Summing up single cell behaviors

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Development relies on orchestrated cell behaviors. The striking precision and robustness observed in a wide range of developmental processes have puzzled biologists for a long time. In interesting contrast, molecular interactions and single cell behaviors are often variable and error-prone (1,2). Many signaling molecules and cell-cell interactions have been identified to regulate these behaviors and consequently affect development. However, it remains unclear how the actions of single cells are summed up at the population level to produce the phenotypes under either normal or mutated conditions. This gap of understanding holds back the engineering of stem cells into useful tissues and organs.

To understand how the summing-up occurs, the processes must be closely observed and then analyzed at single cell resolution in a systematic and quantitative manner. Zebrafish embryos are suitable for this goal because of their transparency, accessibility, and largely conserved developmental processes shared with other vertebrates. Using this model system and the emerging tools that enable acquisition and processing of "in toto" datasets (3-5), my graduate thesis focused on two challenges the embryo - or the collective cell community - has to solve in its course of becoming a fish: the first is to make a thin envelope, and the second is to raise a French flag.

In the early blastula fish embryo, the single layer of cells on the surface becomes a squamous epithelium that serves as a permeability barrier and contributes to gastrulation movements (6). This enveloping layer (EVL) contains cells that adopt a highly flattened shape. To understand how this cell shape is created, I imaged the cells as they become more flattened on average over time and extracted the division patterns in the form of a lineage tree (7). This analysis suggests that the change of cell number on the surface is mainly responsible in explaining the flattening: the fewer cells there are the more "stretched-out" each of them is in order to fully cover the embryonic surface. The cell number increases by divisions that keep both daughters on the surface (the S-S divisions), while the cells get smaller. In the population, some cells undergo S-S divisions while others undergo S-D divisions (one daughter leaving the surface). The fraction of S-S over S-D divisions thus regulates cell number and consequently the cell shapes.

To find out how a cell chooses its division type (S-S or S-D), I tracked single cells throughout the cell cycle and realized that the division type is correlated to cell shape: a flattened cell will undergo S-S whereas a columnar cell will undergo S-D. This means that the cell shape distribution of the population essentially determines the division pattern. We then constructed a mathematical model to simulate this evolving relationship between cell number and cell shape. Interestingly, this model predicts that the flattening process will be robust to changes in parameters such as total embryonic surface area or cell volume. The model also suggests that by changing the relationship parameter between cell shape and division choice, epithelial cell shapes ranging from columnar to squamous can be obtained. Both predictions stood our experimental tests (7).
In another line of research, I looked at the classic problem of morphogen patterning in the neural tube. In this case, the inductive signal sonic hedgehog (Shh) secreted by ventral structures such as the notochord and floor plate induces differently fated neural progenitors that appear as a multi-stripe "French flag" pattern (8). The amount of Shh signaling, which goes down as the distance from the source increases, determines fates (i.e., motoneurons, different types of interneurons). This prevailing model does not explain how the process is robust to the cell movements and signaling noise that are pervasive during neural tube patterning and morphogenesis. To address this question, I captured zebrafish neural tube formation in its entirety (9). These ~12 hour long datasets recorded overnight have a time resolution of 2 minutes allowing tracking of single neural progenitors throughout their specification and migration. These tracks give me access to the detailed cell history of reporter expression, position, division and other parameters. Putting the tracks together, I found that progenitors of different fates are specified early and extensively mixed as the neural tube undergoes morphogenesis. The clean-cut stripe pattern then emerges by cell-cell interactions leading to sorting after Shh laid down a rather noisy, mixed initial pattern (9).

The existence of cell sorting would mean that the requirement of signaling precision could be relaxed - given that a cellular correction mechanism is in place. We tested this prediction by comparing the reporter expression dynamics in single progenitors (10). Indeed, some progenitors that show nearly identical Shh reporter dynamics end up choosing different fates, whereas some that show very different signaling converge at the same fate.

In conclusion, my graduate work reveals how the interactions between cells enable the population to sum up the behaviors of individuals - who are poorly informed and only follow simple rules - to achieve robust and precise solutions to developmental goals. In the enveloping layer, each cell chooses its division outcome by measuring its own shape, which is regulated by the previous division outcome and cell-cell mechanical coupling (see Figure, A). This feedback cycle steadily links the thinning of the tissue - a global property - to the division orientation threshold which is a single cell property changeable by molecular regulation. In the neural tube, cells are inevitably specified in "wrong" locations in the presence of signaling noise and cell movement, but are able to self-correct via cell-cell interaction, possibly by affinities that are associated with fates (see Figure, B). This logic implements the precision of patterning in the pattern itself, thus eliminating the influence of positional and molecular noise. These works identify the super-cellular level mechanisms and logic that sum up behaviors of single cells. By exploiting the intercellular interactions the embryos overcome the constraint of limited single cell functions to promote precise and robust development in the ever changing environment.
(A) The flattening of surface epithelium of zebrafish embryos through an interplay between cell shape and divisions, see also ref.7. i. Organization of the system, the blue cells are surface cells lining along the exterior (marked by dashed line), the red cells are deep cells. The surface cells have different shapes and together the shapes form a distribution. ii. During cell cycles, the surface cells choose division orientations based on their shapes, resulting in surface-surface (S-S) divisions that increase cell number on the surface and surface-deep divisions (S-D) where a daughter cell leaves the surface. In this way the cell shape distribution determines cell number change during the cell cycle. iii. After the divisions, the surface cells couple with each other to homogenize cell shapes to arrive at a new distribution. Through this cycle the average shape change of the population (e.g., flattening) occurs as a function of the parameter linking cell shape and division outcome. (B) Cell sorting promotes patterning precision in the ventral neural tube, see also ref. 9. i. The naive progenitors (circles) move around in the forming morphogen field. Both the signal exposure and cell position are noisy. ii. Specification of progenitors based on their signaling exposure history is consequently mixed and the target "French flag" pattern is imprecise initially. iii. Cell sorting rearranges the specified progenitors into a sharp pattern. While its molecular mechanisms remain to be characterized, the cell sorting process offers a
solution to ensure pattern precision in the presence of inevitable noises of signaling and cell position.

References


