ELECTROPHORETIC ANALYSIS OF SPERM BASIC PROTEINS IN **SCHROEDERYCHTHYS CHILENSIS** AND COMPARISON WITH OTHER CARTILAGINOUS FISH.

Carlos Olivares*, Ruby Valdivia**, Nelly Lafuente**, Davis Kulak*** and Harold E. Kasinsky***

ABSTRACT. Electrophoretic analysis of sperm basic proteins in **Schroederychthys chilensis** and comparison with other cartilaginous fish.

The sperm basic nuclear proteins from testis of the elasmobranch fish **Schroederychthys chilensis** show 4 main bands, SC1, SC2, SC3 and SC4 (doublet), migrating faster than evolutionarily conservative histone H4 on an acid/urea polyacrylamide gel. In addition, a fainter band, SC5, migrates more rapidly in the region of salmon protamine. In epididymis of S. **chilensis** band SC5 increases in intensity, band SC1 disappears and bands SC2, SC3 and SC4 remain present. The electrophoretic mobilities of S. **chilensis** sperm basic proteins are compared with those in 2 other elasmobranchs **Scylliorhinus caniculus** and **Squalus acanthias**, as well as the holocephalan fish **Hydrolagus colliei**. The results indicate that there are some similarities amongst the sperm basic proteins of these species of cartilaginous fish.

Key words: Elasmobranchs, Sperms, Basic chromosomal proteins.

* Laboratory of Developmental Biology, Universidad de Talca, Talca, Chile.
** Experimental Cytogenetic Laboratory, Faculty of Odontology, University of Chile, Santiago, Chile.
*** Department of Zoology, University of British Columbia, Vancouver, B. C. Canada, V6T 2A9.

Correspondence should be sent to Dr. C. Olivares, Casilla 123-V, Universidad de Valparaíso, Valparaíso, Chile.

RESUMEN. Análisis electroforético de las proteínas espermáticas básicas en **Schroederychthys chilensis** y comparación con otros peces cartilaginosos.

Las proteínas nucleares básicas del testículo del pez elasmobranquio **Schroederychthys chilensis** muestran 4 bandas principales, SC1, SC2, SC3, SC4, las cuales migran más rapidamente que la histona H4 en electroforesis en geles de poliacrilamida urea-acético. Además, se observa una banda más débil, SC5, la cual migra en la región que lo hace la protamina de salmón. En el epidídimo de S. **chilensis** la banda SC5 aumenta en intensidad, la banda SC1 desaparece y las bandas SC2, SC3 y SC4 se mantienen. Las mobilidades electroforéticas de las proteínas básicas de espermatozoos de S. **chilensis** se comparan con las de los elasmobranquios **Scylliorhinus caniculus** y **Scualus acanthias**, así como también con las del pez holocéfalo **Hidrolagus colliei**. Los resultados señalan que existen algunas similaridades entre las proteínas espermáticas básicas de estas especies de peces cartilaginosas.

INTRODUCTION

Spermatogenesis offers an excellent model to investigate the relationship between changes in nuclear protein composition and the structural and functional transitions that chromatin undergoes during the differentiation of the germinal cell line. Sperm chromatin is generally characterized by a highly compacted structure. In a number of species this is obtained after the elimination of histones and non-histone proteins and their replacement by low molecular weight, higly basic proteins, the protamines (Ando et al. 1973, Bloch 1969).

In contrast with the structural conservation of nucleosomal core histones during evolution (Isemberg 1979) protamines differ considerably from one species to another (Bloch 1976). In several fish, sperm DNA is associated with typical protamines: proteins richs in arginine (60 - 80%) and having a very low content or the absence of acidic and hydrophobic amino acids. In other species, as separated phylogenetically as cartilaginous fish, mammals, octopus and insects (Chevaillier 1983, Kasinsky et al. 1985, Kasinsky 1987 and Subirana 1983) basic proteins have been described similar to protamines but containing cysteine. This permits crosslinking of the protamine through -S-S- bonds in the final stages of spermiogenesis, making the sperm chromatin more compact.

In this paper, we analyze electrophoretically the testicular and epi-

didymal basic nuclear proteins from the elasmobranch **Schroederychthys chilensis** and compare their electrophoretic properties with those of sperm basic proteins in other cartilaginous fish.

MATERIALS AND METHODS

Sperm were collected from ripe testis and epididymis of a single representative of the elasmobranch **Schroederychthys chilensis** obtained at the "Instituto de Oceanología", Montemar, Valparaíso, Chile. Testicular sperm were obtained from testicular ampullae opposite the germinal zone. Phase microscopy showed sperm predominantly but some late spermatids may also have been present. Epididymis was cut into small pieces and shaken gently until sperms were released. All stages of purification were carried out at 4ºC. Testicular and epididymal sperm were homogenized in a Potter-Elvehjem homogenizer in buffer A: 150 mM KCl, 0.34 M sucrose, 20 mM Tris-HCl, pH 7.5 and 0.5 mM phenylmethylsulphonyl fluoride (PMSF). After centrifugation at 5000 x g for 10 minutes the cells were washed twice with the same buffer A plus 0.4% Triton X-100 for 15 minutes and centrifuged. Then the testicular sperms were put into 8 M urea, 1.4 B-mercaptoethanol (B-MET) and incubated with magnetic stirring for 10 minutes. Epididymal sperms were placed in a similar medium and incubated for 15 minutes. The pellet was washed twice with buffer A and extracted overnight with two volumes of 0.25 M HCl. After centrifugation, the supernatant was adjusted to 20% (w/v) with solid trichloroacetic acid (TCA). Proteins were precipitated overnight at 0ºC, pelleted, washed with acetone-HCl, then acetone and finally air dried.

S. **chilensis** sperm basic proteins were analyzed by electrophoresis on 15% polyacrylamide slab gels, pH 3.2, 6.25 M urea, 15 x 15 x 0.3 cm, according to Panyim & Chalkley 1969. After electrophoresis, gels were stained with 0.2% (w/v) amidoblack in 30% (v/v) methanol/7% (v/v) acetic acid for 5-8 hours, destained in several changes of 30% methanol/7% acetic acid for 5-8 hours and photographed. The mobilities of S. **chilensis** sperm basic proteins relative to marker protamine were compared with those of the elasmobranch dogfish **Scyliorhinus canicula** (Chauvière **et al.** 1983, Gusse & Chevallier 1978, 1981, Gusse **et al.** 1983 and Sautière **et al.** 1984), and **Squalus acanthias** (Bois **et al.** 1980), as well as the holocephalan ratfish **Hidrolagus colliei** (Bois & Kasinsky 1974 and Kasinsky **et al.** 1985). Either salmon or herring protamine purchased from Sigma Chemical Co., St. Louis, MA., U.S.A., was used as a marker protamine. S. **canicula** epididymal sperm basic proteins (Gusse **et al.** 1983) were a gift of Drs. M. Gusse and P. Chevallier. **Tetrapygus niger** histones were a gift of Dr. M. Imschenetzky. Testis-specific basic proteins from S. **acanthias** (Kasinsky **et al.** 1985) and H. **colliei** (Kasinsky **et al.** 1985) were

obtained using the micromethod of Loule & Dixon 1972 to prepare nuclei and extracted with 0.4% M HCl/5% (v/v) B-MET.

RESULTS

Five basic nuclear proteins present in testicular sperm of **Schroederychthys chilensis** were nominated SC1 to SC5 in Fig. 1, lane 2. Band SC4 is a doublet, perhaps due to post-translational modification. In the case of epididymal sperm only four components fig. 3 occur, SC2 to SC5 (Fig. 2, lane 2). These proteins migrate more rapidly than the sperm-specific histones of the sea urchin **Tetrapygus niger** (Fig. 2, lane 1) and except SC5, more slowly than salmon protamine (Fig. 2, lane 3). Band SC4 is present as a single component and somatic histones are almost completely absent in the epididymis (Fig. 2, lane 2).

A similar profile of testis-specific basic proteins to that of S. **chilensis** (Fig. 3A, lane 2), judging from the electrophoretic mobility relative to herring protamine (Fig. 3A, lane 4 and Table 1). Basic protein SC4 in S. **chilensis** comigrates with the basic protein D6 isolated from S. **acanthias** (Fig. 3, lane 2 and 4) and with the protamine Z3 in S. **caniculus** (Gusse & Chevallier 1981). Likewise basic proteins SC2 and SC3 comigrates with S. **canicula** keratinous protamines Z1 and Z2 respectively, as well as with proteins D4 and D5, respectively, in S. **acanthias** testis. Basic protein SC5 appears to be the S. **chilensis** equivalent of keratinous protamine S4 in S. **canicula**. Basic protein SC3 also has

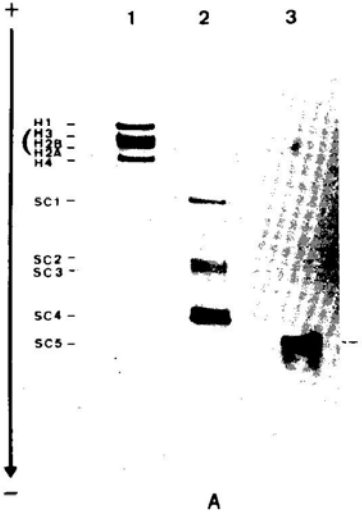

Fig. 1. Electrophoretic profiles of basic proteins from testicular sperm of **Schroederychthys chilensis**. The direction of electrophoresis is from top to bottom. H1 = very lysine-rich histone; H3, H2B, H2A, H4 = core histones; SC = basic proteins from sperm of S. chilensis; P = salmon protamine.

Lane 1. Sperm histones of the sea urchin **Tetrapygus niger**

Lane 2. Basic proteins from testicular sperm of S. **chilensis**.

Lane 3. Salmon protamine.

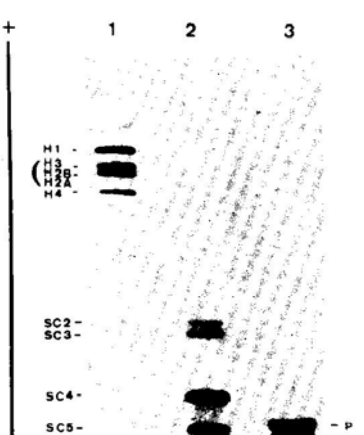

Fig. 2. Electrophoretic profiles of basic proteins from epididymal sperm of **Schroederychthys chilensis.**

Lane 1. Sperm histones of **T. niger.**
Lane 2. Basic proteins from epididymal sperm of **S. chilensis.**
Lane 3. Salmon protamine.

a similar relative electrophoretic mobility to that of basic proteins R4 and R5 in the holocephalan ratfish **Hydrolagus colliei** (Table 1).

DISCUSSION

The electrophoretic pattern obtained for the basic proteins from sperm of **Schroederychthys chilensis** is very similar to that of the lesser spotted dogfish **Scyliorhynus canicula** described previously by Gusse & Chevaillier 1978. The gel patterns only differ in the number of intermediate proteins: only one (SC1) in **S. chilensis** but two (SC1 and SC2) in **S. canicula.** This may be due to differences in the stages of sperm maturation as protein S2 disappears before S1 in **S. canicula** (Gusse & Chevaillier 1981). Also, in **S. chilensis,** during the final maturation of spermatozoa in the epididymis, protein SC1 is completely eliminated (Fig. 2). At this stage, the electrophoretic profile for basic proteins of **S. chilensis** is close to that described for **S. canicula** by Gusse & Chevaillier 1978, 1981, both representatives of the family Scyliorhinidae in the order Carcharhiniformes

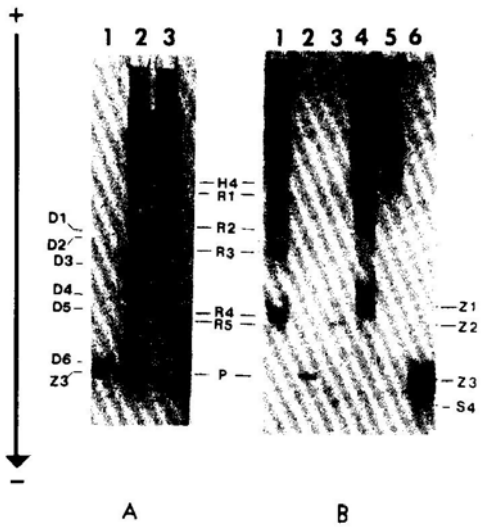

Fig.3. Electrophoretic profiles of testis- or epididymis-specific proteins in the elasmobranch fishes **Scylliorhinus caniculus** and **Squalus acanthias** and the holocephalan fish **Hydrolagus colliei**. The direction of electrophoresis is from top (+) to bottom (−). H4 = evolutionarily conservative core histone H4; Z1, Z2, S4 = sperm-specific keratinous protamines of S. **caniculus**; Z3 = protamine of S. **caniculus** sperm; D1-D6 + testis-specific basic proteins of S. **acanthias**; R1-R5 = testis-specific basic proteins of H. **colliei**; P = herring protamine.

Gel A

Lane 1. Protamine Z3 purified from **S. caniculus** epididymis and vas deferens by Gusse **et al.** 1983, using 0.25 M HCl extraction.

Lane 2. Testis-specific basic proteins extracted from the dogfish S. **acanthias** with 0.4 M HCl/5% B-MET.

Lane 3. Testis-specific basic proteins extracted from the ratfish H. **colliei** with 0.4 M HCl/5% B-MET.

Gel B

Lane 1. H. colliei testis-specific basic proteins.
Lane 2. S. caniculus protamine Z3
Lane 3. S. caniculus scylliorhinines Z1, Z2, S4 extracted from epididymis and vas deferens (Gusse et al. 1983) with 10mM DTT and alkylated with 12.5 mM iodo acetamide.
Lane 4. S. acanthias basic proteins from an inmature testis.
Lane 5. H. colliei somatic histones from heart cell suspension extracted with 0.4 M HCl/5% B-MET.
Lane 6. Herring protamine.

(Compagno 1973). Likewise, the testis-specific basic proteins from the spiny dogfish Squalus acanthias have similar electrophoretic mobilities with those from S. chilensis and S. caniculus. The spiny dogfish is a representative of a different family of elasmobranchs; Squalidae corresponding to the Squaliformes (Compagno 1973). This electrophoretic similarity in the sperm basic proteins of elasmobranch fishes is further supported by evidence obtained from cytochemical studies in different cartilaginous fishes, including Squalus (Bois et al. 1980) Scyliorhinus (Gusse & Chevaillier 1978) and the lognose skate Raja rhina (Bois & Kasinsky 1976), as well as, the holocephalan ratfish Hydrolagus colliei (Bois & Kasinsky 1976). In each organism somatic histones are replaced by protamine and keratinous protamine during spermiogenesis.

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TABLE 1. Relative electrophoretic mobilities of sperm basic proteins in **Schroederychthys chilensis** and three other cartilaginous fish.

Species	Basic Protein	Rp (a)	
		Testis	Epididymis
Elasmobranchii:			
Scroederycthys chilensis (b)	SC1	0.55	---
	SC2	0.76	0.73
	SC3	0.78	0.76
	SC4	0.90-0.93	0.93
	SC5	1.00	1.01
Scyliorhinus caniculus (c)	Z1	---	0.72
	Z2	---	0.78
	Z3	---	0.94
	S4	---	1.01
Squalus acanthias (d)	D1	0.52	---
	D2	0.54	---
	D3	0.62	---
	D4	0.72	---
	D5	0.78	---
	D6	0.94	---
Holocephali:			
Hydrolagus colliei /d)	R1	0.41	---
	R2	0.51	---
	R3	0.59	---
	R4	0.77	---
	R5	0.79	---

(a) Rp = relative electrophoretic mobility. Rp of salmon or herring protamine = 1.00; Rp of core histone H4 = 0.36. 15% polyacrylamide gel as described by Panyim & Chalkley 1969, 15 cm, amidoblack staining.

(b) Extracted with 0.25 M HCl (See Fig. 1, 2).

(c) Samples were gift of Drs. M. Gusse & P. Chevaillier, as prepared by Gusse **et al.** 1983 from sperm of epididymis and vas deferens is extracted with 0.25 M HCl. Scylliorhinines Z1, Z2, S4 were reduced with 10 mM dithiothreitol (DTT) and alkylated with 12 mM iodoacetamide before acid extraction.

(d) Nuclei prepared from testis by the micromethod of Louie & Dixon 1972 were extracted with 0.4 M HCl/5% B-MET. (See Fig. 3).