Modeling Pattern Formation: Counting to Two in the Drosophila Egg

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The EGF receptor pathway patterns the Drosophila egg and specifies the position of its dorsal appendages. A new mathematical analysis of this patterning network has highlighted its crucial features and provided novel insights into the spatial and temporal kinetics controlling patterning.

During development, fields of equivalent cells differentiate into an organized pattern of distinct tissue types. A common patterning principle that employs morphogen gradients is repeatedly used. Morphogens are signaling proteins that can induce several distinct cell fates in a concentration-dependent manner. The diffusion of a morphogen from a localized source generates a concentration gradient which induces position-dependent cell fates across the developing field.

Since the concept of morphogen gradients was first put forward by Lewis Wolpert over thirty years ago [1], numerous morphogens have been identified across all developmental systems studied. The emerging in vivo situation, however, is significantly more complex than the elegant gradient mechanism originally proposed [2,3]. It appears that, although patterning is indeed induced by graded activation of a signaling pathway, this gradient is not determined solely by diffusion of the morphogen. In certain cases, the actual diffusion of the morphogen is tightly regulated and involves its interaction with other proteins. Determination of the activity gradient generally employs a network of proteins. Such networks may include both activators and inhibitors of the signaling pathways, and in many cases they display positive or negative feedback loops resulting from transcriptional or post-transcriptional regulation.

Patterning networks may display complex spatial and temporal dynamics. It is precisely these dynamics, however, that generate the eventual organ shape. Rigorous mathematical tools are thus required to elucidate the properties of a network, analyze its behavior in detail and verify the consistency of proposed molecular mechanisms with the accumulating genetic and phenotypic data. Such approaches are still missing, but a recent paper by Shvartsman et al. [4] presents an important advance in developing quantitative methodologies for investigating patterning systems. The authors chose to study the molecular mechanisms responsible for positioning of the paired organ in the egg — the ‘dorsal appendages’ — that supplies the developing Drosophila embryo with oxygen. This system provides an excellent example of how a simple stimulus can define a complex pattern in development. The molecular network that mediates this patterning is arguably one of the best-studied developmental networks at the genetic and biochemical levels.

Development of the Drosophila egg chamber is an intricate, highly orchestrated chain of events that take

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Patterning is achieved through the action of a conserved signaling cassette composed of several ligands with activating or inhibiting functions. The cardinal ligand used in most systems is Spitz, a homolog of the signaling molecule TGFα. Spitz is uniformly expressed in most developing tissues, but its activity is tightly regulated by the Rhomboid protein, which is required for processing of the inactive Spitz precursor into its active form [10–12]. Rhomboid may function as the protease that cleaves Spitz [13]. Regulation of Rhomboid expression is thus the key for spatial and temporal control of EGF receptor activation.

EGF receptor activation during oogenesis is unique in that the major activating ligand Gurken is oocyte-specific. Gurken mRNA is localized adjacent to the oocyte nucleus, but its protein product triggers EGF receptor activation in the adjacent follicle cells. The specification of the dorsal-appendage-forming cells is initiated following the migration of the oocyte nucleus to the future dorsal-anterior corner of the egg. At this stage, Rhomboid expression in the follicle cells is induced by EGF receptor activation [14–16]. A positive feedback loop is thus realized; localized EGF receptor activation by Gurken leads to Rhomboid expression, which in turn activates Spitz processing, amplifying EGF receptor activation in the follicle cells.

Localized activation of EGF receptor in the dorsal-most follicle cells by Gurken triggers the patterning system. The eventual pattern, however, consists of prominent EGF receptor activation in two distinct domains corresponding to the position of the future dorsal appendages, while lower activation is observed in the dorsal-most cells. How is the subdivision between the appendage and inter-appendage fates generated?

One possibility is that the promoter of Rhomboid responds in a dynamic manner to graded EGF receptor activation in the follicle cells, to generate patterns of expression that will give rise to different numbers of dorsal appendages, as calculated by Shvartsman et al. [4]. An alternative option is that the feedback loops of EGF receptor activation contain sufficient self-organizing properties to convert one peak of signaling to two peaks separated by a valley [15]. This mechanism involves an additional player in the EGF receptor signaling cassette, an inhibitory secreted ligand termed Argos [18]. Expression of Argos is induced by EGF receptor activation, but only in cells receiving the highest levels of activation [19]. Thus, the combined activation of EGF receptor by Gurken and Spitz leads to induction of Argos expression in a narrow row representing the cells receiving maximal signaling. This local induction of Argos may generate the ‘signaling valley’ in the inter-appendage region (Figure 1).

While the possibility that patterning the dorsal appendages can be accounted for by the self-organizing dynamics of EGF receptor signaling itself is intriguing, the consistency of the above mentioned mechanism with all the available genetic and biochemical data could not be rigorously evaluated in the absence of a quantitative framework. Shvartsman et al. [4] have now provided such a quantitative framework. The authors formulated a reaction–diffusion based model corresponding to the EGF receptor signaling cassette, and solved
the model’s equations numerically for a wide range of parameters (the kinetic rate constants and diffusion coefficients). Using this in silico approach, the authors were able to explore the range of parameter space where proper patterning is established and maintained, and highlight its behavior for parameters outside this region.

Shvartsman et al. [4] confirmed the consistency of the model. Importantly, the reported analysis considered not only the establishment of the two-peaked pattern, but also its stability. In addition, they characterized the range of parameter space where proper patterning is achieved. Intriguingly, it turned out that a stable two-peaked pattern could only be obtained when the inhibitor Argos diffuses at a significantly faster rate than the activator Spitz. A similar relationship between the relative diffusion range of Argos and Spitz during Drosophila eye development was suggested earlier by Freeman [20].

Outside the parameter range supporting a two-peaked pattern, the model predicts several classes of qualitatively different solutions. Each solution is characterized by a different number of peaks in the signaling profile. Discrete changes in the number of dorsal appendages were indeed observed experimentally as a result of various genetic perturbations (Figure 2). The mathematical analysis accounts for most of the observed phenotypes within a unified framework. Moreover, it raises the possibility that the four-appendage egg structure observed in other related species [17] has evolved by a small modification of the same basic patterning mechanisms.

While genetic perturbations result in an abnormal number of dorsal appendages, under normal circumstances the number of position of the appendages is remarkably fixed. This stability is maintained despite the expected biological and external fluctuations, most notably temperature changes. Such robustness is a general property of patterning networks and may be an underlying principle determining their design. Quantitative analysis of morphogen gradient systems could shed light on the mechanisms that ensure the generation of precise patterns, despite quantitative fluctuations in parameters of the underlying patterning network.

References