From pluripotency to differentiation: laying foundations for the body pattern in the mouse embryo

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A central question in contemporary stem and developmental biology and modern medicine is how developmental potential becomes progressively restricted as development proceeds. How totipotency—namely the ability to give rise to both embryonic and extraembryonic tissues and, in an ideal situation to the entire organism—is lost. How do cells choose between alternative fates, and how do they stereotypically organize themselves into developing tissues? Today, one can strip differentiated cells of their identity by inducing pluripotency, but it is still largely unknown how pluripotency emerges in its native context within the early embryo. Indeed, the only true pluripotent cell population—the epiblast—exists in vivo within the early mammalian embryo.

Regenerative medicine holds enormous promise for patients with degenerative diseases and tissue lesions. However, the foundations for safe and effective treatments depend on a deep understanding of the mechanisms of lineage commitment and specification, and the optimal environment for maintaining the phenotypic stability of pluripotent stem cells, and the specific lineages that can be directed to differentiate from them. These foundations will, in large part, be laid through experimental investigation of factors affecting pluripotency and lineage specification during development, in embryos and in stem cell culture paradigms.

Recently, there has been a reinvigorated interest in studies of the earliest stages of mammalian development. Studies on early mammalian embryos, exemplified by the mouse, have seen a new beginning. We have begun to lay a molecular and cell biological framework of the critical events surrounding early development of the mouse embryo. In part, this reflects renewed interest in cellular differentiation and reprogramming with a focus on pluripotent cell states, and the potential for directed cellular differentiation. Experimental and conceptual progress has been galvanized by the availability of new experimental approaches for following the contributions of individual cells to development of the mouse embryo, coupled to a molecular understanding of the very first cell fate decisions, and the cellular events that precede them. This rebirth of interest in the earliest stages of mouse development has given new insights into our understanding of how cells evolve specific fates and how they are assigned to particular lineages, of the changes in cellular properties that facilitate fate decisions, and of signalling pathways that control these events. It is now timely to link these early events to later developmental processes that immediately precede and include gastrulation, the process during which the three founder tissues of the body are established.

This collection of review articles and hypotheses presents a contemporary overview of our current understanding of the earliest stages of mouse development, and the biology of embryo-derived pluripotent stem cells. The focus is upon the role of signalling pathways, transcriptional and epigenetic mechanisms, and the activity of genetic networks that contribute to cell lineage commitment and tissue morphogenesis.

It is within the very earliest embryonic stages that fate decisions send cells in the direction of either pluripotency or differentiation. In the first set of articles, the authors address the earliest assignments of cells to particular lineages, the
changes in cellular properties that facilitate fate decisions and the participating signalling pathways. When and how the cells first become different is of great importance in all model systems, and this knowledge has been lacking in mammalian development as mammalian embryos are difficult to study in this respect—they can compensate for perturbations. Takaoka & Hamada [1], experts in early mouse development and whose work allowed establishment of symmetry breaking events leading to development of the anterior–posterior (AP) axis and left–right asymmetry, present a new hypothesis of how pre-patterning might become established in the mammalian embryo leading to the first cell fate specification—formation of the inner cell mass and trophectoderm. This hypothesis suggests that epigenetic asymmetry in zygotes, such as histone methylation and DNA methylation between maternal and paternal genomes, might explain pre-patterning and how previously discovered effects of the inheritance of animal and vegetal parts of the zygote bias cell fate from the 4-cell stage onwards.

The pluripotent epiblast represents the founding population of the entire embryo, whereas the extraembryonic tissues, primitive endoderm and trophectoderm, both support its development. Boroviak & Nichols [2] discuss how following the early cleavage divisions and formation of the blastocyst, cells of the inner cell mass lose totipotency. They describe how developing epiblast cells transiently attain the state of naive pluripotency, and acquire competence to self-renew in vitro as mouse embryonic stem cells (ESCs).

ESCs possess the capacity to indefinitely self-renew in culture. Morgani & Brickman [3] raise the question of whether ESCs, which have been historically defined as pluripotent, can, under certain conditions, acquire a state of totipotency. Can they revert to an earlier stage of embryonic development from which they are derived? These authors discuss cell state transitions between pluripotency and totipotency in culture, and the signalling and transcriptional networks that control them.

Following on, Hermitte & Chazaud [4], whose work revealed the paradigm shifting salt-and-pepper distribution of lineage-restricted progenitor cells within the inner cell mass of the blastocyst, bring us back to the embryo to provide an overview of recent findings that lead to different hypotheses concerning the execution of the second cell fate decision—formation of the pluripotent epiblast and the second extraembryonic tissue, the primitive endoderm. It is now clear that this decision is not set up by cell position, as thought for so long, but rather by pre-specification of cells within the inner cell mass that will sort to their final positions. Recent studies have cast more light on the roles of fibroblast growth factor (FGF) signalling, cell heterogeneity and cell polarity on the pre-specification of cell fate and cell sorting at the time when cells are faced with a choice to commit to a pluripotent epiblast fate.

Emergent epiblast exhibits a state of naïve pluripotency. Kalkan & Smith [5] discuss how, for normal development to proceed, the naïve state must be changed for lineage specification to occur. They discuss how in vitro differentiation of naïve ESCs cultured under defined conditions provides a system for interrogating this critical transition, and discuss the incipient road map of events.

The peri-implantation stage of development has previously been hidden from view, a conceptual ‘black box’. However, the recent development of culture methods and ways of imaging cells in situ in embryos is bringing a thus far missing view of cellular and molecular events that link the pre- and postimplantation periods of embryonic development, and are beginning to reveal how cells establish their fates at this stage. Zernicka-Goetz and co-workers take us from the early preimplantation cell fate decisions through implantation in the first steps towards understanding the mechanism of how a simple ball of blastocyst cells transforms into the more complex structure of the egg cylinder [6]. They present a new hypothesis that a formation of the epiblast rosette at the time of implantation is the primordium that, with the help of extraembryonic tissues, provides the essential foundation for the future body in mammalian embryos.

Papanayotou & Collignon [7], whose work led to discovery of the role of Activin/Nodal signalling in the early mouse embryo, discuss the role of this signalling pathway at pre- and early postimplantation stages of development, and how it controls the expression of target genes in ESCs. They review the TGFβ-related ligands that determine the activity of Activin/Nodal signalling, and the Smad2/3-dependent mechanisms underlying developmental progression. These recent studies indicate a role for Nodal signalling earlier than previously expected.

The body plan of the developing embryo is established, and the cardinal axes (AP, dorsal–ventral and left–right) are elaborated at early postimplantation stages of development. Establishment of the AP axis is governed by complex series of interactions between the various adjacent tissues of the early embryo. A key role is played by a specialized population of migratory epithelial cells, referred to as the anterior visceral endoderm (AVE), which are derived from the primitive endoderm of the blastocyst. Stower & Srinivas [8] discuss our current understanding of the formation and function of the AVE.

Diffusible growth factors, including members of the FGF, Wnt, bone morphogenetic protein and Hedgehog families, emanating from localized areas travel through the extracellular space and reach their target cells to specify the cell fate, and coordinate tissue formation. Matsuo & Kimura-Yoshida [9] discuss the current understanding of mechanisms by which these growth factors travel great distances to their target cells to control signalling activity within the early mouse embryo. They discuss recent studies which reveal that heparan sulfate proteoglycans that are located on the surface of cells, and within the extracellular matrix, play crucial roles in regulating the extracellular distribution of growth factors, and thereby are likely to provide an additional level of regulation in growth factor-mediated signalling.

The pluripotent epiblast is transformed into the three definitive germ layers (ectoderm, mesoderm and endoderm), as well as the primordial germ cells (PGCs), at gastrulation. Surani and co-workers discuss how unlike the majority of epiblast cells which undergo differentiation towards somatic cell lineages, PGCs initiate a unique cellular programme [10]. They discuss the concept of enhancer function in relation to the factors which govern a PGC identity, namely how these PGC-specific factors work by suppression of somatic differentiation genes, and concomitantly regulate the re-expression of pluripotency and germ-cell-specific genes.

Tam and colleagues, whose work was key in compiling the fate map of the mouse gastrula, and in understanding critical signalling events leading to development of endoderm, discuss how the formation of definitive endoderm in embryoid bodies follows a similar process to germ layer formation as from the epiblast, requiring an initial de-epithelialization event and
subsequent re-epithelialization [11]. This requires the activity of both TGFβ signalling at the formative phase of endoderm differentiation and specifically of Nodal, which cannot be substituted for by Activin A, which is commonly used as its in vitro surrogate.

Parfitt & Shen [12] extend the discussion of how development of the mammalian embryo is coordinated from blastocyst to gastrulation stages. They review how regulatory networks constructed for different stem cell types relate to the corresponding networks in vivo within the embryo. They also highlight several studies that have contributed to our current understanding of the molecular regulation of the blastocyst–gastrula transition.

The endoderm is one of three definitive germ layers generated at gastrulation. Cells descended from the endoderm will constitute the multipotent progenitors of the respiratory and digestive tracts, the endocrine glands and auditory and urinary systems. In the final review, Hadjantonakis and co-workers discuss their unexpected insights that lead to revision of our thinking on the cellular dynamics that drive the initial phases of endoderm formation [13]. The authors show that segregation between the embryonic and extraembryonic lineages may not be as strict as previously believed, by suggesting that the endoderm is composed of cells derived from both epiblast-derived definitive endoderm and so-called extraembryonic endoderm, descended from the primitive endoderm.

References