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Insect Signaling Centers

Klaus Sander and the Double Gradient Model of *Drosophila*

Classic embryological experiments demonstrated that there are at least two "organizing centers" in the insect egg. One is the anterior organizing center, the other the posterior organizing center. Klaus Sander (1968; 1975) postulated that these two organizing areas form two gradients, one initiated at the anterior end and the other at the posterior end. Each of these gradients forms its own structures at the poles and interacts with the other gradient to form the central portion of the embryo. Sander based this model on experiments that involved ligating the embryo at various times during development and transplanting regions of polar cytoplasm from one region of the egg to another. First, if he moved cytoplasm from the posterior pole more anteriorly, he obtained a small embryo anterior to the posterior pole plasm, while extra segments, not organized into an embryo, formed behind it. Second, if he ligated the egg early in development, separating the anterior from the posterior region, one half developed into an anterior embryo and one half developed into a posterior embryo, but neither half contained the middle segments of the embryo. The later in development the ligature was made, the fewer middle segments were missing. Thus, it appeared that there were indeed gradients emanating from the two poles during cleavage and that these gradients interacted to produce the positional information determining the identity of each segment.

The possibility that mRNA is responsible for generating the anterior gradient was suggested in a series of experiments by Kalthoff and Sander (1968). They found that when the anterior portion of the *Smittia* (midge) egg was exposed to ultraviolet light at wavelengths capable of inactivating RNA (265 and 285 nm), the resulting embryo lacked its head and thorax. Instead, the embryos developed two abdomens and telsons (tails) with mirror-image symmetry: telson-abdomen-abdomen-telson. Further evidence that RNA is important in specifying the anterior portion of the fly embryo was obtained by Kandler-Singer and Kalthoff (1976), who submerged *Smittia* eggs in solutions containing various enzymes and then punctured the eggs in specific regions. Double abdomens resulted when RNase was permitted to enter the anterior end. Other enzymes did not cause this abnormality, nor did RNase effect this change when it entered other regions of the egg. Thus, Sander’s laboratory postulated the existence of a gradient at either end of the egg, and it seemed likely that the egg sequestered an RNA that generated a gradient of anterior-forming material.

Interestingly, the double gradient model of *Drosophila* body axis formation mirrored the double-gradient model proposed for amphibian axis formation, which had been proposed in the 1950s, but which was achieving experimental confirmation around the same time.

Christiane Nüsslein-Volhard and *Drosophila* Embryogenesis

Why were Christiane Nüsslein-Volhard and Eric Wieschaus the ones who discovered the importance of maternal effect genes to the formation of the body axis? According to Michael Ashburner's (1993) thoughtful history of the field, these experiments could have been done forty years earlier if anyone had wanted to do so. All it required was "some standard genetics, a mutagen, and a dissecting

microscope, all available in the 1930s." Why hadn't anyone earlier searched the *Drosophila* genome for mutations that prevented early *Drosophila* development?

According to Evelyn Fox Keller (1996), there were conceptual and cultural reasons for the delay, and there were also conceptual and cultural reasons for why the undertaking was eventually done by a German woman with a background in biochemistry.

Why the delay?

First, it is important to recall that the *Drosophila* embryo had not been an object of intensive study. Up until the 1970s, embryologists tended to study amphibians, sea urchins, and chicks animals with large eggs whose cells could be transplanted. Similarly, geneticists interested in multicellular animals stuck very closely to *Drosophila* where the genetics had been well studied and the techniques for breeding the animals were well characterized.

Second, *Drosophila* geneticists claimed that there was no difference between the gene action early in development and later in development. In both cases, genes made RNAs, and the RNAs made proteins. Therefore, almost all *Drosophila* geneticists studied those genes which affected traits in the adult flies. These were the easiest to detect.

Third, those who did study *Drosophila* development had a rough time. Work was difficult, labor-intensive, and slow. "Death" is a difficult phenotype to analyze. Donald F. Poulson was an important *Drosophila* embryologist in the 1930s and 1940s, but he had a difficult time attracting graduate students into his laboratory. Poulson had even suggested a critically important experiment—injecting the cytoplasm from a wild-type oocyte into the egg of a maternal mutant and seeing if it would cure the defect—but he never did it. The state of the field can be gauged by the fact that until *The Development of Drosophila melanogaster* was published in 1993, Poulson's reviews were still being used by researchers.

Fourth, there was a conceptual barrier, in that development was seen as being either regulative (conditional) or mosaic (autonomous). When developmental geneticists started looking at embryos in the early 1970s, they were disappointed when they did not find cytoplasmic determinants of the mosaic eggs they assumed would be there. Even when Alan Garen and Walter Gehring (1972) showed that wild-type cytoplasm could rescue a lethal maternal-effect phenotype, this line of research was abandoned.

Fifth, there is what Keller calls the "discourse of gene action". The nucleus was the center of gene action, not the cytoplasm. Whereas embryology was seen to be cytoplasmic and genetics to be nuclear, it was difficult to see any value in studying inheritance that appeared to work through the cytoplasm.

Why were Nüsslein-Volhard and Wieschaus the ones to do the work?

Keller sees Nüsslein-Volhard at the nexus of several strands of social, political, and scientific development. "Even if neither the questions she posed nor the techniques she employed were new, the possibilities that faced her in the mid-1970s—as a woman, as a German, as a recent Ph.D. in biochemistry and molecular biology turning to the study of development (and more specifically, to *Drosophila*embryogenesis)—these were conspicuously new."

Nüsslein-Volhard was born at the height of World War II, and after the War, she attended an all-girls' school in Frankfurt, West Germany. She learned to love Goethe, mathematics, and biology. When she entered the University in Frankfurt, she soon became bored with her biology classes and switched to mathematics and physics. After two years, she felt that physics was "too dry" and was

excited about entering a new program in biochemistry that was starting in Tübingen. Tübingen became a center for young German biologists who were interested in the physical bases of life, and it was the center of a new institute, headed by Alfred Gierer, one of the few German biologists who had gone to the United States (in his case, MIT and CalTech) and who had seen the revolution in molecular biology that had come to America. Molecular biology at that time was new to Germany.

Again, Nüsslein-Volhard seemed to be disappointed. She did not want to spend the rest of her life sequencing DNA. Gierer convinced her that developmental biology was a place where she could make a difference and a field that needed her expertise. She decided that she wanted to combine genetics with embryology, and this meant learning both embryology and genetics. Moreover, she decided that the most interesting place was that "no man's land"—the *Drosophila* embryo. In 1973, she met Walter Gehring, one of the few continental Europeans who knew both molecular biology and development. He had just finished his work at Yale in Garen's laboratory, showing that wild-type cytoplasm could rescue maternal effect mutants. However, Gehring's interest had turned to the later stages of *Drosophila* development and to developing new molecular techniques. Nüsslein-Volhard worked in Gehring's laboratory in Basel and learned how to do genetic crosses from a postdoctoral fellow, Jeanette Holden, and learned *Drosophila* embryology from a graduate student, Eric Wieschaus.

Wieschaus had been a graduate student of Poulson's, so he was one of the few persons who actually knew what was known about early *Drosophila* development. He met Gehring while at Yale, and he went to Basel to finish his project with him. Wieschaus was a perfect counter to Nüsslein-Volhard. While Nüsslein-Volhard was "an intense, driven young woman with the imprimatur of molecular biology, " Wieschaus was "a gentle, apparently easy-going and soft-spoken man who had been a conscientious objector during the Vietnam War..." According to Keller, they formed a quick and long-lasting friendship. "He taught her to look at embryos, how to do transplantations. She provided enthusiasm, drive, and a single-minded focus on the onset of embryonic pattern."

Meanwhile, Nüsslein-Volhard tried to streamline the entire process of producing and analyzing embryos. If they were to do a "saturation mutagenesis " screen, they were going to have to look at thousands of fly embryos and determine immediately whether they had pattern deformities. First, she found that if they used a particular oil that other investigators had used to protect dechorionated eggs from drying out, it made the opaque chorion transparent. This got around the technically difficult and time-consuming process of taking the chorion off the embryo (a process that often destroyed the embryo itself). Second, if she let the females lay their eggs on a medium containing apple juice (instead of the usual dark grape juice) and poured the oil onto the plate, she could analyze the embryos directly on the plate. A third innovation was a huge time-saver—a method of dropping eggs from one female into forty labeled vials at a time. What had taken earlier investigators years could now be done in weeks. The screen was done by them at a time when no one else would have done it.

Keller identifies several interacting strands that come together to enable Nüsslein-Volhard and Eric Wieschaus to do this work. From her identity as a molecular biologist, Nüsslein-Volhard drew confidence, a kind of arrogance, and a cultural style. As a German, she had imbibed a tradition, shared by the French, of finding interest in complexity ("the more complicated the more interesting it was"). Moreover, Nüsslein-Volhard came from a family with an "artistic temperament," a particular visual acumen that, she claimed, was needed to recognize small deviations from pattern. Also, this work would have been impossible for her had she not come into the field at a moment in time when opportunities for women in science were just beginning to appear and if she had not teamed up with somebody competent who would work with her.

Interestingly, to American women scientists, Nüsslein-Volhard is a heroine. Her role in their success is and has been significant in several ways. First, in the example she sets, and more substantively, in the women she trained. Three out of her first four post-docs were women, and at least two of them have gone on to be leaders of the field. Others who came later also owe a great debt to her

mentorship, and even some senior women have found inspiration in her laboratory. But she is no heroine to German feminists. Those who know of her at all are more likely to see her as an enemy than as an ally. Her ambition, her phenomenal drive, her all-consuming investment in her research, are anathema to a generation of feminist scientists in Germany who have become known for their advocacy of a kinder, gentler, and more "relevant"—in a word, a "greener"— science. Fiercely opposed to genetic engineering, they see her as a member of the bio-tech establishment. And they complain bitterly of her intolerance of any interference with the scientific work of those in her lab incurred by family obligations. Even the day-care center she worked so hard to establish at the MPI for Developmental Biology in 1990 (one of the first such examples in western Germany) meets with their disapproval: only partially subsidized by the Max Planck Society, the costs seem prohibitive to them (Keller, 1997).

Nüsslein-Volhard's opportunity was a function of her particular location in cultural, scientific, and political time; her individual contribution derived from her ability to make good use of this opportunity. She was a bricoleur par excellence, in part because of the extent to which she was able to make opportune use of local alliances-- allying herself with those strains in feminism that accord with, and those women who share, her goals. Perhaps as feminist analysts of science, we can learn to do the same. Such strategic alliances may not accord with the utopian vision some of us once adhered to, but it may be more realistic—certainly, it was, in this case at least, manifestly effective.

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