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A genome-wide survey of the evolutionarily conserved Wnt pathways in the sea urchin *Strongylocentrotus purpuratus*

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Abstract

The Wnt pathways are evolutionarily well-conserved signal transduction pathways that are known to play important roles in all Metazoans investigated to date. Here, we examine the Wnt pathway genes and target genes present in the genome of the echinoderm *Strongylocentrotus purpuratus*. Analysis of the Wnt genes revealed that eleven of the thirteen reported Wnt subfamilies are represented in sea urchin, with the intriguing identification of a Wnt-A ortholog thought to be absent in deuterostomes. A phylogenetic study of the Frizzled proteins, the Wnt receptors, performed throughout the animal kingdom showed that not all Frizzled subfamilies were present in the metazoan common ancestor, e.g. Fz3/6 emerged later during evolution. Using sequence analysis, orthologs of the vast majority of the cellular machinery involved in transducing the three types of Wnt pathways were found in the sea urchin genome. Furthermore, of about one hundred target genes identified in other organisms, more than half have clear echinoderm orthologs. Thus, these analyses produce new inputs in the evolutionary history of the Wnt genes in an animal occupying a position that offers great insights into the basal properties of deuterostomes.

Keywords: Sea urchin; Genome survey; Wnt; Frizzled; Canonical; Planar cell polarity (PCP); Wnt/calcium

Introduction

The Wnt pathways are evolutionarily conserved signaling pathways that regulate multiple aspects of metazoan development. They are required throughout development, from early axis specification to organogenesis (e.g. Logan and Nusse, 2004; Cadigan and Nusse, 1997; Katoh, 2005; Veeman et al., 2003a). The Wnt pathways operate by three distinct mechanisms referred to as the canonical pathway, the planar cell polarity (PCP) pathway and the calcium/Wnt pathway (Fig. S1). Of these three signaling, the canonical Wnt pathway is the best characterized. Its activation results in entry of β-catenin into the nucleus of the cell where in concert with a TCF/Lef family member it activates transcription of target genes (Nusse, 1999). The PCP pathway operates through small G proteins such as RhoA and Rac to activate target genes, usually through the inducement of an AP-1 transcriptional complex (Hwang et al., 2005; Veeman et al., 2003b). The third Wnt pathway, the calcium/Wnt pathway, utilizes other small GTPases to control calcium-dependent molecules, including CamKII and PKC, leading to the transcriptional activation of its target genes (Kohn and Moon, 2005). Each of the three Wnt pathways is activated in the same way by the binding of a Wnt ligand to a Frizzled receptor. Transduction of the ligand–receptor interaction subsequently involves more than a hundred proteins that relay and modify the signal on the way to target actuation in a Wnt-pathway-specific manner. Proteins in the canonical Wnt pathway regulate the stability and movement of the key transcriptional activator β-catenin (Logan and Nusse, 2004). This pathway is principally required in many organisms for axis specification and endoderm differentiation (Croce and McClay,

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2006; Imai et al., 2000; Wikramanayake et al., 2004; Zorn et al., 1999). The PCP pathway is not commonly associated with tissue specification but is known instead to control cytoskeletal rearrangements and cell movements such as convergent extension (CE) occurring during gastrulation in deuterostomes (Croce et al., 2006; Heisenberg et al., 2000; Kilian et al., 2003; Wallingford et al., 2002). Finally, the calcium/Wnt pathway also acts on cellular behavior regulating cell adhesion and movement. However, this pathway functions through a different set of proteins that modulate the intracellular concentrations of free calcium and cyclic guanosine monophosphate (cGMP) (Wang and Malbon, 2003). To date, in addition to the main Wnt pathways components, more than eighty other molecules have been reported as modifiers that amplify, degrade, stabilize or alter the trajectory of the signals (Klein and Mlodzik, 2005; Logan and Nusse, 2004; Wang and Malbon, 2003). Most of these proteins have been identified in fully sequenced genomes, including human, ascidian and flies, though some of them have also been reported from individual studies in other more basal organisms such as cnidarians. Here, we present the first exhaustive report of the Wnt pathways genes in the phylum Echinodermata.

Emerging just after the protostome/deuterostome divergence, the Echinodermata offer insights into the earliest and the most basic of the deuterostome animals (Fig. 1A). This phylum contains diverse non-chordate marine organisms including sea urchins. Due to its phylogenetic position, the newly available genomic sequence of *Strongylocentrotus purpuratus* provides, therefore, key material for evolutionary history analyses. In this study, using bioinformatics techniques, we report the identification of the signaling components and the target genes of the three Wnt pathways in the *S. purpuratus* genome. The results clearly show that the entire three Wnt pathways are highly conserved with more than 95% of the more than 100 genes in the pathway represented in the sea urchin genome. Furthermore, in cases where there are multiple members within the same gene family, such as the Wnt family, the sea urchin genome reveals many deuterostome-like properties but also contains, as might be expected from its basal position, genes that are absent in more derived deuterostome groups but that are present in cnidarians and/or protostomes.

Materials and methods

Resources of genes sequences

The genomic sequence of *S. purpuratus* was deciphered and provided by BCM-HGSC [Baylor College of Medicine-Human Genome Sequencing Center] (http://www.hgsc.bcm.tmc.edu/blast/blast.cgi?organism=Spurpuratus). Additional genomic resources were available from several ESTs databases all accessible online at http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=7668. Proteins sequences for Wnt pathways related genes and targets from other animals were obtained from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html) (Benson et al., 2005), Ensembl (http://www.ensembl.org/index.html) (Hubbard et al., 2005), Pfam (http://www.sanger.ac.uk/Software/Pfam/) (Bateman et al., 2004), StellaBase (http://www.stellabase.org/) (Sullivan et al., 2006), Aniseed (Ascidian Network for In Situ Expression and Embryological Data) (http://crfb.univ-mrs.fr/aniseed/index.php) and by BLAST searches (Altschul et al., 1997) at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast/). Sea urchin sequences were all identified by blastp and blastn, using as guidance against genomic databases confirmed genes identified from a range of diverse animal species. All predicted sequences were reciprocally blasted against non-redundant NCBI databases leading to the identification of the best human hit, which was reblasted against the *S. purpuratus* genomic sequences for bi-directional best-hit analysis (see Tables S1, S3 and S4).

Phylogenetic analysis

Proteins alignments were carried out using ClustalX (Thompson et al., 1997) and saved as nexus files. Phylogenetic trees were generated using four complementary methods when needed. First, neighbor-joining trees (Saitou and Nei, 1987) were run with the PAUP 4.0 program (Swofford, 1998) and with 5000 bootstrap replicates. Second, unweighted maximum parsimony reconstructions were performed for each tree with a heuristic search of 1000 replicates. Third, maximum likelihood trees were computed using RAxML VI-1.0 (Stamatakis et al., 2005) and employing the Jones–Taylor–Thornton model of amino acid substitution, with otherwise default settings. Finally, Bayesian trees were performed with Mr. Bayes v.3.1.1 (Huelsenbeck et al., 2001; Ronquist and Huelsenbeck, 2003), and analyses were run for 500,000 generations and node probabilities were calculated after a burn-in of 50,000 generations. All trees were displayed with TreeView X 0.5.0, saved as svg files and colored and converted into JPG with Adobe Illustrator. Accession numbers of all sequences used for those trees are available in Tables S1 and S2.

Embryonic expression and RT-PCR

Embryonic expression of annotated sequences was confirmed using the tiling array database (Samanta et al., in press). For the Wnt genes, additional RT-PCR was performed from total RNA from various developmental stages. After extraction using the method of Cathala et al. (1983), cDNA was synthesized with a TaqMan kit from Clontech. RT-PCRs were carried out using standard protocol with specific primers designed against *S. purpuratus* Wnt predicted sequences.

Results

The activators of the Wnt pathways: Wnt and Frizzled proteins

Wnt family

The Wnt signaling molecules are a large family of secreted glycoproteins characterized by an invariant pattern of 22 to 24 highly conserved cysteine residues usually present in the last 70 C-terminal amino acids (Van Ooyen et al., 1985). In humans, nineteen Wnt proteins have been identified that define twelve distinct subfamilies named from Wnt-1 to Wnt-11 and Wnt-16 (Miller, 2002) (Fig. 1B). The Cnidaria, a basal non-bilaterian phylum thought to more closely reflect the eumetazoan ancestor (Fig. 1A), possesses 14 Wnt orthologs that sort into twelve distinct subfamilies, one of which, Wnt-A, does not have a human representative (Kusserow et al., 2005) (Fig. 1B). In sea urchin, prior to the availability of the genome, four members of the Wnt family (Wnt-1, -4, -5 and -8) had been identified. Each of these genes is expressed in embryos and of these, Wnt-8 has been shown to be required for endoderm specification during embryogenesis (Ferkowicz et al., 1998; Wikramanayake et al., 2004).

In silico analysis of the *S. purpuratus* genomic sequence revealed the presence of eleven Wnt genes, including Wnt-1, -4, -5 and -8 (Table S1). To determine whether these genes were expressed, EST and tiling data were evaluated (Samanta et al., in