$\delta^{13}C$ values for benthic and planktonic foraminifera between cores 22 and 34. Individuals of the species *Globigerapsis index* were used to obtain the planktonic foraminiferal values from these samples, whereas in other samples mixed planktonic species have been used (*5*). It has been found that there are definite species-dependent departures from isotopic equilibrium in planktonic foraminifera (*18*), so that surface water $\delta^{13}C$ values cannot be derived from planktonic foraminiferal measurements even if the depth habitat of the species is known (and it is not for extinct species). Polyspecific samples of calcareous nannofossils may provide a more reliable indication of surface $\delta^{13}C$ changes than planktonic foraminifera (*19*). Measurement of changes in surface water carbon isotopic composition with time may yield useful information on changes in oceanic productivity.

The reliability of nannofossils as indicators of surface water paleotemperatures can be tested by growing pure cultures of coccolithophorids at varying temperatures (*20*). Preliminary oxygen isotope data for samples of these species indicate that during coccolith growth, oxygen isotopes are incorporated in equilibrium with the growth medium, when compared with the empirically determined paleotemperature curve (*21*). Similar isotopic results have been obtained for cultures of *Emiliania huxleyi* (*22*).

Our evidence indicates that $\delta^{18}O$ values obtained from well-preserved polyspecific samples of calcareous nannofossils can be used to estimate surface water paleotemperatures. The $\delta^{13}C$ values appear to reflect the depth of growth for benthic and planktonic foraminifera and calcareous nannofossils when compared with $\delta^{13}C$ profiles for today's oceans. These preliminary results add a new and potentially important method to the paleoclimatologist's arsenal for studying changes in the world's oceans. Many deep-water carbonate cores spanning critical intervals in the Mesozoic and Cenozoic lack planktonic foraminifera but often contain calcareous nannofossils, and these now can be used for detailed isotope paleotemperature studies.

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References and Notes

1. E. Martini, in *Proceedings of the 2nd Plankton Conference, Rome 1970*, A. Farinacci, Ed. (Tecnoscienza, Rome, 1971), vol. 2, p. 739; A. McIntyre and R. McIntyre, in *The Micropaleontology of Oceans*, B. M. Funnell and W. R. Riedel, Eds. (Cambridge Univ. Press, Cambridge, England, 1971), p. 253; A. McIntyre, A. W. H. Bé, M. B. Roche, *Trans. N.Y. Acad. Sci* **32**, 720 (1970).
2. W. H. Berger, *Deep-Sea Res.* **20**, 917 (1973).
3. M. Black, *Endeavour (Engl. Ed.)* **24**, 131 (1965).
4. The δ values are per mil deviations from the Pee Dee belemnite (PDB) isotopic standard: $\delta = [(R/R_{std}) - 1] \times 1000$, where R is either $^{13}C/^{12}C$ or $^{18}O/^{16}O$ in the sample and R_{std} is the corresponding ratio in the standard [H. Craig, *Geochim. Cosmochim. Acta* **12**, 133 (1957)].
5. N. J. Shackleton and J. P. Kennett, in *Initial Reports of the Deep Sea Drilling Project* (Government Printing Office, Washington, D.C., 1975), vol. 29, pp. 743–755.
6. A. R. Edwards and K. Perch-Nielsen, in *ibid.*, pp. 469–539.
7. J. P. Kennett *et al.*, in *ibid.*, pp. 1155–1169.
8. ———, *Science* **186**, 144 (1974).
9. S. V. Margolis and J. P. Kennett, *ibid.* **170**, 1085 (1970).
10. A. McIntyre, A. W. H. Bé, R. Preikstas, *Prog. Oceanogr.* **4**, 3 (1967). Samples containing more than 10 percent nonnannofossil calcium carbonate after centrifugation were not isotopically analyzed, and the next closest samples where coccolith separations of high purity could be obtained were used. The most common calcareous biogenic contaminants were fragments of planktonic foraminifera. Nannofossil isotope analyses were not performed on samples from below core 33 from site 277 because of diagenetic changes.
11. N. J. Shackleton and N. D. Opdyke, *Quant. Res. (N.Y.)* **3**, 39 (1973). Reproducible isotopic results were achieved with each 25-mg sample by roasting in vacuo for 1 hour between 450° and 500°C to remove volatile organic components and char protecting organic films. The carbonate was then converted to CO_2 gas by reaction with 100 percent H_3PO_4 at 25°C. The gas was then analyzed with a Nuclide 3-60 RMS mass spectrometer, and the results are expressed as the per mil deviation from the PDB isotopic standard (*4*). The gas from several samples was analyzed twice by mass spectrometry, and for some samples two separate acid extractions were performed. Based on these experiments, the analytical reproducibility is at least $\pm$ 0.10 per mil.
12. R. G. Douglas and S. M. Savin, *Geol. Soc. Am. Publ. 6* (1974), p. 714.
13. T. Saito and J. Van Donk, *Micropaleontology* **20**, 152 (1974).
14. P. Kroopnick, *Deep-Sea Res.* **21**, 211 (1974).
15. H. Craig, *J. Geophys. Res.* **75**, 691 (1970).
16. P. H. Roth and W. H. Berger, *Cushman Found. Foraminiferal Res. Spec. Publ. 13* (1975), p. 87.
17. P. Kroopnick, *Earth Planet. Sci. Lett.* **22**, 397 (1974).
18. N. J. Shackleton *et al.*, *Nature (Lond.)* **242**, 177 (1973); R. G. Douglas and S. M. Savin, *J. Foraminiferal Res.*, in press; S. M. Savin and R. G. Douglas, *Geol. Soc. Am. Bull.* **84**, 2327 (1973).
19. N. J. Shackleton, personal communication.
20. Pure cultures of *Emiliania huxleyi* and *Cricospaera elongata* obtained from J. B. Jordan (Scripps Institution of Oceanography) and *Cricosphaera carterae* obtained from R. L. Guillard (Woods Hole Oceanographic Institution) have been successfully cultured at constant temperatures ($\pm$ 0.5°C) ranging from 10° to 27°C using modified and buffered natural seawater, and light conditions recommended for optimum growth and plate production [R. L. Guillard and J. H. Ryther, *Can. J. Microbiol.* **8**, 299 (1962); K. M. Wilbur and N. Watanabe, *Ann. N.Y. Acad. Sci.* **109**, 82 (1963); W. F. Blankley, thesis, University of California, San Diego (1971)]. *Cricosphaera carterae* cultures have been harvested near the end of the logarithmic growth phase. After addition of distilled water and centrifugation to burst the cells, the concentrated carbonate plates were roasted at 500°C to remove residual organic matter before CO_2 extraction (*11*).
21. S. Epstein, R. Buchsbaum, H. A. Lowenstam, H. C. Urey, *Geol. Soc. Am. Bull.* **64**, 1315 (1953); H. Craig, in *Stable Isotopes in Oceanographic Studies and Paleotemperatures*, E. Tongiorgi, Ed. (Laboratori di Geologia Nucleare, Consiglio Nazionale della Ricerche, Pisa, 1965), p. 1.
22. A. McIntryre, personal communication.
23. The Deep Sea Drilling Project, a joint project of five oceanographic institutions in the United States, funded by the National Science Foundation, is responsible for collection of the Leg 29 cores analyzed here. We thank N. J. Shackleton, J. P. Kennett, W. H. Berger, and P. H. Roth for helpful suggestions. Supported by NSF grants GD-34270, GD-37514, and IDO71-04202.

18 April 1975

Hybridization Analysis of Histone Messenger RNA: Association with Polyribosomes During the Cell Cycle

Abstract. *Hybridization of cell cycle stage-specific polyribosomal RNA's to histone complementary DNA indicates that histone messenger RNA sequences are present on polyribosomes of Hela S_3 cells only during the period of DNA replication.*

A functional relation between histone synthesis and DNA replication is suggested by the fact that the synthesis of these proteins and their deposition on the DNA is restricted to the S phase of the cell cycle (*1, 2*). Further support for the coupling of histone and DNA synthesis comes from the observation that inhibition of DNA replication results in a rapid shutdown of histone synthesis (*2, 3*). It has previously been shown, with the use of cell-free protein synthesizing systems derived from reticulocytes and Ehrlich ascites cells, that the RNA isolated from polyribosomes of S phase HeLa cells supports the synthesis of histones (*4*). These findings indicate that translatable histone messenger RNA's (mRNA) are associated with polyribosomes exclusively during the S phase of the cell cycle. However, the possibility still exists that histone mRNA's are components of the polyribosomes during other periods of the cell cycle, but have in some way been rendered nontranslatable. Such a possibility would have important implications for the mechanism operative in the regulation of histone gene expression. Therefore, in order to establish that the mRNA's for histones are associated with polyribosomes only during S phase, we examined G_1, S, and G_2 polyribosomal RNA's for their ability to hybridize with histone complementary DNA (cDNA).

[^{3}H]DNA complementary to the five classes of histone mRNA's was synthesized as follows. The 7*S* to 12*S* RNA was isolated from the polyribosomes of S phase HeLa S_3 cells and material containing polyadenylic acid [poly(A)] was removed

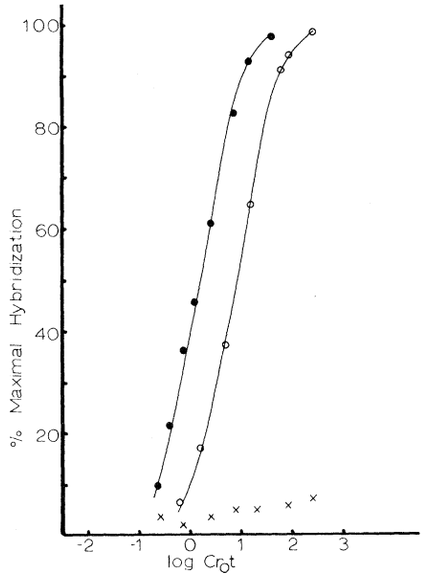

Fig. 1. [^{3}H]cDNA (27,000 dpm/ng) and unlabeled RNA were hybridized at 52°C in sealed glass capillary tubes containing in a volume of 15 μl: 50 percent formamide, 0.5M NaCl, 25 mM Hepes buffer (pH 7.0), 1 mM EDTA, 0.37 ng of cDNA, and 3.75 or 7.5 μg of polysomal RNA from HeLa S_3 cells at the following growth stages; G_1, ×; S, •; or G_2, o. Samples were removed at intervals and incubated for 20 minutes in 2.0 ml of 30 mM sodium acetate, 0.3M NaCl, 1 mM $ZnSO_4$, and 5 percent glycerol (pH 4.6) containing S_1 nuclease (*9*) at a concentration sufficient to degrade at least 96 percent of the single-stranded nucleic acids present. The amount of radioactive DNA resistant to digestion was determined by trichloroacetic acid precipitation. $C_{r_0}t$ = mole of ribonucleotides times seconds per liter. Cells were synchronized as described (*7*). S and G_2 phase cells were obtained by synchronization with two cycles of 2 mM thymidine block. S phase cells were harvested for 3 hours after release from the second thymidine block at which time 98 percent of the cells were in S phase. G_2 cells were harvested 7.5 hours after release from thymidine. G_1 cells were obtained 3 hours after selective detachment of mitotic cells from semiconfluent monolayers; 97 percent of the cells were in the G_1 phase of the cell cycle, and S phase cells were not detected. Polyribosomal RNA was isolated as reported (*5*).

by oligodeoxythymidylate (oligo dT) cellulose chromatography. Poly(A) was added to the 3′OH termini of the histone mRNA's with an ATP-polynucleotidylexotransferase (ATP, adenosine triphosphate) isolated from maize seedlings. The polyadenylated mRNA's were then transcribed in the presence of ^{3}H-labeled deoxycytidine triphosphate (dCTP) and ^{3}H-labeled deoxyguanosine triphosphate (dGTP) with RNA-dependent DNA polymerase from Rous sarcoma virus with dT_{10} as a primer. The mean sedimentation coefficient of the cDNA in alkaline sucrose was 6.1S, which corresponds to a molecule comprising 400 nucleotides. A comparison of the kinetics of the histone cDNA–histone mRNA hybridization reaction with that of the globin cDNA–globin mRNA hybridization reaction indicates that the sequence complexity of the histone cDNA probe is sufficient to account for all five histone mRNA's. The synthesis and properties of this [^{3}H]cDNA have been reported (*5*, *6*).

The extent of hybrid formation between histone cDNA and total polysomal RNA of G_1, S, and G_2 HeLa cells is compared in Fig. 1. The hybridization observed between S phase polyribosomal RNA and the cDNA indicates the presence of histone-specific sequences associated with the polyribosomes of S phase cells. In contrast, the absence of G_1 polyribosomal RNA hybridization demonstrates that histone mRNA sequences are not components of G_1 polyribosomes. A comparison of the kinetics of the hybridization reaction between S phase polyribosomal RNA and cDNA ($C_{r_0}t_{1/2}$ = 1.8) with the hybridization kinetics of the histone mRNA–cDNA reaction ($C_{r_0}t_{1/2} = 1.7 \times 10^{-2}$) indicates that histone mRNA sequences account for 0.9 percent of the S phase total polysomal RNA. This figure is consistent with the situation in vivo where approximately 10 percent of the protein synthesis during S phase is histone synthesis (*7*).

Determination of the presence or absence of histone mRNA sequences on G_2 polysomes is complex. The kinetics of the hybridization reaction between G_2 polyribosomal RNA and histone cDNA ($C_{r_0}t_{1/2}$ = 8.5) suggests that the amount of histone mRNA sequences present on the polyribosomes of G_2 cells is 21 percent of that present on S phase polyribosomes. However, the data in Fig. 2 clearly indicate that 20 percent of the G_2 cell population consists of cells which are undergoing DNA replication (S phase cells). It is therefore reasonable to conclude that the histone mRNA sequences present in the G_2 polyribosomal RNA are due to the presence of S phase cells in the G_2 cell population. This implies that histone mRNA sequences are not associated with polyribosomes during the G_2 phase of the cell cycle. Unfortunately to date, no effective methodology is available for obtaining a pure population of G_2 phase HeLa S_3 cells to definitively establish this point.

Our results demonstrate that histone mRNA sequences are associated with polyribosomes of HeLa S_3 cells only during the S phase of the cell cycle. Taken together with the recent observations that histone mRNA sequences are transcribed from chromatin of S phase cells and not from chromatin of G_1 phase cells (*6*), these findings support the hypothesis that regulation of histone gene expression resides at

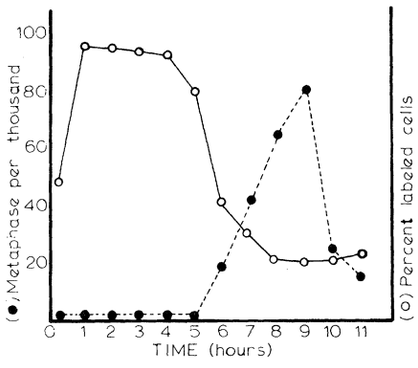

Fig. 2. Percentage of cells in DNA synthesis and the mitotic index at various times after release of HeLa S_3 cells from two cycles of 2 mM thymidine block. Cells were labeled with 5 μc of [^{3}H]thymidine per milliliter for 15 minutes, and the percentage of cells in DNA synthesis was determined autoradiographically (*10*). The mitotic index was determined from the autoradiographic preparations.

the transcriptional level in exponentially growing HeLa cells. Undoubtedly transcriptional control of histone gene expression is operative in many biological systems. However, there is evidence that posttranscriptional regulation of histone synthesis may also occur under certain conditions, for example in echinoderm embryos during the early stages of development (*8*).

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References and Notes

1. J. Spalding, K. Kajiwara, G. Mueller, *Proc. Natl. Acad. Sci. U.S.A.* **56**, 1535 (1966).
2. E. Robbins and T. W. Borun, *ibid.* **57**, 409 (1967).
3. T. W. Borun, M. D. Scharff, E. Robbins, *ibid.* **58**, 1977 (1967); D. Gallwitz and G. C. Mueller, *J. Biol. Chem.* **244**, 5947 (1969).
4. D. Gallwitz and M. Breindl, *Biochem. Biophys. Res. Commun.* **47**, 1106 (1972); M. Jacobs-Lorena, C. Baglioni, T. W. Borun, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2095 (19
5. T. W. Borun, F. Gabrielli, K. Ajiro, A. Zweidler, C. Baglioni, *Cell* **4**, 59 (1975).
6. C. L. Thrall, W. D. Park, W. H. Rashba, J. L. Stein, R. J. Mans, G. S. Stein, *Biochem. Biophys. Res. Commun.* **61**, 1443 (1974).
7. G. S. Stein, W. D. Park, C. L. Thrall, R. J. Mans, J. L. Stein, *ibid.* **63**, 945 (1975); G. S. Stein, J. L. Stein, C. L. Thrall, W. D. Park, in *Chromosomal Proteins and Their Role in the Regulation of Gene Expression*, G. S. Stein and L. K. Kleinsmith, Eds. (Academic Press, New York, 1975), pp. 1–17.
8. G. S. Stein and T. W. Borun, *J. Cell Biol.* **52**, 292 (1972).
9. M. Farquhar and B. McCarthy, *Biochem. Biophys. Res. Commun.* **53**, 515 (1973); A. Skoultchi and P. R. Gross, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2840 (1973).
10. V. Vogt, *Eur. J. Biochem.* **33**, 192 (1973).
11. R. Baserga and D. Malamud, *Autoradiography* (Hoeber, New York, 1969), p. 17.
12. Supported by NSF grant GB38349, NIH grant GM20535, and AEC grant ORO-3982-38.

28 March 1975