that paraspeckles formed *de novo* around the reporter locus were indistinguishable from the endogenous counterparts. First, more than 95% of the newly formed bodies were enriched in four paraspeckle proteins examined; second, reporter mRNAs containing hyperedited repeat elements that specifically associate with paraspeckles were found in the newly assembled bodies and third, three core paraspeckle proteins exhibited similar recovery profiles after photobleaching in *de novo* formed and endogenous bodies. Live-cell imaging of paraspeckles after blocking general transcriptional activity using a reversible inhibitor, specifically shutting down the transcription of the reporter Men ε/β gene by removing doxycycline, and analysing cells on entry into and exit from mitosis during which transcription is blocked, all support the idea that the maintenance of paraspeckles requires active transcription of Men ε/β ncRNAs that remain immobilized around the gene locus.

Having established that RNA provides the scaffold that recruits the proteins that form nuclear bodies, the next question is how individual subunits assemble to produce a body. Single components in a complex may either associate in a defined order, or interact randomly in a self-organized manner (Fig. 2). The Dundr lab reported previously the first direct evidence that Cajal nuclear bodies are self-organized structures[15]. Tethering experiments (Fig. 1c) revealed that immobilization of any individual core protein of the Cajal body was sufficient to nucleate *de novo* formation of a complete nuclear body[15]. Similarly, the new study shows that tethering individual histone locus body proteins directly to the LacO array induces histone locus body assembly[8]. But a different model is reported for paraspeckles, as their assembly cannot be efficiently triggered by immobilization of a single protein subunit[8,9].

The results of both studies are consistent with a seeding model in which nascent transcripts serve as platforms to recruit proteins that interact with the RNA to assemble a nuclear body. However, whether RNA seeding is followed by self-organized or ordered stepwise assembly of the remaining nuclear body components remains to be determined for each type of body. Future live-cell imaging studies performed with higher temporal resolution are needed to track in real-time the assembly of single subunits into multi-component subcellular domains.

COMPETING FINANCIAL INTERESTS
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1. Carmo-Fonseca, M., Mendes-Soares, L. & Campos, I. *Nat. Cell Biol.* **2,** E107–E112 (2000).
2. Marzluff, W. F., Wagner, E. J. & Duronio, R. J. *Nat. Rev. Genet.* **9,** 843–854 (2008).
3. Rino, J. & Carmo-Fonseca, M. *Trends Cell Biol.* **19,** 357–384 (2009).
4. Clemson, C. M. *et al. Mol. Cell* **33,** 717–726 (2009).
5. Sasaki, Y. T., Ideue, T., Sano, M., Mituyama, T. & Hirose, T. *Proc. Natl Acad. Sci. USA* **106,** 2525–2530 (2009).
6. Sunwoo, H. *et al. Genome Res.* **19,** 347–359 (2009).
7. Valgardsdottir, R. *et al. Nucleic Acids Res.* **36,** 423–434 (2008).
8. Shevtsov, S. P. & Dundr, M. *Nat. Cell Biol.* **13,** 167–173 (2011).
9. Mao, Y. S., Sunwoo, H., Zhang, B. & Spector, D. L. *Nat. Cell Biol.* **13,** 95–101 (2011).
10. Robinet, C. C. *et al. J. Cell Biol.* **135,** 1685–1700 (1996).
11. Dundr M. *et al. J. Cell Biol.* **179,** 1095–1103 (2007).
12. Janicki, S. M. *et al. Cell* **116,** 683–698 (2004).
13. Bongiorno-Borbone L. *et al. Cell Cycle* **7,** 2357–2367 (2008).
14. Tsukamoto, T. *et al. Nat. Cell Biol.* **2,** 871–878 (2000).
15. Kaiser, T. E., Intine, R. V. & Dundr, M. *Science* **322,** 1713–1717 (2008).

Proclaiming fate in the early mouse embryo

Magdalena Zernicka-Goetz

In the mouse embryo, the first differences between cells that result in distinct lineages have long been thought to arise only as a consequence of differential cell positioning at relatively late preimplantation stages. Differences in Oct4 transcription factor kinetics between cells at the 4–8-cell stage are now shown to be predictive of future lineages, providing further evidence for much earlier initiation of cell fate decisions.

Cells in the early mouse embryo have long been considered to be identical. This is in large part because of their developmental plasticity and therefore the ability of cells to change their fate on, for example, transplantation to a new position[1]. Moreover, the cells of the early embryo seem to be morphologically identical, with nothing to distinguish them other than their inner or outer positions acquired not earlier than at the 16- and 32-cell stages. These inner and outer cell populations are widely accepted as progenitors of the pluripotent inner cell mass (ICM) and extra-embryonic trophectoderm lineages, respectively. However, a more recent series of studies indicate that cells at much earlier stages may have molecular differences able to affect their fate[2]. It was first found that cells in the 2- and 4-cell stage embryo can already display a bias to contribute progeny either to the embryonic or abembryonic parts of the blastocyst[3–6]. Subsequently it was discovered that underlying this bias is a tendency for individual cells to divide either symmetrically, and so contribute only to extra-embryonic trophectoderm, or asymmetrically, and so contribute progeny to both ICM and extra-embryonic trophectoderm[7]. This developmental bias was correlated with molecular heterogeneities between cells evident as early as the 4- and 8-cell stages[8,9]. In contrast, others have maintained the long-standing idea that cell lineage identity develops only much later and as a consequence of random (stochastic) processes[10–12]. Undoubtedly, the noisy gene expression of the early mouse embryo could enable its developmental adaptability, but does a mask of morphological uniformity of the early embryo's cells hide the stirrings of differential identity? The answer to this question must lie in the molecular machinery that will ultimately dictate cell fate. On page 117 of this issue, Plachta *et al.*[13] report differences in the kinetic behaviour of the key cell fate transcription factor Oct4 between cells of 4- and 8-cell stage embryos, and show that these differences are predictive of subsequent cell fate in the mouse embryo.

Magdalena Zernicka-Goetz is in the Gurdon Institute, The Henry Wellcome Building of Cancer and Developmental Biology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QR, UK.
e-mail: m.zernicka-goetz@gurdon.cam.ac.uk

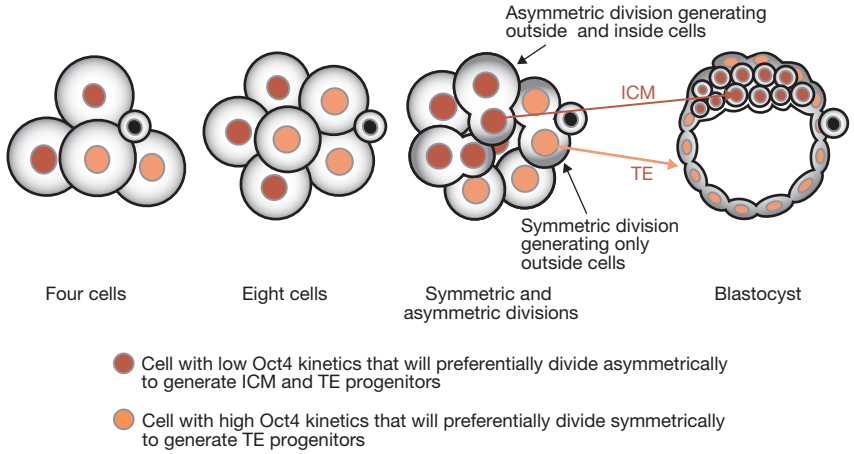

Figure 1 Oct4 kinetics at the 4–8 cell stage influence cell fate decisions. A schematic of four stages of early mouse development reveals how different Oct4 kinetics among morphologically similar blastomeres may bias their division orientation and, as a result, influence final fate at the blastocyst stage. Cells with low Oct4 kinetics will preferentially divide asymmetrically to contribute cells to two lineages in the blastocyst: the inner cell mass (ICM) and trophectoderm (TE). Cells with high Oct4 kinetics will preferentially undergo symmetric divisions to contribute only to trophectoderm outer cells. Cell with black nucleus indicates a second polar body.

The authors use time-lapse confocal microscopy imaging to quantify the fluorescent decay after photo-activation (FDAP) of photo-activatable GFP–Oct4, and thus follow the kinetics of nuclear export, import and retention of Oct4 in 4- and 8-cell stage cell nuclei. The non-invasive nature of this technique is an important aspect of this study, as it sidesteps the difficulties imposed by the ability of mouse embryo cells to regulate their development and adopt new fates in response to experimentation. Plachta *et al.*[13] discovered that although morphologically indistinguishable, the 4- and 8-cell stage cells fell into two distinct categories with respect to their Oct4 kinetics that seemed to reflect differences in the accessibility of Oct4 to its DNA-binding sites. One category was characterized by low rates of nuclear export/import and a highly immobile fraction of Oct4 protein in the nucleus, whereas high rates of export and import and highly mobile Oct4 protein was seen in the second group, which also displayed lower levels of nuclear Oct4. To determine whether these differential Oct4 kinetics reflected a bias in cell fate, the authors followed the lineages of cells displaying different Oct4 kinetics *in vivo*. This revealed that cells characterized by the highly immobile nuclear fraction of Oct4 preferentially underwent asymmetric cell divisions and so contributed significantly more cells to the ICM. In contrast, the progeny of cells with faster Oct4 export/import dynamics divided preferentially symmetrically and contributed to the trophectoderm. Thus, these differences in Oct4 kinetics between morphologically identical cells at the 4- and 8-cell stages were predictive of the later cell lineages, and therefore challenge the belief that the first cell fate decision point is coincident with the differential positioning of cells.

The importance of uncovering such early differences between the cells of the mouse embryo is the insight it provides into the molecular basis of the decision-making processes. It is therefore instructive to examine how these findings relate to previous work also indicating molecular differences between cells at similarly early stages. One striking finding of the present study is that it identifies differences in the kinetic behaviour of Oct4, rather than absolute differences in Oct4 expression levels, as predictive of cell fate. Indeed, early embryos inherit substantial levels of maternal Oct4 protein, indicating that *de novo* expression cannot be the only driver of its action. However, the presumption must be that these kinetic differences will at some point herald changes in the levels of other important cell fate molecules. Thus, it will be interesting to explore the link between Oct4 kinetics and other fate-determining transcription factors, such as Cdx2, which is essential for trophectoderm formation. This is because cells with the highest Oct4 dynamics at the 4–8 cell stages contribute predominantly to trophectoderm, as do cells with the highest Cdx2 levels at the 8-cell stage[9]. Moreover, Cdx2 and Oct4 function antagonistically[14] and as different levels of Cdx2 transcripts and protein are already detectable between cells at the 8-cell stage[9,15], this would imply that the differential kinetics of Oct4 may already influence *Cdx2* expression at this stage. It would also be informative to explore the relationship between Oct4 kinetics and the earliest quantifiable molecular difference that has been described between the cells of the mouse embryo — the differential methylation of histone H3 at arginines 17 and 26 at the 4-cell stage[8]. As high levels of this histone methylation favour the pluripotent state, this epigenetic modification could well be the harbinger of reduced nuclear Oct4 protein mobility biasing cells to a pluripotent ICM fate.

Plachta *et al.*[13] do not explore the origins of the differences in Oct4 kinetics between cells. It will therefore be of great interest to see how their results correlate with previous studies that distinguished between individual cells not by their outward appearance, but through stereotyped patterns of cleavage divisions[16,17]. This finding enabled the generation of chimaeras from individual 4-cell stage cells of uniform type and these chimaeras exhibited different degrees of developmental success depending on the origin of their cells[17]. Furthermore, levels of histone H3 arginine methylation were found to vary in accord with the developmental success shown by these chimaeras[8]. Although these differences can be correlated to the pattern by which the zygote was divided during cleavage, thus far the physical indication of what biases development in this way remains unknown. It is possible that some remaining physical manifestations of the asymmetric meiotic divisions, or modification of the actomyosin cytoskeleton in response to fertilization affects both the timing and planes of cell divisions[3,4,18,19]. Accordingly, rather than being an absolute determinant of cell fate, these changes could be sufficient to tip the balance of the fate decision along a particular path of development, possibly executed by such dynamic mechanisms as described in this issue by Plachta *et al.*[13].

Noisy gene expression in the early mouse embryo could provide the basis for its developmental adaptability. Developmental bias, however, may well be crucial for efficient embryonic development. This is because within a short and well-defined time the embryo has to make a distinct structure that will be able to implant into the uterus and whose extra-embryonic tissues will not

simply be a cradle for its stem cells but will also have to provide essential signalling information to direct further development. In contrast, cultured embryonic stem cells do not face such time or space constraints and seem to be able to respond and differentiate in a rather haphazard way to noisy levels of gene expression[20]. Indeed, it is difficult to imagine how the embryo could achieve its developmental goals within the confines of the preimplantation timeframe if the cell fate decisions were to be made as a result of purely stochastic events. Therefore, even a vestige of the oocyte's obvious organization and asymmetry might be able to harness the stochastic noise and turn it into information-carrying sounds. This might have been exploited for efficient mammalian development, crucial for the genetic fitness of a species.

Collectively, a growing number of studies point to molecular differences between cells of the mouse embryo that give rise to distinct cell lineages arising as early as the 4-cell stage. This places the onset of decision-making events well ahead of the differential positioning of cells on the inside or outside of the embryo at the 16- and 32-cell stages that have been previously proposed to be the first event affecting cell fate. Therefore, the basis for the first cell fate decisions in normal development is laid before cells acquire their regional identities, although these choices are not fixed at these very early stages. In this sense, the later events that establish the blastocyst structure as cells are internalized may be viewed as affirmations of much earlier cell fate choices. From this perspective, the work of Plachta *et al.*[13], in identifying the kinetic heterogeneities associated with Oct4 and their consequences for subsequent cell fate, offers new support to this changing view of early mouse development.

COMPTING FINANCIAL INTERESTS
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1. McLaren, A. *Mammalian Chimeras*. (Cambridge University Press, 1976).
2. Zernicka-Goetz, M., Morris, S. A. & Bruce, A. W. *Nat. Rev. Genet.* **10,** 467–477(2009).
3. Gardner, R. L. *Development* **128,** 839–847 (2001).
4. Piotrowska, K. & Zernicka-Goetz, M. *Nature* **409,** 517–521 (2001).
5. Piotrowska, K., Wianny, F., Pedersen, R. A. & Zernicka-Goetz, M. *Development* **128,** 3739–3748 (2001).
6. Fujimori, T., Kurotaki, Y., Miyazaki, J. & Nabeshima, Y. *Development* **130,** 5113–5122 (2003).
7. Bischoff, M., Parfitt, D. E. & Zernicka-Goetz, M. *Development* **135,** 953–962 (2008).
8. Torres-Padilla, M. E., Parfitt, D. E., Kouzarides, T. & Zernicka-Goetz, M. *Nature* **445,** 214–218 (2007).
9. Jedrusik, A. *et al. Genes Dev.* **22,** 2692–2706 (2008).
10. Motosugi, N., Bauer, T., Polanski, Z., Solter, D. & Hiiragi, T. *Genes Dev.* **19,** 1081–1092 (2005).
11. Louvet-Vallee, S., Vinot, S. & Maro, B. *Curr. Biol.* **15,** 464–469 (2005).
12. Dietrich, J. E. & Hiiragi, T. *Development* **134,** 4219–4231 (2007).
13. Plachta, N., Bollenbach, T., Pease, S., Fraser, S. E. & Pantazis, P. *Nat. Cell Biol.* **13,** 117–123 (2011).
14. Niwa, H. *et al. Cell* **123,** 917–929 (2005).
15. Ralston, A. & Rossant, J. *Dev Biol.* **313,** 614–629 (2008).
16. Gardner, R. L. *Hum. Reprod.* **17,** 3178–3189 (2002).
17. Piotrowska-Nitsche, K., Perea-Gomez, A., Haraguchi, S. & Zernicka-Goetz, M. *Development* **132,** 479–490 (2005).
18. Gray, D. *et al. Curr. Biol.* **14,** 397–405 (2004).
19. Plusa, B. *et al. Nature* **434,** 391–395 (2005).
20. Canham, M. A., Sharov, A. A., Ko, M. S. H. & Brickman, J. M. *PLoS Biol.* **8,** e1000379 (2010).