Researchers Develop Improved Method to Visualize Biologic Molecules

How are biologic molecules arranged inside the embryo so that embryonic development occurs reliably every time? Princeton researchers, led by Thomas Gregor, an assistant professor of physics and the Lewis-Sigler Institute for Integrative Genomics, and Shawn Little, a postdoctoral fellow in the laboratory of Professor Eric Wieschaus in the Department of Molecular Biology, have developed a new method to better understand how an embryo's basic molecular makeup helps ensure that the embryo's development occurs reliably every time. The results of this research into the fruit fly Drosophila introduce a method for making precise measurements of biologic units (so-called mRNA molecules) that play a key role in development. The findings will be published next week in the online, open access journal PLoS Biology.

Embryonic development is the remarkable phenomenon wherein an organism starts as a single cell -- the fertilized egg -- and somehow manages to convert that one cell into many cells of different types. All these different cells organize themselves into diverse tissues and organs that together perform all the functions needed to sustain life. Even more remarkable is the observation that the whole process happens so reliably: For any given animal, all developing embryos of the same age are essentially indistinguishable. And even at the bigger scale, we all have five fingers, and we know very few people who have four or six. Development is a surprisingly well functioning, well organized natural phenomenon, and very few engineering toys have to be able to match such accuracy.

In this paper, Little and Gregor developed a powerful and sensitive method to see messenger RNA (mRNA) in embryos of the fruit fly *Drosophila melanogaster.* They applied this method to understand how early development is influenced by the spatial arrangement of mRNA for a gene called 'bicoid.' Bicoid protein is found in a concentration gradient extending far into the posterior. High levels at the anterior end activate anterior specific genes, and low levels closer to the posterior activate posterior genes. Little and Gregor's method showed that the mRNA is present in a nearly unchanging spatial distribution along the anterior-posterior axis during embryonic development. Just about all (more than 90 percent) of the mRNA is confined to the anterior 20 percent of the embryo. This unchanging distribution is remarkable, because the global architecture of the embryo changes continuously during early embryogenesis as the future cells divide rapidly and repeatedly to give rise to 6,000 cells from one. No prior study has been capable of globally documenting bicoid mRNA at this level, and therefore provided the most accurate description possible of its spatial distribution revealing, for example, that the gradient formed by the Bicoid protein does not depend entirely of the bicoid mRNA, but also requires movement of the protein.

"In summary, our paper introduces a novel method of mRNA quantification to the world of Drosophila," Gregor said. "We have used the method to further the quantitative understanding of the bicoid morphogen gradient, which remains the prime example of morphogen-mediated embryonic patterning. We anticipate that the power of our method will be widely applicable to quantitative analyses of many interesting developmental

processes of embryonic patterning and mRNA metabolism, both in the context of fruit fly embryos and in higher organisms such as mammals."

In addition to Gregor and Little, other researchers involved in the study included Wieschaus, a Howard Hughes Medical Institute Investigator at Princeton's Department of Molecular Biology, former Princeton physics graduate student Gašper Tkačik, now at the University of Pennsylvania, and former Princeton undergraduate Thomas Kneeland, who graduated with a degree in physics in 2010.

Funding: This study was funded by Princeton University (http://www.princeton.edu), Howard Hughes Medical Institute (http://www.hhmi.org), and partially supported by Searle Scholars Award 10-SSP-274 to TG (http://www.searlescholars.net/person/547). GT was supported in part by NSF grants EF-0928048 and BIO-0928048. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests statement: The authors declare that no competing interests exist.

Citation: Little SC, Tkacˇik G, Kneeland TB, Wieschaus EF, Gregor T (2011) The Formation of the Bicoid Morphogen Gradient Requires Protein Movement from Anteriorly Localized mRNA. PLoS Biol 9(3): e1000596. doi:10.1371/journal.pbio.1000596