Trudi Schüpbach

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Genetic Screens to Analyze Pattern Formation of Egg and Embryo in *Drosophila*: A Personal History

Trudi Schüpbach

Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544, USA; email: schupbac@princeton.edu

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Abstract

In *Drosophila* development, the axes of the egg and future embryo are established during oogenesis. To learn about the underlying genetic and molecular pathways that lead to axis formation, I conducted a large-scale genetic screen at the beginning of my independent career. This led to the eventual understanding that both anterior-posterior and dorsal-ventral pattern information is transmitted from the oocyte to the surrounding follicle cells and in turn from the follicle cells back to the oocyte. How I came to conduct this screen and what further insights were gained by studying the mutants isolated in the screen are the topics of this autobiographical article.

INTRODUCTION

For *Drosophila* geneticists, one of the enjoyable tasks in large-scale mutagenesis experiments is thinking of names that imaginatively describe each new mutant phenotype. At the beginning of my career as an independent scientist, I conducted a large-scale mutagenesis screen to isolate female-sterile and maternal-effect mutations on the second chromosome of *Drosophila*. The screen was successful and led to the identification of many loci with striking phenotypes affecting oogenesis and early embryonic development. What name would best describe maternal-effect mutations that eliminate the germplasm in the developing egg such that the resulting progeny are sterile and the original mutant female therefore remains grandchildless? We thought of royal families that died out because of lack of offspring—Tudor was the first that came to mind; Vasa, Staufen, and Valois followed soon after. How to name mutations that cause the eggs laid by the female to be elongated and more or less uniformly patterned in the circumferential dimension? *gurken*, which means "cucumber" in German, was soon followed by *zucchini*, *aubergine*, *okra*, *squash*, and later, *cornichon*, as devised by Siegfried Roth. Finding a descriptive name for mutants and thus for the corresponding genes confers a certain emotional attachment to those genes, as probably many geneticists working with flies or zebrafish or mice would agree.

I discuss how I came to be faced with a large number of such maternal-effect and female-sterile mutants. A successful screen raises further challenges; namely, how does one subsequently choose which mutants to work on? These decisions are inevitably somewhat personal, and in my case, they were heavily influenced by my scientific background and the perspective of developmental genetics in the 1980s. One exciting part of any screen is discovering many different phenotypes that go beyond expectations. The analysis of these phenotypes, and eventually the molecular determination of the corresponding genes, opened a large window into the maternal contribution to the establishment of pattern and polarity of the egg and embryo. Moreover, many of the mutations provided entry points into the analysis of signaling processes, cell biological factors, and novel processes that were unanticipated, such as the germline defense against retrotransposons. In the following sections, I describe what initially led me to conduct this screen, what particular group of genes my laboratory and I then chose to study, how we designed further screens, and in what directions the mutants took us over the years. Since no scientist is independent of their scientific environment, I also try to set the experiments in the historical background in which they were conducted.

SCIENTIFIC BEGINNINGS

I started my scientific career at the University of Zurich in Switzerland in 1969, fully determined to become a biology major. Before university, I had attended an all-girl middle and high school, a six-year course sequence that in Zurich is called the gymnasium. In those early courses, our teachers encouraged us to do well in science and math and we were able to undertake small projects in various science laboratories, something I greatly enjoyed. As an undergraduate, once I had passed the exams that allowed me to declare biology as my major, I was fortunate to be admitted to the biology project laboratory held in a set of trailers outside the Zoological Institute. Here we were given a permanent laboratory space for two semesters, and we were presented every four to six weeks with a new set of animals and instructions on how to conduct experiments. We cut *Hydra* into pieces and watched them regenerate; we crossed *Drosophila* that unbeknownst to us carried attached-X chromosomes, defying the normal expectation of X-linked inheritance; and we worked with other unusual chromosomal aberrations. We were given access to lobsters and could use various neurotoxins to perform electrophysiological measurements. The exciting part was that we were allowed to spend as much time in the laboratory as we wanted, to repeat

experiments that did not work right away, and to design some experiments on our own with only moderate guidance from some advanced graduate students. After this experience I knew that I wanted to continue doing experiments. I was not sure that I was good enough to become an actual experimental scientist, but I already thought that getting a Master's degree and then signing up for a PhD would be wonderful. If a career as a scientist was not possible, I could become a science teacher after obtaining my PhD; I enjoyed teaching. For that reason, I was relatively unconcerned about my future and was happy to choose the laboratory in which to do my Master's and PhD work with an eye not on future employability but instead on what fascinated me most. This was clearly genetics, in particular developmental genetics, which was (and still is) one of the strengths of what was then called the Department of Zoology at the University of Zurich.

DOCTORAL STUDIES AND BEYOND: GENETIC MOSAICS AND SEX DETERMINATION

In the 1960s and early 1970s, the department chair was Ernst Hadorn. Hadorn was a student of Fritz Baltzer, a successful developmental biologist who had studied with Theodor Boveri and worked with Hans Spemann. Hadorn later joined Curt Stern's laboratory for a brief period, where he was introduced to *Drosophila*. Hadorn was well known for his analysis of lethal mutations and, later, for his studies of imaginal disk transplantations and regeneration (23, 24). Hence, the lectures in my undergraduate biology courses involved a lot of classical developmental biology and genetics. Although I enjoyed his lectures immensely, I decided not to continue my thesis work with Professor Hadorn in part because he did not seem very supportive of female students and, more importantly, because I had become fascinated with the genetic approaches that were then just beginning to be used in the laboratory of Rolf Nöthiger. Nöthiger was a close friend of Antonio García-Bellido and shared with him the conviction that the analysis of genetic mosaics could provide unique insights into many aspects of development. Both Nöthiger and García-Bellido had been visiting fellows at the California Institute of Technology working with Edward Lewis, and while there they had also met Alfred Sturtevant, who was a pioneer in the analysis of genetic mosaics. The García-Bellido laboratory in Madrid perfected the study of mosaics and eventually discovered compartments and cell linage restrictions in the imaginal disks of *Drosophila* (16), a major breakthrough in the analysis of developmental growth.

When I began my PhD project in 1974, I was excited about the use of genetic mosaics to study the development of the female genital disk. One of the standard techniques at the time for generating mosaics involved the loss of an unstable ring-X chromosome, which converted initially female XX embryos into gynandromorphs consisting of large patches of male and female tissues. To study the female genital disk, I needed mosaics in which all tissues were female. I therefore started with triplo-X individuals, the result of a cross between an attached-X female and a ring-X-carrying male. Thus, in contrast to regular female/male gynandromorphs that are produced by the loss of the ring-X chromosome in combination with a normal X chromosome, these mosaics were female/female. To follow cell lineages in these mosaics, I dissected the genitalia of the mosaic females and stained them for a histochemical marker to detect mosaicism. Out of curiosity, I also maintained the ovaries along with the genitalia in the staining procedure. This made me realize that we actually knew very little in terms of developmental dynamics of ovarian development. While I finished a characterization of the genital disk itself (51), I turned more and more to the study of ovarian development as an exciting part of my thesis.

My interest in ovarian development was furthered stimulated by two postdoctoral fellows, János Szabad and Eric Wieschaus (my future husband), who had joined the Nöthiger laboratory while I was starting my thesis work. The two came from different backgrounds: János from

Soviet-dominated Hungary and Eric from the United States, although he had already spent three years in Basel, Switzerland, as Walter Gehring's graduate student at the Biozentrum. On Saturdays in the laboratory, when the cafeteria and other easy sources of food at the university were closed, both János and Eric introduced us to food from their home countries prepared on Bunsen burners in weekly cooking sessions that would nowadays never be allowed in functioning laboratories. During those shared meals, we discussed our experiments and papers we had read that week. I soon learned that social aspects like sharing food or going on joint laboratory outings, be it skiing or hiking, are important in furthering a free interchange of ideas in science as well as making life fun in the laboratory. Both János and Eric were immersed in science, full of ideas, and never daunted at performing difficult experiments. Being a part of a laboratory with intense discussions, where people appreciate each other's experiments and give honest advice and feedback, not only was an enjoyable part of my laboratory experience but also contributed to my growing self-confidence as a scientist.

My advisor, Rolf Nöthiger, was very liberal in letting graduate students propose and perform experiments that were not necessarily part of their original thesis plans. While I was constructing a fate map of the female genital disk using the gynandromorph technique, I also analyzed the mosaic patterns of the ovaries of the same animals and tried to deduce cell numbers and growth dynamics from the results (50). In 1976 Eric Wieschaus and János Szabad were using X-ray-induced mitotic recombination to test stem cell dynamics in the ovary (63), and it was useful to compare observations and to discuss how and why results might differ depending on the technique. We also thought of a way to generate and analyze mosaicism in the larval hypoderm, and from these results we were able to make predictions about the behavior of epidermal precursors during embryonic development (56).

The meetings I was allowed to attend as a graduate student were a further important early influence on my career. In particular, Antonio García-Bellido, together with Peter Lawrence and my advisor, Rolf Nöthiger, began to organize small meetings between the three laboratories but soon included other European *Drosophila* scientists as participants. In those small meetings results were discussed that were not yet publication ready, and although I often had difficulties following our Spanish colleagues' rapid description of their research, I nevertheless found the meetings enlightening and was encouraged that our own experiments could be so thoroughly vetted and discussed on a broader level. These meetings also forged friendships, for instance, with Gary Struhl and Peter Lawrence as well as with Gerd Jürgens and Ruth Lehmann, that were to last for all of my career. I am still grateful to Rolf Nöthiger for allowing me to attend these meetings even though I was only a graduate student.

In the late 1970s the main focus of the Nöthiger laboratory shifted from the more descriptive aspects of imaginal disk development to an analysis of sex determination. For me this opened a new set of questions, particularly with respect to ovarian development. In experiments done at the Biozentrum, J. Lawrence (Larry) Marsh and Eric Wieschaus had transplanted germ cells homozygous for a mutation in the *transformer* gene that switched female somatic cells to male fates, and found that it did not affect the development of female germ cells (31). This raised the possibility that the other known sex determination genes might also work only in somatic cells, and that the germline might use a different set of genes in determining their sex.

Having spent my days analyzing mosaic ovaries and thinking about sex determination, I was eager to learn how to transplant pole cells, the germline precursors. Visits to the Biozentrum, which were initiated with the help of Eric Wieschaus, allowed me to learn this technique and bring it to Zurich. Rolf Nöthiger was greatly in favor of letting me transplant germ cells mutant for various sex determination genes and test whether they played a role in the sex determination of the germline as well (45). Transplanting the cells from one embryo to the other was great fun.

The embryos, under the microscope, were beautiful in their simplicity, and taking the pole cells up into the needle and then injecting them into the host embryo was satisfying. The results I obtained with mutations in *Sex-lethal* were particularly interesting, as those mutant germ cells developed into multicellular cysts that resembled neither female nor male differentiated germline cells (46). The result demonstrated a level of germline autonomy, which was, however, also influenced by the somatic environment in which those germ cells found themselves. This nonautonomy was different from the imaginal disk cells that had always tested as strictly cell autonomous in mosaic situations. During these experiments I also had the great experience of interacting with Thomas (Tom) Cline, who had discovered *Sex-lethal* and who spent a summer in Zurich, where we had lively discussions and an exchange of ideas, forging a relationship that grew into an important friendship after I moved to Princeton, New Jersey.

I was able to stay in Rolf Nöthiger's laboratory for two more years after I defended my thesis, which was helpful because I had a child, my daughter Ingrid, from a first, brief marriage, and my parents, who lived in Zurich, were dedicated to helping me with child care. In the 1970s and 1980s, there were no child care centers at the university, and good child care would have been financially difficult on a graduate student stipend. I was fortunate that my parents were supportive and went out of their way to allow me to work on my thesis. Laboratory work is also to some extent flexible and makes it possible, for instance, to work odd hours. I would usually return to the laboratory at eight or nine at night to work for three or four more hours while my daughter was sleeping, supervised by my parents, but then in turn spend more time with her in the morning and late afternoon. It was supportive of Rolf Nöthiger to allow me great flexibility in my schedule—combining science with child-rearing requires support and flexibility, especially at the beginning of a young scientist's career (when money is scarce).

DEVELOPING A PLAN FOR A FEMALE-STERILE SCREEN

Meanwhile, Eric Wieschaus had taken a position at the European Molecular Biology Laboratory in Heidelberg, Germany, which was about four hours from Zurich by train. There, he and Christiane (Janni) Nüsslein-Volhard began their famous Heidelberg screens for embryonic lethal mutations (37). About once or twice a month, Eric or I traveled to visit each other over the weekend and greatly enjoyed sharing our results and discussing future steps (**Figure 1**). Janni had already become a good friend during her time in Basel and Freiburg. When I had on occasion visited her there, it was exciting to learn firsthand about the two maternal mutations, *bicaudal* and *dorsal*, that she was studying (34, 36). Now with the zygotic lethal screens underway in Heidelberg, the science of embryonic patterning became even more fascinating to think about. Since I was immersed in the question of sex determination in the germline and knew that *Sex-lethal* mutations caused multicellular ovarian cysts, I decided that it might be useful to screen the leftover nonlethal lines from Eric and Janni's mutagenesis screen. My hope was to find viable lines with phenotypes similar to those of *Sex-lethal* that might identify other genes in the germline sex determination pathway. The first Heidelberg screen, centered on the second chromosome of *Drosophila*, had been conducted using an isogenized *cn bw* chromosome, which leads to white-eyed flies when homozygous. After balanced lines had been established, Janni and Eric could use the absence of homozygous white-eyed flies to determine whether they had induced a lethal mutation on the chromosome (38). The lines in which the homozygous chromosome carriers survived were not interesting from a zygotic lethal standpoint, but those lines were perfect to test for multicellular ovarian cysts, for female sterility, and for potential maternal effects visible in the progeny. So I spent some time dissecting and testing such homozygous females; this was a useful approach that yielded unexpected and intriguing phenotypes, and Eric routinely checked those lines further even after I returned to

Figure 1

Trudi Schüpbach and Eric Wieschaus at a conference in Greece in 1980. Photo provided by Trudi Schüpbach.

Zurich. We soon realized that this approach would uncover new pathways central for patterning the egg and the embryo and might thus assume an importance far beyond mutations that affected cyst formation in the ovary. The first alleles of *gurken*, *torso*, *tudor*, *BicC*, *kelch*, and some other phenotypes resulted from this somewhat unplanned screen, and indicated that a larger screen for such mutations would be exciting and insightful.

PRINCETON: THE LARGE SCREEN FOR MATERNAL-EFFECT AND FEMALE-STERILE MUTATIONS

In 1980, it became clear to Eric and me that the next step in our careers should involve finding a place where we could both live and work together. When Eric accepted a job offer from Princeton University, I applied to the Swiss National Science Foundation for a postdoctoral fellowship to join his laboratory. I proposed the screen for female-sterile mutations on the second chromosome of *Drosophila* that I later carried out, and in the proposal I used the few examples, such as *tudor* and *gurken*, as an indication that the screen should yield new and fascinating phenotypes. However, this was the first, but not the last, time that I came across a panel of reviewers who were not happy with the proposal of a screen. I was interviewed in person by a group of scientists at the headquarters of the Swiss National Science Foundation in Bern, and they tried to persuade me that I should not perform a screen but concentrate on the mutations that were already in hand, choose one, and analyze the mutant phenotype in depth. This was, after all, how genetics in *Drosophila* had been done successfully to that point. A genome-wide screen was not considered a good way to start out as a postdoctoral fellow. Fortunately, despite their misgivings, they did grant me the fellowship.

A major challenge in maternal-effect screens is that female-sterile and maternal-effect mutations are about ten times less frequent than lethal mutations; that is, under standard mutagenesis doses, most chromosomes carrying a new female-sterile mutation will also carry an unrelated lethal mutation that will kill homozygotes before they can be tested for fertility. Thus, a large number of lines have to be set up and taken to the third generation to produce an adequate number of homozygous females. Hand-selecting homozygous females in the third generation to test for phenotypes of eggs and embryos thus becomes tedious. To avoid hand-sorting flies, I devised a scheme that would involve dominant female-sterile mutations as well as a dominant temperature-sensitive mutation on a balancer chromosome (48).

These tests allowed me to embark on the big mutagenesis experiments a few months later. Moving ahead was even more urgent, as I was soon expecting our second daughter, and wanted to have the screen done before her arrival the following summer. Every week I mutagenized a few hundred males and mated them to females and then proceeded to establish lines from single F_1 males that were easily transferred through two more generations, using high temperature to kill the unwanted progeny while keeping viable lines at 18°C until the tests on the F_3 females were completed. Combining the mutations from the Heidelberg prescreen with those from Princeton, we screened over 18,000 lines and retained in the end 528 with interesting phenotypes, which ranged from blocking early oogenesis to producing abnormal eggs and affecting specific aspects of embryogenesis (48, 49).

Janni had moved to Tübingen from Heidelberg and had started to assemble an outstanding group of postdocs and graduate students who performed a parallel screen on chromosome 3 of *Drosophila*. We were in constant contact and exchanged results and ideas as well as mutants. All of us were interested in how pattern and polarity arise in the embryo, and the Nüsslein-Volhard laboratory quickly embarked on analyzing many of the mutants that appeared to strictly affect the embryonic pattern. Kathryn Anderson concentrated on the genes affecting the dorsoventral polarity of the embryo, notably the *Toll* gene, in which dominant mutations had been isolated by Eric and Janni during their Heidelberg screens. Kathryn's genetic studies and her analysis involving cytoplasmic transplantations yielded exciting and novel results (4, 5) that built the foundation for understanding dorsoventral polarity of the embryo. Ruth Lehmann began her outstanding work on the pole plasm and abdominal patterning mutants such as *oskar* and *nanos*, and Hans Georg Frohnhöfer studied *bicoid* and its effects on anterior patterning (27, 35).

SIGNALS BETWEEN GERMLINE AND FOLLICLE CELLS PATTERN EGG AND EMBRYO

I myself became particularly interested in mutations that affected not only the embryonic pattern but also the pattern of the eggshell. Although produced by the follicle cells during oogenesis, the pattern of the eggshell closely aligned with the pattern of the future embryo that would subsequently develop inside that egg. For instance, the micropyle of the egg, which is formed by specialized follicle cells, is always placed at the anterior tip of the egg, where the head of the embryo will form. Similarly, the dorsal appendages of the outer eggshell derive from two lateral anterior patches of follicle cells flanking the dorsal midline of both the eggshell and the embryo. Some coordination between the outside, i.e., the follicle cells of the developing egg chamber, and the egg's cytoplasmic pattern was therefore to be expected. But how would this occur, and how were the major axes of the egg and embryo established in the first place?

A mutation in the gene *fs(1)K10* that dorsalized the eggshell, leading to an expanded ring of dorsal appendage material around the ventral side of the egg, had already been described. It also produced a partially dorsalized embryo that showed an expansion of dorsal cuticle structures and

a loss of more ventral structures (62). The screens that I had conducted on the second chromosome yielded two more dorsalizing mutations, *cappuccino* and *spire*, as well as several mutations that produced an opposite phenotype in which dorsal appendages were reduced, often fused on the midline, or, in extreme alleles, were lost completely. The embryos developing inside such eggs mirrored the pattern of the mutant eggshell. Inside the ventralized eggshells, for example, the embryos lacked dorsal and lateral pattern elements, formed ventral cuticle structures on the dorsal side, and showed a greatly expanded mesoderm, the ventralmost structure of the insect embryo. The gene that produced the most reliable and extreme ventralized mutant phenotype was *gurken*, with seven alleles recovered in the screens. I tested the requirement for wild-type gene expression by transplanting pole cells, and also by using mitotic recombination, and found that the wild-type *gurken* gene was required in the germline. As long as the germline was wild type, both the eggshell, as produced by the follicle cells, and the embryo developing inside the egg were normal (47). This indicated that, similar to *fs(1)K10*, patterning processes in the germline were necessary to structure the cytoplasm of the egg derived from that germ cell. However, since the overlying eggshell pattern produced by wild-type follicle cells matched the patterning status of the egg, there had to be communication between the oocyte and the surrounding follicle cells.

The analysis became even more exciting when I transplanted pole cells between embryos that were mutant or wild type for the gene *torpedo*. Homozygous *torpedo* females produced ventralized eggs similar to those produced by *gurken* females, but here the mosaics indicated that the requirement was in the somatic cells, not in the germline (47). Since we were already postulating a signal from the germline to the follicle cells, it was logical to think that *torpedo* might encode a factor that was in the receiving part of the pathway, allowing the follicle cells to respond to this unknown signal from the germline. Seeing ventralized embryos produced by mosaic mothers, in which follicle cells mutant for *torpedo* were surrounding a wild-type oocyte, demonstrated that there must also be a signal from the follicle cells back to the oocyte that was instrumental in setting up the pattern of the embryo. This was further underscored by epistasis experiments involving the *dorsal* gene, which tested as farthest downstream in the pathway and active in the germline.

This dual signaling between germline and follicle cells was unexpected and would not have been detected if we had identified only one of the genes in the pathway. The finding illustrates the value of large-scale saturation screens, even though establishing and testing lines and mapping genes and assigning them to complementation groups require a lot of time before one can pursue new projects based on the mutants. Because saturation screens produce groups of genes with similar phenotypes, they point to pathways and processes rather than single activities and thus allow more general conclusions to be drawn about the process. For a geneticist it is also valuable to have more than one allele per gene to study, as sometimes hypomorphic alleles provide a graded series of phenotypes and thus can give further insights, rather than work with only a single allele. In addition, isolating mutations in several genes that affect the process of interest makes it possible to choose which gene seems the most promising to focus on. Among the ventralizing group, *gurken* mutants provided the most extreme and fully penetrant phenotype. *torpedo*, on the other hand, was the only gene in the ventralizing group that was required in the follicle cells and was particularly interesting from that standpoint. Therefore, these two genes became the next focus of the laboratory.

MOLECULAR ANALYSIS IDENTIFIES EPIDERMAL GROWTH FACTOR RECEPTOR SIGNALING INVOLVED IN DORSOVENTRAL PATTERNING

I obtained a grant from the National Science Foundation after completing the mutagenesis, with the grant panel recognizing the potential of the mutants that I had isolated. Once the exciting

results on *gurken* and *torpedo* were published, I applied to the National Institutes of Health for a grant to extend the analysis to the molecular level. In 1987, I held a position as research biologist in the department; at that time it was still possible to obtain a grant, even in this untenured position. When the grant was funded, I was able to hire two postdoctoral fellows, James (Jim) Price and Lynn Manseau, both of whom had molecular backgrounds and took on the task of identifying genes at the molecular level. An undaunted graduate student, Robert Clifford, also joined the group and proceeded to isolate and characterize many more alleles of *torpedo* (10). This was a time when molecular biology in *Drosophila* was still cumbersome, and our cloning strategy for *torpedo* was to obtain some entry DNA into the region and chromosomally walk from there to *torpedo*. A good candidate for an entry point was the *Drosophila Epidermal growth factor receptor* (*Egfr*), an oncogene homologous to the human gene and cloned by William Wadsworth and colleagues (61). Although our initial plan had been to use Egfr merely as an entry point, Jim and I were aware of the possibility that *torpedo* in fact might be the *Drosophila* gene that encodes Egfr, given that we were expecting it to act in a receiving pathway. This possibility was supported by the behavior of probes hybridized to salivary gland squashes of deficiency and inversion chromosomes Jim had isolated that failed to complement *torpedo*. It became clear that the *Egfr* gene was affected by these chromosomal aberrations and therefore likely corresponded to the *torpedo* gene. Meanwhile, Robert Clifford found that most of the new alleles of *torpedo* were homozygous lethal and produced an embryonic phenotype that Janni and Eric had called *faint little ball* (*flb*) in their screen (38). Complementation tests showed that *torpedo* was allelic to the *flb* alleles they had obtained. Some combinations of the alleles were viable but showed other abnormalities, such as missing wing veins and roughened eyes. Our results indicated that the *torpedo/Egfr* gene was involved in several growth and patterning events throughout development. I had therefore been lucky to isolate a viable allele that nevertheless showed an egg and embryo phenotype strong enough for analysis.

As sometimes curiously happens in science, at exactly the same time, the laboratory of Benny Shilo at the Weizmann Institute in Israel had undertaken an analysis of the *Drosophila Egfr* gene. Benny's graduate student, Eyal Schejter, had performed mutagenesis screens in the region of this gene and had identified mutations in the *Egfr* gene itself. He showed that such mutations cause embryonic lethal phenotypes, but because the phenotypes were difficult to analyze, he applied to an EMBO course on *Drosophila* embryonic development that Janni and Eric were organizing. This gave me the opportunity to talk to Eyal, and after discussion with Benny Shilo, we decided to publish our results together in the same journal (41, 44). This was a good outcome because our publications essentially complemented and supported each other's findings, and because it was the start of a long friendship with both Eyal and Benny that brought many intellectual exchanges and personal interactions over the years.

The discovery that *torpedo* encodes the Egfr in flies was followed by our demonstration that *gurken* encodes a TGFα-like ligand (32). I was soon invited to conferences that were focused on cancer genetics. I found that sometimes members of the cancer community for some reason did not appreciate findings made in flies and were often not willing to share insights and freely discuss results. Obviously, not everyone in the field took this attitude, but still, I much preferred to attend meetings involving the *Drosophila* or the wider developmental community, where a much more open and friendlier attitude prevailed. Our analysis of the *Drosophila* Egfr pathway continued to produce results that ultimately were important for understanding human cancer. For instance, Li-Mei Pai, a postdoc in the laboratory, identified mutations in the *Drosophila Chl* gene that she and Laura Nilson had isolated in a new screen, and showed that the product is required in the follicle cells to limit the activity of the Egfr (39). Her analysis provided important insights into signaling thresholds and gradient establishment, with implications for cancer genetics.

In the *Drosophila* community our results were much appreciated, and I was soon involved in lively exchanges with various scientists from various laboratories. I had met two pioneers of ovarian analysis, Anthony (Tony) Mahowald and Allan Spradling, shortly after we moved to the United States. Both Tony and Allan were extremely welcoming to this European newcomer and were happy to exchange thoughts and results. Several postdocs and graduate students in the Spradling laboratory also became good colleagues and friends. They had performed a P-element insertion mutagenesis for ovarian phenotypes, and I was happy to share some of the mutants we had isolated in genes that they were interested in. Terry Orr-Weaver, Lynn Cooley, Celeste Berg, Denise Montell, and many other members of the Spradling group were instrumental in analyzing various cell biological aspects of ovarian development and defining new and uncharted aspects of oogenesis, and I was always particularly happy when the mutants from our screen could be helpful in their analysis.

REGULATION OF THE SIGNAL IN THE GERMLINE

One of the exciting aspects of science is that solving one question almost always leads to many more questions, often venturing into fields that one might not have initially chosen for study. When F. Shira Neuman-Silberberg cloned *gurken*, she found that the *gurken* messenger RNA (mRNA) was localized to one corner of the developing oocyte, in close proximity to the oocyte nucleus (32). In midoogenesis, the oocyte nucleus moves from an initially central position at the posterior of the small egg chamber to an asymmetric cortical position at the anterior. The site where the nucleus anchors determines the future dorsal side of the egg and embryo. Gurken protein adjacent to the nucleus signals to the overlying follicle cells and activates the Egfr in a restricted set of follicle cells (**Figure 2**). The downstream ERK pathway then activates several genes in these follicle cells that pattern the dorsal eggshell while repressing expression of the gene *pipe* (52). David Stein demonstrated in elegant experiments how this asymmetrical expression of *pipe* on the ventral side initiates a new signal that sets up the dorsal-ventral axis of the embryo via processing of Spätzle and activation of Toll (53, 54). The tightly localized *gurken* mRNA at the top of this hierarchy started a new line of inquiry in my laboratory, where we investigated mRNA localization. Amanda Norvell and later Jennifer Goodrich studied the role of the RNA binding proteins Squid and Hrb48 in this process (21, 33), Guene Thio made transgenes to identify localization sequences within the *gurken* mRNA (57), and Angela Jaramillo eventually generated *gurken* transgenes with MS2 stem loops that could act as binding sites for fluorescently labeled MCP proteins (25). These constructs allowed us to show that *gurken* RNA enters the oocyte from the nurse cells and initially accumulates along the posterior cortex in a dynamic and exchangeable way, but becomes stably anchored at the dorsal-anterior corner after nuclear migration. We also identified genes involved in the translational control of *gurken* mRNA, notably *squid*, *encore*, and *cup* (11, 33, 59), leading to more biochemical approaches (28).

ANTERIOR-POSTERIOR PATTERNING IS ALSO DEPENDENT ON EGFR SIGNALING

Siegfried Roth joined my laboratory in the early 1990s as a postdoctoral fellow. He had done his graduate work in the Nüsslein-Volhard laboratory, where he had studied the functions of *dorsal* (an NF-κB homolog) and *cactus* (an IκB homolog), a gene in which I had isolated the first alleles in the second chromosome screen (48). In his studies on *cactus*, Siegfried had isolated alleles of a separate gene that he called *cornichon* ("cucumber" in French), as it had the same phenotype as *gurken*. When he joined my laboratory, he cloned the gene and found that it encoded a protein with some

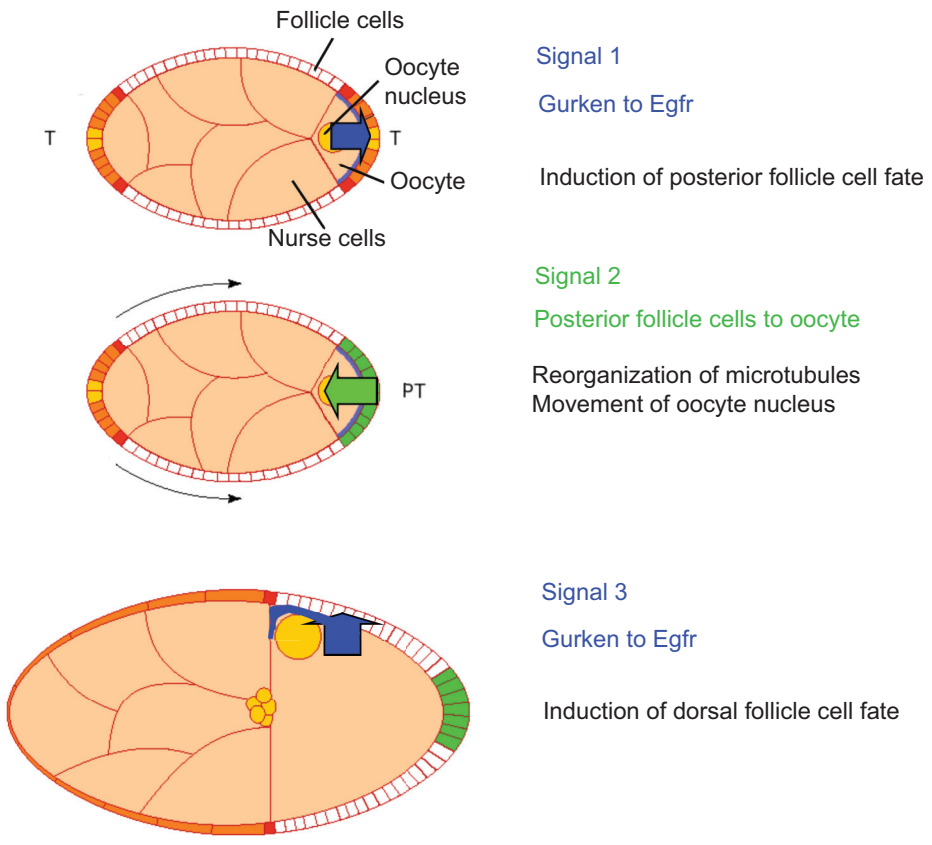

Figure 2

Schematic of Gurken–Egfr signaling during oogenesis. Signal 1: In the egg chamber at stage 6 to 7, activity of the JAK/STAT pathway has defined sets of terminal (T) follicle cells (*yellow*, *orange*, *red*). The oocyte nucleus is positioned in the center of the oocyte. Gurken RNA and protein (*blue*) accumulate at the posterior of the oocyte and Gurken signals to the adjacent follicle cells via the Egfr to take on a posterior cell fate (*blue arrow*). Signal 2: Around stage 7 of oogenesis the newly induced posterior (PT) follicle cells (*green*) send a still unidentified signal back to the oocyte (*green arrow*) that causes a change in the microtubule polarity in the oocyte and leads the oocyte nucleus to move toward the anterior of the oocyte. The anterior terminal (AT) follicle cells move toward the posterior at stage 9 of oogenesis (*black arrows*). Signal 3: Wherever the nucleus contacts the anterior cortex, it will be anchored there and Gurken RNA and protein (*blue*) will accumulate in this anterior cortical region in the vicinity of the nucleus. Gurken signals via the Egfr to the lateral follicle cells and induces them to take on a dorsal cell fate. Images adapted from Reference 60 with permission.

homology to a gene in yeast involved in secretion (42). Being a careful and insightful scientist, Siegfried uncovered that *cornichon*—as well as *gurken* and *torpedo*—actually already functions in an earlier signaling process between the germline and the follicle cells. When the oocyte is still small, Gurken protein is secreted from the oocyte and taken up by the follicle cells at the posterior pole of the egg chamber via the Egfr. This early Gurken-to-Egfr signal induces a polar cap of follicle cells to take on a posterior follicle cell fate, and these posterior follicle cells then send a signal back to the oocyte that serves to repolarize the microtubule cytoskeleton inside the oocyte. The signal also causes the oocyte nucleus to migrate away from the posterior-central position to its

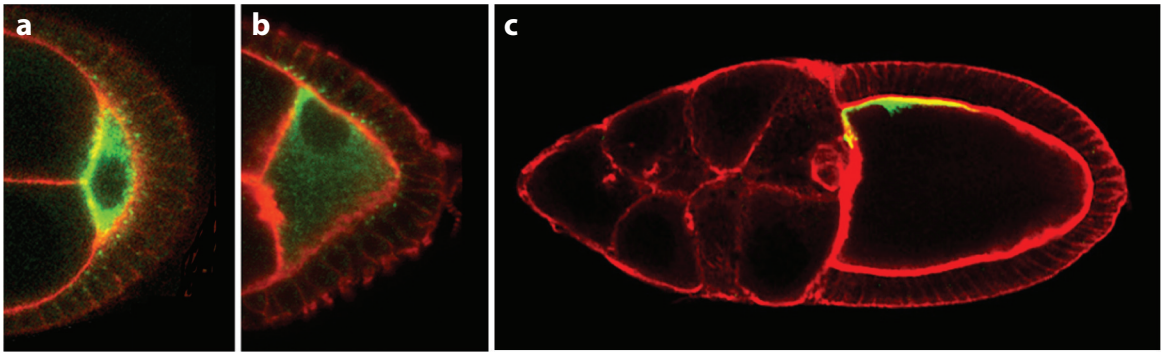

Figure 3

Gurken distribution in oogenesis. Gurken protein is shown in green, actin cytoskeleton in red, and overlap in yellow. (*a*) Egg chamber at stage 6. The oocyte nucleus is in the center of the developing oocyte. Gurken protein accumulates within the oocyte and is also visible after its secretion and uptake via binding to the Egfr into vesicles inside the responding follicle cells at the posterior of the egg chamber. These follicle cells will take on a posterior cell fate. (*b*) Egg chamber at stage 8. Activation of the Egfr in the posterior follicle cells has led to a second (unknown) signal from the follicle cells that causes the oocyte nucleus to move to an asymmetric anterior position within the oocyte. This position defines the dorsal side of the egg chamber. Gurken protein now accumulates in this dorsal-anterior region and is taken up into vesicles in the adjacent follicle cells via the Egfr. A difference between the amount of Gurken internalized by the dorsal versus the ventral follicle cells is visible. (*c*) Egg chamber at stage 10. The oocyte has grown dramatically, but the oocyte nucleus has remained at its dorsal-anterior location. Gurken protein reaches continuous high levels on the dorsal side and induces lateral sets of follicle cells to become dorsal-anterior follicle cells, which will form the operculum and dorsal appendages.

final asymmetric anterior-dorsal location (**Figure 3**). This exciting result showed that both the anterior-posterior and the dorsoventral axes of the egg and embryo were established via signaling between the germline (the oocyte) and the somatic follicle cells and that this axis establishment involved Gurken in the germline and the Egfr in the follicle cells at two different stages of egg chamber development (42). Amazingly, Acaimo González-Reyes, who was a postdoctoral fellow in the laboratory of Daniel St Johnston at the time, came to the same conclusion starting from an analysis of some other genes that caused a ventralized phenotype (20), and fortunately we were able to publish our results at the same time.

SIGNAL PRODUCTION IS REGULATED BY A MEIOTIC CHECKPOINT

The discovery that Gurken functioned in both anterior-posterior and dorsal-ventral patterning was for me a perfect example of a common feature of scientific progress—that it matters often not so much what you first set out to do (cloning a gene) as what you observe along the way and whether you can elevate an initial odd observation to a main project that has general significance. An initially even more puzzling finding was encountered by a graduate student, Amin Ghabrial, and a postdoc, Robert (Rob) Ray, when they molecularly identified the genes *okra* and *spindle B* (*spn-B*). For both of these genes, homozygous mutant females produced ventralized eggshells and embryos, which led us to believe that they might act directly in the Gurken–Egfr pathway. However, the sequence of the corresponding genes showed that *okra* and *spn-B* encode the *Drosophila* homologs of the yeast proteins Rad54 and Rad51, respectively (17). Both of these proteins were key factors in the homologous repair of double-strand DNA breaks (DSBs), and so we were faced with studying DNA repair when we had set out to understand more about control of signaling in development. We knew, of course, that these repair genes are fundamentally involved in recombination during oogenesis, but the formation of synaptonemal complexes and meiotic recombination in *Drosophila* oogenesis occur in the germarium, which comprises the earliest stages of oogenesis,

days before the dorsoventral signaling process takes place in the later stages of oogenesis. So how could we think about this finding? Throughout my career I have been fortunate in attracting smart and talented graduate students and postdocs to the laboratory, and Amin Ghabrial was clearly one of these deep thinkers. He reasoned that unrepaired DNA breaks would presumably activate a checkpoint, and this checkpoint might halt development—or slow down certain processes in development—which might lead to a delay or deficiency in the accumulation of *gurken* RNA or protein. He tested this hypothesis and showed that Gurken protein did not accumulate to normal levels, whereas the *gurken* RNA was present at seemingly normal levels. He then demonstrated that mutations in *mei-41* (an ATR homolog) and *mei-W68* (a Spo11 homolog) could suppress the ventralized phenotype of the eggshell (18). This proved that there indeed exists a meiotic checkpoint activated by unrepaired DSBs in oogenesis. When this checkpoint is active, *gurken* translation remains downregulated. This finding further suggested that the wild-type oocyte would normally be able to halt *gurken* translation during the time of meiotic recombination and allow it to be upregulated only after all the DSBs were repaired. In the repair mutants, however, when the breaks persist for a longer period of time, Gurken translation is not upregulated at the normal time and signaling to the follicle cells is impaired. Uri Abdu, a postdoctoral fellow, followed up these findings further and showed that the gene *chk2* acts downstream of *mei-41* in this process (1). Martha Klovstad and Uri Abdu then went on to demonstrate that the *Drosophila* homologs of the human genes *HUS1* and *BRCA2* were also involved in the meiotic checkpoint activation (3, 26).

In the original second chromosome screen, I had isolated several mutations that caused a variable ventralized phenotype similar to that of *okra* and *spn-B*. I had given the corresponding genes on the second chromosome names of vegetables that vaguely resembled cucumber shapes (e.g., *aubergine*, *zucchini*, *squash*) while the Nüsslein-Volhard group in Tübingen had named this group of genes the "spindle genes" and distinguished them by letters (e.g., A, B, C). After finding that *okra* and *spn-B* encoded DNA repair genes, we wanted to know whether the other genes in this phenotypic class would also encode members of the repair pathway. Indeed, we found that *spindle D* encodes another Rad51 homolog (2). However, another surprise arose when Attilio Pane, a postdoc in the laboratory, together with Kristina Wehr, a graduate student, identified the molecular nature of *zucchini* and *squash* and discovered that they encoded proteins with RNA nuclease homologies (40). Sharon Chen, another graduate student in the laboratory, investigated the gene *cutoff*, in which weak alleles also produced a ventralized eggshell phenotype, and she and Attilio then demonstrated that retrotransposons were upregulated in *cutoff*, *zucchini*, *aubergine*, and *squash* mutant ovaries (9).

In the early 2000s it became understood that a surveillance process guards the *Drosophila* germline against attack by retrotransposons (6–8, 58). As in all genomes of higher eukaryotes, many retrotransposons are present in the genome, and they tend to become active in the germline, which allows them to be transmitted at higher and higher numbers to the progeny. However, the germline has evolved a counterprocess, involving PIWI proteins such as Piwi and Aubergine, to attack both the mature retrotransposons and their transcription (7, 43). Our observations now put *cutoff*, *zucchini*, and *squash* into this surveillance pathway. Since we had already observed that the ventralized phenotype of *aubergine* mutations was suppressed by mutations in *chk2*, we tested the other mutations for suppression by *chk2* and found this to be the case for those mutations as well. These genetic results showed that the germline is using Chk2 as a general node to monitor DNA damage caused by mutations in DNA repair genes or by mutations in Piwi pathway proteins. When activated, Chk2 downregulates translation of *gurken* RNA, which acts as a developmental signal, to prolong the time that DNA could potentially be repaired. A failure to accumulate high levels of Gurken leads to a ventralization of the eggshell, as this signaling process is particularly

sensitive to Gurken levels. Hence, the most noticeable phenotype of the Piwi and DNA repair mutants is a ventralization of egg and embryo.

FOLLICLE CELL SCREENS PROVIDE INSIGHT INTO CELL BIOLOGY

One limitation of maternal-effect and female-sterile screens is that phenotypes can be detected only if homozygous mutant females survive to adult stages. Genes that have earlier vital functions tend to go undetected, since strong alleles kill the homozygous carrier at earlier stages. To isolate such missing genes, we used mitotic recombination both in the germline and in the follicle cells, as this technique allows the heterozygous females to survive but allows homozygous mutant clones to be tested in the adult. These screens yielded many new mutations, although it soon became clear that several of these genes did not encode core components of the signaling processes. Instead, they affected the cell biology of the mutant cells, such that their impact on production, transmission, and reception of the signals was indirect. Their analysis about the underlying mechanics of the cell signaling process still proved insightful. Yan Yan and Natalie Denef, for example, in a screen on the X chromosome, isolated mutations in constituents of V-ATPase, the vacuolar proton pump responsible for vesicle acidification in the endocytic pathway, and found that they affected Notch signaling, blocking the third cleavage of Notch (64). Notch signaling is a timing signal for the follicle cells (13, 30) and cooperates with Egfr signaling to establish anterior-posterior germline polarity via induction of posterior follicle cell fates. The unexpected finding that the V-ATPase blocks Notch signaling told us that acidification of transport vesicles is important in the release of Notch-INTRA. As another example, Yi Sun, a graduate student, found that a myosin light chain phosphatase, PP1β, also affected posterior signaling from the follicle cells back to the germline (55). This finding raised the possibility that mechanical forces in the posterior follicle cells have a role in the signaling process. Natalie Denef showed that the *crag* gene is involved in the restricted polarized secretion of extracellular matrix proteins (12), a process that was then further studied by Olivier Devergne (14, 15), leading us from signaling to more cell biologically focused studies.

Large-scale mutagenesis screens therefore can sometimes lead to the study of processes and aspects of biology that may deviate from the original focus of the screen. But they often open new avenues of research and offer the members of the laboratory the great chance of developing their own independent projects. While it is true that sometimes a mutant phenotype may be hard to interpret and difficult to relate to the molecular nature of the gene, at least the mutant phenotype, when analyzed carefully, can tell us that this gene has an important role in a novel process. It can also be disappointing when a student finds that their gene has no homology and the molecular analysis gives no easy clue about its biochemical function. Looking back, however, I think those students would agree that such unknown genes often provide a starting point for truly unique discoveries that will be of importance to other scientists in the future.

NEW APPROACHES AND FINAL THOUGHTS

Starting in the mid-2000s I have also greatly enjoyed working with a colleague here at Princeton, Stanislav (Stas) Shvartsman, who has a background in chemical engineering and modeling of signaling pathways. His analyses of signaling through the Egfr and other aspects of oogenesis and embryonic development have challenged me in novel ways (19, 22, 29). As long as one moves mostly in one's circle of developmental biologists and geneticists, one feels comfortable, as everyone knows more or less what is possible and does not dwell on proposing experiments that are judged not feasible. But if one encounters scientists from other fields, such as physicists or engineers or computer scientists, they will often ask questions that seem at first naïve but then

require a rethinking and discussion in directions that one would not have normally gone. Often these questions point to new horizons of more quantitative approaches that are nowadays becoming possible and will undoubtedly give new insights into the original problem of patterning and polarity establishment in development.

Today, when many genomic approaches are used to probe development, it is probably rather obvious why it is important to conduct large-scale mutant screens that attempt a good level of saturation in order to identify as many genes as possible that affect a particular aspect of biology. However, an individual student or postdoctoral fellow may still question why they should invest a few years to perform such a genetic screen and determine complementation groups, map the genes, and characterize many phenotypes at once, all before embarking on a specific analysis of a few genes. The big advantage of the full screen is that it allows researchers to establish pathways by grouping genes with the same or similar mutant phenotypes. This was already well known from screens in bacteria and yeast when we started out, but to apply a full screen to development was seen as risky back in 1981. Nevertheless, the Heidelberg screens for zygotic mutations had demonstrated the value of saturation, indicating that the screens should be done at a large-scale level. In addition, the smaller prescreen had shown that interesting new phenotypes could be obtained at a reasonable frequency, and had allowed different genetic schemes to be tested, before starting on a large-scale screen.

Genomic approaches can also yield numerous candidate genes involved in a particular process or developmental stage. However, it is still an arduous task to test many of these candidates for function. Having a mutant phenotype, especially one supported by multiple alleles, predicts that the associated gene is important for the process/developmental transition affected, whatever its molecular function turns out to be. Obviously, there are limits to discovery in a genetic screen if genes are redundant or if they are hard to target, but usually a well-designed screen will at least give first anchor points into a process. From there, one can branch out using other methods to obtain interactors and further build the pathway. For this reason, large-scale forward genetic screens should not be considered a method of the past. There is still so much to discover in biology and I am confident that unbiased genetic screens will yield many more surprises and discoveries in the future.

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LITERATURE CITED

1. Abdu U, Brodsky M, Schüpbach T. 2002. Activation of a meiotic checkpoint during *Drosophila* oogenesis regulates the translation of Gurken through Chk2/Mnk. *Curr. Biol.* 12:1645–51
2. Abdu U, González-Reyes A, Ghabrial A, Schüpbach T. 2003. The *Drosophila spn-D* gene encodes a RAD51C-like protein that is required exclusively during meiosis. *Genetics* 165:197–204
3. Abdu U, Klovstad M, Butin-Israeli V, Bakhrat A, Schüpbach T. 2007. An essential role for *Drosophila* Hus1 in somatic and meiotic DNA damage checkpoints. *J. Cell Sci.* 120:1042–49
4. Anderson KV, Bokla L, Nüsslein-Volhard C. 1985. Establishment of dorsal-ventral polarity in the Drosophila embryo: the induction of polarity by the *Toll* gene product. *Cell* 42:791–98
5. Anderson KV, Jürgens G, Nüsslein-Volhard C. 1985. Establishment of dorsal-ventral polarity in the Drosophila embryo: genetic studies on the role of the *Toll* gene product. *Cell* 42:779–89
6. Aravin AA, Lagos-Quintana M, Yalcin A, Zavolan M, Marks D, et al. 2003. The small RNA profile during *Drosophila melanogaster* development. *Dev. Cell* 5:337–50
7. Aravin AA, Naumova NM, Tulin AV, Vagin VV, Rozovsky YM, Gvozdev VA. 2001. Double-stranded RNA-mediated silencing of genomic tandem repeats and transposable elements in the *D. melanogaster* germline. *Curr. Biol.* 11:1017–27
8. Brennecke J, Aravin AA, Stark A, Dus M, Kellis M, et al. 2007. Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila. Cell* 128:1089–103
9. Chen Y, Pane A, Schüpbach T. 2007. *cutoff* and *aubergine* mutations result in upregulation of retrotransposons and checkpoint activation in *Drosophila*. *Curr. Biol*. 17:637–42
10. Clifford RJ, Schüpbach T. 1989. Coordinately and differentially mutable activities of *torpedo*, the *Drosophila melanogaster* homolog of the vertebrate EGF receptor gene. *Genetics* 123:771–87
11. Clouse KN, Ferguson SB, Schüpbach T. 2008. Squid, Cup, and PABP55B function together to regulate *gurken* translation in *Drosophila*. *Dev. Biol*. 313:713–24
12. Denef N, Chen Y, Weeks SD, Barcelo G, Schüpbach T. 2008. Crag regulates epithelial architecture and polarized deposition of basement membrane proteins in *Drosophila. Dev. Cell* 14:354–64
13. Deng WM, Althauser C, Ruohola-Baker H. 2001. Notch-Delta signaling induces a transition from mitotic cell cycle to endocycle in *Drosophila* follicle cells. *Development* 128:4737–46
14. Devergne O, Sun GH, Schüpbach T. 2017. Stratum, a homolog of the human GEF Mss4, partnered with Rab8, controls the basal restriction of basement membrane proteins in epithelial cells. *Cell Rep*. 18:1831–39
15. Devergne O, Tsung K, Barcelo G, Schüpbach T. 2014. Polarized deposition of basement membrane proteins depends on phosphatidylinositol synthase and the levels of phosphatidylinositol 4,5-bisphosphate. *PNAS* 111:7689–94
16. Garcia-Bellido A, Ripoll P, Morata G. 1973. Developmental compartmentalisation of the wing disk of *Drosophila*. *Nat. New Biol*. 245:251–53
17. Ghabrial A, Ray RP, Schüpbach T. 1998. *okra* and *spindle-B* encode components of the *RAD52* DNA repair pathway and affect meiosis and patterning in *Drosophila* oogenesis. *Genes Dev*. 12:2711–23
18. Ghabrial A, Schüpbach T. 1999. Activation of a meiotic checkpoint regulates translation of Gurken during *Drosophila* oogenesis. *Nat. Cell Biol.* 1:354–57
19. Goentoro LA, Reeves GT, Kowal CP, Martinelli L, Schüpbach T, Shvartsman SY. 2006. Quantifying the Gurken morphogen gradient in *Drosophila* oogenesis. *Dev. Cell* 11:263–72
20. González-Reyes A, Elliott H, St Johnston D. 1995. Polarization of both major body axes in *Drosophila* by *gurken-torpedo* signalling. *Nature* 375:654–58
21. Goodrich J, Clouse KN, Schüpbach T. 2004. Hrb27C, Sqd, and Otu cooperatively regulate *gurken* RNA localization and mediate nurse cell chromosome dispersion in *Drosophila* oogenesis. *Development* 131:1949–58
22. Goyal Y, Jindal GA, Pelliccia JL, Yamaya K, Yeung E, et al. 2017. Divergent effects of intrinsically active MEK variants on developmental Ras signaling. *Nat. Genet.* 49:465–69
23. Hadorn E. 1951. Developmental action of lethal factors in Drosophila. *Adv. Genet.* 4:53–85

24. Hadorn E. 1968. Transdetermination in cells. *Sci. Am.* 219:110–14
25. Jaramillo AM, Weil TT, Goodhouse J, Gavis ER, Schupbach T. 2008. The dynamics of fluorescently labeled endogenous *gurken* mRNA in *Drosophila. J. Cell Sci.* 121:887–94
26. Klovstad M, Abdu U, Schüpbach T. 2008. Drosophila *brca2* is required for mitotic and meiotic DNA repair and efficient activation of the meiotic recombination checkpoint. *PLOS Genet.* 4:e31
27. Lehmann R, Nüsslein-Volhard C. 1986. Abdominal segmentation, pole cell formation, and embryonic polarity require the localized activity of *oskar*, a maternal gene in Drosophila. *Cell* 10:141–52
28. Li W, Klovstad M, Schüpbach T. 2014. Repression of Gurken translation by a meiotic checkpoint in *Drosophila* oogenesis is suppressed by a reduction in the dose of *eIF1A. Development* 141:3910–21
29. Lim B, Dsilva CJ, Levario TJ, Lu H, Schüpbach T, et al. 2015. Dynamics of inductive ERK signaling in the *Drosophila* embryo. *Curr. Biol.* 25:1784–90
30. López-Schier H, St Johnston D. 2001. Delta signaling from the germline controls the proliferation and differentiation of the somatic follicle cells during *Drosophila* oogenesis. *Genes Dev.* 15:1393–405
31. Marsh JL, Wieschaus E. 1978. Is sex determination in germ line and soma controlled by separate genetic mechanisms? *Nature* 272:249–51
32. Neuman-Silberberg FS, Schüpbach T. 1993. The Drosophila dorsoventral patterning gene *gurken* produces a dorsally localized RNA and encodes a TGFα-like protein. *Cell* 75:165–74
33. Norvell A, Kelley RL, Wehr K, Schüpbach T. 1999. Specific isoforms of Squid, a *Drosophila* hnRNP, perform distinct roles in Gurken localization during oogenesis. *Genes Dev.* 13:864–76
34. Nüsslein-Volhard C. 1977. Genetic analysis of pattern-formation in the embryo of *Drosophila melanogaster*: characterization of the maternal-effect mutant *Bicaudal. Wilhelm Roux Arch. Dev. Biol.* 183:249–68
35. Nüsslein-Volhard C, Frohnhöfer HG, Lehmann R. 1987. Determination of anteroposterior polarity in Drosophila. *Science* 238:1675–81
36. Nüsslein-Volhard C, Lohs-Schardin M, Sander K, Cremer C. 1979. A dorso-ventral shift of embryonic primordia in a new maternal-effect mutant of *Drosophila. Nature* 283:474–76
37. Nüsslein-Volhard C, Wieschaus E. 1980. Mutations affecting segment number and polarity in *Drosophila. Nature* 287:795–801
38. Nüsslein-Volhard C, Wieschaus E, Kluding H. 1984. Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. I. Zygotic loci on the second chromosome. *Wilhelm Roux Arch. Dev. Biol.* 193:267–82
39. Pai L-M, Barcelo G, Schüpbach T. 2000. *D-cbl*, a negative regulator of the Egfr pathway, is required for dorsoventral patterning in *Drosophila* oogenesis. *Cell* 103:51–61
40. Pane A, Wehr K, Schüpbach T. 2007. *zucchini* and *squash* encode two putative nucleases required for rasiRNA production in the *Drosophila* germline. *Dev. Cell* 12:851–62
41. Price JV, Clifford RJ, Schüpbach T. 1989. The maternal ventralizing locus *torpedo* is allelic to *faint little ball*, an embryonic lethal, and encodes the Drosophila EGF receptor homolog. *Cell* 56:1085–92
42. Roth S, Neuman-Silberberg FS, Barcelo G, Schüpbach T. 1995. *cornichon* and the EGF receptor signaling process are necessary for both anterior-posterior and dorsal-ventral pattern formation in Drosophila. *Cell* 81:967–78
43. Saito K, Nishida KM, Mori T, Kawamura Y, Miyoshi K, et al. 2006. Specific association of Piwi with rasiRNAs derived from retrotransposon and heterochromatic regions in the *Drosophila* genome. *Genes Dev.* 20:2214–22
44. Schejter ED, Shilo BZ. 1989. The Drosophila EGF receptor homolog (DER) gene is allelic to *faint little ball*, a locus essential for embryonic development. *Cell* 56:1093–114
45. Schüpbach T. 1982. Autosomal mutations that interfere with sex determination in somatic cells of *Drosophila* have no direct effect on the germline. *Dev. Biol.* 89:117–27
46. Schüpbach T. 1985. Normal female germ cell differentiation requires the female X-chromosome-autosome ratio and expression of *Sex-lethal* in *Drosophila melanogaster*. *Genetics* 109:529–48
47. Schüpbach T. 1987. Germline and soma cooperate during oogenesis to establish the dorso-ventral pattern of egg shell and embryo in *Drosophila melanogaster*. *Cell* 49:699–707
48. Schüpbach T, Wieschaus E. 1989. Female sterile mutations on the second chromosome of Drosophila melanogaster. I. Maternal effect mutations. *Genetics* 121:101–17

49. Schüpbach T, Wieschaus E. 1991. Female sterile mutations on the second chromosome of Drosophila melanogaster. II. Mutations blocking oogenesis or altering egg morphology. *Genetics* 129:1119–36
50. Schüpbach T, Wieschaus E, Nöthiger R. 1978. A study of the female germline in mosaics of *Drosophila*. *Wilhelm Roux Arch. Dev. Biol*. 184:41–56
51. Schüpbach T, Wieschaus E, Nöthiger R. 1978. The embryonic organization of the genital disc studied in genetic mosaics of *Drosophila melanogaster*. *Wilhelm Roux Arch. Dev. Biol*. 185:249–70
52. Sen J, Goltz JS, Stevens L, Stein D. 1998. Spatially restricted expression of *pipe* in the *Drosophila* egg chamber defines embryonic dorsal-ventral polarity. *Cell* 95:471–81
53. Stein D, Cho YS, Stevens LM. 2013. Localized serine protease activity and the establishment of *Drosophila* embryonic dorsoventral polarity. *Fly* 7:161–67
54. Stein D, Roth S, Vogelsang E, Nüsslein-Volhard C. 1991. The polarity of the dorsoventral axis in the Drosophila embryo is defined by an extracellular signal. *Cell* 65:725–35
55. Sun Y, Yan Y, Denef N, Schüpbach T. 2011. Regulation of somatic myosin activity by protein phosphatase 1β controls *Drosophila* oocyte polarization. *Development* 138:1991–2001
56. Szabad J, Schüpbach T, Wieschaus E. 1979. Cell lineage and development in the larval epidermis of *Drosophila melanogaster*. *Dev. Biol*. 73:256–71
57. Thio G, Ray R, Barcelo G, Schüpbach T. 2000. Localization of *gurken* in *Drosophila* oogenesis requires elements in the 5′ and 3′ region of the transcript. *Dev. Biol.* 221:435–46
58. Vagin VV, Sigova A, Li C, Seitz H, Gvozdev V, Zamore PD. 2006. A distinct small RNA pathway silences selfish genetic elements in the germline. *Science* 313:320–24
59. Van Buskirk C, Hawkins N, Schüpbach T. 2000. Encore is a member of a novel family of proteins and affects multiple processes in Drosophila oogenesis. *Development* 127:4753–62
60. Van Buskirk C, Schüpbach T. 1999. Versatility in signalling: multiple responses to EGF receptor activation during *Drosophila* oogenesis. *Trends Cell Biol*. 9:1–4
61. Wadsworth SC, Vincent WS III, Bilodeau-Wentworth D. 1985. A *Drosophila* genomic sequence with homology to human epidermal growth factor receptor. *Nature* 314:178–80
62. Wieschaus E, Marsh JL, Gehring W. 1978. *fs(1)K10*, a germline-dependent female sterile mutation causing abnormal chorion morphology in *Drosophila melanogaster*. *Wilhelm Roux Arch. Dev. Biol*. 184:75–82
63. Wieschaus E, Szabad J. 1979. The development and function of the female germ line in *Drosophila melanogaster*: a cell lineage study. *Dev. Biol*. 68:29–46
64. Yan Y, Denef N, Schüpbach T. 2009. The vacuolar proton pump (V-ATPase) is required for Notch signaling and endosomal trafficking in *Drosophila*. *Dev. Cell* 17:387–402