# THE OCCURRENCE OF SPERM ISOHISTONES H2B IN SINGLE SEA URCHINS

Marie STRICKLAND, Walter N. STRICKLAND and Claus VON HOLT*

*Chromatin Research Unit, Council for Scientific and Industrial Research, Department of Biochemistry, University of Cape Town, Private Bag, Rondebosch, Cape Town 7700, Republic of South Africa*

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## 1. Introduction

The complete amino acid sequences of three H2B histones from the sperm cells of *Parechinus angulosus* have been reported [1–3]. These proteins were purified from pooled samples of sperm. The sperm from animals collected at various locations all contained $H2B_1$ but varied in the relative amounts of $H2B_2$ and $H2B_3$. This prompted an investigation to determine whether the 3 isohistone genes for the sperm cell-specific histones H2B are expressed simultaneously in one organism. Such information may help to reveal the significance of the occurrence of these isohistones.

## 2. Materials and methods

Sea urchin (*Parechinus angulosus*) were collected from 5 sites at the Atlantic shore line (sites 1–5) and 4 locations on the Indian shore line of the Cape Peninsula (sites 6–9). Histones were extracted as in [1,4] either by selective extraction [4] or by total extraction with 0.25 M HCl. Selective extraction of histones H2B or molecular sieve fractionation of total or partial extracts [5] does not change the relative concentration of histone H2B variants. The Triton slab-gel electrophoretic method was based on that in [6]. The dimensions of the gel were 100 × 100 × 1.8 mm. The final concentration of components in the running gel were acrylamide 15% (w/v): *N,N'*-methylene bis-acrylamide 0.1% (w/v): Triton X-100 0.37% (w/v): thiodiglycol 0.1% (v/v); and the appropriate concentration of urea. The gel was polymerised in sunlight with 0.25% (w/v) riboflavin. Pre-electrophoresis was performed on all gels, except the urea-gradient gel slabs. The sample wells were then formed in a gel containing half the acrylamide and bis-acrylamide concentration but with all other components as for the running gel. Gels were run 16 h at 16 mA/slab then stained with 0.25% (w/v) Coomassie blue in 50% methanol–10% acetic acid–water, and destained in 10% methanol–10% acetic acid–water (all v/v). We found that the use of riboflavin to initiate polymerization is advantageous because under such regime we did not observe methionyl residue oxidation common when persulfate is used instead. The addition of thiodiglycol provided additional methionyl protection [7].

## 3. Results and discussion

In Triton–urea gel electrophoresis the urea concentration critically affects the mobility of histones [6]. This is demonstrated for the particular isohistones under investigation in fig.1. It appears that 3.8 M urea is optimal to separate a mixture of sperm histones.

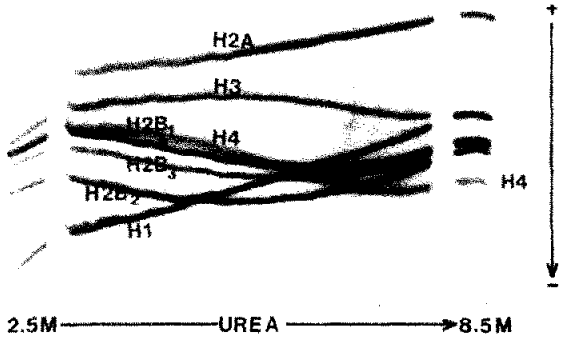


Fig.1. Gel electrophoresis of sperm histones from *Parechinus angulosus* in a transverse urea gradient (2.5–8.5 M), in the presence of 0.37% (w/v) Triton X-100.

* To whom correspondence should be addressed



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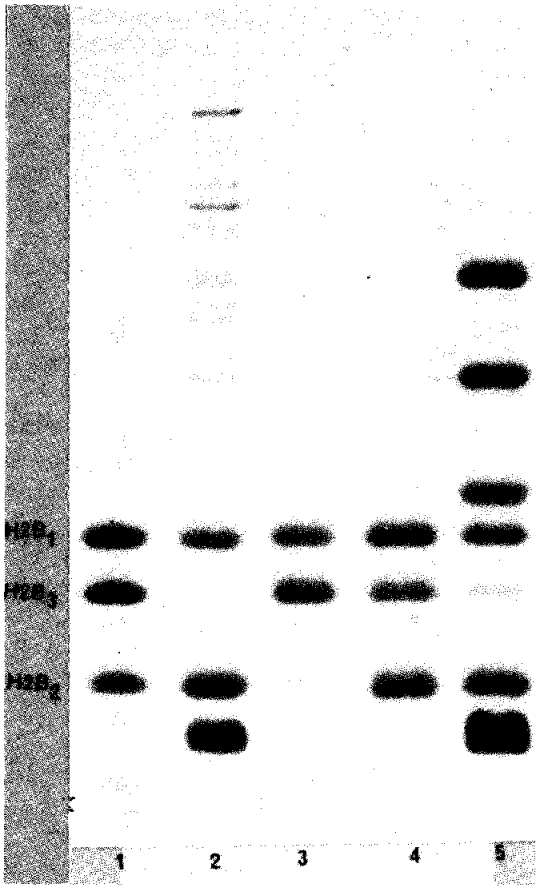


Fig.2. Electrophoresis at 3.8 M urea and 6 mM Triton X-100 of histone extracts from pooled sperm samples: (1) mixture of reference histones; (2) collection site 6; (3) collection site 4 partially purified H2B-fraction; (4) collection site 1; (5) collection site 2 (total histones).

To identify a suitable sea urchin population harbouring all 3 isohistone genes samples were taken from various sites on the Atlantic and Indian shore lines, sites 1–5 and 6–9, respectively.

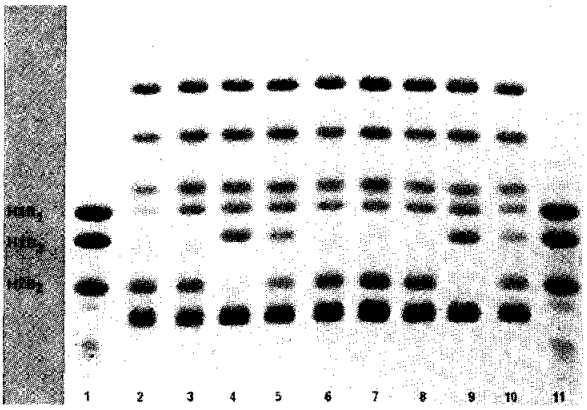


Fig.3. Electrophoresis at 3.8 M urea and 6 mM Triton X-100 of total histone extracts from sperm of individual sea urchins collected at collection site 5. Lanes (1,11) contain a mixture of reference histones.

In histone extracts from sperm pooled from several animals (fig.2) histone $H2B_1$ is present in all samples but the concentrations of $H2B_2$ and $H2B_3$ varied. Samples from sites 6–9 contained only histone $H2B_2$ in addition. $H2B_3$ was not apparent in the pooled sample. Samples from site 4 contained additional $H2B_3$, site 2 samples contained additional $H2B_2$ and those from site 1 contained all 3 isohistones. From this it appeared that animal groups from certain sites expressed all 3 isohistone genes, whereas in other populations the genes for isohistone $H2B_3$ or isohistone $H2B_2$ are either absent or rare. Subsequently the occurrence of the isohistones in individual animals from various sites was investigated. The sea urchin sperm cells of single animals always contain $H2B_1$ and in addition either $H2B_2$ or $H2B_3$ or a mixture of both (fig.3). Histones were then extracted from sperm individually collected from 126 sea urchins. Table 1 confirms the occurrence of the occasional simultaneous expression of all 3 isohistone genes. It appears

Table 1
Distribution of sperm cell isohistones $H2B_1$ $H2B_2$ and $H2B_3$ in individual sea urchins collected at different sites

| Collection site | Number of animals | Number of animals containing isohistone type | | | |
|---|---|---|---|---|---|
| | | $H2B_1$ | $H2B_2$ | $H2B_2 + H2B_3$ | $H2B_3$ |
| 3 | 32 | 32 | 28 | 2 | 2 |
| 4 | 35 | 35 | 0 | 5 | 30 |
| 5 | 28 | 28 | 14 | 7 | 7 |
| 6 | 31 | 31 | 29 | 2 | 2 |

that sea urchins from specific sites may preferentially express either histone $H2B_2$ of $H2B_3$ in addition to the omnipresent $H2B_1$. We had shown that a different sea urchin species *Psammechinus miliaris* also harbours 2 sperm isohistones H2B with distinctly different primary structures [8]. Similarly, 4 sea urchin species collected at the Bay of Naples contained sperm histone H2B variants by SDS gel electrophoretic criteria [9].

The main structural difference in *Parechinus angulosus* between $H2B_1$ on the one side and $H2B_2$ and $H2B_3$ on the other consists in the nature of the repeating pentapeptide occurring in the N-terminal region and the frequency of reiteration of these pentapeptides or their mutated derivatives [10]. In the $H2B_1$ isohistone the pentapeptide has the sequence:

Pro–Thr–Lys–Arg–Ser

whereas in the $H2B_2$ and $H2B_3$ the pentapeptide has the structure:

Pro–Arg–Lys–Gly–Ser

In addition the nature and sequence of the first 45 N-terminal residues in the isotypes 2 and 3 are more similar to each other than to those in the isotype 1. This raises the possibility that the isotype 2 and 3 assume one type of secondary and higher structure which is slightly different from that taken on by the isotype 1.

The occurrence of different types of isohistones may confer on the sperm chromatin advantages in a given type of environment. In this context it may be significant that the isotype 3 occurs mainly on the Atlantic shores with lower water temperature. However the expression of isohistones H2B in sperm cells may be due to another reason. Sperm cell chromatin exhibits no transcriptional activity and contains only a completely repressed set of haploid tightly packed DNA. The haploid set differs from cell to cell only with respect to the presence of the X or Y characteristic. It remains to be shown whether a particular sperm cell isohistone occurs exclusively in one or the other sperm cell types.

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