underestimated if the model grid resolution is coarser than the 1-km spacing that they use in their simulations.

Larour *et al.*'s fine-scale treatment of the feedbacks between glacier retreat and solid-Earth processes shows that if projections of sea level rise ignore these feedbacks, they will be systematically too high. However, their results also show that these feedbacks are not likely to be very important over the coming century, but only on a time scale of a few centuries. This is because a very substantial mass loss must occur before the quantitative differences in model behavior become meaningful. In a sensitivity experiment, the authors find that by the year 2100, solid-Earth feedbacks would reduce mass loss from the Thwaites Glacier by only about 1%. By the year 2350, these feedbacks would reduce grounding line retreat by about 40% and would reduce Thwaites Glacier's contribution to sea level rise by more than 25%. Recently revised estimates of the mantle viscosity under the West Antarctic Ice Sheet (*11*), not accounted for by Larour *et al.*, imply that these numbers are conservative, probably by about a factor of 2.

For those concerned about potentially catastrophic sea level rise, the results of Larour *et al.* might be taken as welcome news. But it is important to recognize that Larour *et al.* do not make a specific prediction. There are too many unknowns about the topography of the glacier bed at the finest spatial scales, the process of glacier calving, and how winds and ocean currents will change. Rather, the results should serve as a guide to the magnitude and sign of the uncertainty in existing predictions, and as a road map for future research. Accounting for solid-Earth feedbacks suggests that although the greatest effects may be delayed by a few decades, Antarctic ice sheet retreat remains virtually certain. ■

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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 971 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 stem 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 labeling that neural crest cells are multipotent when they leave the neural tube and that 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 of 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 down-regulate the expression of neural tube markers and increase the expression of neural crest cell–specific genes. This indicates 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 rather 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*). Although the idea that EMT in the neural crest is not an abrupt process has been previously suggested (*12*), Soldatov *et al.* provide molecular evidence from single-cell RNA-sequencing data combined with spatial transcriptomics and lineage tracing in mouse neural crest differentiation. This allowed the identification of substages of EMT during delamination, characterized by specific marker expression.

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 possible fates as a result of their cellular history. This history is defined by the internal and external events that the cell has experienced, such as the autonomous activation of genes as well as signals from neighboring cells. Progenitor cells initially coactivate 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 affect the transcriptome. Cells then up-regulate one program and down-regulate the other after a decision point (bifurcation). 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 interacts with extrinsic cues to shift the bias into a particular cell fate. This model challenges the cur-

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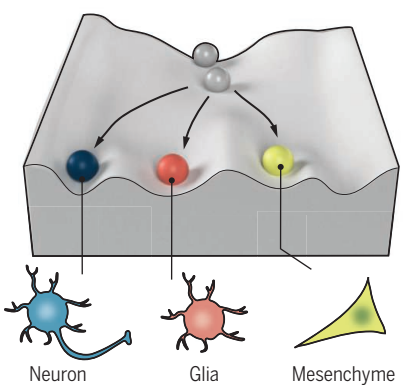
rent view in which neural crest cells abruptly activate only one of many alternative cell fate programs, leading to cell differentiation.

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*).

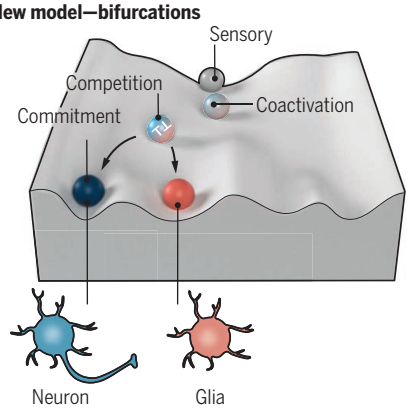
Models of cell differentiation

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

Classical model



New model—bifurcations



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. The possibilities of applying 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 also 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 in 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 diseases. The door is open to unraveling many of the mysteries that have surrounded the neural crest for more than 150 years. ■

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CANCER

Mutated clones are the new normal

Measuring and understanding the dynamics of clonal cell populations is key for cancer prevention

By **Cristian Tomasetti**

Cancers are formed by the expansion of harmful "mutational clones," which are cell populations carrying the same DNA mutations. How harmful they are depends on which mutated genes they contain. On page 970 of this issue, Yizhak *et al.* (*1*) add to the evidence that normal tissues are not so normal after all. Examining a substantial number of healthy tissues from almost 500 individuals, they found a large number of acquired (somatic) DNA mutations—some of which are typically associated with cancer—and mutational clones of macroscopic size, in the absence of cancer pathology. The presence in normal tissues of clonal cell populations, with mutations in cancer-associated genes, is informative about how a tumor evolves from the first mutation to a benign growth and, finally, to cancer.

Initial evidence that suggested the presence of several mutations in healthy cells came from mathematical modeling and statistical analyses of cancer DNA sequencing data to compare the mutational load in human cells before and after tumorigenesis (*2*). Independently of the specific estimates, the analysis provided indirect evidence that a substantial fraction of the somatic mutations found in cancers would have been present in those cells even in the absence of cancer (*2*). Subsequently, targeted DNA sequencing studies of normal tissues provided direct evidence for the presence of large numbers of somatic mutations and large numbers of microscopic mutational clones in the small sets of cancer-associated genes that were analyzed in

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Cell fate decisions during development

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