Victor Ambros

The 2006 Genetics Society of America Medal

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In 1990, Victor Ambros faced a conundrum. The search for the heterochronic gene *lin-4* had led him to a 700-bp DNA fragment. This fragment could rescue a *lin-4* mutant but contained no apparent open reading frame (ORF). The few small ORFs that could be detected were either not conserved in other nematodes or not essential for rescue. Two years and several RNAse protection experiments later, Victor Ambros was forced to conclude that the *lin-4* gene product was not a protein at all, but a very small ~20-base RNA—the first microRNA!

Geraldine Seydoux

THE 2006 GSA Medal is awarded to Victor Ambros in recognition of his seminal discovery of microRNAs and his many contributions to the field since. Victor did his graduate work at MIT with David Baltimore, where he studied mechanisms of poliovirus replication. In 1979, Victor joined the lab of Bob Horvitz, who had recently returned to MIT after 5 years at the MRC where he had helped launch Sydney Brenner's new model system, *Caenorhabditis elegans*. Among the many mutants that Bob Horvitz brought back from the MRC was *lin-4(e912)*. *lin-4* mutants have complex lineage defects with many cells repeating division patterns characteristic of early larval stages. *lin-4* mutants cannot lay eggs, so Victor decided to screen other egg-laying defective mutants for similar "heterochronic" defects. The screen yielded mutations in three new loci: *lin-14*, *lin-28*, and *lin-29*. Remarkably, while some mutations caused "retarded" phenotypes similar to *lin-4*, others caused "precocious" phenotypes, with many cells skipping ahead to cell division patterns characteristic of older larval stages. *lin-14*, in particular, could mutate to both phenotypes, depending on whether the alleles caused loss of function or gain of function. These findings not only identified the first genes regulating developmental timing in a multicellular organism, but also hinted at how mutations in key genes might underlie heterochronic variation between species (Ambros and Horvitz 1984).

In 1985, Victor joined the faculty in the Department of Cellular and Developmental Biology at Harvard University. Taking advantage of the opposite phenotypes of retarded and precocious mutants, Victor set out to dissect the epistatic relationships among *lin-4*, *lin-14*, *lin-28*, and *lin-29*. With keen insight, he focused his analysis on a single, easy-to-score, stage-specific event: the larval-to-adult switch (L/A switch). At the transition between the last larval stage and adulthood, certain cells in the skin of the worm cease division, fuse with one another, and produce a characteristic ridged structure in the cuticle (alae). Victor constructed double, triple, and quadruple mutants and scored the L/A switch in each combination. The results were published in a single-author article (Ambros 1989), now renowned as a classic "epistasis" study, a favorite among teachers of genetics! This publication laid out the core hierarchy of the *C. elegans* heterochronic pathway: *lin-4* negatively regulates *lin-14* and *lin-28*, *lin-14* and *lin-28* inhibit *lin-29*, and *lin-29* activates the L/A switch (Ambros 1989).

With the genetics sorted out, it was time to determine the molecular identities of the heterochronic genes. Cloning in the pregenomic era was not an easy task and the article reporting the cloning of *lin-14* (a collaboration among Gary Ruvkun, Victor Ambros, Bob Horvitz, and the future leaders of the *C. elegans* genome project, Alan Coulson, Bob Waterston, and John Sulston) was an elegant demonstration of how to use linked RFLPs to clone genetic loci in *C. elegans* (Ruvkun *et al.* 1989). Soon after, Victor set his sights on *lin-4*, the negative regulator of *lin-14*. At the time, *lin-4* was defined by only a single allele, raising the possibility that *lin-4* might be

a small gene or, worse, a complicated rearrangement. Nothing, however, had prepared Victor and his lab for what followed. By the spring of 1992, Rosalind Lee and Rhonda Feinbaum in Victor's lab had mapped *lin-4* down to a 60-nt hairpin RNA (*lin-4L*). A smaller ~20-nt species *lin-4S* had also been detected but was initially dismissed as an artifact, until Feinbaum found a second *lin-4* allele that turned out to be a single-base-pair change in the *lin-4S* sequence. But how could such a small RNA regulate *lin-14* and *lin-28*?

In the meantime, the lab of Gary Ruvkun had continued the molecular analysis of *lin-14* and shown that *lin-14* gain-of-function mutations affect conserved sequences in the *lin-14* 3′-UTR. Gary and Victor exchanged the *lin-14* and *lin-4* sequences and, on the same day in June 1992, both realized that *lin-4S* was complementary to a repeated sequence in the *lin-14* 3′-UTR. They immediately shared their discovery over the phone and published their findings back to back the following year in an exemplary demonstration of open collaboration and fair play (Lee *et al.* 1993; Wightman *et al.* 1993).

The cloning of *lin-4* did not immediately trigger the microRNA frenzy that we know today. For many years, *lin-4* remained an oddity, an interesting but nematode-specific quirk. Undaunted, Victor and his lab continued the analysis of *lin-4* and its targets. In 1997, postdoctoral fellow Eric Moss showed that *lin-4* regulates *lin-28* through a 3′-UTR element similar to the ones found in *lin-14*, demonstrating that *lin-4* regulates more than one mRNA (Moss *et al.* 1997). Two years later, postdoctoral fellow Phil Olsen discovered that *lin-4* blocks translation of the *lin-14* mRNA while still on polysomes, suggesting that *lin-4* inhibits a step after translation initiation (Olsen and Ambros 1999). It was not until the Ruvkun lab cloned a second microRNA (*let-7*) and showed it to be conserved across many species (Pasquinelli *et al.* 2000; Reinhart *et al.* 2000), and until the Ambros lab and others discovered scores of microRNAs in *C. elegans* and other animals (Lee and Ambros 2001), that the microRNA world came into its full glory. Today microRNAs are recognized as major players in the regulatory programs that orchestrate the development of organisms as diverse as plants and mammals. Since their discovery through their roles in developmental timing in *C. elegans*, microRNAs have been implicated in many other processes from cell proliferation to cell death and from hematopoiesis to neuronal patterning (Ambros 2004). The spotlight on microRNAs and their connection to RNA interference has led to a renewed interest in the many RNA species that exist in cells.

Victor Ambros, now a professor at Dartmouth in his hometown of Hanover, New Hampshire, is a soft-spoken and unassuming scientist, always eager to give credit to his collaborators. A strong supporter of the *C. elegans* community, he has organized four East Coast *C. elegans* meetings and the International *C. elegans* Conference in 1993, which he will lead again in 2007. He recently received the 2005 Lewis S. Rosenstiel Award in Basic Medical Science from Brandeis University. Victor Ambros's accomplishments exemplify what is most compelling about genetics: the power to discover what nobody knew existed—starting with a handful of interesting mutants.

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