HISTONE GENE EXPRESSION IN INTERSPECIES HYBRID ECHINOID EMBRYOS[1]

D. P. Easton, J. P. Chamberlain,*[2] A. H. Whiteley, and H. R. Whiteley*

Department of Zoology, the Department of Microbiology,* and the Friday Harbor Laboratories
University of Washington, Seattle, Washington 98195

Received February 6,1974

The histones synthesized by three different interspecies hybrid echinoid embryos have been examined. In all three crosses, the species-specific F1 histone of the paternal parent is found in the chromatin of the hybrid. These findings provide direct evidence for the involvement of newly transcribed mRNA in the synthesis of this protein. F1 histone is a unique protein in that it is the only paternal protein detected in these hybrids. The possibility that the synthesis of histones is controlled differently from that of other proteins is discussed.

INTRODUCTION

Interspecies hybrid embryos of echinoids have been valuable for the investigation of the role played by the genome in the control of morphogenesis and biochemical differentiation (1). Studies of hybrids, together with studies of physically and chemically enucleated embryos (see Tyler for review (2)), have indicated that early aspects of development are mediated through information programmed into the cytoplasm of the egg before fertilization. These and other studies have shown hatching to be a time of significant new genetic input. Nonetheless, nuclei are active in the synthesis of RNA early in cleavage, as initially shown by Nemer (3) and Wilt (4). Much of this newly synthesized RNA was found to accumulate in a special class of slowly sedimenting polyribosomes (5,6) subsequently implicated in histone synthesis (7,8). Histone message has also been found in the RNA stored in the

[1]Supported by funds from a PHS training grant HD-00266 under which D.E. was a trainee, an NSF grant to A.H.W., and an American Cancer Society grant, VC-46A to H.R.W.: H.R.W. is a recipient of Research Career Award GM-K6-422.

[2]Present address: Department of Zoology, University of Michigan, Ann Arbor, Michigan 48104.

unfertilized eggs (9,10) in a discrete class of ribonucleoprotein particles (11). That histones are synthesized from both preformed egg RNA and from RNA newly transcribed during cleavage is indicated by reports that actinomycin D partially blocks histone synthesis during cleavage (Lindsay and Ruderman and Gross, personal communications).

The specific classes of histones encoded in the stored and newly transcribed messages have not been identified. We have examined the histones in the chromatin of interspecies hybrids produced in three different crosses. Species-specific F1 histones were found, demonstrating that the mRNA for this class of histone is newly synthesized. This protein appears during cleavage and is the only protein so far ascribable to the paternal genome of these hybrids.

METHODS AND MATERIALS

The embryos of the sea urchins Strongylocentrotus purpuratus (PP) and S. droebachiensis (DD), the sand dollar Dendraster excentricus (DeDe) and the hybrids (Dendraster ♀ x S. purpuratus ♂ (DeP) and Dendraster ♀ x S. droebachiensis ♂ (DeD) were cultured as described by Whiteley and Whiteley (12). S. droebachiensis ♀ x Dendraster ♀ hybrids (DDe) were obtained by heavy over-insemination. Before preparation of chromatin, excess sperm were removed from the embryos by thorough washing.

Nuclei were isolated essentially by the procedure of Roeder and Rutter (13) and chromatin was prepared from the nuclei as described by Easton and Chalkley (14). Histone was extracted from the chromatin with 0.5 N H_2SO_4 for 30 minutes and was precipitated by dialysis against 95% ethanol. Acrylamide gel electrophoresis was used for the high resolution separation of histones by the method of Panyim and Chalkley (15). The gels contained 2.5M urea. Stained gels were scanned using a Gilford spectrophotometer equipped with a scanning accessory device.

RESULTS

The electrophoretic pattern of the histones from all of the embryos were basically similar to those described previously for Arbacia punctulata (14)

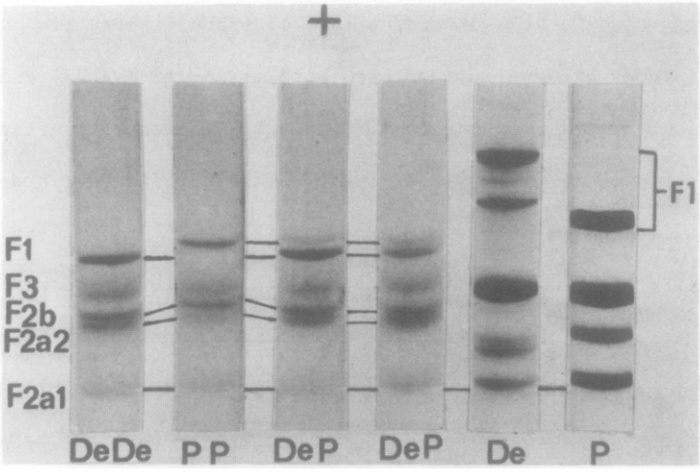

Figure 1. An electrophoretic comparison of the histones from Dendraster hatched blastula (DeDe), S. purpuratus hatched blastula (PP), Dendraster ♀ x S. purpuratus ♂ hatched blastula (DeP), and DeP blocked gastrula. The sperm from both parental species, De and P, have F1 histones with slower electrophoretic mobility than any of the embryonic histones. The S. purpuratus-specific embryonic histone F1 appears in the hybrid patterns, as is indicated by the lines.

and the bands have been assigned to histone fractions by the criteria and using the nomenclature applied there.

As shown in Figure 1, the electrophoretic patterns of histones from Dendraster and S. purpuratus hatched blastulae differ in three respects. The Dendraster F1, F2b and F2a2 histones migrate more rapidly than the corresponding histones from S. purpuratus whereas F3 and F2a1 histones have the same mobilities in the two species. Each of these patterns remains qualitatively the same throughout development. In each species the F1 histones extracted from sperm chromatin have substantially less electrophoretic mobility than their counterparts from embryo chromatin (Figure 1) confirming Ozaki (16) and Easton and Chalkley (14). The electrophoretic patterns of histones from DeP hatched blastulae and from 45-hour-old larvae of these hybrids both contain a band with mobility identical to that of PP F1 histone, in addition to the maternal F1 protein. This band has no counterpart in the histone patterns from the sperm of either species.

As shown in Figure 2, it is possible to duplicate the histone pattern of

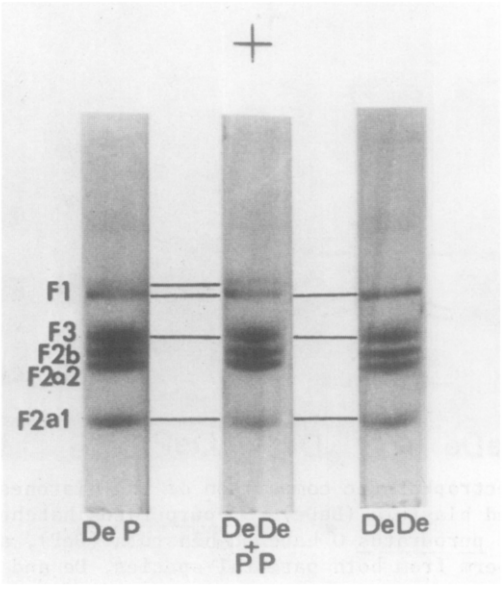

Figure 2. Co-electrophoresis of Dendraster and S. purpuratus embryonic histones. The hybrid (DeP) histones are compared with a mixture of DeDe and PP histones, and the histones of the maternal parent (DeDe). The pattern of the hybrid histones is identical to the mixture. The F2b-F2a2 regions of the three gels are indistinguishable.

the hybrid by co-electrophoresis of whole histone from both parental embryos. For this experiment, the relative concentrations of the two F1 histones were adjusted to give bands with the same stainability as those from the hybrid, as judged from scans of the stained gels. From these observations, we conclude that the species-specific F1 histone of the paternal parent is present in the chromatin of the hybrid embryo along with the maternal F1 histone. The two other species-specific histones, F2b and F2a2, cannot be resolved into their parental forms in preparations from the hybrid embryos (Figure 1), nor can they be resolved in gels from the mixing experiment of Figure 2, either when examined visually or by scanning. Longer gels which are capable of higher resolution also fail clearly to resolve these bands. Consequently it cannot be decided whether the paternal forms of F2b and F2a2 are present or absent in the hybrid chromatin.

The synthesis of paternal F1 histones has been examined in an additional

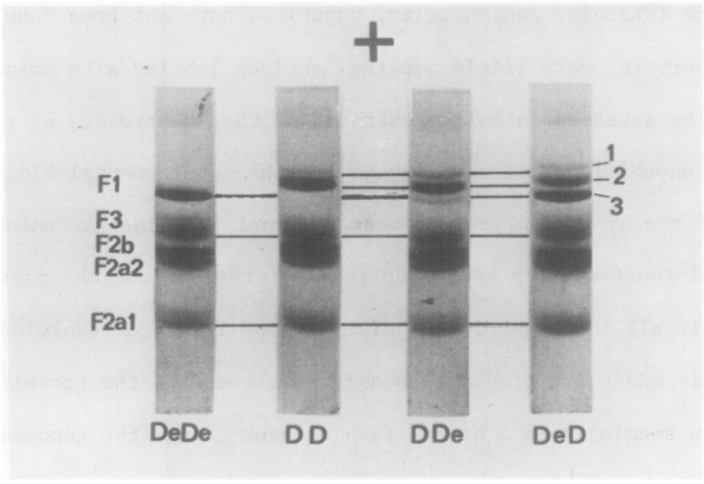

Figure 3. A comparison of the histones from Dendraster (DeDe), S. droebachiensis (DD), S. droebachiensis ♀ x Dendraster ♂ (DDe) hybrid and the reciprocal cross (DeD), all at the hatched blastula stage. The F1 histones marked 1 and 2 are S. droebachiensis-specific histones while the band marked 3 is the Dendraster-specific histone. All three F1 histones appear in both hybrids.

echinoid combination, S. droebachiensis (D) and D. excentricus (De), which has the advantage that the hybridization can be carried out in both directions. In DD embryos the F1 histone contains both a major and a minor component (Figure 3), similar to Arbacia punctulata (14). In both hybrids, DeD and DDe, the F1 histones characteristic of both parents are present (Figure 3).

In all three hybrids examined, the relative intensities of the paternal F1 histone bands are lower than the maternal F1 bands. Scans of the stained gels of histones from hatched larvae indicate that the percentage of the total F1 represented by the paternal protein ranges from 18% in DeP to 30% in DeD.

DISCUSSION

The DeP hybrid has been shown to be a true hybrid in the sense that the genetic information of both parents is present in its genome (12). Nevertheless, the hybrid is blocked in its morphogenesis and arrests as a mid-gastrula. The hybrid makes transcrips from reiterated genes shared by both parents as well as from genes which are different in the two species. Paradoxically, paternal proteins, including esterases (17) malate dehydrogenases (18), and

hatching enzyme (Whiteley and Whiteley, unpubl.), have not been found in this hybrid. Furthermore, very little paternal antigen labeled with amino acids in vivo could be detected in saline extracts of the "gastrulae" of the hybrid (Chamberlain, unpubl.). Both the reasons for the developmental block and for the failure of the hybrid to produce most paternal proteins are unknown.

The F1 histones clearly are exceptional proteins since the paternal forms are produced in all three hybrids studied, in one instance involving reciprocal crosses. Their mRNAs are transcribed and translated and the proteins assembled into chromatin seemingly in a normal fashion even though the genomes and the cytoplasm in which they are functioning are enormously disparate in evolutionary terms. It is not known whether they associate only with paternal DNA. The appearance of paternal histones exclusive of other proteins suggests that the regulation of these two types of proteins is different. Observations of Adesnik and Darnell (19) indicate that the processing, adenylation and transport of histone mRNA proceed differently from other mRNAs and the difference we observe between the synthesis of histones and other proteins might be explained at this level.

The results reported here provide direct evidence that newly transcribed histone mRNA serves as a template for histone synthesis during cleavage since the message for the paternal F1 could have come from no other source. The content of the paternal F1 histone is lower (18-30% of total F1) than the maternal F1 content. This finding is consistent with the hypothesis that the amount of histone present is proportional to the amount of mRNA available for translation. In the case of the hybrids both stored (9, 10) and newly transcribed mRNA would be available for the synthesis of maternal F1 histone, while only newly transcribed mRNA could be translated into paternal F1. It is possible that mRNAs for the other four paternal histones are present in the hybrids but cannot be detected by the methods used.

The histones are remarkably stable both from an evolutionary (20, 21) and metabolic standpoint (22, 23) and have not been accessible for genetic or other

experimental analysis. The echinoid hybrids described here provide an opportunity for studies of their genetic control.

REFERENCES

1. Chen, P.S., in Weber, ed., The Biochemistry of Animal Development New York, Academic Press (1967) Vol. 2 pp 115-191
2. Tyler, A., in Locke, ed., Control Mechanisms in Developmental Processes, Devel. Biol. Suppl. (1) New York Academic Press (1967) pp 170-266
3. Nemer, M., Proc. Natl. Acad. Sci. U.S. 50, 230-235 (1963)
4. Wilt, F.H., Biochem. Biophys. Res. Comm. 11, 447-455 (1963)
5. Infante, A.A. and Nemer, M., Proc. Natl. Acad. Sci. U.S. 58, 681-688 (1967)
6. Rinaldi, A.M. and Monroy, A., Devel. Biol. 19, 73-86 (1969)
7. Nemer, M. and Lindsay, D.T., Biochem. Biophys. Res. Comm. 35, 156-160 (1969)
8. Kedes, L.H. and Gross, P.R., Nature 223, 1335-1339 (1969)
9. Farquhar, M.N. and McCarthy, B.J., Biochem. Biophys. Res. Comm. 53, 515-522 (1973)
10. Skoultchi, A. and Gross, P.R., Proc. Natl. Acad. Sci. U.S. 70, 2840-2844 (1973)
11. Gross, K.W., Jacobs-Lorena, M. Baglioni, C. and Gross, P.R., Proc. Natl. Acad. Sci. U.S. 70, 2614-2618 (1973)
12. Whiteley, A.H. and Whiteley, H.R., Devel. Biol. 29, 183-198 (1972)
13. Roeder, R.G. and Rutter, W.J., Biochemistry 9, 2543-2553 (1970)
14. Easton, D. and Chalkley, R., Exptl. Cell Res. 72, 502-507 (1972)
15. Panyim, S. and Chalkley, R., Arch. Biochem. Biophys. 130, 337-346 1969)
16. Ozaki, H., Devel. Biol. 26, 209-216 (1971)
17. Ozaki, H., Ph.D. Dissertation, Univ. of Washington, Seattle, WA (1966)
18. Ozaki, H. and Whiteley, A.H., Devel. Biol. 21, 196-215 (1970)
19. Adesnik, M. and Darnell, J.E., J. Mol. Biol. 67, 397-406 (1972)
20. Delange, R.J., Fambrough, D., Smith, E.L. and Bonner, J., J. Biol. Chem. 244, 5669-5679 (1969)
21. Panyim, S., Bielk, D., and Chalkley, R., J. Biol. Chem. 246, 4206-4215 (1971)
22. Balhorn, R., Oliver, D., Hohmann, P., Granner, D., and Chalkley, R., Biochemistry 11, 3915-3921 (1972)
23. Hancock, R., J. Mol. Biol. 40, 457-466 (1969)