Cell, Vol. S116, S1–S5, January 23, 2004 Copyright ©2004 by Cell Press

The Bicoid Morphogen Papers (I): Account from CNV

Commentary

Christiane N. Nüsslein-Volhard*
Max-Planck-Institut für Entwicklungsbiologie
Spemannstrasse 35
D-7400 Tubingen
West Germany

Background

When I had finished my thesis (dealing with bacterial transcription) in the Max-Planck-Institut für Virusforschung in Tübingen in 1973, I looked around for a topic and laboratory for my postdoctoral work. Stimulated by discussions with people in Alfred Gierers hydra group, I got interested in embryonic pattern formation. Because of its ability to regenerate a complete organism from tiny pieces, hydra served as a promising model for embryonic development. Based on experiments with hydra, Lewis Wolpert (1969) had proposed the concept of positional information according to which different positions in a developing field are determined by a concentration gradient of a factor, a so-called morphogen. This gradient concept seemed very attractive as it explains an increase in complexity in space by the quantitative differences of just one substance. A physical model describing how such gradients could be established and maintained was developed in Tübingen by Gierer and Meinhardt (1972). I cannot pretend that I fully grasped the importance of these concepts, but in a mysterious way they fascinated me. At the time, the concept of gradients was not widely accepted. This was because such morphogens had not yet been identified. There were plenty of reasons why it was so difficult to isolate morphogens, or indeed any factor that would instruct embryonic tissue to develop a particular structure. In the assays that people had tried, extracts to be tested for their biological activity were added to fragments of embryos (or stumps of the body column in the case of hydra) that were deprived of the hypothetical factor. One problem with such an assay seemed to be that the factor may never have been completely absent from the embryo fragment, but upon operation may even redistribute or regenerate and thus cause erratic results. Furthermore, the coarse experimental interferences upset delicate balances within the developing embryo, causing artifacts that were difficult to distinguish from "real" effects.

In my search for a new topic, I was also influenced by the work of the Tübingen group of Friedrich Bonhoeffer and Heinz Schaller on the genetics of bacterial DNA replication. They carried out a large-scale screen for mutants that were temperature sensitive in replication and identified several new genes, among them DNA E. This turned out to be the replicating enzyme, called polymerase III, while a mutant of the Kornberg DNA polymerase I replicated normally. This work demonstrated how a mutation can cleanly and specifically eliminate one protein without affecting anything else. In vitro complementation provided an assay for the isolation of the gene product (Nüsslein et al., 1971). This convinced me of the powers of a genetic approach. I screened the literature about combining embryology with genetics and soon found *Drosophila*.

In the early seventies, some promising papers on *Drosophila* embryonic development were published. In one famous experiment, Illmensee and Mahowald (1974) demonstrated a transplantable activity localized at the posterior pole, which could induce pole cell formation at the anterior. Even a mutant, *grandchildless*, was described that lacked pole plasm and pole cells, albeit in another *Drosophila* species. It seemed feasible to identify more genes encoding such factors by screening for maternal mutations that affected the informational content of the egg. A mutant embryo lacking a morphogenetic factor might be rescued by the injection of extracts from wild-type embryos and thus provide an assay for the isolation of the factor, which would be much more specific than was possible with operations on normal embryos. Most excitingly, Garen and Gehring (1972) reported a rescue of a maternal mutant, *deep orange,* by cytoplasmic transplantation. I joined the lab of Walter Gehring at the Biozentrum in Basel in 1975 with the long-term goal to isolate morphogens in *Drosophila*. Here I met Eric Wieschaus, who had just finished his thesis with Gehring.

Bicaudal

At that time, *Drosophila* genetics was largely dealing with mutants affected in the structures of the adult fly. A small number of embryonic mutants, however, had been collected by scientists from the lab of Donald Poulson at Yale. These and other embryonic mutants were described in a comprehensive review by Ted Wright (1970). Among those was *bicaudal*, isolated by Alice Bull (1966). Its striking phenotype displayed two abdominal ends in mirror symmetry, while anterior abdomen, head, and thorax were lacking. Similar posterior pattern duplications in insects had been generated by Klaus Sander (1960), who had identified an activity localized at the posterior pole of a leaf hopper egg that could induce posterior pattern at the anterior. On the basis of these experiments, Sander had postulated a center of activity localized at the posterior pole in insect eggs and determined the pattern at a distance via the formation of a gradient of a morphogen. In fact, the *bicaudal* phenotype could best be described by such a gradient with a high point at the posterior pole that was duplicated at the anterior in the mutant. Unfortunately, *bicaudal* mutant females often produced very few if any embryos displaying the phenotype, and therefore it was hard to explain the function of the *bicaudal* gene in forming such a hypothetical gradient. More importantly, some of the intermediate patterns of the *bicaudal* mutant did not make sense in terms of a gradient model. These were the embryos comprised of just one abdomen with normal polarity, topped abruptly at the anterior with a telson, the most posterior structure. Such a discontinuous pattern is incompatible with a gradient model, as it cannot explain such "jumps."

*Correspondence: christiane.nuesslein-volhard@tuebingen.mpg.de

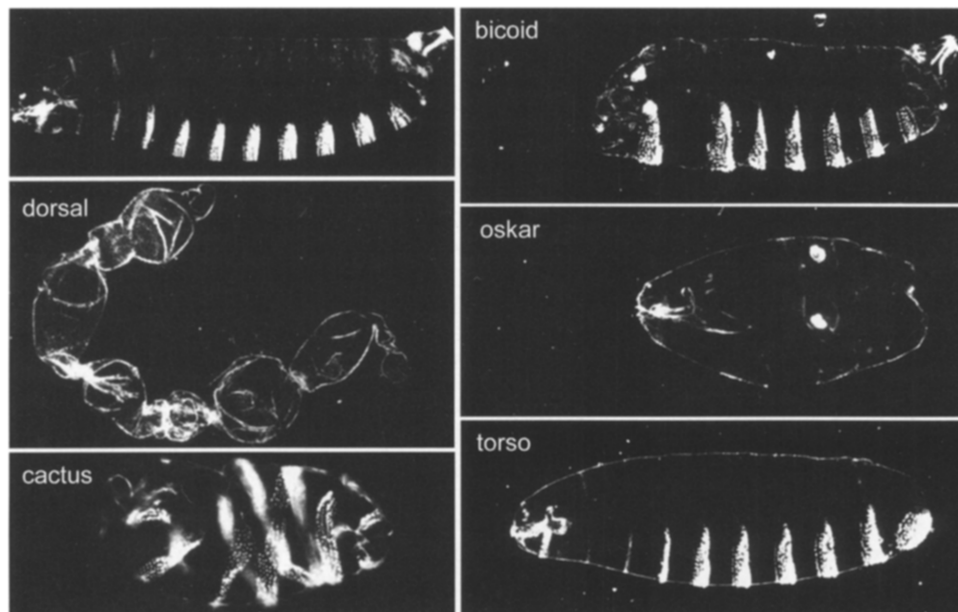

Figure 1. Phenotypes of Maternal Mutants

Maternal Mutants

When the original *bicaudal* mutant was recovered and reinvestigated, its very variable phenotype and unreliable penetrance discouraged any attempts to study it further (Nüsslein-Volhard, 1977). New maternal mutants had to be isolated, as probably many more genes were involved. We and others started collecting more maternal mutants from various sources (Figure 1). The published screens for maternal mutants of the X chromosome and third chromosome described several interesting phenotypes (Gans et al., 1975; Zalokar et al., 1975; Rice and Garen, 1975), which however were difficult to interpret and did not seem to affect pattern formation. One of the first informative maternal mutants was *dorsal*, isolated by chance in Basel in a screen for *bicaudal* alleles. *dorsal* has a clean, penetrant, and nonvariable phenotype, which is also dosage dependent. The phenotypic series suggested the existence of a gradient with a maximum at the ventral side of the egg determining the dorsoventral axis. Taken together with the *bicaudal* phenotype, this implied that the AP and DV axes were set up independently by two gradients oriented at right angles to each other (Nüsslein-Volhard, 1979; Nüsslein-Volhard et al., 1980).

Screens for maternal mutants are very difficult because they require two generations of inbreeding until homozygous females can be obtained that can be tested for the production of abnormally patterned embryos. While developing screening protocols for maternal mutants in our lab at the EMBL in Heidelberg, Eric Wieschaus and I also started looking at zygotic mutants affecting segmentation, which we obtained from various sources. The observed phenotypes turned out to be so interesting that we decided to first do large-scale screens for zygotic mutants because they were easier than maternal screens and extremely rewarding. Indeed these projects resulted in a large and very exiting collection of patterning mutants (Nüsslein-Volhard and Wieschaus, 1980). We did not lose sight of the maternal mutants, however, and while doing the zygotic screen of the second chromosome, we grew the homozygous F2 flies from viable lines to adulthood and investigated their progeny for maternal effects. This screen resulted in the isolation of alleles of *torso, gurken*, and *tudor*. By chance we also picked the first dominant *Toll*, *BicC*, and *BicD* alleles, as well as an *easter* allele that shared the phenotype with *dorsal*. This was very encouraging, but the phenotypes were puzzling and for the time being quite difficult to interpret.

When Eric Wieschaus and I each set up our independent lab after leaving the EMBL, we both undertook large-scale maternal screens. In Princeton, Trudi Schüpbach investigated the second chromosome, while the Tübingen group screened the third chromosome. In my Tübingen group in the Friedrich Miescher Laboratories of the Max-Planck-Society, Kathryn Anderson and Gerd Jürgens were postdocs, while Ruth Lehmann and Hans Georg Frohnhöfer joined a little later as graduate students. Our screening protocol was designed by Gerd Jürgens, who was interested in homeotic transformations caused by maternal effect mutations. The screen turned out to be quite difficult and, in contrast to the Princeton screen (Schüpbach and Wieschaus, 1989), was never published as a whole. However, it provided us with an overwhelmingly rich yield of exiting mutants; we isolated alleles of most of the *dorsal* group genes, together with *torso like*, *oskar*, *pumilio*, and finally *bicoid*.

One striking result that became obvious when we exchanged information with the Princeton screen group was that there was a much smaller set of observed phenotypes than identified genes, and several of these shared a common or at least a similar phenotype. There was the large group of genes with a dorsalized phenotype, like *dorsal*, beside the ventralized phenotypes of *Toll* and *cactus*. Then there was a group of mutants that lacked the abdomen resembling the zygotic *knirps*

mutants, and in most of them, also the pole plasm and pole cells were lacking. This group including *vasa, valois, oskar, pumilio, nanos, staufen,* and *tudor* was initially named the "maternal gap genes," and later called the posterior group. The *torso* phenotype displaying anterior and posterior truncations was shared by *trunk* and *torsolike*. Finally, there was an odd collection of mutants with anterior defects that included *exuperantia, swallow,* and *bicoid*. While the phenotypes of *exuperantia* and *swallow* are quite similar, *bicoid* was unique. In strong *bicoid* alleles, all structures normally derived from the anterior half of the fate map, acron, head, and thorax are lacking and replaced by a telson. The anterior abdomen also may show segmentation defects. Weak alleles share with *exu* and *swa* the anterior defects of head and acron, but do not display posterior duplications. In contrast to *bicaudal*, the *bicoid* phenotype is penetrant and quite constant for any particular allele.

Three groups of genes determining the anterior-posterior pattern (plus those of the dorsoventral pattern), each with several components, seemed to be enough to provide work for decades. The *torso* phenotype had not been seen before in experimental embryology and provided an enigma. The telson was a puzzle as it was absent in *torso*, present in the posterior group phenotype, and duplicated at the anterior in *bicoid*. Without further assumptions, none of the three groups suggested a gradient mechanism, and there was no simple alternative interpretation. Although we had isolated so many good mutants, I got quite depressed because it was difficult to see how we could ever reach an understanding of the three systems. It seemed that we had to clone all the genes and do extensive biochemistry with novel and perhaps strange proteins of unknown functions. Although I had studied biochemistry and was experienced in molecular biology from my thesis work, the prospect was by no means appealing to me. One should remember that cloning genes was still very very difficult at the time. This was when I first thought about working with zebrafish.

Cytoplasmic Transplantation

One better make sure to pick the most important gene among its group before diving into genomic walks. But how to find out? We tackled the problem by characterizing each individual gene to learn to distinguish them, to attribute them with "personalities," as Kathryn Anderson put it at the time. The aim was to order the genes in a hierarchical context and predict a possible molecular function where possible. In addition to a thorough genetic analysis, the most successful approach that we used for this purpose was cytoplasmic transplantation between wild-type and mutant embryos. By that time, it had become clear that the path to the isolation of a morphogen was via cloning the gene encoding it and not via transplantation assays. Nevertheless, such transplantation experiments could yield some important information regarding distribution and requirement of the gene products. In the case of dorsal, where the technique was first employed, the phenotype could be partially rescued by the injection of wild-type cytoplasm, and this rescuing activity was slightly more effective when injected at the ventral side (Santamaria and Nüsslein-Volhard, 1983). Similar experiments with the new dorsal group mutants carried out by Kathryn Anderson lab resulted in the important notion that it frequently was the respective mRNA stored in the egg that rescued the mutant phenotype (Anderson and Nüsslein-Volhard, 1984). But in the cases of the dorsal group of genes, the RNA did not appear to be localized.

Ruth Lehmann and Hans Georg Frohnhöfer worked on the posterior group (RL) and on the mutants affecting anterior pattern including the *torso* group (HGF). Although of very different personalities, both Ruth and Hans Georg were excellent experimentalists and performed the transplantation experiments with great rigor and skill. While Ruth usually was very enthusiastic and excited us with her ideas and new findings, Hans Georg often would spend days without talking and when asked would mumble something about technical disasters. But every now and then, he would, with a wry smile, display a spectacular result.

In sets of ingenious experiments, Ruth and Hans Georg transplanted cytoplasm from various regions to various regions in chosen combinations of mutant and wild-type embryos. Eventually, they sorted out the problem of the telson: When cytoplasm was let to leak out of the posterior pole of a wild-type embryo, the abdomen would show defects while the telson remained present. Following leakage of anterior cytoplasm from wild-type embryos, a telson appeared instead of an acron. These findings together with results from double mutants could be explained by assuming that the telson is determined by the torso group, quite independently of the abdomen, which is determined by the posterior system (Figure 2). The terminal structure at the anterior, the acron, depends on both *bicoid* and *torso*, and in the absence of *bicoid*, a telson instead of an acron would form. According to this interpretation, telson duplications do not reflect a polarity change and therefore, in *bicoid* embryos, there is no jump of a posterior gradient but rather a switch from acron to telson, similar to a homeotic transformation (Frohnhöfer et al., 1986; Nüsslein-Volhard et al., 1987).

Bicoid

The general notion about patterning in insect eggs assumed a posterior gradient source, and Hans Meinhardt (a next door neighbor in Tübingen) explained to us how this could exert long-range effects on the anterior pattern. Therefore Hans Georg initially transplanted posterior plasm into *bicoid* embryos, which gave negative result. He then tried anterior cytoplasm and was immediately successful as this can rescue the mutant phenotype. This suggested that there is an additional gradient with a maximum at the anterior pole (Figure 2). This novel gradient was absent in *bicoid* embryos, but not affected in *torso* embryos, although their head phenotype closely resembles those of weak *bicoid* alleles.

Hans Georg's experiments showed that there is an activity localized in the anterior 10%–15% of a wild-type *Drosophila* egg, which is dependent on the gene dosage of *bicoid*, suggesting that this is the *bicoid* mRNA itself. When transplanted into the anterior tip of a mutant embryo, it rescues the phenotype, while it induces anterior pattern including polarity reversals when transplanted into the middle or posterior of the egg. The long-range effect suggests that the product diffuses away from the RNA source and forms a concentration gradient with a maximum at the anterior tip of the egg. This results in

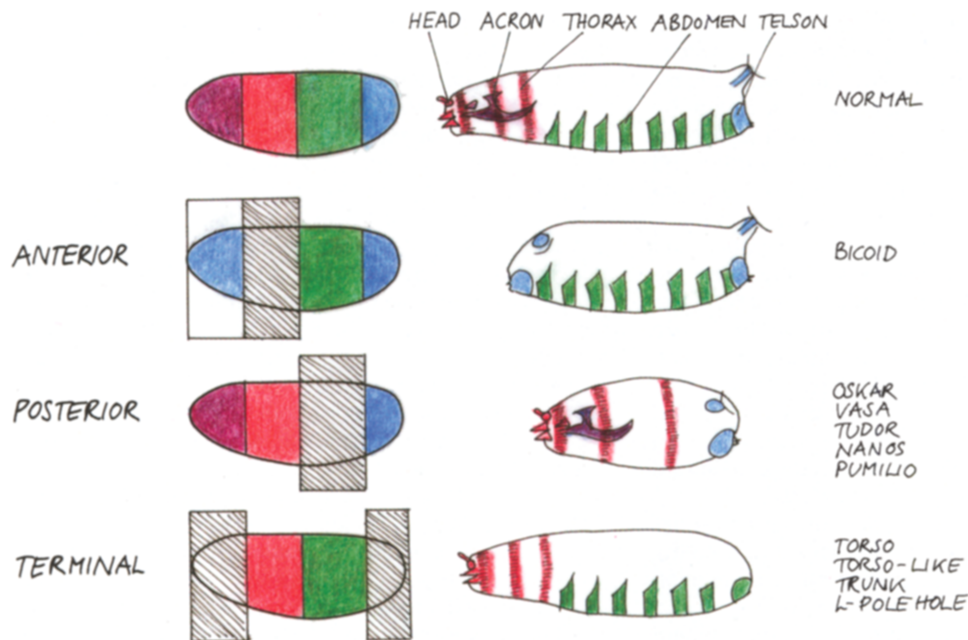

Figure 2. Anterior-Posterior Patterning Systems in the *Drosophila* Egg

the determination of the serial anterior pattern, with high concentrations determining head structures and lower concentrations thorax. In addition, Hans Georg's transplantation experiments showed that high *bicoid* concentrations inhibit abdomen formation (Frohnhöfer and Nüsslein-Volhard, 1986). Mutants of the two other genes of the anterior group, *exuperantia* and *swallow*, shared the anterior defects of the weak *bicoid* alleles; however, they displayed much larger thoracic anlagen than normal. Hans Georg's experiments indicated that in these mutant embryos, the *bicoid* RNA is not localized at the anterior, but spreads out to more posterior regions. Therefore, high levels of protein required for head formations would not be reached at the anterior, but instead regions of lower concentrations would be enlarged (Frohnhöfer and Nüsslein-Volhard, 1987). When the molecular parameters of *bicoid* were solved, all these predictions came true.

The bicoid gene was cloned in the lab of Marcus Noll as a by product of cloning the segmentation gene *paired* because *bicoid* shares a small DNA stretch, the paired-repeat, with *paired* itself and a group of other genes. The distribution of the RNA transcript showed a striking localization at the anterior pole (Frigerio et al., 1986). Thomas Berleth in the Tübingen lab proved this gene to be *bicoid* by constructing transgenic flies in which an extra copy of the cloned gene rescued the *bicoid* phenotype. The presence of a homeobox predicted *bicoid* to be a transcription factor. A sharp localization of the RNA was seen in wild-type embryos while in *exu* and *swa* embryos the RNA was distributed in a shallow gradient (Berleth et al., 1988). To be able to really see and not just imagine the localized RNA, a possible gradient source, was an excitement that is hard to describe. At this time, it was very clear to us that we most likely never again in our scientific careers would have such an exciting time. The most important issue, however, was still to come: to see the gradient and show that it determined position by concentration.

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