SEA URCHIN SATELLITE DNA. ITS ISOLATION, SOME PROPERTIES AND RELATIVE AMOUNT AT DIFFERENT STAGES OF SEA URCHIN DEVELOPMENT

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1. Introduction

It has been shown recently [1–3] that DNA isolated from sea urchin cells contains a certain amount of satellite DNA with buoyant density in CsCl density gradient considerably higher than that of the main band DNA. Although different buoyant density values were obtained for the main band using DNA preparations isolated from various species of sea urchins, a small amount of a "heavy" satellite DNA with relatively invariable density was found in all samples studied (table 1).

Table 1
Buoyant density values of the main and satellite bands for various sea urchin species.

Species	Main buoyant density	Satellite buoyant density	Ref.
Lytechinus pictus	1.693	1.719	1
Lytechinus variegatus	1.695	1.722	2
Arbacia punctullata	1.698	1.717	3
Strongylocentrotus purpuratus	1.696	1.718	3
Arbacia lixula	1.698	1.718	This work

Recently Patterson and Stafford [2] described preparation of sea urchin DNA samples highly enriched in satellite DNA, and reported hybridization of this DNA with ribosomal RNA. In our present paper results are presented concerning heavy satellite DNA isolation using a different method, and some physicochemical characterization of this DNA. Preliminary data are also included on the variation of the satellite DNA content in DNA preparations isolated from sea urchin cells in different developmental stages.

2. Methods

Preparations enriched in satellite DNA were obtained from sea urchin (Arbacia lixula) sperm DNA which was isolated by a modification of Marmur's method [4], using chromatographic fractionation already described [5]. Because of a much lower satellite content in sea urchin DNA as compared with calf thymus DNA [5] the fractionation procedure consisting of DNA elution from a MAK* column by a NaCl concentration gradient was repeated three times. Before the last elution the DNA sample was heated in SSC at 92° for 10 min to achieve partial denaturation of the main band DNA and so to improve the separation of native satellite DNA [5]. Preparative CsCl density gradient centrifugation [1] was used for isolation of DNA from lysates of cells in early developmental stages.

* *Abbreviations:*
MAK: methylated albumin–kieselguhr;
SSC : standard saline citrate solution (0.15 M NaCl + 0.015 M trisodium citrate).

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Analytical CsCl density gradient centrifugation was carried out at 44770 rpm for 20 to 24 hr. Buoyant densities were calculated using the density 1.710 for *E. coli* DNA.

3. Results and discussion

The gradual increase of the percentage of satellite DNA in the sample after individual fractionation steps was followed by CsCl density gradient centrifugation (fig. 1). In fig. 1 curve a corresponds to whole sea urchin sperm DNA; curves b and c show CsCl density gradient profiles after one or three fractionation steps, respectively. The final sample (curve c) contains 40% satellite DNA of a density 1.718.

Denaturation and renaturation behaviour of the satellite DNA is reported on fig. 2. After alkaline denaturation of the native enriched sample (curve a) both peaks corresponding to satellite and main band DNA were displaced to higher densities, as expected for the

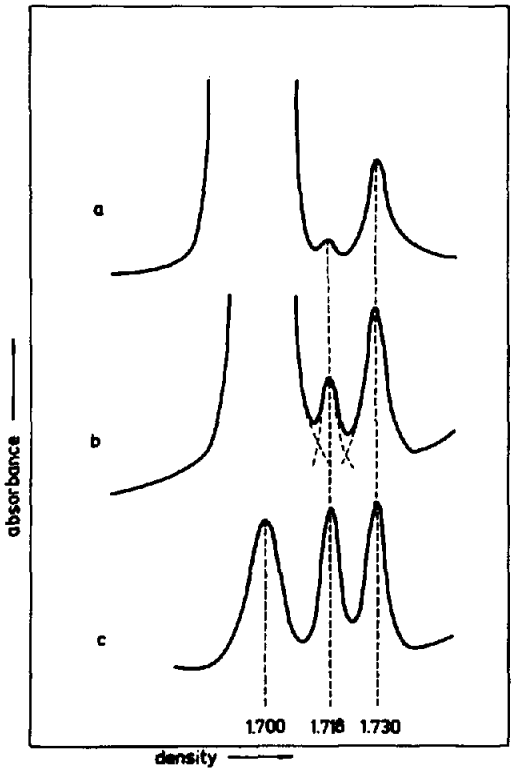

Fig. 1. Gradual enrichment of the sample with satellite DNA, followed by CsCl density gradient centrifugation.
Curve a: Sea urchin sperm DNA, 85 μg DNA, ∿ 0.1% satellite DNA.
Curve b: Sea urchin sperm DNA after one fractionation step on MAK column, 34 μg DNA, 1% satellite DNA.
Curve c: Sea urchin sperm DNA after three fractionation steps (with partial denaturation), 2.5 μg DNA, 40% satellite DNA.
The band centered at density 1.730 is Streptomyces chrysomallus DNA merker.

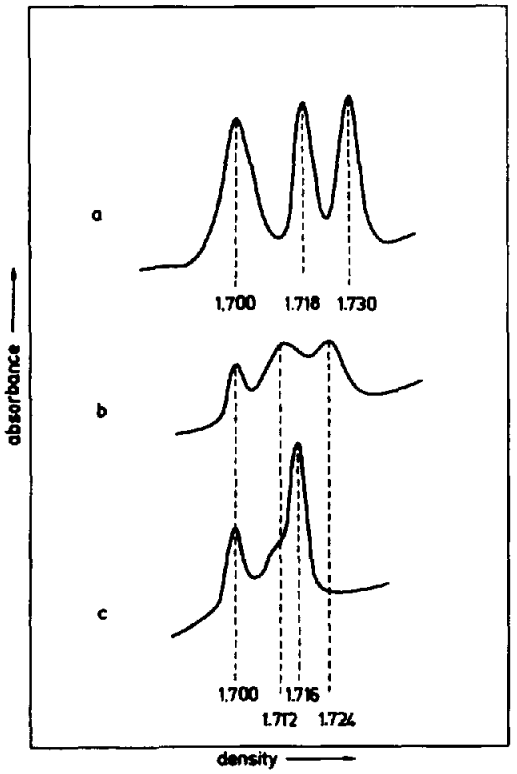

Fig. 2. Denaturation and renaturation of satellite DNA followed by CsCl density gradient centrifugation.
Curve a: Native sample enriched by satellite DNA. The band centered at 1.730 is Streptomyces chrysomallus DNA marker.
Curve b: The same sample denatured by alkali (the solution adjusted to 0.1 M NaOH at a concentration 7 μg/ml DNA, left for 5 min and neutralized by 2 M NaH_2PO_4). The band centered at density 1.700 is T_4 phage DNA marker.
Curve c: The denatured sample annealed at 70° for 5 hr in cation concentration (Na^+) = 0.45. The band centered at density 1.700 is T_4 phage DNA marker.

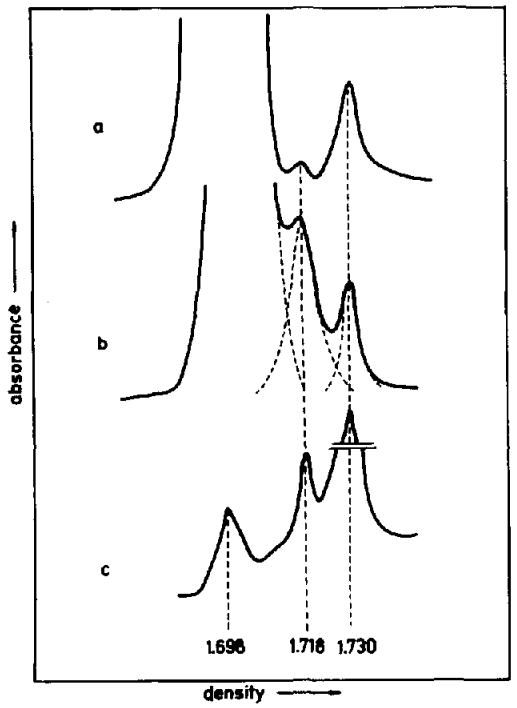

Fig. 3. Relative amount of satellite DNA in DNA preparations isolated from sea urchin cells (Arbacia lixula) in different developmental stages.
Curve a: DNA isolated from sea urchin sperm, ∿ 0.1% satellite DNA.
Curve b: DNA isolated from a culture of cells grown to the swimming blastula stage (1000 cells), 3.6% satellite DNA.
Curve c: DNA isolated from a culture of cells in 8 cells stage, ∿40% of satellite DNA.
The band centered at density 1.730 is Streptomyces chrysomallus DNA marker.

denaturation of a double stranded DNA (curve b). An aliquot of the denatured sample was annealed at elevated temperature in higher ionic strength and analyzed by CsCl gradient centrifugation (fig. 2, curve c). On annealing native buoyant density of the satellite DNA was restored as expected when complete renaturation was achieved, but the main band displayed essentially no change in buoyant density. The apparent density of the renatured satellite DNA band was even lower (1.716) than that of the native one (1.718), but this anomaly is obviously due to overlapping of both bands in the annealed sample. Complete renaturation of the satellite DNA indicates a high degree of homogeneity and probably also the presence of reiterated sequences.

Fig. 3 shows a comparison of the relative amounts of satellite DNA (density 1.718) in the DNA preparations isolated from different developmental stages of sea urchin (Arbacia lixula) cells. A very low content of satellite in sea urchin sperm DNA is indicated by curve a (approximately 0.1%). In DNA isolated from the cells grown to the swimming blastula stage in a relatively well synchronized culture (about 1000 cells from one fertilized egg) the satellite content is more than one order of magnitude higher (3.6%). The satellite DNA content in cells after 3 replication cycles (8 cells from one fertilized egg) is again much higher and amounts to approximately 40% of total sea urchin DNA. The amount of satellite DNA in even earlier developmental stages (4 cells) appeared to be of similar magnitude, but the percentage could not be determined with enough precision because of the very low amount of total DNA isolated.

The decrease of the relative amount if satellite DNA of density 1.718 during cell division reveals a gradual dilution of this DNA by replicating chromosomal DNA and indicates therefore the extrachromosomal nature of this DNA. However, the heavy satellite DNA is probably not of cytoplasmic origin (mitochondrial DNA), because cytoplasmic DNA that was shown to be in excess in sea urchin egg [1] bands at a density about 1.703. In our case the cytoplasmic DNA band could not be distinguished from the main band nuclear DNA (density 1.698) but the presence of a component with a higher density is indicated by the skewed shape of the band (fig. 3, curve c). More probably sea urchin satellite DNA may be similar to extrachromosomal DNA containing amplified ribosomal genes found in oocytes of amphibians and some other animals [6].

References

[1] L.Pikó, A.Tyler and J.Vinograd, Biol. Bull. 132 (1967) 68.
[2] J.B.Patterson and D.W.Stafford, Biochemistry 9 (1970) 1278.
[3] J.Šponar and P.Doty, unpublished results.
[4] R.P.Klett and M.Smith, in: Methods in Enzymology, Vol. 12, part B, eds. K.Moldave and L.Grossman (Academic Press, New York, London, 1968) p. 212.
[5] H.Votavová, J.Šponar and Z.Šormová, European J. Biochem. 12 (1970) 208.
[6] D.D.Brown and I.B.Dawid, Science 160 (1968) 272.