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Proteomic Studies of Various Organs and Tissues of the Frilled Shark *Chlamydoselachus anguineus*

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Feico M.A.H. Schuurmans Stekhoven, Tsung-Han Lee, Pei-Jen Wang, Nian-Hong Jang-Liaw, Sho Tanaka, and Andrew R. Bottrill (2012) Proteomic studies of various organs and tissues of the frilled shark *Chlamydoselachus anguineus*. *Zoological Studies* **51**(8): 1248-1269. In a search for the presence or absence of the Cl^- channel phospholemman in 9 different tissues from organs of the most ancient shark on earth (*Chlamydoselachus anguineus*), we came to the conclusion that these did not contain the Cl^- channel we sought. On the other hand, a large variety of proteins involved in cellular signaling and transport regulation were detected (36.7% of all analyzed protein components), suggesting that in its long history, *C. anguineus* has developed proteins chemically and mechanistically similar to those of modern sharks. http://zoolstud.sinica.edu.tw/Journals/51.8/1248.pdf

Key words: *Chlamydoselachus anguineus*, Proteomics, Localization, Function.

In a previous study on a variety of shark orders and families (Schuurmans Stekhoven et al. 2010), we established that the chloride channel phospholemman, thought to be involved in osmoregulation, is not a standard component of a shark's rectal gland. Although in the orders of Squaliformes and Lamniformes, a phospholemman is consistently present, in the orders of Orectolobiformes and Carcharhiniformes, it is only present in a single family in each of these 2 orders (in the Orectolobidae and Triakidae, respectively). Remarkably, each of them (*Orectolobus ornatus* and *Triakis scyllium*) belonged to the phylogenetically oldest forms tested. All members of the Carcharhinidae and Sphyrnidae studied, which belong to the so-called modern sharks, lacked the channel in their rectal glands.

To determine the situation at the other end of the phylogenetic scale, we studied the occurrence of the phospholemman in the frilled shark *Chlamydoselachus anguineus* Garman 1884, which belongs to the Chlamydoselachidae of the Hexanchiformes (Compagno 1999).

To avoid the chance of missing the presence of the phospholemman in the 16-18-kDa protein range, which is the range of the cytoskeleton-bound form (Kelly et al. 2004), we included proteins of this mass range in our analyses. When it turned out that the Cl^--channel phospholemman did not show up in our analyses, we decided to perform a literature study on the intracellular location and function of the proteins that did show up in the

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*To whom correspondence and reprint requests should be addressed. E-mail:F.Stekhoven@gmail.com proteomic analyses, rather than determine the reason for the absence of the phospholemman in the various tissues.

Tissues and organs that were investigated for the presence of the phospholemman were the same as those tested before: the rectal gland, stomach, brain, spleen, heart, gills, kidneys, and colon (Schuurmans Stekhoven et al. 2003), but this time using a different shark species (*C. anguineus*, vs. *Squalus acanthias*) and a different technique: mass spectrometry de novo sequencing (Schuurmans Stekhoven et al. 2010) instead of antibody staining on blots (Schuurmans Stekhoven et al. 2003). It turned out that many of the sequenced proteins were related to teleost fishes that developed later than the Chondrichthyes, even to Amphibia, like the African (*Xenopus laevis*) and western clawed frogs (*X. tropicalis*), and also birds and bats. In addition, the various sequences related to cellular signaling and thus to advanced events of biochemical process control, indicate modern rather than earlier developed systems. This again would fit in with the concept that *C. anguineus* (like modern sharks) lost its potential for using the chloride channel phospholemman as a control protein for osmoregulation.

MATERIALS AND METHODS

The shark (*Chlamydoselachus anguineus*) and its organs

Three members of this species (all female) were caught by trailnet fishery, 2 in waters around Taiwan, and one in Suruga Bay (Japan). The animals were transported on ice to respective local laboratories, where the organs were excised. For the Taiwanese samples, the rectal gland (0.41, 0.59 g), stomach (16.1, 29.8 g), brain (1.75, 2.5 g), spleen (9.1, 10.3 g), heart (3.0, 3.85 g), gills (19.8, 23.2 g), liver (24.9, 31.2 g), and kidneys (4.1, 11.5 g) were removed. At Tokai Univ., Japan, only lightweight organs were removed: the rectal gland (1 g), brain (3.45 g), kidneys (13 g), and heart (not weighed). The materials were transported in screw-capped tubes, filled with 25% glycerol in 50 mM triethanolamine-HCl (pH 7.0) at -80°C in a plastic foam box with ice bags of that temperature (shipped from Taiwan) or in a thermos, filled with -80°C ice, and placed in a foam box with ice bags of -80°C as above (shipped from Japan). Upon arrival in the Animal Ecology and Ecophysiology Department in Nijmegen, organs and tissues in their screw-capped tubes were stored in a freezer at -20°C.

Subfractionation of the organs

Organs were stripped of their protective tissues or sheets and homogenized (10-20 strokes) with a motorized Potter-Elvehjem homogenizer (home-made Potter-Elvehjem homogenizer) at 0-4°C in medium containing 50 mM mannitol, 2.5 mM K^+-Hepes, 1 mM K^+-EDTA at pH 7.6, and 0.5 mM phenylmethylsulfonyl fluoride as an anti-autolysis agent (Schuurmans Stekhoven et al. 2003).

With the heart, stomach, gills, kidneys, and colon, only the internal cell layers could be used and had to be scraped from the outer layers with a scalpel before homogenization. The stomach and colon were emptied of their food remains, which were green in the colon, before scraping off the internal cell layers.

After homogenization, the homogenates were subjected to fractional centrifugation at 4°C, except for the liver homogenate, which was run at 10°C in order to avoid solidification of the liver oil, which demonstrated a melting range of 0.4-5.2°C as determined by differential scanning calorimetry (DSC; Mettler Toledo, Tiel, The Netherlands). This phase transition agreed with the melting range of 3.98 ± 0.59°C for glycerol trioleate (Knothe and Dunn 2009). Cooling from 20 to 0°C caused an opposite phase transition at 12.2°C, which matched the melting point of 12.6°C of oleic acid (Knothe and Dunn 2009).

The speed of fractionated centrifugation was 1200 g for 10 min, yielding F1 (fraction 1). The supernatant was centrifuged at 9000 g for 10 min to yield F2 (fraction 2), and the supernatant of fraction 2 was centrifuged for 90 min at 10^5 g to yield fraction 3 (F3). The liver (*C. ang.* from Taiwan) and kidney (*C. ang.* from Suruga Bay) did not yield an F3 fraction, apparently due to cohesion with one or two of the other fractions (see “Results and Discussion”).

Further procedures

Procedures of protein determination, electrophoresis with staining and destaining of the gel, and mass spectrometric (MS) identification of the protein bands after excision from the gel were identical to those in our previous publication (Schuurmans Stekhoven et al. 2010). Results of the latter identification with the apparent molecular mass on the gel, accession no., sequence, and data with references to intracellular localization and function are presented in tables 2-14 using the following abbreviations: *C. ang.*, *C. anguineus*; T. sea, sea around Taiwan; S. bay, Suruga Bay. The tables are arranged in sequence from the head (brain) to tail (rectal gland).

RESULTS AND DISCUSSION

Most of previous information on *Chlamydoselachus anguineus* was related to morphology, such as the brain pattern (Masai 1961, Masai et al. 1981), cranial anatomy (Allis 1923), teeth and dermal denticles (Gudger and Smith 1933), reproductive biology (Nishikawa 1898, Tanaka et al. 1990), the male urogenital system (Gilbert 1943), and food habits (Kubota et al. 1991). In more-recent publications (after 1990) biochemical data, like the presence of a 2nd immunoglobulin (Kobayashi et al. 1992), and the cytochrome b gene (Kitamura et al. 1996) were predominant. Therefore it was reasonable to begin a more-extensive and varied biochemical study of this animal, using a proteomics approach provided by MS identification (see Schuurmans Stekhoven et al. 2010).

Differences with the Squalidae (structural aspects)

Immediately after the start of this study, it became apparent that several tissues had an internal protein structure that differed from that of the well-known *S. acanthias*. This is evident in table 1, which demonstrates protein ratios for fractions F1-F3. Most striking in this list is the absence of an F3 fraction from the liver of *C. ang.* T. sea, combined with a high ratio number for F1. The same was true for the kidney sample of *C. ang.* S. bay. Other differences were F1 ratio numbers (exceeding 6.5) for *C. ang.* T. sea brain of 7.8, *C. ang.* T. sea heart of 9.4, *C. ang.* T. sea kidney of 21.5, and *C. ang.* T. sea colon of 19.4. Remarkably similar F1: F2: F3 ratios were scored by rectal glands of *C. anguineus* with respect to *S. acanthias* (Table 1). Standard errors for these were 0.7 for F1, 0.035 for F2, and 0.0 for F3.

The above-described coherences may at least

Table 1. Protein ratios of centrifugal fractions F1-F3 for the successive tissues (brain to rectal gland) of *C. anguineus* from the Taiwanese sea (T. sea) or Japanese Suruga bay (S. bay) in comparison to earlier data (cf. Schuurmans Stekhoven et al. 2003) for *Squalus acanthias* from the North sea (here published for the 1st time)

Genus + catch location	Tissue	F1:F2:F3
C. ang. T. sea	brain	7.8:2.8:1.0
C. ang. S. bay	brain	4 :2.2:1.0
S. acanth. North sea	brain	2.7:1.0:1.0
C. ang. T. sea	gill	6.5:1.0:1.0
S. acanth. North sea	gill	6.1:0.5:1.0
C. ang. T. sea	heart	9.4:3.3:1.0
S. acanth. North sea	heart	3.5:0.3:1.0
C. ang. T. sea	liver	13.5:1.0:0
S. acanth. North sea	liver	1.6:0.5:1.0
C. ang. T. sea	stomach	6.8:0.8:1.0
S. acanth. North sea	stomach	2 :0.8:1.0
C. ang. T. sea	spleen	1.7:1.5:1.0
S. acanth. North sea	spleen	2.5:0.3:1.0
C. ang. T. sea	kidney	21.5 :11.2:1.0
C. ang. S. bay	kidney	27.5:1.0:0
S. acanth. North sea	kidney	4.5:0.7:1.0
C. ang. T. sea	colon	19.4:3.9:1.0
S. acanth. North sea	colon	1.3:0.2:1.0
C. ang. T. sea	rectal gland	1.6:0.9:1.0
C. ang. S. bay	rectal gland	2.1:0.9:1.0
S. acanth. North sea	rectal gland	3.9:0.8:1.0

partly be understood by interactions of membranes with the cytoskeleton. Evidence is presented in table 9 (kidney F1) by L-plastin as an additional construct in the organization of actin filaments, moesin, in table 10 (kidney F2) as a crosslinker between the plasmalemma and microtubules and filamin in table 11 (kidney F3), linking actin filaments to the plasma membrane. The relatively high F1/F3 ratio in the brain of *C. ang.* T. sea can also be explained by a possible anchoring of actin filaments to the plasma membrane by the myelin basic protein (Table 2). In the colon (Table 12), α-actinin was active in ligating actin (microfilaments) to the cell membrane. The F1 liver sample contained contractin α, which helps support the plasma membrane (Table 6). In addition, the relatively high lipid content of the liver (see "Materials and Methods") also interacted and glued proteins together. A phenomenon related to this is the Ca^{2+}-myristoyl switch (Braunewell and Klein-Szanto 2009), although in our case, the lipids involved were a mix of glycerol trioleate and oleic acid (see "Materials and Methods").

Intracellular signaling and transport regulators

To control cellular metabolism, cellular signaling, in which GTPases and their control

Table 2. Brain (*C. ang.* T. sea) F3 fraction: localization and function of the 8.1-13.2 kDa proteins

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
40S ribosomal protein (9.3 kDa, NP_998199, E^{52}-R^{63})	ER	protein synthesis
Guanine nucleotide binding protein γ-2 (9.3 kDa, NP_001005038, A^{33}-K^{46})	Plasmalemma or Golgi system	regulation of protein transport from trans- Golgi network to plasma membrane (Irannejad and Wedegaertner 2010)
Ki-ras-1 protein (9.3 kDa, ACO50969, Q^{150}-R^{161})	plasmalemma, Golgi complex, recycling endosomes (Lopez-Alcala et al. 2008)	cell proliferation and regulated apoptosis via activation of PI3K (phosphoinositide kinase 3) (Rosseland et al. 2008), showing similarity with the Rho c activation of Rho-kinase dependent phosphorylation of LIM-kinase, which in turn activates Cofilin in its depolimerisation of actin (Bravo-Cordero et al. 2011, cf. *C. anguineus* S. bay, brain)
Myelin (8.1 kDa, Q91439, D^{133}-K^{143})	axon sheet	increase of impulse propagation (cf. brain S. bay)
Myelin basic protein (13.2 kDa, P98190, K^{105}-K^{116})	Plasmalemma (oligodendrocyte), cytoskeleton (actin filaments, microtubules), nucleus (Boggs 2006)	possible role in anchoring actin filaments to the membrane and sensing signals transmitted through the membrane (Boggs 2006)
β-globin (8.1 kDa, ACY03393, Y^{42}-K^{60})	part of haemoglobin (red cell membrane, Shaklai et al. 1977)	involved in oxygen transport
Histone H4 (13.2 kDa, XP_002595673, T^{81}-K^{92})	plasmalemma and nucleus (cf. heart, stomach, spleen, kidney and rectal gland)	antimicrobial agent (Smith et al. 2010) and component of nucleosome core (Stryer 1995, p. 980)
14 kDa transmembrane protein (13.2 kDa, Q91499, V^{59}-R^{67})	plasmalemma	receptor, causing interferon induced intracellular signaling (cf. gill and rectal gland)
Synaptosomal associated protein 25-A (SNAP-25A, 13.2 kDa, Q5TZ66, I^{60}-K^{69})	plasmalemma	t-snare, cooperating with syntaxin 1 in exo- and endocytosis of neurotransmitters (cf. *C. anguineus* Suruga bay-brain)
Ubiquitin (9.3 kDa, ACO11160, T^{12}-K^{27})	Plasmalemma and cytoskeleton (microtubules, Hicke and Dunn 2003, Murti et al. 1988)	involved in the ubiquitin-proteasome pathway (Hegde 2010)
Betaine homocysteine methyl-transferase (8.1 kDa, ABF066 73, L^{12}-K^{30})	Cytoskeleton (microtubules, Sandu et al. 2000)	conversion of betaine + homocysteine → dimethylglycine + methionine

Animals (common names) belonging to the accession numbers. NP_998199 + Q5TZ66: zebrafish; ACO50969: European sea bass; P98190: dusky shark; XP_002595673: Florida lancelet; ABF06673: gilthead seabream; NP_001005038: western clawed frog; Q91439: spiny dogfish; ACY03393: spiny mouse; Q91499: marbled electric ray; ACO11160: sea louse.

mechanism operate via an exchange factor GEF and activating protein GAP, plays an essential role in intracellular trafficking. We summarized these and other transport proteins, such as those involved in transmembrane (including ion) transport, and compared them to other, often mere enzymatic or structural proteins, present in the membrane fractions of the shark's tissue components.

The following components were involved in the signaling + transport category (with their table numbers in parentheses): Ki-ras-1 protein (Table 2); SNAP-25A (Table 2); 14-kDa transmembrane protein (Tables 2, 4, 13); G-protein, γ-2 (Tables 2, 8); Skp1 (Table 3); VILIP-1 (Table 3); Arf1 (Tables 3, 5, 7, 11, 12); VAMP1 (Table 3); cellubrevin 3 (Tables 3, 14); syntaxin-binding protein (Table 3); SCP-2 sterol transfer protein (Tables 3, 7); α-synnuclein (Table 3); VDAC (Tables 3, 4, 5, 7, 8, 9, 10, 11); AP-2 complex subunit σ (Table 3); Rho c and Rho c-like (Tables 3, 7, 12); Rab-1a (Tables 6, 7, 9, 10, 12); Rab-1b (Table 7); Rab10 (Table 3); Rab11b (Table 7); V-type proton ATPase, subunits a and d1 (Table 8), a (Table 10), a, b1, or b2 (Table 11); ADP/ATP translocase (Tables 4, 5, 6, 7, 10, 14); aralar (Tables 5, 9, 11); ER Ca^{2+}-ATPase 2 (Table 8); myosin light polypeptide 6 (Table 6); myosin regulatory light chain 2 (Table 12); dynein light chain (Table 13); ARP1 actin-related protein 1 homolog A (Table 6); cell division control protein 42-like protein (Tables 8, 12); K^+-transporting ATPase α-chain 1-like (Table 7); Na^+/K^+-ATPase α-subunit (Tables 9, 10, 11, 13, 14); $Na^+/K^+/2\ Cl^-$ cotransporter (Tables 13, 14); SAM50 (Table 7); flotillin-1 (Tables 9, 11); flotillin-2a (Table 10); neurocalcin-δA (Table 12); RNA-binding protein FUS (Table 12); 14-3-3 protein (Table12); and nucleoside diphosphate kinase (Tables 12, 14). Summation yielded78 components, which in turn accounted for 36.3% of all determined components, reflecting an influential contribution to a cell's biochemical survival.

Some highlighted processes as displayed by the analyses

During our analyses, we encountered a number of processes that underlie the up-to-date biochemical situation for *C. anguineus*.

1. The synaptic vesicle cycle uses synaptosomal-associated protein 25-A (SNAP 25-A) as a t-snare (Table 2) in conjunction with syntaxin (t-snare) and cellubrevin (v-snare, Table 3).

2. The tubulovesicular recycling process of H^+/K^+-ATPase is via the apical plasma membrane, including HCL secretion, followed by endocytosis and subsequent transport by early and recycling endosomes, or degradation (Forte and Zhu 2010). Rab 1a and Rab 11b (Table 7) are involved in this process. Rab 11b is more or less specific to the recycling process, but Rab 1a was also included in tables 6 (F1 liver fraction), 9 (F1 kidney fraction), and 10 (F2 kidney fraction) as a recruiter of the kinesin-like protein, Kifc1, in early retrograde

Table 3. Brain (*C. ang.* S. bay) F3 fraction: localization and function of the 8.7-19 kDa proteins

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
Barrier to autointegration factor (BAF, 8.7 kDa, ACI69910, A^{43}-K^{54})	inner nuclear membrane; in the interphase also in nuclear interior + cytosol (Shimi et al. 2004; Segura-Totten et al. 2002)	interaction with DNA and LEM proteins (Lap 2β, emerin and Man 1) are critical for chromatin decondensation and nuclear envelope growth
Ras-related protein Rab-10 (8.7 kDa, ACM08424, A^{162}-R^{172})	transGolgi network + early endosomes	modulator of Golgi → sorting endosomes protein transport (Kaddai et al. 2008)
Transcription elongation factor B polypeptide 1 (S-phase kinase-associated protein-1, Skp1, 12 kDa, ADO28002, L^{21}-K^{32})	plasmalemma (Boyer et al. 2004), early endosomes and trans-Golgi network (Hermand 2006) and proteasomes (Hegde 2010)	kinase dependent catalysis of ubiquinilated protein breakdown (Hermand 2006), e.g. in the process of synaptic plasticity (Hegde 2010) involving proteasomes; recycling of protein (snare Snc1) from early endosomes to trans-Golgi network, back to the plasma membrane (Hermand 2006)
Visinin-like protein 1 (VILIP -1= neuronal Ca^{2+}-sensor protein, 19 kDa, ACI34363, V^{119}-K^{130})	via Ca^{2+}-induced myristoyl switch to plasmalemma, nuclear membrane, Golgi system (Spilker et al. 2002) and clathrin- coated vesicles (Blondeau et al. 2004)	membrane trafficking, endo- and exocytosis, and modulation of various membrane- bound receptors (Braunewell and Klein Szanto 2009)

Table 3. (Continued)

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
ADP-ribosylation factor-1 (Arf 1, 19 kDa, NP_001158642, Q^{127}-K^{141})	cis/trans Golgi & plasmalemma (Chavrier and Goud 1999; D'Souza-Schorey and Chavrier 2006)	protein trafficking via COPI or clathrin coated vesicles from ER to Golgi and plasmalemma (cf. heart, stomach, kidney and colon)
Rac-1 (19 kDa, ADK54936, Y^{154}-R^{163})	plasmalemma (lamellipodia) + nuclear membrane (Michaelson et al. 2001)	remodeling of actin cytoskeleton (Hall 1998)
Vesicle-associated membrane protein 1 (VAMP1= synaptobrevin, 8.7 kDa, NP_998563, A^{70}-K^{86})	plasmalemma	v-snare in synaptic vesicle cycle, cooperating with syntaxin 1 and SNAP-25 (t-snares) in exo- and endocytosis (Chen and Scheller 2001)
Cellubrevin 3 (8.7 kDa, NP_001135278, A^{51}-K^{67})	plasmalemma	v-snare in neuronal support tissues (glial cells and astrocytes) and expressed in glialcells with the t-snares syntaxin and SNAP-25 (Chilcote et al. 1995); in astrocytes with the t-snare syntaxin only (Parpura et al. 1995)
Syntaxin binding protein 1 (munc 18-1, 8.7 kDa, NP_001093335, V^{30}-R^{39})	plasmalemma	Catalyst of VAMP2/syntaxin 1+SNAP-25 mediated synaptic vesicle fusion with the plasma membrane for transmitter release or fusion of syntaxin 1 alone (Shen et al. 2007)
AP-2 complex subunit σ (AP2S1, 14.5 kDa, ADO 28112, V^{110}-R^{124})	plasmalemma	functions in clathrin-dependent synaptic endocytosis (Jarousse and Kelly 2001; Slepnev and De Camilli 2000)
SCP-2 sterol transfer protein (14.5 kDa, NP_001006093, L^{130}-K^{139})	Plasmalemma (lipid raft and caveolae)	controls multiple lipid signaling pathways (Schroeder et al. 2007, cf. stomach)
α-Synnuclein (12 kDa, ACO 14186, I^{106}-K^{121})	synaptic vesicles and plasmalemma	modulator of synaptic vesicle cycle in endo- and exo- cytosis of neurotransmitters (Oaks and Sidhu 2011)
Voltage-dependent anion selective protein 2 (VDAC, 14.5 kDa, NP_001089399, W^{75}-K^{93})	mitochondrion and plasmalemma	pore forming protein in the mitochondrial outer membrane, release of cytochrome c, but also in the plasma membrane (NADH: ferricyanide reductase, Lawen et al. 2005), functioning in controlled cell death. Further VDAC interacts with the dynein light chain (Tctex1) and the heat shock protein PBP74 (Schwartzer et al. 2002), which are involved in intracellular transport (cf. gill, heart, stomach, spleen and kidney)
Myelin membrane protein (19 kDa, P98190, K^{105}-K^{116})	axon sheet	increase of impulse propagation
Single-stranded DNA binding protein 1 (SSBP1, 14.5 kDa, XP_002197530, N^{57}-R^{71})	Mitochondria (Tiranti et al 1995)	DNA replication
Neuronal migration protein doublecortin-like (DCL, 12 kDa, XP_002938827, S^{79}-R^{89})	neuroblastoma cell lines: cytoskeleton (microtubules)	involved in mitotic spindle stability and length (Vreugdenhil et al. 2007)
β-tubulin (12 kDa, ABY48069, M^{363}-K^{379})	cytoskeleton	component of microtubules
γ-aminobutyric acid receptor- associated protein-like 2 (GABARAP, 14.5 kDa, NP_001158581, V^{36}-K^{46})	trans-Golgi network, cytoskeleton (microtubules), endosomes and postsynaptic membrane	involved in the binding of the γ-aminobutyric acid receptor (GABA_AR to the postsynaptic membrane (Coyle and Nikolow 2003)
Rho-related GTP binding protein (Rho c, 19 kDa, ADO27925, Q^{51}-R^{68})	cytoskeleton (microtubules) (Wheeler and Ridley 2004) and invadopodia (Bravo-Cordero et al. 2011)	vesicle transport (Wheeler and Ridley2004) and indirect control of LIM-kinase dependent actin depolimerisation by Cofilin (Bravo-Cordero et al. 2011, cf. stomach and colon)

Animals (common names) belonging to the accession numbers. ACI34363 + ACI69910 + ACM08424 + NP_00135278: Atlantic salmon; NP_001158581 + NP_001158642: rainbow trout; NP_998563 + NP_001006093: zebrafish; ADO27925 + ADO28002 + ADO28112: blue catfish; NP_001089399 + NP_001093335: African clawed frog; XP_002938827: western clawed frog; NP_001232379 (XP_002197530): zebra finch (finch is a song bird); ADK54936: large yellow croaker; ACO14186: northern pike; P98190: dusky shark; ABY48069: spiny dogfish.

vesicular transport and thus could similarly be involved in this tubulovesicular recycling process.

3. The 14-kDa transmembrane protein, known as interferon-inducible transmembrane protein, acts as an activator of antiviral signaling activities within cells of the brain (Table 2), gills (Table 4), and rectal gland (Table 13), of which the latter 2 tissues are intimately in contact with the external environment. In addition, this signaling component was also encountered in our previous study on the phospholemman of the rectal gland of a few Squaliform sharks (a 14.4-kDa band in *Deania calcea* and a 13.5-kDa band in *Etmopterus princeps*), meaning that its presence in the body is fairly widespread. Further, the interferon-inducible transmembrane protein is not limited to the Chondrichthyes, but is also present in teleosts and mammals (Wan and Chen 2008). The intracellular protein kinase C activation site, S^{51}, is 8 residues to the left of our tryptic fragment V^{59}-R^{67}.

4. Plasmalemmal α-enolase causes dehydration of 2-phosphoglycerate, binding of proteases what could lead to infections and invasions and metastasis. The enzyme, which was bound to the plasmalemma and cytoskeleton, was found in the gills (Table 4) and kidney (F2 fraction, Table 10). Infection is caused by conversion of plasminogen to plasmin after binding to the C-terminal lysine residues of α-enolase (Diaz-Ramos et al. 2012). In tumor cells, α-enolase is upregulated and supports

Table 4. Gill (*C. ang.* T. sea) F3 fraction: localization and function of the 10-20 kDa proteins

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
40S ribosomal protein S18 (20 kDa, AAP20213, I^{14}-K^{22})	ER	protein synthesis
Histone H2A (10 kDa, CAF 98838, A^{57}-R^{71})	plasmalemma (Schuurmans Stekhoven et al. 2004) and nucleus	antimicrobial agent (Schuurmans Stekhoven et al. 2004), and component of nucleosome core, involved in peptide-mediated gene transfer (Balicki et al. 2002) (cf. heart, kidney and rectal gland)
14 kDa transmembrane protein (10 & 14.5 KDa, Q91499, K^{58}-R^{67} & V^{59}-R^{67})	plasmalemma	receptor that causes interferon induced intracellular signaling (Yap et al. 1986), eventually on the transcriptional level (Pfeffer and Colamonici 1991) (cf. brain and rectal gland)
Voltage-dependent mitochondrial anion selective channel 2 (20 kDa, NP_001089399, W^{75}- K^{93})	mitochondrion and plasmalemma (cf. brain, heart, stomach, spleen and kidney)	(cf. brain, heart, stomach, spleen and kidney)
Cell division control protein 42 (Cdc42) homolog precursor (14.5 & 20 kDa, ACI69167, T^{108}- R^{120})	plasmalemma and cytoskeleton (Uniprot Knowledgebase)	
α-enolase (14.5 kDa, ACN10247, A^{33}-R^{50})	plasmalemma and cytoskeleton (Liu and Shih 2007)	dehydration of 2-phosphoglycerate to phosphoenolpyruvate, and binding of proteases, leading to infection and invasions, metastasis (Liu and Shih 2007, cf. kidney F2)
Ras protein (RhoA-like, 10 kDa, ACY03275, Q^{52}-R^{68})	cell cortex and cleavage furrow in cytokinesis (Nishimura & Yonemura 2005)	Mitosis under control of ECT2 RhoGEF) and MgcRacGAP (RhoGAP) (Nishimura & Yonemura 2005; Yüze et al. 2005)
Ras-related protein Rap-1b precursor (14.5 kDa, ACI69891, I^{152}-R^{163})	microvesicles from plasmalemma or endosomal membrane (cf. liver and kidney)	
Guanine nucleotide binding protein G(i), α2 subunit (20 kDa, NP_001136187, E^{146}-R^{162})	plasmalemma, centrosomes and cleavage furrow (Cho and Kehrl 2007)	involved in mitosis, in particular the orientation of the mitotic spindle (Woodard et al. 2010)
ADP/ATP translocase 2 (10 kDa, ACI69327, Y^{81}-K^{92})	mitochondrion	ATP/ADP exchange (cf. heart, liver, stomach, kidney and rectal gland)

Animals (common names) belonging to the accession numbers. ACI69167 + ACI69327 + ACI69891 + ACN10247 + NP_001136187: Atlantic salmon; ACY03275: white spotted bamboo shark; AAP20213: red sea bream; Q91499: marbled electric ray; CAF98838: spotted green puffer fish; NP_001089399: African clawed frog.

anaerobic proliferation (the Warburg effect) (Diaz-Ramos et al. 2012, see Warburg 1955). This does not imply that a high glycolysis rate unavoidably causes cancer. α-Enolase protein expression is carcinogenic, and it attaches to the cell membrane.

5. Worthy of note was the presence of glutamine synthetase and mitochondrial aspartate aminotransferase in the liver (Table 6), which are involved in the synthesis pathway of urea as an osmotic stabilizer in the high-osmotic environment of seawater.

CONCLUSION

In an overview of our data, considering the absence of the phospholemman in all tissues and organs tested, and the many regulatory and transport-directed systems that *Chlamydoselachus anguineus* incorporates, we consider *C. anguineus* a well-adapted elasmobranch fish, even though its history goes back to the Permian period (220-280 million years ago; mya) when it split off from the Neoselachians). For comparison, the existence of teleosts, which comprise 70% of the reference sequences, began 110-160 mya (Volff 2005). Only

Table 5. Heart (*C. ang.* T. sea) F3 fraction: localization and function of the 7.9-18.2 kDa proteins

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
ADP-ribosylation factor 1 (Arf 1, 18.2 kDa, NP_001158642, H^{79}-R^{96})	cis/trans Golgi & plasmalemma (cf. brain, stomach, kidney F3 and colon)	protein trafficking via COPI or clathrin coated vesicles from ER to Golgi and plasmalemma (cf. brain, stomach, kidney F3 and colon)
Histone H2A (13.2 kDa, XP_689030, A^{31}-R^{39})	plasmalemma (Schuurmans Stekhoven et al. 2004) and nucleus (Stryer 1995) (cf. gill, kidney and rectal gland)	antimicrobial agent (Schuurmans Stekhoven et al. 2004), and component of nucleosome core, involved in peptide-mediated gene transfer (Balicki et al. 2002)
Histone H4 (11.0 + 14.4 kDa, CAM12247, I^{47}-R^{56} + D^{69}-K^{78})	plasmalemma and nucleus (cf. brain, stomach, spleen, kidney and rectal gland)	antimicrobial agent (Smith et al. 2010) and component of nucleosome core (Stryer 1995, p. 980)
Ubiquitin (7.9 kDa, ACN 09894, T^{12}-K^{27})	plasmalemma and cytoskeleton (microtubules) (Hicke and Dunn 2003, Murti et al. 1988)	involved in the ubiquitin- proteasome pathway (Hegde 2010, cf. brain)
Voltage-dependent anion channel 2 (13.2 + 14.4 kDa, NP_001016193, W^{75}-K^{93})	mitochondrion and plasma membrane	cf. brain, gill, stomach, spleen and kidney
ATP synthase α-subunit (13.2 + 14.4 kDa, ACI33076, N^{89}-K^{103} + G^{104}-K^{123})	mitochondrion	ATP synthesis
ATP synthase β-subunit (7.9 + 11.0 kDa, ADH51553, L^{73}-R^{87} + I^{122}-R^{133})	mitochondrion	ATP synthesis
ADP/ATP translocase 2 (11.0 + 14.4 + 18.2 kDa, ACN11489, G^{280}-K^{294} + G^{280}-K^{295})	mitochondrion	transport of ATP from matrix to intermembrane space in exchange for ADP (cf. gill, liver, stomach, kidney F2 and rectal gland)
Cytochrome oxidase, subunit 2 (18.2 kDa, ADF50357, I^{45}-K^{60})	mitochondrion	ultimate electron acceptor in respiratory chain and reductor of $O_2 \rightarrow H_2O$
Mitochondrial carrier protein (Aralar, 18.2 kDa, CAG01991, S^{687}-R^{703})	mitochondrion (inner membrane)	involved in Ca^{2+} dependent glutamate/aspartate exchange (cf. kidney F3)
Malate dehydrogenase (7.9 kDa, ACI68220, V^{27}-K^{45})	cytoplasm and mitochondrion	dehydrogenation of malate to oxaloacetate in the citric acid cycle (mitochondria) and to pyruvate in the cytoplasm (Stryer 1995, p. 620)
Citrate synthase (7.9 + 13.2 kDa, AAI66040, E^{418}-R^{430})	mitochondrion (Taylor et al. 1978)	formation of citrate from oxaloacetate and acetyl-CoA
α-Actin (11 kDa, ACN66629, D^{294}-R^{314})	cytoskeleton (microfilaments)	transport track for myosin + support of cell stability (cf. colon)

Animals (common names) belonging to the accession numbers. ACI33076 + ACI68220 + ACN09894 + ACN11489: Atlantic salmon; ADF50357: Atlantic cod; ACN66629: European sea bass; AAI66040 + CAM12247 + XP_689030: zebrafish; CAG01991: spotted green pufferfish; NP_001016193: western clawed frog; NP_001158642: rainbow trout; ADH51553: Indian short-nosed fruit bat.

Table 6. Liver (*C. ang.* T. sea) F1 fraction: localization and function of the 14.4-43.6 kDa proteins

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
60S ribosomal protein L22 (16.6 kDa, NP_001032766, I^{70}-K^{80})	ER	protein synthesis
60S ribosomal protein L30 (14.4 kDa, ACO13275, L^{45}-R^{56})	ER	protein synthesis
Ribosomal protein S13/S18 (16.6 kDa, XP_002609941, A^{56}-R^{66})	ER	protein synthesis
Ribosomal protein S3 (17.4 kDa, CBL52984, E^{28}-R^{40})	ER	protein synthesis
Histone H2A.x-like (14.4 + 16.6 kDa, XP_002935049, N^{90}-K^{100} + V^{101}-K^{119})	nucleus	part of nucleosome core, involved in DNA double strand break repair (Pehrson 2004, Bilsland and Downs 2005)
Small nuclear ribonuclear protein Sm D2 (16.6 kDa, ADO28399, R^{24}-K^{42})	nucleus	involved in splicing (cf. spleen, colon and rectal gland)
Ras-related Rap-1b precursor (17.4 kDa, ACO14317, S^{150}-R^{163} + I^{152}-R^{163})	microvesicles from plasmalemma or endosomal membrane (Choi et al. 2007, cf. gill, and kidney F3)	
Fe-binding zinc finger, CDGSH type(MitoNEET, 14.4 kDa, NP_001014327, H^{103}-K^{118})	Mitochondrial outer membrane	catalyst of complex 1- driven state 3 respiration (Wiley et al. 2007)
ATP synthase α-subunit (14.4 kDa, NP_001070823, N^{88}-K^{102})	mitochondrion	ATP synthesis
ADP/ATP translocase (34.7 kDa, NP_001188059, D^{11}-K^{23})	mitochondrion	transport of ATP from matrix to intermembrane space in exchange for ADP (cf. gill, heart, stomach, kidney F2 and rectal gland)
Succinate-CoA ligase GDP-forming α-subunit (34.7 kDa, NP_001153970, M^{275}-K^{286})	mitochondrion	component of citric acid cycle
Glutamine synthetase (43.6 kDa, AAD34721, V^{6}-R^{26})	mitochondrion	involved in synthesis of urea as osmotic stabiliser, via carbamoyl phosphate synthetase III (Anderson 1980)
Mitochondrial aspartate aminotransferase (43.6 kDa, NP_001187511, K^{80}-K^{88})	mitochondrion	involved in the amino- group transfer from aspartate to α-ketoglutarate; the resulting glutamate can then enter the glutamine synthetase + carbamoyl-P synthetase pathway to form another molecule of urea (Anderson 1980)
Acetyl-CoA C-acetyltransferase (thiolase II, 43.6 kDa, XP_002611764, V^{364}-R^{383})	mitochondrion	ketone body degradation (Staack et al. 1978)
Ras-related protein Rab-1a (17.4 kDa, ACN11413, F^{28}-K^{46} + K^{129}-K^{137})	early and late endocytic vesicles from rat liver (Mukopadhyay et al. 2010)	recruitment of the kinesin- like protein (Kifc1) in early retrograde vesicular transport (Mukopadhyay et al. 2010, cf. stomach, kidney and colon)
Myosin light polypeptide 6 (16.6 kDa, ACQ59015, V^{95}-R^{110})	cytoskeleton	subunit of myosin, involved in vesicle transport and cell shape (Pollard and Cooper 2009)
Capping protein (actin filament) muscle Z-line, β (34.7 kDa, NP_001080116, S^{73}-R^{92} + L^{169}-K^{181})	cytoskeleton	capping barbed ends of actin filaments (Wear and Cooper 2004)
Tropomyosin α-1 chain (34.7 kDa, ADO27865, L^{113}-R^{125})	cytoskeleton	regulation of actin myosin interaction (Gunning et al. 2008)
ARP1 actin related protein 1 homolog A, contractin α (43.6 kDa, NP_001086482, V^{47}-K^{61} + T^{293}-K^{308})	cytoskeleton (microtubules and cell cortex)	subunit of dynactin, involved in ER to Golgi microtubule-based protein transport (Appenzeller-Herzog and Hauri 2006), and mechanical support of the plasma membrane (Garces et al. 1999)
Non-specific lipid transfer protein (SCPx, 14.4 kDa, NP_957159)	peroxisomes	thiolytic cleavage in the β-oxidation process of branched-chain fatty acids (Fernandusse et al. 2006)

Animals (common names) belonging to the accession numbers. NP_001014327 + NP_001032766 + NP_001070823 + NP_957139: zebrafish; ACN11413: Atlantic salmon; NP_001153970: rainbow trout; ACO13275 + ACO14317: northern pike; ACQ59015: sablefish; XP_002609941 + XP_002611764: Florida lancelet; CBL52984: sweetfish; NP_001187511 + NP_001188059: channel catfish; AAD34721: horn shark; ADO27865 + ADO28399: blue catfish; NP_001080116 + NP_001086482: African clawed frog; XP_002935049: western clawed frog.

Table 7. Stomach (*C. ang.* T. sea) F3 fraction: localization and function of the 3.6-21.9 kDa proteins

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
Ribosomal protein S13 (15.1 kDa, NP_001016602, L^{28}-K^{39})	ER	protein synthesis
ADP-ribosylation factor-1 (Arf 1, 15.1 kDa, NP_001158642, Q^{127}-K^{141})	cis/trans Golgi & plasmalemma (cf. brain, heart, kidney and colon)	protein trafficking via COPI or clathrin coated vesicles from ER to Golgi and plasmalemma
Ras-related protein Rab-1b (21.9 kDa, NP_001158692, V^{103}-K^{117})	Plasmalemma (Paradela et al. 2005; Ghosh et al. 2008) and Golgi matrix (Weide et al. 2001)	activation of COPI coated vesicle transport from ER to Golgi (Alvarez et al. 2003) and involvement in cargo sorting in ER exit sites (Slavin et al. 2011)
Histone H4 (3.6 kDa, XP_002595673, T^{81}-K^{92})	Plasmalemma and nucleus (cf. brain, heart, spleen, kidney and rectal gland)	antimicrobial agent (Smith et al. 2010) and component of nucleosome core (Stryer 1995, p. 980)
Sterol carrier protein 2b (12.0 kDa, NP_001006093, L^{130}-K^{139})	Plasmalemma (lipid raft and caveolae)	controls multiple lipid signaling pathways (cf. brain)
K^+-transporting ATPase α-chain 1-like (21.9 kDa, XP_002942857, N^{121}-R^{131})	plasmalemma	involved in H^+/K^+-exchange (excretion of protons, Forte and Zhu 2010)
Voltage-dependent anion- selective channel protein 1 (12.0 kDa, ACI34288, L^{257}-K^{266})	Mitochondrion and plasma membrane	cf. brain, gill, heart, spleen and kidney
F1 ATPase β subunit (3.6 kDa, ACO57569, L^{72}-R^{86})	mitochondrion	oxidative phosphorylation
ADP/ATP translocase 2 (3.6 kDa, NP_001188059, Y^{81}-K^{92})	mitochondrion	transport of ATP from matrix to intermembrane space in exchange for ADP (cf. gill, heart, liver, kidney and rectal gland)
Creatine kinase (6.5 kDa, P00566, L^{342}-K^{358})	mitochondrion	stabilisation of contact between inner and outer membrane (Speer et al. 2005)
Cytochrome *c* oxidase subunit Va precursor (6.5 kDa, AAQ14278, W^{47}-K^{63})	mitochondrion	subunit participating in final step of respiratory chain
Sorting and assembly machinery component 50 homolog (SAM50, 6.5 kDa, ACN10877, I^{457}-R^{468})	mitochondrion (outer membrane)	involved in the uptake and assembly of mitochondrial precursor proteins (Kozjak et al. 2003)
Actin (3.6 kDa, XP_00259 0163, S^{240}-R^{255})	cytoskeleton, microfilaments	cell support + providing trafficking routes in signal transduction
β-Actin (6.5 kDa, AAS169 21, D^{21}-R^{41})	similar to 3.6 kDa fragment	cf. 3.6 kDa actin fragment
Myosin regulatory light polypeptide 9 (Myl 9, 15.1 kDa, ADO28411, A^{19}-K^{36})	cytoskeleton	required for stability of the myosin heavy chain and cellular integrity (Park et al. 2011)
Calmodulin, EF-hand (15.1 kDa, AAX61134, E^{22}-K^{43})	cytoskeleton	activator of myosin light chain kinase (Walsh et al. 1982)
Rho c-like protein (21.9 kDa, ABC00799, Q^{10}-R^{26})	Cytoskeleton (microtubules) and invadopodia (cf. brain and colon)	vesicle transport, and indirect control of LIM-kinase dependent actin depolimerisation by Cofilin (cf. brain and colon)
GTP binding protein Rab 1a (12.0 kDa, AAK94004, S^{77}-K^{87})	tubulovesicles	involved in tubulovesicle recycling
Ras-related protein Rab 11b (21.9 kDa, ADO28731, N^{146}-K^{166})	tubulovesicles	involved in tubulovesicle recycling (Lapierre et al. 2007; Forte and Zhu 2010)

Animals (common names) belonging to the accession numbers. AAS16921: blue shark; ADO28731 + NP_001188059: channel cat fish; AAK94004: smaller spotted catshark; P00566: marbled electric ray; ADO28411: blue catfish; XP_002590163 + XP_002595673: Florida lancelet; AAQ14278: common carp; AAX61134: Mozambique tilapia; NP_001158642 + NP_001158692: rainbow trout; ABC00799: lemon damsel; ACO57569: long-jawed mudsucker; NP_001006093: zebrafish; ACI34288 + ACN10877: Atlantic salmon; NP_001016602 + XP_002942857: western clawed frog.

Table 8. Spleen (*C. ang.* T. sea) F3 fraction: localization and function of the 7.9-21.9 kDa proteins

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
40s ribosomal protein S13 (14.1 kDa, NP_001133064, G^{79}-K^{93}) and S28 (7.9 kDa, NP_998199, E^{52}-R^{63})	ER	protein synthesis
Ca^{2+}-ATPase 2 (7.9 kDa, NP_957259, V^{437}-K^{451})	ER	Cellular signalling (Thastrup 1990)
Peptidyl-prolyl cis-trans-isomerase B (cyclophilin B) (21.9 kDa, NP_001134342, V^{96}-R^{109})	ER	protein folding catalyst (Bose et al. 1994)
Endoplasmin (21.9 kDa, NP_937853, V^{416}-K^{428})	ER	Ca^{2+} binding protein, possibly involved in protein folding (Rowling et al. 1994)
Small nuclear ribonucleoprotein D1 polypeptide (7.9 kDa, NP_001085322, N^{49}-R^{61})	nucleus	involved in splicing (cf. liver, colon and rectal gland)
G-protein, γ-2 (7.9 kDa, NP_001087324, A^{33}-K^{46})	Plasmalemma or Golgi system	regulation of protein transport from trans-Golgi network to plasma membrane (Irannejad and Wedegaertner 2010)
Rap-1b (9.5 kDa, ADO28745, I^{153}-R^{164})	Plasmalemma (Maridonneau-Parini and de Gunzburg 1992; Mollinedo et al. 1993)	Ca^{2+} ATPase effector (Lacabaratz-Porret et al. 1998; cf. kidney)
Histone H4 (14.1 kDa, CAM12247, T^{81}-K^{92})	Plasmalemma and nucleus (cf. brain, heart, stomach, kidney and rectal gland	antimicrobial agent (Smith et al. 2010), and component of nucleosome core (Stryer 1995, p. 980)
Cytochrome *c* oxidase, subunit 5A (9.5 kDa, ACI66570, L^{90}-R^{98})	mitochondrion	electron transport
ATP synthase α- and β-subunit (14.1 kDa, NP_001070823, H^{305}-K^{315}; 14.1 kDa, ACH85277, V^{249}-R^{261})	mitochondrion	ATP synthesis
Voltage dependent anion selective channel protein 2 (VDAC) (21.9 kDa, NP_001089 399, W^{75}-K^{93})	mitochondrion and plasma membrane	pore forming protein in the mitochondrial outer membrane (release of cytochrome *c*), but also in the plasma membrane (NADH: ferricyanide reductase, Lawen et al. 2005), functioning in controlled cell death. Further VDAC interacts with the dynein light chain (Tctex1) and the heatshock protein PBP74 (Schwartzer et al. 2002), which are in volved in intracellular transport (cf. brain, gill, heart, stomach, kidney)
V-type proton ATPase, subunit d1 (21.9 kDa, NP_955914, L^{275}-K^{287})	cellular vacuoles	involved in cellular trafficking, exocytosis and endocytosis, and interaction with the cytoskeleton (Marshansky and Futai 2008; cf. kidney)
Vacuolar ATPase, subunit A (7.9 kDa, ACI33042, V^{45}-R^{56})	cellular vacuoles	
Cell division control protein 42-like protein (9.5 kDa, ADO29025, T^{108}-R^{120})	Cytoskeleton (Briggs and Sachs 2003)	leads to formation of filopodia (membrane protrusions, Nobes and Hall 1995) in response to extracellular signals (Lundquist 2009; cf. colon)
β-Tubulin (14.1 kDa, ABY48069, M^{363}-K^{379})	cytoskeleton	component of microtubules (cf. brain)
Chaperonin, containing TCP1 (TRiC, Feldman et al. 1999) (21.9 kDa, NP_001086080, I^{118}-K^{127})	cytoskeleton	forms a complex with actin and tubulin, leading to folding (Lewis et al. 1996). It also prevents renal van Hippel-Lindau (VHL) carcinoma formation (Feldman et al. 1999)

Animals (common names) belonging to the accession numbers. ABY48069: spiny dogfish; ADO28745 + ADO29025: channel catfish; ACH85277 + ACI33042 + ACI66570 + NP_001133064 + NP_001134342: Atlantic salmon; CAM12247 + NP_937853 + NP_955914 + NP_957259 + NP_998199 + NP_001070823: zebrafish; NP_001085322 + NP_001086080 + NP_001087324 + NP_001089399: African clawed frog.

Table 9. Kidney (*C. ang.* S. bay) F1 fraction: localization and function of the 6.9-17.4 kDa proteins

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
T-complex protein 1 subunit δ (TCP-1= CCT, 10.2 kDa, NP_001133482, D^{302}-K^{314})	nucleus, cytoskeleton (microtubule organizing centre), cytoplasm (Souès et al. 2003)	chaperonin, involved in protein folding (Souès et al. 2003; Yam et al. 2008)
Histone 2A (10.2 kDa, XP_002607033, A^{60}-R^{74})	plasmalemma (Schuurmans Stekhoven et al. 2004) and nucleus	antimicrobial agent (Schuurmans Stekhoven et al. 2004) and component of the nucleosome core, involved in peptide mediated gene transfer (Balicki et al. 2002, cf. gill, heart and rectal gland)
Interleukin enhancer binding factor 2 (ILF2, 13.8 kDa, ABP35586, V^{197}-R^{206})	nucleus (Yang et al. 2011)	1. regulating in conjunction with ILF3 the transcription of interleukin 2 (IL-2), involved in the immune system; 2. RNA splicing; 3. translational initiation (Yang et al. 2011)
Histone H4 (6.9 kDa, XP_002595673, K^{80}-K^{92})	plasmalemma and nucleus (cf. brain, heart, stomach, spleen, rectal gland)	antimicrobial agent (Smith et al. 2010) and component of nucleosome core
Flotillin-1 (Reggie-2, 10.2 kDa, ACN10783, F^{127}-K^{133})	plasmalemma and cytoskeleton (Langhorst et al. 2005), late endosomes and lysosomes (Riento et al. 2008)	involved in Fyn kinase activated endocytosis of membrane components (Riento et al. 2008)
Na$^+$/K$^+$-ATPase α-subunit (13.8 kDa, ADO60023, Q^{421}-K^{439})	plasmalemma	Na$^+$(3)/K$^+$(2) exchange transport
L-plastin (10.2 kDa, CAZ69804, N^{57}-R^{65})	plasmalemma, cytoskeleton (ruffling membranes and spike-like structures, Al Tanoury et al. 2010)	additional construct and catalyst in the organisation of actin filaments
Voltage-dependent anion-selective channel protein 2 (VDAC, 6.9 kDa, CBN81505, L^{97}-K^{109})	mitochondrion and plasma membrane	cf. brain, gill, heart, stomach, and spleen
60 kDa heat shock protein, mitochondrial precursor (preHsp60, 6.9 kDa, ACN11370, G^{43}-K^{58})	mitochondrion; following cleaving the target sequence Hsp60 partially returns to ER, plasmalemma and peroxisomes (Soltys and Gupta 1996)	protein folding and protection against stress, like heat (Hendrick and Hartl 1993)
Single stranded DNA binding protein, mitochondrial precursor (6.9 + 13.8 kDa, ACQ59073, N^{51}-R^{65})	mitochondrion	involved in transcription (cf. kidney F2 fraction and rectal gland)
Calcium binding mitochondrial carrier protein Aralar 1 (10.2 kDa, NP_997947, G^{393}-K^{405})	Mitochondrion (inner membrane, cf. heart and kidney F3)	involved in Ca^{2+}-dependent glutamate/aspartate exchange (Palmieri et al. 2001)
Mammalian mitochondrial aldehyde dehydrogenase 2 (13.8 kDa, CAA28990, V^{209}-K^{225})	mitochondrion	oxidation of acetaldehyde + NAD$^+$ + CoA $\rightarrow$ acetyl CoA + NADH + H$^+$
H$^+$-transporting ATP synthase F1 complex β-subunit (17.4 kDa, ACH85277, T^{180}-K^{192})	mitochondrion	ATP synthesis
Triose phosphate isomerase (17.4 kDa, ACO14153, V^{159}-K^{173})	possibly cytoskeleton in presence of reaction partners fructose-bisphosphate aldolase and/ or glyceraldehyde 3-phosphate dehydrogenase (Stephan et al. 1986)	transformation of dihydroxyaceton phosphate into glyceraldehyde 3-phosphate
Actin (13.8, 17.4 kDa, ACM77787, L^{217}-K^{239})	cytoskeleton, microfilaments (cf. heart, stomach, kidney F2 + F3, colon and rectal gland)	cell support+ providing trafficking routes in signal transduction (cf. heart, stomach, kidney F2 + F3, colon and rectal gland)
Phosphotriesterase-related protein (PTER, 6.9 kDa, NP_001080454, I^{215}-K^{225})	proximal tubules of kidney (Davies et al. 1997)	hydrolysis of phosphotriesters, including detoxification (Vanhooke et al. 1996)
Ras-related protein Rab-1a (17.4 kDa, NP_001007162, E^{138}-K^{153})	early and late endocytic vesicles (cf. liver, stomach, kidney F2 and colon)	recruitment of the kinesin-like protein (Kifc1) in early retrograde vesicular transport (cf. liver, stomach, kidney F2 and colon)

Animals (common names) belonging to the accession numbers. XP_002595673 + XP_002607033: Florida lancelet; ACH85277 + ACN10783 + ACN11370 + CAZ69804 + NP_001133482: Atlantic salmon; ABP35586: greasy grouper; ADO60023: fire clownfish; CBN81505: European seabass; ACQ59073: sable fish; ACO14153: northern pike; NP_997947 + NP_001007162: zebrafish; ACM77787: Japanese flying squid; NP_001080454: African clawed frog; CAA28990: human.

Table 10. Kidney (*C. ang.* S. bay) F2 fraction: localization and function of the 6.6-36.3 kDa proteins

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
Hypoxanthine-guanine phosphoribosyl transferase (HPRT, 10.2 + 13.8 kDa, NP_001187366, V^{116}-K^{128} + N^{129}-K^{141})	cytoplasm and nucleus (cf. kidney F3 fraction)	conversion of hypoxanthine to IMP and guanine to GMP (cf. kidney F3 fraction)
Regucalcin (SMP30, 36.3 kDa, NP_001187297, F^{102}-R^{112})	cytosol and nucleus (Nakagawa and Yamaguchi 2006)	gluconolactonase, e.g. in pentose phosphate pathway (step 2, Stryer 1995), yielding ribose-5P, involved in the synthesis of nucleotides and nucleic acids
Peroxiredoxin 1 (Prx-1, 36.3 kDa, NP_001134858, L^{159}-K^{168})	cytoplasm and nuclei (Wang et al. 2010)	kinase (Cdc2) controlled breakdown of cell damaging hydrogen peroxide and peroxinitrite (Rhee et al. 2005)
Selenium binding protein 1 (SBP1, 10.2 kDa, NP_956864, I^{104}-R^{114})	cell membrane protrusions (Miaguchi 2004), Golgi system (Porat et al. 2000, Dikiy et al. 2007)	activation of von Hippel-Lindau protein (PVHL) interacting deubiquinating enzyme 1 (VDU1) (Jeong et al. 2009)
Ras-related protein Rap-1b (6.6 kDa, NP_001187514, I^{153}-R^{164})	plasmalemma (cf. spleen)	Ca^{2+} ATPase effector (cf. spleen)
Na^{+}, K^{+}-ATPase α-subunit (17.8 kDa, CAG77578, E^{541}-R^{556})	plasmalemma	Na^{+}(3)/K^{+}(2) exchange transport
Moesin (6.6 kDa, NP_001167294, I^{238}-R^{246})	plasmalemma (filopodia) and cytoskeleton (Furthmayer et al. 1992)	crosslinks plasma membrane and microtubules; counteracts retroviral infection (Naghavi et al. 2007)
Enolase 3-2 (10.2 kDa, NP_001133193, G^{16}-K^{28})	plasmalemma & cytoskeleton (cf. gill)	reaction step in glycolysis + protease binding (cf. gill)
Flotillin -2a (Reggie 1a, 36.3 kDa, NP_001133508, M^{145}-K^{155})	cf. Flotillin 1 (cf. kidney F1, Suruga bay)	cf. Flotillin 1 (cf. kidney F1, Suruga bay)
Voltage-dependent anion channel 2 (10.2 kDa, P81004, W^{74}-K^{92})	mitochondrion and plasmalemma (cf. brain, gill, heart, stomach and spleen)	cf. brain, gill, heart, stomach and spleen
Single-stranded DNA-binding protein, mitochondrial precursor (6.6 kDa, ACQ59073, N^{51}-R^{65})	mitochondrion	involved in transcription (cf. rectal gland Suruga bay)
Adenine nucleotide translocator (6.6 kDa, ADI58835, Y^{82}-K^{93})	mitochondrion	transport of ATP from matrix to intermembrane space in exchange for ADP (cf. gill, heart, liver, stomach and rectal gland)
ATP synthase α-subunit mitochondrial precursor (13.8 kDa, ACI33076, E^{442}-R^{463})	mitochondrion	
ATP synthase H^{+} transporting F1 complex β-subunit (17.8 kDa, ACH 85277, V^{249}-R^{261})	mitochondrion	ATP synthesis
L-lactate dehydrogenase B chain (13.8 kDa, Q9YI05, M^{234}-K^{244})	mitochondrion (Matisson et al. 1972; Lemire et al. 2008), peroxisomes (Baumgart et al. 1996)	oxidation of lactate to pyruvate or reduction of pyruvate to lactate (Baumgart et al. 1996)
V-type H^{+}-ATPase, catalytic subunit A (6.6 kDa CBN81966, F^{203}-R^{212})	cellular vacuoles	involved in cellular trafficking, exo- and endocytosis, and interaction with the cytoskeleton (cf. spleen)
Ras related protein Rab-1a (17.8 kDa, ACN11075, K^{97}-K^{105} + N^{122}-K^{138})	early and late endocytic vesicles (cf. liver, stomach, and colon)	recruitment of the kinesin-like protein (Kifc1) in early retrograde vesicular transport (cf. liver, stomach, and colon)
β-Actin-1 or -2 (36.3 kDa, ADV 76250 or 76251, D^{292}-R^{312})	cytoskeleton (microfilaments)	cell support + providing trafficking routes in signal transduction (cf. stomach, and rectal gland)
Histidine ammonia-lyase (10.2 kDa, NP_001098736, Q^{329}-K^{342})	undefined	conversion of L-histidine into urocanic acid and ammonia; plays a role in protection against zinc and nickel toxicity(Murphy et al. 2011)
Antithrombin III (36.3 kDa, AAL73207, S^{157}-R^{165})	blood plasma	anticoagulation factor

Animals (common names) belonging to the accession numbers. CAG77578: spiny dogfish; ADI58835: white spotted bamboo shark; NP_001187297 + NP_001187366 + NP_001187514: channel catfish; ACH85277 + ACI33076 + ACN11075 + NP_001133193 + NP_001133508 + NP_001134858 + NP_001167294: Atlantic salmon; NP_956864: zebrafish; CBN81966: European sea bass; ADV76250 + ADV76251: cobia; ACQ59073: sable fish; P81004 + NP_001098736: African clawed frog; AAL73207: tuatara.

a relatively few sequences (9.3%) are related to amphibians (African and western clawed frog), which originated in an earlier period, the Devonian, 400 mya (Pidwirny 2012). Even smaller was the contribution (3.3%) by the more-ancient lancelet from the Cambrian period (550 mya, Holland et al. 2008). Hence, proteochemical comparability of *C. anguineus* with the teleostei appears to be justified. This comparability can be explained in 2 ways: (1) *C. anguineus* proteins have evolved together with those of the teleostei such that the protein amino acid sequences are similar; and (2) teleostei protein amino sequences did not evolve, but remained conserved with regard to the

Table 11. Kidney (*C. ang.* T. sea) F3 fraction: localization and function of the 7.2-19 kDa proteins

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
ADP-ribosylation factor 1 (Arf1, 7.2 kDa, ACI33932, D^{118}-K^{127})	cis/trans Golgi & plasmalemma (cf. brain, heart, stomach, and colon)	protein trafficking via COPI or clathrin coated vesicles from ER to Golgi and plasmalemma
Hypoxanthine-guanine phosphoribosyl transferase (HPRT, 14.4 kDa, NP_998151, F^{74}-K^{83})	cytoplasm and nuclei (Aronin et al. 1999)	conversion of hypoxanthine to IMP, and guanosine to GMP via transfer of 5-phosphoribosyl group from 5-P-ribosyl 1 pyrophosphate to the purine (part of RAS: renin/angiotensin system, Schefe et al. 2006)
Histone H4 (7.2 kDa, CAM 12247, K^{79}-K^{91})	Plasmalemma and nucleus (cf. brain, heart, stomach, spleen, and rectal gland)	antimicrobial agent (Smith et al. 2010) and component of the nucleosome core (Stryer 1995, p. 980)
Na$^+$/K$^+$-ATPase α1b-subunit (7.2 kDa, CAQ13999, E^{537}-R^{553})	plasmalemma	Na$^+$(3)/K$^+$(2) exchange transport
Ras-related protein Rap-1b precursor (11 kDa, ACM08557, S^{17}-K^{31})	plasmalemma (cf. gill and liver)	
Flotillin 1 (Reggie 2, 11kDa, CAL49374, A^{111}-K^{123})	Plasmalemma and cytoskeleton (Langhorst et al. 2005), late endosomes and lysosomes (Riento et al. 2008)	intramembranal scaffold, binding extracellular proteins, and leading them to internalisation upon activation by FYN kinase (cf. kidney F1 Suruga bay, Riento et al. 2008)
Filamin A (7.2 & 14.4 kDa, XP_001922206, L^{78}-K^{88} + L^{174}-K^{181})	plasmalemma, cytoskeleton (actin filaments)	linking actin networks to cell membranes, thus providing a scaffold for cytoskeletal proteins and multiple signaling proteins (Kim and McCulloch 2011)
Voltage-dependent mitochondrial anion selective channel 2 (19 kDa, NP_001089399, W^{75}-K^{93})	Mitochondrion and plasmalemma (cf. brain, gill, heart, stomach and spleen)	pore forming protein in the mitochondrial outer membrane and plasmalemma, functioning in apoptosis, interacting with the dynein light chain (Tctex1) and heatshock protein (PBP74), involved in trafficking (cf. brain, gill, heart, stomach and spleen)
Guanine nucleotide binding protein G(i), α2 subunit (19 kDa, NP001136187, E^{146}-R^{162})	plasmalemma, centrosomes and cleavage furrow (cf. gill)	involved in mitosis, in particular the orientation of the mitotic spindle (cf. gill)
Ca^{2+}-binding mitochondrial carrier protein (Aralar, 14.4 kDa, ACI34077, S^{588}-R^{604})	Mitochondrion (inner membrane)	involved in Ca^{2+}-dependent glutamate/aspartate exchange (Palmieri et al. 2001, cf. heart)
ATP-synthase β-subunit (14.4 kDa, NP_001083034, V^{279}-R^{291})	mitochondrion	ATP synthesis
Vacuolar H$^+$-ATPase, subunit a, b1 or b2 (19 kDa, CAD88270, AAD55091, AAC78641, F^{370}-R^{387}, A^{428,438}-K^{448,458})	cellular vacuoles (cf. spleen)	involved in cellular trafficking, exocytosis, and interaction with the cytoskeleton (cf. spleen)
β-actin (11 kDa, ACY01916, D^{263}-R^{283})	Cytoskeleton (microfilaments, cf. stomach and rectal gland)	cell support + providing trafficking routes in signal transduction
Pyruvate kinase (11 kDa, NP_001135175, M^{73}-K^{88})	Cytoskeleton (microtubules, Walsh et al.1989)	conversion of phosphoenol pyruvate to pyruvate

Animals (common names) belonging to the accession numbers. ACI33932 + ACI34077 + ACM08557 + NP_001135175 + NP_001136187: Atlantic salmon; CAM12247 + CAQ13999 + NP_001083034 + NP_998151 + XP_001922206: zebrafish; CAD88270: marbled electric ray; AAC78641 + AAD55091: European eel; ACY01916: northern bluefin tuna; CAL49374: western clawed frog; NP_001089399: African clawed frog.

Table 12. Colon (*C. ang.* T. sea) F3 fraction: localization and function of the 14.4-42.6 kDa proteins

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
60S ribosomal protein L30 (14.4 kDa, ACQ58213, K^{57}-K^{68})	ER	protein synthesis
ADP-ribosylation factor 1 (20.9 kDa, NP_001158642, H^{79}-R^{96} + Q^{127}-K^{141})	cis/trans Golgi & plasmalemma (cf. brain, heart, stomach and kidney)	cf. brain, heart, stomach and kidney
Neurocalcin-δA (VILIP-3, 25.1 kDa, NP_001107882, S^{119}-K^{130})	trans-Golgi network, cytoskeleton, clathrin coated vesicles	involved in vesicular protein transport (Ivings et al. 2002)
Proteasome subunit α type-1 (32.4 kDa, NP_001187433, N^{4}-R^{18}) type-3 (32.4 kDa, NP_001187447, H^{73}-R^{86})	nucleus, ER and cis- Golgi (Palmer et al. 1996; Rivett 1998)	ER-associated degradation of misfolded or improperly secreted proteins+ some ER integral membrane proteins (Rivett 1998)
Small nuclear ribonucleoprotein Sm D2 (14.4 kDa, ADO28399, R^{24}-K^{42})	nucleus	involved in splicing (cf. liver, spleen and rectal gland)
Histone H2A.Z (14.4 kDa, ADO27735, A^{103}-K^{116})	nucleus	regulates transcription (Santisteban et al. 2000)
RNA-binding protein FUS (14.4 kDa, NP_957377, L^{347}-K^{362})	nucleus and spreading initiation centres in cellular matrix (Zinszner et al. 1997; de Hoog et al. 2004)	shuttling of RNA between nucleus and cytoplasm (Zinszner et al. 1997) + rate control of cell spreading (de Hoog et al. 2004)
Ras-suppressor protein 1 (Rsu-1, 32.4 kDa, NP_001085943, H^{249}-K^{259})	plasmalemma, final component of the PIP (PINCH- ILK-PARVIN) complex (Dougherty et al. 2005; Kovalevich et al. 2011)	involved in the formation of focal adhesion complexes (Dougherty et al. 2005)
14-3-3 protein ε (32.4 kDa, ACI34249, A^{197}-K^{215})	plasmalemma, mitochondrion, nucleus	regulation of enzymatic activity; regulation of subcellular localisation; inhibition of protein-protein or protein-DNA interaction; protection against dephosphorylation or proteolytic degradation; stabilisation of multiprotein complexes (Fu et al. 2000; Obsil and Obsilova 2011)
Nucleoside diphosphate kinase (20.9 kDa, P27950, Y^{66}-K^{84})	perinuclear plasma and granules, cytoskeleton (microtubules), ER, plasmalemma (cf. rectal gland Suruga bay)	multifunctional protein (cf. rectal gland Suruga bay)
Rho-related GTP binding protein Rho c (20.9 kDa, CBN80760, Q^{52}-R^{68})	Cytoskeleton (microtubules, Wheeler and Ridley 2004) and invadopodia (Bravo-Cordero et al. 2011, cf. Brain and stomach)	vesicle transport and indirect regulation of actin depolimerisation (cf. brain and stomach)
Myosin regulatory light chain 2, smooth muscle isoform (20.9 kDa, CBN82082, A^{18}-K^{35})	Cytoskeleton (microtubules)	involved in cytokinesis (Matsumura 2005; Burgess 2005)
Cell division control protein Cdc42 small GTPase (25.1 kDa, NP_001188177, N^{167}-K^{183})	Cytoskeleton (cf. spleen)	formation of filopodia in response to extracellular signals (cf. spleen)
α-actinin-1 (32.4 kDa, NP_001161758, N^{173}-K^{184})	Cytoskeleton (microfilaments)	involved in ligating actin to the cell membrane (focal adhesions, Pavalko and Burridge 1991) and to each other (Sjöblom et al. 2008)
α-actin (42.6 kDa, ABN58897, H^{37}-K^{47} + S^{236}-R^{251} + D^{289}-R^{309})	Cytoskeleton (microfilaments, cf. heart)	transport track for myosin + support of cell stability (Pollard and Cooper 2009)
α-actin (42.6 kDa, ABN58891, V^{93}-K^{110})	Cytoskeleton (microfilaments)	idem
actin (42.6 kDa, CBY39717, E^{220}-K^{244})	idem	idem
GTP binding protein Rab 1a (25.1 kDa, AAK94004, E^{30}-K^{45} + N^{46}-K^{62})	endocytic vesicles (cf. liver, stomach, and kidney)	vesicle transport (cf. liver, stomach and kidney)

Animals (common names) belonging to the accession numbers. P27950: nurse shark; AAK94004: smaller spotted catshark; ACQ58213: sablefish; NP_001187433 + NP_001187447 + NP_001188177: channel catfish; ADO27735 + ADO28399: blue catfish; NP_957377 + NP_001107882 + NP_001161758: zebrafish; CBN80760+CBN82082: European seabass; NP_001158642 : rainbow trout; ACI34249: Atlantic salmon; ABN58891 + ABN58897: Nile tilapia; NP_001085943: African clawed frog; CBY39717: zooplankton.

sequences of *C. anguineus*.

It is hard to choose from these 2 possibilities. Even when we look at phylogenetic relationships, it turns out that the order position may change depending on the biochemical or biological characteristics tested. So changing the position of the Lamniformes from being more ancient than the Squaliformes to being more recent than the Squaliformes depends on the macromolecules used for comparison (cytochrome *b* + 16S RNA vs. cytochrome a$_3$-1, Schuurmans Stekhoven et al. 2010). Also *C. anguineus* was changed to a post-Squalimorphi position upon changing the comparison of mitochondrial DNA to a morphological comparison (Tanaka et al. 2011). Thus on this basis, it is not possible to tell which came first and which came later. We prefer to take the presence of the Cl$^-$ channel phospholemman as a indication of ancientness, although the presence and absence of the channel may also be described by "rise and fall of phospholemman in sharks". Hence, it may have never evolved in *C. anguineus*.

The absence of a phospholemman was not caused by an inability of the MS to trace its presence, since it could detect it in all Squaliformes

Table 13. Rectal gland (*C. ang.* T. sea) F3 fraction: localization and function of the 11-18.6 kDa proteins

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
Ribosomal protein S11 (18.6 kDa, XP_002596229, I^{129}-R^{141})	ER	protein synthesis
Ribosomal protein S13/S18 (16.2 kDa, XP_002609941, A^{56}-R^{66})	ER (cf. liver)	protein synthesis
60S ribosomal protein L22 (18.6 kDa, NP_001032766, I^{70}-K^{80})	ER (cf. liver)	protein synthesis
Histone H2A (13.8 kDa, AAI46738, A^{22}-R^{30})	plasmalemma (Schuurmans Stekhoven et al. 2004) and nucleus	antimicrobial agent (Schuurmans Stekhoven et al. 2004) and component of nucleosome core, involved in peptide mediated gene control (cf. gill, heart and kidney)
MacroH2A variant (18.6 kDa, NP_001085741, G^{97}-R^{108})	nucleus	fine control of regulation of gene expression (Araya et al. 2010)
14 kDa transmembrane protein (11 kDa, Q91499, V^{59}-R^{67})	plasmalemma	interferon inducible transmembrane protein (cf. brain and gill)
Histone H4 (11 kDa, XP_002595673, I^{47}-R^{56} + K^{80}-K^{92})	plasmalemma and nucleus (cf. rectal gland *C. anguineus* S. bay, brain, heart, stomach, spleen and kidney)	antimicrobial agent (Smith et al. 2010) and component of nucleosome core
Na$^+$,K$^+$-ATPase α-subunit (13.8 kDa, CAG77578, I^{275}-R^{286}; 16.2 kDa, P05025, S^{446}-K^{458})	plasmalemma	Na$^+$(3)/K$^+$(2) exchange transport
Na$^+$/K$^+$/2Cl$^-$ symporter (18.6 kDa, P55013, E^{838}-R^{851})	plasmalemma	Na$^+$, K$^+$ and Cl$^-$ homeostasis
ATP synthase α-subunit (16.2 kDa, NP_001070823, H^{305}-K^{315})	mitochondrion	ATP synthesis
ATP synthase β-subunit (13.8 kDa, AAH95620, T^{202}-K^{214}; 16.2 kDa, D^{284}-R^{299})	mitochondrion	ATP synthesis
Dynein light chain (11 kDa, ACO08201, N^{61}-K^{71})	Cytoskeleton (microtubules)	motile processes: mitosis, localisation of the Golgi apparatus, trafficking of membrane vesicles and other intracellular particles: lysosomes, endosomes, and vesicular transport from early to late endosomes (Holzbaur and Vallee 1994; Lippincott-Schwartz 1998; King 2000)
β-Actin (13.8 kDa, ACR83576, K^{138}-R^{159})	Cytoskeleton microfilaments (cf. stomach and kidney)	cell support + providing trafficking routes in signal transduction (cf. stomach and kidney)

Animals (common names) belonging to the accession numbers. CAG77578 + P55013: spiny dogfish; Q91499: marbled electric ray; P05025: Pacific electric ray; XP_002595673 + XP_002596229 + XP_002609941: Florida lancelet; AAH95620 + AAI46738 + ACR83576 + NP_001032766 + NP_001070823: zebrafish; ACO08201: rainbow trout; NP_001085741: African clawed frog.

Table 14. Rectal gland (*C. ang.* S. bay) F3 fraction: localization and function of the 8.3-16.6 kDa proteins

Protein + protein band (kDa + Acc. No. with sequence)	Localization	Function
40s ribosomal protein S13 (16.6 kDa, NP_001154143, G^{79}-K^{93})	ER	protein synthesis
40s ribosomal protein S29 (8.3 kDa, NP_001134590, Y^{26}-R^{32})	ER	protein synthesis
60s ribosomal protein L23 (16.6 kDa, ACO08568, L^{52}-K^{66})	ER	protein synthesis
Ribosomal protein L19 (14.8 kDa, AAN73354, L^{146}-R^{154})	ER	protein synthesis
Small nuclear ribonuclear protein (11.5 kDa, ADO29194, G^{64}-R^{75})	nucleus	involved in splicing (Lerner et al. 1980)
Sm D2 (14.8 kDa, ACO08505, G^{93}-R^{101})	nucleus	involved in splicing (cf. liver, spleen, and colon)
$Na^+/K^+/2Cl^-$ cotransporter (11.5 kDa, CAB86885, E^{73}-R^{86})	plasmalemma	Na^+, K^+ and Cl^- homeostasis
Na^+/K^+-ATPase, α-subunit (11.5 kDa, CAG77578, S^{451}-K^{463}; 14.8 kDa, 16.6 kDa, BAJ13363, V^{218}-R^{232})	plasmalemma	$Na^+(3)/K^+(2)$ exchange transport
Histone H4 (11.5 kDa, XP_002595673, T^{81}-K^{92})	Plasmalemma and nucleus (cf. brain, heart, stomach, spleen and kidney)	antimicrobial agent (Smith et al. 2010) and component of nucleosome core (Stryer 1995, p.980)
Cellubrevin 3 (13.2 kDa, NP_001135278, A^{51}-K^{67})	Plasmalemma in colon and rectum	v-snare in neuroendocrine cells (Kämper 2007)
Ubiquitin-conjugating enzyme E2N (16.6 kDa, NP_001154123, S^{131}-R^{141})	plasmalemma	transthiolation of ubiquitin prior to transacylation of a lysine side chain of the substrate via a ubiquitin ligase for degradation by a proteasome (Hicke and Dunn 2003; Hegde 2010)
Glucagon family neuropeptides precursor (GLP-1, 8.3 kDa, ACQ 58189, K^{114}-K^{122})	glial cell membrane	reduces intestinal motility (McDonagh et al. 2007)
Nucleoside diphosphate kinase (Nm23/NDPKA, 16.6 kDa, AAG14350, Y^{68}-K^{86})	perinuclear plasma and granules, cytoskeleton (microtubules), ER, plasmalemma (Bosnar et al. 2009)	multifunctional, a.o support of microtubule polymerisation and vesicle transport, activation of Cdc42 and Rac1 in the formation of filopodia and lamellipodia (Bosnar et al. 2009)
G protein γ 7 (8.3 kDa, XP_002198263, N^{45}-K^{60})	Plasmalemma (Mervine et al. 2006, $\beta 1 \gamma 7$) and recycling endosomes (Hynes et al. 2004)	modulator of cyclin-dependent kinase inhibitor ($p27^{Kip1}$) expression (Shibata et al. 1999)
ATP synthase β-subunit (8.3 kDa, ACH85277, V^{249}-R^{261})	mitochondrion	ATP synthesis
Chaperonin 10 kDa subunit (Hsp10, 11.5 kDa, CAG02594, K^{57}-R^{64})	mitochondrion, red blood cells and secretory granules (Sadacharan et al. 2001)	protein folding as cochaperone for Hsp60
ADP/ATP translocase 2 (13.2 kDa, ADO29492, Y^{81}-K^{92})	mitochondrion	transport of ATP from matrix to intermembrane space in exchange for ADP (cf. gill, heart, liver, stomach and kidney)
cytochrome *c* (13.2 kDa, P00022, T^{41}-K^{54})	mitochondrion	electron transport
Isocitrate dehydrogenase 2-2 (13.2 kDa, NP_001133197, L^{289}-K^{299})	mitochondrion	citric acid cycle
Single-stranded DNA-binding protein, mitochondrial precursor (14.8 kDa, ACQ59073, N^{51}-R^{65})	mitochondrion	involved in transcription

Animals (common names) belonging to the accession numbers. CAG77578: spiny dogfish; AAN73354 + CAB86885: smaller spotted catshark; XP_002595673: Florida lancelet; ACO08505 + ACO08568 + NP_001154123 + NP_001154143: rainbow trout; ACH85277 + NP_001133197 + NP_001134590 + NP_1135278: Atlantic salmon; BAJ13363: cherry salmon; AAG143504 + ADO29194 + ADO29492: channel catfish; ACQ58189 + ACQ59073: sablefish; CAG02594: spotted green pufferfish; P00022: common snapping turtle XP_002198263: zebrafish.

(Schuurmans Stekhoven et al. 2010). Other Cl^- channels, like the cystic fibrosis transmembrane conductance regulator (CFTR, Lu et al. 2010) and CLC-K chloride channels (Liantonio et al. 2006) were also not detected in our studies. Yet another Cl^- transporter ($Na^+/K^+/2Cl^-$ cotransporter) was found in rectal glands of *Furgaleus macki* and *Hemitriakis japanica* of the Triakidae, and *Etmopterus princeps* of the Squaliform (Schuurmans Stekhoven et al. 2010), and in the present paper in tables 13 and 14.

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