Exploring intermediate cell states through the lens of single cells

Adam L. MacLean, Tian Hong, and Qing Nie

Abstract
As our catalog of cell states expands, appropriate characterization of these states and the transitions between them is crucial. Here we discuss the roles of intermediate cell states (ICSs) in this growing collection. We begin with definitions and discuss evidence for the existence of ICSs and their relevance in various tissues. We then provide a list of possible functions for ICSs with examples. Finally, we describe means by which ICSs and their functional roles can be identified from single-cell data or predicted from models.

Keywords
Intermediate state; Transition state; Cell plasticity; Hybrid cell type; EMT; Cell differentiation; Cell lineage; Multistability

Introduction
Studying single cells in high resolution has led to many advances, including new ways to characterize and understand cell states. These can be persistent and accompanied by well-defined functions – commonly referred to as cell types – or they might occupy less well-characterized roles in an atlas of cells (see the Human Cell Atlas project [1]). These latter cell states are referred to as intermediate, hybrid, or transition states in various contexts. Single-cell studies have advanced our ability to probe these states, but require new computational methods and theoretical models for analysis, as they are typically high dimensional (tens of thousands of genes measured in thousands of cells). With rapidly improving experimental techniques, more complex landscapes of cell states will be investigated and revealed, making development of appropriate tools even more important.
Characterizing the heterogeneity present within and between cell states is crucial to understanding them and defining their boundaries; here models accelerate progress, as cell states can be defined as attractors on a potential landscape. Below we will discuss the role of noise in cell states: how biology both accounts for it and exploits it, in various contexts.

Intermediate cell states (ICSs) can be defined in terms of cellular phenotype, i.e. the quantifiable characteristics of a cell, which include gene expression, protein abundances, post-translational modifications, and cell morphology. We consider any state that lies between two traditionally defined cell types (i.e. cell states that have accompanying functions) to be intermediate (Figure 1A) and we refer to a generic intermediate cell state as an ICS of Type 0. These cell types may be distinguished from each other by either quantitative or qualitative measurement. While heterogeneity within a given cell state may also be functionally relevant, we limit our discussion here to cell states with distinct functions.

ICSs become particularly important when they mediate transitions, which can have distinct meanings in different contexts (Figure 1B). ICSs can be ‘lineage siblings’ (Type 1), i.e. share a hierarchical level with terminal states. Other ICSs occupy distinct hierarchical levels from terminal states and potentially also between themselves (Types 2 and 3). ICSs can also exhibit more complex lineage relationships (Type 4).

In the following discussion, we seek to characterize ICSs and discuss how they may be predicted conceptually, either from models or data; we do not however provide specific methods with which to identify ICSs. For comparative purposes, we focus on three biological systems and the roles of ICSs in each. These are: the epithelial-to-mesenchymal transition (EMT); hematopoietic progenitor cell differentiation; and CD4$^+$ T cell lineage specification. The ICSs in these systems can be classified with the definitions above (Figure 1B) (EMT: Types 2 & 3; Hematopoietic stem/progenitor cell states: Types 2–4; CD4$^+$ T cells: Type 1).

The existence of intermediate states

EMT

Epithelial and mesenchymal cells are distinguished by cellular function, morphology, migratory behavior and transcriptional programs. During embryonic development, epithelial cells undergo a transition to a mesenchymal state, a process known as epithelial–mesenchymal transition (EMT). This transition is associated with the loss of cell–cell junctions and cell polarity, and the acquisition of migratory and invasive properties. The EMT is reversible: mesenchymal-to-epithelial transition (MET) may occur in development and other physiological conditions, and is important for the morphogenesis of internal organs [2,3]. The EMT-MET system thus appears to be highly dynamic in response to either intrinsic signals or the microenvironment. Complex signaling and transcriptional networks [2,4] control this plasticity of cellular phenotypes.

Initial characterization of EMT indicated a binary decision between E (epithelial) and M (mesenchymal) states. While the notion of a direct transition is useful and parsimonious, it
cannot explain key observations regarding partial phenotypes exhibiting both E and M characteristics, during morphogenesis or cancer progression. These data have stimulated mathematical modeling and quantitative experimentation to characterize partial EMT. Modeling studies have revealed that complex EMT regulatory networks govern the existence and stability of multiple ICSs [5–9], for example two EMT ICSs displaying distinct differentiation propensities [5]. Experiments have found evidence for these states in the mammary epithelium, both naturally and signal-induced [5], in agreement with experiments showing multiple ICSs in similar systems [10–13]. These systems approaches have led to a new paradigm for EMT involving multiple transitional stages [14]. Intermediate EMT states can be classified as Type 3 in Figure 1B, where they serve as ‘waypoints’ assisting with cellular plasticity, but recent association of EMT ICSs with stemness leads to the hypothesis that these states may be more undifferentiated (Type 2) [15]. The differential stability and dynamic behaviors of these intermediate may be critical to morphogenesis, wound healing and disease progression [14].

**Hematopoietic progenitor cells**

Hematopoiesis proceeds by an archetypical stem cell process: a rare cell population with the capacity to self-renew indefinitely gives rise to the many differentiated cell types of the blood system. Characterization of the differentiation paths traveled by hematopoietic stem cells (HSCs) led to the construction of a hematopoietic lineage tree consisting of multiple lineage-restricted progenitor cell populations controlling successive bifurcations (Type 4 in Figure 1B), leading eventually to the various distinct and specialized cell lineages [16]. Whereas some progenitor cell populations represent cell types, others may be ICSs; our characterization of these is incomplete. Furthermore, each of these lineages may still contain (unipotent) progenitor cells and have capacity for further specification and complex cell fate dynamics involving ICSs (e.g. the CD4⁺ T cell lineage). Experiments mostly based on cell surface marker expression via flow cytometry led to this view of hematopoiesis; but differentiation culture experiments were performed at a population level and thus unable to resolve single progenitor cell fate decisions. Modeling studies have been able to delineate the landscapes and cell states of stem and progenitor populations, as well as the timings of cell fate decisions and some of the regulatory networks that control these decisions [17–22]. In the case of multiple steady states, generalized stability analysis can map out the global stability properties of the landscape and thus define the basins of attraction in parameter space [23]; progenitor cell states tend to lie in shallower wells than stem or differentiated cell states [24]. These studies have, until recently, focused for the most part on population dynamics. Significant plasticity/heterogeneity within progenitor cell populations has been hinted at in the past [25], but only recently have we become able to probe these phenomena in detail.

Single-cell analysis and models have led to dramatic changes in our characterization and understanding of cell states during hematopoietic differentiation. Previously well-defined intermediate progenitor states were revealed to be – rather than a single population – mixtures of heterogeneous cell populations [26,27]. Thus rather than a bifurcating tree-like lineage, a fan-like lineage has been proposed with fewer intermediate progenitor states and earlier lineage restriction (Figure 1B Type 1). Single-cell differentiation assays provide
functional means to test the composition of these controversial states, and have found that, in agreement with gene expression data, the megakaryocytic-erythrocytic progenitor population is not in fact composed of bipotent progenitor cells, but rather consists of lineage-restricted erythroid or megakaryocyte progenitors, along with cells exhibiting high plasticity (see Function 4) [28]. In addition, new ICSs have emerged, for example a “multi-lineage” state associated with the monocytic-granulocytic cell fate choice that might act as a primed state in which cells can become “trapped” if they do not receive the appropriate transcriptional cues [29].

If we consider the landscape of hematopoietic differentiation, these new data suggest we must go beyond Waddington’s classical bifurcating valleys [30]; instead, saddle points might lead to three or more new cell states [31]. This additional complexity presents both a challenge and an opportunity for modelers. These results also have implications for non-hematopoietic tissues such as the skin, which have simpler differentiation trajectories (fewer – or perhaps no – bifurcations), but still pass through intermediate progenitor states en route to terminal differentiation. As we begin to interrogate these lineages in greater detail [32], our understanding of the existence and nature of ICSs may again be subject to change.

**CD4^+ T cells**

CD4^+ T cells play an essential role in the adaptive immune response, exhibiting a remarkable diversity of transcriptional programs and functions. They coordinate an immune response by releasing cytokines specific to the immune activity and to their own identity. Differentiation of CD4^+ T cells is triggered by pathogenic challenges to an organism, which are followed by antigen-presentation to the naïve (undifferentiated) CD4^+ T cells, influenced by surrounding cytokines [33]. These antigen and cytokine signals determine the fates of the CD4^+ T cells.

Previous experimental and modeling studies focused on signaling networks controlling fate determination of CD4^+ T cells, with the underlying assumption that cells adopt discrete and mutually exclusive transcription programs upon differentiation [34–38]. However, mutually exclusive differentiation has been challenged by numerous observations in the past decade: multiple studies have found intermediate (hybrid/double-positive) CD4^+ T cells, which are generated together with the ‘terminal’ cells and stably maintained (Type 1, Figure 1B). For example, cells expressing both RORγt (master regulator of Th17) and Foxp3 (master regulator of Treg) exist in human and mouse, and can be stably maintained in culture under non-polarizing conditions [39–41]. Other ICSs, e.g. Th1–Th2 and Th1–Th17 cells, can be found in various physiologically relevant conditions [42–47]. The formation of these ICSs can be explained by modeling the core transcriptional networks [48–50]. In addition to the identification of cells that express key factors of two lineages, stable cellular states with varying lineage-defining factors have been reported for CD4^+ T cells [51,52]. Eizenberg-Magar et al. found that CD4^+ T cells combine cytokine signals and choose their fates in a linear continuum in vitro, although the stability of these states in vivo has not been examined [52]. Th1 cells have been shown to have stable quantitative memory in terms of the cytokine production rates [51]. These observations suggest that many possible cellular states exist between the extrema of the CD4^+ T cell phenotypic spectrum. The stability of some of these
ICSs has been demonstrated, however the fates of CD4+ T cells are plastic, and transitions between states can occur during immune responses [53–56]. The relative contributions of cells undergoing lineage transitions between ICSs are less clear.

The three examples discussed above are among many biological systems where ICSs exist. In fact, the ICS may appear ubiquitously in developmental processes involving gradual cell fate determination [57–60]. In mature systems, ICSs may appear between cell types that possess sufficient plasticity, including cancer cells, and those systems which involve dynamics among multiple subpopulations [61,62].

The role of noise in intermediate states

The plastic nature of epithelial and mesenchymal cell states (along with ICSs) highlights the importance of studying the dynamics of this system in fine detail. Intercellular signaling molecules such as TGF-β and BMP influence EMT-MET transitions, but it is not clear how intracellular noise influences the transition dynamics. Stochastic simulations suggest that ICSs have different differentiation propensities, driven by fluctuations in gene expression, and that noise can trigger transitions into an ICS from a terminal state [5]. In addition, an ICS allows noise-induced switching more easily, such as the transition from a main state to an ICS, then to another main state. Such noise-induced switching is beneficial in cell fate specification [63,64].

Stochasticity plays a central role in hematopoietic progenitor cell dynamics, and new states have been gained and lost according to different measurement and analysis techniques [26,29,65]. Mathematical models have been used to study stochastic hematopoietic dynamics in various ways [66–68], but have yet to describe single-cell fate decision dynamics. Experimentally, techniques are improving to observe cell-to-cell heterogeneity [28], highlighting the importance of these effects during hematopoietic differentiation.

Stochastic fate choices for multiple CD4+ T cell lineages and corresponding ICSs have been observed and modeled [41,48–50]. Stochastic cytokine production has been reported in transitioning Th1–Th2 intermediate cells [69], however, differentiated Th1 cells have stable states in terms of their levels of cytokine production, and stochastic switching between the quantitative states is very limited [51]. Whether stochasticity contributes significantly to the long-term behavior of intermediate phenotypes remains an open question.

Possible functions of intermediate states

Here we discuss known or predicted functions of ICSs under five headings, and give examples for each. We consider two generic cell types (A and B) and the transitions that occur between them, which could be due to lineage dynamics or to metaplasticity.

1. The ICS controls bidirectional transitions between cell types

If cell types A and B are separated by a gap in phenotypic space, then an ICS might enable a transition from A to B. Or, if the transition A → B exists, but the transition B → A does not, an ICS might enable this reverse transition. An ICS could also have a negative effect on
the transition $A \rightarrow B$, by either reducing the rate or halting the transition completely, thus acting as a sink.

In EMT, using landscape theory to characterize the kinetic paths of a three-state EMT system [70], we found that transitioning through an ICS may be required when an EMT-inducing signal is not sufficient to convert E to M directly. In hematopoiesis, the existence of ICSs that were previously thought to drive bifurcations during differentiation has been challenged [26,27], leading to new roles for ICSs with greater lineage bias but reduced multipotency (perhaps precluding these states from performing function 4 below) [29]. Since new roles for ICSs in facilitating differentiation have yet to be carefully defined (a challenge exacerbated by the speed with which our understanding of the hematopoietic landscape is changing [59,71,72]), much work remains to be done; here modeling studies will likely play a crucial role. In culture or pathogenic settings, dedifferentiation can occur; such reversibility is also widespread for metastatic transitions: the role of ICSs in reversible (or reverse) transitions is likely important but remains to be well defined.

2. The ICS exhibits a hybrid phenotype

For cell types A and B with distinct functions, an ICS can display a hybrid phenotype containing characteristics of phenotype A and phenotype B. There could exist a transition $A \rightarrow B$, or $B \rightarrow A$, or there could be no transition.

Several studies have found that intermediate CD4$^+$ T cells produce cytokines of mixed lineage signatures and functions [40,43,45], and the dual-function of Th1–Th2 intermediate cells in limiting immunopathologic inflammation [47]. It has been proposed that the ICSs can serve as ’moderators’ that help to avoid damage from extreme immune responses [73]. It has been suggested that intermediate EMT cells may exhibit a hybrid function with regards to collective migration; a key feature of invasive cancer cells requiring both cell-to-cell contact and the ability to migrate. As for hematopoiesis, in terms of the stemness axis, progenitor ICSs are – at least in part – by definition hybrid states, exhibiting mixed stem-like and differentiated cell characteristics [28,29].

3. The ICS controls size fluctuations of cell populations

An ICS between cell types A and B can regulate the variance of cell types A and B. This regulation might act to stabilize the cell populations via ICS transitions, or the ICS can be used by the system to increase the variance without changing the mean.

Models have predicted that multiple ICSs can facilitate the attenuation of fluctuating cell populations [74]. By absorbing some of the noise, these additional states serve as ‘buffers’ against environmental fluctuations, thereby preventing imbalances of cells in terminal states. These homeostatic properties could be hijacked by cancer; their misregulation leading to increased invasiveness. Recent experiments have shown that key genes – which may regulate ICSs and induce transitions – control fluctuations without affecting the mean of the gene expression state [75]. The level of temporal fluctuations is determined by a critical quantity called the Signed Activation Time [76,77], which may be regulated by ICSs. Increasing cell heterogeneity, made possible by ICSs, can also enable faster regeneration and reduce the variability in desired cell populations [78].
4. The ICS expands the reach of a cell type

In various cell transition processes, including differentiation, reprogramming/dedifferentiation, or direct reprogramming, the number of cell states accessible to a cell at any given time can change. The ICS can act to change this cell type “accessibility”, which can, in different contexts, be thought of as potency, stemness, or potential to broadcast information [79]. Increasing accessibility implies greater potency/stemness, while decreasing accessibility may correspond to/result from differentiation.

The bipotent characteristics of intermediate EMT states have led to suggestions that these states have higher stemness and invasiveness than terminal states. This association of the ICS with stemness has been found in cancer cell lines, and the signature genes associated with the intermediate state are correlated with poor cancer prognosis [15]. However, the functional role of multiple ICSs under normal physiological conditions is less clear. In hematopoiesis, controlling cell state accessibility during transitions is closely linked to the traditional functions of progenitor cell states, however (as discussed above) some of these functions have recently been called into question. The landscape of hematopoietic differentiation, which leads to many diverse cell types, must be controlled at points of lineage restriction/fate choice. Mutual inhibition (toggle-switch) models can control bifurcations [19,22,66], but if more complex decisions occur (see Ref. [29]), larger transcriptional networks may be required [5,31].

5. The ICS as a cell ledger

The ICS can be used to record information regarding cell dynamics, for example acting as a checkpoint during differentiation, requiring a certain threshold to be met by counting progenitor cells before differentiation proceeds.

For example, mesenchymal stem cell states exhibit memory in response to mechanical stimuli, thus providing a means to record cell counts [80]. In early T cell development, multiple intermediate stages may serve as checkpoints that ensure proper transcriptional programs and sufficient cell expansion are achieved [59,81]. In particular, DN1 (the earliest intermediate stage of T cell development in thymus) cells need to undergo multiple cell divisions before progress to the DN2 stage [82]. This regulation of differentiation competence by cell expansion is critical for T-cell homeostasis, and it could represent a more general strategy for maintaining cell populations through ICSs.

Modeling and computational approaches to find intermediate states

In many cases, prior knowledge of the gene regulatory networks of a biological system provides a good starting point for the identification of ICSs. Typically, models describe interactions among genes, protein abundances, post-translational modifications, or chromatin states, and are characterized by a system of equations (discrete or continuous). The challenge then becomes finding and classifying all the steady state solutions of the system that correspond to cell states [83]. Locations (in state space) and stability properties (e.g. stable, meta-stable, unstable) are critical quantities to consider for ICSs [84].
One minimal condition for finding ICSs is the existence of five distinct steady state solutions, three of which need to be stable [49]. Network topologies that give rise to such multistability typically contain a core mutual inhibitory circuit with additional positive feedback loops on one or more genes. For systems of a small number of genes, nullclines and numerical bifurcation analysis are applicable for analysis [5,50], while for systems of a large number of genes, more sophisticated computational tools are required. Interestingly, in some cases, stable ICSs may emerge only in a stochastic model of the system [85]. Exploration of stochastic dynamical systems requires effective numerical methods that can deal with temporal stiffness in the rate constants [86–88].

When the cell state space is defined in high dimensions (e.g. by expression of many genes), statistical methods are needed to identify ICSs. Dimensionality reduction and hierarchical clustering are common strategies for quantifying the distances among cells and defining the state space in lower dimensions [10,89]. Additional metrics can be used to define a linear spectrum of phenotypes so that the possible states in the spectrum can be scored quantitatively [10,90].

**Single-cell analysis approaches to find intermediate states**

Single-cell transcriptomic data provide a flexible and unbiased way to search for ICSs in gene expression state space. For example, single-cell analysis has shown that metastatic mammary epithelial cells exhibit strong phenotypic variations [91], and indicates the involvement of one or more ICSs, but their relationships with stem cell subpopulations and EMT/MET need to be further analyzed. Bifurcation of Th1 and Tfh cells during *Plasmodium* infection has been discovered via single-cell sequencing [92], and multiple ICSs during CD4+ T cell differentiation have been suggested [93], however, these studies did not address a central question: how are mature CD4+ T cell ICSs distributed on a gene expression landscape? Future single-cell analysis will help to resolve this picture of mixed phenotype states [94,95]. Single-cell analysis of hematopoietic progenitors has led to dramatic changes in the understanding of the cell state landscape as discussed above, with corrections made to our previous (false) perceptions of some progenitor cell states [26–28], and the discovery of others [29].

The existence of an ICS from single-cell data can be predicted by analysis methods. In Figure 2A, a hypothetical low-dimensional projection of single cell data (e.g. via RNA sequencing) is shown, colored by subpopulation identity. These subpopulations could be determined by biological markers or predicted by clustering [96] or energy-landscape based methods [97]. Three possible means to identify ICSs from such a dataset are shown (labels (i)–(iii)). Cluster C2, of class (i), is defined by its distinctness in pseudotime (Figure 2B), suggestive of a state involved in phenotype transitions. Class (ii) (Cluster C5) appears as a distinct subpopulation between other subpopulations, and may also represent an intermediate state. Finally, type (iii) represents a mixture of subpopulations (C6 and C7), which could also predict an ICS.

It can be particularly challenging to identify an ICS with transient properties, especially since most single-cell data give only a snapshot of time; cells can be analyzed in pseudotime.
to give hints regarding transience [65] (populations occupying very little of pseudotime may be considered transient). Although preserving distances among clusters of cells in the projections can be useful for subsequent clustering analysis [98], possible ICSs might be lost by using these methods because of the potential bias towards separation of major clusters. If the number of cells in the intermediate state is significantly smaller than the terminal states [71] (a rare cell subpopulation), classical clustering or dimension reduction methods are insufficient, and new computational tools are needed to reveal ICSs and their connections to terminal states [71,99].

Conclusions

Accelerated by advances in single cell technologies, our ability to characterize cell types is expanding, and new forms of phenotypic diversity are revealed, including the intermediate cell states (ICSs) that lie between traditional categories of cells. ICSs re-ignite debate over how we should define cell types and cell type transitions [100]. Due to a lack of quantitative tools, cells were previously classified based on their morphologies and cell surface marker expression, which can lead to ambiguity and inaccuracy. Single-cell transcriptomics lead to definition of cell types using dimensionality reduction and clustering techniques, but it remains challenging to standardize these methods across multiple biological systems. The existence of many ICSs becomes suggestive of a ‘continuum’ of cellular phenotypes [14,52]. Although this could be a useful model for understanding certain systems, the implementation of this idea for defining cell types requires more generalized method development.

Given the importance of ICSs in induced cellular reprogramming and differentiation [101–103], and their potential to regulate phenotypic switches, we are poised to undergo a transformation in our ability to control cell differentiation [104,105]. Success in these endeavors relies on our ability to provide suitable theoretical models of cell dynamics, and may lead to a renewal of the definitions we give for the cell states that define us.

Acknowledgments

This study was partially supported by a NSF grant DMS1562176, NIH grants R01GM107264, R01NS095355, R01GM123731, and a grant funded by the Jayne Koskinas Ted Giovanis Foundation for Health and Policy joint with Breast Cancer Research Foundation (to Q. Nie).

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Regev A, Teichmann SA, Lander ES, Amit I, Benoist C, Birney E, Bodenmiller B, Campbell P, Carninci P, Clatworthy M, Clevers H, Deplancke B, Dunham I, Eberwine J, Eils R, Enard W, Farmer A, Fugger L, Gottgens B, Hacohen N, Haniffa M, Hemberg M, Kim S, Klennerman P, Kriegstein A, Lein E, Linarsson S, Lundberg E, Lundeberg J, Majumder P, Marioni JC, Merad M, Mhlanga M, Nawijn M, Netea M, Nolan GP, Pe’er D, Phillipakis A, Ponting CP, Quake S, Reik W, Rozenblatt-Rosen O, Sanes J, Satija R, Schumacher TN, Shalek A, Shapiro E, Sharma P, Shin JW,
Stegle O, Stratton M, Stubbington MJT, Theis FJ, Uhlen M, van Oudenaarden A, Wagner A, Watt F, Weissman J, Wold B, Xaver R, Yosef N, Human Cell P. Atlas meeting, the human cell atlas. eLife. 2017; 6:503.

2. Nieto MA. Epithelial plasticity: a common theme in embryonic and cancer cells. Science. 2013; 342:e27041.

3. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009; 139:871–890. [PubMed: 19945376]

4. Nieto MA. The ins and outs of the epithelial to mesenchymal transition in health and disease. Annu Rev Cell Dev Biol. 2011; 27:347–376. [PubMed: 21740232]

5**. Hong T, Watanabe K, Ta CH, Villarreal-Ponce A, Nie Q, Dai X. An Ovol2-Zeb1 mutual inhibitory circuit governs bidirectional and multi-step transition between Epithelial and Mesenchymal states. PLoS Comput Biol. 2015; 11:e1004569. Modeling predicts two intermediate EMT states with distinct differentiation propensities; existence of these intermediate states is demonstrated experimentally. [PubMed: 26554584]

6*. Lu M, Jolly MK, Levine H, Onuchic JN, Ben-Jacob E. MicroRNA-based regulation of epithelial-hybrid-mesenchymal fate determination. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110:18144–18149. Predicts an intermediate EMT state governed by microRNA-mediated regulatory circuits. [PubMed: 24154725]

7. Tian X-J, Zhang H, Xing J. Coupled reversible and irreversible bistable switches underlying TGFβ-induced epithelial to mesenchymal transition. Biophys J. 2013; 105:1079–1089. [PubMed: 23972859]

8. Zhang J, Tian XJ, Zhang H, Teng Y, Li R, Bai F, Elankumaran S, Xing J. TGF-β -induced epithelial-to-mesenchymal transition proceeds through stepwise activation of multiple feedback loops. Sci Signal. 2014; 7:ra91–ra91. A combined experimental and dynamical systems theory-based study on a breast epithelial cell line that reveals an intermediate EMT state. [PubMed: 25270257]

9. Jolly MK, Tripathi SC, Jia D, Mooney SM, Celiktas M, Hanash SM, Mani SA, Pienta KJ, Ben-Jacob E, Levine H. Stability of the hybrid epithelial/mesenchymal phenotype. Oncotarget. 2016; 7:27067. [PubMed: 27008704]

10. Huang RY, Wong MK, Tan TZ, Kuay KT, Ng AH, Chung VY, Chu YS, Matsumura N, Lai HC, Lee YF, Sim WJ, Chai C, Pietschmann E, Mori S, Low JJ, Choolani M, Thiery JP. An EMT spectrum defines an anoikis-resistant and spheroidogenic intermediate mesenchymal state that is sensitive to e-cadherin restoration by a src-kinase inhibitor, saracatinib (AZD0530). Cell Death Dis. 2013; 4:e915. [PubMed: 24201814]

11. Tam WL, Weinberg RA. The epigenetics of epithelial-mesenchymal plasticity in cancer. Nat Med. 2013; 19:1438–1449. [PubMed: 24202396]

12. Baulida J, Garcia de Herreros A. Snail1-driven plasticity of epithelial and mesenchymal cells sustains cancer malignancy. Biochim Biophys Acta. 2015; 1856:55–61. [PubMed: 26050961]

13. Jordan NV, Johnson GL, Abell AN. Tracking the intermediate stages of epithelial-mesenchymal transition in epithelial stem cells and cancer. Cell Cycle. 2011; 10:2865–2873. [PubMed: 21862874]

14. Nieto MA, Huang RY, Jackson RA, Thiery JP. EMT: 2016. Cell. 2016; 166:21–45. [PubMed: 27368099]

15. Grosse-Wilde A, Fouquier d'Herouel A, McIntosh E, Ertaylan G, Skupin A, Kuestner RE, del Sol A, Walters KA, Huang S. Stem-ness of the hybrid epithelial/mesenchymal state in breast cancer and its association with poor survival. PLoS One. 2015; 10:e0126522. [PubMed: 26020648]

16. Wang LD, Wagers AJ. Dynamic niches in the origination and differentiation of haematopoietic stem cells. Nature Reviews Molecular Cell Biology. 2011; 12:643–655. [PubMed: 21886187]

17. Mangel M, Bonsall MB. Phenotypic evolutionary models in stem cell biology: replacement, quiescence, and variability. PLoS One. 2008; 3:e1591. [PubMed: 18270578]

18. Manesso E, Teles J, Bryder D, Peterson C. Dynamical model-ling of haematopoiesis: an integrated view over the system in homeostasis and under perturbation. J R Soc Interface. 2013; 10https://doi.org/10.1098/rsif.2012.0817
19. Chickarmane V, Enver T, Peterson C. Computational modeling of the hematopoietic erythroid-myeloid switch reveals in-sights into cooperativity, priming, and irreversibility. PLoS Comput Biol. 2009; 5:e1000268. [PubMed: 19165316]

20. Roeder I, Glauche I. Towards an understanding of lineage specification in hematopoietic stem cells: a mathematical model for the interaction of transcription factors GATA-1 and PU.1. J Theor Biol. 2006; 241:852–865. [PubMed: 16510158]

21. Buzi G, Lander AD, Khammash M. Cell lineage branching as a strategy for proliferative control. BMC Biol. 2015; 13:13. [PubMed: 25857410]

22. Marr C, Strasser M, Schwarzfischer M, Schroeder T, Theis FJ. Multi-scale modeling of GMP differentiation based on single-cell genealogies. FEBS J. 2012; 279https://doi.org/10.1111/j.1742-4658.2012.08664.x

23*. MacLean AL, Kirk P, Stumpf MPH: Cellular population dynamics control the robustness of the stem cell niche. Biology Open. 2015; 4:1420–1426. . Provides means to analyze the global stability properties of a model of multiple steady states corresponding to both terminal and intermediate states. [PubMed: 26453624]

24**. Mojtahedi M, Skupin A, Zhou J, Castaño IG, Leong-Quong RYY, Chang H, Trachana K, Giuliani A, Huang S. Cell fate decision as high-dimensional critical state transition. PLoS Biol. 2016; 14:e2000640. Proposes a framework for the analysis of cell fate landscapes via attractor states. [PubMed: 28027308]

25. Bell JJ, Bhandoola A. The earliest thymic progenitors for T cells possess myeloid lineage potential. Nature. 2008; 452:764–767. [PubMed: 18401411]

26. Notta F, Zandi S, Takayama N, Dobson S, Gan OL, Wilson G, Kaufmann KB, McLeod J, Laurenti E, Dunant CF, McPherson JD, Stein LD, Dyr O, Dick JE. Distinct routes of lineage development reshape the human blood hierarchy across ontogeny. Science. 2016; 351:aab2116–aab2116. [PubMed: 26541609]

27. Paul F, Arkin Ya, Giladi A, Jaitin DA, Kenigsberg E, Keren-Shaul H, Winter D, Lara-Astiaso D, Gury M, Weiner A, David E, Cohen N, Lauridsen FKB, Haas S, Schlitzer A, Mildner A, Ginhoux F, Jung S, Trumpp A, Porst BT, Tanay A, Amit I. Transcriptional heterogeneity and lineage commitment in myeloid progenitors. Cell. 2015; 163:1663–1677. [PubMed: 26627738]

28. Psaila B, Barkas N, Iskander D, Roy A, Anderson S, Ashapal VS, Lichtenberg J, Loaiza S, Bodine DM, Karadimitris A, Mead AJ, Roberts I. Single-cell profiling of human megakaryocyte-erythroid progenitors identifies distinct megakaryocyte and erythroid differentiation pathways. Genome Biol. 2016; 17:387.

29*. Olsson A, Venkatasubramanian M, Chaudhri VK, Aronow BJ, Salomonis N, Singh H, Grimes HL. Single-cell analysis of mixed-lineage states leading to a binary cell fate choice. Nature. 2016; 537:698–702. Single-cell hematopoiesis study reveals new intermediate states governing cell fate decisions with the ability to ‘trap’ cells in undifferentiated wells. [PubMed: 27580035]

30. Waddington C. The strategy of the genes: a discussion of some aspects of theoretical biology. London: Allen & Unwin; 1957.

31. Anderson MZ, Porman AM, Wang N, Mancera E, Huang D, Cuomo CA, Bennett RJ. A multistate toggle switch defines fungal cell fates and is regulated by synergistic genetic cues. PLoS genetics. 2016; 12:e1006353. [PubMed: 27711197]

32. Liu Z, Wang L, Welch JD, Ma H, Zhou Y, Vaseghi HR, Yu S, Wall JB, Alimohamadi S, Zheng M, Yin C, Shen W, Prins JF, Liu J, Qian L. Single-cell transcriptomics reconstitutes fate conversion from fibroblast to cardiomyocyte. Nature. 2017; 551:100–104. [PubMed: 29072293]

33. Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations. Annu Rev Immunol. 2009; 28:445–489.

34. Höfer T, Nathansen H, Löhning M, Radbruch A, Heinrich R. GATA-3 transcriptional imprinting in Th2 lymphocytes: a mathematical model. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:9364–9368. [PubMed: 12087127]

35. Yates A, Callard R, Stark J. Combining cytokine signalling with T-bet and GATA-3 regulation in Th1 and Th2 differentiation: a model for cellular decision-making. J Theor Biol. 2004; 231:181–196. [PubMed: 15380383]
36. Mendoza L. A network model for the control of the differentiation process in Th cells. Biosystems. 2006; 84:101–114. [PubMed: 16386358]
37. van den Ham H-J, de Boer RJ. From the two-dimensional Th1 and Th2 phenotypes to high-dimensional models for gene regulation. Int Immunol. 2008; 20:1269–1277. [PubMed: 18765426]
38. Zhou L, Chong MMW, Littman DR. Plasticity of CD4+ T cell lineage differentiation. Immunity. 2009; 30:646–655. [PubMed: 19464987]
39. Lochner M, Peduto L, Cherrier M, Sawa S, Langa F, Varona R, Riemthaler D, Si-Tahar M, Di Santo JP, Eberl G. In vivo equilibrium of proinflammatory IL-17+ and regulatory IL-10+ Foxp3+ RORγt+ T cells. J Exp Med. 2008; 205:1381–1393. [PubMed: 18504307]
40. Voo KS, Wang Y-H, Santori FR, Boggiano C, Wang Y-H, Arima K, Bover L, Hanabuchi S, Khalili J, Marinova E. Identification of IL-17-producing FOXP3+ regulatory T cells in humans. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:4793–4798. [PubMed: 19273860]
41. Zhou L, Lopes JE, Chong MMW, Ivanov II, Min R, Victora GD, Shen Y, Du J, Rubtsov YP, Rudensky AY. TGF-β-induced Foxp3 inhibits TH17 cell differentiation by antagonizing RORγt function. Nature. 2008; 453:236–240. [PubMed: 18368049]
42. Mus A, Cornelissen F, Asmawidjaja PS, van Hamburg JP, Boon L, Hendriks RW, Lubberts E. Interleukin-23 promotes Th17 differentiation by inhibiting T-bet and FoxP3 and is required for elevation of interleukin-22, but not interleukin-21, in autoimmune experimental arthritis. Arthritis & Rheumatology. 2010; 62:1043–1050.
43. Lexberg MH, Taubner A, Albrecht I, Lepenies I, Richter A, Kamradt T, Radbruch A, Chang HD. IFN-γ and IL-12 synergize to convert in vivo generated Th17 into Th1/Th17 cells. Eur J Immunol. 2010; 40:3017–3027. [PubMed: 21061434]
44. Zheng J, Liu Y, Qin G, Lam KT, Guan J, Xiang Z, Lewis DB, Lau YL, Tu W. Generation of human Th1-like regulatory CD4+ T cells by an intrinsic IFN-γ-and T-bet-dependent pathway. Eur J Immunol. 2011; 41:128–139. [PubMed: 21182084]
45. Hegazy AN, Peine M, Helmstetter C, Panse I, Fröhlich A, Bergthaler A, Flatz L, Pinschwer DD, Radbruch A, Löhnig M. Interferons direct Th2 cell reprogramming to generate a stable GATA-3+ T-bet+ cell subset with combined Th2 and Th1 cell functions. Immunity. 2010; 32:116–128. [PubMed: 20079668]
46. Abromson-Leeman S, Bronson RT, Dorf ME. Encephalitogenic T cells that stably express both T-bet and RORγt consistently produce IFNγ but have a spectrum of IL-17 profiles. J Neuroimmunol. 2009; 215:10–24. [PubMed: 19692128]
47. Peine M, Rausch S, Helmstetter C, Fröhlich A, Hegazy AN, Kühl AA, Greveling CG, Höfer T, Hartmann S, Löhnig M. Stable T-bet+ GATA-3+ Th1/Th2 hybrid cells arise in vivo, can develop directly from naive precursors, and limit immunopathologic inflammation. PLoS Biol. 2013; 11:e1001633. [PubMed: 23976880]
48. Hong T, Oguz C, Tyson JJ. A mathematical Framework for understanding four-dimensional heterogeneous Differentiation of CD4+ T cells. Bull Math Biol. 2015; 77:1046–1064. [PubMed: 25779890]
49. Hong T, Xing J, Li L, Tyson JJ. A mathematical model for the reciprocal differentiation of T helper 17 cells and induced regulatory T cells. PLoS Comput Biol. 2011; 7:e1002122. [PubMed: 21829337]
50*. Hong T, Xing J, Li L, Tyson JJ. A simple theoretical framework for understanding heterogeneous differentiation of CD4+ T cells. BMC Syst Biol. 2012; 6:66. A core signaling motif governing the formation of the intermediate state is identified, and used to build models that make specific predictions about intermediate states. [PubMed: 22697466]
51. Helmstetter C, Flossdorf M, Peine M, Kupz A, Zhu J, Hegazy AN, Duque-Correa MA, Zhang Q, Vainshtein Y, Radbruch A. Individual Th2 helper cells have a quantitative cytokine memory. Immunity. 2015; 42:108–122. [PubMed: 25607461]
52. Eizenberg-Magar I, Rimer J, Zaretsky I, Lara-Astiaso D, Reich-Zeliger S, Friedman N. Diverse continuum of CD4+ T-cell states is determined by hierarchical additive integration of cytokine signals. Proceedings of the National Academy of Sciences of the United States of America. 2017 201615590.
53. Zhu J, Paul WE. Peripheral CD4+ T-cell differentiation regulated by networks of cytokines and transcription factors. Immunol Rev. 2010; 238:247–262. [PubMed: 20969597]
54. Krawczyk CM, Shen H, Pearce EJ. Functional plasticity in memory T helper cell responses. J Immunol. 2007; 178:4080–4088. [PubMed: 17371962]
55. O’Shea JJ, Paul WE. Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. Science. 2010; 327:1098–1102. [PubMed: 20185720]
56. Locksley RM. Nine lives: plasticity among T helper cell subsets. J Exp Med. 2009; 206:1643–1646. [PubMed: 19635860]
57. Mingueneau M, Kreslavsky T, Gray D, Heng T, Cruse R, Ericson J, Bendall S, Spitzer MH, Nolan GP, Kobayashi K. The transcriptional landscape of [alpha][beta] T cell differentiation. Nat Immunol. 2013; 14:619–632. [PubMed: 23644507]
58. Moris N, Pina C, Martinez Arias A. Transition states and cell fate decisions in epigenetic landscapes. Nat Rev Genet. 2016; 17:693–703. [PubMed: 27616569]
59. Rothenberg EV, Kueh HY, Yui MA, Zhang JA. Hematopoiesis and T-cell specification as a model developmental system. Immunol Rev. 2016; 271:72–97. [PubMed: 27088908]
60. Bayraktar OA, Doe CQ. Combinatorial temporal patterning in progenitors expands neural diversity. Nature. 2013; 498:449–455. [PubMed: 23783519]
61. Li C, Wang J. Quantifying the landscape for development and cancer from a core cancer stem cell circuit. Canc Res. 2015; 75:2607–2618.
62+. Gupta PB, Fillmore CM, Jiang G, Shapira SD, Tao K, Kuperwasser C, Lander ES. Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. Cell. 2011; 146:633–644. Introduces models to describe the role of stochastic cell state transitions in tissue maintenance and cancer progression. [PubMed: 21854987]
63. Holmes WR, de Mochel NSR, Wang Q, Du H, Peng T, Chiang M, Cinquin O, Cho K, Nie Q. Gene expression noise enhances robust Organization of the early mammalian blastocyst. PLoS Comput Biol. 2017; 13:e1005320. [PubMed: 28114387]
64. Zhang L, Radtke K, Zheng L, Cai AQ, Schilling TF, Nie Q. Noise drives sharpening of gene expression boundaries in the zebrafish hindbrain. Mol Syst Biol. 2012; 8:463–466. [PubMed: 23010996]
65. Wang S, MacLean AL, Nie Q. Low-rank similarity matrix Optimization identifies subpopulation structure and Orders single cells in pseudotime. bioRxiv. 2017:168922.
66. Roeder I, Horn M, Glauche I, Hochhaus A, Mueller MC, Loeffler M. Dynamic modeling of imatinib-treated chronic myeloid leukemia: functional insights and clinical implications. Nat Med. 2006; 12:1181–1184. [PubMed: 17013833]
67. Székely T, Burgare K, Mangel M, Bonsall MB. Stochastic dynamics of interacting haematopoietic stem cell niche line-ages. PLoS Comput Biol. 2014; 10:e1003794. [PubMed: 25188267]
68. Lei J, Mackey MC. Stochastic differential delay equation, moment stability, and application to hematopoietic stem cell regulation system. SIAM J Appl Math. 2007; 67:387–407.
69. Fang M, Xie H, Dougan SK, Ploegh H, van Oudenaarden A. Stochastic cytokine expression induces mixed T helper cell states. PLoS Biol. 2013; 11:e1001618. [PubMed: 23935453]
70. Li C, Hong T, Nie Q. Quantifying the landscape and kinetic paths for epithelial-mesenchymal transition from a core circuit. Phys Chem Chem Phys. 2016; 18:17949–17956. [PubMed: 27328302]
71. Grün D, Lyubimova A, Kester L, Wiebrands K, Basak O, Sasaki N, Clevers H, van Oudenaarden A. Single-cell messenger RNA sequencing reveals rare intestinal cell types. Nature. 2015; 525:251–255. [PubMed: 26287467]
72. Villani A-C, Satija R, Reynolds G, Sarkizova S, Shekhar K, Fletcher J, Griesbeck M, Butler A, Zheng S, Lazo S, Jardine L, Dixon D, Stephenson E, Nilsson E, Grundberg I, McDonald D, Filby A, Li W, De Jager PL, Rozenblatt-Rosen O, Lane AA, Haniffa M, Regev A, Hacohen N. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science. 2017; 356 eaah4573.
73. Huang S. Hybrid T-helper cells: stabilizing the moderate center in a polarized system. PLoS Biol. 2013; 11:e1001632. [PubMed: 23976879]
74. Ta CH, Nie Q, Hong T. Controlling stochasticity in epithelial-mesenchymal transition through multiple intermediate cellular states. Discrete Continuous Dyn Syst - Ser B (DCDS-B). 2016; 21
75. Sosnik J, Zheng L, Rackauckas CV, Digman M, Gratton E, Nie Q, Schilling TF, Krumlauf R. Noise modulation in retinoic acid signaling sharpens segmental boundaries of gene expression in the embryonic zebrafish hindbrain. eLife. 2016; 5:e14034. [PubMed: 27067377]
76. Chen M, Wang L, Liu CC, Nie Q. Noise attenuation in the ON and OFF states of biological switches. ACS Synth Biol. 2013; 2:587–593. [PubMed: 23768065]
77. Wang L, Xin J, Nie Q. A critical quantity for noise attenuation in feedback systems. PLoS Comput Biol. 2010; 6:e1000764. [PubMed: 20442870]
78*. Lei J, Levin SA, Nie Q. Mathematical model of adult stem cell regeneration with cross-talk between genetic and epigenetic regulation. Proceedings of the National Academy of Sciences of the United States of America. 2014 201324267. Demonstrates via modeling that cell-to-cell heterogeneity can provide robustness benefits for tissue regeneration.
79**. Potoyan DA, Wolynes PG. Stochastic dynamics of genetic broadcasting networks. Phys Rev E. 2017; 96:052305. Proposes an intriguing role for intermediate states: as facilitators of information broadcasting, thus enabling transcriptional networks to act over biological timescales. [PubMed: 29347696]
80. Peng T, Liu L, MacLean AL, Wong CW, Zhao W, Nie Q. A mathematical model of mechanotransduction reveals how mechanical memory regulates mesenchymal stem cell fate decisions. BMC Syst Biol. 2017; 11:55. [PubMed: 28511648]
81. Yui MA, Rothenberg EV. Developmental gene networks: a triathlon on the course to T cell identity. Nat Rev Immunol. 2014; 14:529–545. [PubMed: 25060579]
82. Manesso E, Chickarmane V, Kueh HY, Rothenberg EV, Peterson C. Computational modelling of T-cell formation kinetics: output regulated by initial proliferation-linked deferral of developmental competence. J R Soc Interface. 2013; 10 20120774.
83. Huang S. Cell lineage determination in state space: a systems view brings flexibility to dogmatic canonical rules. PLoS Biol. 2010; 8:e1000380. [PubMed: 20520792]
84*. Guantes R, Poyatos JF. Multistable decision switches for flexible control of epigenetic differentiation. PLOS Comput Biol. 2008; 4:e1000235. Identifies a core network motif that can govern stable intermediate states. [PubMed: 19043543]
85. Li C, Wang J. Quantifying Waddington landscapes and paths of non-adiabatic cell fate decisions for differentiation, reprogramming and transdifferentiation. J R Soc Interface. 2013; 10 20130787.
86. Lo W-C, Zheng L, Nie Q. A hybrid continuous-discrete method for stochastic reaction–diffusion processes. Open Science. 2016; 3:160485.
87. Zhang J, Nie Q, Zhou T. A moment-convergence method for stochastic analysis of biochemical reaction networks. J Chem Phys. 2016; 144:194109. [PubMed: 27208938]
88. Rackauckas C, Nie Q. Adaptive methods for stochastic differential equations via natural embeddings and rejection sampling with memory. Discrete Continuous Dyn Syst - Ser B (DCDS-B). 2017; 22
89. Huang B, Lu M, Jia D, Ben-Jacob E, Levine H, Onuchic JN. Interrogating the topological robustness of gene regulatory circuits by randomization. PLoS Comput Biol. 2017; 13:e1005456. [PubMed: 28362798]
90. Tan TZ, Miow QH, Miki Y, Noda T, Mori S, Huang RYJ, Thiery JP. Epithelial-mesenchymal transition spectrum quantification and its efficacy in deciphering survival and drug responses of cancer patients. EMBO Mol Med. 2014; 6:1279–1293. [PubMed: 25214461]
91. Lawson DA, Bhakta NR, Kessenbrock K, Prummel KD, Yu Y, Takai K, Zhou A, Eyob H, Balakrishnan S, Wang C-Y. Single-cell analysis reveals a stem-cell program in human metastatic breast cancer cells. Nature. 2015; 526:131–135. [PubMed: 26416748]
92. Lönnberg T, Svensson V, James KR, Fernandez-Ruiz D, Sebina I, Montandon R, Soon MSF, Fogg LG, Nair AS, Lilgeito U. Single-cell RNA-seq and computational analysis using temporal mixture modelling resolves Th1/Th2 fate bifurcation in malaria. Science immunology. 2017; 2
93. Proserpio V, Piccolo A, Haim-Vilmovsky L, Kar G, Lönnberg T, Svensson V, Pramanik J, Natarajan KN, Zhai W, Zhang X. Single-cell analysis of CD4+ T-cell differentiation reveals three
major cell states and progressive acceleration of proliferation. Genome biology. 2016; 17:103. [PubMed: 27176874]

94. Proserpio V, Mahata B. Single-cell technologies to study the immune system. Immunology. 2016; 147:133–140. [PubMed: 26551575]

95. Miragaia RJ, Teichmann SA, Hagai T. Single-cell insights into transcriptomic diversity in immunity. Current Opinion in Systems Biology. 2017; 5:63–71.

96. Satija R, Farrell JA, Gennert D, Schier AF, Regev A. Spatial reconstruction of single-cell gene expression data. Nat Biotechnol. 2015; 33:495–502. [PubMed: 25867923]

97*. Jin S, MacLean AL, Peng T, Nie Q. scEpath: Energy landscape-based inference of transition probabilities and cellular trajectories from single-cell transcriptomic data. Bioinformatics. 2018. https://doi.org/10.1093/bioinformatics/bty058bty058. . Presents a method for predicting transition probabilities between cell states via energy landscapes and a statistical physics-based approach.

98. Van der Maaten L, Hinton G. Visualizing data using t-SNE. J Mach Learn Res. 2008; 9:2579–2605.

99. Shaffer SM, Dunagin MC, Torborg SR, Torre EA, Emert B, Krepler C, Beqiri M, Sproesser K, Braddock PA, Xiao M. Rare cell variability and drug-induced reprogramming as a mode of cancer drug resistance. Nature. 2017; 546:431–435. [PubMed: 28607484]

100. Shahbazi MN, Scialdone A, Skorupska N, Weberling A, Recher G, Zhu M, Jedrusik A, Devito LG, Noli L, Macaulay JC, Buecker C, Khalaf Y, Ilic D, Voet T, Marioni JC, Zernicka-Goetz M. Pluripotent state transitions coordinate morphogenesis in mouse and human embryos. Nature. 2017; 122:881.

101. Hayashi K, de Sousa Lopes SMC, Tang F, Surani MA. Dynamic equilibrium and heterogeneity of mouse pluripotent stem cells with distinct functional and epigenetic states. Cell stem cell. 2008; 3:391–401. [PubMed: 18940731]

102. Unternaehrer JJ, Zhao R, Kim K, Cesana M, Powers JT, Ratanasirintrawoot S, Onder T, Shibue T, Weinberg RA, Daley GQ. The epithelial-mesenchymal transition factor SNAIL paradoxically enhances reprogramming. Stem cell reports. 2014; 3:691–698. [PubMed: 25316190]

103. Liu X, Sun H, Qi J, Wang L, He S, Liu J, Feng C, Chen C, Li W, Guo Y. Sequential introduction of reprogramming factors reveals a time-sensitive requirement for individual factors and a sequential EMT–MET mechanism for optimal reprogramming. Nat Cell Biol. 2013; 15:829–838. [PubMed: 23708003]

104*. Briggs JA, Li VC, Lee S, Woolf CJ, Klein A, Kirschner MW. Mouse embryonic stem cells can differentiate via multiple paths to the same state. Elife. 2017; 6:e26945. Introduces models of path dependence relevant to intermediate states and their impact on stem cell differentiation. [PubMed: 28990928]

105. Li Q, Hutchins AP, Chen Y, Li S, Shan Y, Liao B, Zheng D, Shi X, Li Y, Chan W-Y. A sequential EMT-MET mechanism drives the differentiation of human embryonic stem cells towards hepatocytes. Nat Commun. 2017; 8:15166. [PubMed: 28466868]
Figure 1. Identities of intermediate cell states (ICSs)
(A) An ICS (green, asterisk) refers to any phenotypic state lying between traditionally defined cell types (yellow or blue); generic ICSs are referred to as Type 0. (B) ICSs can facilitate cell state transitions in many ways, occupying the same (Type 1) or distinct (Types 2&3) hierarchical levels as other cell states. Complex lineage transitions can be mediated by ICSs (Type 4).
Figure 2. Methods to predict the existence of intermediate cell states (ICSs) from single-cell data

(A) Single cell data projection (e.g. via t-distributed stochastic neighbor embedding (t-SNE)), with cells labeled by subpopulation (C1 to C7). (B) Cells ordered in pseudotime (an unobserved dimension that measures the progress of cell state transitions) by subpopulation. Three classes of ICS are postulated from these data (others are by all means possible): class (i) – distinct in pseudotime (C2), may indicate transitioning state; class (ii) – distinct on a low-dimensional projection (C5); class (iii) – mixture of subpopulations (C6 & C7).