PHOSPHORYLATION OF NONHISTONE CHROMATIN PROTEINS DURING SEA URCHIN DEVELOPMENT

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SUMMARY

The phosphorylation of nonhistone chromatin proteins during development was studied in the sea urchin, Strongylocentrotus purpuratus. The rate of phosphorylation was found to be maximal during gastrula, slightly lower during prism and almost 70% lower in pluteus stage embryos. Analysis of the phosphorylated nonhistone chromatin proteins by SDS-acrylamide gel electrophoresis showed significant variations in the labeling pattern during different stages of development. A specific protein which is actively phosphorylated during gastrula and prism stages is nearly absent from the pluteus stage.

Nonhistone chromatin proteins are believed to play a key role in the regulation of gene expression in eukaryotic organisms (1,2). They were found to stimulate transcription of DNA and chromatin in cell free systems (3-6) and to respond to gene-activating stimuli in vivo (7-9). Nonhistone chromatin proteins have recently been implicated in changing the transcriptional capacity of the genome during the cell cycle (10-11). Specific changes in these proteins have been reported during differentiation in the slime mold (12) and during development in sea urchins (13). A significant fraction of the nonhistone chromatin proteins is extensively phosphorylated and exhibits many properties expected of molecules involved in the regulation of gene transcription. For example, phosphoproteins alter the rate of RNA synthesis in vitro (3,14-17), they are heterogeneous and tissue specific (14,18), they recognize and bind specifically to homologous DNA (14,19),

and changes in their phosphorylation correlate with differences in gene activity in a variety of systems (20-24).

In order to explore the possibility that the phosphorylation of chromatin nonhistone proteins has a regulatory function during development, we have begun an investigation on the phosphorylation of these proteins during various stages of development in the sea urchin. The present experiments demonstrate the active phosphorylation of a stage specific protein in sea urchin embryos. The results suggest that phosphorylated nonhistone proteins may be involved in the control of differentiation.

METHODS

Sea urchins (Strongylocentrotus purpuratus) were purchased from Pacific Biomarine Co., Venice, Calif. Procedures for obtaining mature gametes, fertilizing the eggs and growing the embryos were followed as described by Johnson and Hnilica (25). At selected stages, embryos were concentrated by low speed centrifugation, suspended in sea water to an average density of 50,000 embryos/ml and pulse labeled for 1 hour with $^{32}P_i$ (10-12 μCi/ml). Nuclei were isolated (25) and chromatin prepared as described by Spelsberg and Hnilica (26). The purified chromatin was extracted once with 0.1 M NaCl and the phosphorylated nonhistone proteins isolated according to the procedure of Gershey and Kleinsmith (27). The phosphoproteins were separated by electrophoresis in a 10% acrylamide gel containing sodium dodecyl sulfate (SDS) as described by Weber and Osborn (28). Gels were sliced at 0.5 mm or 1 mm intervals and counted in a liquid scintillation spectrometer.

RESULTS

Densitometer tracings of gels obtained by electrophoresis of chromatin phosphoproteins from late gastrula (39 hrs), prism (45 hrs), and pluteus (72 hrs) stages are shown in Figure 1. The major protein peak of gastrula and prism is almost absent from the pluteus protein pattern. This protein, migrating at 23 mm, is actively phosphorylated when gastrula and prism

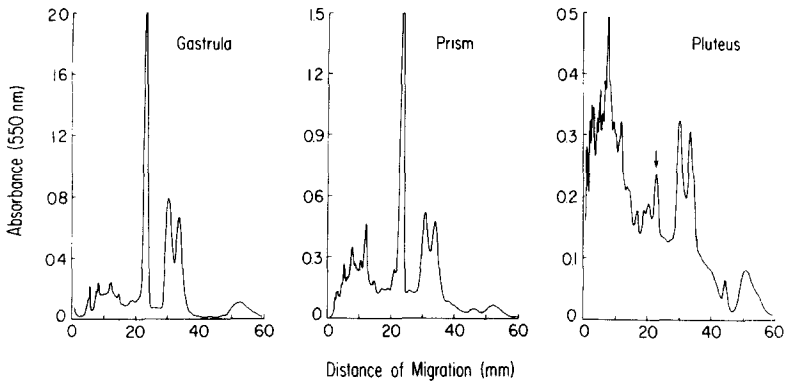

Figure 1. Comparison of protein patterns of nonhistone chromatin phosphoproteins from sea urchin embryos at different stages of development. Embryos were grown in artificial sea water (Instant Ocean from Aquarium Systems, Wickliff, Ohio) at 16°C and stages were varified by microscopic inspection.

Phosphoprotein samples of 10-20 µg from each stage were electrophoresed in 10% SDS-acrylamide gels, stained with Coomassie Brilliant Blue (28), and scanned on a Gilford Spectrophotometer at 550 nm. The arrow indicates the position of a protein peak which is present in gastrula and prism but greatly reduced in the pluteus stage.

stage embryos are incubated for 1 hour with $^{32}P_i$ but only weakly phosphorylated (if at all) in pluteus stage embryos (Figure 2). The amount of radioactivity in this region of the gel drops from 24% of the total cpm recovered in the gastrula to 16% of the total in prism and finally accounts for less than 4% of the total in pluteus. The specific activities of phosphoproteins isolated from each stage are shown in Figure 2. Between gastrulation and pluteus, the overall rate of phosphorylation drops at least 70%.

DISCUSSION

These data provide evidence for the active phosphorylation of nonhistone chromatin proteins in sea urchin embryos and demonstrate a significant change in the amount and in the phosphorylation of specific nonhistone chromatin proteins during early development. Although no direct relationship is shown here between phosphorylation and a change in gene expression, the results are consistent with such a relationship. The rate and extent of phosphorylation is very high during the gastrula and prism stages of develop-

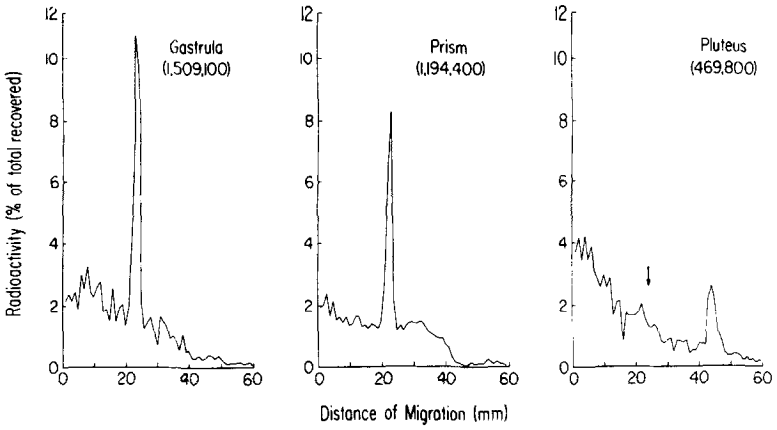

Figure 2 Comparison of ^{32}P labeling patterns of nonhistone chromatin phosphoproteins from sea urchin embryos at different stages of development. Embryos were labeled for 1 hour with ^{32}P at the appropriate stages. Phosphoproteins were electrophoresed in 10% SDS-acrylamide gels. The gels were sliced at 1.0 mm intervals and counted in a liquid scintillation spectrometer.

Radioactivity in each slice is expressed as a percentage of the total radioactivity recovered from the gel. Note that the major phosphorylated peak in gastrula and prism is essentially absent in the pluteus stage (arrow). The numbers in parentheses are the specific activities in counts/min/mg of the total phosphoprotein preparation.

ment which are characterized by rapid cellular differentiation. In contrast, the phosphorylation of nonhistone proteins is much lower in the fully differentiated, free-swimming pluteus stage. The gastrula represents a period of intense gene activity: the nucleolus appears, the embryo synthesizes its own ribosomes and undergoes complex morphogenetic movements. During this period a specific nonhistone protein is actively phosphorylated. After gastrulation, the level of phosphorylation drops. In the free-swimming pluteus which is a fully differentiated larval stage, this protein is not only greatly reduced in quantity, but weakly phosphorylated as well.

The great complexity of the nonhistone protein in chromatin makes it difficult to correlate specific protein species with changes in genetic function. Adequate techniques for the resolution and identification of individual proteins are needed. This observation that certain nonhistone

proteins are actively phosphorylated during specific developmental stages should permit a more detailed investigation of the possible role of specific nuclear proteins in altering the restriction of individual genes during cellular differentiation.

Work is currently underway to extend these findings and clarify the significance of nonhistone protein phosphorylation during development.

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