

Drosophila embryo development in Lund

The Accursed Gradient

For 20 years, the dogma has been that the Bicoid protein produces a morphogenic gradient across the early *Drosophila* embryo. Recently, however, Stefan Baumgartner's group from Lund University proved that, in fact, the *bicoid* mRNA forms the gradient before being translated into Bicoid protein – a result he, in principle, published 22 years ago.

Organisms that sexually reproduce start life from a single cell, the fertilized oocyte. This cell divides and divides to finally produce a huge number of differentiated cells. But how does a cell become specialised? How does it know whether to become a part of the head or an inner organ? Essentially, how do the shapes of tissues, organs and eventually of the entire organism arise?

For decades, research in developmental biology has shed light on these questions and much of our understanding of the molecular processes in this field has been obtained from the fruit fly *Drosophila melanogaster*. In this model system, many control genes have been identified that define the spatial patterns of cell types and the body structure of the fruit fly. Results from research in *Drosophila* have also provided more general key information on the molecular principles of development, as many of the basic mechanisms are similar in higher organisms.

Front or rear

In textbooks on developmental biology, the processes of axis formation are described in detail: at the beginning of *Drosophila* embryonic development, 13 nuclear divisions occur without cell division, creating a syncytium. Even at this early stage, the embryo – or even the oocyte, before fertilization – receives instructions through various factors about where its anterior and posterior ends will be located. In this interplay of factors, special molecules play a central role: the morphogens. These molecules influence and control gene activities and cellular processes in a concentration-dependent manner. In other words, a morphogen switches on particular genes at different threshold concentrations, thereby in-

itiating a new pattern of gene expression along the axes of the embryo. A number of morphogens act directly as transcription factors that regulate the activities of a variety of target genes. Moreover, products of morphogen-activated genes that also encode transcription factors regulate, in turn, the expression of other target genes and so on, thus generating a regulatory cascade.

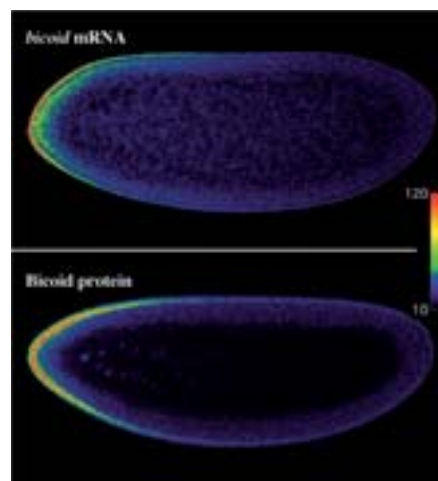
One of the most famous genes involved in this process is the *bicoid* (*bcd*) gene which is required for the development of the larval head and thorax in *Drosophila* embryos. Its mRNA is produced in the mother's ovaries and localised to the anterior tip of the oocyte. After fertilisation, the *bcd* mRNA is translated into Bcd protein, which begins to form a morphogenic gradient with the Bcd protein concentration, declining exponentially from the anterior towards the posterior pole. Thus, it provides the initial posi-

tional information for “anterior” and “posterior” and differentially activates downstream segmentation genes, in particular, the so-called gap genes that also encode transcription factors.

“The Bcd protein was the first morphogen that has been identified and hence serves as a paradigm for morphogen action,” says Stefan Baumgartner from the Department of Experimental Medical Sciences at Lund University, Sweden. Located in the Section for Developmental Biology, his group studies the molecular basis of early *Drosophila* development.

A localisation problem

The idea of the Bcd protein gradient can be traced back to studies done in the lab of the German Nobel Laureate Christiane Nüsslein-Volhard at the Max-Planck-Institute for Developmental Biology in Tübingen. “Janni, the sobriquet she is known for in the scientific community, elucidated the role of the *bcd* gene for axis formation of the early *Drosophila* embryo,” Baumgartner remembers. She postulated in 1986 that a morphogenic gradient of the *bcd* gene product exists in the early embryo. In 1988, she and her then doctoral student, Wolfgang Driever, indeed demonstrated a concentration gradient for the Bicoid protein (*Cell* 54, 83-93 and 95-104). The protein was translated from a *bcd* mRNA, which was claimed to be strictly localised at the anterior tip of embryos, as it had been shown in oocytes. This localised *bcd* mRNA was to serve as the source of the Bcd protein and its subsequent distribution in an exponential concentration gradient along the anteroposterior axis through diffusion and dispersed degradation. This was the birth of the so-called SDD model, referring to localised synthesis, diffusion and spatially uniform degradation



A *bcd* mRNA gradient refutes the SDD model. The upper picture shows the distribution of the *bcd* mRNA, which reveals a gradient resembling that of the protein at a similar stage (lower picture).

of the Bcd protein. Since then, biology students have absorbed this as a fundamental dogma of developmental biology.

The crux, however, is that there seems to be a serious error in the model. “The *bcd* mRNA is not strictly localised at the anterior tip of the embryo,” Stefan Baumgartner says. His group and co-workers from the University of Zurich, Switzerland, and Stony Brook University, USA, recently published an article showing that the mRNA itself forms a concentration gradient along the embryonic cortex. This was seen to fall off exponentially from the anterior pole of the embryo at the syncytial blastoderm stage and to parallel the Bcd protein distribution at all stages (*Development* 136: 605-14). “Using a sensitive fluorescent in situ hybridisation technology and confocal microscopy, we demonstrated that the *bcd* mRNA and protein patterns behave very similarly,” Baumgartner describes. “This evidently demonstrates that the Bcd protein gradient arises through a *bcd* mRNA gradient that is translated into a Bcd protein gradient, rather than through diffusion of the protein, from a *bcd* mRNA source located at the anterior pole of the embryo,” he proceeds. “We propose that the *bcd* mRNA gradient is formed by a novel mechanism involving quasi-random active transport along a cortical microtubular network,” the scientist adds. Taken together, the results of Baumgartner and co-workers refute the SDD model.

With these results, a 20 year-old paradigm can be buried and replaced by an “active RNA transport and synthesis” model, as called for by Baumgartner and colleagues. Too bad about the dogma – and for the students, who now have to re-learn - but that’s often how it is in science.

A kind of *déjà vu*

However, there is yet another interesting detail in the story. “It was not for the first time that we published the existence of a *bcd* mRNA gradient,” Stefan Baumgartner points out. Previously, in 1986, when he was a doctoral student in Markus Noll’s lab in the Biozentrum at the University of Basel, the *bcd* mRNA gradient was noticed and published (*Cell* 47, 735-746). The Swiss had already cloned the *bcd* gene a year earlier. “At that time, we used a radioactively labelled probe for our in situ hybridisation experiments to examine the spatial pattern of the *bcd* transcripts,” the biologist remembers. In the oocyte, they found high concentrations of the *bcd* transcripts at the anterior end. “During cleavage stages of

the embryo, we saw that the concentration of the *bcd* mRNA fell off as a gradient towards the posterior end,” Baumgartner describes, “Thus, not the protein but the *bcd* mRNA gradient is the first morphogen gradient identified!”

Observed but ignored

“At that time, we were in close contact with Janni and her team.” Considering that the problem of morphogenic gradients was solved by the identification of a *bcd* mRNA gradient encoding a transcription factor, Noll donated all important molecular reagents and sequencing information of the *bcd* gene to the Tübingen group, which



Stefan Baumgartner re-proved a 22 year-old, disregarded result

eventually resulted in a joint publication. What remains puzzling, however, is why the gradient of the *bcd* mRNA was not recognised again in studies during the following years. In the meantime, non-radioactive mRNA detection methods became available, e.g. using digoxigenin-labelled probes and a blue colour reaction as the signal. Applying those, short exposure of the colour reaction to the substrate can give the false impression that the *bcd* mRNA is tightly associated with the tip and thus fits the SDD model. “If you stop the reaction after 30 minutes, which is normal for the detection of non-abundant transcripts, you can clearly see the gradient.” But Stefan Baumgartner goes further, “In some publications, a *bcd* mRNA gradient could indeed be observed, but was simply missed or ignored.”

Although Baumgartner realised that the SDD model, proclaimed two years af-

ter the 1986 Swiss publication, contradicted their published results, it took him more than 20 years to pick the topic up again. The biologist had meanwhile been working on other subjects such as the extracellular matrix and its function in *Drosophila* development. “Markus Noll and I always had plans to correct the model during the last 20 years, but we felt that we had already published it once and that others in the field would eventually vindicate our earlier model. Since then, I only touched on this issue when lecturing about it.”

A watchful student

It was a 4th term Swedish student, who persuaded Baumgartner to come back to the field. In 2006, during a practical course, the student saw embryos exhibiting *bcd* mRNA gradients in the microscope and questioned him why this contrasted so drastically with what was taught by textbooks. Prompted by this embarrassing experience, he decided to re-examine the old data, using the most modern techniques. “I took a sabbatical year in 2008 and went to Zurich, to Markus Noll, to carry out the studies together with him.” And the biologists confirmed what they had found 22 years ago.

“In 2007, Eric Wieschaus, co-Nobel Laureate with Christiane Nüsslein-Volhard, and his colleagues demonstrated that the Bcd protein cannot diffuse rapidly enough to fit the SDD model and to reach the gradient profile observed after 90 minutes,” Baumgartner summarises (*Cell* 130: 141-52). This was just another hint that something was wrong but still nobody questioned the SDD model. “Our proposed idea of transport along microtubules is fast enough to establish the *bcd* mRNA gradient and consequently the Bcd protein gradient in the time window available. Thus, it can solve all difficulties Wieschaus and colleagues encountered”.

Nevertheless, as plausible as it may sound, to overthrow a theory, maintained for such a long time and which so many believed in, is not easy. “We sent our manuscript to *Cell* to be published as an article under ‘Matters Arising’ but *Cell* refused to even send it out for review,” says Baumgartner. “This was probably not for scientific reasons,” the 51-year old biologist adds sadly. Despite this setback Baumgartner and his colleagues were eventually successful in publishing their data.

Plenty of work now seems to lie ahead: rewriting the developmental biology textbooks for starters. SUSANNE DORN