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Molecular asymmetry in the 8-cell stage *Xenopus tropicalis* embryo described by single blastomere transcript sequencing

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ABSTRACT

Correct development of the vertebrate body plan requires the early definition of two asymmetric, perpendicular axes. The first axis is established during oocyte maturation, and the second is established by symmetry breaking shortly after fertilization. The physical processes generating the second asymmetric, or dorsal–ventral, axis are well understood, but the specific molecular determinants, presumed to be maternal gene products, are poorly characterized. Whilst enrichment of maternal mRNAs at the animal and vegetal poles in both the oocyte and the early embryo has been studied, little is known about the distribution of maternal mRNAs along either the dorsal–ventral or left–right axes during the early cleavage stages. Here we report an unbiased analysis of the distribution of maternal mRNA on all axes of the *Xenopus tropicalis* 8-cell stage embryo, based on sequencing of single blastomeres whose positions within the embryo are known. Analysis of pooled data from complete sets of blastomeres from four embryos has identified 908 mRNAs enriched in either the animal or vegetal blastomeres, of which 793 are not previously reported as enriched. In contrast, we find no evidence for asymmetric distribution along either the dorsal–ventral or left–right axes. We confirm that animal pole enrichment is on average distinctly lower than vegetal pole enrichment, and that considerable variation is found between reported enrichment levels in different studies. We use publicly available data to show that there is a significant association between genes with human disease annotation and enrichment at the animal pole. Mutations in the human ortholog of the most animally enriched novel gene, *Slc35d1*, are causative for *Schneckenbecken dysplasia*, and we show that a similar phenotype is produced by depletion of the orthologous protein in *Xenopus* embryos.

1. Introduction

The first steps of vertebrate development are guided by maternal mRNAs and proteins deposited during oogenesis. A subset of these maternal factors is likely to control asymmetry for correct axial development, and polymorphisms or deficiencies in these may lead to defects in body patterning. An understanding of which of these genes are asymmetrically expressed in the oocyte and early embryo, and also implicated in human disease, may help to direct research more effectively into the genetic causes of disease. *Xenopus* is an ideal model system both for the discovery of asymmetric and early acting genes, and the investigation of their role in development.

Bilaterian animals require the early definition of two asymmetric, perpendicular axes for correct development. The process of axial differentiation in the embryo is controlled by two main mechanisms: the action of maternal determinants transmitted to specific blastomeres, and cellular interactions mediated by various signaling molecules (Koga et al., 2012). Understanding the specific molecular determinants of these processes remains an important question in developmental biology. In many organisms, these early events are accomplished by the localization or sequestration of maternally synthesized proteins and mRNA (Danilchik et al., 2006). Mechanisms vary in non-vertebrate species (Gonczy and Rose, 2005; Kugler and Lasko, 2009; Steinhauer and Kalderon, 2006), but in vertebrates, the animal–vegetal axis is set up during oocyte maturation, and the dorsal–ventral axis is established at or shortly after fertilization (Croce and McClay, 2006).

In *Xenopus*, the oocyte develops with radial symmetry around the animal–vegetal axis between the darkly pigmented animal pole and the lightly pigmented vegetal pole (Kageura, 1997). Fertilization can only take place in the animal hemisphere, which ensures that axial symmetry is broken in a stereotypical manner.

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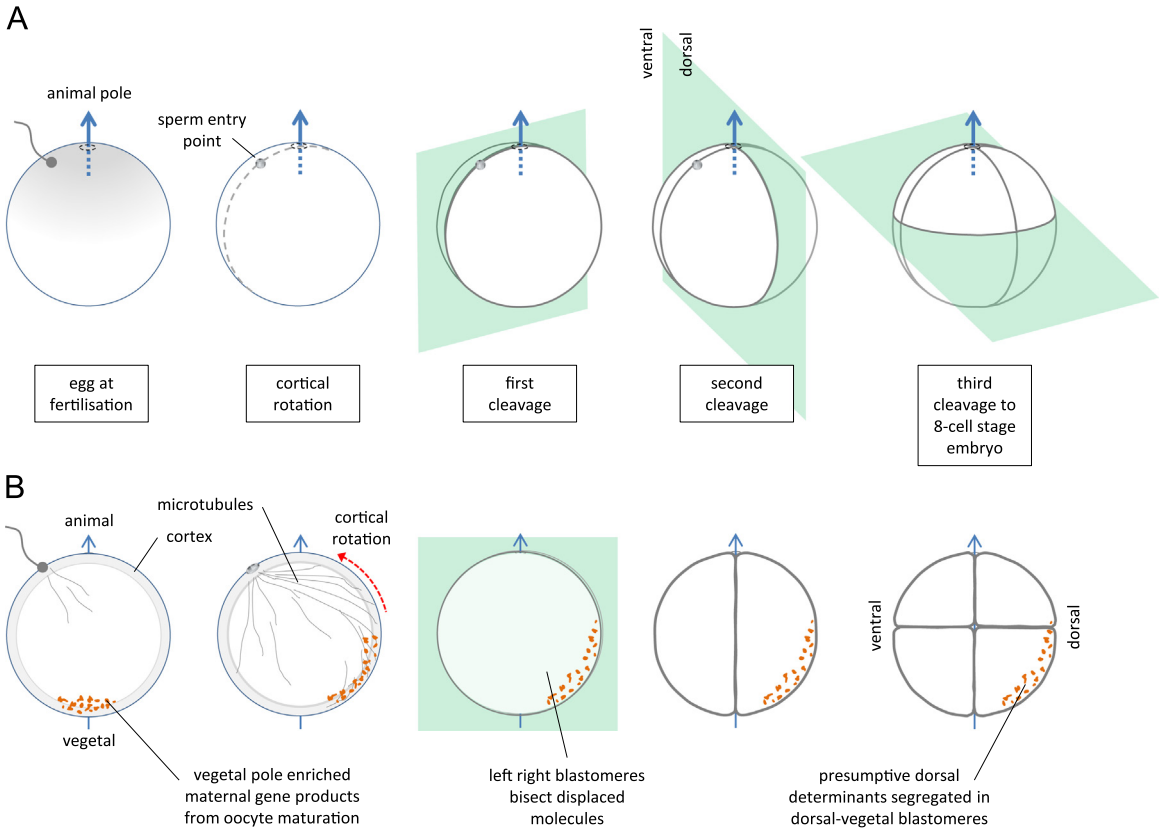

Fig. 1. Cortical rotation and the segregation of maternal gene products in the cleavage stage embryo. A. Orientation of early cell divisions. The first cleavage plane is determined by the sperm entry point (SEP) and the animal–vegetal (A–V) axis. The second cleavage is orthogonal to the first but still contains the A–V axis; dorsal blastomeres are defined in the hemisphere opposite the SEP. The third cleavage is slightly above the equator. B. Displacement of maternal gene products. The egg contains vegetally localized maternal gene products. A microtubule network is set up at fertilization and the outer layer, or cortex, of the single cell embryo rotates to displace the vegetal cortical region away the SEP. Maternal gene products are displaced by movement with the cortex or by vesicle trafficking into the presumptive dorsal hemisphere, and are further segregated by subsequent cell divisions.

Sperm entry initiates processes that reorganize the molecular content of the embryo, and the position of the sperm entry point defines the orientation of the other axes with reference to the animal–vegetal axis (Fig. 1A). Partway through the first cell cycle, the outer cortex of the embryo is rotated 30–40° degrees relative to the core cytoplasm; the vegetal polar region moving away from the sperm entry point (Gerhart et al., 1989). This *cortical rotation*, either directly or indirectly, transports molecules from the poles of the animal–vegetal axis to more equatorial regions (Houston, 2012) (Fig. 1B), which locates the dorsal end of the dorsal–ventral axis in the opposite hemisphere to the sperm entry point (Danilchik et al., 2006).

The cleavage plane of the first cell division is defined by the plane of cortical rotation. The second cleavage plane, orthogonal to the first but still containing the animal–vegetal axis, segregates the more dorsally distributed molecules from the more ventral ones. The third cleavage plane, slightly above the equator towards the animal pole, further compartmentalizes the segregated molecules (Denegre et al., 1998) (Fig. 1B). On the dorsal side, this segregation forms the basis for the Nieuwkoop center in the region of the vegetal–dorsal blastomeres, and later the Spemann organizer (De Robertis and Kuroda, 2004; Houston, 2012). In this scheme, the first cleavage determines the left and right halves of the animal, and while these exhibit superficial mirror symmetry, substantial left–right internal asymmetry develops over time. Although this has been investigated for some years, the molecular origins and timing of the development of left–right asymmetry are not well understood (reviewed in Blum et al., 2014; Coutelis et al., 2014).

Blastomere isolation and removal experiments have been carried out with *Xenopus* embryos. These have shown that separated halves of 2-cell stage embryos, and 8-cell stage embryos containing some combination of ventral and dorsal blastomeres, can still reach tailbud stage, whilst purely ventral halves give rise to cell masses lacking head or axial structure (Kageura and Yamana, 1983, 1984). Furthermore, the ability of transplants from oocytes or early embryos to induce the formation of a secondary axis in later stage embryos is evidence that some cytoplasmic dorsal determinants are localized early in specific regions of the embryos (Fujisue et al., 1993; Gallagher et al., 1991; Hainski and Moody, 1992; Kageura and Yamana, 1986).

The nature and identities of these dorsal determinants are not currently well defined. Previous experiments have shown that these putative cytoplasmic factors may be mRNA or proteins (Klein and King, 1988; Miyata et al., 1987; Shiokawa et al., 1984). Further, it has been argued that cytoplasmic polyadenylation may be the mechanism for the change in dorsal inducing activity of total RNA isolated from the dorsal lineage between the 8- and 16-cell stages (Pandur et al., 2002). Differential polyadenylation along the dorsal–ventral axis has been proposed to account for an observed dorsal enrichment of Wnt11 protein (Schroeder et al., 1999), and observed vesicle trafficking has been proposed to account for dorsal enrichment of Disheveled protein (Miller et al., 1999).

The process of mRNA segregation in the maturing oocyte has been well studied (King et al., 2005; Kloc et al., 2001, 2002; Melton, 1987), and numbers of mRNAs with an asymmetric distribution on the animal–vegetal axis are published (Cuykendall and Houston, 2010; Houston, 2013; King et al., 2005).