DNA methylation pattern changes during development of a sea urchin

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Cytosine methylation of developmentally regulated genes of the sea urchin *Strongylocentrotus purpuratus* was studied by using restriction-endonuclease digestion and Southern blotting. The single-copy bindin gene, the family of five cytoplasmic actin genes and the 400-fold-repeated set of five early histone genes were mostly unmethylated, but some sites exhibited partial methylation that varied throughout development. This shows that in echinoderms the methylation of DNA is not confined to the non-transcribed portion of the genome, as previously believed [Bird, Tagart & Smith (1979) Cell 17, 889–901], and may play a role in transcriptional regulation.

INTRODUCTION

Methylation of DNA is a significant factor in the regulation of gene expression in eukaryotes. Manipulation of the level of DNA methylation *in vivo* and *in vitro* changes the pattern of gene expression. A negative correlation between methylation and transcription has been amply documented for vertebrate and plant genes (see [1–3] for reviews). The situation is much less clear-cut in lower eukaryotes. In a number of species DNA methylation is undetectable [4,5]; in some lower eukaryotes low levels of DNA methylation are apparently correlated with certain aspects of genome functioning [6,7]. Echinoderms belong to a third group, in which the degree of DNA methylation is comparable with that of the vertebrates, but it is believed to be limited to the non-transcribed portion of the genome and to remain constant throughout development, and consequently seems to have no regulatory role [8]; 5-azacytidine treatment, however, inhibits echinoderm development in a manner suggestive of such a role [9].

In the present paper we report evidence that subtle changes in the pattern of methylation of individual genes do indeed accompany development of an echinoderm, the sea urchin *Strongylocentrotus purpuratus*.

MATERIALS AND METHODS

S. purpuratus embryos were grown and nuclei were isolated as described [10]. Purified DNA was digested for 3 h with 2 units of a restriction endonuclease/ μ g of DNA. Two isoschizomer pairs were used to investigate cytosine methylation: *HpaII* and *MspI* recognize the sequence CCGG and are respectively sensitive and insensitive to 5-methylation of the inner C residue; *Sau3AI* and *MboI* recognize the sequence GATC and are respectively sensitive and insensitive to 5-methylation of C.

Digested DNA was electrophoresed in 1.6%-agarose gels at 6 μ g per lane and was electrotransferred on to Zeta-Probe membranes (Bio-Rad). Membranes were hybridized and washed as suggested by the manufacturer.

Three cloned sequences were used as hybridization probes: pCO2A, containing the whole genomic repeat of the early histone genes [11]; pSpCRI, containing the bindin cDNA ([12]; Joe Minor, personal communication); and pSpG17, containing a genomic non-muscle actin gene [13], hybridizing with five divergent copies of the actin genes.

Hybridization probes were labelled with 32 P by using a random-primer kit (Boehringer).

RESULTS AND DISCUSSION

In eukaryotes, methylation of DNA is almost exclusively at the 5-position of cytosine in the dinucleotide CpG. Such a modification can be conveniently studied for individual genes by digestion of genomic DNA with restriction-endonuclease isoschizomer pairs differing in their sensitivities to cytosine methylation, followed by electrophoretic separation of the digests and hybridization with cloned gene-specific probes. The presence of bands unique to only one of the isoschizomers indicates methylation (partial or complete) of the recognition sequence of the restriction endonuclease.

We used this approach to investigate the methylation status of several genes of the sea urchin *S. purpuratus* differing in their respective expression patterns and their copy numbers. The genes chosen were: the early histone gene repeat, present in approx. 400 copies, transcribed only in morula [14]; cytoplasmic actin genes, 5 copies of which show varied expression patterns in the early embryo [15]; and the unique bindin gene, transcribed solely in testes [12].

Fig. 1 shows the methylation status of the early histone genes in morula, hatching blastula, mesenchyme blastula and adult (represented by sperm) DNA. Pairs of lanes for each of these stages were loaded with DNA digested with methylation-sensitive/methylation-insensitive isoschizomers. For each pair of lanes the patterns are very similar, indicating that most of the cutting sites are not methylated; several bands (indicated by arrows), however, are unique to the left-hand lanes in each pair. Their presence indicates methylation of the restriction-endonuclease cutting sites. As no band is completely absent from the left-hand lanes, generated by the methylation-sensitive enzymes, it may be concluded that no site is completely protected from digestion, i.e. these sites are methylated only in some of the 400 copies of the histone gene repeat.

No general correlation between transcriptional activity and methylation pattern of the histone genes is evident: a site is methylated only in morula, where the transcription of these genes is maximal, and in hatching blastula, where the transcription has just been terminated (Fig. 1a), another is methylated only in the mesenchyme blastula, where the genes are silent (Fig. 1b, upper arrow) and still another exhibits varying degrees of methylation in all the stages studied (Fig. 1b, lower arrow).

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Fig. 1. Methylation of the early histone genes in developing embryo and sperm

DNA from morula (lanes 1 and 2), hatching blastula (lanes 3 and 4), mesenchyme blastula (lanes 5 and 6), and sperm (lanes 7 and 8) was digested with: (a) endonucleases *HpaII* (lanes 1, 3, 5 and 7) and *MspI* (lanes 2, 4, 6 and 8), or (b) endonucleases *Sau3AI* (lanes 1, 3, 5 and 7) and *MboI* (lanes 2, 4, 6 and 8). pCO2A [11] was used as the radioactive probe for hybridization.

Fig. 2. Methylation of the bindin gene in developing embryo and sperm

The filter from Fig. 1(a) was rehybridized with pSpCRI ([12]; Dr. Joe Minor, personal communication).

The presence of bands of significantly different intensities within any single lane, and the differences in the band patterns among different DNA preparations are due to the sequence heterogeneity of the individual copies of the early histone gene

Fig. 3. Methylation of the cytoskeletal actin genes in embryo and sperm

DNA from mesenchyme blastula (lanes 1 and 2) and sperm (lanes 3 and 4) was digested with *HpaII* (lanes 1 and 3) and *MspI* (lanes 2 and 4) and was hybridized with pSpG17 [13].

repeat within a single genome and to the differences among the repeats of different individuals [11].

Similar analysis was performed for the unique testis-specific bindin gene [12] (Fig. 2) and for cytoskeletal actin genes, five copies of which show strong sequence homologies, but differ in their respective expression patterns during embryogenesis [15] (Fig. 3). Similarly to the results obtained for the early histone

genes, the overall level of methylation was very low, but certain sites did exhibit methylation in some copies of these genes. For the actin genes this probably indicates the methylation of only certain copies of the gene; for the unique bindin gene apparently only certain cells of the developing embryo exhibit methylation.

All these data show that, contrary to the current paradigm [8], DNA methylation in echinoderms concerns also transcribed sequences and is not constant throughout development. The relationship between this modification and gene expression remains to be established.

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