18 hours at 37°C. The resulting mixture had α_1-antitrypsin concentration and function of approximately half the original levels in the Pi MM sample; the Pi type of the mixture was MM.

The other major protein fractions in the patient's serum were normal, as determined by agarose electrophoresis; immunoglobulins G, A, and M were found to be normal on immunoelectrophoresis.

While the Pi ZZ variant of α_1-antitrypsin deficiency is relatively common, with an incidence of approximately 1 out of 1400 individuals in one large study (*16*), the Pi$^-$ variant described here has not been previously encountered; other investigators have proposed its existence, on the basis of finding Pi MM individuals with unusually low levels of α_1-antitrypsin.

RICHARD C. TALAMO
CAROL E. LANGLEY
Children's Service, Massachusetts General Hospital, Boston 02114
CHARLES E. REED
Department of Medicine, University of Wisconsin School of Medicine, Madison 53706
SOHEI MAKINO
School of Medicine, University of Tokyo, Tokyo, Japan

References and Notes

1. M. K. Fagerhol and C.-B. Laurell, *Progr. Med. Genet.* **7**, 96 (1970).
2. S. Eriksson, *Acta Med. Scand.* **177** (Suppl. 432), 1 (1965).
3. R. C. Talamo, J. D. Allen, M. G. Kahan, K. F. Austen, *N. Engl. J. Med.* **278**, 345 (1968).
4. H. L. Sharp, R. A. Bridges, W. Krivit, E. F. Freier, *J. Lab. Clin. Med.* **73**, 934 (1969).
5. J. F. T. Glasgow, A. Hercz, H. Levison, M. J. Lynch, A. Sass-Kortsak, *Amer. J. Med.* **54**, 181 (1973).
6. M. K. Fagerhol and H. E. Hauge, *Acta Allergol.* **24**, 107 (1969).
7. B. F. Erlanger, N. Kokowsky, W. Cohen, *Arch. Biochem. Biophys.* **95**, 271 (1961).
8. C.-B. Laurell, *Anal. Biochem.* **15**, 45 (1966).
9. M. K. Fagerhol, in *Proteolysis and Pulmonary Emphysema*, C. Mittman, Ed. (Academic Press, New York, 1972), pp. 145–149.
10. R. C. Talamo, C. E. Langley, B. W. Levine, H. Kazemi, *N. Engl. J. Med.* **287**, 1067 (1972).
11. J. J. Scheidegger, *Int. Arch. Allergy Appl. Immunol.* **7**, 103 (1955).
12. O. Ouchterlony, *Progr. Allergy* **5**, 1 (1958).
13. E. R. Giblett, *Genetic Markers in Human Blood* (Davis, Philadelphia, 1969), p. 320.
14. S. Makino and C. E. Reed, *J. Lab. Clin. Med.* **75**, 742 (1970).
15. F. Kueppers and R. J. Fallat, *Clin. Chim. Acta* **24**, 401 (1969).
16. M. K. Fagerhol, *Acta Pathol. Microbiol. Scand.* **70**, 421 (1967).
17. We acknowledge the assistance of M. T. Larson for performance of blood typing studies, M. K. Fagerhol for his studies on this family, and J. Ross for his expert technical assistance. Partially supported by PHS grants HE 14479, AI 00313, and AI 08106; contract 71-2217 from the National Heart and Lung Institute; and PHS career development award AI 31937 to R.C.T.

2 January 1973

Nonhistone Chromosomal Protein Synthesis: Utilization of Preexisting and Newly Transcribed Messenger RNA's

Abstract. *Treatment of HeLa S_3 cells with actinomycin D during mitosis suppresses the synthesis of several major classes of nonhistone chromosomal proteins during the subsequent period before DNA replication, but allows the synthesis of other species of these proteins. Such results are consistent with the utilization of preexisting, as well as newly transcribed, messenger RNA's for nonhistone chromosomal protein synthesis during the prereplicative phase of the cell cycle.*

Evidence is rapidly accumulating which suggests that nonhistone chromosomal proteins are responsible for the regulation of gene expression in eukaryotic cells (*1*) and, specifically, in the control of transcription during the cell cycle (*2*). A regulatory function for nonhistone chromosomal proteins in continuously dividing cells and in quiescent cells that are stimulated to proliferate is supported by significant variations in the rates of synthesis (*3–6*), turnover (*7*), and phosphorylation (*8*) of these proteins during defined periods of the cell cycle. The synthesis and phosphorylation of specific molecular-weight classes of nonhistone chromosal proteins has also been observed during defined periods of the cell cycle (*3–6, 8*). Furthermore, unlike the histones, whose synthesis is restricted to S phase (the period of DNA synthesis in the cell cycle) and is tightly coupled to DNA replication (*9*), synthesis of nonhistone chromosomal proteins is independent of concomitant DNA synthesis (*5, 6*). More direct evidence that nonhistone chromosomal proteins are involved in the regulation of DNA-dependent RNA synthesis during the cell cycle comes from studies showing that chromatin reconstituted with cell cycle stage–specific nonhistone chromosomal proteins exhibits a template activity characteristic of the native chromatin (*2*), and recent results suggest that nonhistone chromosomal proteins regulate transcription during the cell cycle by mediating the binding of histones to DNA (*10*). The aim of the present study was to determine whether the nonhistone chromosomal protein synthesis that occurs during the prereplicative phase of the cell cycle in continuously dividing HeLa S_3 cells requires the synthesis of new messenger RNA, or whether preexisting messenger RNA templates are utilized.

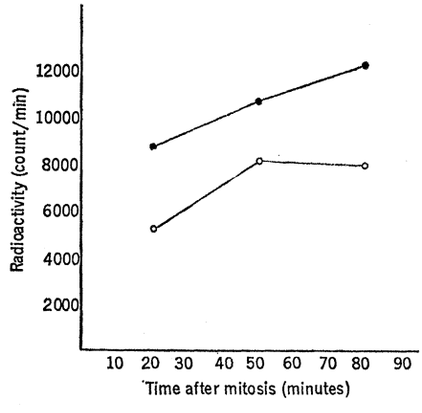


Fig. 1. Incorporation of L-[^{3}H]tryptophan into chromatin 20, 50, and 80 minutes after selective detachment of mitotic cells. The cells were pulse-labeled for 30 minutes, and chromatin was isolated as described in the text. The data are expressed as counts per minute per 50 μg of DNA. Control, solid circles; actinomycin D–treated, open circles.

Mitotic HeLa S_3 cells were detached selectively from semiconfluent monolayers, and actinomycin D (*11*) was added immediately to a final concentration of 2 μg/ml. Controls were not treated with the antimetabolite. Procedures for growth and synchronization of the cells have been described (*5*); in the present experiments, 97 percent of the detached cells were in mitosis, as determined by phase-contrast microscopy. At 20, 50, and 80 minutes following mitosis, as the cells progressed through early G_1 (the phase of the cell cycle preceding DNA replication), 5×10^7 control cells and cells treated with actinomycin D were pulse-labeled for 30 minutes at a concentration of 5×10^6 cells per milliliter in tryptophan-free Eagle's minimal essential spinner medium containing 2 percent fetal calf serum and L-[^{3}H]tryptophan (10 μc/ml) (2.18 c/mmole, New England Nuclear). The actinomycin D–treated cells were labeled in the presence of the inhibitor (2 μg/ml). Cells were harvested by centrifugation at 600*g* for 3 minutes and washed four times in 80 volumes of Earle's balanced salt solution at 4°C. Nuclei and chromatin were then prepared as described (*5*), the entire procedure at 4°C. The cells were lysed in a solution contain-

ing 80 m*M* NaCl, 20 m*M* ethylenediaminetetraacetic acid, and 1 percent Triton X-100, *p*H 7.2; and nuclei were pelleted by centrifugation at 1000*g* for 5 minutes. The nuclei were washed three times with the same solution and twice with a solution containing 0.15*M* NaCl and 0.01*M* tris(hydroxymethyl)-aminomethane, *p*H 8.0, and lysed in distilled water. Chromatin was then pelleted by centrifugation at 12,000*g* for 15 minutes, resuspended in distilled water, and pelleted by centrifugation at 12,000*g* for 15 minutes.

Figure 1 shows an increased rate of L-[^{3}H]tryptophan incorporation into HeLa S_3 chromosomal proteins during G_1, a result suggesting an accelerated rate of synthesis. Since histone synthesis is restricted to S phase and these basic chromosomal polypeptides lack tryptophan residues, the incorporation of L-[^{3}H]tryptophan into chromosomal proteins 20, 50, and 80 minutes after mitosis solely reflects the synthesis of nonhistone chromosomal proteins. Also, prior treatment with actinomycin D during mitosis at a concentration that suppresses DNA-dependent RNA synthesis to less than 0.5 percent of controls is ineffective in totally inhibiting the early G_1 increase in the synthesis of nonhistone chromosomal proteins (Fig. 1). Inasmuch as there is a disaggregation of polyribosomes and a cessation of RNA synthesis during mitosis, these results indicate that the G_1 nonhistone chromosomal proteins are synthesized, at least in part, on messenger RNA templates transcribed prior to mitosis and reactivated in G_1. Such results are consistent with a report by Hodge *et al.* (*12*) that rapidly labeled polyribosome-associated RNA made prior to mitosis persists into the subsequent G_1 period.

We then determined whether the decreased synthesis of nonhistone chromosomal proteins which occurs early during G_1, in cells first treated with actinomycin D during mitosis is restricted to defined classes of these polypeptides. Chromatin isolated from control and actinomycin D–treated cells labeled with L-[^{3}H]tryptophan as previously described was fractionated according to molecular weight by electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate (SDS) (*13*). Figure 2 indicates that whereas some degree of actinomycin D sensitivity is evident in a number of the nonhistone chromosomal proteins synthesized 20, 50, and 80 minutes following mitosis, those low-molecular-weight nonhistone chromosomal proteins that migrate between fractions 70 and 90 are completely inhibited by the antimetabolite. In Fig. 2, a to f, there are significant differences in specific classes of nonhistone chromosomal proteins synthesized and associated with chromatin at various times early during G_1, and such heterogeneity is particularly apparent in the low-molecular-weight polypeptides sensitive to actinomycin D.

These results suggest that in continuously dividing HeLa S_3 cells, several defined classes of early G_1 nonhistone chromosomal proteins are synthesized on stable messenger RNA templates transcribed before mitosis and reactivated during G_1, while other nonhistone chromosomal proteins appear to require the transcription of messenger RNA during the prereplicative phase of the cell cycle for their synthesis. Previous reports have indicated actinomycin D insensitivity of the initial increased synthesis of nonhistone chromosomal proteins early during G_1 in quiescent cells stimulated to proliferate (*3*), the synthesis of unique classes of G_1 nonhistone chromosomal proteins (*3–6*), the influence of G_1 nonhistone chromosomal proteins on the transcriptional capacity of the genome (*2*), and the possible presence of a nucleoplasmic nonhistone chromosomal protein pool (*6*). One can therefore speculate

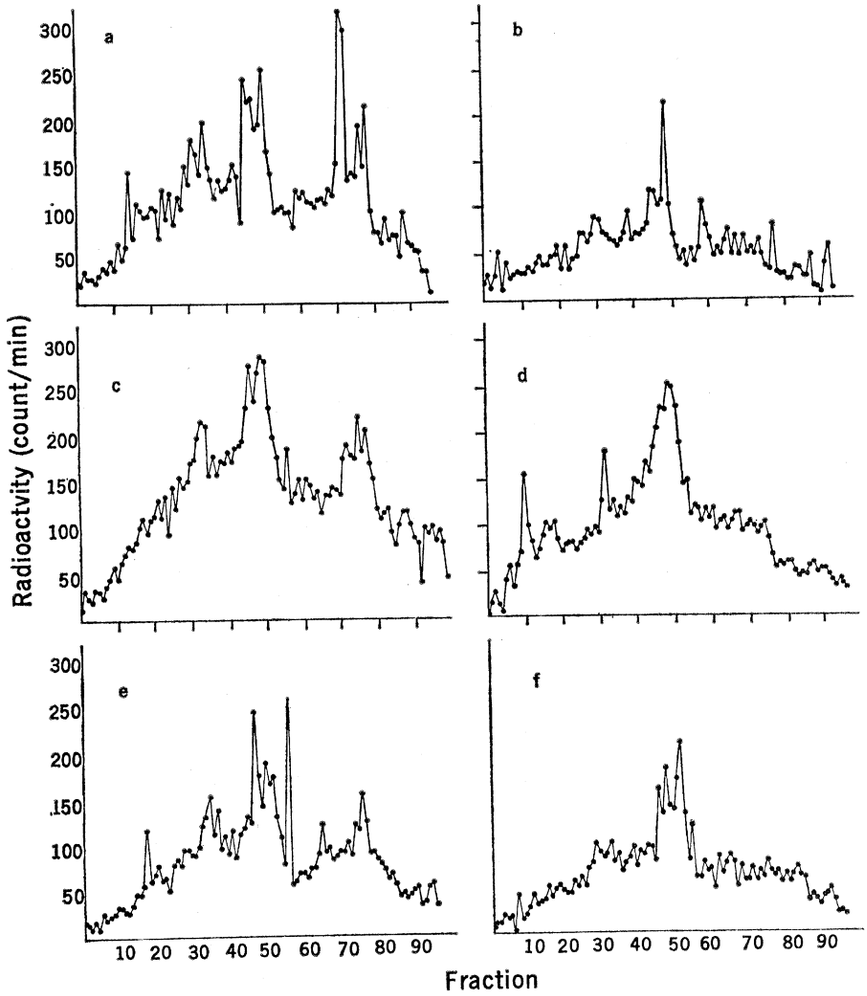


Fig. 2. Effect of actinomycin D on SDS-polyacrylamide gel electrophoretic profiles of L-[^{3}H]tryptophan-labeled chromosomal proteins. HeLa S_3 cells (5×10^7) were pulse-labeled for 30 minutes at various times after mitotic selective detachment, and chromatin was isolated as described in the text. Chromatin was solubilized in a solution containing 1 percent SDS and 0.01*M* sodium phosphate, *p*H 7.0, and dialyzed for 12 hours against the same solution. Electrophoresis of 100 μg of protein was carried out at room temperature on cylindrical 7.5 percent polyacrylamide gels measuring 0.6 by 15 cm. The details of the procedure have been reported (*5*). Treatments and intervals between detachment and start of pulse-labeling were as follows: (a) No drug, 20 minutes; (b) actinomycin D, 20 minutes; (c) no drug, 50 minutes; (d) actinomycin D, 50 minutes; (e) no drug, 80 minutes; and (f) actinomycin D, 80 minutes.

that although the nonhistone chromosomal proteins synthesized and associated with chromatin during G_1 may be responsible for regulating the expression of genetic information during this phase, the synthesis of these "regulatory" macromolecules may be controlled at the transcriptional as well as at the translational level.

GARY S. STEIN
DAVID E. MATTHEWS
Department of Biochemistry, University of Florida, Gainesville 32601

References and Notes

1. R. S. Gilmour and J. Paul, *FEBS Lett.* **9**, 242 (1970); *ibid.*, p. 435; T. C. Spelsberg and L. Hnilica, *Biochem. J.* **120**, 435 (1970); T. Y. Wang, *Exp. Cell Res.* **53**, 288 (1968); M. Shea and L. J. Kleinsmith, *Biochem. Biophys. Res. Commun.* **50**, 473 (1973); C. Teng, C. Teng, V. G. Allfrey, *J. Biol. Chem.* **246**, 3597 (1971); C. W. Dingman and M. B. Sporn, *J. Biol. Chem.* **239**, 3483 (1964).
2. G. S. Stein and R. Baserga, *Advan. Cancer Res.* **15**, 287 (1972); G. S. Stein and J. L. Farber, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 2918 (1972); G. S. Stein, S. C. Chaudhuri, R. B. Baserga, *J. Biol. Chem.* **247**, 3918 (1972).
3. G. S. Stein and R. B. Baserga, *J. Biol. Chem.* **245**, 6097 (1970).
4. ———, *Biochem. Biophys. Res. Commun.* **41**, 715 (1970); R. Levy, S. Levy, S. Rosenberg, R. T. Simpson, *Biochemistry* **12**, 224 (1973).
5. G. S. Stein and T. W. Borun, *J. Cell Biol.* **52**, 292 (1972).
6. G. S. Stein and C. L. Thrall, *FEBS Lett.*, in press.
7. T. W. Borun and G. S. Stein, *J. Cell Biol.* **52**, 308 (1972); G. Rovera and R. Baserga, *J. Cell. Physiol.* **77**, 201 (1971).
8. R. Platz, G. S. Stein, L. J. Kleinsmith, *Biochem. Biophys. Res. Commun.* **51**, 735 (1973); L. J. Kleinsmith, V. G. Allfrey, A. E. Mirsky, *Science* **154**, 780 (1966).
9. E. Robbins and T. W. Borun, *Proc. Nat. Acad. Sci. U.S.A.* **54**, 409 (1967); J. Spalding, K. Kajiwara, G. Mueller, *ibid.* **56**, 1535 (1966).
10. G. S. Stein, G. Hunter, L. Lavie, in preparation.
11. Actinomycin D was supplied by H. B. Woodruff of the Merck Institute of Therapeutic Research, Rahway, New Jersey.
12. L. D. Hodge, E. Robbins, M. D. Scharff, *J. Cell Biol.* **40**, 497 (1969).
13. J. Maizel, *Science* **151**, 988 (1966).
14. Supported by grant DRG-1138 from the Damon Runyon Memorial Fund for Cancer Research, by NSF grant GB38349, and by American Cancer Society grant S73 UF-6.

26 February 1973

Sand Dollar: A Weight Belt for the Juvenile

Abstract. *Juvenile sand dollars* (Dendraster excentricus) *selectively ingest heavy sand grains from the substrate and store them in an intestinal diverticulum which may function as a weight belt, assisting the young animal to remain in the shifting sandy environment. The sand disappears from the diverticulum when the animal reaches the length of 30 millimeters.*

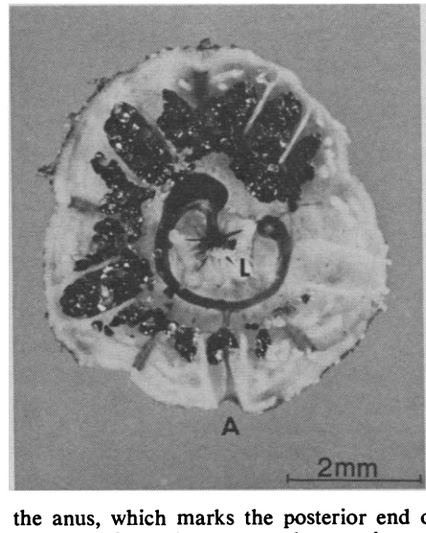
Gregory (*1*) described an intestinal diverticulum in a sand dollar, *Echinarachnius parma*, and noted that this organ was distended with sand in juveniles but not in adults. Similar situations have been observed in several other sand dollars (*2*). This suggests the question, What is the adaptive significance for the young animal of storing sand in the diverticulum? I report here that in the Pacific sand dollar, *Dendraster excentricus*, the juvenile stores specifically heavy sand grains in the diverticulum, thus increasing the body weight, enabling the animal to maintain its position in the sandy substrate.

Thirty-seven young specimens of *Dendraster* (5 to 32 mm long) and ten adult specimens (70 to 80 mm long) were collected from Puget Sound, at the south side of Alki Point, Seattle, Washington, in August 1972, and were fixed immediately in 75 percent ethanol. Each animal was then weighed (alcohol wet weight), and was dissected by removing the aboral half of the test. In this way the entire digestive tract and the diverticulum were exposed (Fig. 1).

All animals examined possessed the diverticulum but only the juveniles, when less than 30 mm long, contained sand. The diverticulum sand in all juveniles was extracted and the dry weight was measured. The amount of sand in each animal varied from 0.2 to 12 mg; it equaled 0.2 to 23 percent of the total body weight. Animals about 9 to 10 mm long contained more sand than both bigger and smaller ones. Small animals in general contained proportionally more sand (Fig. 2).

Grain types were separated and identified for sand from diverticula and from the substrate. The results are summarized in Table 1. There are eight grain types in the substrate; only two of these were found in the diverticula. The interesting point is that 78 percent of the diverticulum sand is iron oxide, which is heaviest of all grain types but constitutes only 9.8 percent of the substrate sand. This indicates that young sand dollars store heavy sand grains selectively. The density of the sand dollar itself is about 2 g/cm^3, which is less than half of the density of the ingested sand. Therefore the storage of heavy sand in the diverticulum will considerably increase the density of the animal. This may be necessary for the survival of the juveniles in the shifting substrate. In other words, the young sand dollar has, in fact, equipped itself with a weight belt which enables it to remain in the sand. Moreover, it should be noted that the diverticulum with its radial pouches is essentially located at the anterior half of the animal. This arrangement may be important for enabling the animal to maintain an oblique position with the anterior end downward, which is the natural position of the adult (*3*).

At present I can offer no explanation



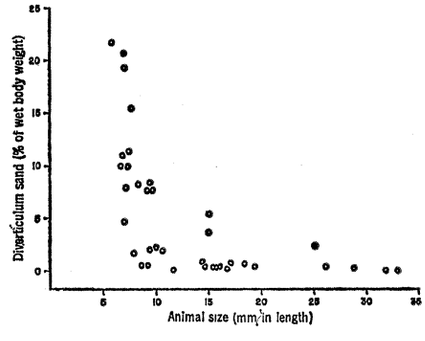


Fig. 1 (left). Aboral view of a young sand dollar after removal of the aboral half of the test, showing Aristotle's lantern (*L*), the digestive tract, and the intestinal diverticulum, which is filled with sand. The dark sand grains are iron oxide and the clear ones are feldspar and quartz. Area *A* is the anus, which marks the posterior end of the animal. The length of the animal was measured from the anus to the anterior margin of the test by crossing the mouth. The length is slightly greater than the width. Fig. 2 (right). Diverticulum sand expressed as percentage of wet body weight and also as a function of the size of the animal.