Fate mapping the mouse embryo

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ABSTRACT The use of clonal analysis to obtain a fate map of the epiblast of the mouse embryo and to investigate cell distribution during gastrulation and early neurulation is described in a personal reminiscence. A revised fate map of the epiblast at 6.5 days gestation is provided, and the development of 3-dimensional, quantitative image analysis techniques outlined.

KEY WORDS: primordial germ cells, gastrulation, epiblast, mouse embryo

Lineage analysis by Richard Gardner and his associates in the 1970’s had established that extraembryonic lineages were segregated in the mouse embryo before implantation and that all the foetal lineages, including the germline, were derived from the primitive ectoderm (epiblast) (Gardner, 1978). In spite of the inaccessibility to manipulation of the mammalian embryo after implantation, pioneering work with postimplantation rodent embryos using ectopic transplants (Skreb et al., 1976), explants (Snow, 1981), and transplants within the embryo followed by whole embryo culture (Beddington, 1981) was beginning to answer questions about potency and commitment during gastrulation.

In 1982, I had the opportunity to spend a year in Roger Pedersen’s laboratory in San Francisco. Roger and Hannah Balakier had microinjected horse-radish peroxidase (HRP) iontophoretically into single blastomeres to trace cell lineage in the preimplantation mouse embryo (Balakier and Pedersen, 1982). He now thought the time ripe to extend the technique to investigate postimplantation cell fate and lineage. With Juanito Meneses we started by tracing the spread of clones generated from endoderm along the A-P axis during gastrulation (Lawson et al., 1986, 1987). With the introduction of fluorescent dextrans, it was possible to reliably inject internal, single epiblast cells (from which all the foetal tissues are derived) with a mixture of HRP and dextran, using the dextran to immediately identify the position of the injected cell and HRP to identify the descendants after culture. The immediate, striking result was the characteristic distribution of clones in the epiblast: there was extensive mingling with unlabelled cells in the epiblast epithelium and the descendants in individual clones were directed round the egg cylinder towards the primitive streak, with little proximodistal spread (Fig. 1).

The collaboration continued for some years, with short visits to San Francisco to inject and culture embryos, and long periods in Utrecht to analyse the results. The analysis was laborious, involving manually stacking photographic transparencies of the sectioned embryo in order to count the number of clonal descendants and visualise their positions and relationships in the different germ layers. The results of each reconstruction were projected on to a schematic sagittal section of an embryo with average dimensions. The cumulative data provided a fate map of the mouse epiblast at the onset of gastrulation (Lawson et al., 1991), which has been extended and added to over the years (Fig. 2) and demonstrated that the topological relationship of the presumptive germ layers were the same as in other vertebrates, in spite of the awkward geometry of the mouse conceptus. The resolution of clonal analysis also made clear that the germ layer fate of individual cells was not fixed in the epiblast: regions of overlap of prospective germ layers on the fate map were due to cells with descendants in more than one germ layer.

Advances in image analysis techniques and, in particular, the national funding in the Netherlands of a programme to develop computer based techniques for 3D image analysis, gave us the opportunity to represent the clonal data more accurately, with the aim to create a visual, statistical representation of the transition of the pregastrula epiblast containing ~600 cells to the onset of neurulation with ~14,000 cells and including the substantial growth which accompanies gastrulation in the mouse embryo. Although a basis was laid for automatic, objective stacking of histological sections, and for the identification and plotting of labelled cells within an ellipsoid model representing the form of the embryo up to the neural plate stage (Verweij, 1993), the dynamic, integrated, statis-
cally valid 4D visualisation has not yet been realised. (See further Verbeek et al., 1999 in this issue). However, by building on these programmes, and using some interactive procedures, the positions of labelled descendants of epiblast in the 3D volume of the early neural system (Fig. 3) have been measured by Rienk van der Ploeg. This mapping indicated that the anteroposterior spatial relationship in the epiblast at the onset of gastrulation has been transformed into a dorsoventral one in the neurectoderm by early somite stages: lineage relationship in the neurectoderm is closer rostrocaudally than dorsoventrally, except between ventral midbrain and ventral hindbrain.

The finding that descendants of anterior epiblast were often located at the base of the allantois and the posterior part of the embryo near the endoderm, where the primordial germ cells are located at the headfold stage, suggested that precursors of the germline might arise anteriorly. Although this idea turned out to be a red herring, clonal analysis using fixable fluorescent dextran as lineage label did eventually establish the position of the precursors of the primordial germ cells in the extreme proximal epiblast at the onset of gastrulation (Fig. 2) and the fact that the germline is not committed at this stage. From the quantitative analysis, it was possible to estimate the size of the founding population of PGCs and the time of lineage restriction (Lawson and Hage, 1994).

Intracellular injection of a nonreplicating label clearly has limited usefulness for long-term lineage analysis, but still has a future in revealing whether changing patterns of gene ex-
pression during gastrulation are lineage based, and for answering questions about altered cell fate and cell behaviour in mutant embryos (Faust et al., 1998).

References


