Lineage commitments: emphasis on embryonic–extraembryonic interfaces

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The EMBO Workshop on 'Lineage Commitments: Emphasis on Embryonic–Extraembryonic Interfaces', held in May 2011, demonstrated that embryonic and extraembryonic tissues play early and significant interacting roles that mutually promote each other's further and correct deployment within the mammalian conceptus. Highlighted here are those presentations that directly addressed embryonic–extraembryonic interfaces in building the mammalian fetus.

An Zwijsen, Susana Chuva de Sousa Lopes and Anne Camus did an excellent job of gathering together an international group of scientists in Leuven, Belgium, despite the imminent threat of Icelandic volcanic ash. Ultimately, all the participants arrived safely for the EMBO Workshop on 'Lineage Commitments: Emphasis on Embryonic–Extraembryonic Interfaces', held in May this year, and the meeting was a great success.

Interactions of EVE, ExE and epiblast

Key interacting tissues in the mouse conceptus, the focus of most contemporary mammalian studies, are shown in Fig 1. During the course of development, the primitive endoderm or hypoblast (Fig 1A) produces extraembryonic visceral endoderm that, according to its location, is called either extraembryonic or embryonic (Fig 1B). Embryonic visceral endoderm (EVE) exhibits unique morphological (Fig 1B) and expression profiles (Rivera-Perez, 2007). Distal EVE shifts its position to become a fixed anterior domain, called the anterior visceral endoderm (AVE; Fig 1C; Thomas & Beddington, 1996); signalling from the AVE prohibits posterior identity and formation of the primitive streak (PS), while establishing a permissive role in anterior development. The PS appears later (Fig 1D), when it indicates the presence of the antero-posterior axis.

Nodal, a member of the transforming growth factor- β (TGF- β) family of signalling proteins and possible master regulator of embryonic and extraembryonic body pattern, is involved in signalling between the

epiblast EVE and extraembryonic ectoderm (ExE). In the opening Keynote lecture, Liz Robertson (U. Oxford, UK) summarized her results on the activity of Nodal, the main message of which was that the strength of Nodal signalling regulates the development of the three primary germ layers from the epiblast. Robertson's most recent work indicates a novel role for *Eomes*, a Nodal target, in specifying cardiac mesoderm and in disconnecting the process of mesoderm formation from the epithelial-to-mesenchymal (EMT) transition, thought to be the province of the PS. This triggered an interesting debate about the formation of the PS and its role in the EMT. Anne Foley (Cornell U., USA) proposed that XEN cells, derived from extraembryonic visceral endoderm (XVE; Fig 1B), might be a valuable tool to elucidate those presumptive AVE signals that maintain and pattern cardiomyocytes in the early embryo. Jérôme Collignon (Institut Jacques Monod, France) described HBE, a conserved Nodal enhancer that interacts with the molecular machinery involved in pluripotency in various embryonic stem cells (emerging on E3.5–E5.5; Fig 1A,B), linking Nodal to the undifferentiated state. Daniel Constam (U. Lausanne, Switzerland) developed a transgenic biosensor to monitor paracrine convertase activities secreted by the extraembryonic ectoderm (Fig 1B,C); GFP fusions of these proteases were shown to move to the adjacent epiblast where they sufficiently activated Nodal to suppress precocious neural differentiation and induce mesoderm and endoderm. Yoh

Wada (Osaka U., Japan) showed that embryos mutant in *mVAM2*, which encodes a regulator of membrane transport, exhibited fragmented endosomes in visceral endoderm that resulted in ectopic activation of bone morphogenetic protein (BMP) signalling and a disorganized embryo. Pantelis Georgiades (U. Cyprus) provided compelling evidence that extraembryonic ectoderm influences epiblast (Fig 1B) behaviour beyond the induction of gastrulation and thus proper fetal development, possibly through Nodal- and *Bmp4*-mediated *Ets2* activities.

Using time-lapse microscopy and three-dimensional protein imaging, Shankar Srinivas (U. Oxford, UK) made the case that the movement of visceral endoderm from distal to anterior (Fig 1B,C) occurs by intercalation through the simple epithelium of the EVE under the influence of Wnt-PCP (planar cell polarity) signalling. José Belo (U. Algarve, Portugal) described a large-scale screen on CER1P-EGFP transgenic mouse embryos, the latter of which allowed him to sample specific regions of embryonic visceral endoderm (Fig 1C); about half of the genes isolated (101 messages) relevant to this process were not functionally characterized. Having selected three of these novel AVE genes, Belo is now conducting well-defined biological studies in both *Xenopus* and mouse to identify function.

Kat Hadjantonakis (Memorial Sloan-Kettering Cancer Center, USA) had previously shown that the fetal gut is composed of a combination of EVE and definitive endoderm from the PS (Kwon *et al*, 2008).

Fig 1 | Development of the mouse conceptus (E4.5–E7.5). Tissues derived from the trophoblast and/or trophectoderm of the blastocyst are coloured blue or grey; all others are derived from the inner cell mass of the blastocyst (E3.5, not shown). (A) Embryonic day 4.5: implantation. Hypoblast, or primitive endoderm, is an extraembryonic tissue not to be confused with definitive endoderm, one of the three primary germ layers formed during gastrulation from the primitive streak. (B) Embryonic days 5.0–5.5. Hypoblast or primitive endoderm gives rise to (i) extraembryonic visceral endoderm (XVE), so-called because it is found in the extraembryonic region of the conceptus, (ii) embryonic visceral endoderm (EVE), which is also extraembryonic but called embryonic because it overlies the future embryo and fetus (the epiblast), and (iii) parietal endoderm (pE). Polar trophectoderm (trophoblast; TE) gives rise to extraembryonic ectoderm (ExE), which in turn gives rise to the chorionic ectoderm. (C) Anterior visceral endoderm (AVE) and posterior visceral endoderm (PVE) are thought to be key induction tissues during this stage (see text). (D) The appearance of the primitive streak (PS) at the embryonic–extraembryonic junction overtly defines the posterior side of the future fetus. It elongates anteriorly to reveal the antero-posterior midline axis. (E–G) Sequence leading to the exocoelom (EC), chorion and amnion. Mesoderm (red) enters the extraembryonic region, displacing posterior ExE from contact with the XVE (E). Extraembryonic mesoderm cavitates, forming the exocoelomic cavity (EC) while producing the amnio-chorionic fold (ACF; F; see text). By E7.5 (G), the ACF fuses at its anterior edge to create the exocoelom, chorion and amnion. The posterior end of the primitive streak has extended into the extraembryonic region, creating the allantoic bud and allantoic core domain (ACD). Distal ACD, proximal ACD and posterior primitive streak (pPS) of the embryo proper were fate-mapped (see text). Primordial germ cells (PGCs) are indicated as a small cluster of cells within the proximal midline of the ACD. Other designations, mural TE (A), trophoblast giant cells and proamniotic cavity (B), anterior primitive streak (aPS), node and ectoplacental cavity (EPC) (G) are indicated for completeness, but were not discussed in the text. Although the pE is not indicated in panels (C–G), it is nonetheless present, as indicated in panel (B). The original figure was provided by Paulo Pereira (K.U. Leuven, Belgium) and redrawn inhouse.

Focusing on endoderm-defective *Sox17* mutants, she presented new evidence leading to a definitive model for extracellular matrix-dependent endoderm egression and intercalation. This model suggests that dispersion of EVE and intercellular communication through gap junctions might relay information leading to embryonic turning and heart looping.

The posterior region

The properties of the PS (Fig 1C–G) continue to be an area of intense study in vertebrate biology. Before the overt appearance of the streak (Fig 1D), the hypoblast (the chick equivalent of mouse EVE) promotes local intercalation in the epiblast that positions and shapes the streak (Voiculescu *et al.*, 2007). Viktoriya Halacheva (U. Göttingen, Germany) presented time-lapse stereotypic cell movements toward and within the midline of the pre-streak rabbit gastrula—equivalent to the stages shown in Fig 1B,C—providing support for a conserved mechanism of streak formation across amniote species. Contrary to the prevailing view of the primacy of extraembryonic ectoderm in induction of the PS, Jaime Rivera (U. Massachusetts, USA) provided tantalizing evidence that posterior visceral endoderm (PVE; Fig 1C) actively directs mouse streak formation through Wnt3 signalling at least a day earlier than currently thought, and roughly simultaneously with AVE formation.

Many major extraembryonic components originate from the poorly studied posterior region, including the allantois, exocoelom, amnion and chorion. In addition, primordial germ cells (PGCs) are thought to segregate here from the epiblast (Fig 1G). Christoph Viebahn (U. Göttingen) raised the question of whether mammalian PGCs universally reside within a posterior median extraembryonic niche (Fig 1G; McLaren, 1999) or whether that niche is specific to rodents; he found PGCs scattered across the postero-lateral embryonic–extraembryonic junction in non-rodent mammalian species. The idea of a posterior stem-cell domain encompassing more than just PGCs was addressed by Karen Downs (U. Wisconsin-Madison, USA), who provided evidence that the PS extends its reach into the precursor umbilical tissue, or allantois, where, with visceral endoderm, it establishes a putative stem-cell reservoir called the allantoic core domain (ACD, Fig 1G; Downs, 2009). Maria Mikedis

(U. Wisconsin-Madison) fate-mapped this region, and found that not only does it contribute to mesoderm and endoderm—a property of the streak—but that the region containing Stella-positive putative PGCs contributed to derivatives of all three primary germ layers, suggesting that Stella is not restricted to the PGCs. Monika Bialecka (Hubrecht Institute, The Netherlands), provided evidence that *Cdx2*-null embryos exhibit a reduction in PGC numbers along with a foreshortened allantois, suggesting that *Cdx2* is required for maintaining the ACD, and thus, a broader stem-cell reservoir than just the PGCs.

The exocoelom, amnion and chorion

In the second Keynote lecture, Allen Enders (U. California Davis, USA) revealed evidence of a surprising diversity in the formation of extraembryonic tissues relative to the embryo. For example, in different species, the amnion—which surrounds the fetus and cushions it through secretion of amniotic fluid (Fig 1G; Dobrev *et al.*, 2010)—forms by folding, cavitation, or a combination of the two. Mouse amnion formation has been controversial. Now, work by Paulo Pereira (K.U. Leuven, Belgium) showed that the mouse amnion forms by a single cavitating fold—the amniochorionic fold (ACF; Fig 1E,F). The cavitation of the ACF is achieved through the coalescence of extracellular spaces. Closure of the ACF, close to the anterior margin of the egg cylinder, leads to the separation of the amnion, chorion and exocoelom as distinct structures (Fig 1G). As explained by Pereira, these results make an invaluable contribution toward elucidating the Smad5-mediated antagonism of the Nodal autoregulatory pathway in the amnion, where it prevents ectopic streak formation.

As with the amnion, formation of the chorion is associated with the ACF (Fig 1F,G). Although the derivatives of chorionic ectoderm will become major mediators of fetal–maternal exchange, the precise origin of chorionic ectoderm is not known. Tracing single cells, Kirstie Lawson (MRC Human Genetics Unit, Edinburgh, UK) demonstrated that chorionic ectoderm is derived from the ExE that lies closest to the epiblast (Fig 1B,C), and that most of the chorionic midline is formed from descendants of cells that were in contact with, or very close to, the epiblast. These results add new insight into the developmental relationship between the exocoelom, amnion and

chorion, and further underscore the importance of embryonic–extraembryonic interactions in specific cell-fate decisions within these lineages.

Trophoblast

Whether extraembryonic tissues—such as trophoblast and extraembryonic endoderm, which are both visceral and parietal endoderm (Fig 1A,B)—have distinct ‘signatures’ to ensure that they do not contribute cells to the fetus, is a question that has remained unresolved in mammalian biology. Now, by using sensitive epigenomic methods, Myriam Hemberger (Babraham Institute, UK) showed that DNA methylation is critical to epigenetic reinforcement of the first cell-lineage decisions leading to the segregation of the trophoblast from the inner cell mass (around E3.5; not shown in Fig 1). Importantly, Hemberger showed that such decisions might even extend to the primitive endoderm (Fig 1A) and its derivatives in the extraembryonic endoderm (Fig 1B), as judged from the analysis of stem cells representative of each lineage. Potential interactions of the fetus and the mother at the trophoblast interface are a topic that might be expanded in a subsequent workshop. Cees Oudejans (VU University Medical Center, The Netherlands) showed that pre-eclampsia, a dangerous and poorly understood complication of pregnancy, has an early-onset familial form.

New concepts and technologies

Mark Henkelman (U. Toronto, Canada) presented the use of optical projection tomography (OPT) to characterize mouse embryos. Fluorescent or brightfield images are acquired from multiple views of a rotated fixed sample, which are then reconstructed into a three-dimensional image. This technique has been used to visualize the complex three-dimensional relationship between the venous and arterial circulatory systems in mouse embryos (Walls *et al.*, 2008). Given how little is known about vascular relationships in the mammal, OPT will be crucial for elucidating complex mispatterning in mouse mutants defective in vascular formation and signalling. Jerry Rhee (Children’s Memorial Research Center, Chicago, USA) presented data suggesting that haematopoietic stem-cell maturation initiates near the posterior embryonic–extraembryonic junction, at a specific site where the primitive streak, allantois and visceral yolk sac endoderm meet (Fig 1G). To

interrogate single-cell behaviours at this site, Rhee combined a series of static optical sections with Netlogo software (<http://ccl.northwestern.edu/netlogo/docs/>)—a freely available programmable modelling environment for simulating natural and social phenomena that might prove useful to many studies presented at this workshop.

Conclusions and future directions

In the final Keynote lecture, Claudio Stern (U. College London, UK) summarized his view of the hypoblast. In a thought-provoking lecture based on years of study of the chick, Stern proposed that during the course of evolution, freedom from intracellular nutrition—that is, yolk—led to the slowing of development; amniote species lost synchrony of early cell division, the mid-blastula transition and intracellular localization of maternal components as a primary method for establishing global polarity in the embryo. Extraembryonic tissues thereby emerged to provide not only these missing functions, but also innovations for building the body axis.

Thus, the hypoblast acquired a major role in establishing embryonic spatial coordinates, such as positioning the PS, or shifting the site of gastrulation from the periphery of a disc (for example, anamniotes) to the midline. He also stressed that the PS represents axial–lateral fates of the mesoderm, that is, axial at the tip and lateral at the posterior end, rather than the anterior–posterior axis of the embryo.

In closing, this workshop integrated a critical mass of investigators actively working in the emerging and important field of embryonic–extraembryonic interactions. Allen Enders's myriad examples of extraembryonic tissue diversity in placental mammals were a stark reminder that scientists cannot risk putting all of their (fertilized) eggs into the mouse basket; the examination of a wider number of species will ultimately shed light on human development and manifold human diseases. Perhaps we can strive to make this happen sooner rather than later; in any case, well before the Grímsvötn volcano erupts again!

ACKNOWLEDGEMENTS

I thank all of the participants highlighted in this report for their constructive criticisms of and contributions to the manuscript; sincere apologies are extended to those scientists whose wonderful talks were not included. Special thanks go to Paulo Pereira for his contribution of Fig 1.

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Published online 23 September 2011

EMBO reports (2011) **12**, 987–990.
doi:10.1038/embor.2011.186