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# Transdifferentiation: do transition states lie on the path of development?

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## Introduction

The direct conversion of one differentiated cell fate into another identity is a process known as transdifferentiation (Td). It has been hypothesized, however, that most, if not all, converting cells go through an intermediate state and that the 'direct' aspect of Td is not as clear-cut as previously thought [27]. During Td, cells were thought to pass through transitional states resembling those seen during development. For many examples of Td this holds true as, for instance, fibroblasts undergoing Td to neurons pass a progenitor-like state which, at the level of their gene expression profile, partly resembles a state seen during normal development [41]. Transitional states that are different to those in development also occur. For example, during pancreatic alpha- to beta-cell conversion, mixed-state cells express markers for both alpha- (glucagon) and beta-cells (insulin) following beta-cell ablation *in vivo* [40].

Understanding the states that cells pass through during Td is crucial given its potential application in regenerative medicine and disease modeling. However, one important question pertaining is whether cells pass through an intermediate state and, whether such cells could undergo uncontrolled conversion or proliferation and pose a risk for patients. To answer this, researchers have begun to analyse the transcriptomes of donor, converting and target cells to explore the mechanisms of Td. By doing so, one can also identify the barriers that prevent Td and, if conversion is occurring, the degree to which the original fate is lost. These efforts have been greatly assisted by the development of single cell RNA-sequencing (RNA-seq) technologies. Studies can now compare transitional states occurring during Td to those found along the path of development at a single cell level.

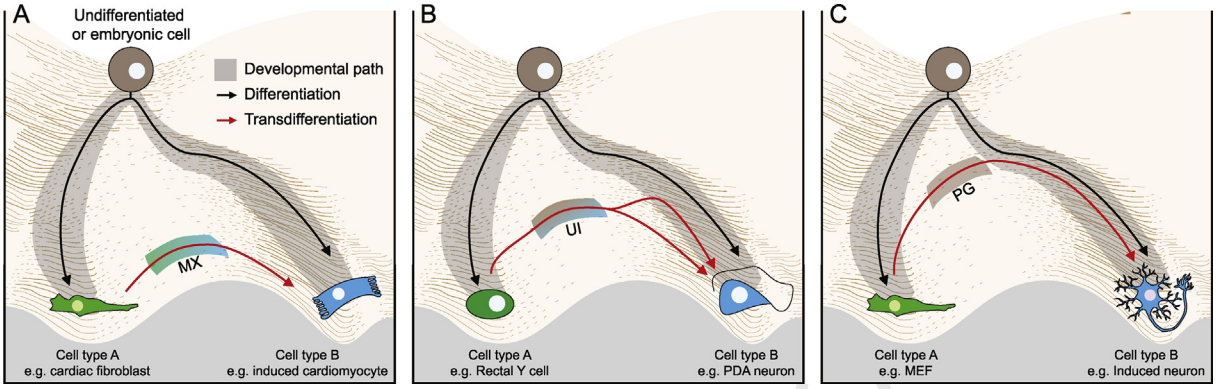
Here, we review several key examples of Td that were studied in a range of model systems and organisms. We propose that cells pass either through a mixed, unspecific intermediate or progenitor-like state during the course of Td, which, to varying degrees, resemble states seen during development (Figure 1).

It is important to note that the Td transition states defined here broadly reflect a complex process and cells undergoing Td may lie at the intersection of these definitions. The nature and complexity of transitional states observed during Td are likely to be influenced by donor- and target-cell types, as well as the environmental context. We also briefly discuss the role of terminal selector transcription factor(s) (TFs) and highlight the need to carefully assess the degree to which the original fate is turned off during Td. Overall, our understanding of Td at the single-cell level will be crucial for characterising transitional states observed during Td.

## Mixed states during Td

With the concomitant loss of one fate and the gain of another, a converting cell will pass through a mixed state where both fates are present. How a cell navigates this change in identity, and emerges as a differentiated cell with a new fate, is quite remarkable given the fact that this state would be likely absent during development. Moreover, many Td events are initiated by only a single or few TFs which initiate a program leading to target fate acquisition. For example, expression of Mef2c, Gata4 and Tbx5 (MGT) leads to Td of mouse cardiac fibroblasts to induced cardiomyocytes (iCMs) via several states defined by Liu et al., as intermediate fibroblast, pre-iCM and iCM [19]. Single-cell transcriptome analysis suggests that the pre-iCM state is unstable and represents a 'mixed' state where both cardiomyocyte and fibroblast-specific markers are expressed [19]. This observation was mirrored at the protein level. After induction of mouse fibroblast to iCM conversion upon MGT expression [36], a mild decrease,

**Figure 1**




Converting cells can deviate from the path of development. Cells undergoing Td can pass through (A) mixed 'MX' (B) unspecific intermediate 'UI' or (C) progenitor-like 'PG' transition states. Figures are modified versions of the Waddington landscape [4].

of fibroblast-specific genes Col1a1 and Col1a2 was observed at the 48–72 h time point [36]. If these cells access developmental programs to achieve conversion, one would expect to detect markers for a progenitor-like state. During fibroblast to iCM conversion, the early cardiac progenitor marker Isl1 and the pan-cardiovascular progenitor marker Mesp1 were not activated, suggesting that Td did not pass through a progenitor-like state [11]. However, in other cases of Td, a mixed state with precursor-like properties exists. For instance, endogenous 'hybrid' $CD4^+$ T cells produce cytokines characteristic of different lineages and, from these cells, multiple cell fates arise as reviewed elsewhere [20].

Interestingly, a mixed state during Td might be the result of a mechanism where the original fate is switched off passively. That is, as the new fate is established, cells fail to maintain the original fate and it 'fades' away over time. This likely occurs because the gene expression program of the target fate dominantly recruits the transcriptional machinery to genes specific to its own fate. To address this hypothesis and tease apart mechanisms of Td, mixed states must be characterized in more detail with single-cell resolution.

## Unspecific intermediate states during Td

During Td, cells may lose their original identity prior to acquiring a new fate and this can be interceded by an intermediate state that does not resemble donor nor target fates. Unspecific intermediate states may display aspects of stemness, but do not revert completely to a stem cell-like state. In the nematode *Caenorhabditis elegans*, cells can be traced easily due to its transparent body and highly invariant lineage. In-depth characterization of an endogenous Td event has begun in the Jarriault laboratory where a post-mitotic and functionally differentiated epithelial Y cell of the rectum disengages from the rectal tube, migrates and finally converts to a motor neuron termed PDA [13,48]. During Td, the Y cell de-differentiates, passes through an intermediate state and then redifferentiates into a motor neuron termed PDA. The observed intermediate state does not show marker expression for either the rectal Y (LIN-26) nor the neuronal PDA fate (*cog-1*) [34]. To test whether these cells reverted to a bona fide pluri or multipotent state, they were challenged with transient expression of the cell fate-inducers *hlh-1* (muscle), *end-1* (endoderm), *lin-26* (epithelial) and *unc-30* (GABAergic neurons), but no detour to a new identity was observed [34]. Interestingly, it was later shown that NODE (Nanog and Oct4-associated deacetylase) activity was required for Y cell-to-PDA Td in *C. elegans* [15]. The homologs of NODE complex members, including CEH-6/Oct4 and SOX-2/Sox, are known pluripotency factors in mammals [39]. It is therefore possible, that intermediate cells observed during Y cell-to-PDA Td have reverted to a progenitor-like state with restricted potential, but are distinct from bona fide progenitor-like intermediates.

Another interesting example is the CCAAT/enhancer binding protein alpha (CeBPα) induced conversion of pre-B cells into macrophages. During this Td, cell-surface marker combinations that are characteristic for hematopoietic stem and progenitor cells, such as c-Kit and FMS-like tyrosine kinase 3, were essentially not observed and expression of pluripotency factors Oct4, Nanog and Sox2 was not detected [5]. Cells undergoing pre-B cell-to-macrophage Td show expression of genes specific for the B cell (*Cd19*) and macrophage (*Mac1*) fates being present and, therefore, also display aspects of a mixed transitional state [5,46].