MOLECULAR AND CELLULAR BIOLOGY, Aug. 1983, p. 1537–1539
0270-7306/83/081537-03$02.00/0

Vol. 3, No. 8

Analysis of Transcription During the Cell Cycle in Toluenized *Chlamydomonas reinhardi* Cells

THOMAS DALLMAN, MANUEL ARES, JR.,† AND STEPHEN H. HOWELL*
Biology Department, C-016, University of California, San Diego, La Jolla, California 92093

Received 14 February 1983/Accepted 25 May 1983

A toluene-permeabilized cell system was established to examine the transcription of certain RNAs regulated during the cell cycle in *Chlamydomonas reinhardi*. The incorporation of [α-^{32}P]UTP into RNA which hybridizes to specific cloned cDNA, such as β-tubulin, indicates that the cell cycle pattern of RNA accumulation may be controlled, in part, by differential transcription.

The cell cycle in light-dark-synchronized cultures of *Chlamydomonas reinhardi* is marked by the periodic synthesis of certain cell cycle-regulated proteins (4) and the stage-specific appearance of their corresponding mRNAs. Of note is the burst of α- and β-tubulin synthesis (5, 9) and the coordinate appearance of the tubulin mRNAs (1, 2) at about the time of division. Also, the 32-kd precursor of the light-harvesting chlorophyll *a*/*b*-binding protein (LHCP), a major chloroplast membrane component, is synthesized, and its mRNA is accumulated in a wave-like manner during the mid-light phase of the cycle (4, 6), at a time when cells are actively producing components of the photosynthetic apparatus.

These observations have led us to ask whether the pattern of mRNA accumulation for cell cycle-regulated proteins is due to differential transcription during the cell cycle or some other process such as differential turnover. To address this issue, we developed a permeabilized cell transcription system to permit us to assess the amount of ongoing transcription at particular cell cycle stages. We chose to permeabilize cells rather than isolate nuclei because nuclei are not fully intact during division.

C. reinhardi (strain 137C mt+) were grown synchronously in a 12-h light to 12-h dark illumination cycle. At the appropriate stage, 250 ml of cells was harvested and washed with toluene buffer (10 mM Tris-hydrochloride (pH 8), 1 mM dithiothreitol, 0.5 mM EDTA, 10 mM KCl). Cells were suspended at 5×10^7 cells per ml and gently shaken for 15 min at room temperature with toluene buffer containing 1% toluene. The cell suspension was divided into appropriate samples (usually containing 2×10^7 cells), and the toluenized cells were collected by centrifugation. Toluenized cells incorporated [α-^{32}P]ATP or UTP into RNA linearly for about 30 min (data not shown). To enhance nucleotide incorporation through the activity of RNA polymerase II, cells were incubated with increasing concentrations of NH_4^+ from 0 to 400 mM (Fig. 1A). At 150 mM NH_4^+, total nucleotide incorporation was reduced to 60% of that lacking NH_4^+, but 45% of the remaining incorporation was α-amanatin sensitive (Fig. 1B). Nucleotide incorporation at 150 mM NH_4^+ was further optimized for Mg^{2+} (Fig. 1C) and K^+ (Fig. 1D) concentrations, which in the standard reaction were 20 and 50 mM, respectively.

The efficiency of UTP incorporation at different stages during the cell cycle was examined in light-dark-synchronized cultures. Incorporation expressed on a per cell basis declined progressively from L2, the beginning of the light period, to D2, the time of nuclear and cell division, and then rose sharply (Fig. 2). The incorporation pattern appears to reflect a reduced transcriptional capacity during division.

The transcription of specific genes was examined during the cell cycle by hybridizing RNA labeled in cells toluenized at different cell cycle stages to various cloned cDNAs. Plasmid pMAD251 is a *Chlamydomonas* cDNA apparently derived from a multigene family and hybridizes to two major abundant RNA species of 950 and 820 bases (M. Ares, Ph.D. thesis, University of California, San Diego, 1982). These RNAs are not cell cycle regulated but are present in relatively unchanging amounts during the cell cycle when expressed relative to total RNA (Ares, Ph.D. thesis). The pattern of hybridization of [^{32}P]UTP-labeled RNA to pMAD251 DNA (Fig. 3) reflects, by and large, the overall efficiency of RNA synthesis (Fig. 2). RNA hybridizable to pMAD251 is synthesized extensively in late-dark-phase to early-light-phase cells (G_1 cells) and declines as cells move into

† Present address: Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510.

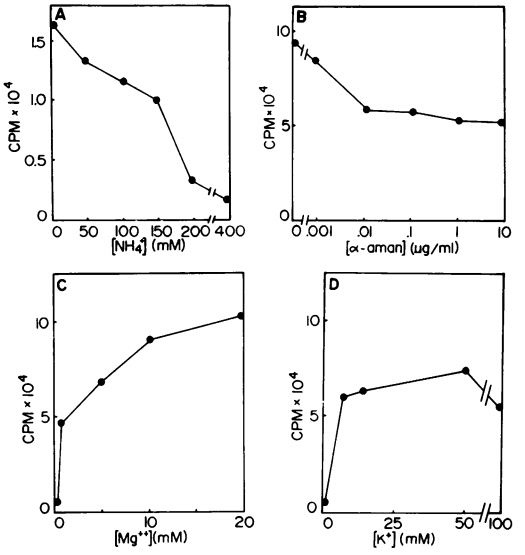

FIG. 1. Optimization of conditions for ribonucleotide incorporation. Toluene-treated cells (2×10^7) were suspended in a standard 1× reaction mix (200 μl) containing, except as indicated, 10 mM HEPES (pH 7.9); 0.1 mM EDTA; 10% glycerol; 1 mM dithiothreitol; 150 mM $(NH_4)_2SO_4$; 50 mM KCl; 20 mM $MgCl_2$; 0.2 mM CTP, GTP, and UTP; 1 (A) or 4 (B through D) μCi of 5′ [α-^{32}P]ATP (400 Ci/mmol). Cells were incubated for 30 min at 30°C. Incorporation was measured by precipitating with 5% trichloroacetic acid, collecting the precipitate on glass fiber filters, and counting in a scintillation counter.

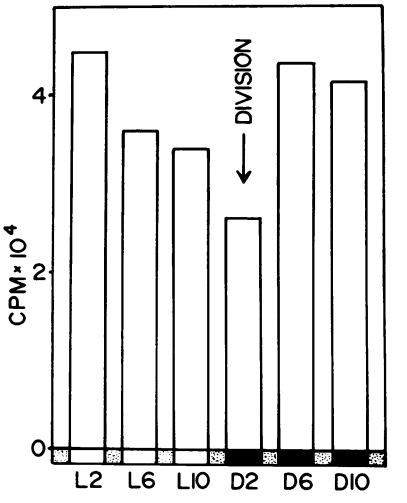

FIG. 2. Incorporation of 5′ [α-^{32}P]UTP during the cell cycle in toluenized cells. Cells were synchronized with a 12-h-light (L) and 12-h-dark (D) illumination cycle. Reaction conditions were as indicated in the legend to Fig. 1, except that 2 μCi of 5′ [α-^{32}P]UTP was used. Time of cell division is indicated.

division. The difference in the synthesis pattern of pMAD251-specific RNA and its unchanging presence during the cell cycle is easily reconciled if it is assumed that pMAD251-specific RNAs are stable during the cell cycle.

The accumulation of RNA specific for another *Chlamydomonas* cDNA plasmid, pMAD25, is highly regulated during the cell cycle. The mRNA represented by this cDNA is an abundant 1.8-kilobase species and is apparently derived from a single gene (Ares, Ph.D. thesis). The accumulation of pMAD25 RNA rapidly reaches a peak late in the dark phase, D10 (or L22 in continuous light), and declines to its lowest ebb just before cell division (L10) (2). The synthesis pattern in toluenized cells (Fig. 3) and the RNA accumulation pattern match each other fairly well both in terms of timing and extent of variation (20-fold). Hence, the pattern of appearance of pMAD25 RNA may be transcriptionally controlled.

The same cannot be said for the synthesis of β-tubulin and LHCP mRNAs. Plasmid pT2 contains chicken β-tubulin cDNA (kindly provided by Don Cleveland; 3) and hybridizes to two *C. reinhardi* β-tubulin messages (7) of 2.3 and 2.35 kilobases which accumulate coordinately at the time of division, D2, in light-dark cells. The synthesis of β-tubulin or pT2-specific RNA in toluenized cells (Fig. 3) rises somewhat gradually near division, peaks at D6, and falls again. The extent of variation in the synthesis of β-tubulin RNA during the cell cycle as assayed in

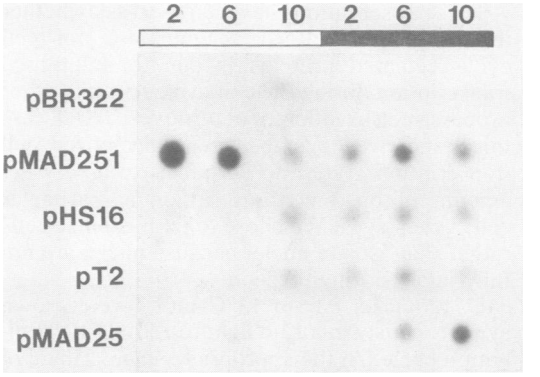

FIG. 3. Hybridization to cloned DNAs of ^{32}P-labeled RNA extracted from cells toluenized at different cell cycle stages. RNA was extracted by the method of Ares and Howell (1), from 10^8 cells which were labeled in a 5× reaction mix as in Fig. 1. RNA was hybridized in formamide-containing buffers according to Wahl et al. (8) to an excess of plasmid DNAs (4 μg per dot) loaded onto nitrocellulose filters by using a dot-blot manifold. Under these conditions, the autoradiographic signal represents the relative amount of ongoing transcription of a specific gene at that particular cell cycle state, expressed on a per cell basis.

toluenized cells is only twofold, whereas the changes in accumulation are ~8 to 10-fold (1). For LHCP mRNA, the pattern of synthesis in toluenized cells is much like that of β-tubulin but completely different from its own pattern of accumulation (6). Plasmid pHS16 hybridizes to a 1.2-kilobase LHCP mRNA (6) and to a single *C. reinhardi* nuclear gene. (P. Imbault, U. Johanningmeier, and S. H. Howell, manuscript in preparation). LHCP RNA accumulates in a wavelike pattern reaching a peak in the mid- to late light phase and showing a 10- to 20-fold variation in accumulation during the cell cycle.

To conclude, we have asked whether the pattern of accumulation of cell cycle-regulated RNAs can be explained by differential gene transcription during the cell cycle as assayed in toluenized cells. For pMAD25-specific RNA, the notion is supported—the synthesis and accumulation patterns are easily reconciled. For β-tubulin mRNAs, the timing, not the extent of in vitro synthesis, is close to what would be anticipated if the accumulation pattern was due solely to changes in transcription. For LHCP- or pHS16-specific RNA, neither the timing nor the extent of in vitro transcription is as expected. If the toluenized cell assay is an accurate reflection of ongoing transcription, then the cell cycle-regulated pattern of the appearance of the messages may not be entirely controlled at the level of transcription. Perhaps differential stability of these RNAs during the cell cycle more profoundly influences their pattern of appearance. Alternatively, the toluene treatment of cells may differentially disrupt the ongoing transcriptional machinery for various genes. In using this assay, one assumes that cells harvested at different cell cycle stages have been caught in the act of transcribing specific genes and that the synthesis observed in toluenized cells reflects the prior loading of genes with RNA polymerases.

This work was supported by the National Science Foundation.

LITERATURE CITED

1. **Ares, M., and S. H. Howell.** 1982. Cell cycle stage-specific accumulation of mRNAs encoding tubulin and other polypeptides in *Chlamydomonas*. Proc. Natl. Acad. Sci. U.S.A. **79**:5577–5581.
2. **Brunke, K. J., E. E. Young, B. U. Buchbinder, and D. P. Weeks.** 1982. Coordinate regulation of the four tubulin genes of *Chlamydomonas reinhardi*. Nucleic Acids Res. **10**:1295–1310.
3. **Cleveland, D. W., M. A. Lopata, R. J. MacDonald, N. S. Cowan, W. J. Rutter, and M. W. Kirschner.** 1980. Number and evolutionary conservation of α- and β-tubulin and cytoplasmic β- and γ-actin genes using specific cloned cDNA probes. Cell **20**:95–105.
4. **Howell, S. H., J. W. Posakony, and K. R. Hill.** 1977. Cell cycle program of polypeptide labeling in *Chlamydomonas reinhardtii*. J. Cell Biol. **72**:223–241.
5. **Piperno, G., and D. J. L. Luck.** 1977. Microtubule proteins of *Chlamydomonas reinhardtii*. J. Biol. Chem. **252**:383–391.
6. **Shepherd, H. S., G. Ledoigt, and S. H. Howell.** 1982. Regulation of light-harvesting chlorophyll-binding protein (LHCP) mRNA accumulation during the cell cycle in *Chlamydomonas reinhardi*. Cell **32**:99–107.
7. **Silflow, C. D., and J. L. Rosenbaum.** 1981. Multiple α- and β-tubulin genes in *Chlamydomonas* and regulation of tubulin mRNA levels after deflagellation. Cell **24**:81–88.
8. **Wahl, G., M. Stern, and G. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. U.S.A. **76**:3683–3687.
9. **Weeks, D. P., and P. S. Collis.** 1979. Induction and synthesis of tubulin during the cell cycle and life cycle of *Chlamydomonas reinhardi*. Dev. Biol. **69**:400–407.