptions were performed in a total volume of 100 μl containing 25 μl Transcription Buffer II (0.1 M Tris, pH 8, 0.1 M KCl, 10 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM spermidine, 2 mM spermine, 6 mM b-mercaptoethanol, 2 mM $CaCl_2$), 10 μg/ml creatine phosphokinase, 25 mM creatine phosphate, 0.5 mM each of ATP, CTP, and GTP, 100 μCi 32P-UTP (800 Ci/mmol, Amersham), 40 units of RNasin and 50 μl of nuclear suspension. Incubation was for 30 minutes at 28°C.

The reaction was stopped by adding 10 units of DNase and incubating 10 minutes at 25°C. An equal volume of 0.1 M Tris, pH 7.5, 0.5% SDS and proteinase K (final concentration 0.1 mg/ml) was added and incubated at 37°C for 20 minutes. Samples were extracted once with phenol-chloroform and once with chloroform. Incorporated radioactivity was collected by passing the aqueous phase over pre-packed Sephadex G-50 RNA columns (Boehringer Mannheim Biochemicals).

Slot Blot Hybridization

Linearized plasmid DNA was immobilized on nitrocellulose, following denaturation in 0.1 N NaOH, neutralization with 1 volume 1M Tris, pH 7.5 and the addition of 1 volume 20× SSPE. Bluescript vector (Stratagene) served as background control. Prehybridization was performed at 45°C for at least 4 hours in 50% formamide, 5× Denhardt's solution (1× Denhardt's solution = 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinyl pyrrolidone), 3× SSC, 20 mM Phosphate buffer, pH 7, 1% SDS, and 250 μg/ml degraded herring sperm DNA. Fresh hybridization solution containing 1×10^7 cpm/ml was added and incubated at 45°C for 72 hours. Filters were washed 4× at room temperature for 15 minutes and 2 times at 75°C for 30 minutes in 2× SSC, 0.5% SDS. Autoradiography was performed with an intensifying screen at −70°C using pre-flashed film. Autoradiographs were quantitated densitometrically and by liquid scintillation counting of excized slots. Since equal counts were used in the hybridizations, to express differences in transcription on a per cell basis, any differences in the incorporation per nucleus were used to correct the differences quantitated directly from the blots.

RESULTS

Run-on transcription with isolated macronuclei

The run-on transcription assay was optimized for the following components: the concentrations of $CaCl_2$, KCl, $MgCl_2$, $MnCl_2$ and RNasin; the presence or absence of polyamines (spermidine, spermine and putrescine) or creatine phosphate and creatine phospho kinase; incubation temperature of the run-on reaction; and culture density when harvested. The rate of run-on transcription using the optimized conditions described is approximately 0.03 pmol UMP/μg DNA per minute for log cell nuclei and 0.01 pmol UMP/μg DNA per minute for starved or conjugating cell nuclei. A rate of 2 pmol UMP/μg DNA per minute can be achieved if 0.5 mM cold UTP is added. 90% of the TCA precipitable counts are sensitive to Actinomycin D (50 μg/ml).

Table 1. Pattern of expression of Class 1 genes.

CLONE	GENE	Ref.	GROWING mRNA	GROWING Transc.	STARVED mRNA	STARVED Transc.	CONJUGATING mRNA	CONJUGATING Transc.
BC11		9	-	-	+	+	+	+
pC1.2	cnjA	10, 11	-	-	-	-	+	+
pC2.1	cnjB	10, 11	-	-	-	-	+	+
pC5.5	ngoA	10, 11	-	-	+	+	+	+
pC7.1	cnjC	8, 11	-	-	-	-	+	+
pmBrC9a		12	-	-	-	-	+	+

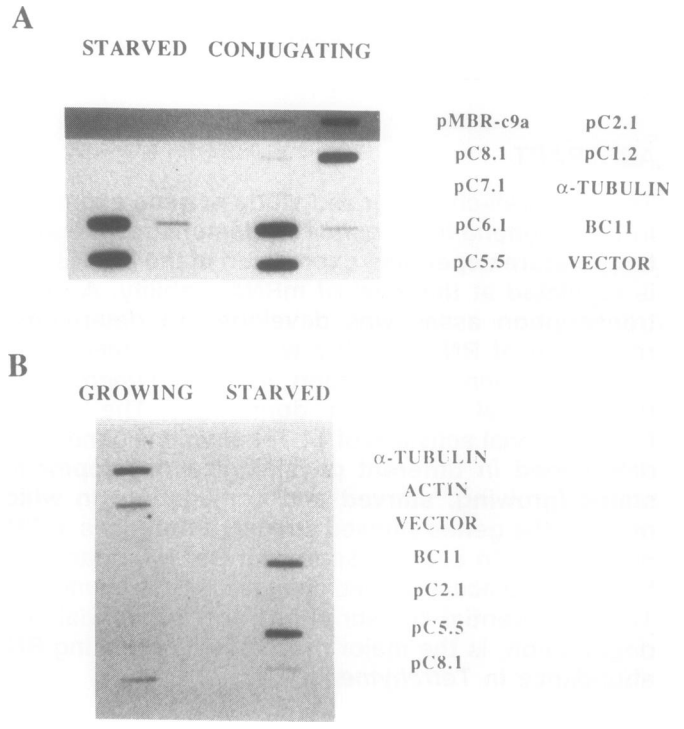

Figure 1. In A, hybridization of run-on transcripts isolated from starved or conjugating cell nuclei to 1 μg of the indicated plasmid DNA bound to filters. In B, hybridization of run-on transcripts isolated from growing or starved cell nuclei to 1 μg of plasmid DNA bound to filters.

Table 2. Pattern of expression of Class 2 genes.

CLONE	GENE	Ref.	GROWING / STARVED mRNA	GROWING / STARVED Transcription
pTub5	α−Tubulin	a	16	12.3
p539-2	Actin	b	10.8	13.9
pI210	Histone H1	13	10	4.7
pGEM/H4ITT	Histone H4-I	14	18	3.4
pGEM/H4IITS	Histone H4-II	14	29	6.4
pC6	SerH3	10, 11	10.4	1.2
pC8.1	cupC	10, 15	7.4	6.4
pRP-9	rDNA	16	3	2.4

a. K.E. McGrath, personal communication.
b. D.P. Heruth, personal communication.

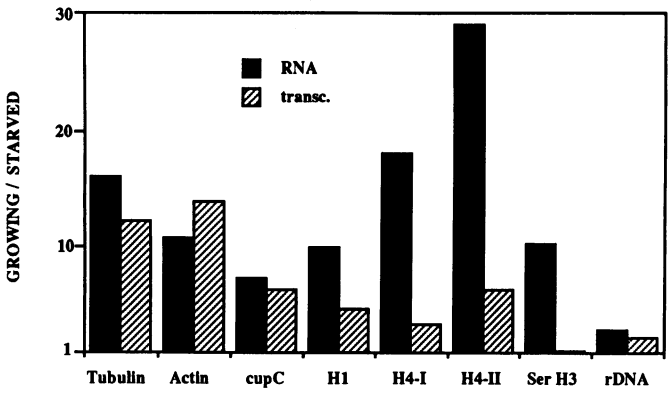

Figure 2. Ratios of RNA abundance and transcriptional activity between log and starved cells. Transcription (striped boxes) was measured by the run-on assay. Relative message abundance (filled boxes) between log and starved cells was obtained from the references listed in Table 1.

Most *Tetrahymena* genes are regulated at the level of transcription

The run-on assay was used to investigate the transcriptional activity of 14 genes, which can be divided into 2 categories by their pattern of message abundance (Table 1). The first class is comprised of those genes which code for messages which are not detectable during some physiological cell state. This group includes the clones coding for genes possessing messages that are either starvation-induced (BC11, pC5−5) or conjugation-specific (pC1, pC2, pC7, pMBrC9a). Nuclei were isolated from growing, starved and conjugating cells (4 hours post mixing), and RNA labelled in vitro by extension of transcripts initiated in vivo was hybridized to plasmid DNA bound to filters. Figure 1A shows an analysis of the products of the run-on reaction. In every case, the absence of message in a particular physiological state coincides with the absence of detectable levels of transcription in nuclei isolated from cells in that state (Table 2).

The second category of genes is comprised of those genes whose RNAs, though always present, change markedly in abundance between growing and starved cells. The run-on assay was used to determine the transcriptional activity of the 8 genes which fall into this class: the single a-tubulin gene; the actin gene; the histone H1 gene; the 2 histone H4 genes; Ser H3; cupC (an isoleucyl-tRNA synthetase); and the ribosomal RNA gene. Nuclei were isolated from growing and starved cells, and run-on transcripts were hybridized to plasmid DNA bound to filters (figure 1B). In all cases but 1 changes in message abundance correlate with changes in transcriptional activity (figure 2). The increase in transcriptional activity for tubulin, actin, cupC and rDNA corresponds closely to the increase observed in the RNA accumulation. For the 3 histone genes, though transcription rates do increase as the message increases, the increases are not sufficient to account for the total increases in message abundance. The single exception to the observation that an increase in message is accompanied by an increase in transcription rate, was for Ser H3. For this gene the message increased greater than 10 fold between growing and starved cells while the transcription rate did not change significantly.

DISCUSSION

An optimized run-on transcription assay was used to determine the transcriptional activity of 14 *Tetrahymena* genes as a function of cell state. Two major classes of genes were analyzed. The first consists of genes whose products are not always detectable in different physiological cell states. Except for cnjC (pC7.1) which appears to code for a subunit of RNA polymerase II (8), the function of these genes is not yet known. For all 6 of these inducible genes, transcriptional regulation appears to play the primary role in the regulation of gene expression.

The second class of genes examined possess RNAs that are always detectable, yet change in abundance. The genes examined encode structural proteins (tubulin and actin), nuclear proteins (histone H1 and H4II), an isoleucyl-tRNA synthetase gene (cupC), a surface protein (Ser H3) and the rRNA gene transcribed by RNA polymerase I. In all cases but 1 (Ser H3), there was an increase in transcriptional activity between log and starved cells which corresponded with the increase in message abundance. For tubulin, actin and pC8, transcriptional control appears to play the primary role in the regulation of gene expression, since increases in mRNA accumulation and transcription rates are similar. The data for the histone genes indicates that, though regulated partly by transcription, these genes may also be under post-transcriptional mechanisms of control. The apparent increase in histone message stability in growing cells may be related to the replication of DNA which is only occurring in these cells.

Ser H3 is the only gene whose transcription rate stayed constant when its message level changed. As described earlier (1), this gene is known to be regulated at the level of mRNA stability, which changes with temperature. Hence, this gene may be similarly regulated between log and starved cells by a change in message stability.

In summary these studies demonstrate that, in *Tetrahymena* as in other eukaryotes, regulation of transcription is the major level at which RNA abundance is regulated.

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