Somatic cell reprogramming into pluripotent stem cells using transcriptional factors or chemical compounds has been shown to include an intermediate cell state with epithelial features. Two recent papers show that this intermediate state can be redirected to create other differentiated cell types—specifically hepatocytes and neurons—using chemical cocktails. These results shed new light on a critical intermediate in cell fate conversion with mechanistic and practical implications.

It was not that long ago that cellular differentiation was thought to be a unidirectional process: Cells that became neurons were fated to remain neurons and so forth. However, pioneering research since the first cloned animal Dolly was born in 1996 has shown us that cell fate is not deterministic, and cells can be converted into different states through diverse reprogramming strategies (1). For example, oocyte-mediated reprogramming, using nuclear transfer technology, enables conversion of differentiated cells into totipotent cloned embryos; these embryos can develop into cloned animals in vivo or used to create embryonic stem cells in vitro. Although nuclear transfer embryonic stem cells have been successfully generated using human cells, ethical issues involved in using human oocytes have limited applications in regenerative medicine. One alternative strategy to derive similarly reprogrammed cells is based on the ectopic expression of transcription factors (TFs),2 such as the four TFs collectively known as the Yamanaka factors, which can induce differentiated cells into pluripotent stem cells (iPSCs) that can be further differentiated into desired cell types in vitro for cell replacement therapies and drug screening. TFs can also be applied to directly convert one type of somatic cells into another. This strategy, called transdifferentiation, can bypass the pluripotent stage, thus avoiding the tumorigenicity arising from acquisition of pluripotency. However, TF-based reprogramming has its own safety concerns, as application of this strategy would require the insertion of retroviral vectors and could potentially reactivate exogenous transcription factors, leading to unintended outcomes. To circumvent these problems, recent efforts have been focused on small molecule compounds, which have been used to convert somatic cells into pluripotent stem cells and other functional differentiated cells (2). Chemical reprogramming has several advantages over other methods, including structural versatility and the comparatively low costs of making the molecules, and the ability to create a highly controlled dosing schedule (2, 3). However, chemical treatments also require a long time course and yield only low efficiency conversions, especially in chemically induced transdifferentiation.

To achieve higher conversion rates, a better mechanistic understanding of the reprogramming process is needed. Previous studies have shown that somatic reprogramming induced by Yamanaka factors is a multistep process, in which induction of the mesenchymal-to-epithelial transition (MET) is the initiation step of reprogramming (Fig. 1) (4–7). The MET leads to unstable intermediate cells with epithelial features that are amenable to reprogramming. Interestingly, methods that induce a MET can enhance the efficiency of iPSC generation, indicating that the MET is a rate-limiting step during TF-induced iPSC generation. Chemical reprogramming of somatic cells into iPSCs (ciPSCs) pass through a similar intermediate step, in which cells in a unique epithelial extra-embryonic endoderm (XEN)-like state emerge and can be stably captured in culture (Fig. 1) (8). Importantly, chemical compounds that direct the cell transition through a XEN-like state can dramatically promote ciPSC generation. However, it was not known whether this intermediate state would also facilitate transdifferentiation. Two recent studies explore this hypothesis, finding that the intermediate epithelial-like state is also critical for chemically induced transdifferentiation.

Cao et al. (9) began their study by developing a chemical mixture that could efficiently convert mouse embryonic fibroblasts into epithelial-like cells through the MET process. To this end, they composed a chemical formula including small molecules or growth factors that can inhibit TGFβ signaling pathway (involved in reinforcement of fibroblast-like characteristics), activate epithelial characteristics, or have previously been shown to help promote cell fate conversion (4, 8). Interestingly, they found that this chemical recipe could enable chemically induced epithelial-like cells to acquire endoderm progenitor cell markers such as SOX17 and GATA4/6. Further optimization of their chemical mixture and induction process by examining the mechanistic con-
sequences of treatment with each compound improved the ratio of epithelial-like cells as measured using SOX17. With a refined protocol in hand, they demonstrated efficient reprogramming of mouse embryonic fibroblasts into endodermal progenitor cells (ciEPCs), which could be captured in culture as stable cell lines that self-renew in vitro for >30 passages without changing endodermal features. Cao et al. (9) further showed that ciEPCs could be efficiently converted into albumin-producing hepatocytes (ciHeps) that could rescue mice from liver failure. Finally, the authors confirmed the generality of their strategy by using the same mixture to convert adult fibroblast cells from mouse embryonic fibroblasts into endodermal progenitor cells (ciPSCs) and sustain the ability to differentiate into functional cells from both ectoderm and endoderm layers. The potential explanation might be that ciEPCs, as derivatives of the MET process, specifically express endodermal and epithelial markers, which limit their differentiation potential, whereas XEN-like cells, initially identified as the intermediate state of ciPSC derivation, express master genes controlling cell fate choices that can lead toward different germ layers and cell lineages.

In combination, these results raise a multitude of interesting questions that remain to be addressed. For example, would a similar improvement of the ratio of intermediate cells in an epithelial state during reprogramming also enhance TF-based transdifferentiation (Fig. 1)? Particularly because Cao et al. (9) and Li et al. (10) used different chemical combinations, it will be intriguing to explore further cocktails, including combining TFs and chemicals to further improve the reprogramming efficiency. What are the mechanisms that underlie cell fate conversion into intermediate cells with different differentiation potentials by different molecular combinations? A comparative study using the two chemical cocktails reported will not only aid us in elucidating the differences but may also provide clues for further improving the efficiency of chemical reprogramming. Can chemicals convert somatic cells into stable intermediate epithelial-like cells that can differentiate into functional cells from the ectoderm or mesoderm layers? Can human somatic cells be efficiently converted into a stable intermediate state by chemicals, leading to efficient transdifferentiation into functional cells? These chemical tools will undoubtedly help guide us to new insights into cellular development and new opportunities in human medicine.

Figure 1. An intermediate state with epithelial features is critical for cell fate conversion induced by transcription factors and chemicals. See text for details. The dashed and solid boxes represent intermediate state cells and stable cells captured from intermediate state cells, respectively. Question marks mean that these states are not clarified yet.

References
1. Smith, Z. D., Sindhu, C., and Meissner, A. (2016) Molecular features of cellular reprogramming and development. Nat. Rev. Mol. Cell. Biol. 17, 139–154
2. Xie, X., Fu, Y., and Liu, J. (2017) Chemical reprogramming and transdifferentiation. Curr. Opin. Gen. Devel. 46, 104–113
3. Li, X., Yang, Y., Yang, Z., and Deng, H. K. (2017) Chemical reprogramming of mouse fibroblasts. Curr. Opin. Gen. Devel. 46, 6–7
4. Li, R., Liang, J., Ni, S., Zhou, T., Qing, X., Li, H., He, W., Chen, J., Li, F., Zhuang, Q., Qin, B., Xu, J., Li, W., Yang, J., Gan, Y., Qin, D., Feng, S., Song, H., Yang, D., Zhang, B., Zeng, L., Lai, L., Esteban, M. A., and Pei, D. (2010) A mesenchymal-to-epithelial transition initiates and is required for the nuclear reprogramming of mouse fibroblasts. Cell Stem Cell 7, 51–63
5. Samavarchi-Tehrani, P., Golipour, A., David, L., Sung, H. K., Beyer, T. A., Datti, A., Woltjen, K., Nagy, A., and Wrana, J. L. (2010) Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming. Cell stem cell 7, 64–77
6. Polo, J. M., and Hochedlinger, K. (2010) When fibroblasts MET iPSCs. Cell stem cell 7, 5–6
7. Skrypek, N., Goossens, S., De Smedt, E., Vandamme, N., and Berx, G. (2017) Epithelial-to-mesenchymal transition: epigenetic reprogramming driving cellular plasticity. Trends Genet. 10.1016/j.tig.2017.08.004
8. Zhao, Y., Zhao, T., Guan, J., Zhang, X., Fu, Y., Ye, J., Zhu, J., Meng, G., Ge, J., Yang, S., Cheng, L., Du, Y., Zhao, C., Wang, T., Su, L., Yang, W., and Deng, H. (2015) A XEN-like state bridges somatic cells to pluripotency during chemical reprogramming. Cell 163, 1678–1691
9. Cao, S., Yu, S., Chen, Y., Wang, X., Zhou, C., Liu, J., Wang, D., Ye, J., Qing, Y., Chu, S., Wu, L., Guo, L., Li, Y., Shu, X., Chen, J., Liu, J., and Pei, D. (2017) Chemical reprogramming of mouse embryonic and adult fibroblast into endoderm lineage. J. Biol. Chem. 292, 19122–19132
10. Li, X., Liu, M., Ma, Y., Du, X., Jing, J., Wang, L., Xie, B., Sun, D., Sun, S., Jin, X., Zhang, X., Zhao, T., Guan, J., Yi, Z., Lai, W., Zheng, P., Huang, Z., Chang, Y., Chai, Z., Xu, J., and Deng, H. (2017) Direct reprogramming of fibroblasts via a chemically induced XEN-like state. Cell Stem Cell 21, 264–273.e7