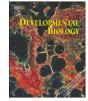
Developmental Biology 344 (2010) 463-470

Contents lists available at ScienceDirect



**Developmental Biology** 

journal homepage: www.elsevier.com/developmentalbiology



# Early Embryo Patterning

## Program/Abstract # 160 A role for apical membrane polarization in the generation of trophectoderm fate

Robert O. Stephenson, Janet Rossant Department of Developmental & Stem Cell Biology, Hospital for Sick Children, Canada Department of Molecular Genetics, University of Toronto, Canada

Despite more than half a century of research, the mechanisms underlying the divergence of the first two cell lineages in the mouse, the trophectoderm (TE, which later forms the placenta) and the inner cell mass (ICM, which later forms the embryo and the volk sac), are not completely understood to this day. Recent work has identified transcription factors important for the commitment of cells to these 2 different lineages. However, these transcription factors are expressed in all cells at the same time early on and the means by which their expression becomes restricted to different cell populations is unclear. The purpose of this study was to determine the relationship between blastocyst morphogenesis and restriction of TE specific transcription factor expression. We found that E-cadherin mutants, that lack cell adhesion and do not form a normal epithelium, still generated cells with either TE or ICM specific gene expression patterns. In comparison to wild type embryos however, more cells in mutant embryos appeared to express TE specific genes. Furthermore, expression of TE specific genes in mutant cells was associated with apical membrane polarization. We also found that disruption of apical membrane polarization leads to a reduction in TE specific gene expression in wild type embryos. These results are consistent with an important role for apical membrane polarization in TE fate.

doi:10.1016/j.ydbio.2010.05.201

## Program/Abstract # 161 The preferential incorporation of H2A.X into chromatin in early preimplantation embryos involves its C-terminal domain Buhe Nashun, Fugaku Aoki

Dept. of Integ. Biosci., Univ. Tokyo, Kashiwa, Chiba, Japan

Histone H2A has several variants that have specific roles in regulating gene expression. Previously, we showed that the canonical histone H2A and its variants H2A.Z, macroH2A, and H2A.X were deposited in the nuclei of full-grown oocytes in mice, but only histone H2A.X was abundant in the pronuclei of one-cell embryos after fertilization, in contrast to the low level of histone H2A and the absence of H2A.Z and macroH2A. Here, we report that the 23 C-terminal amino acids of H2A.X are involved in its preferential incorporation into chromatin after fertilization and in the regulation of preimplantation development. Flag-tagged mRNA microinjection experiments revealed that Flag-H2A.X was actively incorporated into the nucleus after fertilization, whereas Flag-

H2A incorporation was limited, and Flag-H2A.Z and Flag-macroH2A incorporation were minimal. By contrast, when the 23 C-terminal amino acids of H2A.X were fused to histone H2A, H2A.Z, and macroH2A, the resultant fusion proteins were actively incorporated into the nucleus after fertilization, indicating that the C-terminal amino acids of H2A.X function specifically to target this protein into chromatin in embryos after fertilization. Furthermore, the forcible incorporation of H2A.Z or macroH2A with the 23 C-terminal amino acids into chromatin had a detrimental effect on the progression of preimplantation development, suggesting that the selective incorporation of H2A.X, regulated by its C-terminal domain, is involved in preimplantation development.

#### doi:10.1016/j.ydbio.2010.05.202

#### Program/Abstract # 162

**Using the brainbow to trace lineages in early mouse development** Inna Tabansky<sup>a</sup>, Ryan W. Draft<sup>a,b</sup>, Jacqueline Rosains<sup>d</sup>, Jean Livet<sup>e</sup>, Jeff W. Lichtman<sup>c,d</sup>, Joshua R. Sanes<sup>c,d</sup>, Kevin C. Eggan<sup>b</sup> <sup>a</sup>Depts of MCB and SCRB, Harvard University, Cambridge, MA, USA <sup>b</sup>Stowers Biomedical Institute, Cambridge, MA, USA <sup>c</sup>CBS, Harvard University, Cambridge, MA, USA <sup>d</sup>Dept of MCB, Harvard University, Cambridge, MA, USA <sup>e</sup>INSERM, Institute de Vision and UPMS University of Paris, Paris, France

The mouse preimplantation embryo is highly regulative with regard to cell fate determination, but some experiments have suggested that there may be a hidden bias in the contribution of cleavage-stage blastomeres to the trophectoderm (TE) and inner cell mass (ICM). Investigation of this possibility has been hindered by technology. We employed the brainbow system to determine contribution of different blastomeres to the TE/ICM in the blastocyst. We are able to individually label the blastomeres by combining the brainbow with an inducible Cre system. The size of cell clones observed at the blastocyst stage is consistent with expectations based on the developmental stage at which Cre induction occurred. Based on our analysis, recombination occurs for 12-24 hours following Cre induction. We analyzed the contribution of cleavage-stage blastomeres to the TE/ICM by performing Cre induction in embryos at the 4-8 cell stage or the 8-16 cell stage and allowing them to develop into blastocysts. We found that at both the 4-8 cell stage, and the 8-16 cell stage, most blastomeres contribute primarily to the TE. We also observed that contributions of 8-16 cell stage blastomeres to the TE or ICM are more variable than the contributions of 4-8 cell stage blastomeres to these lineages. In the future, this system can be used to trace multiple cell lineages during development beyond the blastocyst stage.

doi:10.1016/j.ydbio.2010.05.203