

# Julia Zeitlinger Profile: Unraveling the Complex Transcriptional Networks of Genomes

By Geoffrey Montgomery



In late 1998, while HFSP fellow Julia Zeitlinger was in the last year of her graduate studies at the European Molecular Biology Laboratory (EMBL) in Heidelberg, the molecular biologist Richard A. Young came to the EMBL to give a talk. Young's Whitehead Institute lab had long been a leader in defining the molecular components of the general transcription apparatus—the giant, multi-protein enzymatic machinery that zips along DNA peeling off RNA transcripts—and now the lab was deploying a powerful new post-genomic tool in its analysis: yeast DNA microarrays, also known as gene expression chips.

Zeitlinger was not especially interested in the general transcription machinery that had been the traditional focus of the Young lab. Indeed, her primary interest lay in the opposite direction. What she most wanted to know was not how genes in general were transcribed, but instead how specific genes were transcribed in response to specific cellular signals. How were the specific, intricate molecular links in these complex signaling and transcriptional networks woven together? “I think the beauty of biology is the complexity,” says Zeitlinger, “and that the challenge today is how do we deal with that complexity.” And Young's 1998 lecture opened her eyes to a new way of unraveling the complexity of molecular networks, in which DNA microarrays and the remarkably powerful armamentarium of yeast molecular genetics could be combined to look at “not just one component of a network, but all the components.”

When she went to visit Young's lab in 1999, she found that in fact the lab was moving beyond the study of general transcription machinery to the study of transcriptional networks—exactly the area she was fascinated by. “I was very lucky to come to the right place at the right time,” she says now. After deciding to go to Young's lab for post-doctoral studies, she applied for an HFSP fellowship because “I thought HFSP was the best fellowship I could apply for.” In addition to a generous stipend, HFSP offered research money to be used at the fellow's own discretion. “And this made me feel like, yes, they really want to make sure I can do what I want to do.”

Zeitlinger had not anticipated doing biology early in life. As a child she liked to build little machines and solve newspaper puzzles, but there were no scientists in her family. In school she felt biology was too descriptive: what was the pleasure, she thought, in memorizing the names of plants? “I was interested in math, in chemistry,” she says, “because that was logic.” Math and chemistry offered intellectually challenging puzzles, a logical system at the core of numbers and of physical matter.

But at school she eventually found math and chemistry too dry for her taste, and she became oriented towards human biology, with an eye towards doing medical research. She enrolled in a special theoretical medicine program in Marburg, Germany as an undergraduate. But Zeitlinger had grown up in bustling, divided Berlin, and she found the small, quiet city of Marburg rather boring by comparison. She completed her undergraduate studies in human biology at King's College in London, and then moved to the EMBL in Heidelberg, attracted by both the high quality of the EMBL's researchers and by its international environment, in which scientists from many countries are brought together in one place.

In London, Zeitlinger's scientific focus had once again changed. "I got interested in developmental biology," she says. She was fascinated by anatomy, by the visible structure of animal and human bodies, "but I always wanted to know: how does that structure develop? I felt that development was a more logical, mechanistic way of understanding anatomical structure." Development provided not just a description of visible body structures but an attempt to explain the invisible forces that gave rise to bodies. At London, she did experiments with zebrafish, marveling at how the cells of their beautifully transparent embryos blossomed into creatures with gills and fins. And at the EMBL, she worked on *Drosophila* development, and did genetics, "which I loved so much. Because genetics is so logical---it had exactly the sort of logic that I liked about math and chemistry. And so I saw that you have that logic in biology too."

In her graduate studies of *Drosophila* embryogenesis, Zeitlinger felt that genetics defined the logical framework and the scientific questions, and "molecular biology for me was more the tools for genetics, a way to manipulate things and do experiments."

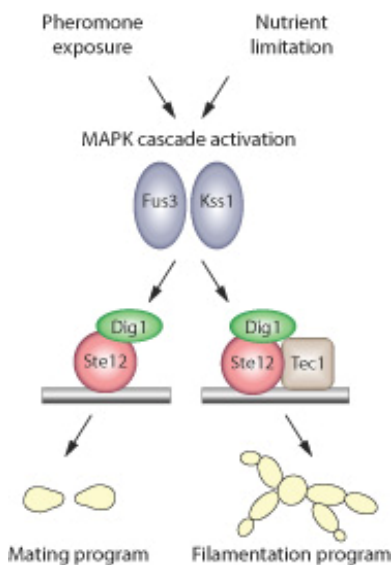
Yet despite the combined power of *Drosophila* genetics and molecular biology, at the end of her graduate studies Zeitlinger felt that she had come face to face with a biological black box she had no tools to open. She discovered that a particular transcription factor, D-fos, was crucially involved in controlling a key morphogenetic movement, called dorsal closure, early in fly embryogenesis [Zeitlinger and Bohmann, *Development* 126:3947-56 (1999)]. Yet this same transcription factor was involved in triggering many other developmental events in other *Drosophila* tissues and in other organisms; indeed, the fos transcription factor had been discovered as a mammalian oncogene. "It is one of the most responsive transcription factors known," says Zeitlinger. "When cells in all kinds of tissues are exposed to all kinds of stimuli, then this transcription factor is turned on." Zeitlinger had shown that a classic molecular signaling cascade, the MAP kinase pathway, was involved in activating D-fos transcription. "So you had this signaling cascade, you had a transcription factor, but it didn't quite explain why they were specifically doing this morphogenetic movement. Because this transcription factor could do other things in other developmental contexts. That's why I felt: this is the black box. ....And that was my motivation for going into yeast."

Richard Young's 1998 talk suggested to her that a new way of doing systems biology was dawning in yeast, in which DNA microarrays offered for the first time a means of studying large-scale, complex molecular networks: a way of opening up the black box containing the intricate signaling and transcriptional circuitry of a cell. In her first year in Young's Whitehead lab, Zeitlinger helped develop a major new method of analyzing transcriptional networks called "genome-wide location analysis." [Ren, B. et al.. *Science* 290:2306-9 (2000)]. In this method, all the protein transcription factors bound to the genome are cross-linked to DNA, freezing the cells in a certain transcriptional state. The cells are opened, their DNA fragmented, and then specific antibodies to nearly all the cell's transcription factors are used to pull out each DNA fragment to which a particular transcription factor is bound. The sequences and genomic locations of these DNA targets are then identified by a kind of DNA microarray unavailable for higher organisms: an intergenic microarray that contains not protein-coding gene sequences, but the DNA sequences between genes. Included in these intergenic region are critical control sequences, called promoters, which regulate transcription through the integrative action of the transcription factors that the promoter binds. Deciphering the transcriptional regulatory code [Michelson, PNAS 99:546-8 (2002)] somehow embedded in promoters and other control sequences (such as the enhancers found in higher organisms) is a central challenge now faced in functional genomic studies.

During her HFSP fellowship, Zeitlinger made new and fundamental inroads into the problem of transcriptional networks and signaling specificity, work published in *Cell*. [Zeitlinger et al., *Cell* 113:395-404 (2003)] In this study, she analyzed how a single transcription factor elicits completely different behaviors in living yeast cells: mating, performed in response to pheromone signals; and filamentation, in which yeast cells grow out and colonize new areas in response to starvation signals. Zeitlinger showed how, in response to the two different signals governing mating and filamentation, the same transcription factor Ste12 will bind to two different sets of promoters and regulate the expression of two different sets of genes. Dependent on MAPK signaling, Ste12 will either bind alone or in partnership with another transcription factor, Tec1, which has its own DNA binding domain and therefore directs Ste12 to different promoters. Thus Zeitlinger has taken the problem of specificity in complex signaling and transcriptional networks down to the level of DNA regulatory sequences in a genome.



Zeitlinger was pleasantly surprised at the elegance with which yeast used the same signaling and transcription molecules to follow such disparate life-paths as mating and filamentation. “I worked on a transcription factor in *Drosophila*,” she says, “and I always wanted to know: how can a single transcription factor do so many things? And when you look at *Drosophila*, you say this is just so complex, you’ll never understand it. But when I actually looked in a single cell, you can understand it. If you pick the right thing to look at, it can actually be quite simple. And that was kind of satisfying as well as surprising.”



Nevertheless, the regulatory codes of even the lowly yeast genome is far from being deciphered. Recent studies of transcriptional networks and regulatory codes by the Young lab [*Science* Lee et al 298: 799-804 (2002); *Nature* Harbison et al 431:99-104 (2004)], which were groundbreaking enough to be reported in the *The New York Times*, have shown that a surprisingly large number of different transcription factors are bound to many yeast promoter DNA sequences---presenting the prospect of a combinatorial code for gene regulation more complex than most yeast biologists had anticipated. This combinatorial code will be orders of magnitude more complex still in humans, whose genomes contain vastly larger intergenic regulatory sequences responsible for regulating the different functions and behavior of the many different types of cells in the human body and brain. Even in single-celled yeast, it is usually impossible to predict from a promoter’s DNA sequence alone which transcription factors will bind to this sequence, not to mention the on-off logic by which these factors interact to switch on transcription of an adjoining gene.

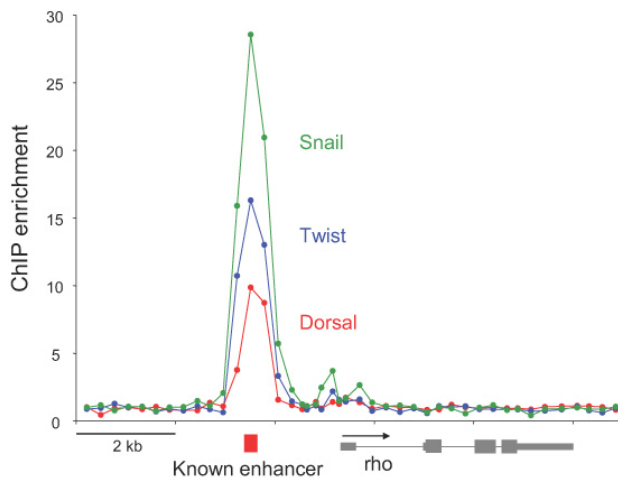
“Some transcription factors have long and specific DNA sequences they bind to” says Zeitlinger; these are the binding sites which often can be identified by DNA-sequence analysis alone. “But other transcription factors are kind of promiscuous. They bind DNA together in a combinatorial fashion that has aspects we don’t understand, like the spacing between binding sites and the state of the chromatin....Ideally—we don’t know whether it’s possible—but ideally we’d like to have the sequence and predict how the promoter will behave *in vivo*. That’s still a major challenge for the future.”

One of the major goals of HFSP is to promote deeper interactions between biologists and computational scientists in addressing fundamental problems of biological complexity, interactions which

Zeitlinger has already been involved in during her studies of transcriptional networks. “One problem is that the interactions [between biologists and computational scientists] can be kind of frustrating for both sides,” she says, “and that’s partly why, I think, it’s not being done much.” Computational scientists and biologists lack a common language and a common grounding in the detailed empirical knowledge of biological systems. “If I see the results from a computational person,” says Zeitlinger, “I can say, wow, this really makes sense, because I know what this gene is doing and how it relates to this other gene. For me, it’s satisfying. But for the computational person, it’s just a gene and another gene. So they don’t see the beauty of it in a way... Because biology requires a lot of knowledge, a lot of learning things by heart.”

At the same time, Zeitlinger can identify with the mindset of computational scientists. “They are often more interested in their mathematical puzzles. And I can understand that, because I also had that bent with math and chemistry when I was younger. I was more interested in something defined and logical, and the beauty of biology I only discovered later on.” Zeitlinger has found that in order to attract the excitement of computational scientists, it is best to frame her biological problems in terms of a “precise puzzle. You say, here are the inputs and outputs of this genetic system. Here are some of the rules we think the cell is using. And then they say, Hah, okay, now we can play with those rules.”

Zeitlinger’s 2003 *Cell* paper on Ste12 already has become an oft-cited classic among genomic scientists, in good measure because of the strong biological foundation of knowledge about yeast mating, filamentation and signaling pathways that underpinned her work. After finishing her HFSP fellowship, Zeitlinger has extended the methodology she helped pioneer in yeast back to the higher, multicellular organism that first whetted her interest in transcriptional networks: the *Drosophila* fruitfly.



“The complexity of transcriptional regulation in *Drosophila* will be much higher than in yeast. But I think *Drosophila* is a great system, because [as in yeast] there’s so much known biologically.” There is now a burgeoning post-genomic effort called the *Drosophila* Encode Project which aims to decipher the function of every base-pair in the *Drosophila* genome, whether protein-coding or regulatory, as a key step towards deciphering the biological “meaning” of every base-pair in the human genome. “The *Drosophila* Encode Project fits very well within the theme of what I’m trying to do, and also within the theme that it’s a good time to move to higher organisms. Of course there are very interesting

questions [about transcriptional networks and regulation] to pursue in mammals, but I think *Drosophila* will be a lot more tractable.” The black box of complex living genomes that so tantalized Zeitlinger as a graduate student is now beginning to be opened.

### Update June 2008

Julia Zeitlinger has successfully pursued her career goals and is now an Assistant Investigator at the [Stowers Institute for Medical Research](#) in Kansas City, Missouri, where she is continuing her analysis of transcriptional networks in *Drosophila*. Her two initial studies of these networks, using a *Drosophila* DNA microarray of her own design, were published in 2007<sup>1</sup>.

<sup>1</sup> Zeitlinger et al. *Genes Dev.* (2007) 21:385-90 "Whole-genome ChIP-chip analysis of Dorsal, Twist, and Snail suggests integration of diverse patterning processes in the *Drosophila* embryo."

In June 2008 Julia was named a Pew Scholar in Biomedical Sciences by the Pew Charitable Trusts. This honour carries an award of \$240,000 over four years.

In September 2008 she was selected for the prestigious NIH Director's New Innovator Award.

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Zeitlinger et al. Nat Genet. 2007 39:1507-11 "RNA polymerase stalling at developmental control genes in the *Drosophila melanogaster* embryo."