Genomic and molecular control of cell type and cell type conversions

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Abstract

Organisms are made of a limited number of cell types that combine to form higher order tissues and organs. Cell types have traditionally been defined by their morphologies or biological activity, yet the underlying molecular controls of cell type remain unclear. The onset of single cell technologies, and more recently genomics (particularly single cell genomics), has substantially increased the understanding of the concept of cell type, but has also increased the complexity of this understanding. These new technologies have added a new genome wide molecular dimension to the description of cell type, with genome-wide expression and epigenetic data acting as a cell type ‘fingerprint’ to describe the cell state. Using these genomic fingerprints cell types are being increasingly defined based on specific genomic and molecular criteria, without necessarily a distinct biological function. In this review, we will discuss the molecular definitions of cell types and cell type control, and particularly how endogenous and exogenous transcription factors can control cell types and cell type conversions.

Keywords: Cell type, Transcription factor, Epigenome, Transdifferentiation

1. Defining cell type

The cells of an organism are made up of a limited number of ‘cell types’ that are reused in different tissues and combine to form organs and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems. For example macrophages, phagocytic immune cells, are found throughout the body, as are connective tissues and systems.
were discriminated based on their tissue of origin: bursa of Fabricius-derived lymphocytes (bone marrow-derived in mammals) became B cells, and thymus-derived lymphocytes became T cells. However, B and T cells only became recognized as distinct cell types in the 1960s as B cells were definitively identified as the source of the humoral (i.e. antibody) immune response, whilst T cells were initially recognized as ‘B cell helpers’ a few years later. The widespread adoption of monoclonal antibody technology led to a burst of activity in defining further T cell types. The cluster of differentiation (CD) antibodies are a set of defined monoclonal antibodies against a variety of cell surface targets. Two CD antibodies can separate T cells into two distinct cell types: CD4+ T helper cells and CD8+ T cytotoxic cells. T helper cells play a supporting role in immune responses, whilst T cytotoxic cells perform cytotoxic killing of virus-infected cells, importantly, their cell morphology is basically identical and they can only be discriminated by their biological activity and cell surface markers. Further application of monoclonal antibodies and careful flow cytometry experiments divided T helper cells into a wide range of other T helper cell types.

For example, naïve T helper cells, that have not encountered their antigen are defined by the absence of CD25, whilst experienced (those that have encountered their antigen) T helper (Th) cells differentiate into four major types, namely, Th1, Th2, Th17, and Tregs (regulatory T cells), along with many more less well characterized T helper cell types. Importantly, these cell types are not just finer definitions of sub-populations, but each T helper cell type has a distinct biological function. The four best characterized T helper cell types are Th1, Th2, Th17 and Treg cells, which are important in responding to intracellular pathogens, helminth infection, extracellular pathogens, and maintaining self-tolerance, respectively. However, many more T helper cell types have been discovered (e.g. Th9, Th3, TR1, Th22, Thb, nTreg, etc.).

These new T helper cell types have less clear biological roles, but take part in a range of specific activities, including airway inflammation, allergic reactions, B cell responses and immune-related diseases, amongst other roles.

T and B cells were originally defined based on the organ they were first purified from, and the tissue of origin can have a strong influence on cell type. For example, gene expression microarrays of macrophages purified from different tissues showed greater overall variation in gene expression patterns, when compared to other immune cells, or compared to just other lymphoid cells. Dendritic cells (DCs), antigen-presenting cells of the immune system, highlight the opposite problem of separating cell types. DCs and macrophages are challenging to experimentally separate accurately, as they share many of the same cell surface markers. Consequently, there is argument about the difference between macrophages and DCs, and a model has been put forward that suggests DCs and macrophages are a ‘spectrum’ cell type, with phagocytic cells (macrophages) on one end and antigen-presenting cells (DCs) on the other, with several cell types sitting in the middle of the spectrum, each possessing more or less macrophage or DC character. Molecular characterization suggests that, from the perspective of gene expression at least, macrophages and DCs can be distinguished based on a unique gene expression signature, and macrophages and DCs respond differently to inflammatory stimuli. Yet, arguments over the differences between these cell types remains.

3. Heterogeneity in embryonic stem cells; defining cell type by biological function

One of the better studied cell types are mouse embryonic stem cells (mESCs), which are derived from early embryos, and maintain the ability to regenerate a full mouse. Although mESCs have many similarities with the inner cell mass (ICM) of the early blastocyst, particularly in the activity of key transcription factors such as OCT4, SOX2, KLF4 and NANOG, there remains debate about their exact origin and cell type. As the ICM converts to mESCs the cells undergo many gene expression changes. mESCs as a cell culture were thought to be relatively homogenous, yet careful study of mESCs revealed small numbers of cells in a typical cell culture with altered gene expression profiles. In mESCs, the expression level of the essential pluripotency gene Nanog naturally fluctuates, and about 5–20% of mESCs express very low levels. In culture, mESCs cycle Nanog on and off, which helps prime mESCs to differentiate, and so these cells have a distinct phenotype and arguably cell type. Nanog is by no means the only example of heterogeneity in mESCs. STELLA, a marker of primordial germ cells, is expressed in 20–30% of mESCs, and those cells with STELLA more closely resemble the ICM, whilst those without STELLA express developmentally later epiblast-specific genes. Indeed, there are multiple cell types contained within a typical mESC culture, including small numbers of cells with radically different biological function. Normally, mESCs very rarely contribute to extraembryonic tissues, such as the trophectoderm (placenta) or primitive endoderm. However, mESC cultures contain about 15% of cells that are Hhex+ (a homeobox protein that specifically marks endoderm), and these cells can contribute to extraembryonic tissues in mouse chimeras. Although the Hhex+ and Hhex-mESC’s gene expression signature is nearly identical, they have different biological potential, and so can be considered a distinct cell type. One caveat is that these Hhex+ cells still contribute to the epiblast and embryo proper, so it is not a pure population of cells. A rarer subset of cells within mESC cultures express the endogenous retrovirus MERVL. MERVL is specifically expressed at the 2 cell stage of embryonic development, and using a MERVL-Tomato reporter, the ~2% of mESCs that express MERVL can contribute to extraembryonic tissues, although again, the MERVL+ cells can also contribute to the embryo proper, and the cells can interconvert between MERVL+ and MERVL- cells, suggesting instability in their cell type. It was initially thought that these MERVL expressing cells closely resemble the 2 cell (2C) stage of the embryo, where MERVLs are also specifically expressed, however, recent single cell RNA-seq data suggests these 2C-like cells may more closely resemble the blastocyst, so their ultimate identity remains unclear. Ultimately, the relationship between all of these heterogeneous cell types or sub-cell types within mESC cultures remains unclear.
Hhex+ cells to contribute to extraembryonic tissues, it is unclear how they are related to each other, along with other potential cell types revealed by single cell genomics.35

Ultimately mESCs are an in vitro artifact, a ‘trapped’ version of the blastocyst ICM that can grow indefinitely, but still maintain pluripotency. It is possible to capture many additional embryonic cell types, of which some appear to represent earlier timepoints in the developmental process. One such cell type are ‘Extended pluripotent stem cells’ (EPSCs), that can contribute to extraembryonic tissues, and have distinct gene expression compared to mESCs.44 Other embryonic cell types appear to be developmentally later than mESCs, such as Epiblast stem cells (EpiSCs), that more closely resemble the developing epiblast and have a primitive endoderm-like gene expression signature.45,46 and lack Esrrb activity.47 The similar but distinct EpiLCs (epiblast-like cells), lack the endoderm-like gene expression signature,45,46 and lack Esrrb activity earlier than mESCs, such as Epiblast stem cells (EpiSCs), that more closely resemble the developing epiblast and have a primitive endoderm-like gene expression signature.45,46 and lack Esrrb activity.47 The similar but distinct EpiLCs (epiblast-like cells), lack the endoderm-like gene expression signature found in EpiSCs, and are instead biased towards a primordial germ cell fate.48,49

Finally, region-selective EpiSCs (rsEpiSCs) are biased to colonize just the posterior part of the developing embryo, suggesting an even later developmental phenotype than EpiSCs.50 These and other embryonic cell types indicate that at specific stages, with the right conditions, transient cell types can be captured and maintained in vitro.51

4. A continuum of cell states in the transitions between cell types

It is challenging to define at what point two cell types are distinct, and where two cells are simply at one end of a continuum. Single cell data suggests that cells can transit through stages where the cell type-signatures of both origin and destination cells are simultaneously present. For example, in developing lung, some cells express markers for both alveolar type 1 and 2 cells simultaneously,52 and early in the embryo some cells simultaneously express genes for the primitive endoderm and epiblast.53 Consequently, identifying cell types in developmental processes is challenging. Potentially there is a continuum of expression as cells pass through developmental stages, and at any one point along that process the cell is not stable and may collapse into a more stable and distinct cell type (Fig. 2). Single cell mass spectrometry of cell surface markers in developing human B cells revealed a continuous spectrum of B cell development stages, rather than specific barriers,54 something similar was seen for in vitro differentiation of cells to neurons,55 and in in vitro transdifferentiation of cells to myoblasts.56 This calls into question the existence of cell types during development and, instead of development proceeding in jumps across energy barriers to local energy minima (or distinct cell types), cells develop in a continuous manner with intermediate stages where cells can continue to choose their developmental outcome (Fig. 2). Crucially, as cells differentiate to alternate cell types they lose developmental potential, and consequently most, if not all, adult cells cannot transfertiate.57 There appear to be many epigenetic blocks that lock cells into a specific cell type and limit the cells capability to dedifferentiate and transfertiate.58

A major candidate for the control of cell type is transcriptional control, which may act to lock cells into a cell type.

5. Transcriptional control of cell type

Cell type is thought to be controlled through the activity of transcription factors (TFs), that respond to either internal or external cellular cues.59 TFs bind to DNA and regulate gene expression, and interact with local chromatin to control cell type. Although a comprehensive model describing exactly how TFs perform these feats remains frustratingly elusive.60

TFs can be expressed in both a cell type-specific and cell type-independent manner. Many, about 60%, of TFs are cell type-specific.61 Cell type-specific TFs can function as ‘master regulators’, a class of TF that can specify cell type in the absence of any other activity. The prototypical example is MyoD (Myf4), which when overexpressed converts fibroblasts to myoblasts,62 activating an entire gene expression program in the absence of specific external cues (Fig. 3A).

However, a single master regulator for each cell type seems to be a relatively rare phenomenon, and often the same TF can act in multiple cell types. For example, knocking down Gata3 in mouse embryos leads to a failure to establish mature blastocysts, likely due to a trophectoderm defect,63 as when Gata3 is overexpressed in mESCs it drives them to a trophectoderm cell fate.64 Yet, despite its importance in the early embryo, Gata3 is also a critical factor in the specification CD4+ T cell cells.65 A further difficulty with the idea of master regulators is tremendous degeneracy in the DNA sequences that individual TFs use to bind to DNA. For example, the homeodomain TFs all bind to a similar version of the same sequence of DNA,66 despite the involvement of homeodomain proteins in a wide range of developmental processes. This is not restricted to just one family of TFs, as almost all TF families bind to very similar DNA motifs,67 leading to the vexing issue of finding cell type-specific activity between different TFs that bind to the very similar sequences of DNA. One solution is for pairs (or more) of TFs to combine together to specify a developmental process. For example, the combination of OCT4-SOX2 is critical for pluripotency,68 but OCT4-SOX17, binding to a slightly different DNA motif acts to specify primitive endoderm,69 whilst another OCT/POU-family containing complex, BRN2-SOX2, specifies neural progenitors22 (Fig. 3B). Complex cell type specific assembly of TF complexes is not limited to OCT/POU-SOX factor pairs, as GATA1, GATA2 and PU.1 can assemble on a variety of specific DNA motifs to direct erythroid and neutrophil cell fates.71 TF–TF pairing appears to be widespread; a systematic analysis of genome-wide TF binding discovered 603 potential constrained TF–TF pairs,72 suggesting a combinatorial code that adds complexity to regulate the diversity of cell types and biological processes.

TFs that have a cell type-independent pattern of expression might not seem a promising area to explore for cell type-specific control, but these TFs can also exert specificity in the correct setting. Around 30% of TFs are cell type-independent at both the RNA,73 and protein level.61 It might seem that these TFs are involved in basal cell activities, and indeed many are,23 but cryptically, many cell type-independent TFs can have highly cell type-
specific function. For example, STAT3, despite being expressed almost uniformly in cells and tissues, specifies pluripotency in mESCs, an anti-inflammatory response in macrophages, a pro-inflammatory response in dendritic cells, and has a critical role in T helper 17 cells type differentiation, amongst many other cell type-specific roles (Fig. 3C). Ultimately, many TFs have widely overlapping functions in multiple cell types, and as yet, no comprehensive model of TF cell type control exists.

6. Exogenous expression of transcription factors can drive conversion of cell type

TFs have been instrumental in the forced conversion of one cell type to another. The earliest use of a TF to drive transdifferentiation was the transfection of Myod1 (MyoD), to convert cells to myoblasts, and Cebpa and Cebpβ to convert B cells into macrophages. However, the most dramatic demonstration of the power of TFs was the conversion of fibroblasts to mESCs using just four TFs: OCT4, SOX2, KLF4 and c-MYC. Since this breakthrough many other transdifferentiation protocols have been discovered, along with the use of small molecules to convert cell type, for example the conversion of fibroblasts to neurons or fibroblasts to mESCs. Intriguingly, many of the small molecules used in these protocols directly interfere with epigenetic control, such as DZNep (methylilation inhibitor), VPA (histone deacetylase inhibitor), or Tranylcypromine (histone demethylase and monoamine oxidase inhibitor), indicating that epigenetic control is a major factor in the determination of cell type.

Transdifferentiation protocols mediated by TFs nonetheless remain relatively few, and there are many target cell types we would like to in vitro differentiate, but cannot. Consequently, there has been a lot of activity in designing systematic computational approaches to predict candidates and improve existing approaches. Many approaches attempt to identify cell type-specific genes, as these are relatively easy to identify, and their specific presence in a cell type is often (although by no means always), indicative of function. Computational efforts to identify transdifferentiation factors has included modelling development onto patterns of gene expression, and approaches to discover ‘core’ TFs that are both cell type-specific and expressed at high levels. Magnify used a cell ontology tree to map cell type-specific genes against their developmental pattern and so identify TFs specific to a developmental lineage. Magnify also includes nearest neighbour protein–protein interactions to overcome limitations in discovering cell type-independent TFs. This technique was very successful in discovering previously known transdifferentiation TFs, and was used to predict and then validate TFs that transdifferentiated keratinocytes into endothelial cells. CellNet describes another approach using vast amounts of microarray data to build cell type-specific gene regulatory networks, and then to apply these networks to predict cell type-specific regulatory modules, and so candidate TFs for transdifferentiation. CellNet set out to solve a common problem in transdifferentiation and differentiation experiments where the differentiated cells fail to completely silence the gene expression program of the originating cell type, and remain immature. CellNet was successfully used to improve the transdifferentiation of B cells to macrophages, and also identified an alternate colon cell fate for cells that were transdifferentiating to hepatocytes. Pairs of TFs often antagonize each other’s function, hence pairs of TFs with opposing gene expression in two cell types could be used to predict master regulators of lineages. Methods that combine gene expression data with epigenetic data have also been successful in predicting transdifferentiation TFs. Another approach extended the discovery of cell type-regulatory modules by looking at gene expression in other mammalian species, and discovered many primate-specific long non-coding RNAs (lncRNAs) with putative cell type-specific functions. Indeed, lncRNAs are also expressed in a cell type-specific pattern, and are good candidates for cell type-specific control. However, a comprehensive explanation of how TFs, lncRNAs, and other non-coding RNAs can control cell type, and why transdifferentiation is rare in the adult organism remains unclear. One possible solution to this problem are developmental landscapes and cellular pathways that describe the routes cells can traverse to alter cell type.

7. Developmental landscapes

The concept of cell type is a powerful and attractive model to explain how a limited supply of information can encode a wide
array of complex developmental patterns. Development is a highly-ordered process marked by major stages of cell differentiation during gastrulation that establish the three somatic germ lineages of the mesoderm (blood, muscle), endoderm (lung, digestive tract) and ectoderm (skin, brain). As cells take part in development they differentiate to specific cell types and lose developmental competency. What mechanistically underlies these cell type conversions in development is less well understood. What is clear is that combinations of interconnected genes form ‘gene regulatory networks’ (Fig. 4A), and the genes and proteins regulate each other at multiple levels to maintain a semi-stable developmental competency. What mechanistically underlies these cell type conversions in development is less well understood. What is clear is that combinations of interconnected genes form ‘gene regulatory networks’ (Fig. 4A), and the genes and proteins regulate each other at multiple levels to maintain a semi-stable cell type. But what ultimately builds Waddington’s epigenetic landscape, and Cook’s islands, still lack robust biological mechanisms that can explain all aspects of cell type stability separated by expanses of unstable sea (Cook’s islands) (Fig. 4B). Gene regulatory networks underlying cell types may function as ‘attractors’ for cell types to cluster around, similar to probabilistic cell state maps that can construct landscapes for cells, or cellular network entropy, or landscapes constructed based on molecular similarity. Ultimately, conceptual ideas such as Waddington’s epigenetic landscape, and Cook’s islands, still lack robust biological mechanisms that can explain all aspects of cell type control. Specifically, why there is a limited number of cell types at all, why there are so many unstable intermediary states between cell types, why some cell types are stable and some are not, and why there are strict limits placed on cell type transdifferentiation. Nonetheless, it is becoming possible to model the organization of cell type on a large scale, even if we cannot as yet understand the process in detail. New technologies will continue to be applied to this problem; its deeper understanding will have important implications for understanding cellular regeneration and ultimately tissue regeneration and how these techniques can be applied to the understanding of development and human disease.

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