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Review

Characterization and modulation of gene expression and enzymatic activity of delta-6 desaturase in teleosts: A review

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ABSTRACT

There is currently considerable interest in understanding how the biosynthetic pathways of highly unsaturated fatty acids (HUFA) are regulated in fish. The aim is to know if it is possible to replace fish oils (FO), rich in HUFA, by vegetable oils (VO), poor in HUFA and rich in their 18 carbon fatty acid precursors, in the feed of cultured fish species of commercial importance. Thus many studies have focussed on delta-6 desaturase (Δ6D) since it is the rate-limiting enzyme involved in HUFA biosynthesis from precursors. The aims of this paper were (i) to review and compare the structure, function, and tissue distribution of the Δ6D gene in teleosts and (ii) to review the effect of nutrition and environment on the modulation of Δ6D gene expression and on the activity of this enzyme in teleosts. Most existing studies have clearly shown that Δ6D is modulated by nutrition and environment in freshwater fish. This modulation allows the control of lipid metabolism and the maintenance of cell membrane functionality. Δ6D gene expression and enzymatic activity were higher in fish fed VO diets than in those fed FO diets, irrespective of their life cycle in seawater or freshwater; this concurs with expectations regarding the compensation for HUFA deficiency in VO. However, the magnitude of these increases was not great enough to maintain HUFA tissue content. Such a decrease in tissue content may result either from competition between substrates for Δ6D or from the inhibition of subsequent steps in HUFA biosynthesis, such as elongation or delta-5 desaturation activity. Other studies showed that Δ6D enzyme activity is stimulated at low temperatures and low salinities, which keep cell membranes fluid. In salmonids, Δ6D would then play an important role in the adaptation to salinity changes at parr–smolt transformation. In marine fish, similar nutritional and environmental modulations may occur, although conflicting data exist. A low expression of the Δ6D gene or the involvement of genetic, environmental, or hormonal factors could explain why Δ6D appears to be barely functional in marine fish. This review shows that, despite the large number of studies investigating the regulation of Δ6D, little is known about the molecular mechanisms involved. Furthermore, the nutritional and environmental regulation of other enzymes involved in HUFA biosynthesis still need to be investigated to obtain a better understanding of the regulation of HUFA biosynthetic pathways in teleosts, ultimately leading to improvements in fish production.

Contents

Abbreviations: CLA, conjugated linoleic acid; Δ6D, delta-6 desaturase; Δ5D, delta-5 desaturase; DM, dry matter; FA, fatty acid; FO, fish oil; HUFA, highly unsaturated fatty acid; LA, linoleic acid; LNA, linolenic acid; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; RXR, retinoic acid X receptor; SRE, sterol regulatory element; SREBP, sterol regulatory element binding protein; VO, vegetable oil.

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1. Introduction

The nutritional benefits associated with seafood are related to the presence of 20- and 22-carbon highly unsaturated fatty acids (n–3 and n–6 HUFA), such as ecosapentaenoic acid (EPA, 20:5n−3), docosahexaenoic acid (DHA, 22:6n−3), and arachidonic acid (AA, 20:4n−6) (Simopolous, 1991; Lauritzen et al., 2001; Delarue et al., 2003, 2004). These nutritional benefits are one of the reasons for the increasing worldwide demand for fish and shellfish. Because HUFAs are the major phospholipid component of cell membranes, they perform a variety of important physiological functions in vertebrates. In fish, they play key roles in ontogenesis, growth, survival, pigmentation, and resistance to stress and disease as well as in the development and functionality of the brain, vision, and nervous system (for a review, see Sargent et al., 2002).

Fish, like mammals, are unable to synthesize HUFA *de novo* from n−3 and n−6 series (EPA, DHA, and AA), which is not the case with the n−9 series HUFAs. Fish therefore require a dietary supply either of the final molecules or of their precursors (Watanabe, 1982; Sargent et al., 2002; Fig. 1). Fish are able to bioconvert 18-carbon fatty acids (C18 FA) that are found in food into n−3 HUFAs (Sargent et al., 2002; Fig. 1). The bioconversion from linoleic acid (LA; 18:2n−6) to AA and from linolenic acid (LNA; 18:3n−3) to EPA and DHA involves desaturations at the delta-6 and delta-5 positions in the carbon backbone as well as an intermediate 2-carbon chain elongation step (Fig. 1). Synthesis of DHA from EPA requires the elongation of EPA to 22:5n−3 and 24:5n−3, which is then converted by delta-6 desaturase (Δ6D) to 24:6n−3; the chain is finally shortened to DHA in peroxisomes (Buzzi et al., 1996, 1997; Sargent et al., 2002).

The Δ6D enzyme has been widely studied in vertebrates, including fish, since it is the rate-limiting enzyme involved in HUFA biosynthesis. This enzyme is responsible for the first step of the desaturation/elongation process in HUFA synthesis, converting LNA (18:3n−3) and LA (18:2n−6) to 18:4n−3 and 18:3n−6 respectively (Fig. 1; Brenner, 1981a,b). Δ6D is also involved in the synthesis of DHA (22:6n−3)

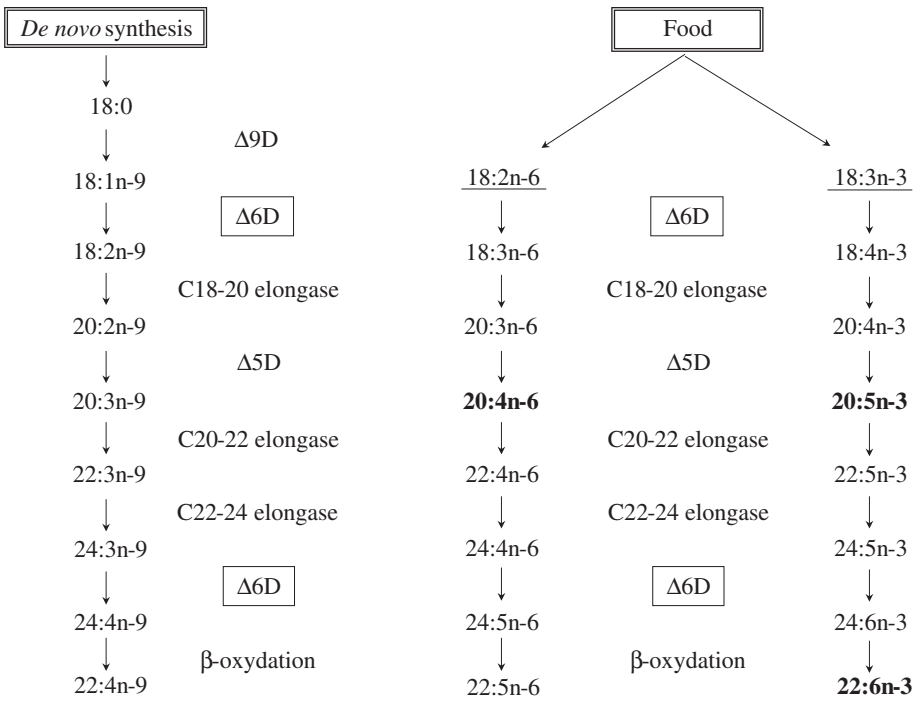

Fig. 1. HUFA biosynthesis pathways in vertebrates, including fish, and the different enzymes involved. HUFAs can be synthesised *de novo* (n−9 series), or from precursor C18 FA brought by food (n−3 and n−6 series). Underlined C18 FA are essential in freshwater fish, while n−6 and n−3 HUFAs written in bold case are the main essential FA (AA 20:4n−6, EPA 20:5n−3 and DHA 22:6n−3) in seawater fish. This figure shows the role of the delta-6 desaturase (Δ6D) as the rate-limiting enzyme responsible of the first step of bioconversion of C18 FAs brought by food into n−6 and n−3 HUFAs. Delta-9 and delta-5 are written Δ9D and Δ5D.

from EPA (20:5n−3). In fish, it is still unclear if one or two enzymes are involved in both reactions (Sargent et al., 2002). However, in humans (de Antueno et al., 2001) and mice (D'Andrea et al., 2002), the same enzyme serves for both reactions.

The extent to which fish can desaturate/elongate C18 FAs to HUFAs varies with species (Sargent et al., 1995, 1999, 2002; Tocher et al., 2006a,b). Freshwater species, such as carp, tilapia, and trout—which are mainly herbivorous or omnivorous—have a recognized capacity to desaturate C18 FAs from the diet (LA [18:2n−6] and LNA [18:3n−3]) into AA, EPA, and DHA (Owen et al., 1975; Henderson and Tocher, 1987; Buzzi et al., 1997; Sargent et al., 2002). In consequence, C18 FAs are essential for HUFA synthesis in freshwater fish (Fig. 1). In contrast, marine species such as turbot, seabream, and sea bass, which are carnivorous, are assumed to have a weak capacity to bioconvert C18 precursors into HUFAs and hence require the preformed HUFAs in their diet (Owen et al., 1975; Kanazawa et al., 1978; Mourente and Tocher, 1994; Sargent et al., 2002). Therefore, preformed HUFAs are themselves essential FAs for seawater fish (Fig. 1). Anadromous fish such as salmon begin their life in freshwater and migrate to the sea before returning to freshwater to breed. Their desaturase profile appears to be that of freshwater fish (Owen et al., 1975; Bell et al., 1997). Although long recognized, the reasons for the low desaturation capacity in marine species remain unclear. It could result from an adaptation to a carnivorous lifestyle (Mourente and Tocher, 1994; Henderson et al., 1995; Sargent et al., 1995), as has been suggested for terrestrial carnivores (Brenner, 1974; Rivers et al., 1975; Sinclair et al., 1979). Consumption of a piscivorous diet, naturally rich in HUFA, could have resulted in an evolutionary down-regulation of the desaturase enzymatic activity that is required for the conversion of C18 FA to HUFA (Sargent et al., 2002).

There has recently been considerable interest in the regulation of Δ6D gene expression and its activity, with the aim of better understanding the regulation of HUFA biosynthetic pathways in commercially important cultured fish species. With the decline of traditional fisheries, aquaculture supplies an increasing proportion of fish produced for human consumption (Tidwell and Allan, 2002). Paradoxically, the predominant protein and lipid sources in the diets for aquaculture fish production come from fish meal and oil (FO) from marine fisheries. However, worldwide supplies of FO have reached their sustainable limits, forcing the feed industry to look for alternative lipid sources (Sargent and Tacon, 1999; Tacon, 2004; Pike, 2005). The only sustainable alternatives to FO are vegetable oils (VO). Therefore, there is a need to produce fish that are able to utilize and metabolize VO. Vegetable products are rich in C18 FAs but do not contain n–3 HUFAs, such as EPA and DHA. It has been widely demonstrated that the dietary FA composition is mirrored in fish tissues (Sargent et al., 2002; Bell et al., 2003a,b; Francis et al., 2007), thus the substitution of FOs by VOs in feeds would have a detrimental impact on the nutritional quality of the final farmed product (Seierstad et al., 2005). However, the lipid composition of organisms not only depends on the dietary lipid ingested, but also on the capacity of species to transform these lipids through desaturation and elongation pathways (Clandinin et al., 1983; Holman, 1986; Lands, 1991). The activity of desaturases is highly dependent upon membrane FA compositions since these are membrane-bound enzymes (Stubbs and Smith, 1984; Spector and Yorek, 1985; McMurchie, 1988; Merrill and Schroeder, 1993). Therefore, the dietary lipid composition may modulate the ability of species to desaturate FAs in order to adjust and maintain the n−3 HUFA content in tissues (Brenner, 1981a,b; Buzzi et al., 1996; Tocher et al., 1997). There is currently considerable interest in understanding the regulation of biosynthetic HUFA pathways in fish to determine the effectiveness with which the FAs in VOs can be utilized by commercially important cultured fish species (Mourente and Dick, 2002; Sargent et al., 2002). In this context, many studies have focussed on the modulation at both the molecular and enzymatic levels of the Δ6D rate-limiting enzyme involved in HUFA biosynthesis.

The aims of this paper were (i) to review and compare the structure, tissue distribution, and function of the Δ6D gene and (ii) to review the effect of nutritional and environmental modulation on Δ6D gene expression and Δ6D enzymatic activity in teleosts.

2. Characterization of Δ6D in teleosts

2.1. Structure and tissue expression

Fatty acid desaturases are membrane-bound enzymes that contain three histidine boxes and two transmembrane domains. Δ5D and Δ6D also present an N-terminal cytochrome b5-like domain, which includes a heme-binding motif H-P-G-G (Fig. 2). In teleosts, these regions are perfectly aligned with those of the Δ5D and Δ6D of other vertebrates, and desaturases in freshwater species present eight to ten additional amino acid residues in the N-terminal extremity. Fish Δ6D has about a 65% homology with the human homologue FADS2 (Table 1). Moreover, the characterized Δ5D of Atlantic salmon (*Salmo salar*) and the putative Δ5D from masu salmon (*Oncorhynchus masou*) are more similar to the FADS2 protein than to the human homologue FADS1, probably because a high percentage of identity (>90%) exists between Δ5D and Δ6D of the same species (Table 1).

The tissue expression of fish Δ6D has been studied in rainbow trout *Oncorhyncus mykiss* (Seiliez et al., 2001), Atlantic cod *Gadus morhua* (Tocher et al., 2006a), cobia *Rachycentron canadum* (Zheng et al., 2009), and sea bass *Dicentrarchus labrax* (González-Rovira et al., 2009). For each of these species, high levels of expression have been found in brain, liver, intestine, and kidney (Seiliez et al., 2001; Tocher et al., 2006a). González-Rovira et al. (2009) recorded the highest Δ6D expression in the heart of sea bass. Moreover, Northern blot studies have shown the presence of highly expressed desaturase transcripts in both rainbow trout (Seiliez et al., 2001) and sea bream *Sparus aurata* (Seiliez et al., 2003). In each case, the size of the lower transcript corresponds to that of the desaturase-like cDNA while the physiological function of the upper transcript remains unknown.

2.2. Functional characterization

Many fish desaturases have been functionally characterized by heterologous expression in the yeast *Saccharomyces cerevisiae* (Table 1). This technique involves the amplification of the desaturase cDNA coding sequence and the subsequent ligation of the amplified product in a yeast expression vector. The *S. cerevisiae* host is then transformed with the resulting plasmid construct and the expression of the transgene is induced by galactose addition (Hastings et al., 2001). Cultures are grown in the presence of desaturase substrates, and the resultant FAs are analyzed using gas chromatography coupled with mass spectrometry. This analysis allows the identification of the specific desaturase activity when exogenous substrates are desaturated in the Δ6 and/or Δ5 positions.

The first characterized fish desaturase was the bifunctional Δ5/Δ6 zebrafish enzyme (Hastings et al., 2001). Apart from this protein, which possesses the capacity to desaturate both types of substrates, other fish desaturases that have been characterized are either unifunctional (cod; Tocher et al., 2006a) or present a major desaturase activity with a residual capacity to desaturate in another position (Table 1). Even though this technique is semiquantitative because of some limitations in comparing desaturase capacities among substrates with different carbon numbers (de Antueno et al., 2001), it is possible to determine if expressed heterologous enzymes have a preference for substrates of the same chain length belonging to the n−3 or n−6 pathway. In this sense, fish desaturases preferentially

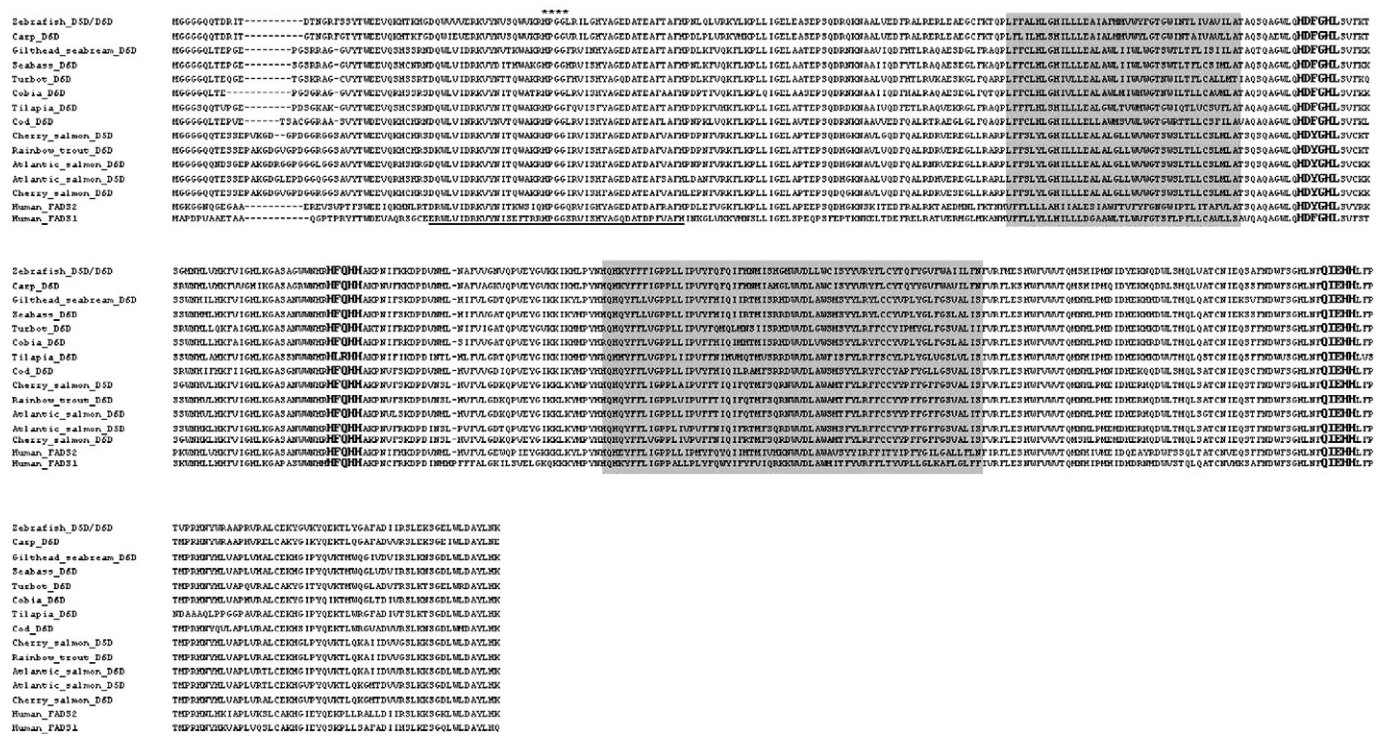

Fig. 2. Comparison of the deduced amino acid sequences of the fatty acyl desaturase from fish and human. The amino acid sequences were aligned using ClustalX. The cytochrome b5-domain is underlined. Putative transmembrane domains are shown in shaded areas and the three histidine-rich regions are bolded. The asterisks indicate the heme-binding motif (HPGG).

desaturate n−3 substrates (Table 1), while the functional characterization of Δ6D from mammals, fungi, and mosses (Aki et al., 1999; Cho et al., 1999a; Kajikawa et al., 2004) has shown that these enzymes are equally active with both n−3 and n−6 substrates. The only exception is the family *Primula* sp., which prefers the n−3 substrates (Sayanova et al., 2003).

3. Nutritional and environmental modulation of Δ6D gene expression and enzymatic activity in teleosts

3.1. Nutritional modulation

The nutritional modulation of Δ6D has been well described in mammals (Christiansen et al., 1991; Ulmann et al., 1992; Cao et al., 1995; Cho et al., 1999a,b). For example, diets rich in DHA reduce microsomal Δ6D activity in rat liver (Garg et al., 1988; Christiansen et al., 1991; Ulmann et al., 1992; Cao et al., 1995). Concerning teleosts, the nutritional modulation of Δ6D has been extensively studied to test whether desaturase activity could compensate for the reduction of tissue HUFA levels due to the inclusion of dietary VOs.

3.1.1. Freshwater fish

Although not always attaining statistical significance, several studies have shown that Δ6D gene mRNA levels were higher in freshwater fish fed VOs (low HUFA content) than in those fed FOs (high HUFA content). For example, higher Δ6D mRNA levels were measured in the liver and red muscle of Atlantic salmon fed diets in which 75% of FOs had been replaced by a blend of rapeseed, palm,

Table 1
Available Δ5D and Δ6D genes in fish. An asterisk denotes a desaturase sequence that has not been functionally characterized.

Species	Type	GenBank accession number	No of amino acid residues	% Identity			n−3 preference	Other desaturase residual activity	Functional characterization
				Intraspecific	Human				
					FAD2	FADS1			
Atlantic salmon (*Salmo salar*)	Δ6	AY458652	454	–	65	54	Yes	Yes	Zheng et al. (2005b)
Cherry salmon (*Oncorhynchus masou*)	Δ6*	AB070444	454	–	63	53	–	–	–
Rainbow trout (*Oncorhynchus mykiss*)	Δ6	AF30191	454	–	65	54	Yes	Yes	Zheng et al. (2004a)
Seabream (*Sparus aurata*)	Δ6	AY055749	445	–	65	52	Yes	Yes	Zheng et al. (2004a)
Cod (*Gadus morhua*)	Δ6	DQ054840	447	–	65	51	Yes	No	Tocher et al. (2006a)
Turbot (*Scophthalmus maximus*)	Δ6	AY546094	445	–	63	58	Yes	Yes	Zheng et al. (2004a)
Cobia (*Rachycentron canadum*)	Δ6	FJ440238	442	–	66	52	Yes	Yes	Zheng et al. (2009)
Sea bass (*Dicentrarchus labrax*)	Δ6	EU647692	445	–	65	52	–	–	Santigosa et al. (2010)
							Yes	–	González-Rovira et al. (2009)
Nile tilapia (*Oreochromis niloticus*)	Δ6*	AB069727	445	–	?	?	–	–	González-Rovira et al. (2009)
Carp (*Cyprinus carpio*)	Δ6	AF309557	444	–	?	?	Yes	Yes	Zheng et al. (2004a)
Atlantic salmon (*Salmo salar*)	Δ5	AF478472	454	91	64	54	Yes	Yes	Hastings et al. (2005)
Cherry salmon (*Oncorhynchus masou*)	Δ5*	EU098126	452	94	65	52	–	–	–
Zebrafish (*Danio rerio*)	Δ5/Δ6	AF309556	444	–	64	58	Yes	Bifunctional	Hastings et al. (2001)

and linseed oils in a 3.7:2:1 ratio compared to fish fed HUFA-rich diets containing only FO as a lipid source (Zheng et al., 2005a,b). Similar results were obtained in salmon and rainbow trout fed diets containing linseed oil as the lipid source compared to those fed fish oil as the sole lipid source (Seiliez et al., 2001; Zheng et al., 2004b).

Moreover, replacing FOs by VOs consistently resulted in increased Δ6D activity in freshwater fish (Bell et al., 1997, 2001, 2002; Tocher et al., 1997, 2000, 2001a,b, 2002, 2003a,b, 2004, 2006b; Zheng et al., 2005a,b); this was also seen in mammals (Brenner, 1981a,b). In Arctic charr (*Salvelinus alpinus*), the conversion by Δ6D of [1-^{14}C]-labelled LA (18:2n−6) and LNA (18:3n−3) to 18:3n−6 and 18:4n−3, respectively, was higher in fish fed diets containing only VOs compared to those fed commercial diets containing FO (Olsen and Ringo, 1992). Buzzi et al. (1996) demonstrated an increase in hepatic Δ6D activity when feeding rainbow trout on a diet containing olive oil compared to a diet containing FO. Similar results were obtained in caecal enterocytes of this species when crude palm oil replaced FO in the diet (Tocher et al., 2004; Fonseca-Madrigal et al., 2005). In Atlantic salmon, the desaturation of 18:3n−3 and 18:2n−6 in isolated hepatocytes was up to 2.5-fold higher in fish fed a diet containing VO (1:1 blend of linseed and rapeseed oils) rich in 18:2n−6 and 18:3n−3 compared to those fed a diet containing FO (Tocher et al., 1997). Several comparative studies on salmonids reared in freshwater showed that Δ6D activities were up to 2.5-fold (Bell et al., 1997), 2.4-fold (Tocher et al., 2000), and 2.8-fold (Tocher et al., 2001a) greater in fish fed VO compared to fish fed FO. More recently, a 4-fold increase in desaturation levels was measured in the caecal enterocytes of salmon fed a VO blend (1:1 rapeseed and linseed oils), than in fish fed FO (Tocher et al., 2003a). Turchini et al. (2006) demonstrated the ability of freshwater Murray cod (*Maccullochella peelii peelii*) to desaturate 18:2n−6 and 18:3n−3 when fed a diet containing either canola or linseed oil. Similar results were further obtained in this species when replacing FO with a blend of VOs (Francis et al., 2007).

Such molecular and enzymatic data from freshwater fish can be interpreted as an inhibition of Δ6D by n−3 HUFAs, as has been shown in mammals (Garg et al., 1988; Christiansen et al., 1991; Ulmann et al., 1992). This is in accordance with Leger et al. (1981), who first reported that a high dietary level of 22:6n−3 inhibits the desaturation of both 18:2n−6 and 18:3n−3 in rainbow trout. On the contrary, Ruyter et al. (2000) showed that the percentage of 18:3n−6—the Δ6D desaturation product formed from [1-^{14}C] labelled 18:2n-6—was twice as high in the hepatocytes of Atlantic salmon fed the FO diet than in those fed VO. This indicates that the high percentage of 22:6n−3 in the FO diet did not have the expected result of radically inhibiting the desaturation of 18:2n−6. This could be explained by the higher 18:2n−6/18:3n−3 ratio found in the FO diet (2.8) compared to the VO diet (0.5). This higher ratio probably promoted a higher desaturation rate of 18:2n−6 to 18:3n−6 in fish fed the FO diet than in those fed the VO diet (Ruyter et al., 2000).

Several studies in freshwater fish reported that Δ6D gene expression could not only be inhibited by dietary n−3 HUFA content but also stimulated by dietary-conjugated LA (CLA; i.e., positional and geometric isomers of LA 18:2n−6; Twibell et al., 2000; Kennedy et al., 2005, 2006). For example, Atlantic salmon fed a diet that included 2% CLA exhibited increased Δ6D gene expression in the liver (Kennedy et al., 2006). This was particularly evident in the diet containing only 17% FO (i.e., the low-oil diet; the high-oil diet contained 34% FO); measurements showed a 2-fold increase in the Δ6D mRNA level. This was correlated with an increase in HUFA synthesis in these fish and is in accordance with Twibell et al. (2000), who reported an increase in EPA and DHA in response to dietary CLA in hepatic and muscle tissues of hybrid striped bass (*Morone chrysops, saxatilis*). On the contrary, Leaver et al. (2006) reported that CLA had no effect on Δ6D mRNA levels in the liver or intestine of Atlantic salmon. Moreover, Kennedy et al. (2005) reported that dietary CLA had no significant effect on either liver or muscle FA compositions in salmon. In mammals, conflicting data exist regarding CLA effects on Δ6D. Some studies have indicated a suppression of enzymatic activity after CLA feeding (Eder et al., 2002), while others showed marked induction of gene expression and enzymatic activity (Peters et al., 2001; Takahashi et al., 2003).

3.1.2. Seawater fish

As in freshwater, the Δ6D mRNA levels in Atlantic salmon reared in seawater were significantly higher in fish fed VOs than in fish fed FOs (Zheng et al., 2005a,b). This molecular result was reflected by increasing levels of 20:2n−6 and 20:3n−6 in the liver, indicating metabolism of 18:2n−6.

The nutritional modulation of Δ6D was revealed for the first time in a strictly marine fish, gilthead seabream, by Seiliez et al. (2003). As in freshwater fish, Δ6D gene expression in sea bream liver was higher in fish fed a HUFA-free diet (containing olive oil as the sole lipid source) than in fish fed a HUFA-rich diet (containing FO). While these authors did not measure enzyme activities, a higher production of 18:2n−9 and 20:2n−9 was revealed, indicating higher Δ6D activity in fish fed HUFA-deficient diets. High levels of 18:2n−9 and 20:2n−9 were previously reported in juvenile grey mullet (initial weight = 0.7 g) fed on a fat-free diet for 84 days in sea water (Argyropoulou et al., 1992). Seiliez et al. (2003) also showed an 18:3n−6 increase in seabream fed a HUFA-free diet, which might be a further indication of a Δ6D activity in these fish. Similarly, significantly higher levels of 18:2n−9 and 18:3n−6 and increases of more than 6-fold in Δ6D mRNA levels were reported in larvae of this species that had been fed diets in which FO was totally replaced by rapeseed or soybean oil (Izquierdo et al., 2008). In the same way, Vagner et al. (2007a,b, 2009) showed that Δ6D mRNA levels were higher in sea bass larvae fed a low HUFA diet (0.5% or 0.7% EPA + DHA, % dry matter [DM]; FO replaced with soybean oil) compared to those fed a high HUFA diet (1.7% or 3.7% EPA + DHA, % DM; only FO). This was supported by an increase in 18:3n−6 content in phospholipids. Finally, a new study also showed a significantly higher Δ6D mRNA level in the livers of sea bass fed linseed and rapeseed oils compared to the livers of fish fed FO (González-Rovira et al., 2009). None of these studies reported an increase in 18:4n–3 (the desaturation product of 18:3n−3 by Δ6D). This could be due to the relatively high level of this FA initially present in fish and to the negligible levels of 18:3n−3 in VOs used in HUFA-free diets.

Unlike the results described above on studies done with other oils, González-Rovira et al. (2009) demonstrated that dietary olive oil did not significantly increase Δ6D mRNA levels in sea bass liver compared to FO. Furthermore, contrasting results were obtained with sea bass fed two different blends of VO (60% substitution of FO with two blends of rapeseed, linseed, and olive oils), with the expression of the Δ6D only significantly up-regulated in fish fed one of the blend (González-Rovira et al., 2009). These conflicting data obtained from sea bass could be due to the low expression of Δ6D in the liver of this species. In addition, Δ6D expression was not determined in other tissues following the ingestion of dietary VOs (González-Rovira et al., 2009). Previous studies have demonstrated that the inclusion of VOs did not lead to a significant increase in desaturase activity in sea bass hepatocytes (Mourente and Dick, 2002; Mourente et al., 2005b), while increased activity was reported in pyloric caecal enterocytes of sea bass fed VO blends (Mourente et al., 2005a).

Similar conflicting data have previously been reported in marine fish. Comparable Δ6D mRNA levels were measured in the liver and intestine of adult cod (*G. morhua*) fed diets containing VOs or FOs (Tocher et al., 2006a). This was reflected in unchanged enzyme activities in hepatocytes and enterocytes. These results suggested that the nutritional regulation of Δ6D could vary according to the species or the developmental stage considered (Tocher et al., 2006a).

The occurrence of conflicting data in both Δ6D gene expression and enzymatic activity in marine fish may be due to the lower

levels of expression and enzyme activity recorded in marine fish tissues compared to freshwater fish and salmonids (Tocher et al., 2006a,b; González-Rovira et al., 2009). There may be other environmental, genetic, or hormonal factors that could explain why Δ6D appears to be barely functional in marine fish (González-Rovira et al., 2009).

3.1.3. Variation of Δ6D nutritional modulation in teleosts

3.1.3.1. Variation with time: is it a long-lasting stimulation? Recent studies have attempted to discover whether the nutritional modulation of Δ6D gene expression is a persistent adaptation at the molecular level or a transient acclimation to the nutritional environment. Vagner et al. (2007b, 2009) tested whether it was possible to modulate Δ6D gene expression in sea bass juveniles using nutritional conditioning during the larval phase.

Larvae were fed a low (0.5–0.8% EPA + DHA, % DM) or high (1.7–3.7% EPA + DHA, % DM) HUFA diet from mouth opening (6 days post-hatching) to the end of the larval stage (45 days post-hatching). After a subsequent period (1 to 3 months) of feeding with a commercial diet (2.7% EPA + DHA, % DM), the capacity of juveniles to adapt to a HUFA-restricted diet (0.3–0.5% EPA + DHA, % DM) was tested. Results showed that the Δ6D mRNA level was enhanced in larvae fed a low n−3 HUFA diet and that this effect remained for 30 days in the resulting juveniles fed the n−3 HUFA-restricted juvenile diet. Moreover, a slightly, but significantly, higher DHA content in the polar lipids of pre-conditioned juveniles was measured. Similar results were not found in larvae fed the high HUFA diet or in the resulting juveniles. This indicates that pre-conditioned juveniles could better develop desaturation processes in order to adapt to low dietary HUFA than unconditioned fish. However, the Δ6D mRNA stimulation was transient in pre-conditioned juveniles, with the effect only being detectable for 30 days. This was interpreted as an adaptation of juveniles to a nutritional environment restricted in n−3 HUFA since the low n−3 HUFA diet used during juvenile stage (0.5% EPA + DHA, % DM) was close to the n−3 HUFA requirement of sea bass juveniles, which was reported to be 0.7% EPA + DHA (% DM) by Skalli and Robin (2004). The use of a more restricted n−3 HUFA diet for larvae and juveniles led to a persistent enhancement of the Δ6D mRNA level in juveniles pre-conditioned with a low n−3 HUFA diet during larval stage (Vagner et al., 2009). However, this did not lead to an increase in HUFA tissue content in juveniles, contrary to previous observations (Vagner et al., 2007b). In conclusion, it is not clear if the stimulation of the Δ6D mRNA level by diet is a persistent adaptation at the molecular level or if it is an acclimation to the nutritional environment of juveniles (Vagner et al., 2007b, 2009). One hypothesis raised is that some molecular adaptations are settled during the larval stage in conditioned larvae, and that these molecular pathways could later be modulated by the nutritional environment during the juvenile stage to control HUFA tissue content (Vagner et al., 2007b, 2009).

3.1.3.2. Variation with the Δ6D substrate. Several studies reported that the degree of nutritional modulation of Δ6D enzymatic activity by VO diets depends on the dietary ratio of 18:3n−3/18:2n−6, both of which are Δ6D enzyme substrates (Padley et al., 1986; Tocher et al., 2001a, 2002; Izquierdo et al., 2008). Maximal enzyme efficiency is reached at particular levels of substrate availability (Bell et al., 2002; Francis et al., 2009). For example, Bell et al. (2002) fed Atlantic salmon (*S. salar*) a diet in which the added lipid was either 100% palm oil (PO, 38% 18:1n−9 and 10% 18:2n−6 as Δ6D enzyme substrates) and 0% FO, 75% PO and 25% FO, 50% PO and 50% FO, 25% PO and 75% FO, or 0% PO and 100% FO. The results showed a progressive increase in the [1-^{14}C] 18:3n−3 desaturation product (i.e., 18:4n−3) with added dietary palm oil. However, only fish fed 100% palm oil were significantly different from the other three treatments, with a 10-fold higher activity than fish fed 100% FO. Nevertheless, when palm oil made up more than 50% of the dietary lipid, significant reductions in muscle 20:5n−3, 22:6n−3, and n−3/n−6 FA ratio occurred.

It is well known that Δ6D enzyme in fish has a higher affinity with n−3 than with n−6 FAs (Section 2.2). Several studies have shown that using a high 18:3n−3/18:2n−6 ratio in the diet leads to greater Δ6D activity (Bell et al., 1997, 2001; Tocher et al., 1997, 2000, 2001a, 2002; Li et al., 2008). For example, borage oil, which contains only γ-LNA (i.e., γ−18:3n−3), leads to total desaturation products that are 2-fold higher than those found with other VOs in Atlantic salmon (Tocher et al., 1997). More recently, a study on salmonids found that palm oil (which has a very high 18:3n−3/18:2n−6 ratio) led to hepatic FA desaturation activities ~10-fold higher in fish fed a diet where the lipid was 100% palm oil compared to in those fed a diet containing 100% FO (Bell et al., 2002). However, increases in FA desaturation activities were only 2.4- to 2.8-fold higher in fish fed diets where all FO was replaced by various VOs (Bell et al., 1997, 2001; Tocher et al., 1997, 2000, 2001a,b, 2002). All these results are in accordance with a more recent study indicating that a high 18:3n−3/18:2n−6 ratio up-regulated Δ6D gene expression (Li et al., 2008). The Δ6D mRNA level was significantly higher in *Siganus canaliculatus* fed a diet containing perilla oil as the sole lipid source (rich in α-LNA [α-18:3n−3]) compared to those fed a diet containing safflower oil as the sole lipid source (rich in LA [18:2n−6]). The Δ6D mRNA levels were nevertheless significantly higher in both these groups of fish than in those fed FO as the sole lipid source (Li et al., 2008).

An excess of 18:3n−3 in the diet could block Δ6D gene transcription (Izquierdo et al., 2008). Complete inhibition of Δ6D gene expression was observed in gilthead seabream larvae fed a diet in which FO was totally replaced by linseed oil (Izquierdo et al., 2008). Other studies also showed that an excess of 18:3n−3 could inhibit Δ6D enzymatic activity, preventing desaturation of 18:2n−6 (Bell et al., 1993). Reduced Δ6D activity was measured in sea bass hepatocytes and in Atlantic salmon enterocytes when fish were fed linseed oil containing up to 56% 18:3n−3 with the 18:3n−3/18:2n−6 ratio over 3 (Padley et al., 1986; Tocher et al., 2002; Zheng et al., 2005a,b). Previously, Tocher et al. (2002) found no evidence of dietary 18:2n−6 desaturation products in either the liver or intestinal tissue of Atlantic salmon fed a diet with linseed oil while the stimulation of desaturation pathways was revealed by an increase in 18:4n−3 products.

Previous studies in salmonids suggested that too high a level of 18:2n−6 in VOs may also inhibit the desaturation of 18:3n−3 (Ruyter et al., 2000; Tocher et al., 2001a,b). A higher 18:2n−6/18:3n−3 ratio in FO than in a linseed oil diet led to a higher quantity of 18:2n−6 desaturation products in Atlantic salmon hepatocytes due to the lower percentage of the competing 18:3n−3 (Ruyter et al., 2000). This is in accordance with the higher 18:2n−6 desaturation products measured in rainbow trout and sea bass when the level of competing 18:3n−3 FA was low in the diet (Bell et al., 1996; Vagner et al., 2007b, 2009). However, it is important to note that Tocher et al. (1997) found increased amounts of 18:3n−3 desaturation products in hepatocytes from Atlantic salmon fed diets that contained high levels of n−6 FA. This was explained by the higher affinity of Δ6D with n−3 FA than with n−6 FA (Tocher et al., 1997).

The 18:3n−3 fatty acid could also compete with 24:5n−3 for the Δ6D (Fig. 1). This could explain why no desaturation products were found in hepatocytes of Atlantic salmon fed linseed oil diet (18:3n−3) despite a 18:3n−3/18:2n−6 dietary ratio of nearly two (Ruyter et al., 2000). This ratio should normally favour the conversion of 18:3n−3 into HUFA, but the ratio of 20:5n−3/18:3n−3 was two. Thus, the 20:5n−3 can easily be converted to 24:5n−3, which competes with 18:3n−3 for the Δ6D. The 24:5n−3 newly formed is then converted at the expense of 18:3n−3 (Ruyter et al., 2000). These results are in accordance with those of Yu and Sinnhuber (1976), who demonstrated that more unmetabolized radioactivity was recovered when 18:3n−3 was the substrate than when 18:2n−6 was used.

Ruyter et al. (2000) hypothesized that competition between the Δ6D substrates could partly explain the HUFA deficiency observed in tissues of fish fed high VO dietary contents, while VOs provide sufficient C18 FA substrates for the Δ6D and thus for potential conversion to 20:5n−3 [EPA] and 22:6n−3 [DHA] (Ruyter et al., 2000; Torstensen et al., 2000; Tortensen et al., 2005; Bell et al., 2001, 2002, 2003a,b; Tocher et al., 2001a,b, 2002, 2003a,b; Mourente and Dick, 2002; Seiliez et al., 2003; Zheng et al., 2005a,b; Francis et al., 2007, 2009; Vagner et al., 2007a,b, 2009; Izquierdo et al., 2008). Despite the increase in gene expression and Δ6D activity as well as the increase in Δ6D desaturation products in fish fed VO, an excessively high dietary content of C18 FA may prove counterproductive when the aim is to maximize in vivo n−3 HUFA production (Francis et al., 2009). Furthermore, these HUFA deficiencies could also be a consequence of low activities during subsequent steps of elongation and desaturation by Δ5D. Deficiencies in these steps have been measured in turbot and sea bream cell lines (Ghioni et al., 1999; Tocher and Ghioni, 1999). It is also important to note that, while the Δ6D has been described as the rate-limiting enzyme in the FA biosynthetic pathways (Brenner, 1981a,b), HUFA synthesis can also be limited by Δ5D and elongase since these enzymes are also under nutritional and environmental regulation (Zheng et al., 2004b, 2005a,b; Francis et al., 2007; Izquierdo et al., 2008).

3.1.4. Mechanisms involved in the nutritional modulation of Δ6D

It has remained unclear whether Δ6D nutritional modulation is due more to desaturation product reduction or to increased substrate supply, or if both are involved. Some authors demonstrated that the rate of C18 FA desaturation was more strongly regulated by the competition between C18 FA substrates for Δ6D than by DHA (Ruyter et al., 2000; Francis et al., 2007). However, Tocher et al. (2002) speculated that this rate of desaturation is a direct result of product reduction rather than an increased supply of precursors. Conversely, other authors concluded that both product reduction and increased substrate supply were factors determining hepatocyte FA desaturation activity (Christiansen et al., 1991; Horrobin, 1991; Tocher et al., 2003a,b). Further investigation is required to identify the mechanisms involved.

In mammals, FA desaturases are known to be regulated by two transcription factors: Sterol Regulatory Element Binding Protein-1 (SREBP-1) and Peroxisome Proliferator-Activated Receptors (PPARs) (for a review, see Nakamura and Nara, 2002, 2003). In mammals, desaturases are unique in that they are the only genes whose transcription is activated by both SREBP-1 and PPARs (Nakamura and Nara, 2002, 2003).

SREBP-1 presents two subforms in mammals: SREBP-1a in differentiating cells and SREBP-1c in cells already differentiated. In humans, SREBP-1c would induce Δ6D gene transcription binding to SRE (Sterol Regulatory Element), a promoter region located on the Δ6D gene (Horton et al., 2002; Matsuzaka et al., 2002). This promoter would also be responsible for the inhibition of Δ6D gene transcription by HUFA (Nara et al., 2002). Over-expression of SREBP-1 increases Δ6D gene transcription in mice, while disruption of the SREBP-1 gene reduces Δ6D gene transcription (Matsuzaka et al., 2002).

PPARs are nuclear receptors that have three isoforms in mammals, birds, and amphibians: α, β or δ, and γ (Dreyer et al., 1992). In mammals, each isoform is a product of a separate gene and has a distinct tissue distribution (Desvergne and Wahli, 1999; Escher et al., 2001; Hihi et al., 2002). PPAR α represents the predominant hepatic isoform in mammals. It controls target genes involved in the intestinal absorption of HUFA and in FA β-oxidation that occurs in liver mitochondria and peroxisomes (Gulick et al., 1994). It is also involved in hepatic FA biosynthesis. PPAR β is involved in keratinocyte differentiation in which lipid metabolism is very active (Tan et al., 2002). PPAR γ plays a role in adipogenesis, stimulating adipocyte differentiation and the induction of lipogenic enzymes (Gregoire et al., 1998; Zhang et al., 2006). PPAR ligands are lipids such as HUFA (EPA, DHA, and AA) and CLA, but they also include eicosanoids, prostaglandins, and leukotrienes (Forman et al., 1997; Kliewer et al., 1997; Krey et al., 1997; Leaver et al., 2005). The ligand–PPAR complex binds to the retinoic acid X receptor (RXR), forming a heterodimer. This heterodimer then binds to the regulation zone of target genes involved in FA β-oxidation, called the Peroxisome Proliferator Response Element (PPRE) or PPAR response zone, to module their transcription (Schoonjans et al., 1996). This FA β-oxidation would then lead to a HUFA requirement, which will activate Δ6D gene transcription indirectly. PPAR would also have a direct action on Δ6D gene transcription (Tang et al., 2003). However, the delay in desaturase activation related to the activation of enzymes involved in FA β-oxidation observed in mammal hepatocytes would indicate that Δ6D gene transcription is mainly controlled by the indirect action of PPAR (Tan et al., 2002; Tang et al., 2003). However, the precise mechanisms involved are not well known.

Concerning fish, partial cDNAs for two distinct PPAR β-like proteins have been described from zebrafish (Robinson-Rechavi et al., 2001). In pufferfish (*Fugu rubripes*), a single homolog of the human PPAR β and γ genes and two homologs of the human PPAR α gene were suggested (Maglish et al., 2003). In Atlantic salmon, a complete cDNA sequence similar to PPAR γ has been isolated (Andersen et al., 2000). However, this species may contain up to five PPAR genes, and their precise roles have not been clearly defined (Leaver et al., 2005). Recent work has revealed that three species of marine fish, plaice (*Pleuronectes platessa*), gilthead seabream, and sea bass, share gene sequences of PPAR α, β, and γ that are homologous to those of mammals, with phylogenetic characteristics similar to the mammalian PPAR counterparts; this would also suggest similar molecular roles (Boukavala et al., 2004; Leaver et al., 2005). In contrast, PPAR γ is expressed more widely in seabream and plaice tissues than in mammals whereas PPAR α and β expression profiles are similar to those in mammals. As in mammals, PPARs in these species bind to a variety of natural PPRE.

Recent studies suggested the involvement of PPARs in the modulation of Δ6D gene transcription in fish (Kennedy et al., 2006; Vagner et al., 2009). PPAR α and β mRNA levels were significantly higher in sea bass larvae fed a diet low in n−3 HUFA (0.3−0.5% EPA + DHA, % DM) compared to those fed a diet higher in n−3 HUFA (0.7–3.7% EPA + DHA, % DM; Vagner et al., 2009). Furthermore, the PPAR β mRNA stimulation was maintained when the resulting juveniles were fed a very low n−3 HUFA diet (0.3% EPA + DHA, % DM). This is in accordance with the significantly higher Δ6D mRNA levels measured in these larval and juvenile groups at the same time. These concomitant increases of Δ6D and PPAR mRNA levels in larvae and juveniles suggested that PPAR could be partly involved in the nutritional modulation of Δ6D gene transcription in larval and juvenile sea bass (Vagner et al., 2009). Although the PPAR γ mRNA level was not significantly higher in larvae fed a diet deficient in n−3 HUFA, it was significantly higher in the resulting juveniles. This suggests that PPAR γ could have a role in the stimulation of Δ6D gene transcription measured at the same time (Vagner et al., 2009). Conversely, PPAR mRNA levels were not significantly higher in larvae fed a low n−3 HUFA diet from 0.7 to 0.8% EPA + DHA (% DM) or in the resulting juveniles, even though significantly higher Δ6D mRNA levels were measured in the larvae and juveniles (Vagner et al., 2007a,b, 2009). These results led to the hypothesis that Δ6D gene transcription in sea bass could be stimulated by PPAR when drastic nutritional conditions occurred, and that above a certain dietary HUFA threshold, other mechanisms like those observed in mammals may be implicated, such as SREBP-1 (Vagner et al., 2007a,b, 2009).

This possible involvement of PPAR in the nutritional modulation of Δ6D gene transcription in fish has also been reported in Atlantic salmon (Kennedy et al., 2006). Higher Δ6D and PPAR α and γ mRNA levels as well as higher HUFA synthesis in liver, red muscle, and white

muscle were measured in fish fed a low-oil diet with a highly conjugated LA (CLA) content (Kennedy et al., 2006). This could indicate a role for ligand activation of PPAR α by CLA. This is in accordance with the activation in mammals of Δ6D gene transcription by LA (18:2n−6), which act as high-affinity ligands for PPARα (Moya-Camarena et al., 1999). In contrast, in fish fed high-oil diets, Δ6D expression and HUFA synthesis were repressed, even when similar amounts of CLA were present (Kennedy et al., 2006). This could indicate a possible role of SREBP-1 proteins in Δ6D repression (Kennedy et al., 2006). Consequently, these authors hypothesized that transcription factors equivalent to mammalian PPAR α and SREBP-1c are directly involved in regulating Δ6D in fish via a feedback mechanism (Kennedy et al., 2006).

3.2. Environmental modulation of Δ6D gene expression and enzymatic activity

In addition to nutritional factors, environmental factors such as temperature and salinity have been demonstrated to mediate Δ6D gene expression and enzymatic activity in teleosts (Bell et al., 1997; Tocher et al., 2000, 2004; Zheng et al., 2005a,b).

3.2.1. Effect of temperature

It is well documented that temperature influences the degree of FA saturation in fish membranes (Ninno et al., 1974; de Torrengo and Brenner, 1976; Hazel, 1995; Ruyter et al., 2003; Tocher et al., 2004). Several studies demonstrated that FA in phospholipids are less saturated in fish exposed to low temperatures than in fish exposed to high temperatures so that membrane fluidity is maintained (Sellner and Hazel, 1982; Cossins, 1983; Cossins and Bowler, 1987; Tiku et al., 1996). For example, at 5 °C, the percentage of 16:0 was lower and the percentage of 22:6 n−3 was higher in the total lipid fraction of salmon hepatocytes than at 12 °C, indicating a tendency for a higher production of 22:6 n−3 from [1-^{14}C] 20:4 n−3 at low temperatures (Moya-Falcón et al., 2006). This phenomenon is known as homeoviscous adaptation, and it ensures that membrane function is unaltered during changes in water temperature (Sinesky, 1974; Robertson and Hazel, 1999). However, this notion as related to fish was questioned by Sargent et al. (2002), who considered that the intrinsic structure of DHA was resistant to temperature variation and that its abundance of phospholipid ensures membrane fluidity, whatever the temperature.

Several studies reported that the increase in the degree of FA unsaturation at low temperatures is related to changes in both desaturase and elongase capacities (Ninno et al., 1974; de Torrengo and Brenner, 1976; Schünke and Wodtke, 1983; Hagar and Hazel, 1985; Wodtke and Cossins, 1991). It has been shown in several freshwater fish species that the Δ6D activity decreases when temperature increases (Ninno et al., 1974; de Torrengo and Brenner, 1976; Schünke and Wodtke, 1983; Hagar and Hazel, 1985; Tocher et al., 2004). For example, Δ6D activity was higher in common carp kept at 10 °C than in those kept at 30 °C (Schünke and Wodtke, 1983). The Δ6D activity was also higher in enterocytes and hepatocytes of rainbow trout kept at 5 °C or 7 °C than in trout kept at 20 °C or 15 °C, respectively (Hagar and Hazel, 1985; Tocher et al., 2004). Catfish (*Pimelodus maculatus*) kept at 14–15 °C had higher liver microsomal desaturation activities for oleic, LA, and α-LNA than did fish kept at 29–30 °C (de Torrengo and Brenner, 1976). Similarly, Δ6D activities in liver microsomes have been shown to be 2-fold higher at 16 °C than at 30 °C (Ninno et al., 1974). Furthermore, a variation in the Δ6D activity of catfish has been observed for a temperature variation of ±2 °C (de Torrengo and Brenner, 1976).

De Torrengo and Brenner (1976) demonstrated that the induction timing of Δ6D gene transcription could depend on temperature. When catfish acclimated to 28 °C were shifted to 18 °C, Δ6D activity initially decreased one day after transfer, probably due to a kinetic effect, but subsequently increased one week after transfer, presumably due to the induction of gene transcription. However, when a temperature increase occurred, the response time for gene transcription inhibition was only one day (de Torrengo and Brenner, 1976).

The interaction between water temperature and diet in the regulation of FA desaturation has been investigated in several studies (Ruyter et al., 2003; Tocher et al., 2004; Vagner et al., 2007a,b). The cultured hepatocytes of Atlantic salmon fed with increasing amount of soybean oil in the diet (substrates: 18:3n−3, 18:2n−6 and 24:5n−3) demonstrated more desaturation products when fish were reared at 5 °C than at 12 °C (Ruyter et al., 2003). In rainbow trout acclimatized to 7, 11, or 15 °C and fed for four weeks on diets in which FO was replaced in a graded manner by crude palm oil, both water temperature and diet had significant effects on 18:3n−3 desaturation. The effects of the graded levels of dietary palm oil on desaturation activities were most clearly observed at 11 °C in both hepatocytes and enterocytes (Tocher et al., 2004). Unlike the results from these studies, similar Δ6D mRNA levels were measured at low (16 °C) and high (22 °C) temperatures in sea bass larvae fed a diet deficient in n−3 HUFA (0.7% EPA + DHA, % DM), although these values were significantly higher than Δ6D mRNA levels of fish fed a diet rich in n−3 HUFA (2.2% EPA + DHA, % DM; Vagner et al., 2007a,b). This observation in marine fish is in accordance with the very low expression and functional activity of Δ6D with nutritional modulation recorded in marine fish tissues compared to freshwater fish and salmonids (Tocher et al., 2006a,b; González-Rovira et al., 2009).

Some studies have shown that the influence of temperature on variations in desaturation activity depends on the tissue (Tocher et al., 2004; Skalli et al., 2006). For example, the highest desaturation activity was observed at 7 °C in enterocytes and at 11 °C in hepatocytes in rainbow trout (Tocher et al., 2004).

It is assumed that the adaptive change in the desaturation activity of microsomes with changes in environmental temperature does not greatly modify the final FA composition of the fish since increased Δ6D activity at low temperatures was associated with decreased yield of the desaturation reaction (de Torrengo and Brenner, 1976; Tocher et al., 2004).

3.2.2. Effect of salinity

The regulation of desaturation pathways for HUFA synthesis by salinity has been particularly well demonstrated in relation to parr–smolt transformation of salmonids (Bell et al., 1997; Tocher et al., 2000; Fonseca-Madrigal et al., 2006).

In Atlantic salmon, Δ6D gene expression increased significantly around the time of seawater transfer; it was at its lowest point during the seawater phase, irrespective of diet (Zheng et al., 2005a). Similarly, the euryhaline teleost *S. canaliculatus* showed a 1.56-fold higher Δ6D gene expression in liver at salinities of 10 ppt than at 32 ppt (Li et al., 2008), suggesting that the transcriptional control of Δ6D gene expression is involved in the regulation of the HUFA biosynthetic pathway by ambient salinities.

These results are in accordance with the significant increase in Δ6D activities measured around seawater transfer independent of fish diet ([FO or VO]; Bell et al., 1997; Tocher et al., 2000, 2003b; Fonseca-Madrigal et al., 2006), with a subsequent reduction in activity after transfer to seawater (Bell et al., 1997). However, activities measured were higher in fish fed VO (up to 3-fold) than in those fed FO (Bell et al., 1997; Fonseca-Madrigal et al., 2006). This is in accordance with Li et al. (2008), who showed that the higher Δ6D gene expression in *S. canaliculatus* measured at 10 ppt than at 32 ppt was significantly higher in fish fed a HUFA-free diet (containing safflower and perilla oils as lipid sources) than in those fed a HUFA-rich diet (containing FO as the lipid source). Despite differences in Δ6D activities between fish fed FO and fish fed VO, the levels of AA, EPA, and DHA appeared to converge at seawater transfer in salmonids (Bell et al., 1997). This could indicate that these HUFA

must reach a genetically predetermined level that must be achieved to allow seawater transition to occur, and that this level was attained despite the differences in dietary FA composition (Bell et al., 1997).

Salinity-mediated variations of Δ6D gene expression and activity are in accordance with measurements made in salmonids hepatocytes: HUFA synthesis was higher during the freshwater phase, peaked around seawater transfer, and declined again in the seawater phase (Tocher et al., 2003b; Zheng et al., 2005a; Fonseca-Madrigal et al., 2006). This increase in HUFA synthesis and the regulation of Δ6D gene expression are part of the pre-adaptive processes of smoltification, which prepare the fish for the marine environment (Folmar and Dickhoff, 1980) and may contribute to the increased tissue HUFA levels observed before transfer to seawater (Sheridan

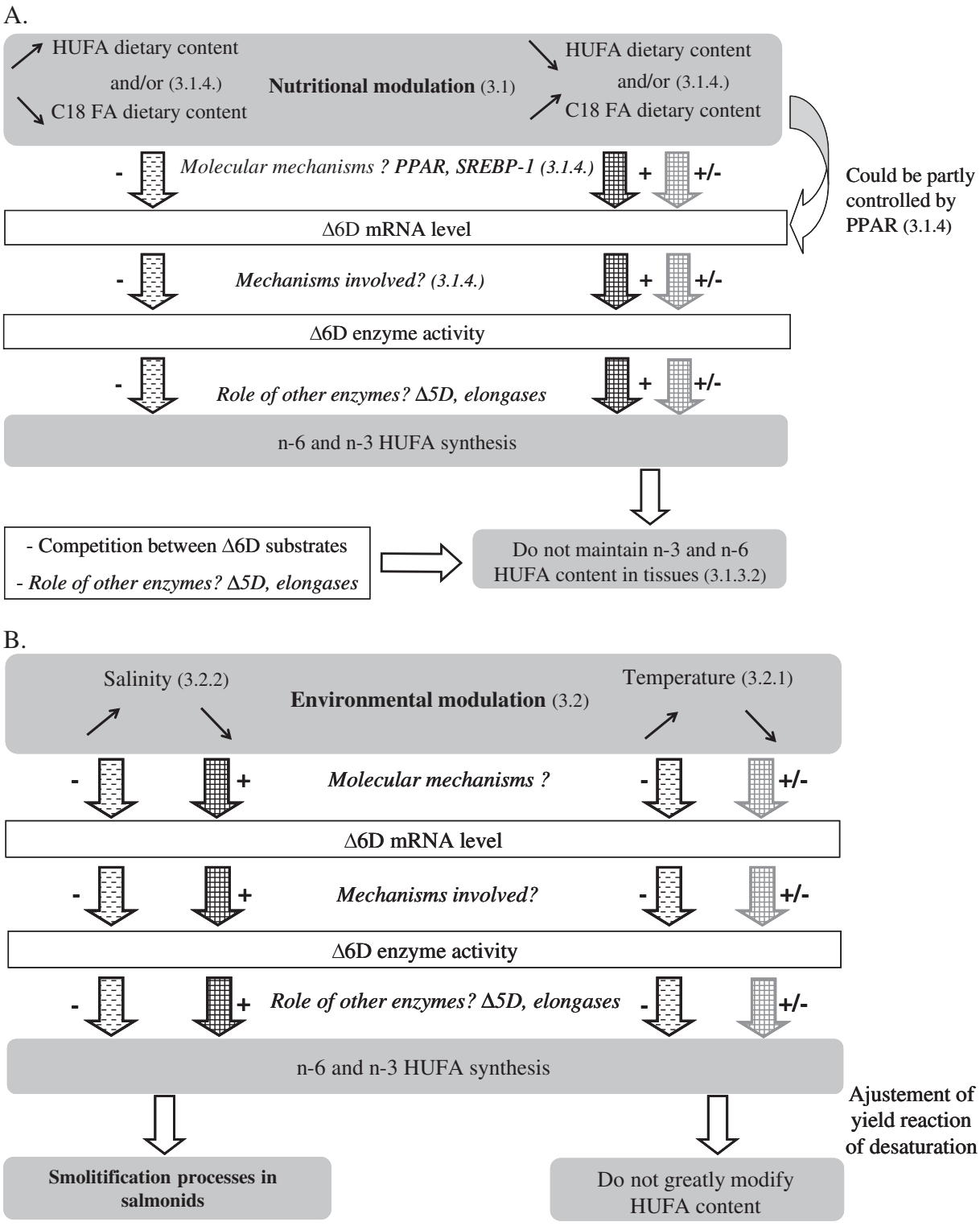

Fig. 3. Summary of nutritional (A) and environmental (B) modulation of Δ6D in teleosts reviewed in this paper. Black arrows indicate the modulation in freshwater fish, while grey arrows indicate a more barely modulation in marine fish. Numbers in brackets report to the chapter in the review. In italic are indicated the obscure points requiring complementary studies.

et al., 1985; Li and Yamada, 1992). In addition, masu salmon that had undergone smoltification but were maintained in freshwater on the same diets underwent a desmoltification process that was accompanied by a return to a "freshwater type" lipid composition (Li and Yamada, 1992). This indicates that lipid metabolism could be modified in response to ambient salinity changes (Li and Yamada, 1992). Several studies have described the importance of HUFA in adaptation to salinity changes: a diet deficient in HUFA can cause reduced ionic permeability in brush border membranes in trout (Di Constanzo et al., 1983) and morphological changes in the gills of turbot (Bell et al., 1985), and can affect the activity of a number of ion pumps and membrane-bound enzymes, including Na^+/K^+-ATPase (Spector and Yorek, 1985; Salem et al., 1988; Gerbi et al., 1994). Moreover, HUFA are substrates for eicosanoid synthesis and prostaglandins, which are known to mediate fluid and electrolyte fluxes in fish gills and kidneys and are therefore important in adaptation to salinity changes (Brown and Bucknall, 1986; Mustafa and Srivastava, 1989). It is important to note that although Δ5D and elongase gene expression also showed temporal variation, it was not correlated with the changes in the HUFA biosynthetic pathway activity (Zheng et al., 2005a). These data suggest that the regulatory mechanisms of HUFA biosynthesis in response to seasonal effects, including smoltification, were primarily due to the regulation of Δ6D expression. This is consistent with Δ6D being the rate-limiting step in the pathway (Brenner, 1981a,b).

Further studies are required to clarify the regulatory process of Δ6D gene expression by salinity. It is known that a large number of endocrine systems, such as cortisol, angiotensin II, growth hormone, and insulin-growth factor, are affected during salinity adaptation. Δ6D gene expression could thus be modulated by salinity through some related mechanisms, such as those influencing hormone production and metabolism, interactions of hormones and their target tissues, and signal transduction (Li et al., 2008).

3.2.3. Effect of other environmental factors

Because photoperiod is the primary environmental cue controlling smoltification, some studies have investigated its influence on HUFA biosynthetic activity during parr–smolt transformation in Atlantic salmon (Bell et al., 1997; Tocher et al., 2000). However, nothing is known about the potential mechanisms involving the regulation of Δ6D by photoperiod (Zheng et al., 2005a).

The nutritional and environmental modulations of Δ6D gene expression and enzymatic activity in teleosts reported in this paper are summarized in Fig. 3.

4. Conclusion

This review showed that fish desaturases are similar to those of mammals and that a high percentage of intraspecific similarity has been described between Δ6D and Δ5D. The first fish desaturase that was characterized was the bifunctional Δ5/Δ6 enzyme in zebrafish. However, other fish desaturases have been found to be unifunctional (in cod) or have a major desaturase activity with a residual desaturate capacity in the other position. Furthermore, fish desaturases have been found to have preferences for n−3 substrates; this is important in the context of the substitution of FOs with VOs in the diets of cultured fish.

The evidence presented clearly shows that Δ6D is under nutritional and environmental regulation in freshwater fish, and it functions to control lipid metabolism and thus maintains cell membrane functionality. Δ6D gene expression and enzymatic activity were higher in fish fed a diet with high VO content (low HUFA and high C18 content) than in those fed diets with high FO content (high HUFA and low C18 content). This increased activity compensates for the HUFA deficiency in VO, irrespective of whether fish are in seawater or freshwater. Moreover, Δ6D gene expression and enzymatic activity are stimulated at low temperatures, thus maintaining the fluidity and consequently the functionality of cell membranes. Δ6D gene expression and enzymatic activity are also stimulated at low salinity, thus they play an important role in salinity adaptation during parr–smolt transformation in salmonids.

Some of these nutritional and environmental modulations of Δ6D may occur in marine fish, although conflicting data exist. The Δ6D enzyme appears to be barely functional in marine fish. This could be explained by a low expression of the Δ6D gene or by the involvement of genetic, environmental, or hormonal factors.

The nutritional modulation observed seems not enough to maintain HUFA tissue content in freshwater or marine fish. This could be due to competition between C18 FA substrates for Δ6D, or to deficiencies in subsequent HUFA biosynthesis steps, such as elongation and/or desaturation at the delta-5 position. Therefore, it seems necessary that all enzymes involved in the HUFA biosynthesis be considered concomitantly in order to better understand its regulation and the precise role played by Δ6D. These kinds of studies will give new and crucial information for aquaculture feed development.

It could also be interesting to investigate the effects of environmental variables other than temperature and salinity, such as photoperiod, ambient oxygen availability, or water pH, on Δ6D gene expression and enzymatic activity. This would allow a better understanding of the effect of controlled parameters on HUFA biosynthetic pathways, and thus on fish flesh quality in aquaculture.

This review showed that while many studies have investigated the nutritional modulation of Δ6D, little is known about the molecular mechanisms involved in its regulation. The stimulation of Δ6D mRNA levels by the nutritional environment seems to be partly controlled by PPARs, but the precise molecular mechanisms involved remain unclear. It is still unclear whether Δ6D nutritional modulation is due more to desaturation product reduction or to increased substrate supply, or if both are involved. Finally, nothing is known about how temperature and salinity affect Δ6D gene expression. Further investigations are required in these fields.

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