Yolk protein hydrolysis and oocyte free amino acids as key features in the adaptive evolution of teleost fishes to seawater

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SARSIA

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The ancestors of teleost fishes lived in freshwater for about 250 million years before returning to the sea during the Jurassic period. The hyposmotic blood of extant marine teleosts is assumed to reflect this freshwater origin. About 100 million years ago the palaeontological record shows a sudden differentiation of the teleosts with a burst of new species evolving. This transition from freshwater to the sea demanded certain osmotic adaptations in order to maintain homeostasis.

These osmotic adaptations especially apply to the embryos since they lack the organs responsible for osmoregulation in the adult fish. At spawning, marine fish eggs must contain a water reservoir to compensate for the passive water loss imposed by the hyperosmotic seawater. The high water content of the yolk of marine teleost eggs reflects this water reservoir. Most extant marine fishes, regardless of systematic affinities, spawn pelagic eggs. A mechanism must have been established during teleost evolution to bring the water into the yolk before the eggs were spawned. Yolk protein hydrolysis and increase in content of free amino acids (FAA) during final oocyte maturation is part of this mechanism in extant marine teleosts with pelagic eggs.

The oocyte FAA pool is generated mainly by hydrolysis of a ~100 kD yolk protein. This provides the osmotic drive for the water uptake into the oocyte. Intriguingly, this pool of FAA in pelagic teleost eggs is remarkably similar regardless of the taxonomic position of the species, implying that the hydrolysed fraction of the yolk protein is evolutionary conserved. This yolk protein is a fragment of the N-terminal end of a derivative of vitellogenin. In the authors' opinion, the establishment of the yolk protein hydrolysis at final oocyte maturation with the resulting increase in the FAA pool and oocyte hydration was a key step in teleost evolution that gave rise to their successful differentiation in the oceans about 100 million years ago.

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THE PROBLEM

When teleost fishes made their re-entry into seawater about 150-200 million years ago after some 250 million years of evolution in freshwater (Long 1995), they had to overcome a formidable problem. This problem relates to their osmoregulation and to their oviparous habit of spawning eggs that develop unattended (physiologically speaking) in the water masses.

The osmolality of the blood plasma of extant teleosts (Evans 1993) is about 300 mOsm in freshwater and just a little higher in seawater. Indeed, the hyposmotic condition of marine teleosts, a condition that is quite unusual among animals, is taken as evidence for the freshwater origin of the teleost group (Evans 1993; Moyle & Cech 1996). Stable internal osmotic conditions seem to be a demand of all teleost cells including the gonadal cells. Thus, even the eggs and the early embryos of extant marine teleosts are as hyposmotic to seawater as the adult fishes (e.g. Guggino 1980; Riis-Vestergaard 1982, 1987; Hølleland & Fyhn 1986; Mangor-Jensen 1987; Hahnenkamp & al. 1993; Tytler & al. 1993).

There are no reasons to believe that the osmotic problem of the newly spawned eggs was any different during the early Mesozoic period when teleosts made their re-entry into the oceans (Fig. 1). Although the adults of the Actinopterygian ancestors of the teleosts with their epidermal armour, fully differentiated organs and physiological mechanisms could gradually adapt to the saline environment and its constraints in the sea, the situation was not so for the newly spawned egg and embryos. These early stages lack the organs and specialised cells that deal with physiological mechanisms involved in the osmotic adaptations to the environment.

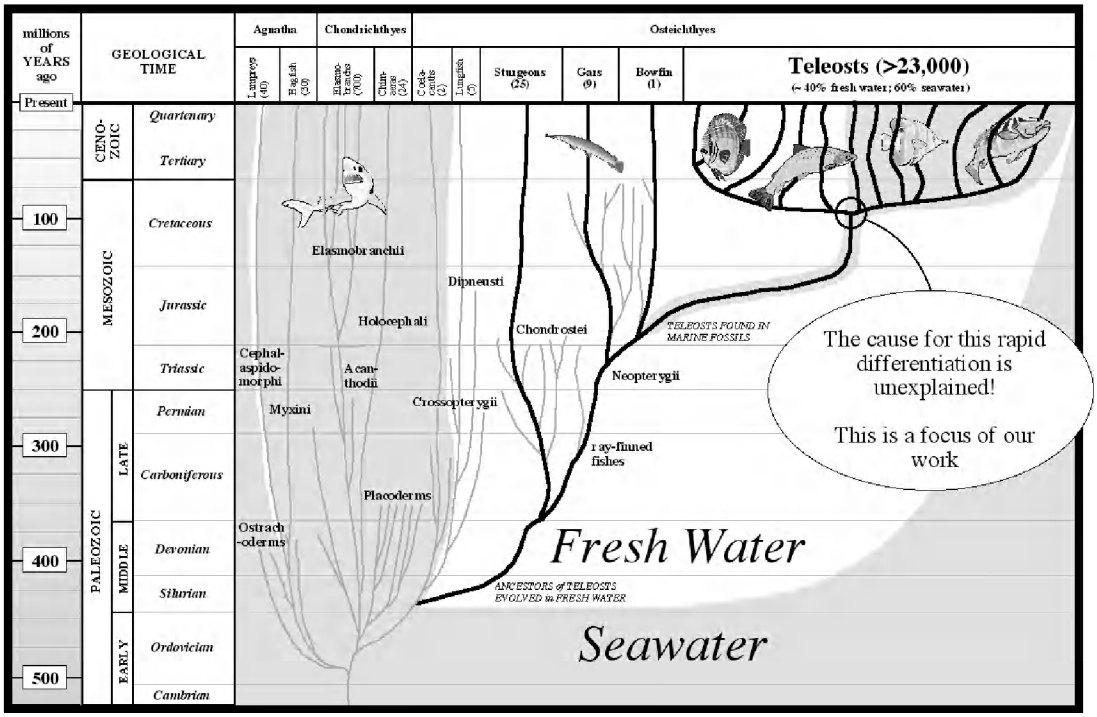

Fig. 1. The palaeontological record for fishes and their assumed relationship to fresh water and seawater. (Adapted from Moyle 1993 and Long 1995)

The teleost egg at spawning must contain the components necessary to resist the demands of the ambient environment. No physiological compensation is possible before the involved cells and organs become differentiated and functional.

During their long evolution, the teleosts have established various strategies of reproduction. Common to all, however, is the development of oocytes from primordial germ cells within a maternal follicle. For oviparous fishes, these germ cells undergo differentiation and deposition of the vital components necessary for the early embryo during its initial cleidoic existence in the external environment after spawning. Water is an important and vital component in this regard since the developing embryo has different demands for an internal water store depending on the osmotic conditions of the ambient medium.

The osmotic implication for the teleost embryo when developing in freshwater is a water influx and a continuous ion loss (Fig. 2). This is the case for extant fishes as it would be for the extinct fishes back in the Devonian period. As an adaptation, eggs of freshwater fishes have a low water content (typically 50-70 %; Kamler 1992), a very low vitelline membrane permeability (Potts & Rudy 1969; Mangor-Jensen 1987), and the dominating solutes are inorganic ions (Na^+, K^+ & Cl^-). Moreover, since the downward flow of freshwater will sweep away any free eggs, freshwater fish eggs are typically demersal; being either embedded in the bottom gravel or attached to the substrata.

In seawater, however, the osmotic problems of the teleost embryo are the reverse of those in freshwater (Fig. 2). In this situation, water is continuously lost to the environment and environmental ions tend to diffuse into the egg. In order for the teleosts to radiate from the freshwater into the marine environment they needed adaptations that would compensate for the effects of the hyperosmotic seawater for their water and ion balance. Probably, the first trials of colonising the saline ocean was by way of excursions into the sea for feeding, and return to the freshwater environment for spawning. This assured that the embryos could rely on the mechanisms and adaptations that already were suited for a life in freshwater. Anadromic fishes such as the salmonids still show this behaviour. They live and feed in the sea but migrate back into freshwater for spawning.

The challenge for the teleosts in order to become truly marine was to solve the osmotic problems for the spawned egg. Most marine fishes, regardless of sys-

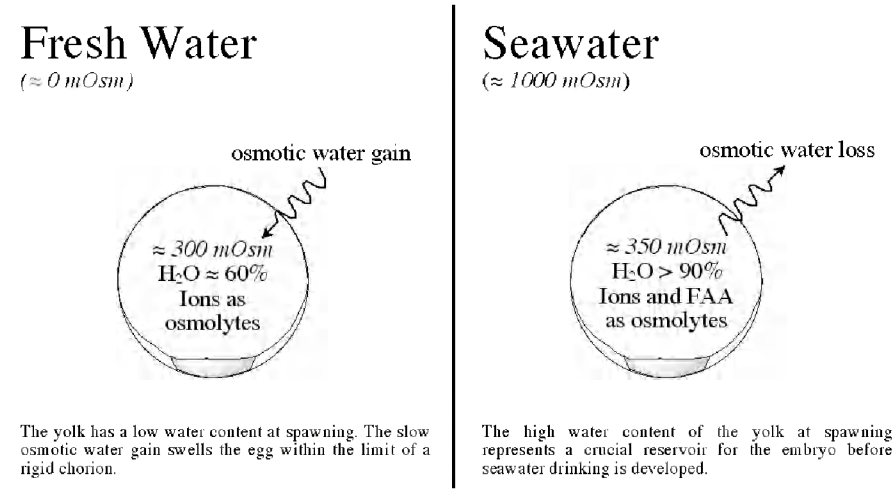

Fig. 2. The osmotic problems of fish eggs in fresh water and seawater. FAA = Free amino acids

tematic affinities, spawn pelagic eggs that are fertilised externally and float individually near the surface of the sea (Kendall & al. 1984). Pelagic marine teleost eggs are endowed with a large reservoir of yolk water, giving the egg a water content at spawning of 92-96 % of the egg wet mass (Craig & Harvey 1987; Finn & al. 1995a; Thorsen & al. 1996). This is significantly higher than that of eggs of freshwater teleosts and reflects an adaptation to life in the hyperosmotic seawater. The water is taken up during final oocyte maturation just prior to ovulation and spawning (Fulton 1898; Milroy 1898; Wallace & Selman 1981; Craig & Harvey 1986; Greeley & al. 1991; Thorsen & Fyhn 1996). The yolk water reservoir is vital to survival of the embryo during its early development in seawater since water uptake by drinking is not yet possible due to lack of the differentiated organs. Thus, the high water content of the yolk seems to be a key feature of teleosts in their adaptation to life in the ocean. This applies equally well to the demersal eggs of marine teleosts although fewer studies have concerned these eggs (Craig & Harvey 1986, Hølleland & Fyhn 1986; McPherson & al. 1989; Thorsen & Fyhn 1991; Greeley & al. 1991; Thorsen & al. 1993). The mechanism(s) of water transfer into the oocyte during final oocyte maturation, and the evolution of these mechanism(s) during teleost phylogeny, are the topics of our present investigation.

A POSSIBLE SOLUTION

Our studies of pelagic eggs of marine fishes have shown the presence of a large pool of free amino acids (FAA) in the egg at spawning (e.g. Fyhn 1989, 1993; Rønnestad & Fyhn 1993; Rønnestad & al. 1999). The FAA pool is almost fully restricted to the yolk sac and disappears in parallel with yolk consumption (Rønnestad & al. 1993; Finn & al. 1995a, 1995b). Furthermore, the FAA seem to originate from the breakdown of a specific ~100 kD yolk protein during final oocyte maturation while the oocytes are swelling due to water uptake just prior to ovulation and spawning (Fyhn 1993; Thorsen & al. 1993; Thorsen & Fyhn 1996; Thorsen & al. 1996). Pioneering work on teleost oocyte hydration and yolk protein changes during final oocyte maturation *in vitro* and *in vivo* were made by Selman and Wallace (e.g. Wallace & Selman 1978, 1981, 1985; Selman & Wallace 1989). Yolk protein hydrolysis during final oocyte maturation in the barfin flounder (*Verasper moseri*) has been studied by Matsubara and co-workers (Matsubara & Sawano 1995; Matsubara & al. 1995; Matsubara & Koya 1997).

By comparative studies (Fig. 3) we have shown that the profiles of the FAA pool are remarkably similar regardless of the taxonomic affinities or the habitat (boreal, subtropical or tropical) of the teleost species (Rønnestad & Fyhn 1993; Rønnestad & al. 1996, 1999). Some differences could, however, be discerned when comparing 23 species from 8 families of 5 suborders of tropical perciform teleosts (Rønnestad & al. 1996). The differences are less within families than between families suggesting genetic and phylogenetic relationships.

SDS PAGE has shown a major yolk protein of ~100 kD as well as other smaller yolk proteins to disappear concurrently with oocyte swelling and the generation of the FAA pool (Fig. 4). Western blots indicate that the ~100 kD protein (termed "FAA-precursor protein") is a fragment of a vitellogenin derivative, probably lipovitellin. Amino acid sequences of seven tryptic fragments of haddock (*Gadus aeglefinus*) FAA-precursor

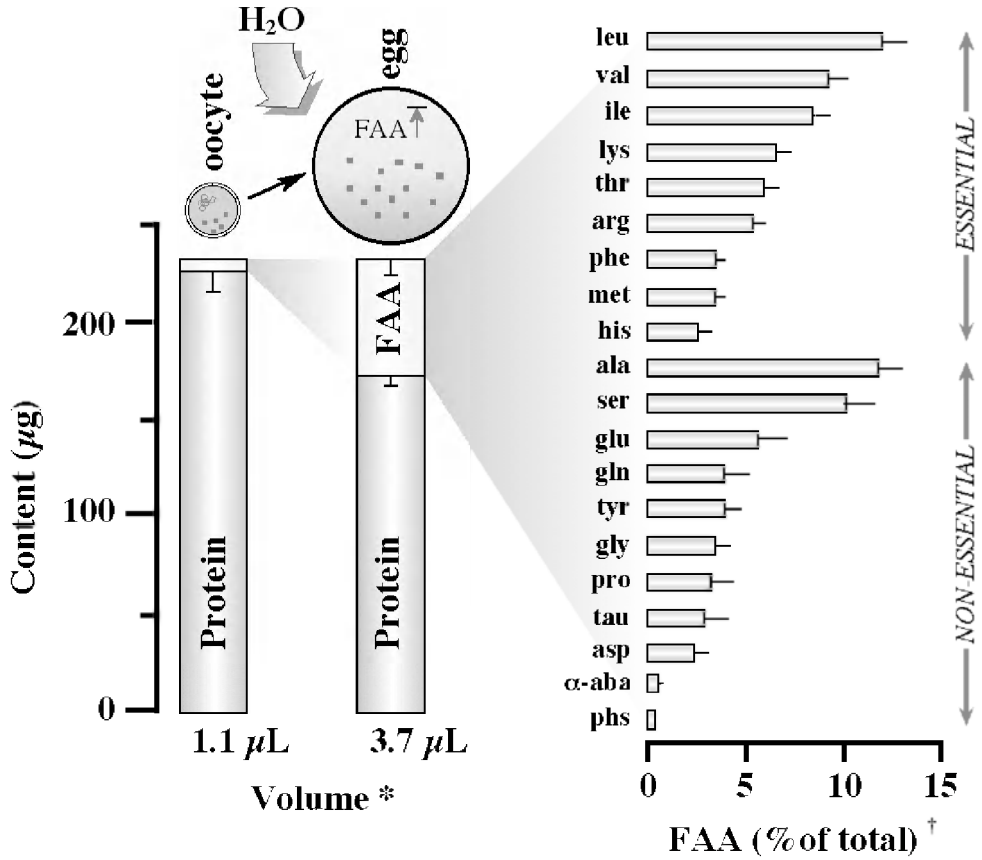

Fig. 3. The changes in yolk protein and free amino acid (FAA) content during final oocyte maturation in marine fishes with pelagic eggs. Data given as mean ± SD. The specific data for FAA refer to mean values for 23 species of teleosts. *Adapted from Thorsen & Fyhn (1996). †Adapted from Rønnestad & al. (1996).

protein, determined by mass spectroscopy, can be aligned with equivalent regions of the rainbow trout and winter flounder vitellogenin sequences (Finn & al. 2000). The sequences all lie within the first 840 amino acids of vitellogenin, indicating that the FAA-precursor protein originates from the N-terminal end of a derivative of vitellogenin. Further analyses are under way.

Since the FAA pool seems to originate from the ~100 kD yolk protein in all tested fishes, the similarity of the FAA pool of the pelagic eggs should reflect a similarity in the amino acid sequence of that protein. Thus, they should all have a similar FAA-precursor protein in their oocytes prior to final maturation. The mechanism of oocyte hydration in marine teleost pelagic eggs by way of hydrolysis of a specific protein followed by osmotic influx of water due to the generated FAA pool, may therefore be of ancient origin among teleosts. Possibly this dates back all the way to the time when the teleost group made the transition from life in freshwater to life in seawater, i.e. some 150-200 million years ago. The similarity of the FAA pool (i.e. the FAA-precursor protein) among all tested fish species points to a common origin of the mechanism and thus, to a bottleneck for the teleosts as they succeeded in overcoming the challenges of preparing the embryo for an independent life in the seawater. The widespread distribution of pelagic eggs with a large yolk FAA pool shows that the underlying mechanism has been a successful adaptation for life in the ocean during teleost phylogeny. We have set out to characterise the physiological elements and the molecular biology of this mechanism among teleost fishes in relation to their evolution.

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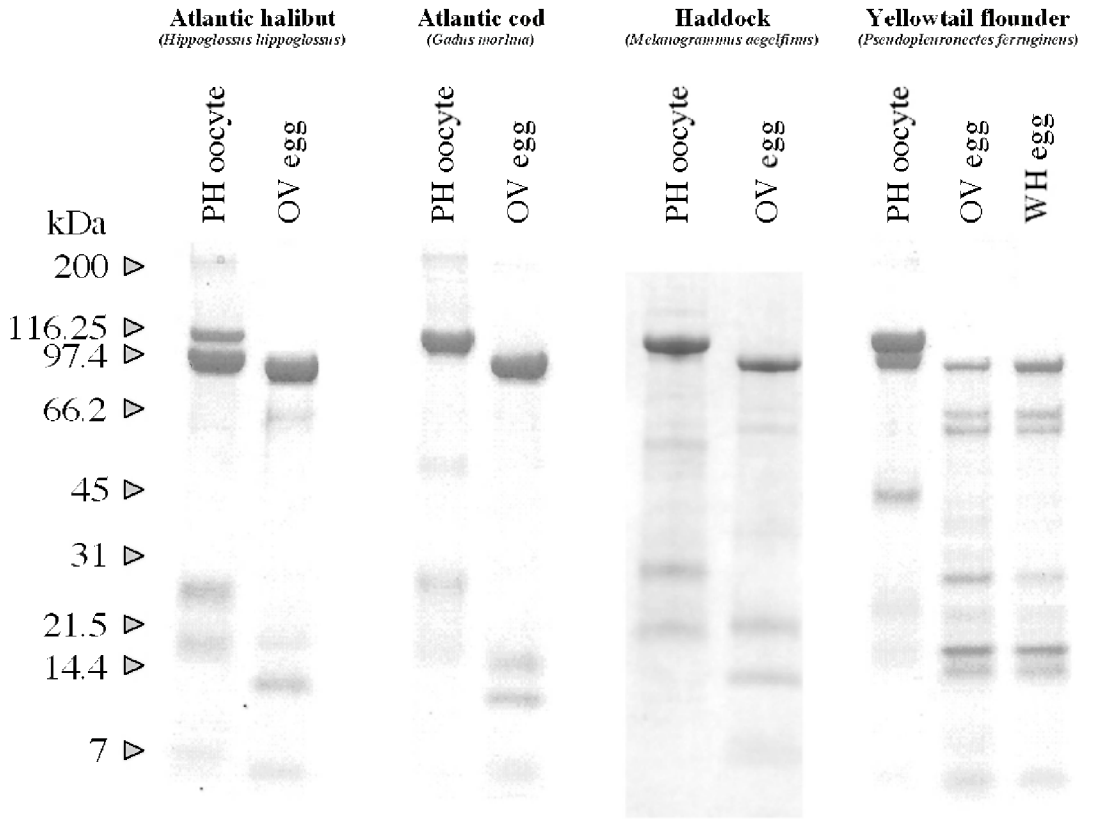

Fig. 4. SDS-PAGE of yolk proteins from prehydrated (PH) oocytes and ovulated (OV) and water hardened (WH) eggs of four North Atlantic marine teleosts with pelagic eggs.

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