

The pathogenesis of IBD involves both environmental factors (smoking, diet, exposure to pollution, and the commensal gut microbiome) and a genetic predisposition, which has been narrowed down to mutations in ~100 genes involved in pathways including epithelial cell and barrier function, and immunity (4). One of these genes is *C1orf106*. Mohanan *et al.* show that the role of the C1orf106 protein is to maintain appropriate amounts of cytohesin 1 protein in mature epithelia, by promoting its ubiquitination and subsequent proteolytic degradation. Cytohesins are activators of the Ras guanine triphosphatase (GTPase) ARF6 (ADP ribosylation factor 6), which directs cytoskeletal remodeling and endocytic internalization of cadherins. When epithelial cells form mature monolayers, ARF6 activity must be down-regulated, to maintain TJ stability. Experimental depletion of C1orf106, which mimics the down-regulation of the mutant form that occurs in IBD patients, leads to abnormally high amounts of cytohesin, thus promoting excessive ARF6 activation (see the figure). This in turn causes increased cadherin endocytosis, which results in higher TJ permeability to small molecules, without detectable changes in TJ protein organization. The passage of some small molecules, notably of bacterial nanoparticles, debris, and other antigenic molecules, can induce an immune response and inflammation, potentially escalating barrier damage (1, 3).

Manfredo Vieira *et al.* show that in organisms predisposed to autoimmune disease, exposure to pathogenic bacteria leads to a severe breakdown of the intestinal epithelial barrier, and eventually bacterial translocation to mesenteric lymph nodes and liver. This exacerbates autoimmune reactions (see the figure).

Thaiss *et al.* show that chronic hyperglycemia can affect barrier function through metabolic and transcriptional reprogramming in intestinal epithelial cells, which is mediated by the glucose transporter GLUT2. This results in enhanced dissemination of bacterial products and intestinal and systemic inflammation, which is common in patients with obesity, diabetes, and metabolic syndrome (see the figure). However, the mechanism by which the intestinal barrier becomes leaky is unclear and requires further investigation.

Because a defective intestinal barrier can lead to leakage of either bacterial antigens or bacteria into the underlying tissue as well as, potentially, into blood and lymph vessels, it can both initiate and maintain inflammation and spread of infection. Thus, therapeutic strategies should address the integrity of the TJ barrier. This can be done through small molecules that target proteins involved in the control of barrier function, when the under-

lying molecular mechanism is known. For example, in a mouse model of T cell-mediated acute diarrhea, pharmacological control of either actomyosin contractility or endocytosis reverses the symptoms (15). Thus, stabilizing C1orf106, as suggested by Mohanan *et al.*, could be a strategy in the subset of IBD patients that carry the mutation, provided that no off-target toxicity results from such stabilization. As additional information becomes available about the molecular mechanisms through which claudins, occludin, tricellulin, cytoplasmic adaptor proteins, and signaling proteins control the leak pathway, new potential direct TJ targets could be identified. For example, claudin isoform expression changes following the differentiation of intestinal cells along the crypt-surface axis, and dietary components, including fiber, favors differentiation and increased expression of TJ proteins. Therefore, promoting intestinal cell differentiation could help to strengthen the otherwise leakier barrier of intestinal crypts.

Another key strategy is to limit the inflammatory responses that occur once bacterial products and bacteria have crossed the epithelial barrier—for example, through immunosuppression and antibodies that target inflammatory cytokines (3). Conversely, because intestinal epithelial homeostasis results from a balance between shedding of damaged and old cells and their replacement by new cells, prevention and therapy of IBD must also include the stimulation of mechanisms that promote epithelial monolayer repair (3). Still, in the presence of massive bacterial invasion through defective epithelial barriers in hosts with a compromised immune system, antibiotic therapy and vaccination may be the most effective approach, as shown by Manfredo Vieira *et al.*, whereas Thaiss *et al.* suggest the control of glycemia as another important factor to consider. Future studies on the complex interplay between microbiome, intestinal epithelium, and immune response will help to refine rational strategies to maintain and repair intestinal barriers. ■

REFERENCES

1. A. Buckley, J. R. Turner, *Cold Spring Harb. Perspect. Biol.* **10**, a029314 (2018).
2. S. M. Krugel *et al.*, *Semin. Cell Dev. Biol.* **36**, 166 (2014).
3. A.-C. Luissint *et al.*, *Gastroenterology* **151**, 616 (2016).
4. B. Khor *et al.*, *Nature* **474**, 307 (2011).
5. V. Mohanan *et al.*, *Science* **359**, 1161 (2018).
6. S. Manfredo Vieira *et al.*, *Science* **359**, 1156 (2018).
7. C. A. Thaiss *et al.*, *Science* **10.1126/science.aar3318** (2018).
8. C. M. Van Itallie, J. M. Anderson, *Annu. Rev. Physiol.* **68**, 403 (2006).
9. C. Zihni *et al.*, *Nat. Rev. Mol. Cell Biol.* **17**, 564 (2016).
10. S. Tsukita, M. Furuse, *J. Cell Biol.* **149**, 13 (2000).
11. D. Günzel, A. S. L. Yu, *Physiol. Rev.* **93**, 525 (2013).
12. A. S. Fanning, J. M. Anderson, *Ann. N. Y. Acad. Sci.* **1165**, 113 (2009).
13. A. I. Ivanov *et al.*, *Bioessays* **27**, 356 (2005).
14. S. Citi *et al.*, *Small GTPases* **5**, e973760 (2014).
15. D. R. Clayburgh *et al.*, *J. Clin. Invest.* **115**, 2702 (2005).

10.1126/science.aat0835

DEVELOPMENT

Diverging roads to the heart

Cardiovascular lineage decisions in the mouse embryo are explored at single-cell resolution

By Robert G. Kelly¹ and Silke R. Sperling^{2,3,4}

Insight into early cardiac lineage diversification is essential to unravel the origins of congenital heart defects, which are among the most frequent birth anomalies. Congenital heart defects commonly affect specific regions of the heart or cardiac cell types (1). Moreover, directed differentiation of pluripotent stem cells into specific cardiac lineages is a pivotal step in modeling heart disease, drug testing, and regenerative therapies. On page 1177 of this issue, Lescroart *et al.* (2) used single-cell RNA sequencing to generate two high-resolution snapshots of gene expression in nascent cardiovascular mesoderm in the early mouse embryo. Their findings capture the transcriptional complexity of pre-cardiac mesoderm and reveal how different lineages contributing to the heart first arise during embryogenesis.

Understanding the regulatory mechanisms that drive cell fate choices during lineage diversification is a central goal of developmental biology. Waddington's epigenetic landscape provides a powerful metaphor for such decisions, in which a cell is represented by a ball rolling across a contoured landscape signifying gene regulatory space (3). By defining the transcriptional content of many individual cells scattered across the landscape, single-cell transcriptomics heralds a revolution in our grasp of how cell fate decisions take place (see the figure). This technique is transforming our perception of biological complexity and has led to the discovery of new cell types and regulatory mechanisms in homeostasis

¹Aix-Marseille University, CNRS UMR 7288, Developmental Biology Institute of Marseille, Campus de Luminy Case 907, Marseille Cedex 9, France. ²Department of Cardiovascular Genetics, Experimental and Clinical Research Center, Charité—Universitätsmedizin Berlin, Berlin, Germany. ³Department of Biology, Chemistry, and Pharmacy, Freie Universität Berlin, Berlin, Germany. ⁴Berlin Institute of Health, Berlin, Germany. Email: robert.kelly@univ-amu.fr

and disease as well as during development (4–7). Furthermore, approaches coupling transcriptomics with epigenomic studies at single-cell resolution hold the promise of a holistic understanding of the gene regulatory networks underlying cell fate decisions.

During embryonic development, cardiac progenitor cells transiently express the transcription factor mesoderm posterior protein 1 (*Mesp1*) at gastrulation (8). Nascent cardiac mesoderm migrates to the anterior lateral region of the embryo, where progenitor cells of the first heart field give rise to the early heart tube. Subsequently, late differentiating multipotent progenitor cells of the second heart field contribute to growth of the heart from adjacent pharyngeal mesoderm. Second heart field cells give rise to the venous and arterial poles of the heart, which are hotspots of congenital heart defects (1). The first and second heart fields segregate before *Mesp1* is expressed (9, 10).

Single-cell RNA sequencing has recently offered insights into the extent of transcriptional diversity in different regions and cell types of the developing heart, providing valuable resources for discovering new pathways and genes involved in cardiac disease (6, 7). Lescroart *et al.* focused on *Mesp1*-expressing cells at the early time points when this gene is expressed in first and second heart field progenitor cells. The new data neatly fit a developmental continuum between single-cell transcriptomes from pregastrulation epiblast cells and later mesoderm, previously generated by this group (11). Using a clustering algorithm to visualize gene expression topology in these combined data sets, the authors identified distinct progenitor cell subpopulations indicative of early lineage diversification. These comprise cardiomyocyte and endothelial progenitor cells, presumably of first heart field origin, as well as cells with a pharyngeal mesoderm genetic signature, defined as posterior and anterior clusters of second heart field cells, the latter including head muscle progenitors. At later stages of development, posterior and anterior second heart field cells contribute to the venous and arterial poles of the heart, respectively. Of particular interest is the finding that the different progenitor cell clusters emerge at the edge of a core of molecularly heterogeneous cardiovascular progenitor cells.

Lescroart *et al.* computed pseudotime trajectories to track progress between pluripotent epiblast and distinct progenitor cell states based on transcriptional profiles. This analysis revealed that anterior and posterior second heart field clusters diverge from common progenitor cells expressing genes enriched in both lineages within the central core. Such multilineage priming has

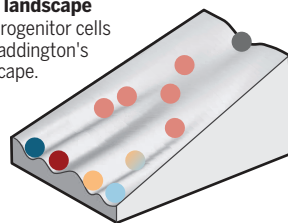
been observed in *Mesp1*-labeled cardiac and pharyngeal muscle progenitor cells in the basal chordate *Ciona intestinalis*, suggesting that this is a conserved feature of cell fate segregation in pharyngeal mesoderm (12). Cells within the core population may thus constitute a transition state between different trajectories, within which extrinsic signaling events influence lineage outcomes (5). Transcriptional heterogeneity in the core population would confer developmental plasticity and robustness in the face of genetic or environmental perturbation, potentially contributing to compensatory mechanisms and phenotypic variability in congenital heart defect patients. Mining the data set generated by Lescroart *et al.* will contribute to systematic approaches to defining the extrinsic and intrinsic regulators controlling sequential fate decisions

Early road map to the heart

The transcriptional complexity of precardiac mesoderm reveals early segregation of lineages contributing to the heart.

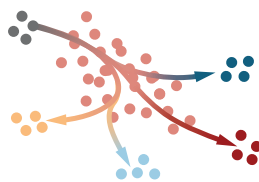
The precardiac landscape

Cardiovascular progenitor cells distributed on Waddington's epigenetic landscape.



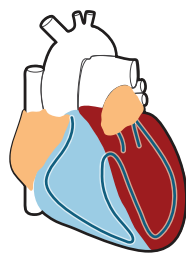
Gene expression topology

Distinct progenitor cell clusters emerge at the edge of a transcriptionally heterogeneous core of nascent cardiovascular mesoderm.



Contributions to heart development

Distinct progenitor cell populations give rise to different regions of the heart and cardiac cell types.



- Pluripotent epiblast cells
- Cardiovascular progenitor cells
- Endothelial progenitor cells
- Early myocardial progenitor cells
- Anterior second heart field cells
- Posterior second heart field cells

in early mesoderm (13). As an example, the authors identified Notch signaling as a *Mesp1*-regulated pathway enriched early in the endothelial versus myocardial trajectory. Furthermore, transcriptome analysis of single cells from mutant embryos showed that *Mesp1* itself controls the transition from pluripotency to progenitor cell specification.

A major challenge of single-cell transcriptomic analysis is mapping cells to the tissue of origin. Using fluorescent in situ hybridization to track selected genes, the authors showed that myocardial and endocardial progenitor cells could be spatially distinguished in nascent mesoderm, as could anterior and posterior pharyngeal territories. Systematic methods have recently been developed to map single cells after RNA sequencing. These exploit high-resolution reference atlases generated from in situ hybridization of a series of landmark genes (14) or spatial transcriptomic approaches, such as single-embryo spatial RNA sequencing (15). The latter technique has been developed at a stage overlapping with the work of Lescroart *et al.* and should permit topological mapping of this new data set. This will allow identification of the niches harboring progenitor cells on divergent trajectories and clarify our understanding of how future regions of the heart and cardiac cell types are patterned in the progenitor population.

Another challenge of such high-throughput data sets concerns distinguishing biological from experimental noise; analysis of additional cells and time points is likely to refine our view of the progenitor population substructure. Integrating single-cell transcriptomic approaches at multiple time points with single-cell epigenomic data sets and lineage history will provide a fine-grained map of the dynamic cardiovascular progenitor cell landscape. This will guide systematic exploration of the mechanisms driving cardiac progenitor cell fate choices in development and disease. ■

REFERENCES

1. J. A. Epstein, *N. Engl. J. Med.* **363**, 1638 (2010).
2. F. Lescroart *et al.*, *Science* **359**, 1177 (2018).
3. C. H. Waddington, *The Strategy of the Genes: A Discussion of Some Aspects of Theoretical Biology* (Allen & Unwin, 1957).
4. C. Trapnell, *Genome Res.* **25**, 1491 (2015).
5. N. Moris *et al.*, *Nat. Rev. Genet.* **17**, 693 (2016).
6. D. M. DeLaughter *et al.*, *Dev. Cell* **39**, 480 (2016).
7. G. Li *et al.*, *Dev. Cell* **39**, 491 (2016).
8. Y. Saga *et al.*, *Trends Cardiovasc. Med.* **10**, 345 (2000).
9. F. Lescroart *et al.*, *Nat. Cell Biol.* **16**, 829 (2014).
10. W. P. Devine *et al.*, *eLife* **3**, e03848 (2014).
11. A. Scialdone *et al.*, *Nature* **535**, 289 (2016).
12. F. Razy-Krajka *et al.*, *Dev. Cell* **29**, 263 (2014).
13. K. M. Loh *et al.*, *Cell* **166**, 451 (2016).
14. R. Satija *et al.*, *Nat. Biotechnol.* **33**, 495 (2015).
15. G. Peng *et al.*, *Dev. Cell* **36**, 681 (2016).

10.1126/science.aat0230

Diverging roads to the heart

Robert G. Kelly and Silke R. Sperling

Science **359** (6380), 1098-1099.
DOI: 10.1126/science.aat0230

ARTICLE TOOLS

<http://science.sciencemag.org/content/359/6380/1098>

RELATED CONTENT

<http://science.sciencemag.org/content/sci/359/6380/1177.full>

REFERENCES

This article cites 14 articles, 3 of which you can access for free
<http://science.sciencemag.org/content/359/6380/1098#BIBL>

PERMISSIONS

<http://www.sciencemag.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of Service](#)

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. 2017 © The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works. The title *Science* is a registered trademark of AAAS.