GTPases Rac and Cdc42 in mediating spindle position. Again, many questions remain, such as identity of the molecular targets of the GTPases and the precise nature of their effects on spindle positioning. Finally, determining to what extent such mechanisms are shared between mouse eggs, *Drosophila* neuroblasts, *C. elegans* zygotes, and budding yeast cells, for example, will be required to define the general principles of cortical polarization and asymmetric cell division.

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Epigenetic Arbitration of Cell Fate Decisions: Tipping the Bias

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Epigenetic modifications of nucleosomal histones are thought to mediate transcriptional states and impose heritable instructions upon differentiation. In a paper of Torres-Padilla and colleagues in *Nature*, protein modification at arginine residues, namely of core histones, is correlated with cell fate determination at the 4-cell stage in the mouse embryo. This represents the first link of global epigenetic instructions associated with specification of early cell lineages.

In mammals, DNA methylation and covalent modifications of the amino terminal tails of nucleosomal histones constitute the majority of epigenetic modifications. Acetylation, methylation, phosphorylation, ADP ribosylation, and ubiquitination of core nucleosomal histones are thought to extend the information content of the underlying genetic code conferring unique transcriptional instructions. The overall influence of these modifications has been termed the 'histone code' (Nightingale et al., 2006; Strahl and Allis, 2000), and these marks are particularly prevalent on lysine and arginine residues. Epigenetic instructions create a dynamic nuclear environment that specifies transcriptional states and comprises the essential components of heritable cellular memory, a hallmark of differentiation. However, an integrated understanding of a mechanistic link to the early events associated to cell fate decision in the early embryo has yet to be established.

In development, reconstitution of the zygote upon fertilization marks the state of ultimate totipotency. Thereafter, subsequent cell divisions are associated with a progressive restriction in totipotency culminating in the first differentiative events and the establishment of the two cell lineages of the blastocyst, inner cell mass (ICM) and trophectoderm (TE).

There has been a recent renewal of interest in the possibility that cell fate is not simply a random process. Results of Zernicka-Goetz and colleagues have contended, not without some controversy, that the timing and positioning of the plane of cleavage of the first 2 cell divisions may be capable of influencing cell fate (Zernicka-Goetz, 2006). In a simplified view, if the division plane of the first dividing blastomere is in a meridional position, the progeny of this "M" blastomere appears more likely to contribute to the ICM than the TE. Investigation of epigenetic marks potentially associated to this critical juncture have included DNA methylation and several histone modifications but have not revealed a mechanistic link to cell fate decisions in the early embryo (Dean et al., 2001; Margueron et al., 2005). In the current study of Torres-Padilla and colleagues (Torres-Padilla et al., 2007), the authors focus their attention to the second cleavage division and find that individual blastomeres differ in their epigenetic make-up as early as the 4-cell embryo.

Specifically, using indirect immunofluorescence, they find that the blastomeres of a 4-cell embryo are nonequivalent as far as histone H3 arginine methylation levels are concerned. Curiously this only applies to 4-cell embryos in the tetrahedral configuration as those forming a planar diamond shape showed no differences between blastomeres. The unequal distribution of histone arginine methylation is specific to H3 as histone H4 methylation which is conferred by a different methyltransferase, PRMT1, does not display this differential pattern.

Moreover, the blastomere exhibiting most prominent H3 arginine methylation seems to be predetermined in its differentiation fate toward the ICM. In an important extension to this observation, the authors then go on to inject CARM1, the methyltransferase that mediates H3 arginine methylation, in one blastomere of 2-cell stage embryos and find that CARM1 overexpression influences the probability of the injected blastomere's allocation toward the ICM lineage (Figure 1). This experiment is elegantly controlled for by generating a catalytic mutant of CARM1 which resulted in a random distribution of the injected blastomere's progeny between ICM and TE, at an indistinguishable frequency from the cell marker alone. This tidy experiment reinforces the idea that the preferential allocation to the ICM arises as a consequence of the methylase function attributed to CARM1.

CARM1 is responsible for the addition of methyl groups to arginines R2, R17, and R26 in histone H3, and these modifications have been recognized as an epigenetic mark that confers overall gene activity. Presumably, transcriptional activation through H3 methylation at key arginine residues mediates a major part of the transcriptional coactivator functions that have been ascribed to CARM1. CARM1 has been isolated as a transcriptional coactivator of nuclear receptors, and it can also cooperate with various transcription factors such as p53, NFκB, LEF1/TCF4, and TIF1a (An et al., 2004; Teyssier et al., 2006; Wysocka et al., 2006). As part of these complexes, it can change the chromatin environment at responsive gene promoters to mediate gene activation. However, CARM1 also has specific nonhistone targets that contribute to the overall emerging functions of protein arginine methylation at various stages of gene regulation including transcription initiation and elongation, splicing, and mRNA transport (Bedford and Richard, 2005). Thus, the role of CARM1 in predetermining cell fate in the early embryo may not solely be due to its function in modifying histones but may also be mediated by modification of the enzyme's nonhistone substrates.

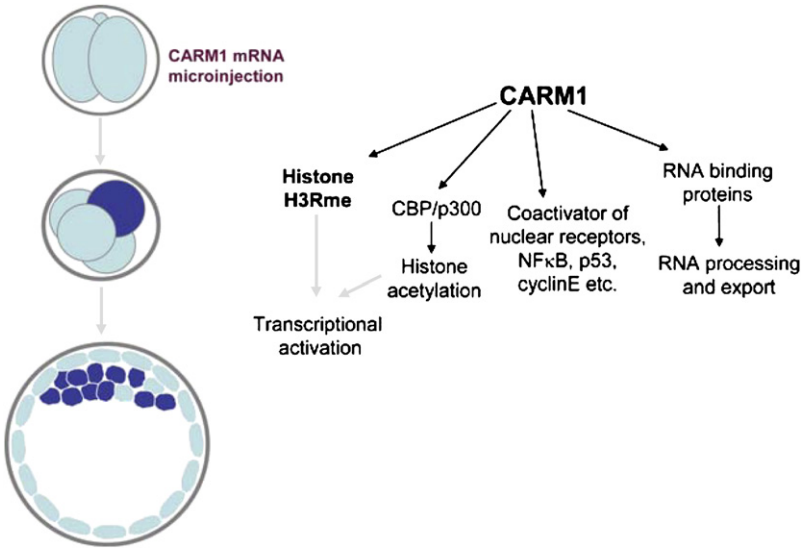

Figure 1. Schematic Representation of Development from the 2-Cell to the Blastocyst Stage

Uneven distribution of the methyltransferase CARM1 and histone H3 arginine methylation at the 4-cell stage results in preferential allocation of the blastomere with highest levels (shown in blue) to the inner cell mass. Such global differences occur normally in development or can be experimentally manipulated by injection of CARM1 mRNA into one blastomere of 2-cell embryos. Preferential blastomere allocation is dependent on the methylase function of CARM1. Examples of the various functions and interaction partners of CARM1 are depicted. CARM1-mediated arginine methylation may maintain pluripotency, and thus influence cell fate, either directly by creating an active chromatin environment, or indirectly through transcriptional coactivation, mRNA processing and transport.

Given these diverse functions, CARM1 and H3 arginine methylation are capable of conferring a state of elevated transcriptional activity and a proliferative advantage to a cell. Differential distribution of this methyltransferase activity between individual blastomeres of 4-cell stage mouse embryos may thus account for the preferential allocation of the blastomere with highest CARM1 and H3 arginine methylation levels to the ICM. This function of CARM1 and/or H3 arginine methylation is also likely to preserve the blastomere's pluripotent potential as demonstrated for example by activation of the pluripotency genes *Nanog* and *Sox2*. An interesting aspect arising from this study is whether stem cells are generally characterized by elevated CARM1 levels, and whether differentiated cells can acquire multi- or even pluripotent features when CARM1 is overexpressed.

The experiments presented by Torres-Padilla et al. do not conclusively lay to rest the controversy of the random versus prepatterned models of blastomere allocation. What they do illustrate, however, is that global epigenetic instructions may already differ between individual cells of very early embryos. These differences have the potential for a bias of transcriptional activity to influence the inclination of a given blastomere to contribute to the ICM and hence ultimately affect the establishment of a polar axis at the blastocyst stage.

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Bcl-2-Regulated Calcium Signals as Common Mediators of Both Apoptosis and Autophagy

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The calcium ion, a major intracellular second messenger, is a known mediator of apoptosis and is regulated by the antiapoptotic protein Bcl-2. A paper by Høyer-Hansen et al. (2007) in the current issue of *Molecular Cell* indicates that calcium also mediates the induction of macroautophagy in a Bcl-2 regulated fashion and identifies a signaling pathway through which calcium exerts its action. These intriguing findings provoke speculation as to how a cell decides to undergo either apoptosis or macroautophagy in response to calcium signals.

A recent paper in *Molecular Cell* by Marga Jäättelä and coworkers (Høyer-Hansen et al., 2007) emphasizes the important role of calcium in formation of the autophagosome and raises the intriguing possibility that the antiapoptotic protein Bcl-2 regulates macroautophagy by modulating calcium homeostasis and signaling. Macroautophagy (hereafter referred to as autophagy) is a tightly regulated process in which cells degrade their own organelles to generate energy sufficient for survival. In normal cells autophagy plays important roles in development (Levine and Klionsky, 2004), but in cancer cells autophagy is subverted to maintain cell survival when faced with nutrient deprivation or therapeutic assault (Degenhardt et al., 2006).

In earlier work, Jäättelä's group discovered that cytoplasmic calcium elevation mediates autophagy in MCF-7 breast cancer cells treated with 1,25-dihydroxyvitamin D_3 and its analog EB1089, or other agents that mobilize intracellular calcium (Mathiasen et al., 2002, Høyer-Hansen et al., 2005). Similar to nutrient deprivation-induced autophagy, calcium-mediated autophagy is dependent upon Beclin-1, a protein critical to autophagosome formation. In their current work, Jäättelä's group identified a signaling cascade that mediates autophagy in response to elevated calcium (Høyer-Hansen et al., 2007). The suggested cascade involves sequential activation of calcium/calmodulin-dependent kinase kinase-β and AMP-activated protein kinase, leading to autophagy through repression of mTOR (mammalian target of rapamycin).

The calcium ion is a major intracellular second messenger whose role in mediating apoptosis is already well established (Orrenius et al., 2003). How then does a cell decide whether to undergo apoptosis, autophagy, or both, in response to calcium elevation? In earlier work from the Jäättelä group using MCF-7 cells, vitamin D compounds induced both autophagy and apoptosis (Mathiasen et al., 2002). But apoptosis is not evident in the present study, even though the calcium-mobilizing agents used to induce autophagy are well-known inducers of apoptosis. Some readers may wonder if the cells employed in the present study were inherently resistant to apoptosis, allowing autophagy to emerge as the predominant cell fate following disruption of calcium homeostasis. This thought may be stimulated by recent evidence that autophagy takes over when apoptosis is blocked in cancer cells (Degenhardt et al., 2006). Future studies will be required to understand better the balance between apoptosis and autophagy and the regulatory factors that govern whether one cell fate predominates over the other.

One intriguing hypothesis, as yet not formally addressed, is that cells make a decision to undergo either apoptosis or autophagy based on the nature of the calcium signal itself. Calcium signaling is a complex process in which information is encoded by the