Asymmetric Cell Division: A CAB Driver for Spindle Movements

To divide asymmetrically, a cell must position the mitotic spindle relative to localized cell fate determinants. Recent work in the early ascidian embryo reveals the function of a single factor that coordinates this act to control cleavage pattern and cell fate determination.

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Division of one cell to produce two daughters that are different is central to animal development. Embryonic cells work endless variations on this basic act in the context of cell fate determination, the maintenance of multipotent stem cell progenitors or the elaboration of complex tissue architecture [1–3]. In every case, the basic idea is essentially the same: A cell must first generate functional asymmetry by localizing specific factors, activities or structures to specific cellular domains; then it must position the mitotic spindle relative to these asymmetries in order to position the cleavage furrow and to apportion the different cellular domains to only one of two daughters.

Much of what we know about mechanisms that position the mitotic spindle during asymmetric cell division comes from studies in a few model organisms — namely budding yeast, *Caenorhabditis elegans* worms and fruit flies [4]. These studies have identified some common themes and some conserved machinery, but they also reveal considerable diversity in how this machinery is configured and how it is put to use. Thus, it is clear that there is much to be gained from looking more closely at asymmetric division in other organisms. One of these, as nicely illustrated by Negishi *et al.* [5], is the humble ascidian *Halocynthia roretzi*.

In the ascidian embryo, where a cell comes from is easily as important for its future fate as who its neighbors are. In ascidian embryos, cell fate specification occurs rapidly in the context of an essentially invariant cleavage pattern. Many of the basic cell types — including epidermis, endoderm, and muscle — are specified by direct inheritance of maternal factors that are localized in the cytoplasm of fertilized eggs [6]. Most of the remaining cells are known to be specified by local inductive interactions between neighboring cells. But even in many of these cases, both signal and response are shaped by inheritance of maternal factors [7,8]. Thus, a central question has been: how does the ascidian embryo organize cleavage patterns with respect to localized cytoplasmic determinants to ensure the robust allocation of cell fates?

Attempts to address this question have focused on a cellular domain called the Posterior Vegetal Cortex/Cytoplasm (PVC) — so called because it forms from vegetal cytoplasm and its position defines the future anterior pole [9]. The PVC consists of an actin-rich cortical layer, connected to a dense network of sub-cortical endoplasmic reticulum (ER) that surrounds an electron dense matrix resembling the germ plasm described in other organisms [10]. More than a dozen maternally supplied mRNAs are enriched within the PVC, many in close association with the ER. Among these are a transcription factor necessary for specification of posterior vegetal somatic cell fates [11], and a homologue of the conserved germline determinant VASA [12]. In an elegant and now classic set of micromanipulation experiments, Hiroki Nishida [9] showed that removal of the PVC before the first cleavage resulted in the complete replacement of posterior vegetal cell fates and cleavage pattern with a mirror duplicate of those of the anterior vegetal quadrant. Transplanting the PVC from one zygote to the presumptive anterior vegetal pole of a zygote whose PVC had been removed caused a complete reversal of the AP axis. Thus factors localized to the PVC determine both cell fate and cleavage pattern.

In subsequent work, Nishida's group found that after the 8-cell stage, the PVC condenses to form a more compact structure, which they named the centrosome attracting body (CAB) [13]. The CAB appears to attract one of the centrosomes during interphase such that the next division produces a small daughter that inherits the CAB as well as the putative germ plasm, and a larger daughter that goes on to make only somatic tissues, such as muscle, mesenchyme and endoderm [13]. The same process occurs repeatedly during three successive cell cycles (Figure 1), culminating in the formation of a single pair of tiny germline precursor cells. The centrosome attraction appears to be mediated by dense bundles of astral microtubules that form between the centrosome and the CAB and that shorten as the two move closer together [14]. This suggests that factors localized to the CAB pull on the distal tips of astral microtubules to exert an attractive force on the centrosome. But the identities of these factors have remained obscure.

In a recent paper in *Current Biology*, Negishi *et al.* [5] from Hiroki Nishida's lab show that a protein called PEM is both associated with the CAB and required for centrosome capture and asymmetric divisions within the posterior vegetal quadrant. While previous work had already shown that PEM mRNA is the most abundant mRNA associated with the CAB, Negishi *et al.* [5] show that the protein itself is also localized, first to the PVC, and then later to the CAB itself. Inhibiting translation of the maternally provided PEM mRNA results in a complete loss of PEM protein and abolishes the highly asymmetric divisions of the posterior vegetal quadrant. The CAB itself forms normally, but the microtubule bundle that connects the CAB to the proximal centrosome is missing, suggesting that

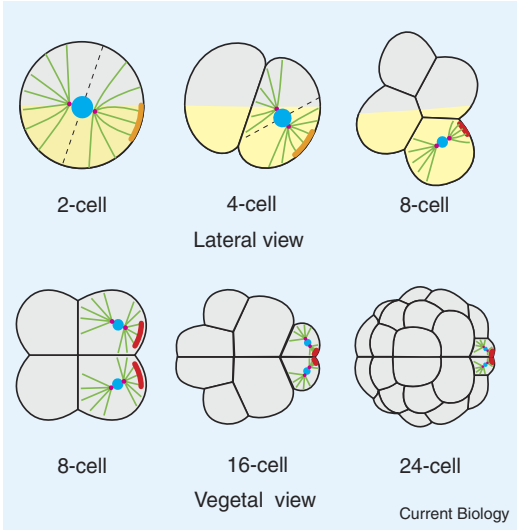

Figure 1. PEM-dependent centrosome capture determines posterior vegetal cleavage patterns in ascidian embryos.

During early cleavages of the ascidian embryo, PEM mRNA and protein localize to the posterior vegetal cytoplasm (orange in 2-cell and 4-cell stage panels) before the 8-cell stage. Later, PEM localizes to the centrosome attracting body (CAB; red). Beginning in the interphase of each cell cycle, an interaction between microtubules and PEM causes a shift in the position of one centrosome/spindle pole (pink). During the second and third cleavages, this shifts the orientation of the cleavage plane (dashed lines) relative to the boundary between animal (gray) and vegetal (yellow) cytoplasm. During later cleavages, this results in production of a smaller posterior daughter that inherits the CAB and germline determinants, and a larger anterior somatic daughter. Modified from [5].

a specific interaction between the CAB and astral microtubules is lost.

Negishi *et al.* [5] go on to show that PEM acts even before formation of the CAB to control spindle position during earlier cleavages. Before the first cleavage, the zygote's cytoplasm is partitioned into distinct animal and vegetal domains [15]. The first cleavage bisects these domains exactly, but during the second and third cleavages, in blastomeres that contain the PVC, one of the centrosome/spindle poles moves towards the PVC, so that the second cleavage plane is not quite perpendicular to the boundary between the animal and the vegetal domain; likewise, the third cleavage plane is not quite parallel to that boundary (Figure 1). Inhibiting PEM translation abolishes these asymmetries. As Negishi *et al.* [5] point out, these observations may help to resolve puzzling anomalies in the ascidian fate map by explaining why some cell types derived from the animal tier have vegetal characteristics and vice versa. More importantly, perhaps, they reveal that centrosome attraction is not a property of the CAB *per se*. Instead, it is an activity that requires PEM and that operates during all early stages. Negeshi *et al.* [5] thus propose that a single mechanism involving PEM-dependent centrosome attraction may account for the overall pattern of cell division within the posterior vegetal quadrant.

How does PEM work? It's too early to say. Simple sequence analysis reveals no obvious homolog in other organisms and only limited conservation within ascidians [5]. A likely scenario, extrapolating from recent studies in other organisms, is that PEM forms part of an adaptor complex that couples microtubule tips to a molecular scaffold, which holds localized determinants in place. For example, in fruit fly embryos, a conserved adaptor system involving heterotrimeric G-proteins, their binding partners Pins and Ins, and the microtubule binding protein Mud links a conserved polarity complex (Par-3/Par-6/aPKC) to astral microtubules to position the mitotic spindle relative to localized cell fate determinants [2]. Mammalian cells appear to use a highly homologous system [3,16]. *C. elegans* embryos use many of the same factors during early asymmetric cleavages, but their interactions differ, and a protein called Lin-5 substitutes for the missing homologue of Mud/NuMA [17]. Recent work shows that ascidian homologues of Par-3/Par-6/Pkc-3/aPKC complex concentrate at the cortex within the CAB and that astral microtubules directly contact this layer [18]. Thus it seems reasonable to suspect that PEM could be an ascidian-specific component of a variant of this same adaptor system.

Of course there are other possibilities. For example, among the mRNAs that localize to the CAB is a putative guanine nucleotide exchange factor for the small GTPase CDC-42 — another binding partner of Par-3/Par-6/aPKC — and a conserved regulator of spindle position and orientation [19]. All of these possibilities remain to be tested, but the identification of a specific factor required for centrosome capture has opened a new door. Given the list of candidate molecules identified in other systems, the fully sequenced and compact ascidian genome, and the growing wealth of molecular genetic approaches to ascidian embryology, we can expect some more definite answers in the very near future.

References

1. Betschinger, J., and Knoblich, J.A. (2004). Dare to be different: asymmetric cell division in Drosophila, C. elegans and vertebrates. Curr. Biol. *14*, R674–R685.
2. Gonzalez, C. (2007). Spindle orientation, asymmetric division and tumour suppression in Drosophila stem cells. Nat. Rev. Genet. *8*, 462–472.
3. Lechler, T., and Fuchs, E. (2005). Asymmetric cell divisions promote stratification and differentiation of mammalian skin. Nature *437*, 275–280.
4. McCarthy, E.K., and Goldstein, B. (2006). Asymmetric spindle positioning. Curr. Opin. Cell Biol. *18*, 79–85.
5. Negishi, T., Takada, T., Kawai, N., and Nishida, H. (2007). Localized PEM mRNA and protein are involved in cleavage-plane orientation and unequal cell divisions in ascidians. Curr. Biol. *17*, 1014–1025.
6. Nishida, H. (2005). Specification of embryonic axis and mosaic development in ascidians. Dev. Dyn. *233*, 1177–1193.
7. Kim, G.J., Yamada, A., and Nishida, H. (2000). An FGF signal from endoderm and localized factors in the posterior-vegetal egg cytoplasm pattern the mesodermal tissues in the ascidian embryo. Development *127*, 2853–2862.
8. Lemaire, P., Bertrand, V., and Hudson, C. (2002). Early steps in the formation of neural tissue in ascidian embryos. Dev. Biol. *252*, 151–169.
9. Nishida, H. (1994). Localization of determinants for formation of the anterior-posterior axis in eggs of the ascidian Halocynthia roretzi. Dev. Biol. *120*, 3093–3104.
10. Sardet, C., Dru, P., and Prodon, F. (2005). Maternal determinants and mRNAs in the

cortex of ascidian oocytes, zygotes and embryos. Biol. Cell *97*, 35–49.
11. Nishida, H., and Sawada, K. (2001). macho-1 encodes a localized mRNA in ascidian eggs that specifies muscle fate during embryogenesis. Nature *409*, 724–729.
12. Fujimura, M., and Takamura, K. (2000). Characterization of an ascidian DEAD-box gene, Ci-DEAD1: specific expression in the germ cells and its mRNA localization in the posterior-most blastomeres in early embryos. Dev. Genes Evol. *210*, 64–72.
13. Hibino, T., Nishikata, T., and Nishida, H. (1998). Centrosome-attracting body: a novel structure closely related to unequal cleavages in the ascidian embryo. Dev. Growth Differ. *40*, 85–95.
14. Nishikata, T., Hibino, T., and Nishida, H. (1999). The centrosome-attracting body, microtubule system, and posterior egg cytoplasm are involved in positioning of cleavage planes in the ascidian embryo. Dev. Biol. *209*, 72–85.
15. Prodon, F., Chenevert, J., and Sardet, C. (2006). Establishment of animal-vegetal polarity during maturation in ascidian oocytes. Dev. Biol. *290*, 297–311.
16. Du, Q., and Macara, I.G. (2004). Mammalian Pins is a conformational switch that links NuMA to heterotrimeric G proteins. Cell *119*, 503–516.
17. Srinivasan, D.G., Fisk, R.M., Xu, H., and van den Heuvel, S. (2003). A complex of LIN-5 and GPR proteins regulates G protein signaling and spindle function in C elegans. Genes Dev. *17*, 1225–1239.
18. Patalano, S., Pruliere, G., Prodon, F., Paix, A., Dru, P., Sardet, C., and Chenevert, J. (2006). The aPKC-PAR-6-PAR-3 cell polarity complex localizes to the centrosome attracting body, a macroscopic cortical structure responsible for asymmetric divisions in the early ascidian embryo. J. Cell Sci. *119*, 1592–1603.
19. Etienne-Manneville, S. (2004). Cdc42–the centre of polarity. J. Cell Sci. *117*, 1291–1300.

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Social Cognition: Overturning Stereotypes of and with Autism

New data suggest that even children with autism are subject to race and gender stereotypes. This result constrains theories of stereotype acquisition and social cognition in autism.

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Stereotypes about the characteristics of individuals based on their group membership, for example race or gender, are a pernicious feature of human society. A prejudiced image of a woman struggling with a math test or a black man as a threat has no basis in reality, but almost everyone in American and European cultures is subject to these stereotypes. Once acquired, such stereotypes are remarkably robust and difficult to overcome (for review, see [1]), despite their unpleasant impact on human social interactions.

There is, however, one group of individuals who one might think would be immune to the influence of stereotypes. Children with autism have profound difficulties with many types of social interaction. They do not orient towards social stimuli [2] and show reduced social behaviour even before the diagnosis of autism can be made [3]. These children fail to engage others by means of joint attention [2] or imitation [4] and have trouble recognising faces [5]. Cognitive studies have revealed that autistic children have specific difficulties with understanding other people's mental states [6], and this 'Theory of Mind' deficit is a core feature of autism. The broad impairment of social cognition in autism might be expected to reduce the autistic child's capacity for learning social information, such as how to treat members of other racial groups, from other people's behaviour. Thus, children with autism should surely be impervious to the detrimental influence of race and gender stereotypes.

In a study published recently in *Current Biology*, Hirschfeld and colleagues [7] used a simple test of children's tendency to stereotype to compare children with autism and their matched typical peers. Surprisingly, the autistic children demonstrated a clear propensity to make judgements based on race and gender stereotypes, just like typical children. Contrary to the naïve prediction, it seems that children with autism do use social stereotypes. Moreover, when children were tested on a novel 'conflict' task which pitted the explicitly stated desires of the characters ('Mary likes playing with trucks') against the implicit stereotypical preference ('girls don't like trucks'), both typical seven year olds and children with autism who passed theory of mind tasks made more judgements based on the character's desires than on stereotypes. In contrast, both typical three year olds and autistic children who failed theory of mind tasks continued to use stereotypes to predict behaviour in the conflict task. These results imply that theory of mind abilities may be important not for acquiring stereotypes, but for overcoming them. Again, the similarity between the typical and autistic groups suggests that stereotype use is not dysfunctional in autism.

These data bring together two fields of social cognition which have not previously interacted, and have interesting implications for both. First, the question of how the autistic child acquires stereotypes is now critical. In order to form a stereotype, a child must be able to classify the people they see as members of a particular social group based on visual features and must then link the group to particular unobserved character traits, which can be attractive (friendly, strong), or unattractive (stupid, ugly). Typical children acquire these abilities early, with awareness of gender roles at age 26 months [8] and the use of racial stereotypes from age 3 years [9]. But the sources of information which children draw on to make links between social groups and