Study finds that two non-coding RNAs trigger formation of a nuclear subcompartment

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The nucleus of a cell, which houses the cell's DNA, is also home to many structures that are not bound by a membrane but nevertheless exist as distinct compartments. A team of Cold Spring Harbor Laboratory (CSHL) scientists has discovered that the formation of one of these nuclear subcompartments, called paraspeckles, is triggered by a pair of RNA molecules, which also maintain its structural integrity.

As reported in a study published online ahead of print on December 19 in Nature Cell Biology, the scientists discovered this unique structure-building role for the RNAs by keeping a close watch on them from the moment they come into existence within a cell's nucleus. The scientists' visual surveillance revealed that when the genes for these RNAs are switched on, and the RNAs are made, they recruit other RNA and protein components and serve as a scaffolding platform upon which these components assemble to form paraspeckles.

The two RNAs described in the study, named MENε and MENβ, are "non-coding" RNAs —a type of RNA that does not serve as a code or template for the synthesis of cellular proteins. The genes that give rise to these non-coding RNAs are now thought to make up most of the human genome, in contrast to the genes that produce protein-coding RNAs, which account for approximately 2% of the human genome.

"We've known for several years that much of the other 98% of the

genome doesn't encode for useless RNA," explains CSHL's Professor David L. Spector, who led the current study. "Various types of non-coding RNAs have been found that regulate the activity of protein-coding genes and cellular physiology in different ways. Our results reveal a new and intriguing function for a non-coding RNA—the ability to trigger the assembly and maintenance of a nuclear body."

The nuclear bodies in question—the paraspeckles—are believed to serve as nuclear storage depots for RNAs that are ready to be coded, or translated, into proteins but are retained in the cell nucleus. Paraspeckles are thought to release this RNA cache into the cell's cytoplasm—the site of protein synthesis—under certain physiological conditions, such as cellular stress. Spector estimates that storing pre-made protein-coding RNA within the paraspeckles and releasing them as needed allows the cell to respond faster than if it had to make the RNA from scratch.

Previous experiments by Spector's team and two other groups indicated that MENε and MENβ RNAs were the critical elements for paraspeckle formation. "What wasn't clear was how the paraspeckles actually form and the dynamics of how the non-coding MEN RNAs help organize and maintain its structure," says Spector.

To address this question, the team developed an innovative approach—spearheaded by CSHL postdoctoral fellow Yuntao (Steve) Mao and graduate student Hongjae Sunwoo—to peer into living cells and capture the real-time dynamics of the interactions among the set of molecules known to be involved in paraspeckle formation. The scientists engineered cells in which each of these players—the MENε/β genes, the newly formed MEN RNAs, and the various paraspeckle protein components—each carried a different colored fluorescent tag. The cells were also genetically manipulated such that the MEN genes could be switched on by exposing the cells to a drug.

The resulting movies shot by the Spector team, showed that within five minutes of switching on the MENε/β gene, individual paraspeckle proteins arrived and assembled at the sites of MEN RNA transcription. As the RNA transcripts accumulated, the fully functional paraspeckles enlarged in tandem and eventually broke away to cluster around the transcription sites.

"Our experiments show that it is the act of MEN RNA transcription alone that triggers paraspeckle formation and sustains them," says Spector. In the absence of transcriptional activity—such as during cell division or when the scientists added drugs that block RNA transcription or specifically switched off the MEN genes—the newly formed paraspeckles fell apart.

This dependency on RNA transcription seems to be unique, as other nuclear compartments such as Cajal bodies can form when one of their components is simply tethered to a site on the genome, which in turn causes other components to coalesce around it. In contrast, says Spector, "Paraspeckles seem to follow a different assembly model in which MEN non-coding RNAs serve as seeding molecules that are driven by transcription to recruit the other components."

More information: Yuntao S. Mao, et al. "Direct visualization of the co-transcriptional assembly of a nuclear body by noncoding RNAs," December 19, 2010 *Nature Cell Biology*.

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