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# Expression Analysis of *jagged* Genes in Zebrafish Embryos

**Elisabetta Zecchin, Alice Conigliaro, Natascia Tiso, Francesco Argenton, and Marino Bortolussi***

**The interaction of transmembrane Delta and Jagged/Serrate ligands with Notch receptors on neighboring cells is critically involved in cell specification during development. In zebrafish, the early expression of *delta* but not of *jagged* genes has been investigated in some detail. We have analyzed the sequence and embryonic expression pattern of the three zebrafish genes *jagged1a*, *jagged1b*, and *jagged2*. These genes, whose transcripts are detectable by in situ hybridization from early somitogenesis, are widely and dynamically expressed in embryos. Coexpression is limited, however, to the notochord and lens (*jagged1a* and *jagged1b*) and to the otic vesicle and pronephros (*jagged1b* and *jagged2*). Conversely, *jagged1a* and *jagged2*, both widely expressed in the central nervous system, are not coexpressed. *jagged2* is also detected in the epidermis, newly formed somites, pharyngeal pouches, and pancreatic exocrine anlage and *jagged1b* in otic placodes and cell clusters close to the pancreatic islet. The similarities of the expression patterns of *jagged* and *delta* genes in zebrafish suggest that the Jagged and Delta ligands are functionally redundant or required in specific combinations in many differentiation processes. *Developmental Dynamics 000: 000–000, 2005.* © 2005 Wiley-Liss, Inc.**

**Key words:** vertebrate; Zebrafish; embryo; *jagged/serrate* genes; notch signaling; in situ hybridization; nervous system; placodes; notochord; somites; pronephros; pancreas

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## INTRODUCTION

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Lateral inhibition, mediated by the evolutionary conserved Notch signaling pathway, is widely involved in developmental processes regulating cell fate specification through local cell interactions. The Notch cascade is triggered by the molecular interaction of transmembrane DSL (Delta, Serrate, Lag-2) ligands with Notch receptors on neighboring cells directing them to an alternative differentiation fate. In vertebrates, the DSL proteins include the Delta and Jagged/Serrate subfamilies. As three *Delta*, two *Jagged/Serrate*, and four *Notch* genes are expressed in tetrapods, several ligand–receptor combinations differing in their affinities and functions may be envisaged. In addition, proteins of the Fringe family that modulate Notch signalling may inhibit or potentiate Notch receptor activation, depending on the particular ligand–receptor combination (Hicks et al., 2000). In zebrafish, the situation appears to be very complex, owing to the occurrence of at least four *delta* (Haddon et al., 1998b; Smithers et al., 2000), three *jagged/serrate* (Haddon et al., 1998a; http://www.zfin.org), and four *notch* (Bierkamp and Campos-Ortega, 1993; Westin and Lardelli, 1997) genes. Thus, the knowledge of the precise expression patterns of the various DSL and Notch proteins is a prerequisite to understanding the role played by DSL/Notch signalling during zebrafish embryogenesis. With regard to the DSL ligands, the embryonic expression patterns of the four zebrafish *delta* genes have been studied in some detail (Haddon et al., 1998b; Smithers et al., 2000), but only the expression of the single *serrateB/jagged2* gene (hereafter called *jagged2*) in the developing ear has been reported to date (Haddon et al., 1998a). Here, we have

Dipartimento di Biologia, Università di Padova, Padova, Italy
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***Correspondence to: Marino Bortolussi, Dipartimento di Biologia, Università di Padova, Via U. Bassi 58/B, 35131 Padova, Italy. E-mail: marino.bortolussi@unipd.it**

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**TABLE 1. Comparison of Amino Acid Sequences**

| | Full-length | | | Extracellular region | | | Cytoplasmic region | | |
|---|---|---|---|---|---|---|---|---|---|
| | zf-Jag1a (1242aa) | zf-Jag1b (1213aa) | zf-Jag2 (1254aa) | zf-Jag1a (29–1070) | zf-Jag1b (28–1064) | zf-Jag2 (28–1084) | zf-Jag1a (1096–1242) | zf-Jag1b (1088–1213) | zf-Jag2 (1108–1254) |
| zf-Jag1a (1242aa) | 100 | 70 | 49 | 100 | 74 | 56 | 100 | 42 | 8 |
| zf-Jag1b (1213aa) | 70 | 100 | 52 | 74 | 100 | 58 | 42 | 100 | 12 |
| zf-Jag2 (1254aa) | 49 | 52 | 100 | 56 | 58 | 100 | 8 | 12 | 100 |
| mm-Jag1 (1218aa) | 66 | 73 | 52 | 70 | 74 | 56 | 37 | 76 | 13 |
| mm-Jag2 (1247aa) | 48 | 51 | 57 | 55 | 56 | 63 | 10 | 17 | 17 |

analyzed the sequences and embryonic expression patterns of the three zebrafish *jagged* genes.

## RESULTS

### Structure and Relationships of Zebrafish Jagged Proteins

The sequences of three zebrafish *jagged* genes, *jagged1a*, *jagged1b*, and *jagged2*, were obtained from available databases. Before this work was carried out, *jagged1a* and *jagged1b* were listed in the database as *jagged1* and *jagged3*, respectively. They were renamed on the basis of the results of the present investigation. The deduced sequences of the three zebrafish Jagged proteins indicate that their domain structure (Fig. 1A) is similar to those of *Drosophila* Serrate and tetrapod Jagged/Serrate proteins (Lissemore and Starmer, 1999). Notably, the extracellular domain of all three zebrafish Jagged proteins is characterized by a conserved DSL motif, an adjacent region of tandemly repeated epidermal growth factor-like (EGF) motifs (with 16 EGF repeats in Jagged1a and Jagged1b, and 15 EGF repeats in Jagged2), and a cysteine-rich segment that is unique to Jagged/Serrate members among DSL proteins (Fig. 1A). Comparison of the deduced amino acid sequences (Table 1) reveals that Jagged1a and Jagged1b are most similar to one another, whereas Jagged2 is more divergent. Moreover, Jagged1a and Jagged1b are more similar to mouse Jagged1, whereas Jagged2 is more similar to mouse Jagged2. Accordingly, the phylogenetic analysis (Fig. 1B) indicates that Jagged1a and Jagged1b group with mouse Jagged1, whereas Jagged2 is more closely related to mouse Jagged2 (Table 1). Similarly to the Delta proteins (Haddon et al., 1998b), the degree of conservation of the extracellular domains is higher than that of the cytoplasmic domains. Nonetheless, the intracellular domains of Jagged1b and mouse Jagged1 are highly conserved, their homology being even slightly higher than that of their extracellular domains. Noteworthy, the six C-terminal amino acids of Jagged1b are identical to those of human Jagged1 (Fig. 1C) shown to constitute a functional PDZ-ligand (Hock et al., 1998; Ascano et al., 2003). A putative PDZ-ligand is also present at the C-terminus of Jagged1a (Fig. 1C).



**Fig. 1.** Domain structure and phylogenetic relationships of zebrafish Jagged proteins. **A:** Schematic diagram of the motif/domain structure of zebrafish Jagged1a, Jagged1b, Jagged2, and DeltaD, obtained with the SMART program (http://smart.embl-heidelberg.de). The cysteine-rich domain encompasses the von Willebrand factor type C-like domain. **B:** Dendrogram of zebrafish (*Danio rerio*, Dr) Jagged1a, Jagged1b, and Jagged2; mouse (*Mus musculus*, Mm) Jagged1, Jagged2, and Dll1 (Delta-like1); and Drosophila (*Drosophila melanogaster*, Dm) Serrate, obtained with the MegAlign program that uses the Clustal W algorithm (Thompson et al., 1994). **C:** C-terminal hexapeptides of zebrafish (Dr) and mouse (Mm) Jagged proteins. The hexapeptide of Jagged1b is identical, whereas that of Jagged1a differs by one amino acid, from the sequence of mouse Jagged1, shown to represent a functional PDZ-ligand (Hock et al., 1998; Ascano et al., 2003). The conserved amino acids in the three hexapeptides are in bold characters. The hexapeptides of zebrafish and mouse Jagged2 are not conserved and neither resemble a PDZ-ligand (Hock et al., 1998; Ascano et al., 2003).

**Fig. 2.** *jagged1a* expression pattern. in situ hybridization of embryos at different developmental stages performed with the *jagged1a* probe. **A:** At the four-somite stage (4s), *jagged1a* is expressed in the notochord (A1) and in scattered cells (arrowheads) in the intermediate neural plate (A2). **B:** At the 12-somite stage (B1, B2) *jagged1a* is expressed in the notochord and in cells located in the intermediate neural keel. **C:** At the 18-somite stage (C1, C2) the notochord is no longer labeled, while in the spinal cord *jagged1a* is expressed in cells located in a mediolateral position where some cells expressing *deltaB* are also observed (C1). **D:** At 24 hpf *jagged1a* is expressed in the lens (D1), the rhombomere borders (D2), and the spinal cord (D3). **E:** At 48 hpf in the central nervous system (E1–E4), *jagged1a*-expressing cells occur in the midbrain, hindbrain, and spinal cord. In the hindbrain (E1, E2, E4), the labeled cells are located dorsally at the anterior and posterior rhombomere borders and in bilateral inner and outer strings of cell clusters. In the caudal hindbrain and spinal cord (E1 and E3), the *jagged1a*-expressing cells are situated medially in bilateral upper and lower stripes. In D2 and E4, the position of the rhombomeres has been determined on the basis of the location of the otic vesicles. The arrows in E4 indicate the boundaries between the rhombomeres. Views are lateral (A1, B1, D3, E1) or dorsal (A2, C1, D1, D2, E4); transverse sections are the level of the trunk (B2, C2), the rhombomeres (E2), and caudal hindbrain (E3). Anterior is to the left in all lateral and dorsal views except for D1, in which it is to the top. is, inner string of *jagged1a*-expressing cell clusters; l, lens; lr, lower row of jagged1a-expressing cells; m, midbrain; n, notochord; nk, neural keel; os, outer string of *jagged1a*-expressing cell clusters; ov, otic vesicle; r1 to r6, rhombomeres 1 to 6; sc, spinal cord; ur, upper row of *jagged1a*-expressing cells.

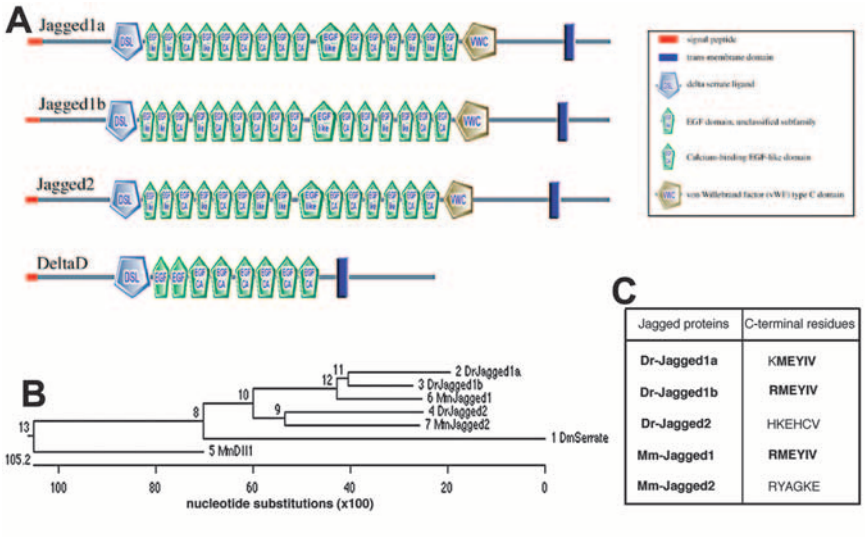
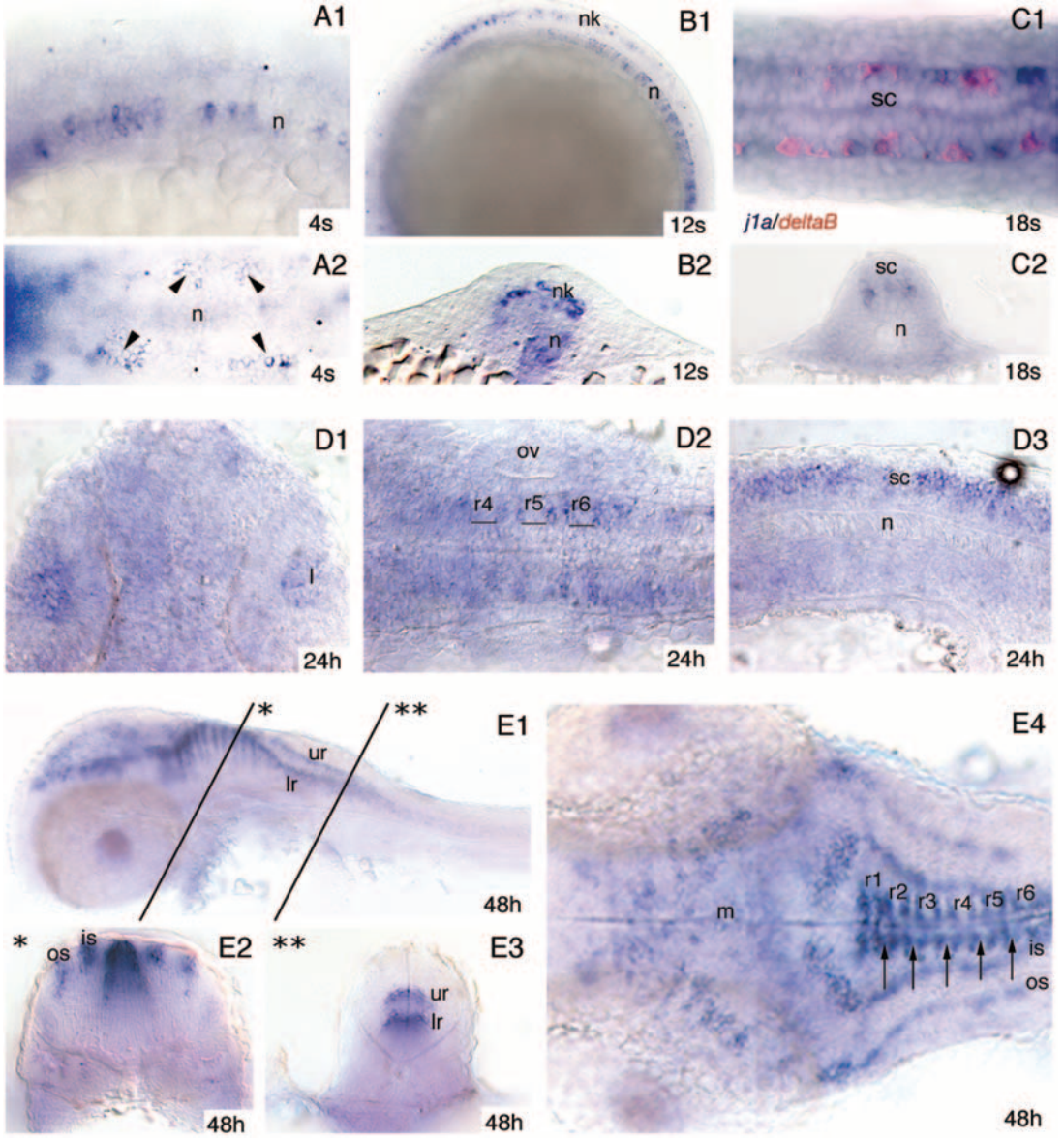



Fig. 1.

Conversely, the C-terminal sequences of zebrafish and mammalian Jagged2 are neither conserved nor resemble a PDZ-ligand (Fig. 1C).

## Expression Pattern of Zebrafish *jagged* Genes

Transcripts of the three *jagged* genes are clearly visible by in situ hybridization during early somitogenesis but are undetectable at earlier stages such as shield and tail bud. This finding suggests that their expression is zygotic and slightly delayed in comparison to that of the *delta* genes, which is detectable by in situ hybridization during epiboly (Haddon et al., 1998b).

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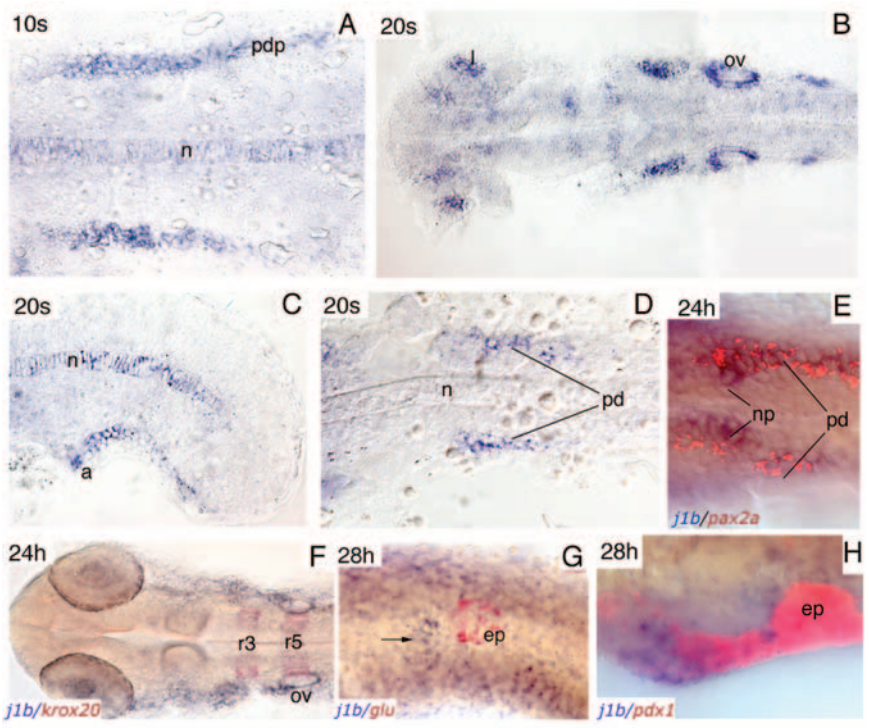


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### *jagged1a*

At the four-somite stage, *jagged1a* is strongly expressed in scattered cells in the notochord (Fig. 2A1). A similar pattern is also observed at the 12-somite stage (Fig. 2B1,B2), but *jagged1a* transcripts are no longer detectable in the notochord at the 18-somite and later stages (Fig. 2C2). In the nervous system, the expression of *jagged1a* is visible at the four-somite stage as a faint labeling in some cells located in an intermediate position along the mediolateral axis of the neural plate (Fig. 2A1,A2). Thereafter, *jagged1a* is expressed in the neural keel at the 12-somite stage (Fig. 2B1,B2), as well as in the spinal cord at the 18-somite (Fig. 2C1,C2) and 24 hours postfertilization (hpf; Fig. 2D3) stages, in a bilateral longitudinal series of cell clusters located halfway along the dorsoventral axis. At the 12-somite stage, the labeled cells are found from the midline to the lateral side of the neural keel (Fig. 2B2), whereas at later stages the *jagged1a*-positive cells are located laterally

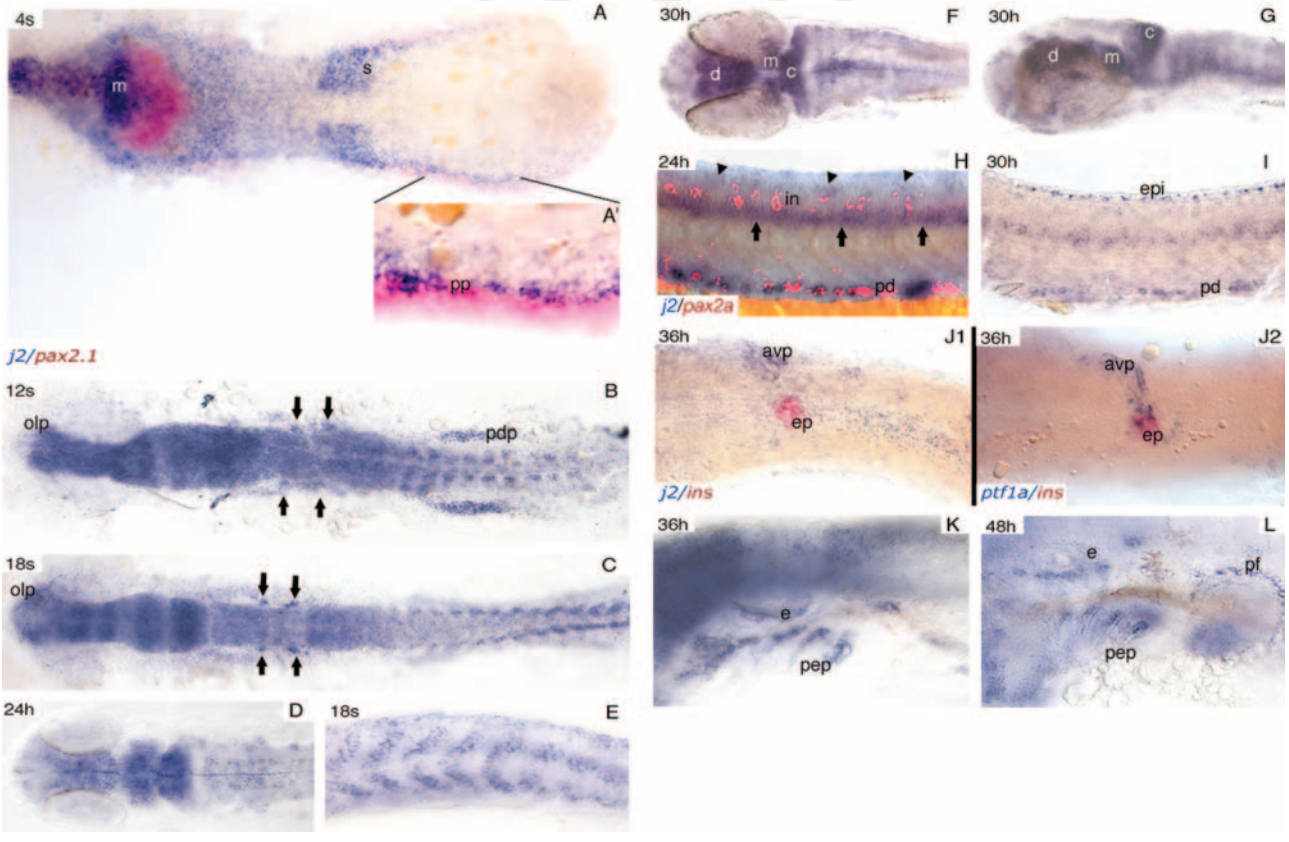


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Fig. 4.

(Fig. 2C2). Coexpression experiments performed at the 4-somite (not shown) and 18-somite stages (Fig. 2C1) show that, in the nervous tissue, *jagged1a*- and *deltaB*-expressing cells are found in same locations. The labeled cells in the spinal cord are grouped in clusters arranged as a string of beads, suggesting a segmental patterning of *jagged1a*-expressing cells (Fig. 2C1,D3). By 24 hpf, *jagged1a* transcripts are also detected bilaterally in the lens (Fig. 2D1), as well as at the anterior and posterior rhombomere borders (Fig. 2D2). At 48 hpf, in both the spinal cord and caudal hindbrain, the labeled cells are arranged in an upper and a lower bilateral row of cell clusters, situated close to the midline (Fig. 2E1,E3). More rostrally in the hindbrain, a series of transverse stripes, consisting of *jagged1a*-expressing cell clusters approximately two cells thick, are situated dorsomedially at the anterior and posterior rhombomere borders (Fig. 2E1,E2, E4). In addition, an inner and an outer string of *jagged1a*-positive cell clusters are situated laterally to the transverse stripes and are in continuation rostrally with groups of labeled cells in the isthmus and dorsal midbrain (Fig. 2E1,E2,E4).

### *jagged1b*

*jagged1b* is initially expressed in the notochord and in a bilateral stripe in the mesoderm (Fig. 3A), matching *pax2a* expression in the pronephric duct primordia (Majumdar et al., 2000). The labeling in the notochord is transient and declines rostrocaudally so that, at the 20-somite stage, only the caudal most cells are labeled (Fig. 3C), and by 24 hpf, *jagged1b* mRNA is no longer detectable in this structure. At the 20-somite and 24-hpf stages, *jagged1b* is expressed in the rostral part of the pronephric ducts and, with regard to the 24-hpf stage, in the nephron primordia as well (Fig. 3D,E). In the latter *jagged1b* is expressed in both the lateral cells that give rise to the tubule, as shown by colabeling with *pax2a* and in medial cells that originate the glomerulus but do not express *pax2a* (Fig. 3E). At the 20-somite stage *jagged1b* is also expressed in the lens, in the otic vesicle, in two large groups of cells located anteriorly and posteriorly to the otic vesicle, and in scattered groups of cells in the brain (Fig. 3B). By 24 hpf, *jagged1b*-positive cells are no longer observed in the brain but still occur, though in a more dispersed pattern, in a lateral region that includes the otic vesicle and extends anteriorly toward the eye (Fig. 3F). At 28 hpf, *jagged1b* transcripts are detected anteriorly and close to the pancreatic islet, as shown by double labeling with either *glucagon* (Fig. 3G) and *insulin* (not shown) probes, in an area that overlaps in part with the *pdx1* expression domain (Fig. 3H).

### *jagged2* F3

At the beginning of somitogenesis, the expression of *jagged2* is visible in the brain primordium, where it is stronger in the prospective midbrain, in the somites, and in the mesoderm (Fig. 4A,A′). At the 12- and 18-somite F4 stages (Fig. 4B and C, respectively), *jagged2* is strongly expressed throughout the brain, in the olfactory placodes and, as previously reported by Haddon et al. (1998a), in two small clusters of cells at the anterior and posterior ends of the otic placodes (Fig. 4B) and vesicles (Fig. 4C). Afterward, *jagged2* expression is no longer detected in the olfactory placodes (Fig. 4D) but persists in the ventral part of the ear (Fig. 4K,L) where it was shown to label the hair cells (Haddon et al., 1998a). At 24–30 hpf in the brain (Fig. 4D,F,G), *jagged2* transcripts become concentrated in the diencephalon, ventral midbrain, cerebellum, and ventromedial part of the rhombomeres. In the spinal cord, *jagged2* transcripts are undetectable at least up to the 18-somite stage but are visible at 24 and 30 hpf (Fig. 4H,I, respectively). In the ventral spinal cord, the *jagged2* probe labels uniformly an area encompassing primary motoneurons (Appel and Eisen, 1998), while dorsally the signal is faint. At



**Fig. 3.** *jagged1b* expression pattern. In situ hybridization of embryos at different developmental stages performed with the *jagged1b* probe. **A:** At the 10-somite stage (10s), *jagged1b* is expressed in the notochord and the pronephros primordia. **B–D:** At the 20-somite stage, *jagged1b*-expressing cells are detected mainly in the lens, otic vesicles, and two bilateral groups of cells situated anteriorly and posteriorly to the latter (B), in the caudal part of the notochord (C), and in the rostral part of the pronephric ducts (D). C: At the 20-somite stage, *jagged1b* transcripts are detected in a group of cells located ventrally and posteriorly to the anus. **E:** At 24 hours postfertilization (hpf), *jagged1b* and *pax2a* are coexpressed in cells of the rostral end of the pronephric duct and lateral part of the nephron primordia, but only *jagged1b* is expressed in the medial cells of the nephron primordia. **F:** At the same stage, *jagged1b* transcripts are also expressed in the region extending from the otic vesicle to the eye. **G,H:** At 28 hpf, the *jagged1b* (arrow) expression domain does not overlap with that of *glucagon* in the pancreatic islet (G), but is in part overlapped with the *pdx1* expression domain (H). Views are dorsal (A,B,F), lateral (C,H), or ventral (D,E,G). Anterior is to the left in all pictures. a, anus; ep, endocrine pancreas; l, lens; n, notochord; np, nephron primordia; ov, otic vesicle; pd, pronephric duct; pdp, pronephric duct primordium; r3, rhombomere 3; r5, rhombomere 5.

**Fig. 4.** *jagged2* expression pattern. In situ hybridization of embryos at different developmental stages performed with the *jagged2* probe. **A:** At the four-somite stage (4s), *jagged2* is expressed in the mesoderm as shown by colabeling with the *pax2a* probe (see inset A′). **B,C:** At the 12- and 18-somite stages, *jagged2* is strongly expressed throughout the brain, in the developing somites (shown at a higher magnification in E), the pronephric duct primordia, and in two clusters of cells (arrows) in the otic placode (B) and vesicle (C). **D–G:** At 24 hours postfertilization (hpf, D) and 30 hpf (F,G), *jagged2* is expressed in the diencephalon, midbrain, and cerebellum, and with regard to the 30-hpf stage, in the ventromedial part of the rhombomeres as well. **H:** At 24 hpf, *jagged2* is expressed strongly and diffusely in the ventral part of the spinal cord (arrows) and faintly in the dorsal part (arrowheads) but not in interneurons located in between detected by *pax2a* labeling. Moreover, *jagged2* is also expressed in scattered cells of the pronephric duct, in which some *pax2a*-expressing cells are also observed. **I:** At 30 hpf, the *jagged2* expression pattern is identical to that observed at 24 hpf, but the picture evidences the expression in the epidermis, which is out of focus in H. **J1,J2:** *jagged2* labeling is observed in the anteroventral pancreatic anlage that expresses *ptf1a* and grows to engulf the endocrine pancreatic islet identified by *insulin* expression. **K,L:** At 36 hpf (K) and 48 hpf (L), *jagged2* is expressed in the ear, pharyngeal pouches, and pectoral fin. Views are dorsal (A–D,F), lateral (E,G–I,K,L), or ventral (J1,J2). Anterior is to the left in all pictures. avp, anteroventral pancreas; c, cerebellum; d, diencephalon; e, ear; ep, endocrine pancreas: epi, epidermis; in, interneurons; m, midbrain; olp, olfactory placode; pep, pharyngeal endodermal pouches; pd, pronephric duct; pdp, pronephric duct primordium; pf, pectoral fin; pp, prospective pronephros; s, somites.

24–30 hpf, a punctuate and evenly distributed *jagged2* expression is also detected in the epidermis (Fig. 4I). *jagged2* expression is not detectable in the presomitic mesoderm (PSM), but is observed in the somites shortly after their formation (Fig. 4A,B). As the maturation of the somites progresses, the expression of *jagged2* increases, becoming restricted to the anterior parts, and then fades out progressively to eventually disappear (Fig. 4B,C,E). At the four-somite stage a faint labeling is observed in the mesoderm (Fig. 4A). At the 12-somite stage *jagged2* is expressed in the pronephric duct primordium (Fig. 4B) and, by 24 and 30 hpf, in single cells along the pronephric ducts (Fig. 4H1,I) but, contrary to *jagged1b*, not in the nephron primordia. By 36 hpf, *jagged2* is expressed in the developing gut in an area overlapping with the expression domain of *ptf1a* (Fig. 4J1,J2), a marker of the anteroventral pancreatic bud that gives rise to the exocrine pancreas (Zecchin et al., 2004). In addition, *jagged2* transcripts are also detected in the developing pectoral fins and, at all stages examined from 24 to 48 hpf, in the epithelium of the pharyngeal pouches (Fig. 4K,L).

## DISCUSSION

The widespread and dynamic embryonic expression of *jagged* genes testifies their involvement in processes leading to the differentiation of a variety of zebrafish organs and tissues derived from all three germ layers.

Two *Jagged/Serrate* genes, *Jagged1/SerateA* and *Jagged2/SerrateB*, have been described to date in tetrapods. From an evolutionary point of view, it appears that *jagged1a* and *jagged1b* are orthologues of the single mouse *Jagged1*, whereas *jagged2* is the orthologue of mouse *jagged2*. This finding is most probably the outcome of a genome duplication event in the lineage of zebrafish that yielded two *jagged* genes from a common precursor with mouse *Jagged1*. This idea is supported by the position of the *jagged1* genes in the zebrafish genome: *jagged1a* is in chromosome 1 between *msxb* and *deltaA*, whereas *jagged1b* is in chromosome 13 between *msxc* and *deltaD* (http://www.zfin.org).

Similarities in the expression domains of the three *jagged* genes occur only at a few sites. Coexpression is essentially observed between *jagged1a* and *jagged1b* in the notochord and the lens, and between *jagged1b* and *jagged2* in the otic vesicle and the pronephros. On the other hand, in several instances the *jagged* genes are expressed in regions in which the *delta* genes have also been shown to be expressed. Thus, *jagged1a* and *jagged2* are expressed in the central nervous system (CNS) together with *deltaA*, *deltaB*, and *deltaD* (Haddon et al., 1998b), whereas similar to *deltaC* (Smithers et al., 2000), *jagged1a* and *jagged1b* are expressed in the notochord during somitogenesis and in the pronephros. Moreover, *jagged2* is coexpressed in the anterior parts of the somites with *deltaD*, whereas *deltaC* is expressed in the posterior parts (Haddon et al., 1998b). However, in contrast to *deltaC* and *deltaD*, which are expressed in the PSM and similar to tetrapod *Delta1* are involved in the oscillator mechanism governing the formation of the somites (Giudicelli and Lewis, 2004), *jagged2* does not appear to participate to this process because its expression starts later in newly formed somites.

The development of the pronephric kidney in zebrafish has been described in detail (Drummond et al., 1998). The pronephros develops from *pax2a*-expressing cells in the intermediate mesoderm. During somitogenesis the pronephric duct primordium grows posteriorly and by 24 hpf has acquired a lumen. At the rostral end of the duct, the lateral and medial cells of the nephron primordium give rise to the tubule and glomerulus, respectively. Here, we show that *jagged* genes are expressed in all structures of the developing pronephros. In fact, *jagged1b* and *jagged2* are both expressed in the developing pronephros during somitogenesis and, at 24 hpf, *jagged1b* is expressed in the nephron primordia and anterior duct, while *jagged2* is expressed more caudally in the duct. Hence, in zebrafish as in *Xenopus* (McLaughlin et al., 2000), Notch signaling appears to be involved in pronephros development.

Although both widely expressed in the CNS, *jagged1a* and *jagged2* show little coexpression. In fact, during somitogenesis up to the 20-somite stage, *jagged1a* is expressed segmentally in the spinal cord and the hindbrain, while *jagged2* is broadly expressed in brain regions but not in the spinal cord. In addition, in the brain of 24 hpf embryos, *jagged1a* is expressed dorsally in the midbrain and hindbrain, whereas *jagged2* is expressed in the diencephalon, ventral midbrain, cerebellum, and ventral part of the rhombomeres. Moreover, at the same stage in the spinal cord, *jagged2* is expressed in ventral and dorsal regions and *jagged1a* is expressed in cells located in-between. Indeed, the location of *jagged1a*-expressing cells in an intermediate position, both along the mediolateral axis in the neural plate, and along the dorsoventral axis in the neural tube, suggests that they correspond to nascent interneurons.

A recent report (Cheng et al., 2004) demonstrates that Notch signalling is involved in zebrafish hindbrain segmentation. It was shown that Notch activation regulates the differentiation and segregation of boundary cells located at the interface of adjacent rhombomeres by inhibiting their premature neuronal differentiation. Moreover, *deltaA* and *deltaD* were found to be expressed in stripes of neuroblasts that flank the rhombomere boundaries, matching the *jagged1a* expression pattern observed in our present investigation. This finding suggests that *jagged1a* is involved together with *deltaA* and *deltaD* in the mechanisms of Notch activation leading to the formation of rhombomere boundaries.

Notch signalling plays a critical role in pancreas development in the mouse (Apelqvist et al., 1999). It was found that repression of the Notch pathway favored the premature differentiation of precursor cells into endocrine cells, resulting in the depletion of precursors that would otherwise proliferate and differentiate into exocrine cells. In zebrafish, the pancreas derives from two anlagen within the *pdx1*-expression domain of the gut: a posterodorsal bud that arises earlier and gives rise to the first endocrine islet and an anteroventral bud that develops later and originates the exocrine tissue in addition to sparse late endocrine cells (Field et al., 2003; Zecchin

et al., 2004). Thus, the occurrence of *jagged1b*-expressing cells close to the islet and that of *jagged2*-expressing cells in the anteroventral bud may contribute to bring about the appropriate levels of Notch activation necessary to regulate the number of cells adopting specific pancreatic fates.

Our present results, together with previous reports concerning the *delta* genes (Haddon et al., 1998a,b; Smithers et al., 2000), suggest that different combinations of Notch ligands are expressed in several cell types in zebrafish embryos. This explanation may reflect a functional redundancy of Delta and Jagged ligands, or account for the expression of several Notch receptors with different affinities for the various ligands. It can also be envisaged that various Delta and Jagged ligands, while delivering their signal by binding to Notch receptors in neighboring cells, also play a distinct functional role in the cell in which they are expressed. In this regard, it is well-known that ligand binding triggers the proteolytic cleavage of Notch receptors, releasing their intracellular domain that translocates to the nucleus where it regulates the expression of target genes in conjunction with CSL DNA-binding proteins (Schroeter et al., 1998; Struhl and Adachi, 1998). But recent investigations have demonstrated that, upon binding to Notch receptors, the Delta and Jagged ligands undergo a similar proteolytic cleavage, releasing their cytoplasmic domains that mediate signalling mechanisms within the ligand-expressing cells (Ikeuci and Sisodia, 2003; LaVoie and Selkoe, 2003). Moreover, human Jagged1 was shown to up-regulate the expression of target genes within the cells in which it is expressed by means of a PDZ-ligand, consisting of its cytoplasmic C-terminal hexapeptide (Ascano et al., 2003). Of interest, a putative PDZ-ligand also occurs at the C-terminus of zebrafish Jagged1a and Jagged1b, the latter being identical to the C-terminal hexapeptide of its human counterpart, thereby hinting at the conservation of a signalling activity of the cytoplasmic domains of Jagged1 ligands in vertebrates. The Delta-Jagged/Notch interaction may thus be a bidirectional signalling mechanism in which the cytoplasmic domain of each protein plays a specific functional role in the cell in which it is expressed (Ascano et al., 2003). In this context, the expression of specific combinations of Jagged and Delta proteins within a single cell may be required to regulate distinct mechanisms involved in its own differentiation, along with the activation of Notch signalling in neighboring cells.

## EXPERIMENTAL PROCEDURES

### Phylogenetic and Domain Structure Analyses

The sequences of the zebrafish *jagged* genes are available from GenBank (www.ncbi.nlm.nih.gov/) (*jagged1a* ID: AF229448; *jagged1b* ID: AF229451; *jagged2* ID: AF229449). The Swissprot accession numbers of the amino acid sequences of the DSL proteins are as follows: zebrafish Jagged1a (NP_571936), Jagged1b (NP_571938), Jagged2 (NP_571937), and DeltaD (NP_571030); mouse Jagged1 (AAF15505) and Jagged2 (AAF16411); *Drosophila* Serrate (NP_524527). Multiple alignments and phylogenetic tree building were carried out using the Clustal W program (Thompson and Gester, 1994). The analysis of the domain architecture of Jagged proteins was performed using the SMART program (http://smart.embl-heidelberg.de).

### Whole-Mount In Situ Hybridization

Embryos were staged according to Kimmel et al. (1995). Whole-mount in situ hybridizations were performed according to Thisse et al. (1993). Double staining was carried out according to Hauptmann and Gester (1994). The following digoxigenin- or fluorescein-labeled (Roche) antisense riboprobes were used: *glu* and *pdx1* (Argenton et al., 1999); *ptf1a* (Zecchin et al., 2004); *pax2a* (Krauss et al., 1991); *deltaB* (Haddon et al., 1998b). The *jagged1a*, *jagged1b* and *jagged2* templates were amplified from genomic DNA and oligos were designed according to sequence data available from GenBank: *jagged1a* (Forward GAGAAGAATTCGGGACTGTGCTTAACCTTAATA and Reverse ACGATTTCGAATAGTCTCGCTTATCACATTCAG, 995 bp), *jagged1b* (Forward GTTCAGAATTCCAGTGCTCTCACAGCTAAAGTA and Reverse CCTGTTTCGAAGGTCTACAGAGAGTTCCTTTGT, 978 bp), and *jagged2* (Forward CTTTTTTGAATTCAGAGGAAGCAGACTGTATTTGT and Reverse CGCCACATTCGAACACACGATCGTATAGGTGTCTA, 815 bp). To simplify the cloning, the oligos contained either a *Eco*RI site at their 5′ end or a *Hin*dIII site at their 3′ end. The polymerase chain reaction fragments were cloned in the pCRII-TOPO plasmid (Invitrogen). The *jagged1a*, *jagged1b*, and *jagged2* antisense probes, thus, were derived from partial 3′ untranslated regions (*jagged1a* and *jagged1b*) and/or coding sequences (*jagged2*). The plasmids were cut and transcribed with *Apa*I/Sp6 (Promega) for *jagged1a*, *Eco*RV/Sp6 (Promega) for *jagged1b*, *Spe*I/T7 (Promega) for *jagged2*.

### Microscopy and Imaging

After in situ hybridization, embryos were post-fixed in 4% buffered *p*-formaldehyde and mounted in 85% glycerol/phosphate-buffered saline (PBS) for microscope observation. Embryos at early somitogenesis were dehydrated with methanol and cleared with a 2:1 mixture of benzylbenzoate:benzyl alcohol. Sections were obtained from embryos embedded in 3.5% agarose in PBS. The agarose blocks were sectioned in PBS using a Leica VT1000S Vibratome. The slices were mounted in 50% glycerol/PBS for microscope observation. Observations were made with a Leica DMR compound/Nomarski microscope and images were acquired with the Leica DC500 digital camera. Image data were processed using the Adobe Photoshop 6.0 software.

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