Rapid exchange of histones H2A and H2B in sea urchin embryo chromatin

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Chromatin from sea urchin blastula and gastrula was partially digested with micrococcal nuclease and separated electrophoretically in two dimensions into various nucleosome fractions and their component histones. After labelling of the embryos with radioactive lysine the various isohistones of the H2A and H2B group in all nucleosome fractions had incorporated approximately twice as much the radioactivity as the histones H2 and H4. The results are interpreted in terms of a labile nucleosome structure during transcription.

Sea urchin embryo *Nucleosome* *Chromatin* *Histone*

1. INTRODUCTION

Chromatin of the developing sea urchin embryo is characterized by the programmed deposition of histone variants. This programme has been described in detail for *S. purpuratus* [1,2] and *P. angulosus* [3–5]. For both species early and late variants have been characterized with the switching over between the two sets of histones at the blastula stage. The early developmental stages are characterized by rapid DNA replication and very active transcription. Replication necessitates the de novo assembly of core particles. The fate of cores in the course of transcription is unknown. It may well differ in polymerase III and polymerase II transcribed regions. The former transcribes core free regions [6] though it has not been established whether the latter are also free from histones. The fate of the core during the transcription by the latter may involve complete or partial dissociation of core subunits. If histone subunits noncomitant with replication and/or transcription, move simultaneously as an octamer, one would expect stoichiometric amounts of de novo synthesized histone subunits to be associated with DNA. If the subunits making up the core move independently from each other the radioactivity of the core histones should differ after a suitable radioactive pulse.

We have labelled the hatching blastula of *Parechinus angulosus* for 30 min with lysine and measured the incorporation into the various histones of soluble chromatin subfractions isolated after partial micrococcal nuclease digestion of chromatin.

2. MATERIALS AND METHODS

2.1. *Preparation of blastula cells*

Sea urchin embryos of the species *P. angulosus* were grown [7] in batches of 500 ml sea water, at a density of 4% (v/v) cells, on a rotray shaker (200 rpm) at 20°C labelled at the appropriate time with 4 mCi [^{3}H]lysine (75 Ci/mmol) and harvested after 30 min. Embryos were washed 5 times with Ca/Mg-free sea water (48 mM NaCl, 11 mM KCl, 2.5 mM $NaHCO_3$, 0.5 mM EDTA) via centrifugation for 5 min at $500 \times g$. To disintegrate the embryos the last pellet was suspended in 80 ml of 0.5 M sucrose in 15 mM NaCl, 65 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 5 mM mercap-

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toethanol, 15 mM Tris-HCl, pH 7.4 (buffer A) [8]. If necessary the disintegration of the embryos into cells was assisted by a few strokes with a loosely fitting Dounce homogenizer. An equal volume of cell lysis buffer was added (buffer A), containing in addition 20 mM EDTA, 2.5 mM EGTA, 0.2 mM PMSF and 0.5% (w/v) Nonidet to liberate nuclei. After a maximum of 5 min lysis the nuclei were sedimented at $3000 \times g$ and washed with 0.25 M sucrose in buffer A containing 0.2 mM EDTA, 0.2 mM EGTA and 0.2 mM PMSF.

2.2. *Preparation of soluble chromatin*

Nuclei were digested for 15 min at 37°C with 40 units micrococcal nuclease per mg DNA at 1 mg DNA/ml in buffer A supplemented with $CaCl_2$ to 1.4 mM. After digestion 40 μl of a 250 mM EDTA solution were added per ml of the digestion mixture, followed by sedimentation of the nuclei at $3000 \times g$ for 3 min. The pellet was homogenized in 10 ml of 0.2 M EDTA, 10 mM Tris-HCl (pH 7.4) with a tight fitting Dounce homogenizer and this dialysed against the extraction buffer overnight. After pelleting, the supernatant was concentrated via ultrafiltration to 4 mg DNA/ml.

2.3. *Electrophoresis*

The soluble chromatin was electrophoresed on a 3–16% wedge gradient gel (acrylamide: bisacrylamide = 70:1, 10 mM triethanolamine, 2 mM EDTA, pH 7.6), polymerized in light with 100 μg riboflavin per 100 ml gel. The same buffer served

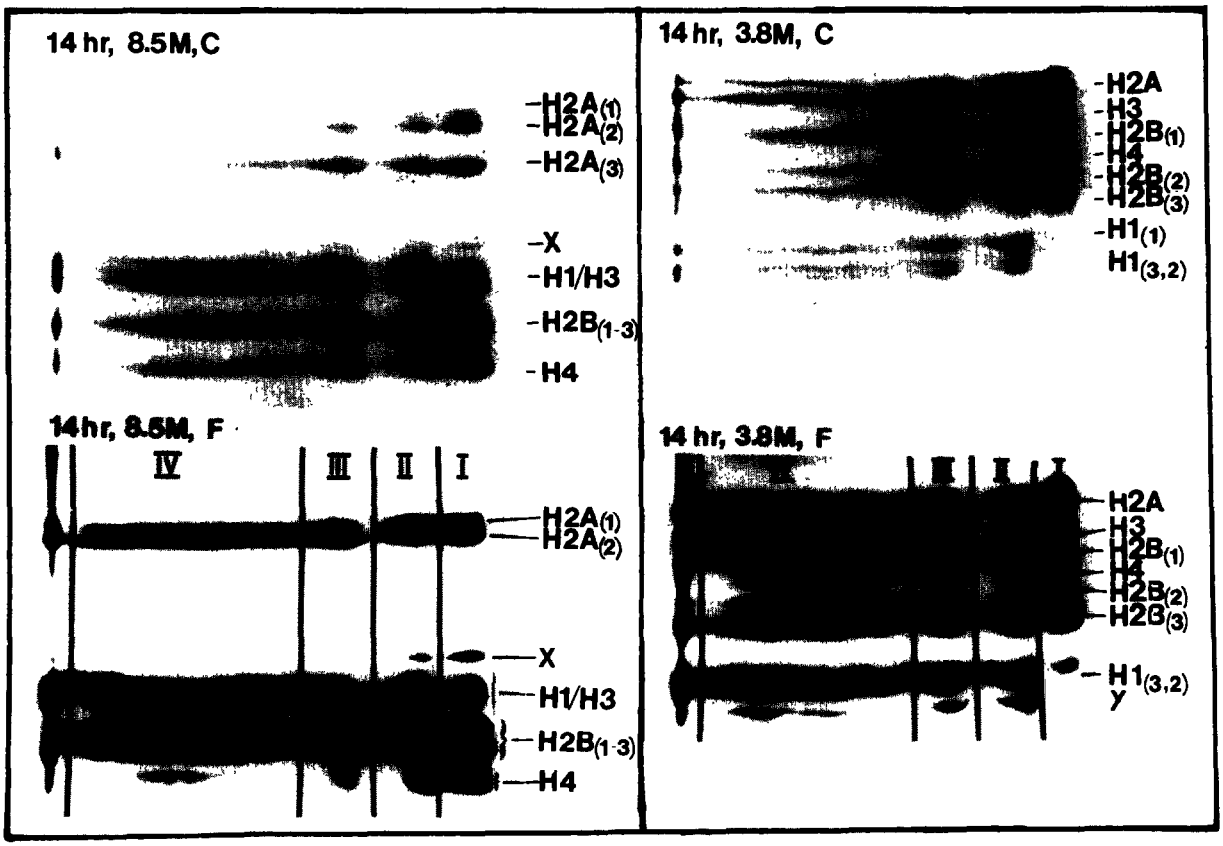

Fig.1. Two dimensional electrophoresis of soluble sea urchin chromatin. Blastula chromatin, partially digested with micrococcal nuclease, was separated in the first dimension in a polyacrylamide concentration gradient and in the second dimension in acetic acid urea-Triton at two urea concentrations (8.5 and 3.8 M) to optimize isohistone separation. The upper frames (C) are the stained gels, the lower frames (F) the fluorograms. Fractions I–IV represent cores, mononucleosomes, dinucleosomes, trinucleosomes and tetra- to hexanucleosomes, respectively.

as electrolyte. The sample was applied in 2 mM EDTA in 5% (v/v) glycerol. For single dimension gels the sample was between 20–40 μg and for two dimensional gels 400 μg. Second dimension gels were run either in the Triton system described by Zweidler [9] or as DNA electrophoresis in agarose gels [10]. For second dimension Triton gels histones were released with a solution of 1 g urea, 90 μl mercaptoethanol and 160 mg protamine sulfate in 1.2 ml water, pH 3.8. Gels were run at 8 and 16 mA constant current over 16 h for 1st and 2nd dimensions, respectively. Staining was with Coomassie blue for proteins, ethidium bromide for DNA and fluorograms were done after soaking in 1 M sodium salicylate in 25% (v/v) ethanol [11]. Dried gels were exposed to preflashed X-ray film [12] over 1–2 weeks. Radioactive fractions were excised and oxidised to water in a sample oxidizer (Packard Instruments) and the tritium activity was determined via liquid scintillation counting. Losses of radioactivity from the bands in the gels during staining and destaining were approx. 5% and during salicylate soaking also 5%.

3. RESULTS

Under the conditions used the nuclease solubilises 15% of the chromatin. Two dimensional gel electrophoresis separates this chromatin into a number of subfractions ranging from core particles to oligosomes comprising up to 6–7 nucleosomes (fig.1). The size distribution of the DNA associated with the chromatin subfractions ranges from approximately chromatosome length to 1 kb. (fig.2).

The protein complement of the electrophoretically separated chromatin subfractions contains the typical isohistones expected for this stage of development. These have been characterized with respect to amino acid composition, partial sequences and electrophoretic mobilities in two detergent systems [13]. From the dye uptake no gross deficiences in the component histones are apparent. In fraction I, Hl is consistently absent. This fraction represents untrimmed cores either pre-existent in chromatin or as an artifact.

The incorporation of lysine into the core histones

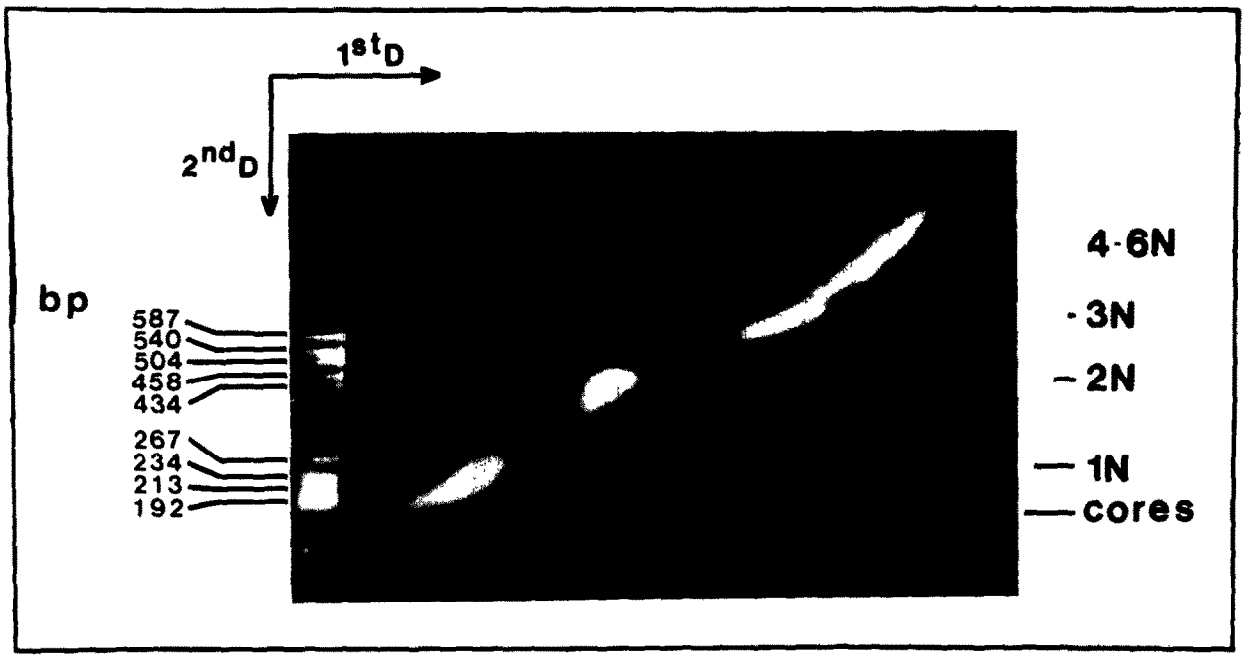

Fig.2. Two dimensional electrophoresis of soluble sea urchin chromatin. Partially nuclease digested blastula chromatin was separated in the first dimension in a polyacrylamide gradient into cores and various oligomers. For the second dimension the DNA was liberated by digestion for 3 h at 45°C with 4×10^{-3}% proteinase K in 1% SDS and run into agarose gels. Size standards were fragments of an Alu 1 digest of pBR 322.

in all chromatin subfractions appears to follow a nonstoichiometric pattern in that H2A and H2B are more highly labelled than the H3 and H4 histones (fig.1). The mononucleosome fraction (II) displays labelling heterogeneity of core histones suggesting nucleosome subpopulations associated with DNA lengths from approx. 210 to 270 bp. All nucleosome-like structures assembled on that stretch of DNA have in common a preponderance of the label in the H2A and H2B fractions. A major subpopulation appears not to contain any freshly synthesized H4. A comparison of the 8.5 and 3.8 M urea 2nd dimension gels (fig.1) indicates that a minor subpopulation containing freshly synthesized H4 has as its H2B complement the isohistone $H2B_2$ which belongs to the early histone set and at the blastula stage only represents a minor histone. The freshly synthesized histones associated with the longer stretches of DNA in subfraction II are mainly of the H2A and H2B type, typical for that stage of development.

The fluorogram labelling pattern is confirmed (fig.3) by direct quantification and normalisation with respect to lysine residues [13]. In all 4 chro-

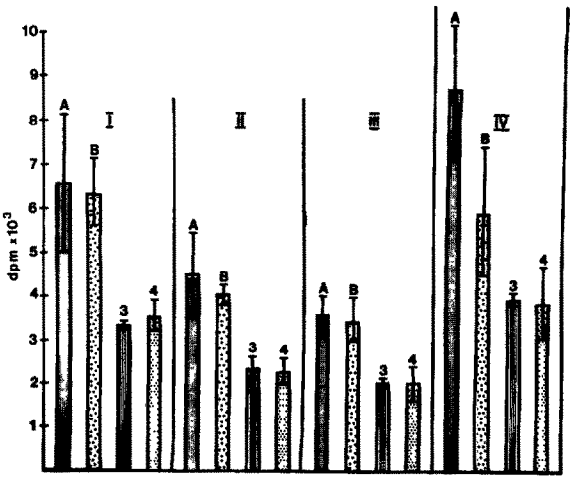

Fig.3. Lysine incorporation into core histones of chromatin subfractions from blastula. The histone containing gel fractions from 2nd dimension gels (see fig.1) were excised and oxidised to water. Their tritium content was determined and normalised with respect to their lysine content [13]. Roman numerals I–IV correspond to fig.1 notation. A,B,3 and 4 are histones H2A, H2B, 3 and 4, respectively, the bars represent averages and standard deviations from 4 gels.

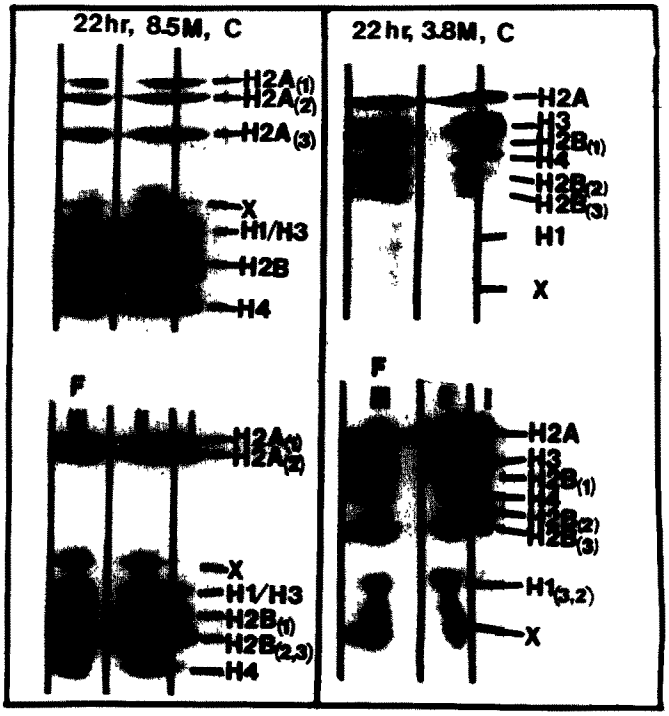

Fig.4. Two dimensional electrophoresis of a chromatin subfraction. Cores, nucleosomes and dinucleosomes were prepared from gastrula (22 h after fertilisation) as in fig.1 and separated in two dimensional gels, notations being the same as in fig.1.

matin subfractions the label in the histones of the H2A and H2B group is considerably higher than the radioactivity in the H3 and H4 subunits. This picture of unequal labelling of the core histones in chromatin subfractions persists also in the gastrula, 22 h after fertilisation (fig.4).

4. DISCUSSION

We have established that the core histones in micrococcal nuclease solubilized chromatin subfractions become unequally labelled. The labelling period extends over 30 min, a time interval too long to reveal differences due to the sequential histone deposition spaced in the order of seconds as they occur in the assembly of cores near the replication fork [14]. Assuming a replication rate at the fork of approx 50. nucleotides per s [15], the position of the first post-fork nucleosomes between 100 and 300 bp downstream of the leading and lagging strand, respectively [16], and a maturation period corresponding to the assembly time of approx. 5–10 nucleosomes [17], then a nucleosome should be fully completed within approx. 40–60 s after its initiation. Thus analysis of sequential processes associated with replication-dependent nucleosome assembly requires very short pulses of radioactivity with extensive chasing. Under our conditions, therefore, the incorporation pattern most likely reflects partial core and nucleosome dismantling with subsequent reassembly concomitant with transcription. The fact that the H2A-H2B pair is on average twice as radioactive as the H3 and H4 subunits, points at a situation in which the former leave the nucleosome more readily to exchange with recently synthesized H2A and H2B subunits.

These experimental results and their interpretation are consistent with the observation of a core subpopulation deficient in one pair of the H2A-H2B dimer which exhibits a higher affinity to RNA polymerase [18]. We conclude that during transcription in the developing sea urchin embryo the nucleosomal structure is modified so as to allow H2A and H2B subunits of the octamer to dissociate reversibly from the nucleosome.

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