DEVELOPMENTAL DYNAMICS 226:155–159, 2003

BRIEF COMMUNICATION

Transitions in the Model of Epithelial Patterning

Michal Přibyl,[1] Cyrill B. Muratov,[2] and Stanislav Y. Shvartsman[1]*

We analyze pattern formation in the model of cell communication in *Drosophila* egg development. The model describes the regulatory network formed by the epidermal growth factor receptor (EGFR) and its ligands. The network is activated by the oocyte-derived input that is modulated by feedback loops within the follicular epithelium. We analyze these dynamics within the framework of a recently proposed mathematical model of EGFR signaling (Shvartsman et al. [2002] Development 129:2577–2589). The emphasis is on the large-amplitude solutions of the model that can be correlated with the experimentally observed patterns of protein and gene expression. Our analysis of transitions between the major classes of patterns in the model can be used to interpret the experimentally observed phenotypic transitions in eggshell morphology in *Drosophila melanogaster*. The existence of complex patterns in the model can be used to account for complex eggshell morphologies in related fly species. *Developmental Dynamics 226:155–159, 2003.* © 2002 Wiley-Liss, Inc.

Key words: EGFR; Spitz; Argos; Rhomboid; Drosophila oogenesis; autocrine loop; feedback; computational analysis; pattern formation; phenotypic transitions; dorsal appendages

Received 28 June 2002; Accepted 6 October 2002

INTRODUCTION

Half a century ago, Alan Turing suggested that chemical blueprints guiding the morphogenesis can arise from the interplay between chemical reactions and diffusion (Turing, 1952). Within this paradigm, pattern formation is self-organized, in a sense that it does not require prepatterns for its initiation and guidance. The importance of self-organized pattern formation in development is still debated (Wolpert et al., 1998; Freeman, 2000; Monk, 2000). The extent to which patterns in development are hard-wired or self-organized is one of the main issues in this debate. Pattern formation, whereby inductive signals strongly interact with secondary refinement processes, is a hybrid between the purely hard-wired and self-organized mechanisms. Here, we analyze such a mechanism in a recently proposed mathematical model of cell communication in *Drosophila* oogenesis (Shvartsman et al., 2002). The model describes the intermediate stages of oogenesis, when the Gurken signal released from the oocyte initiates pattern formation in the overlying follicular epithelium (Fig. 1A; Sapir et al., 1998; Wasserman and Freeman, 1998; Nilson and Schupbach, 1999; Peri et al., 1999).

The primary response, the localized pattern of epidermal growth factor receptor (EGFR) signaling that mirrors the Gurken input, is sequentially amplified and inhibited by the network of feedback loops within the follicular epithelium (Sapir et al., 1998; Wasserman and Freeman, 1998; Peri et al., 1999). As a result of this modulation, the original pattern of EGFR activity is transformed into a pattern with two maxima (Fig. 1B). Cells with the elevated levels of EGFR signaling activate the transcriptional program eventually leading to the formation of the dorsal appendages (Fig. 1C; Deng and Bownes, 1997, 1998; Stevens, 1998; Dobens and Raftery, 2000). The feedback loops modulating the Gurken input rely on secreted EGFR ligands (Fig. 1D,E). The positive feedback is formed by the receptor, its stimulatory ligands (Spitz and Vein), and a ligand-releasing protease Rhomboid (Ruohola-Baker et al., 1993; Wasserman and Freeman, 1998; Lee et al., 2001; Tsruya et al.,

[1]Department of Chemical Engineering and Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey
[2]Department of Mathematical Sciences and Center for Applied Mathematics and Statistics, New Jersey Institute of Technology, Newark, New Jersey
*Correspondence to: Stanislav Y. Shvartsman, Department of Chemical Engineering and Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544. E-mail: stas@princeton.edu

DOI 10.1002/dvdy.10218

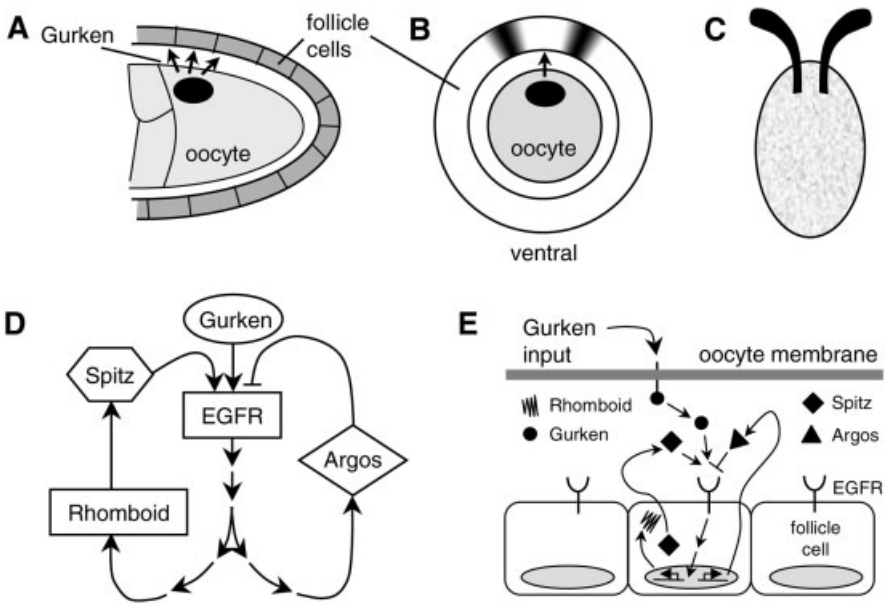

Fig. 1. **A:** Geometry of cell communication at the intermediate stages of oogenesis. Diffusing signal released from the dorsal–anterior cortex of the oocyte stimulates the epidermal growth factor receptor (EGFR) in the overlying follicle cells. **B:** Feedback loops within the follicular epithelium establish a two-peaked pattern of EGFR activity. **C:** This pattern provides a blueprint for the formation of two dorsal appendages. **D,E:** Summary of the regulatory network captured by the model.

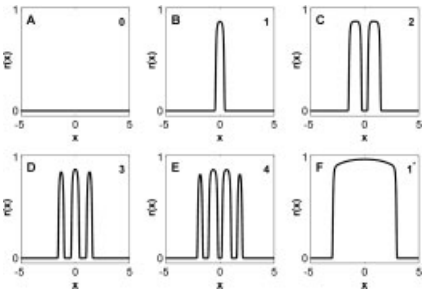

Fig. 2. Six major classes of stationary patterns in the model, represented by the pattern of Rhomboid, $r(x)$.

2002). The negative feedback is composed by the receptor and its inhibitory ligand Argos (Golembo et al., 1996; Morimoto et al., 1996; Wasserman and Freeman, 1998). Argos is the only secreted EGFR inhibitor in *Drosophila* (Perrimon and Perkins, 1997).

Extensive studies of the *Drosophila* EGFR system have enabled the formulation of a mechanistic model of this signaling circuit (Shvartsman et al., 2002). Recently, we have used this model to validate the peak-splitting mechanism of Wasserman and Freeman (Wasserman and Freeman, 1998). We have found that the mechanism is sufficient to convert a single-peaked extracellular input into a two-peaked pattern. The mechanism can also account for several observed phenotypic transitions. Furthermore, we have found that the mechanism predicts the existence of complex signaling patterns with more than two peaks of receptor activity. Here, we report a comprehensive analysis of these complex patterns and discuss the problem of pattern selection.

RESULTS AND DISCUSSION

The mathematical model of the EGFR patterning network, the selection of model parameter values, and the description of numerical methods have been described in a previous study (Shvartsman et al., 2002).

Main Classes of Large Amplitude Patterns in the Model

The analysis of stationary patterns in the model reveals five major classes characterized by the different number of maxima (Fig. 2). In the case of a two-peaked pattern (Fig. 2C), a single-peaked input induces a pattern in which signaling is localized to the two narrow domains with high levels of Rhomboid and Argos production. Signaling is essentially "off" outside of these two domains. The number of appendages corresponds to the number of peaks in the EGFR signaling pattern (Wasserman and Freeman, 1998). Accordingly, different phenotypes can be interpreted in terms of the qualitatively different stationary patterns in the model. We correlate the two-peaked pattern in Figure 2C with the two-striped patterns of *rhomboid* expression and EGFR activity (Ruohola-Baker et al., 1993; Neuman-Silberberg and Schupbach, 1994; Wasserman and Freeman, 1998; Peri et al., 1999). Similarly, the low amplitude pattern in Figure 2A would correspond to the eggshell with no dorsal appendages, whereas the pattern with a single narrow peak in Figure 2B would correspond to the phenotype with a single dorsal appendage. These phenotypes have been robustly observed in fruit flies with defects in genes responsible for the generation of Gurken signal (reviewed in Nilson and Schupbach, 1999). A pattern with a single broad peak (Fig. 2F) can be correlated with strongly dorsalized eggshells with a single broad appendage observed in two experimental systems with the elevated levels of Gurken (Neuman-Silberberg and Schupbach, 1994; Ghiglione et al., 2002).

The patterns with three and four peaks shown in Figure 2D,E indicate the presence of phenotypes with more than two appendages. Although the eggs of *Drosophila melanogaster* have two appendages, eggshells of related fly species may exhibit more complex morphology (Hinton, 1981). Furthermore, genetic manipulations of *Drosophila melanogaster* can also yield more complex phenotypes (Roth et al., 1999). For example, eggs with binuclear oocytes and, hence, two sources of Gurken can have four dorsal appendages. Even more remarkably, manipulation of the level of *dpp* gene yields eggs with three appendages even with a single source of Gurken (Deng and Bownes, 1997). (*dpp*, among its other functions, me-

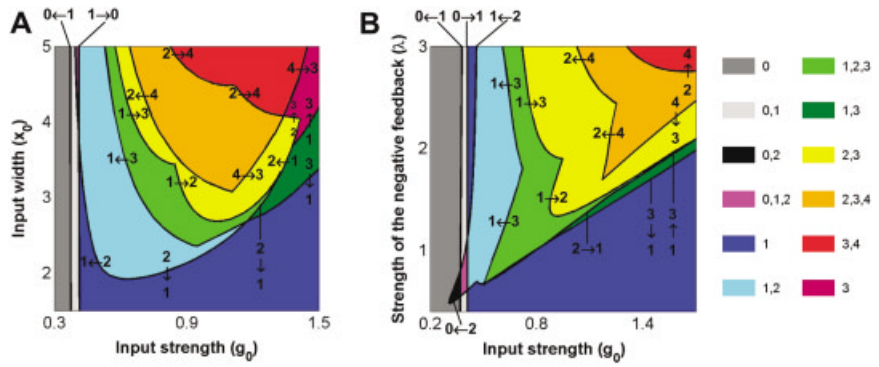

Fig. 3. The classification of the stationary patterns in the model. Each region contains a different combination of stable patterns (see the color map on the right). The lines represent transitions between different patterns. **A:** Two-parameter diagram computed as a function of the input strength (g_0) and width (x_0), $\lambda = 1.6$. **B:** Two-parameter diagram computed as a function of the relative strength of negative feedback (λ) and the input amplitude (g_0), $x_0 = 3$. Parameters: $c_r = 0.4$, $b_r = 0.2$, $c_a = 0.5$, $b_a = 0.05$, $x_0 = 3$, $\epsilon = 0.1$, $\tau_a = 1$, $\tau_s = 0.1$. Notation is described in (Shvartsman et al., 2002).

diates the positive feedback by Rhomboid (Dobens and Raftery, 2000; Peri and Roth, 2000).) The ability of our model to predict patterns with more than two peaks can be interpreted in several ways. First, if it is a real feature of the mechanism, it indicates that the pattern-forming capabilities of the *r-a-s* signaling network may have been conserved across species (Deng and Bownes, 1997; Peri et al., 1999; Shvartsman et al., 2002). Second, it suggests new experiments aimed at creating such phenotypes in *Drosophila melanogaster*.

We have numerically mapped the regions of existence and the transitions between different classes of patterns. Figure 3 presents the two-parameter diagrams computed upon variation of the input parameters and the strength of the negative feedback. Experimentally, such parameter variation can be realized in flies with mutations in the components localizing and releasing Gurken and in those controlling the expression of *argos* (Golembo et al., 1996; Wasserman and Freeman, 1998; Nilson and Schupbach, 1999; Zhao and Bownes, 1999; Hsu and Schulz, 2000). We have found that transitions between different patterns are discontinuous and hysteretic. We believe that it is the abruptness of these transitions that enables the classification of a large number of experimental results in terms of a relatively small number of well-defined phenotypes.

The Problem of Pattern Selection

Qualitatively, different patterns can coexist for a wide range of parameters (Figs. 3, 4A). It is important to consider this result in connection with the problem of pattern selection in dorsal appendage morphogenesis. Specifically, 100% of the eggs laid by the wild-type fruit flies have two dorsal appendages. This finding means that the real system can robustly select the right pattern over a naturally occurring range of Gurken doses and egg chamber geometries. In terms of our model, this evidence would suggest that the two-peaked pattern (Fig. 2C) is invariably selected from several patterns that are realized for a range of input and feedback parameters.

One mechanism that might explain this robustness requires the existence of a region of the parameter space in which the two-peaked pattern is the unique attractor. This is not what we see in our analysis. In fact, the two-peaked patterns always coexist with other large amplitude solutions, either one- or three-peaked (Figs. 3, 4A). An alternative mechanism for pattern selection relies on the input history that guides the system to the "right" pattern. In one scenario of dorsal appendage morphogenesis, the Gurken signal is slowly increasing (Shvartsman et al., 2002); this increase can be realized by the adiabatic increase of the input strength. (A similar scenario relies on the slowly increased strength of the positive feedback (Guichet et al., 2001).)

We have found that the slow variation of the input robustly selects the two-peaked signaling pattern for a wide range of input widths and amplitudes. Specifically, upon slow variation of the input strength, the pattern is transformed between the zero-, the one-, and the two-peaked pattern. The existence of this 1→2 transition is realized over a wide range of model parameters.

We have numerically analyzed the dynamical properties of the inputs that are necessary for inducing the two-peaked patterns. We have parameterized the time dependence of the input amplitude by its asymptotic value, and the time scale on which this value is attained. Focusing on the region where the two- and three-peaked solutions are the only large-amplitude patterns, we have mapped out the dependence of the final pattern on these two parameters (Fig. 4B). We have then computed the domains of asymptotically attained patterns in the space of these parameters (Fig. 4C). In our case, the initial condition of the initial value problem with time-dependent input is fixed to the unique ("off") steady state in the absence of input. The question of pattern selection reduces to determining the eventual outcome (two- vs. three-peaked pattern) of the transient induced by the increasing and saturating input (a model of increasing and saturating Gurken stimulus).

Figure 4C presents the parametric dependence of the steady states asymptotically attained in these transients. The diagram can be summarized as follows. First, slow inputs promote the selection of two-peaked patterns. Second, the domains of reachability of the two- and three-peaked solutions are separated by well-defined boundaries; overstepping these boundaries qualitatively changes the pattern attained in the transient. Third, the domains of

reachability of the three-peaked solutions can be disconnected.

As a consequence of the disconnected nature of the domains of the asymptotically attained patterns, the pattern selected by an increasing and saturating input can exhibit nonmonotonic dependence on the input parameters. An example of such nonmonotonic dependence on the time scale of the input is shown in Figure 4D. We found that the connectedness of reachability domains in the space of inputs parameters depends on the initial conditions for the transient. In particular, when the transient is induced from the steady states realized for a higher value of the input strength (a well-formed one-peak pattern, Fig. 2B), the domain becomes simply connected.

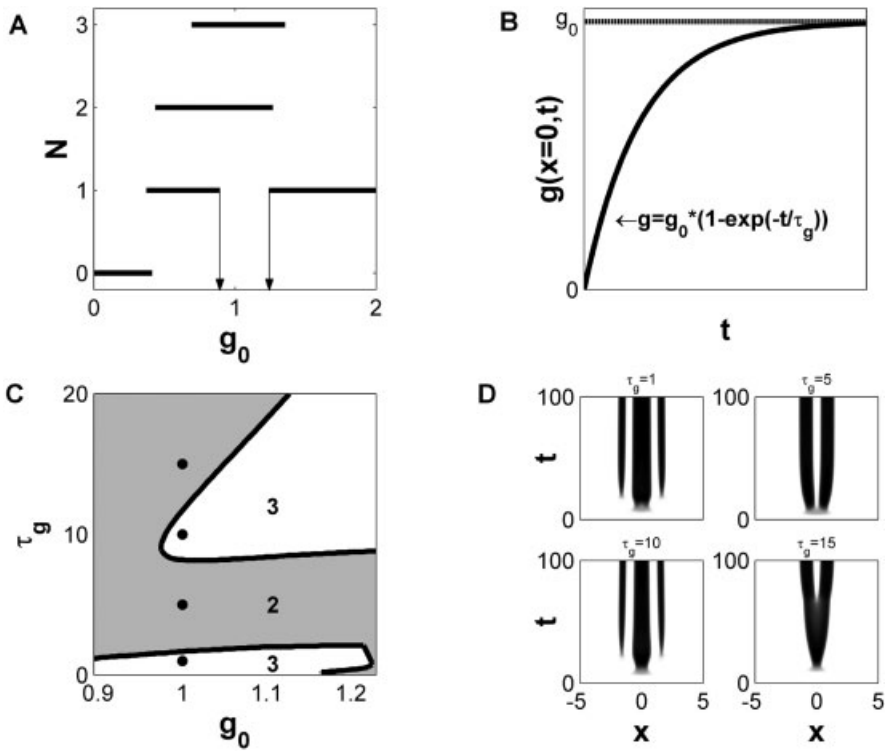

Fig. 4. **A:** Dependence of the number of peaks in the pattern on the amplitude of the input signal. **B:** The time-dependence of the input amplitude is parameterized by the asymptotic value (g_0) and the time scale on which it is attained (τ_g). **C:** Distribution of the different classes of patterns of the model in the space of the input design parameters. The input amplitude is restricted with only two- and three-peaked patterns. Gray and white areas correspond to parameters realizing the two- and three-peaked patterns, respectively. **D:** The spatiotemporal patterns of Rhomboid computed for several values of τ_g and $g_0 = 1$ show the nonmonotonic dependence of the attained pattern on the time scale of the input. The gray scale corresponds to the level of Rhomboid. Parameters: $\lambda = 1.6$, $c_r = 0.4$, $b_r = 0.2$, $c_a = 0.5$, $b_a = 0.05$, $x_0 = 3$, $\epsilon = 0.1$, $\tau_a = 1$, $\tau_s = 0.1$.

SUMMARY AND CONCLUSIONS

Each domain within the two-peaked pattern of EGFR activity marks the appendage-producing cells (Wasserman and Freeman, 1998). The two-peaked solutions can indeed be realized over a wide range of parameters in our model (Shvartsman et al., 2002). Here, we report that, at least within the framework of our model, the two-peaked patterns *always* coexist with other solutions (Fig. 3). The coexistence with the zero- and one-peaked patterns fits well with the phenotypes observed at lowered levels of Gurken input or EGFR activity (Nilson and Schupbach, 1999). The coexistence of the two-peaked patterns with more complex solutions is a robust feature of the mechanism with a single diffusing inhibitor. It suggests that perturbations of the EGFR regulatory network in oogenesis can generate more complex dorsal appendage phenotypes.

Our results motivate future experiments on generation of eggshells with more than two appendages. Currently, there are only two reports of such phenotypes in mutants of *D. melanogaster* (Deng and Bownes, 1997; Reich et al., 1999). Given the wide range of the model parameters for which different patterns coexist, it is important to explore the mechanisms by which the egg can robustly select the wild-type two-appendage morphology over a range of developmental conditions.

The pattern-forming capability of nonlinear reaction–transport mechanisms is rich. Generically, several patterns can coexist for a wide range of model parameters. Recently, this feature has been emphasized in a model of a neurogenic network in *Drosophila* (Meir et al., 2002). There, the lateral inhibition network could simultaneously generate a variety of patterns. It was suggested that this property of the model signals the high evolvability potential of the real network (Kirschner and Gerhart, 1998). Specifically, small variations in the parameters of the module may generate patterns of higher complexity at other stages of development or in related species. In the case of appendage producing network, this versatility might account for wild-type eggshells in related fly species. We suggest that new morphologies may be realized without the introduction of additional molecular components.

The evolvability of regulatory modules must be balanced by their robustness in performing specific tasks (Kirschner and Gerhart, 1998). For the neurogenic network, it was suggested that patterns are selected by variations in initial conditions (Meir et al., 2002). For the dorsal appendage producing network, the dynamic regulation of the Gurken input might be the key to robust selection of the two-peaked phenotype (Fig. 4C). Most of the experimental work in this direction focused on genes regulating nuclear migration and Gurken release (Nilson and Schupbach, 1999; Guichet et al., 2001). An additional layer of control might be provided by the system for the vitelline

membrane production (Waring, 2000; Andrenacci et al., 2001). Although it is believed that the Gurken input to the follicle cells is reduced by this process, its role in patterning of the follicular epithelium is largely unexplored (Stevens, 1998; Waring, 2000). Experiments with transient Gurken stimuli indirectly suggest that dorsal appendages should be fully induced before the onset of the vitelline membrane formation (Guichet et al., 2001). Dorsal appendage morphogenesis tightly depends on the spatial regulation of Gurken. It will be interesting to examine the role of temporal regulation of the same input.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation. S.Y.S. thanks Trudi Schupbach (Princeton), Allan Spradling (Carnegie Institute), Uri Abdu (Princeton), and Doug Lauffenburger (MIT) for many helpful discussions. C.B.M. was partially supported by the NJIT SBR program.

REFERENCES

Andrenacci D, Cernilogar F, Taddel C, Rotoli D, Cavaliere V, Graziani F, Gargiulo G. 2001. Specific domains drive VM32E protein distribution and integration in Drosophila eggshell layers. J Cell Sci 114:2819–2829.

Deng WM, Bownes M. 1997. Two signalling pathways specify localised expression of the broad-complex in Drosophila eggshell patterning and morphogenesis. Development 124:4639–4647.

Deng WM, Bownes M. 1998. Patterning and morphogenesis of the follicle cell epithelium during Drosophila oogenesis. Int J Dev Biol 42:541–552.

Dobens LL, Raftery LA. 2000. Integration of epithelial patterning and morphogenesis in Drosophila oogenesis. Dev Dyn 218:80–93.

Freeman M. 2000. Feedback control of intercellular signalling in development. Nature 408:6810.

Ghiglione C, Bach EA, Paraiso Y, Carraway KL, Noselli S, Perrimon N. 2002. Mechanism of activation of the Drosophila EGF Receptor by the TGFalpha ligand Gurken during oogenesis. Development 129:175–186.

Golembo M, Schweitzer R, Freeman M, Shilo BZ. 1996. argos transcription is induced by the Drosophila EGF receptor pathway to form an inhibitory feedback loop. Development 1996:223–230.

Guichet A, Peri F, Roth S. 2001. Stable anterior anchoring of the oocyte nucleus is required to establish dorsoventral polarity of the Drosophila egg. Dev Biol 237:93–106.

Hinton HE. 1981. Biology of insect eggs. Oxford, New York: Pergamon Press.

Hsu T, Schulz R. 2000. Sequence and functional properties of ETS genes in the model organism Drosophila. Oncogene 19:6409–6416.

Kirschner M, Gerhart J. 1998. Evolvability. Proc Natl Acad Sci U S A 95:8420–8427.

Lee JR, Urban S, Garvey CF, Freeman M. 2001. Regulated intracellular ligand transport and proteolysis regulate egf receptor activation in Drosophila. Cell 107:161–171.

Meir E, von Dassow G, Munro E, Odell GM. 2002. Robustness, flexibility, and the role of lateral inhibition in the neurogenic network. Curr Biol 12:778–786.

Monk NAM. 2000. Elegant hypothesis and inelegant fact in developmental biology. Endeavour 24:170–173.

Morimoto AM, Jordan KC, Tietze K, Britton JS, O'Neill EM, Ruohola-Baker H. 1996. Pointed, an ETS domain transcription factor, negatively regulates the EGF receptor pathway in Drosophila oogenesis. Development 122:3745–3754.

Neuman-Silberberg FS, Schupbach T. 1994. Dorsoventral axis formation in Drosophila depends on the correct dosage of the gene gurken. Development 120:2457–2463.

Nilson LA, Schupbach T. 1999. EGF receptor signaling in Drosophila oogenesis. Curr Top Dev Biol 44:203–243.

Peri F, Roth S. 2000. Combined activities of Gurken and Decapentaplegic specify dorsal chorion structures of the Drosophila egg. Development 127:841–850.

Peri F, Bokel C, Roth S. 1999. Local Gurken signaling and dynamic MAPK activation during Drosophila oogenesis. Mech Dev 81:75–88.

Perrimon N, Perkins LA. 1997. There must be 50 ways rule the signal: the case of the Drosophila EGF receptor. Cell 89: 13–16.

Reich A, Sapir A, Shilo BZ. 1999. Sprouty, a general inhibitor of receptor tyrosine kinase signaling. Development 126:413–447.

Roth S, Jordan P, Karess R. 1999. Binuclear Drosophila oocytes: consequences and implications for dorsal-ventral patterning in oogenesis and embryogenesis. Development 126:927–934.

Ruohola-Baker H, Grell E, Chou TB, Baker D, Jan L, Jan YN. 1993. Spatially localized rhomboid is required for establishing the dorsal-ventral axis in Drosophila oogenesis. Cell 73:953–965.

Sapir A, Schweitzer R, Shilo BZ. 1998. Sequential activation of the EGF receptor pathway during Drosophila oogenesis establishes the dorsoventral axis. Development 125:191–200.

Shvartsman SY, Muratov CB, Lauffenburger DA. 2002. Modeling and computational analysis of EGF receptor-mediated cell communication in Drosophila oogenesis. Development 129:2577–2589.

Stevens L. 1998. Twin peaks: Spitz and Argos star in patterning of the Drosophila embryo. Cell 95:291–294.

Tsruya R, Schlesinger A, Reich A, Gabay L, Sapir A, Shilo BZ. 2002. Intracellular trafficking by Star regulates cleavage of the Drosophila EGF receptor ligand Spitz. Genes Dev 16:222–234.

Turing AM. 1952. The chemical basis of morphogenesis. Philos Trans R Soc Lond B Biol 237:37–72.

Waring GL. 2000. Morphogenesis of the eggshell in Drosophila. Int Rev Cytol 198:67–108.

Wasserman JD, Freeman M. 1998. An autoregulatory cascade of EGF receptor signaling patterns the Drosophila egg. Cell 95:355–364.

Wolpert L, Beddington R, Jessel T, Lawrence P, Meyerowitz E. 1998. Principles of development. Oxford: Oxford University Press.

Zhao DB, Bownes M. 1999. Misexpression of argos, an inhibitor of EGFR signaling in oogenesis, leads to the production of bicephalic, ventralized, and lateralized Drosophila melanogaster eggs. Dev Genet 25:375–386.