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The Bicoid Morphogen Papers (II): Account from Wolfgang Driever

Commentary

Wolfgang Driever*
Developmental Biology Unit
Department of Biology I
University of Freiburg
Hauptstrasse 1
D-79104 Freiburg
Germany

My first contact with *Drosophila* developmental genetics was as a biochemistry student during a practical course that Janni (that's what we call Christiane Nüsslein-Volhard) held in her own lab. I received *cactus* mutants for analysis and was asked to find out what went wrong. It was the first time that I was confronted with complex mechanistic developmental problems involving integration of analyses at organismic, genetic, and molecular levels. My dedication to *Drosophila* pattern formation was ignited.

Two years later, I was really fortunate when Janni gave me the opportunity to carry out my Ph.D. thesis in her lab. As a biochemistry student, she suggested that I could investigate the Bicoid protein. Although the information gained from cloning the *bicoid* gene was spectacular and the localized mRNA made *bicoid* a bona fide localized determinant, it was clear that the long-range patterning effects could only be understood once distribution and function of the Bicoid protein were revealed.

It was an exciting task, and the pressure was high to demonstrate whether Bicoid protein distribution would simply reflect its mRNA localization (thus far-ranging effects would potentially be mediated indirectly through downstream genes) or whether distribution of Bicoid protein built indeed a long-range gradient. If so, would the Bicoid gradient reach way into the posterior abdomen to explain the cuticle patterning defects observed in *bicoid* mutant posterior abdominal segments? The mechanistic implications were tremendous: Either Bicoid serves as an initial switch to turn on relay mechanisms involving other genes that determine anteriorposterior pattern or Bicoid as a single protein acts as a morphogen to initiate pattern and control spatial expression patterns of early gnathal, thoracic, and abdominal genes.

The technology to make antibodies against gene products that are too scarce in their native biological system had just become available through bacterial overexpression vectors. However, to demonstrate the reach of a gradient required the generation of an antibody and use of immunohistology techniques that would provide virtually background-free results, as background would make it difficult to determine where the gradient ends. Fortunately, having Herbert Jäckle and his group in Tübingen in the same building provided an excellent environment for technical advice. Also, Janni, being aware of the technical problems as a biochemist herself, brought from her visits to other labs new expression vectors that were supposed to make more protein and thus cleaner antigen preparations. We decided to make both polyclonal and monoclonal antibodies, and the good expertise with the latter in the Bonhoeffer department raised our hopes for a background-free "supermonoclonal." The Bicoid protein and fusion proteins were quickly expressed, and rabbits as well as mice were injected. Rabbit antibodies were much faster than the monoclonal production, which was handicapped by the hot and humid Tübingen summer air, rich in fungal spores. Only a few weeks later, the first sera were obtained—and they were reactive to Bicoid on Western blots! However, then the problems began: there was a lot of crossreactivity with both bacterial as well as *Drosophila* embryonic proteins, and the first whole-mount immunohistochemistry showed a clear signal just at the anterior tip of the embryo but otherwise homogenous background. Thus, initially, there was no far-reaching gradient visible. Fierce discussions came up with the Tübingen model building community around Hans Meinhard—Bicoid would not be the anterior morphogen and would not have the right features as a transcription factor anyway, but may be just initiating a pattern generator that remains obscure. But, over the months, there was gradual progress: The trick was to achieve rigorous affinity purification of antibodies and to identify just the two bleeds from the dozen immunized rabbits that had a nice polyclonal profile of high-affinity antibodies. And then there were the first whole mounts clearly showing that Bicoid protein was distributed in a gradient detectable from the anterior pole (= 100% egg length) toward at least till 60% egg length—and thus has a distribution profile clearly different from its mRNA localization. Janni was at a conference, and thus I took a series of photographs, afraid that the stain would darken over time and the background would increase. Scientific documentation in 1987 still meant using black and white 35 mm film, and working at the Max-Planck-Institute provided the luxury of an excellent photo shop. Two days later when I picked up the prints, our photographer smiled at me and said I would need more practice at the microscope, but that she did her best to give me nice prints. When I looked at the prints, I was very disappointed—she had in a most artistic way waved away the gradient on the prints, thinking that I had been unable to set homogenous illumination at the scope. Thus, I had no photos when Janni came back to the lab. But fortunately the whole mounts were stable and we looked at them together on the dual viewing scope in the lab—excited that there was indeed a gradient! Immediately, Janni got involved in a long discussion that a gradient only reaching to 60% egg length was by no means sufficient to explain the results Hans-Georg Fronhöfer had obtained about Bicoid activity. If it were to be a regulator of hunchback, the gradient had to reach to 50% egg length—and Hans-Georg and Ruth Lehmann even had evidence that Bicoid should affect *krüppel* and *knirps*. Back to the bench! Finally, further improved immunohistochemistry demonstrated that Bicoid protein was present from the anterior pole, reaching across the embryo,

*Correspondence: driever@biologie.uni-freiburg.de

to at least 20% egg length, and thus the range of the gradient perfectly matched the range of biological activity.

Now came the second challenge: Is there a correlation between Bicoid protein concentration and position in the embryo? To address this question, we decided to compare the Bicoid protein profile quantitatively with changes in pattern formation in embryos from females with a *bicoid* gene dose of one, two, three, or four functional genes. Two good quantitative measures for shifts in pattern were quickly identified: First, the position of a morphological landmark, the head fold. Second, the expression pattern of the pair-rule gene *even skipped*. Both were perfect indicators of pattern at the blastula and early gastrula stages and could easily be quantified by measuring their positions in percent of egg length. But how should we quantify and compare Bicoid protein concentration over the 600 micrometer egg length for different eggs and genotypes? Tobias Bonhoefer in the Cybernetics Max-Planck Institute across the street was one of the few people in Tübingen at that time with expertise in digital image processing, and he taught us the new technologies. When we achieved similar results using both traditional densitometric approaches on slides and digital image processing, we started to get confident—and indeed obtained immunohistochemistry intensity curves and standard deviations as measure for variance in Bicoid distribution very similar to those published 14 years later by Houchmandazah and colleagues (Houchmandazah et al., 2002), who used today's modern confocal imaging technologies. There was a clear correlation between Bicoid concentration and positional identity in the *Drosophila* blastoderm. Our data provided the first clear demonstration of a gradient instructing positional information in an embryonic field and established Bicoid as the first bona fide morphogen. However, in 1988, it also did not escape our attention that varying *bicoid* gene dosage produced shifts in protein concentration that were always more pronounced than was the corresponding shift on the late blastoderm fate map. Thus, it had to be postulated that, while the Bicoid concentration itself was an important factor in determining position, ultimately the decisions about precise gap gene and pair-rule gene expression borders reflected the integration of several inputs. Alternatively, the concentration of Bicoid at any given point could be somehow interpreted by the embryo in a nonlinear fashion. This raised questions whether, for example, the slope of the gradient rather than the absolute value of Bicoid concentration would specify anterior-posterior position in the embryo. However, the measurements of Bicoid distribution in other maternal effect mutations, which change the shape and slope of the gradient, including *exuperantia*, *swallow*, and *staufen*, clearly demonstrated that the absolute concentration, and not the slope of the gradient, determines position. The analysis of Bicoid protein concentration also solved important open issues regarding potential interactions among the three maternal gene systems affecting anterioposterior pattern: As the Bicoid gradient was not altered in mutants for the signal of the terminal system, trunk, or in the posterior system mutants *nanos* and *pumilio*, the three maternal systems appeared to act independently, at least in a sense that terminal and posterior systems did not affect the shape of the anterior morphogen gradient.

It became obvious that our data published in the two *Cell* papers immediately asked for a more detailed analysis of how Bicoid controls target gene expression. As we became aware that other groups (Paul MacDonald and Gary Struhl) had also started to investigate the molecular function of Bicoid, a spectacular scientific race started, which resulted in the publication of a series of papers in the following year (Driever et al., 1989a, 1998b; Driever and Nüsslein-Volhard, 1989; Struhl et al., 1989, 1992). The homeodomain protein Bicoid was found to bind multiple promoter elements of its predicted target gap gene *hunchback* (*hb*). Analysis of transgenic strains carrying *hb* promoter fragments or synthetic consensus Bicoid binding sites fused to reporter genes demonstrated that the Bicoid binding sites indeed mediate most of the activation of the *hunchback* gene. Interestingly, different types of binding sites mediated a significantly different posterior extent of target gene expression. In our interpretation, these data demonstrated that low-affinity binding sites in a promoter restrict target gene expression to anterior domains, while high-affinity binding sites in the promoter enable gene expression far into the abdomen. Thus, we had identified a potential mechanism by which high or low affinities of the binding sites for Bicoid could mediate the morphogen function to set different activation thresholds and thus achieve distinct anterioposterior expression borders for several target genes. Single Bicoid binding sites led to very little target gene activation, and near normal levels were observed only when several binding sites were included in a reporter. Thus, cooperative or synergistic effects must play an important role in Bicoid function. Finally, we teamed up with Mark Ptashne and Jun Ma to demonstrate that Bicoid has transcriptional activator activity on its own—an important fact when considering how potential cooperative effects may contribute to the sharpening of expression borders of target genes. Indeed, the mechanism of how the shallow slope of Bicoid protein concentration was translated into the relatively sharp boarders of gap gene expression remained a challenge for several years. These molecular mechanisms were later studied in more detail by the groups of Jun Ma and Steve Hanes (Ma et al, 1996; Burz et al., 1998). Over the next 10 years, it was very satisfying to see many labs contribute to understanding the complexities of Bicoid function in *Drosophila* patterning—the concept gradually changed from Bicoid being a singular "master morphogen" to Bicoid protein acting as a morphogen in concert with other regulators to contribute to the positioning of the expression boundaries of a number of gap, head, and pair-rule genes (Simpson-Brose et al., 1994). Further, today, we know from the analysis of other flies and insects that, during evolution of long germband insects, Bicoid enabled embryos to "develop on the fast track"—by simultaneously synchronizing and performing decision-making tasks during patterning, which in short germband insects happen only consecutively (Schröder, 2003; Wimmer et al., 2000).

Overall, the "*bicoid* years" were a fascinating scientific era that we enjoyed tremendously. The intellectual challenges and the satisfaction when molecular mechanisms became clear provided a spectacular experience—a

phase in science that had started with the analysis of homeotic genes, then gap genes, and finally Bicoid. It was surprising to have transcription factors play such a dominating role in early patterning, when biochemical pattern generators had been the favorites for decades. But then—transcription factors had been known to be excellent decision makers from bacteria to yeast, and Bicoid taught us a lot about how developmental decisions and patterning can be accomplished at the level of the gene in higher eukaryotes.

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