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Asymmetric developmental potential along the animal–vegetal axis in the anthozoan cnidarian, *Nematostella vectensis*, is mediated by Dishevelled

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Abstract

The relationship between egg polarity and the adult body plan is well understood in many bilaterians. However, the evolutionary origins of embryonic polarity are not known. Insight into the evolution of polarity will come from understanding the ontogeny of polarity in non-bilaterian forms, such as cnidarians. We examined how the axial properties of the starlet sea anemone, *Nematostella vectensis* (Anthozoa, Cnidaria), are established during embryogenesis. Egg-cutting experiments and sperm localization show that *Nematostella* eggs are only fertilized at the animal pole. Vital marking experiments demonstrate that the egg animal pole corresponds to the sites of first cleavage and gastrulation, and the oral pole of the adult. Embryo separation experiments demonstrate an asymmetric segregation of developmental potential along the animal–vegetal axis prior to the 8-cell stage. We demonstrate that Dishevelled (Dsh) plays an important role in mediating this asymmetric segregation of developmental fate. Although *NvDsh* mRNA is ubiquitously expressed during embryogenesis, the protein is associated with the female pronucleus at the animal pole in the unfertilized egg, becomes associated with the unipolar first cleavage furrow, and remains enriched in animal pole blastomeres. Our results suggest that at least one mechanism for Dsh enrichment at the animal pole is through its degradation at the vegetal pole. Functional studies reveal that NvDsh is required for specifying embryonic polarity and endoderm by stabilizing β-catenin in the canonical Wnt signaling pathway. The localization of Dsh to the animal pole in *Nematostella* and two other anthozoan cnidarians (scleractinian corals) provides a possible explanation for how the site of gastrulation has changed in bilaterian evolution while other axial components of development have remained the same and demonstrates that modifications of the Wnt signaling pathway have been used to pattern a wide variety of metazoan embryos.

Keywords: Axial properties; Polarity; Cnidaria; Anthozoa; *Nematostella vectensis*; Dishevelled; Wnt; β-Catenin

Introduction

Most bilaterian eggs possess polarity along the primary axis, the animal–vegetal (AV) axis, which is established during oogenesis. Egg polarity is manifest in the asymmetric distribution of maternal components, including mRNAs (Di Carlo et al., 2004; King et al., 2005; Momose and Houliston, 2007; Nishida, 2005; van Eeden and St Johnston, 1999), proteins (Vinot et al., 2004; Weitzel et al., 2004), organelles (Coffman et al., 2004; Zalokar and Sardet, 1984), and cytoskeletal elements (Egana et al., 2007; Sardet et al., 1992). The relationship between egg polarity and larval/adult axial properties is generally well conserved among bilaterians, such that the anterior of the larva/adult is derived from the region of the animal pole and the posterior from the vegetal pole (reviewed in Martindale, 2005). Despite such widespread conservation between the general axial properties of the egg and the adult, there exists in bilaterians a great degree of variability in terms of the site of gastrulation with respect to both the AV axis of the egg and the anteroposterior (AP) axis of the adult (reviewed in Martindale, 2005). The axial properties of the early embryo determine the site of gastrulation, and gastrulation plays a central role in organizing the axial properties of the adult. It is clear, therefore, that

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understanding the cellular and molecular aspects establishing embryonic polarity and the determination of cell fates in diverse organisms will ultimately provide insight to the evolution of body plan organization.

One of the central and conserved molecular signaling pathways involved in regulating both axis formation and germ-layer segregation in bilaterians is the Wnt/β-catenin, or canonical Wnt, signaling pathway. In several deuterostome embryos, egg polarity is translated into the axial polarity of the adult body plan as asymmetrically localized maternal factors activate Wnt/β-catenin signaling in a restricted region of the cleavage-stage embryo. This results in the stabilization and nuclearization of β-catenin in cells at one region of the embryo and the activation of target genes involved in establishing axial properties (reviewed in Croce and McClay, 2006; Marikawa, 2006). Accumulation of nuclear β-catenin in blastomeres at one pole of the embryo marks the site of gastrulation and specifies the dorsoventral (DV) axis in vertebrates (Larabell et al., 1997; Moon and Kimelman, 1998; Schneider et al., 1996), mediates establishment of the AV axis in lower chordates (Imai et al., 2000; reviewed in Nishida, 2005), and patterns the AV axis in echinoderms (Logan et al., 1999; Wikramanayake et al., 1998). Nuclear localization of β-catenin is also involved in the formation of endomesodermal precursors and the process of germ-layer segregation through the activation of *Wnt* target genes in the endomesodermal gene regulatory network in deuterostomes (Imai et al., 2000; reviewed in Loose and Patient, 2004; Oliveri and Davidson, 2004). Despite all that is known about the downstream effects of canonical Wnt signaling on axis establishment and germ-layer formation, the upstream factors that activate Wnt/β-catenin signaling in the early embryo are not as well characterized. Most recently, a maternally supplied Wnt has been shown to dorsally activate Wnt/β-catenin signaling in *Xenopus* (Tao et al., 2005), however, maternal Wnt ligands that regulate β-catenin nuclearization during early embryogenesis have not been identified in other organisms. In sea urchins, an upstream component in the Wnt/β-catenin pathway, Dishevelled (Dsh), is enriched at the vegetal cortex of unfertilized eggs and early embryos and is necessary for the accumulation of nuclear β-catenin in vegetal blastomeres, axial patterning, and endomesoderm formation (Kumburegama and Wikramanayake, 2007; Weitzel et al., 2004).

The Cnidaria is a phylogenetically important outgroup, separating from other metazoans prior to the origin of the bilaterian assemblage (Collins et al., 2006; Medina et al., 2001). Cnidarians share many critical developmental regulatory genes with bilaterians (Broun et al., 2005; Finnerty et al., 2004; Galliot, 2000; Kortschak et al., 2003; Kusserow et al., 2005; Magie et al., 2005; Matus et al., 2007; Ryan et al., 2007; Scholz and Technau, 2003; Technau et al., 2005), despite important differences in symmetry, axial properties, and germ-layer composition. Early work in hydrozoan cnidarians (Tessier, 1931) suggested that the axial properties of the embryo and adult are specified by a maternally established egg polarity, as in most bilaterians. Recent work in the hydrozoans *Podocoryne carnea* and *Clytia hemisphaerica* (formerly the genus *Phialidium*) supports the notion of localized determinants in the egg being responsible for axis polarity and germ-layer formation (Momose and Houliston, 2007; Momose and Schmid, 2006; Yanze et al., 2001). Conflicting experimental evidence, however, indicates that the single body axis of hydrozoan cnidarians, the oral–aboral (OA) axis, is not specified until the time of first cleavage by the location of the first unipolar cleavage furrow (Freeman, 1980; Freeman, 1981). Regardless of when the OA axis is established, in all hydrozoans examined the site of cleavage initiation becomes the oral pole of the planula. Under normal developmental conditions, the site of first cleavage coincides with the animal pole of the egg. Therefore, as in bilaterians, there is a consistent correlation between the animal pole of the egg and the oral end of the adult (Freeman, 1980; Freeman, 1983; Momose and Houliston, 2007; Momose and Schmid, 2006; Schlawny and Pfannenstiel, 1991; Tessier, 1931). Whether the axial properties found in hydrozoans, a highly diverse class of cnidarians with complex life cycles, are general features shared by all cnidarians is not known as other cnidarian classes have not been studied.

There is increasing evidence that, as with deuterostomes, Wnt/β-catenin signaling is crucial in establishing and maintaining polarity in cnidarian embryos. Past studies have shown that β-catenin is nuclearized in oral blastomeres in early embryos of the anthozoan *Nematostella vectensis*, and signaling by this protein in the canonical Wnt pathway is required for germ-layer segregation (Wikramanayake et al., 2003). More recent studies in two species of hydrozoans have also shown a role for canonical Wnt signaling in axial development. Messenger RNAs for both a Wnt ligand and a Wnt signaling component, Tcf, are localized to the first cleavage furrow in *Hydractinia* and are involved in oral development (Plickert et al., 2006). Additionally, in the eggs of *C. hemisphaerica*, mRNAs for two Wnt receptors, Frizzled 1 (Fz1) and Frizzled 3 (Fz3), are localized to the oral and aboral poles, respectively, and regulate both oral and aboral development, including nuclearization of β-catenin in prospective endoderm during embryogenesis (Momose and Houliston, 2007).

A universal role of the Wnt pathway in axial development and germ-layer segregation during cnidarian development is far from clear however. No Wnt signal has been detected in cleavage stages of *Nematostella* (Kusserow et al., 2005; Lee et al., 2006) or *Clytia* (Momose and Houliston, 2007), and although mRNA for Tcf in *Nematostella* becomes gradually localized to presumptive endoderm, along with nuclear β-catenin (Wikramanayake et al., 2003), it is not asymmetrically localized at first cleavage (Lee et al., 2006). Furthermore, several *Fz* genes are present in the *Nematostella* genome, including a *Fz1* ortholog, but none of them are asymmetrically expressed in the egg (N. Wijesena and A. Wikramanayake, unpublished observations), raising the possibility that asymmetric *Fz* localization is a derived feature of hydrozoan eggs. It is also not clear how the Wnt pathway may be involved in reorganizing the oral–aboral axis in *Clytia* by changing the site of first cleavage (Freeman, 1980; Freeman, 1981).

These studies in hydrozoans have further highlighted the importance of anthozoans for providing insight into the ancestral mechanisms underlying axial organization in developing