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Long intervening non-coding RNAs play pivotal roles in brain development

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Whitehead Institute scientists have identified conserved, long intervening non-coding RNAs (lincRNAs) that play key roles during embryonic brain development in zebrafish. They also show that the human versions of the lincRNAs can substitute for the zebrafish versions, which implies that the functions of these non-coding RNAs have been retained in humans as well as fish.

Until now, lincRNAs have been studied primarily in cell lines rather than at the organismal level, which has precluded research into how lincRNAs affect growth and development.

"These studies show that zebrafish, an animal that is frequently used to study the genetics of animal development, can also serve as a tool to uncover in systematic fashion the functions of lincRNAs," says Whitehead Member David Bartel, who is also a Howard Hughes Medical Institute investigator and a professor of biology at MIT. "This is another case in which a phenomenon in zebrafish provides insight into what's probably happening in humans, as has been established in many studies of protein-coding genes."

Only a minority of RNAs transcribed in a human cell goes on to template protein production, according to a 2007 assessment of the human genome by the Encyclopedia of DNA Elements (ENCODE) Project Consortium, which was funded by the National Human Genome Research Institute. The rest of the RNAs are dubbed non-coding RNAs (ncRNAs), with those located between protein-coding genes and with

lengths of 200 base pairs or longer referred to as lincRNAs.

Despite their prevalence in the cell, lincRNAs have been referred to as the "dark matter" of all the transcribed RNAs because little is known of their functions or mechanisms. One limitation to studying this class of RNAs is their low sequence similarity between species. Unlike protein-coding genes, which are frequently well-conserved between species, lincRNA genes typically have a very small bit of conserved DNA between species, if any. This lack of conservation makes identification of related lincRNAs difficult in closely related species and nearly impossible in distantly related species.

For example, Bartel lab scientists Igor Ulitsky and Alena Shkumatava identified more than 500 lincRNAs in zebrafish but found that only 29 of these have homologs in both humans and mice.

Ulitsky and Shkumatava, who report their findings in this week's issue of the journal *Cell*, tested the function of two of the 29 lincRNAs by knocking them down in zebrafish embryos. Both knockdowns had striking effects on the zebrafish's brain development. Reduction of one of the lincRNAs, which they called cyrano, caused the zebrafish to have enlarged snouts, small heads and eyes, and short, curly tails, while the zebrafish lacking the lincRNA they called megamind had abnormally shaped heads and enlarged brain ventricles.

To test if the human homologs of the cyrano and megamind lincRNAs are functionally equivalent, Shkumatava injected the human versions into the knocked-down zebrafish. Remarkably, the human lincRNAs rescued the zebrafish and restored brain development and head size for both lincRNAs, indicating that the human lincRNAs may have the same role in embryonic development as their zebrafish analogs.

"This work represents a major advance because it provides a framework

for studying lincRNAs, a poorly understood, but abundant class of molecules," says Michael Bender, who oversees RNA processing and function grants at the National Institutes of Health's National Institute of General Medical Sciences, which partially funded the work. "The discovery that human lincRNAs appear to function much like their zebrafish counterparts in embryonic development suggests that the framework will prove valuable in bringing new insights on the roles played by lincRNAs in mammalian organisms."

The zebrafish is already a powerful tool for studying genetics. Whitehead Member Hazel Sive, who collaborated with Bartel and his lab members on the *Cell* paper, uses zebrafish to study brain development and genetic mutations linked to autism.

Says Sive, "The zebrafish is a fantastic, facile system for discovering the mechanisms by which genes work."

"We humans share with zebrafish this subset of ancient, peculiar genes, and the functionality has been retained in them," says Ulitsky. "We can perturb them in zebrafish and then replace them with the human ones and, at least in the lincRNAs we look at, the human ones function to restore proper development."

"Because of this functional conservation of lincRNAs between zebrafish and humans, we're introducing the zebrafish as a new vertebrate tool that could be used basically to uncover the functions of other lincRNAs," says Shkumatava.

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More information: "Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution", *Cell*, December 23, 2011

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