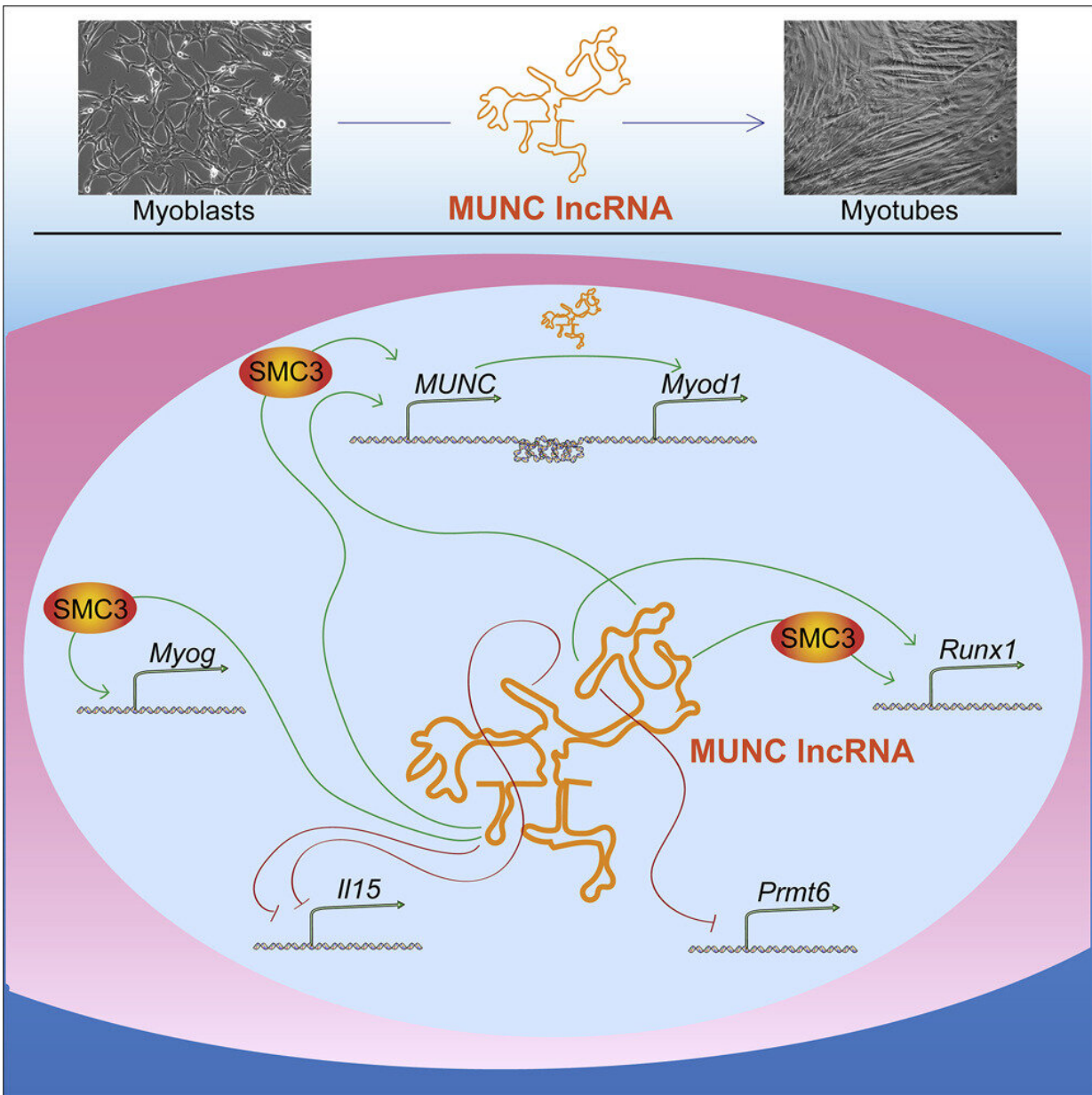


Distinct structural domains in MUNC long non-coding RNA regulate gene expression

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Graphical abstract. Credit: DOI: 10.1016/j.celrep.2022.110361

In a paper in *Cell Reports*, Anindya Dutta, M.D., Ph.D., and colleagues detail the first structure-function study of a long non-coding RNA, or lncRNA, called MUNC lncRNA. Their findings point out the importance of experimentally determining the structure of an lncRNA through a chemical method—rather than computer modeling—to identify structural domains that turn out to have multiple different functions.

An lncRNA is an RNA longer than 200 bases that is not translated into a functional protein. It appears that humans have many tens of thousands of these RNAs that seem to play key roles in [gene regulation](#) through interactions with DNA, RNA or proteins. However, few have been functionally characterized, and even fewer have experimentally determined information for their structure.

The researchers studied MUNC lncRNA in mouse myoblasts, [precursor cells](#) that can differentiate into skeletal muscle cells. MUNC lncRNA is known to play an important role in this skeletal muscle tissue formation through differentiation, a process called myogenesis. The MUNC lncRNA has two isoforms, one with 1,083 nucleotides that has two exons and one intron, and one with 518 nucleotides, where the intron has been cut out and the two exons spliced together.

Knowledge of how lncRNAs function in differentiation is potentially important in understanding and controlling cancer, Dutta says, because cancer cells are dedifferentiated cells. Understanding how they get there and learning how to reverse that could lead to new therapy approaches. Dutta became chair of the University of Alabama at Birmingham Department of Genetics in March 2021, arriving from the University of

Virginia School of Medicine.

In the current study of MUNC lncRNA, the researchers found two important results when they did chemical structural determinations of the two isoforms. First, those structures had multiple structural domains that were not detected by computer predictions. This shows the need to determine structures experimentally. Second, those domains were largely conserved between the two isoforms, indicating the stability of the domains. The domains included six common "hairpins," where the RNA folds back on itself to make a double strand, found in each [isoform](#). The spliced isoform had one additional hairpin and one loop not seen in the unspliced isoform.

Also, when researchers compared the spliced isoform that was chemically treated while still inside the cell versus the spliced isoform that was chemically treated after purification, they saw a difference. The MUNC lncRNA treated inside the cell had two protected regions that the lncRNA treated outside the cell lacked. This may mean those regions were interacting with proteins inside the cell, preventing the chemical reagent from reaching the RNA bases touching the proteins.

Dutta and colleagues next set out to probe the functional importance of these domains.

Both isoforms, when overexpressed in mouse myoblast cells, were promyogenic, meaning they promoted expression of genes involved in muscle cell differentiation, likely through activation of a common set of transcription factors. However, while 645 genes were upregulated by both forms of MUNC, 2,730 other genes were upregulated by only one isoform or the other. Thus, they control different sets of genes.

The researchers made variants of the spliced isoform that either lacked one specific structural domain or had a defined mutation in a single

[domain](#). Each variant was overexpressed in mouse myoblast [cells](#) to see how disruption of individual structural domains affected expression of promyogenic factors. The resulting changes in gene expression revealed that different RNA domains of MUNC were important for binding to and regulating different [genes](#). Thus, they could act independently, suggesting that the spliced MUNC is something like a Swiss Army knife that contains different tools in one pocketknife.

The independence of two of these domains and the overall stability of MUNC was shown another way. Chemical structure analysis was repeated for two of the variants—one with a common-hairpin-1 deletion and the other with a common-hairpin-4 deletion. These two hairpins were examined because both are critical for promyogenic phenotypes. Results revealed that both deletions were surgical, meaning the rest of the secondary structure of the remaining RNA was unchanged.

The researchers also tested how loss or mutation of the domains affected binding of MUNC to two different promoter sites on the chromosome for the gene *Myod1*, which encodes a protein that acts as a master switch for myogenesis. Such binding at either site leads to enhanced *Myod1* gene expression. Experiments showed that three of the hairpins were required for binding at both sites. In addition, six other domains were important for binding, but with a difference—three influenced binding at one site, and the other three influenced binding at the other site. Thus, different domains mediate distinct features of MUNC promyogenic activity.

To add to this complex story, Dutta and colleagues tested whether structure or the sequence of RNA bases was responsible for the functional roles of common-hairpin-1 and common-hairpin-4 in promyogenic gene expression. They did this by making mutants of the two hairpins, where different stretches of RNA base sequence were changed without altering the hairpin structure. The result? The mutants

retained their function of inducing Myod1, indicating that the shape, not the RNA sequence, was key. "Based on the results from eight independent sequence mutants, we conclude that MUNC function attributed to common-hairpin-1 and common-hairpin-4 is primarily driven by structure," Dutta said.

"Overall," Dutta said, "the multi-modal mechanisms of regulation of gene expression by MUNC involve different domains of MUNC, and likely different protein partners. MUNC recruits varying cooperative machinery depending on the genomic context, and this behavior is likely exemplary of other lncRNAs that regulate gene expression."

More information: Roza K. Przanowska et al, Distinct MUNC lncRNA structural domains regulate transcription of different promyogenic factors, *Cell Reports* (2022). [DOI: 10.1016/j.celrep.2022.110361](https://doi.org/10.1016/j.celrep.2022.110361)

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