DEVELOPMENT

Cell fate decisions during development

Cell differentiation involves activation of mutually exclusive genetic programs

By Roberto Mayor

The shape of our nose, the color of our skin, the movement of our gut, all depend on an extraordinary cell type, called neural crest cells, which originate during embryogenesis. Since their discovery in 1868 (*1*), neural crest cells, which are present in all vertebrates, have fascinated developmental biologists (*2*). One of the amazing features of neural crest cells is their extraordinary multipotency: they form cartilage, muscle, neurons, glia, pigment cells, adrenal cells, and so on. (*3*). No other embryonic cell type can differentiate into so many different kinds of cells. However, how this multipotency is achieved is not understood. On page XXX of this issue, Soldatov *et al.* (*4*) clarify some of the mechanisms that explain how the multiplicity of cell types is generated by neural crest cells.

The neural crest is an embryonic cell population that is initially formed in an embryonic tissue layer called the ectoderm. The ectoderm will also form the neural tube, which later becomes the central nervous system. The neural crest is formed adjacent to the neural tube, in a region called the neural plate border, from where cells delaminate, migrate to colonize different tissues and then differentiate (*3, 5*). It has been shown using genetic labelling that neural crest cells are multipotent when they leave the neural tube and their fate is decided after delamination (*6-8*), but how this multipotency is controlled has remained elusive.

Once neural crest cells are formed in the ectoderm, one of the first steps in their development is to delaminate and undergo an epithelial-to-mesenchymal transition (EMT), which is required for their migration (*9*). The classical view for neural crest EMT is that this is an abrupt process that results from the activation of a gene regulatory network (*10*). Soldatov *et al.* show that this is not the case, as they are able to resolve a sequence of stages around delamination, demonstrating that pre-EMT neural crest cells express genes associated with neural plate border and neural tube identity. More advanced neural crest cells downregulate the expression of neural tube markers and increase the expression of neural crest cell-specific genes. These results indicate that the transition from premigratory to migratory neural crest cells is more gradual and complex than initially thought.

This view is consistent with recent reports showing that EMT in cancer cells is not an all-or-nothing process, but a complex event with many steps controlled by different genes: Different cells undergoing EMT activate different aspects of the gene expression program at different times (*11*). The similarities between cancer cell EMT and developmental EMT support the notion that cancer cells hijack the EMT program used during development (*11*). Although the idea that EMT in the neural crest is not an abrupt process has been previously suggested (*12*), Soldatov *et al.* provide solid molecular evidence from single cell RNA-sequencing data (transcriptomics) with spatial and lineage tracing in mouse neural crest differentiation. This approach has allowed the identification of substages of EMT during delamination, characterized by the expression of specific markers.

One of the most intriguing conclusions from Soldatov *et al.* is the identification of specific steps involved in neural crest differentiation, in which progenitor cells undergo binary choices between two possible fates as a result of their cellular history. This history is defined by the set of internal and external events that the cell has experienced, such as the autonomous activation of genes and signals coming from neighbor cells, respectively. Progenitor cells initially co-activate gene expression programs that lead to competing cellular fates (see the figure). These mutually exclusive cell fate programs then compete with each other. This competition is determined by differences in gene expression caused by historical changes that impact the transcriptome. Cells then upregulate one program and downregulate the other after a decision point (bifurcation), and consequently become committed to a specific fate. Thus, by inducing competing gene expression programs the cell fate commitment process starts to look like a sequence of biasing factors that pull the cells in different directions depending on their own history. It is likely that an interplay between these intrinsically developing biases interact with extrinsic cues from the environment to shift the bias into a particular cell fate. This model challenges the current view in which neural crest cells abruptly activate only one of many alternative cell fate programs, leading to cell differentiation.

This revised vision for neural crest cell differentiation is consistent with what has been proposed for the differentiation of other cell types, such as those of the hematopoietic lineage (*13*). The first stable bifurcation identified in neural crest differentiation separates progenitors of the sensory lineage from those of autonomic and mesenchymal fates. This is followed by additional binary decisions that separate autonomic neuronal fate from mesenchymal differentiation. This contrasts with the current view in which a single precursor differentiates directly into specific cell types. In addition, Soldatov *et al.* show that many transcription factors considered as "master regulators" for specific lineages are not expressed at the time of the bifurcation to differentiate into these three lineages. This suggests that the activation of specific gene expression programs around the bifurcation point is triggered not by these master regulators, but by environmental conditions, such as chemical or mechanical cues (*3, 14*).

Soldatov *et al.* also compare cell differentiation between neural crest formed in the head (cephalic) or the trunk of the embryo; showing that after delamination the transcriptional signature that distinguishes the neural crest along the anterior-posterior axis of the embryo is erased, activating cell fate-specific gene expression programs. A mesenchymal program is activated in the cephalic neural crest, whereas a neuronal program is activated in the trunk.

The study of Soldatov *et al.* represents a supreme example of the use of single-cell analysis combined with spatial transcriptomics to address the question of cell differentiation in a heterogeneous cell population, such as the neural crest. Due to this cell heterogeneity, the single-cell analysis becomes essential and the possibilities to apply similar approaches related to different aspects of neural crest development are enormous. For example, it has been proposed that neural crest behavior is different among different species (*15*). Comparing neural crest differentiation across different species could not only provide valuable insights into this conundrum, but it could also

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reveal how neural crest originated during evolution. A comparison between normal neural crest and neural crest taken from embryos in which extracellular signals have been modified will provide valuable information about the role of external cues on neural crest differentiation. In addition, comparing neural crest between normal individuals and patients with neurocristopathies (pathologies associated with defective neural crest development) could clarify the origin of these many diseases. The door is open to unravelling many of the mysteries that have surrounded the neural crest for more than 150 years.

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Models of cell differentiation

The classical versus new model of neural crest differentiation is depicted on a Waddington's landscape. Marbles represent cells differentiating as they roll down the hill.