News & views

Developmental biology

A peek into the black box of human embryology

Alexander Goedel & Fredrik Lanner

The molecular mechanisms involved in human gastrulation, a crucial stage in early embryonic development, have been largely elusive. Gene-expression data from a gastrulating human embryo shed light on this process. **See p.285**

Even for embryology researchers, it is difficult to grasp that the entire human body is derived from a single cell. In the first weeks of life, this cell divides, building all the embryonic as well as the supportive tissues, such as the placenta. Advances in technologies to study individual cells have provided unprecedented mechanistic insights into the initial 'decisions' that determine the fate and lineages of cells in the early embryo, before it implants into the wall of the uterus[1,2]. However, the events that follow implantation have remained a 'black box', with knowledge based mostly on limited historical histological collections, or on experimental work performed on model organisms or *in vitro* model systems[3–6]. On page 285, Tyser *et al.*[7] provide a glimpse into this black box by profiling gene expression in individual cells in a post-implantation human embryo, using a method called single-cell RNA sequencing.

The embryo under study was in the middle of gastrulation, a crucial developmental process in which a layer of cells known as the epiblast gives rise to the embryo's three 'germ' layers (the endoderm, mesoderm and ectoderm) and the body plan is first established. Understanding this process is fundamental to uncovering the causes of congenital diseases, early miscarriage and infertility.

Human reproduction is surprisingly inefficient. Although exact numbers are difficult to estimate, it is thought that approximately 30% of fertilizations are lost before implantation and about 20% fail shortly after implantation, mainly during the transition from gastrulation to organogenesis (the process of organ formation)[8].

The embryo in Tyser and colleagues' study was obtained from the Human Developmental Biology Resource following informed consent by a donor undergoing termination of her pregnancy. The embryo was determined to be male, with the normal number of structurally intact chromosomes, and, through detailed analysis of its shape and size, it was staged to be between 16 and 19 days post-fertilization (a period known as Carnegie stage 7).

At this stage, the embryo consists of an embryonic disc separating two cavities: the amniotic cavity and the yolk sac (Fig. 1). The authors isolated the yolk sac, and then split the embryonic disc and overlying amnion into two parts. They measured levels of various RNA transcripts in a total of 1,195 cells, and ensured that all cells collected were indeed of embryonic, and not maternal, origin by showing the absence of X-inactive specific transcript (*XIST*), which is present in female, but not male, cells. In addition, the cells collected contained transcripts from the Y sex chromosome (which is present in male, but not female, cells).

Using cell-location data, together with measures of the expression of marker genes defining distinct cell types, and a statistical analysis in which cells are grouped according to the similarity of their gene-expression profiles, the authors identified cell populations from various embryonic structures. These structures included the epiblast, the three embryonic germ layers, the amniotic ectoderm (which forms the amniotic cavity), the extra-embryonic mesoderm surrounding the embryo, and the primitive streak, which forms the basis of the body's bilateral symmetry (Fig. 1). The authors also observed the first progenitor cells of a rudimentary blood system and primordial germ cells (PGCs; in

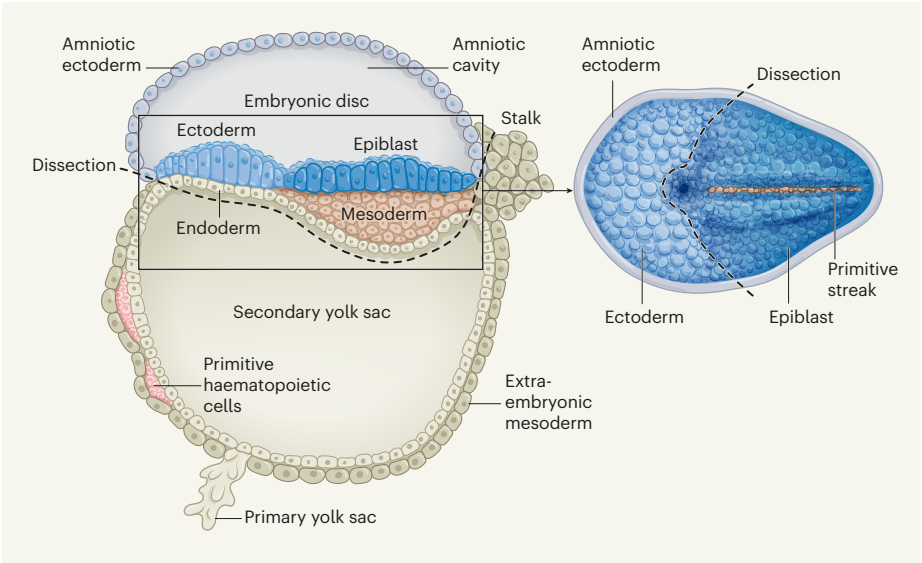

Figure 1 | A human embryo examined during gastrulation. Tyser *et al.*[7] assessed gene expression in individual cells dissociated from a male human embryo that was estimated to be 16–19 days post-fertilization and that was undergoing a developmental process called gastrulation. During this process, a layer of cells called the epiblast gives rise to three 'germ' layers (the ectoderm, mesoderm and endoderm), establishing the body plan. At this stage, the embryo contains two cavities: a cavity formed by the amniotic ectoderm overlying the embryonic disc, and the yolk sac (surrounded by the extra-embryonic mesoderm) on the other side of the disc. Tyser *et al.* dissected the gastrulating embryo into three parts, and used a statistical analysis to 'group' the dissociated cells according to the similarity of their gene-expression profiles. Using this approach, the authors identified cells that make up the various embryonic structures shown here, as well as primitive haematopoietic cells that give rise to a rudimentary blood system, and primordial germ cells (future sperm cells; not shown).

this case, the future sperm cells). (This data set can be browsed and downloaded at http://www.human-gastrula.net)

Analysis of the data set revealed several intriguing findings. First, cells of the amniotic ectoderm and the embryonic ectoderm display highly similar gene-expression profiles. The anatomical organization of the disc-shaped human embryo reflects this similarity, with the ectoderm directly bordering the overlying amniotic sac. This is different from the case in mouse embryos, in which these tissues are spatially separated. Further studies are needed to explore this close relationship between the human amniotic ectoderm and embryonic ectoderm in more detail.

Notably, there was no sign of neural induction, a process in which the embryo's ectoderm starts to form neural tissue, suggesting that this occurs later. Furthermore, using algorithms targeted at the detection of rare cell types, a small population of putative PGCs was identified among the cells of the primitive streak, indicating that the future sperm cells had already been set aside. This is in line with findings from *in vitro* cultures of human embryos showing that such cells are specified as early as day 11 after fertilization, before gastrulation[9]. The authors also found pigmented cells in the wall of the yolk sac that they identified as primitive blood-related cells called erythroblasts and haemato-endothelial progenitors, confirming previous evidence that the production of blood cells in humans begins as early as Carnegie stage 7 (ref. 10).

The availability of human embryos is limited owing to ethical, as well as technical, reasons. Thus, embryonic stem cells that are derived from the inner cell mass of human embryos at about six days after fertilization are a crucial tool in researching early development. However, a long-standing debate exists about the exact *in vivo* equivalent of these human embryonic stem cells. Tyser *et al.* compare their data set with gene-expression data from the epiblast of pre-implantation embryos. They conclude that embryonic stem cells maintained in a 'naive' state are most similar in terms of gene expression to epiblast cells of pre-implantation embryos. By contrast, gene expression in conventional 'primed' embryonic stem cells (which resemble more-mature cells ready to differentiate) is more similar to that in the epiblast of the gastrulating embryo.

This comparison also exemplifies the potential use of the authors' data set as an *in vivo* reference for *in vitro* models of early embryonic development. Cell populations previously identified in macaque embryos cultured to day 14 *in vitro*[11] showed close correlation with those in the human gastrulating embryo, validating their correct identification as epiblast, endoderm, embryonic mesoderm, extra-embryonic mesoderm and amniotic ectoderm. Similar studies have used these data to benchmark the changes in gene expression that occur in different cell types in models of gastrulation, in which aggregates of human embryonic stem cells are organized in concentric layers (see, for example, ref. 12).

Given the rapidly growing interest in this research field, several *in vitro* models that mimic human post-implantation development and gastrulation are emerging. Careful evaluation of their fidelity to their counterpart cell types *in vivo* is warranted, to see whether such models hold the promise of opening this black box of embryology to provide mechanistic insights into this stage of human development.

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Climate science

Constraints on the CO_2 fertilization effect emerge

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Plants offset a large fraction of Earth's carbon dioxide emissions, but estimating the size of this carbon sink relies on differing terrestrial-biosphere models. Combining multiple models with data has now reduced the uncertainty. **See p.253**

The goal of mitigating climate change requires a global commitment to reducing greenhouse-gas emissions to avoid substantial temperature increases. However, achieving this objective is complicated by uncertainty over how much carbon dioxide will be absorbed naturally by trees and other plants. On page 253, Keenan *et al.*[1] have constrained estimates of this uncertainty by drawing empirical links between observations of the current climate and multi-model predictions of the land-carbon sink. The relationships between these observations and predictions are known as emergent constraints.

Plants draw CO_2 from the atmosphere to grow using photosynthesis. Since the Industrial Revolution, the burning of fossil fuels has increased atmospheric CO_2 concentrations from around 280 parts per million (ref. 2) to 416 parts per million in 2020 (ref. 1). The effect of this increase on plants has been the subject of many investigations — from controlled laboratory experiments to large-scale free-air CO_2 enrichment (FACE) studies, which simulate different concentrations of atmospheric CO_2 accessible to plants growing under realistic field conditions[3] (Fig. 1). The consensus is that photosynthesis increases as CO_2 concentrations rise, a phenomenon known as CO_2 fertilization.

A simple conclusion would be that plants naturally compensate for a substantial fraction of the CO_2 emissions caused by the burning of fossil fuels. However, biological processes are complex: we still have much to learn about everything from the inner workings of a leaf to the dynamics of entire ecosystems. The impact of CO_2 fertilization will depend on how the plant ecosystem adapts as the climate changes. For example, the location-specific availability of water and nutrients will modulate CO_2 fertilization and have varying consequences for different species. The net result of these interactions at the global scale will determine the amount of carbon absorbed by plants and, therefore, the extent to which CO_2 fertilization can help to slow global warming.

Each year, there are updates to the status of the global carbon cycle (see, for example, ref. 4). These updates have been used to infer the land-carbon gain by accounting for all emissions, and then subtracting the carbon