GE PRIZE ESSAY

The Molecular Basis of Size Differences

Michael A. Crickmore

Regulation of morphogen signaling controls tissue size.

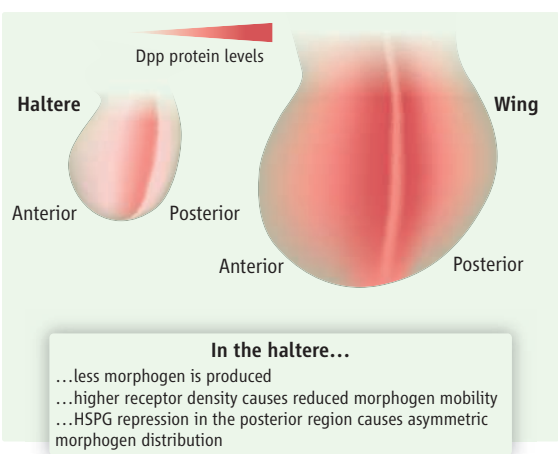
GE Healthcare and *Science* are pleased to present the prize-winning essay by Michael A. Crickmore, a regional winner from North America, who is the Grand Prize winner of the GE & *Science* Prize for Young Life Scientists.

GE & Science Prize for Young Life Scientists

Size differences account for a great deal of the diversity found in the animal kingdom, but we still have much to learn about how sizes are programmed. Generally, the cells of different animals are comparable in size, and all animals begin as a single cell. This leaves the number of cells accumulated as the main determinant of animal size. We can reasonably expect the genes controlling cell number to be conserved among animals. So it seems that size-determining genes must be deployed in the elephant in such a way that it amasses several hundred thousand times more cells than the mouse. Which are these genes and how do they control size? I asked this question in a more experimentally tractable context: How do body parts of a single animal become different sizes? Fingers, toes, and ribs are sets of structures whose members are similar in form but differ in size. Although we know that Hox transcription factors specify the identity of individual fingers, toes, and ribs, little is known about how their individual sizes are programmed.

Fortunately, a similar situation exists in an experimental model system, the fruit fly, *Drosophila melanogaster*. *Drosophila* has two true wings that develop from imaginal discs of 50,000 cells and two smaller balancing organs called halteres that form from imaginal discs of 10,000 cells. We know that all differences between these appendages are specified by the expression of the Hox gene *Ultrabithoax* (*Ubx*) in the haltere and its absence from the wing (*1*). Thus I was able to ask how the expression of this single gene limits the size of the haltere.

My first insight came from removing *Ubx* function from random clusters of haltere cells (*2*). As expected, the resulting *Ubx* mosaic halteres are larger than wild-type halteres. Surprisingly, however, the overgrowth is not restricted to the *Ubx* mutant tissue but spreads to the wild-type tissue as well. This means that *Ubx* does not control haltere size cell-autonomously by, for example, acting directly on cell cycle or apoptotic checkpoints. Rather, I hypothesized that, because of their fundamental and non-autonomous role in the control of tissue growth and patterning, alterations in morphogen signaling might underlie changes in organ size. Indeed, transcription of *decapentaplegic* (*dpp*), a growth-promoting morphogen of the BMP family, is reduced in the haltere in comparison with the wing (*2*). Furthermore, the pattern of Dpp pathway activation is altered between the haltere and the wing, not just quantitatively but also qualitatively (see the figure). In the wing, Dpp travels far from its source to form a broad activity gradient. In the haltere, I found Dpp signaling to be largely restricted to the cells in which it is produced (*2*). Because receptor binding impedes morphogen mobility, I examined the expression pattern of the Dpp receptor *thickveins* (*tkv*) and found it to be strongly up-regulated in the haltere compared with the wing. I was able to show that the Dpp mobility restriction in the haltere is due in large part to transcriptional up-regulation of *tkv*. The alterations in transcription of *dpp* and *tkv* account for much of the reduced size of the haltere relative to the wing (*2*).



Morphogen distribution in the haltere and wing-size regulation. Ubx reduces the size of the haltere imaginal disc relative to that of the wing by decreasing the production and mobility of growth-promoting morphogens (for example, Dpp). *Ubx* impedes morphogen mobility in the haltere by up-regulating the receptor throughout the haltere and repressing an HSPG in the posterior half of the haltere. Reduced morphogen availability causes a reduction in haltere size relative to the wing.

The mechanism by which *Ubx* orchestrates these changes in the haltere is a telling example of selector gene function. *Ubx* converts one *tkv* repressor into a repressor of a second *tkv* repressor, thereby upregulating *tkv* levels in all haltere cells. The resulting elevated receptor density traps secreted Dpp at or near its site of production, leading to high Dpp signaling in the cells that transcribe *dpp*. This triggers a negative autoregulatory loop through which Dpp signaling represses *dpp* transcription, leading to a global reduction in the amount of Dpp in the tissue (*2*). This demonstrates how a fairly subtle modification of a regulatory network, effected by a selector gene, can set off a chain of events that has powerful ramifications for morphogen signaling and organ size.

Hox modulation of components of morphogen signaling pathways seems likely to define a general principle of size regulation (*3*). For example, the Wnt and Hedgehog morphogen pathways are also altered in the haltere relative to the wing (*4, 5*). The mobilities of BMPs, Wnts, Hedgehogs, and other morphogens are promoted by heparan sulfate proteoglycans (HSPGs) (*6*). In the haltere, I found that *Ubx* works with the posterior transcription factor *engrailed* to selectively repress the HSPG *dally* in the posterior compartment. As a consequence, morphogen mobility is severely impaired in the posterior side of the tissue, causing the posterior of the haltere to be smaller than the anterior (*5*) (see the figure).

As is clear from the examples above, it is critical that the production levels of size-regulating genes such as *dpp*, *tkv*, and *dally* be precisely controlled. In the haltere, the same is true of the regulator of the size regulators: Heterozygous *Ubx* mutant

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flies have enlarged halteres (*1*), and flies with extra copies of the *Ubx* locus have shrunken halteres (*7*). While studying how Ubx levels are controlled in wild-type halteres, I found that when Ubx protein levels rise, subsets of overlapping transcriptional input into the *Ubx* promoter become silenced (*8*). This silencing likely buffers against inappropriately high cellular Ubx concentrations and may stabilize Ubx levels against the unpredictability of genetic variation. I found that when *Ubx* enhancer-reporting lab strains are outcrossed to various wild-collected *D. melanogaster* strains, dramatic enhancer silencing is seen in a large fraction of the resulting progeny (*8*). Despite (or perhaps because of) the silencing of subsets of *Ubx* enhancers, Ubx protein levels remain normal in the outcrossed progeny, and their halteres develop perfectly. Only through the use of the sensitive transcriptional reporter lines available in the *Ubx* locus is the chaos beneath this calm surface revealed. It may be that if we look close enough, we will find similar systems operating to generate stereotyped expression levels of many concentration-dependent transcription factors.

In conclusion, my thesis work has shown that alterations in morphogen signaling landscapes underlie differences in tissue sizes. Transcription factors manipulate the extent and intensity of morphogen signaling through the control of morphogen production and mobility. At least some size-controlling genes autoregulate their production levels to ensure reliable size outcomes in the face of natural genetic variation. These findings help explain how size differences are generated and how the genes that orchestrate size changes are themselves regulated. But is the regulation of morphogen signaling capable of explaining size differences as great as those between elephants and mice? Or are the growth effectors downstream of morphogen signaling also differentially tuned to create specific sizes for specific contexts? And how exactly do changes in patterns of morphogen activity translate into changes in size? Sizeable questions, all.

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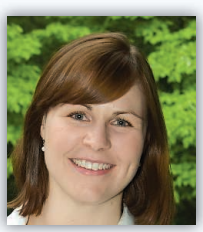
2009 Grand Prize Winner

Michael A. Crickmore, the author of the prize-winning essay, was born in Flint, MI, but as a child he moved with his mother and brother to suburban Los Angeles and finally to Philadelphia. He became serious about science only after college while working as a technician in Ken Irvine's lab at Rutgers University. Inspired by the ideas of Dragana Rogulja, an Irvine lab graduate student studying size control, Dr. Crickmore did his graduate work on size in Richard Mann's lab at Columbia University. But his long-term interests lie in understanding how the brain works, something he is now trying to address as a postdoctoctoral fellow in Leslie Vosshall's lab at The Rockefeller University in New York City, where he lives with his wife Dragana and their little boy Cy.

Regional Winners

Europe: **Michaela Gack** for her essay "Regulation of RIG-I-Mediated Antiviral Innate Immunity." Dr. Gack was born in Coburg, Germany. She studied molecular medicine at the Friedrich-Alexander University (FAU) Erlangen-Nuremberg, Germany, and in September 2005 joined the newly established exchange program between the graduate training program of the FAU Erlangen-Nuremberg and Harvard Medical School (HMS) in Boston. Dr. Gack completed a Ph.D. project in the laboratory of Jae Jung at the New England Primate Center of HMS. Her postdoctoral studies were conducted at the University of Southern California, Los Angeles. Since April 2009, she has been an Independent Instructor at the Department of Microbiology and Molecular Genetics of HMS, where she continues to investigate innate immune responses against viral infections and viral immune evasion mechanisms.



Japan: **Masahiro Kitano** for his essay "Imaging of Rab5 Activity Identifies Essential Regulators for Phagosome Maturation." Dr. Kitano was born in 1980 and grew up in Bieicho, Japan, a town famous for beautiful scenic hills. He attended Kyoto University, where he received a bachelor's degree in pharmaceutical science in 2003 and a master's degree in physical and organic chemistry in 2005. A strong interest in molecular imaging led him to join Michiyuki Matsuda's laboratory at Osaka University, where he developed a biosensor to identify regulators of the phagosome maturation process. Dr. Kitano completed his Ph.D. in September 2008 and is currently studying the dynamics of immune cells *in vivo* as a Special Postdoctoral Researcher in the laboratory of Takaharu Okada at the RIKEN Yokahama Institute.

All Other Countries: **Tommy Kaplan** for his essay "From DNA Sequence to Chromatin Dynamics: Computational Analysis of Transcriptional Regulation." Dr. Kaplan received his B.Sc. in Computer Science and Cognitive Studies from The Hebrew University of Jerusalem, Israel. His Ph.D. research, in computational biology, focused on various aspects of transcriptional regulation, under the supervision of Nir Friedman and Hanah Margalit at The Hebrew University, and in close collaboration with Ollie Rando at Harvard/University of Massachusetts. Since 2002, Dr. Kaplan has been involved in teaching the combined B.Sc./M.Sc. program in Computer Science and Life Sciences at The Hebrew University. Currently, he is a postdoctoral fellow in Mike Eisen's lab at the University of California, Berkeley, where he develops computational models to understand the evolution and control of gene expression during the early developmental stages of fruit fly embryos. In his spare time, Dr. Kaplan enjoys mountain biking, reading, and hiking in Northern California with his wife and two sons.

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