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ScienceDirect
Developmental Biology 300 (2006) 153 – 164
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Genomics and expression profiles of the Hedgehog and Notch signaling pathways in sea urchin development

Katherine D. Walton *, Jenifer C. Croce, Thomas D. Glenn, Shu-Yu Wu, David R. McClay

Developmental, Cellular, and Molecular Biology Group, Duke University, Durham, NC 27710, USA

Received for publication 10 May 2006; revised 18 August 2006; accepted 28 August 2006
Available online 1 September 2006

Abstract

The Hedgehog (Hh) and Notch signal transduction pathways control a variety of developmental processes including cell fate choice, differentiation, proliferation, patterning and boundary formation. Because many components of these pathways are conserved, it was predicted and confirmed that pathway components are largely intact in the sea urchin genome. Spatial and temporal location of these pathways in the embryo, and their function in development offer added insight into their mechanistic contributions. Accordingly, all major components of both pathways were identified and annotated in the sea urchin *Strongylocentrotus purpuratus* genome and the embryonic expression of key components was explored. Relationships of the pathway components, and modifiers predicted from the annotation of *S. purpuratus*, were compared against cnidarians, arthropods, urochordates, and vertebrates. These analyses support the prediction that the pathways are highly conserved through metazoan evolution. Further, the location of these two pathways appears to be conserved among deuterostomes, and in the case of Notch at least, display similar capacities in endomesoderm gene regulatory networks. RNA expression profiles by quantitative PCR and RNA in situ hybridization reveal that Hedgehog is produced by the endoderm beginning just prior to invagination, and signals to the secondary mesenchyme-derived tissues at least until the pluteus larva stage. RNA in situ hybridization of Notch pathway members confirms that Notch functions sequentially in the vegetal-most secondary mesenchyme cells and later in the endoderm. Functional analyses in future studies will embed these pathways into the growing knowledge of gene regulatory networks that govern early specification and morphogenesis.

Keywords: Hedgehog; Notch; Endoderm; Mesoderm; Sea urchin

Introduction

The transducing machinery in both the Notch and the Hh signaling systems is relatively simple compared to the number of modifying components in other signal transduction pathways. Nevertheless the pathways are tightly controlled and modifiers play a clear role as reported in many animal systems. Based on published data, numerous duplications occur in vertebrates, and other duplications or losses are reported in various organisms. All of these changes tend to obscure ancestral function in development. Using a comparative approach animals such as the sea urchin, that occupy a basal position in the deuterostome clade, provide a means of gaining perspective on how these pathways operate in development. The sequencing of the sea urchin genome gives an opportunity to explore these signal transduction pathways in detail. The goal of this project, therefore, was to annotate all members of both signal transduction pathways and then to begin to explore where and how some of the main components of these pathways operate in the sea urchin embryo.

Fig. 1 depicts the main components of both the Hh and the Notch signaling pathways as a combination of molecules known from studies largely in *Drosophila* and vertebrates. Although differences exist between vertebrates and *Drosophila* (reviewed in Huangfu and Anderson, 2006), a generalized Hh pathway can be described as follows. Hh is a secreted protein that is enzymatically modified to make it active (Bumcrot et al., 1995; Chamoun et al., 2001; Lee et al., 1994; Pepinsky et al., 1998; Porter et al., 1995, 1996) and it binds to its cognate receptor Patched (Ptc) (Chen and Struhl, 1996; Marigo et al., 1996; Stone et al., 1996). In the absence of Hh, the receptor Ptc operates as an inhibitor by blocking the ability of Smoothened (Smo) to activate the pathway. In this case the downstream transcription factor cubitus interruptus (Ci, or Gli in vertebrates, note that

* Corresponding author. Fax: +1 919 613 8177.
E-mail address: kdw6@duke.edu (K.D. Walton).

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doi:10.1016/j.ydbio.2006.08.064

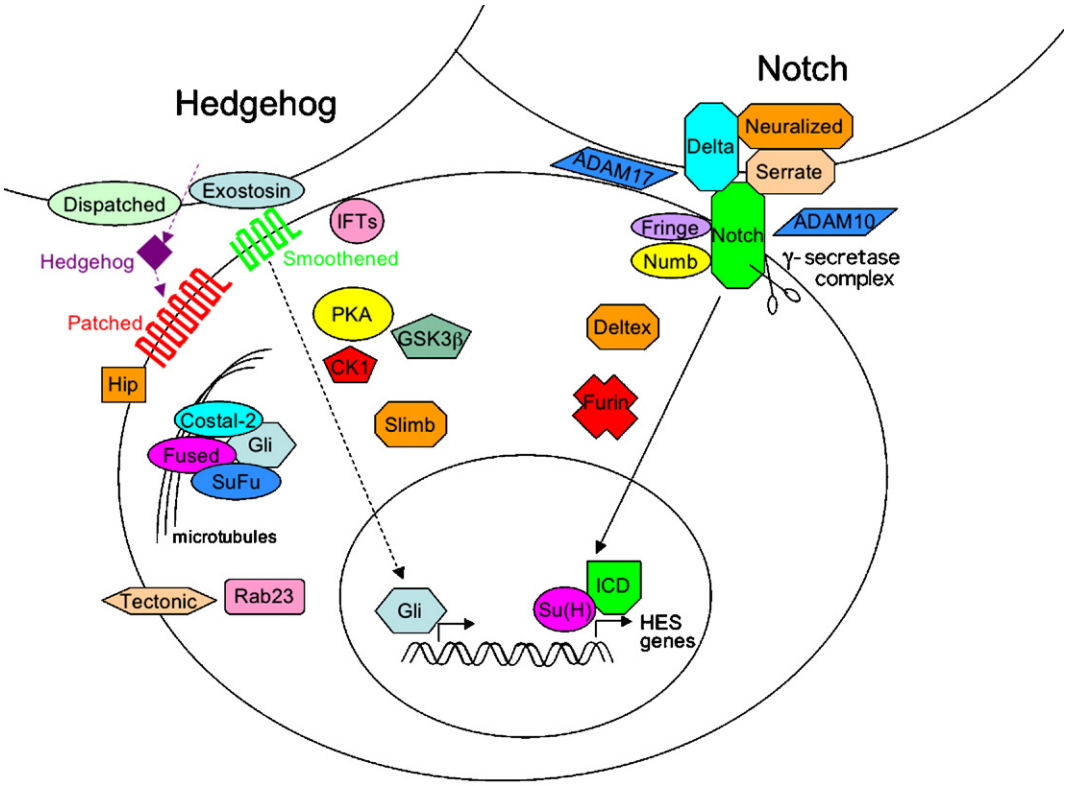

Fig. 1. Diagram of the major components and modifiers of the Hh and Notch signaling pathways that were identified in the annotation of the *S. purpuratus* genome. These components and modifiers are also listed in Supplemental Table 1 along with their other names, Glean3 model/SPU_ numbers, best human genome blast hit and relative level of embryonic expression detected by the tiling array experiment. See the text for a brief functional description of these pathways.

there are 3 forms of Gli in vertebrates: Gli1, Gli2, and Gli3) becomes phosphorylated by PKA, GSK3β and CK1, and is subsequently targeted for processing by the protease, Slimb (Chen et al., 1998; Jia et al., 2002; Jiang and Struhl, 1998; Price and Kalderon, 2002; Theodosiou et al., 1998). Slimb cleaves the full-length 155 kDa Ci to a shortened 75 kDa form and this shortened form translocates to the nucleus where it acts as a repressor (Akimaru et al., 1997; Aza-Blanc et al., 1997; Chen et al., 1999). Gli3 (and Gli2 in some contexts) is also similarly cleaved in vertebrates and it is thought that the ratio of full-length activator forms to shortened repressor forms determines the transcriptional regulatory action (von Mering and Basler, 1999). Costal-2 (Cos2) is a kinesin-like protein which is part of a complex of proteins that act to sequester Ci/Gli in the cytoplasm and promote the cleavage of the full-length Ci in *Drosophila*, however the currently identified vertebrate orthologs of Cos2 appears unable to affect subcellular localization of Glis or promote their cleavage (Chen et al., 1999; Lefers et al., 2001; Methot and Basler, 1999, 2000; Varjosalo et al., 2006; Wang et al., 2000). Suppressor of Fused (Su(Fu)) and Fused are also part of this protein complex (Preat, 1992). Su(Fu) is a weak antagonist of Hh signaling in *Drosophila*, but can act as a potent inhibitor of Hh signaling in vertebrate cells (Varjosalo et al., 2006) while Fused is a kinase thought to inactivate Su(Fu) possibly by direct phosphorylation (Lum et al., 2003; Methot and Basler, 2000).

When Hh is present, it binds to Ptc, alleviating the inhibition of Smo. Smo is then able to antagonize Cos2 activity and Slimb protease activity is prevented. As a result the full-length form of Ci is retained and this molecule proceeds to the nucleus where it activates transcription, often of the same genes that previously were repressed by the smaller Ci protein (Methot and Basler, 2000).

In addition to the major components described above, other modifiers affect the Hh pathway including Dispatched (Disp), Exostosins (Exts), Rab23, Hedgehog-interacting protein (Hip), Intraflagellar transport proteins (IFTs), Tectonic (Tect), SIL, Talpid3 and FKBP8. Disp is required for Hh secretion from Hh releasing cells (Burke et al., 1999). The *Drosophila tout-velu* (*ttv)* genes, which are homologs of the vertebrate *ext* genes, are critical to the movement of the Hh signal between cells, and are required in the receiving cells (Bellaiche et al., 1998; The et al., 1999). Rab23 is a negative regulator of vertebrate Hh signaling and is a member of the small GTP-activated proteins, which are associated with membrane trafficking (Eggenschwiler et al., 2001). Its function appears to be in localizing some factor in Hh signaling that acts between Ptc and Smo and the downstream transcription factor, Gli (Eggenschwiler et al., 2006). Hip, a Hh-binding protein previously thought to be vertebrate specific, is upregulated by Hh signaling and has a negative effect on that signal, thereby providing a feedback mechanism (Chuang and McMahon, 1999). IFTs are required for assembling cilia and flagella (Rosenbaum and Witman, 2002) and are necessary for Gli activity in response to Hh signaling in vertebrates at a step between Smo and Gli (Huangfu and Anderson, 2005; Huangfu et al., 2003; Liu et al., 2005). This requirement for IFTs in Hh