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Tiny RNA may be universal regulator of developmental timing in animals

Discovery by MGH/Harvard scientists may be key developmental insight Researchers at Massachusetts General Hospital (MGH) and Harvard Medical School have discovered a tiny RNA gene that may control developmental timing in creatures as diverse as fish, sea urchins, mollusks, marine worms, flies, nematodes and humans.

The team led by Gary Ruvkun, PhD, of the MGH Molecular Biology Department, a professor of Genetics at Harvard Medical School, showed that a tiny regulatory RNA that controls developmental timing in the *C. elegans* nematode worm is present in the genomes of a wide variety of animals and is regulated in a similar manner.

The discovery that a universally conserved gene may control developmental timing could prove to be a fundamental insight for developmental biology, the study of how multicellular creatures control the complex choreography of cells as they grow from a fertilized egg into an adult organism. The study appears in the Nov. 2 issue of *Nature*.

The team was composed of postdoctoral fellows Amy Pasquinelli, PhD, Brenda Reinhart, PhD, and a worldwide group of biologists who are experts in the biology of marine mollusks, jellyfish, coral, sponges, worms, flies, mice, fish, sea urchins and sea squirts.

By analyzing the genes of this Noah's ark of creatures, the team found that this RNA gene is universal to bilaterally symmetric animals (those in whom the left and right sides of the body are essentially identical) but is not present in more primitive animals, such as sponges and coral, nor in plant or microbial species. An RNA gene is one that ultimately leads to the production of the single-strand molecule RNA instead of a protein.

The discovery of this common feature of developmental timing suggests that this tiny RNA gene evolved almost a billion years ago in animals to regulate the transition from early larval stages to later reproductive phases and that almost all animal species have inherited the gene from this common ancestor.

Because the gene has been conserved over all these years, it is likely to regulate features of development that are common to all animals. The discovery was greatly aided by the now nearly complete human and fruit fly genome sequences.

Earlier this year the Ruvkun team published in the Feb. 24 issue of *Nature* the discovery in *C. elegans* of the *let-7* regulatory RNA, a 21-nucleotide molecule that regulates temporal transitions during the development of the worm.

This regulatory RNA gene, which is switched on as the animal matures from a larval to adult stage and is necessary for this transition, can base pair - that is, form a double strand of RNA - with the messenger RNAs from other timing control gene, which suppresses their activity.

In the current report, the Ruvkun team used genome sequence searches to detect perfect matches to the *let-7* RNA in the *Drosophila* (fruit fly) and human genome databases. They then

showed that humans and flies also express this 21-nucleotide RNA and that in flies and zebrafish these tiny RNA genes are turned on only at late stages of development, just as in *C. elegans*.

In a broad sampling of animals, they found that the let-7 regulatory RNA also is expressed only at later stages in mollusks and annelid worms and is absent in more primitive animals - such as jellyfish, coral, and sponge - and in non-animal species (plants and microbes). The team also found that the major RNA target of let-7 RNA is also conserved in flies and zebrafish, as are the specific elements in the target RNA that form base pairs with the let-7 RNA.

The paper makes a strong case that the let-7 regulatory RNA acts universally in animal development to trigger a major temporal transition, from larval to adult forms, in many species. While humans and other mammals do not have larval stages, it is possible that their let-7 RNA genes could regulate such developmental transitions as the "molting" of baby teeth or the growth of certain tissues at puberty. Defects in developmental timing could figure in a variety of human birth defects.

The let-7 gene is the type that is missed by current methods of genome sequence analysis, Ruvkun explains. For example, estimates of the number of human genes vary from 25,000 to 100,000 mostly because current methods of detecting genes in the vast expanse of chromosomes are very primitive. Most gene-finding computer programs focus on the detection of protein-coding DNA segments, the most common form of gene. But there are thousands of known genes that encode RNA rather than protein products.

Genes that encode RNAs are the most primitive, since before protein-coding genes evolved, life was dominated by RNA genes in what has been dubbed the "RNA World." Even today, protein-coding genes depend on the ribosome, an ancient RNA-based enzyme, for the translation of messenger RNA into protein.

Genes encoding RNAs as short as 21 nucleotides, such as let-7 RNA, are the easiest to miss in the analysis of genome sequences. In the case of the let-7 regulatory RNA, it was genetic analysis in the worm that identified the gene and comparative analysis of genome sequences that revealed its universality.

As more genome sequences emerge, such global comparisons of sequences will reveal other regulatory genes by finding long regions of DNA conserved among disparate animal genomes. These analyses should reveal the remnants of the RNA world that genomes still carry. The paper from the Ruvkun lab also demonstrates how comparison of genome sequence databases from a few very diverse organisms can catalyze discovery in quite distant species.

The discovery of the broad role of the let-7 regulatory RNA may be a fundamental step in developmental biology. An analogous universal control gene called the homeobox, which encodes a protein that binds to DNA, was discovered in fruit flies in 1984. That discovery led to a renaissance in developmental biology in both vertebrate and invertebrates that continues to this day.

Similarly, the discovery of how broadly the let-7 timing RNA is conserved across the animal kingdom - from worms to flies to mice to humans - will trigger further study of how timing is regulated.