



Editorial overview: Cell differentiation and development — wiring principles of transcriptional states, signaling networks and cell fate trajectories

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Sara A. Wickström is an associate professor of tissue architecture at the University of Helsinki, Finland. Her group studies how complex but stereotyped tissues are formed, maintained, and regenerated through local growth, differentiation, and remodeling, using the mammalian epidermis as a model. Her research aims to establish quantitative principles of how multicellular stem cell niches self-organize and how mechanical forces and cellular interactions allow coordination of single stem cell behaviors at the population level and further couple this behavior to the surrounding tissue architecture.

Cells within developing tissues transition through successive states of decreasing pluripotency into a state of terminal differentiation. Detailed understanding of these stereotypic and tightly controlled cell fate transitions that operate within complex, dynamic multicellular environments is critical toward mechanistic understanding of developmental disorders and other devastating diseases including cancer. Recent technological breakthroughs such as single-cell sequencing, elaborate lineage-tracing methods, quantitative high-resolution imaging, and interdisciplinary approaches combining mathematical modeling, computational biology, physics of living matter, and cell biology have enabled precise identification of cell states and provided insights into their differentiation trajectories, plasticity, and principles of self-organization. To highlight these exciting recent developments, we present a collection of reviews that discuss the biological process of differentiation on a range of scales from single molecules to complex signaling networks and lineage trajectories and from emergence of specific cell types to construction of complex organs.

The first reviews of the issue focus on signals, transcription factors, and genetic circuitries that control early embryonic differentiation. In the review by Bleckwehl and Rada Iglesias, they discuss the transcriptional and epigenetic control of primordial germ cell specification. The authors highlight key transcriptional transitions that involve shutting down the naive pluripotency gene expression program for the acquisition of germ line competence, which is followed by a transient formative state where a dedicated network of transcription factors coordinates both the silencing of naive genes and the activation of early postimplantation epiblast markers. These transcriptional changes operate in a tightly regulated, complex landscape of chromatin modifications, and the authors propose that heterogeneity achieved on the epigenetic level could be a key mechanism that would allow targeted specification of a subset of cells.

Continuing on the theme on early embryonic cell-type specification, Ferretti and Hadjantonakis review the specification and diversification of the mesoderm, the most common germ layer of origin for human cell types. The authors discuss emerging data suggesting that mesodermal progenitors are plastic and commit only when reaching their final destinations. On the other hand, organ-specific progenitors are most likely already defined early at the primitive streak, and their fate becomes progressively restricted by integration of signaling cues that converge at this site. The authors also highlight key challenges that deal with linking single-cell transcriptomics and epigenomics data into information on dynamic changes in cell position as well as understanding how sensing of signaling gradients is integrated with input from tissue growth and mechanical forces.

Shifting from a systems perspective in the mesoderm to a central signaling network in the endoderm, Scheibner et al focus on canonical and noncanonical Wnt signaling and its implications in endoderm development and

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Yingzi Yang is a professor of developmental biology at the Harvard School of Dental Medicine. Her laboratory has long-standing interests in understanding the roles of the Wnt, Hedgehog, and Hippo signaling pathways in embryonic development, skeletal biology, and skeletal diseases as well as liver cancer and diseases. Her studies aim to reveal fundamental mechanisms of Wnt, Hedgehog, and Hippo signal transduction and their critical roles in many aspects of embryonic morphogenesis and adult physiology.

subsequent organogenesis of the pancreas. The authors review the critical role of canonical Wnt signaling, which is required already to initiate the formation of the primitive streak and consequently also for the formation of both the endoderm and mesoderm. Subsequently, the specification of the pancreatic anlage and the later segregation of the pancreatic lineages and pancreatic islet formation rely on dynamic, spatiotemporal regulation of Wnt activity and intricate cross talk with other morphogenetic pathways.

The subsequent reviews shift the focus toward organogenesis and in particular highlight the central roles of cell fate plasticity, reprogramming, and transdifferentiation in this process. Cartilage is a mesoderm-derived tissue formed by a unique cell type, the chondrocyte, during embryonic development. Chondrocytes are differentiated from multipotent skeletal progenitor cells and their fate determination elaborates the regulation of molecular networks, which are mediated by sets of transcription factors; of which, Sox9 is central. Lefebvre et al review recent developments on understanding the role of Sox9 in chondrocyte differentiation. The authors highlight new findings on Sox9 function and its transcriptional partners, as well as recent discoveries of Sox9 structure–function relationship, post-translational modifications, and mechanisms of gene regulation.

Tsang and Cheah continue on the timely theme of cartilage and discuss the most recent developments in the field of endochondral bone formation. Endochondral ossification requires a spatiotemporally tightly controlled cascade of chondrocyte proliferation, maturation, and hypertrophy to establish a growth plate, which then through deposition of bone on the cartilage template mediates longitudinal skeletal growth. The authors highlight the very recent identification of skeletal stem cells that are capable of giving rise to both chondrocytes and osteoblasts as well as stromal cells. In addition, they describe the intricate continuum of fate and cell behavioral transitions in the chondrocytes to the bone lineage during ossification and, in particular, the molecular underpinnings of the intriguing transformation step from chondrocytes to osteoblast fate.

Kameneva and Adamyeko discuss lineage trajectories of central and peripheral nervous system progenitors, with a particular focus on technological and computational advances in the field of single-cell sequencing that have facilitated precise identification of neuronal and progenitor cell types and subtypes and uncovered intricate transcriptional and epigenetic changes that occur during neuronal differentiation. In particular, the authors underline the importance of computational methods such as pseudotime-organized developmental trajectories and analyses of RNA kinetics, which in combination with lineage tracing and functional perturbations are starting to allow construction of detailed, time-resolved lineage trees that describe the precise transcriptional events, critical cell fate split points, and the molecular mechanism that drive these transitions and bifurcations.

In addition to transcriptional circuits and intracellular signaling, cell–cell communication plays a critical role in cell fate decisions and differentiation. In this context, Notch signaling, reviewed by Lloyd-Lewis et al, plays a prominent role in coordinating dynamic cellular rearrangements that occur throughout development with cell fate. The authors highlight the extreme context dependency of Notch signaling that operates through well-established lateral inhibition mechanisms to impose differential cell fate to stem cells and their neighbor progenitor cells during development. This binary signaling mode allows dynamic cell rearrangements to generate spatially and temporally restricted cues, dictating engagement or expression of a Notch ligand/receptor.

Continuing on the theme of intercellular signaling pathways, which play essential roles in controlling cell proliferation and fate determination by activating and/or mediating the effects of specific transcription factors, Yi Yu and Xinhua Feng review recent advances in the understanding of the Smad-dependent and Smad-independent TGF- β signaling in cell fate control and cancer. The authors describe how Smad mediates Transforming growth factor- β (TGF- β) signaling by interacting with various fate-determining transcription factors and highlight the intriguing noncanonical functions of Smad in epigenetic and epitranscriptional regulation of gene expression that functions as negative feedback to modulate its canonical roles in transcription. The authors further provide insightful examples of critical functions of TGF- β signaling in immune cell fate determination, double-edged actions in cancer, and the therapeutic potential of these findings.

Another key differentiation pathway is the Hedgehog (HH) signaling pathway, which governs embryonic development by regulating cell division and fate and is itself elaborately controlled. Hu and Song discuss recent advances in HH signaling focusing on the interplay of the HH ligand-binding transmembrane protein Patched (PTCH), a seven-transmembrane protein Smoothed (SMO) that transduces HH signals to intracellular components, and cholesterol. A long-lasting question in HH signal transduction is how PTCH prevents activation of SMO. The authors summarize recent high-resolution structural studies that provide insight into the molecular basis of HH recognition by PTCH and discuss the post-translational modifications of SMO with specific emphasis on the identification of cholesterol as an endogenous ligand of SMO. The authors conclude by providing insightful perspectives into the roles of cholesterol and cellular sterol metabolism in HH signaling.

Signaling pathways controlling cell proliferation, differentiation, and survival in embryonic development play crucial roles also in tumorigenesis. Switching gears from development and regeneration to disease, Zhang and Zhou provide a comprehensive review of recent findings on the Hippo signaling pathway in hepatocellular carcinoma. The Hippo pathway is evolutionarily conserved and plays critical roles in organ size control in development and tumorigenesis in adults. The transcriptional coactivators YAP/TAZ, downstream effectors of the Hippo signaling pathway, have emerged as attractive targets for cancer therapeutics as their abnormal activation has been implicated in several human cancers including hepatocellular carcinoma.

Apart from control of gene expression, signaling to control fundamental cellular processes such as metabolism and autophagy is critical for cell fate decisions and differentiation during development and disease progression. Zhang and Zhao discuss recent progress in understanding how perturbations of autophagy are linked with multiple pathological changes in humans,

including neurodegenerative diseases, cancer, inflammation, and metabolic and developmental disorders. The authors focus on recent findings of genetic associations of core autophagy genes with human diseases. These findings highlight the critical roles of autophagy in human development and physiology as well as the value of human genetic studies in identifying basic regulatory mechanisms in human biology.

Expanding from signaling networks to complex intercellular communication, in the review by Myllymäki and Mikkola on inductive signals in epithelial branching morphogenesis, they discuss how cell fate trajectories are coregulated in conjunction with formation of specific organ structures, with a focus on the mammary and salivary glands. The authors highlight that the generation of these nonstereotypic mature epithelial trees is a complex, integrated process of cell proliferation, branch point generation, and branch elongation. The model that begins to emerge is that inductive signals derived from epithelial–mesenchymal cross talk regulate both cell fate decisions and morphogenetic behaviors, where the branching potential of a cell is likely directly linked to its differentiation state.

As knowledge of the precise molecular mechanisms of differentiation and cell plasticity accumulates, scientists have exploited these molecular wiring diagrams to develop technologies to reprogram cellular identities. In the review by Ofenbauer and Tursun, they discuss strategies for cellular reprogramming from classical induced pluripotent stem cells (iPSCs) systems to *in vivo* reprogramming and transdifferentiation, where somatic cells can be directly converted into another cell type. They discuss not only recent advances in delivering reprogramming factors such as lineage-specific transcription factors, synthetic CRISPR-based transcriptional regulators and small molecules in driving controlled fate conversions both *in vivo* and *in vitro*, but also the current challenges in *in vivo* reprogramming that include induction of senescence, constraints from the microenvironment, and risks of teratogenesis and cancer.

As reprogramming and transdifferentiation *in vivo* are becoming recognized as potentially physiologically important processes, it is necessary to rigorously demonstrate their occurrence with lineage-lineage-tracing techniques. The Cre-loxP genetic system is commonly used for this purpose, but this approach has limitations leading to controversies in multiple fields. Zhou and Zhao discuss a dual genetic approach based on Cre-loxP and another orthogonal recombinase that permits cell tracking at a significantly higher resolution and enables a more precise method for gene manipulation and cell fate control. The authors highlight emerging work to demonstrate how the application of these new approaches has enabled identification of stem cell fate plasticity during tissue repair and regeneration and helped to resolve controversies regarding stem cells in the heart and liver. Importantly, they also provide

outlooks on how the dual genetic approaches can be used to more precisely manipulate cell-specific gene expression.

Better understanding of principles of embryonic development has also shed light on mechanisms of tissue regeneration and cancer. Seto and Eiraku review recent progress in brain organoids derived from iPSCs, which have emerged as important experimental tools to study development and pathogenesis of human brains by recapitulating developmental processes *in vitro*. The authors focus on brain organoids with proper neuronal networks and discuss strategies to construct such organoids guided by what is known about normal brain development. The authors emphasize that incorporation of a blood vascular system into brain organoids to prevent necrosis is a critical hurdle to overcome to drive full maturation of constructed neuronal networks.

Continuing on iPSCs and their potential as valuable models to study human development, Kechele and Wells review recent advances in the generation and application of human endodermal tissues, including the esophagus, lung, pancreas, liver, stomach, small intestine, and colon by directed differentiation of human iPSCs. The authors discuss improvements in transcriptional and functional maturation of tissues, multicellular complexity, and scalability, all of which allow better development and disease modeling, large-scale drug and toxicity screening, and potentially cell-based therapeutic applications.

The *in vitro* organoid-based approaches have shown great potential in tissue repair, but their applications are intrinsically limited in multiple ways, calling for innovative strategies to promote *in vivo* injury repair. The human heart is one of the least regenerative organs, with an adult cardiomyocyte (CM) renewal rate of less than 1%. Massive loss of CMs caused by an injury results in high mortality due to ischemic heart diseases. Deshmukh et al discuss recent studies that have advanced *in vivo* cardiac regeneration based on better understanding of the molecular mechanisms underlying blockage in adult human CM proliferation. The authors summarize cellular and molecular pathways that

regulate CM proliferation and show that cardiac regeneration and repair can be achieved in animal models by stimulating endogenous CM renewal based on identified genetic mechanisms of heart regeneration, providing new insights into therapeutic options for patients with heart failure.

Tissue regeneration guided by principles in development has also been a fruitful strategy in biologically oriented regenerative dentistry. The attempt to regrow a functional tooth will revolutionize current dental practice on repairing damaged or missing teeth. Li et al discuss how understanding the lost *in situ* revitalization capability during evolution provides conceptual breakthroughs in tooth regeneration. The review highlights progress in stem cell-based *in vivo* tooth repair and recent development of *in vitro* production of implantable bioengineered tooth germs by recapitulating the tooth developmental program. The authors also provide a useful road map of challenges to be overcome to achieve full functional tooth regeneration in the near future.

In summary, this issue highlights the leaps in knowledge that, in particular single-cell sequencing, high-resolution imaging and *ex vivo* organ explant cultures and organoids, have facilitated in understanding differentiation trajectories during development, regeneration, and disease. On the other hand, they underline the need for combining these various approaches to obtain real-time information on single-cell transcriptomes and epigenomes with quantitative information on changes in cell shape, mechanics, and position. In addition, the reviews make clear that our understanding of the molecular and cellular mechanisms of human development remains relatively superficial, but in particular, the rapid development in *in vitro* systems and human genetics to address these questions will likely change this in the near future. We hope you enjoy reading these reviews as much as we have.

Conflict of interest statement

Nothing declared.