Two-dimensional gel electrophoresis of proteins released from sea urchin nuclei by limited digestion with DNase I

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The morula and the mesenchyme blastula nuclei contained approx. 30 nuclear proteins which were preferentially released by limited digestion with DNase I, but no proteins were released from sperm nuclei. While most of the proteins released by DNase I digestion were common to the two embryonic stages, 2 and 6 proteins were specific or enriched in morulae and mesenchyme blastulae, respectively.

DNase I digestion *Nuclear protein* *Sea urchin*

1. INTRODUCTION

Analyses of changes in chromatin organization that occur when a gene becomes transcriptionally active are indispensable to understanding the regulatory mechanisms of the stage-dependent gene expression during early embryogenesis. The transcriptionally active genes have been shown to be preferentially digested with DNase I [1,2]. The DNase I hypersensitive sites were shown to be tissue-specific [3–5] and closely correlated to the sequences required for gene expression [6]. Brief treatment of the nuclei with DNase I also released chromosomal proteins from trout testis [7], avain erythrocytes [8,9] and wheat [10]. It has also been shown that one of the chromosomal proteins released from *Drosophila* nuclei by limited digestion with DNase I is preferentially associated with the active region of the chromosomes [11,12].

The previous study suggested that several genes which are inactive in morulae of sea urchin become transcribed when the embroys develop to mesenchyme blastulae [13]. To examine the differences in chromatin structures between morulae and mesenchyme blastulae, nuclei purified from embryos at the two developmental stages were digested with DNase I, and proteins subsequently released from the nuclei were analyzed by two-dimensional gel electrophoresis.

2. MATERIALS AND METHODS

2.1. Embryo culture

Eggs from the sea urchin, *Hemicentrotus pulcherrimus,* were inseminated and cultured at 18°C with gentle stirring. To remove fertilization membrane, eggs were inseminated in the presence of 1 mM 3-amino-1,2,4-triazole [14] and the fertilization membrane thus weakened was removed by passage through a 62 μm nylon mesh.

2.2. Purification of nuclei

Embryos were homogenized in 0.25 M sucrose, 0.1 M NaCl, 2 mM magnesium acetate, 5 mM Tris–HCl (pH 7.4), 2 mM 2-mercaptoethanol, 0.5% (v/v) Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF) by several strokes with a loose-fitting pestle in a Dounce homogenizer. Cell disruption was monitored under phase contrast microscopy and the lysate filtered through a 40 μm nylon mesh. Nuclei were collected by centrifugation at 5000 × g for 10 min followed by further purifi-

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cation at 24000 rpm for 2 h through a step sucrose gradient consisting of 2.2 M sucrose, overlaid by 1.7 M sucrose. Both sucrose solutions contained 0.1 M KCl, 4 mM magnesium acetate, 5 mM Tris–HCl (pH 7.4), 2 mM 2-mercaptoethanol, and 1.7 M sucrose also contained 0.2% (v/v) Triton X-100 and 1 mM PMSF [15].

For purification of sperm nuclei, dry sperm were washed with 4.25% (w/v) KCl and 0.25 M sucrose, 50 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$ containing 1% (v/v) Triton X-100. The nuclei were purified by centrifugation through 2 M sucrose, 50 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$ at 14000 × *g* for 10 min [16,17].

2.3. *DNase I digestion of nuclei and preparation of nuclear proteins*

The purified nuclei were washed twice with and resuspended at 1 mg DNA/ml in 0.25 M sucrose, 10 mM Tris–HCl (pH 7.5), 10 mM NaCl, 1 mM $CaCl_2$ [2]. DNA content was determined by measuring the absorbance at 260 nm of the supernatant obtained by pelleting the nuclei after being boiled in 5% (v/v) perchloric acid for 15 min. One mg DNA/ml gave 28.0 as the absorbance at 260 nm under the condition employed. The nuclei were digested with pancreatic DNase I (Worthington, EC 3.1.4.5) (28 units/ml) at 37°C. Incubation was terminated by the addition of EDTA to a final concentration of 5 mM, and the nuclei sedimented at low speed. The extent of DNA digested as the percentage of the DNA introduced was determined by measuring the release of material absorbing at 260 nm in the supernatant [2]. For the preparation of nuclear proteins, incubation was terminated when the nuclei were digested to release 10% of the nuclear DNA into the soluble fraction. The nuclei were then extracted twice with 0.2 mM EDTA (pH 7.0) [11].

2.4. *Two-dimensional electrophoresis of nuclear proteins*

The released proteins were concentrated 40-fold, and subjected to two-dimensional gel electrophoresis [18]. The first dimension contained 1.6% (v/v) pH 5–7 ampholytes and 0.4% (v/v) pH 3.5–10 ampholites (LKB). Electrophoresis in the second dimension was on slabs containing 10% (w/v) acrylamide. M_r-standards (Pharmacia) were run simultaneously on each gel in the second dimension. The gels were stained with Coomassie brilliant blue followed by silver [19].

3. RESULTS AND DISCUSSION

Fig.1 shows the digestion kinetics of nuclei purified from morula, mesenchyme blastula and sperm. The course of DNA digestion involved a rapid initial phase which leads to 15–20% of the DNA intoduced being digested into the soluble fraction followed by a slow phase. During the rapid initial digestion, the mesenchyme blastula nuclei were more susceptible to DNase I compared to the morula or sperm nuclei. The result that the blastula nuclei tend to be digested with DNase I more rapidly than the sperm nuclei was similar to that of the previous study except for the digestion rate of the blastula nuclei [20].

When approx. 10% of the DNA was digested, the incubation was terminated and proteins extracted from the nuclei. Fig.2 shows that about 30 proteins are released from both the morula and mesenchyme blastula nuclei. The gel patterns of proteins were very reproducible. Since no proteins were released when the nuclei were incubated for the same periods of time in the absence of DNase I (not shown), these proteins were suggested to be preferentially released from the nuclei by digestion with the nuclease. The number of protein species which can be released from the embryonic nuclei by limited digestion with DNase I seems to be more

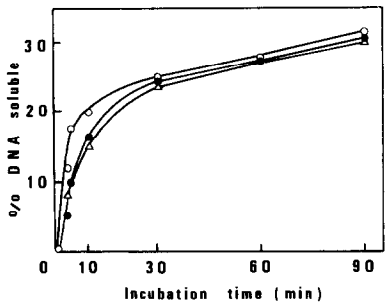

Fig.1. Digestion kinetics of the sea urchin nuclei with DNase I. Purified nuclei (1 mg DNA/ml) were digested with DNase I (28 units/ml) for increasing periods of time at 37°C. The extent of DNA digested into the soluble fraction was determined by measuring the released materials absorbing at 260 nm in the supernatant after pelleting the nuclei. (●—●) Morulae, (○—○) mesenchyme blastulae, (△—△) sperm.

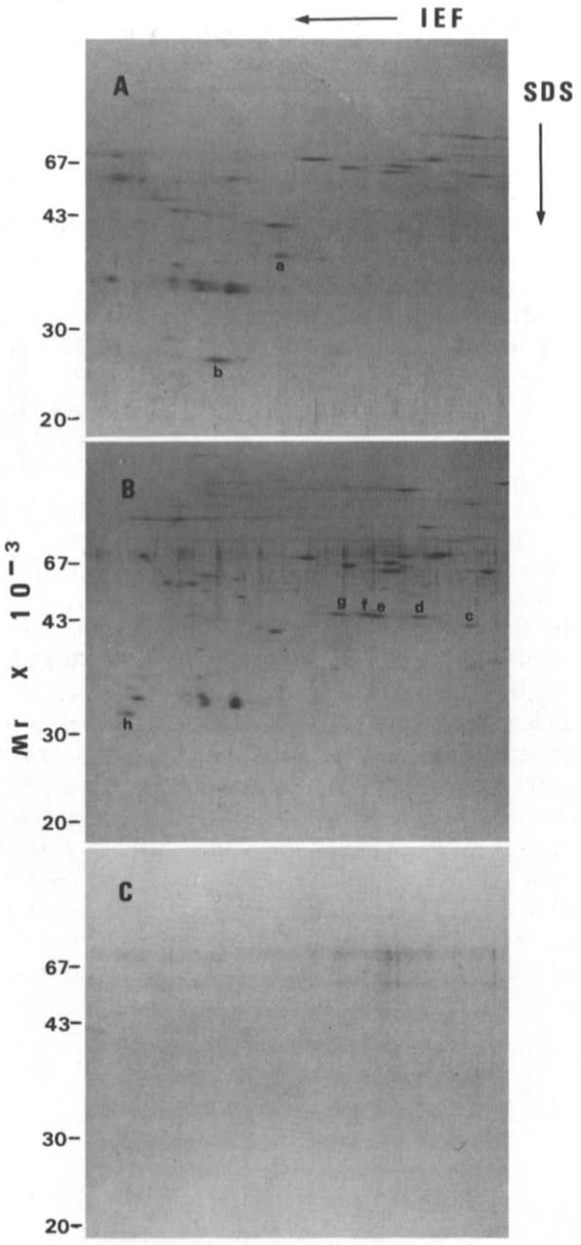

Fig.2. Two-dimensional gel electrophoresis of the proteins released from the nuclei by limited digestion with DNase I. The purified nuclei from (A) morulae, (B) mesenchyme blatsulae and (C) sperm were digested at 37°C with DNase I (28 units/ml), and digestion terminated when 10% of the DNA introduced was digested into the soluble fraction. The released materials were concentrated 40-fold, subjected to two-dimensional gel electrophoresis, and the gels stained with silver. a,b, morula-specific or -enriched spots; c–h, mesenchyme blastula-specific or -enriched spots. Bands in the region 51–65 kDa are artifacts due to 2-mercaptoethanol [23].

than one-tenth of the total nuclear protein species that have been estimated to be approx. 200 in the sea urchin embryos [15].

While most of the proteins released from the morula and the mesenchyme blastula nuclei after limited digestion with DNase I are common, two proteins, indicated by a and b in fig.2A, which are detected in morulae are missing or only faintly detectable in mesenchyme blastulae. Brief treatment of the mesenchyme blastula nuclei with DNase I released 6 proteins, indicated by c–h in fig.2B, which were not released from the morula nuclei. The result that most of the nuclear proteins released from the nuclei of the two embryonic stages by brief treatment with DNase I were common may refect the fact that most of the total nuclear protein species remain unchanged during early embryogenesis [15].

All the proteins released from the embryonic nuclei by limited digestion with DNase I have $M_r >$ 20000 (fig.2), and no nuclear proteins corresponding to high mobility group chromosomal protein found in the sea urchin embryos [22] were released by the brief treatment here of embryonic nuclei with DNase I. This agrees with the result for trout testis [7] but not with that for avian erythrocytes [8,9] or wheat [10].

In contrast to embryonic nuclei, no detectable proteins were released from sperm nuclei (fig. 2c), although sperm DNA was digested similarly to embryonic DNA (fig. 1). This may be due to little, if any, non-histone proteins being present in the sperm nuclei. This has yet to be proven; however, it has been shown that the amount of non-histone proteins decreases to virtually zero in developing trout testis [21].

In summary, the present results show that some proteins which are preferentially released from embryonic nuclei by brief treatment with DNase I were stage-dependent. The functional significance of these proteins is at present unknown. It will be of considerable interest to examine the possible relationship between these nuclear proteins and the stage-dependent gene expression during early embryogenesis.

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