Protein revealed as glue that holds biomolecules within the nucleolus

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Researchers have determined that the protein nucleophosmin (NPM1) serves as glue that holds proteins and RNA together in the nucleolus and showed how NPM1's structure makes it ideal for the job. St. Jude Children's Research Hospital scientists led the study, which appears today in the scientific journal *eLife*.

The nucleolus is a membrane-less structure or organelle that takes up about 25 percent of the nucleus. Membrane-less organelles are transient compartments that are assembled as needed by cells to perform particular functions. These structures form through a process called liquid-liquid phase separation in which proteins and often ribonucleic acids (RNA) condense into liquid-like droplets that fuse and grow.

The largest membrane-less organelle, the nucleolus has been compared to a manufacturing hub, since one of its main functions is to assemble the ribosomes that produce all of the proteins required by cells. The nucleolus is home to a vast array of proteins, RNA and other molecules, but until this study its molecular foundation was unknown. Ribosomes are assembled from RNA and proteins, and the assembly process begins in the nucleolus.

Using a variety of imaging, structural and biophysical laboratory techniques, researchers identified NPM1 as key to the process. Investigators showed that the structure of NPM1 lets it bind to a wide variety of proteins as well as to RNA in different, specific ways that promote phase separation and retain NPM1 and other proteins in the

nucleolus. NPM1 forms networks of interactions with other molecules in the nucleolus, loosely gluing nucleolar components together. The scientists reported that NPM1 did not accumulate in the nucleolus when binding with either proteins or RNA was disrupted.

"The nucleolus performs a specialized function, and NPM1 seems to have evolved to assist in the process by being able to phase separate with these two important and very different types of nucleolar molecules," said corresponding author Richard Kriwacki, Ph.D., a member of the St. Jude Department of Structural Biology. "NPM1 is like the glue that holds different factors required for ribosome assembly within the nucleolus."

NPM1 is an important regulatory protein that is abundant in the nucleolus. NPM1, which is mutated in about 35 percent of adults with acute myeloid leukemia, is known in part for its role in tumor suppression as a binding partner of the tumor suppressor protein ARF.

Proteins are long chains of amino acids whose function is dictated in part by their 3-D shape and flexibility. NPM1 includes segments that fold into a rigid, five-sided pentamer and other segments rich in flexible, negatively charged amino acids that bind transiently to other proteins. This study identified the interactions that stem from these segments and allow NPM1 to form loose networks with multiple proteins and RNAs. These molecular networks are essential for phase separation as well as retention of NPM1 and other proteins in the nucleolus and ribosome assembly.

"There are other proteins in the nucleolus that have some of the same features as NPM1, including the negatively charged amino acid tracts," Kriwacki said. "That suggests that NPM1 is probably not the only protein contributing to phase separation in the nucleolus, but our studies show that it certainly is a very important player."

The findings come amid intense scientific interest in the role liquid-liquid phase separation plays in promoting membrane-less organelle assembly as well as in performing the molecular processes that occur within them.

This study builds on previous work by Kriwacki and his colleagues that demonstrated how under certain conditions the NPM1 pentamer unfolds into a single disordered strand of amino acids. Those investigators also found evidence that the disordered strand prevented binding to proteins like ARF that include short amino acid sequences rich in the amino acid arginine.

In this study, researchers determined that 73 percent of 132 proteins known to bind NPM1 include multiple arginine-rich amino acid segments. In contrast, such segments were present in just 44 percent of all human proteins. The investigators showed that these arginine-rich segments cause proteins to undergo phase separation with NPM1 to form liquid-like droplets. Previous work showed that the unfolding of NPM1 may be one of the molecular mechanisms involved in dismantling the liquid-like structure of the nucleolus during cell division.

Working in several different experimental systems, including mouse cells growing in the laboratory, researchers showed that incorporation within the nucleolus required NPM1 binding with both nucleolar proteins that include the arginine-rich segments and ribosomal RNA. The researchers also identified where on the pentamer protein and RNA binding occurred and how the different bonds promoted condensation into liquid-like droplets. The latter information was uncovered using a variety of laboratory approaches, including single-molecule fluorescence spectroscopy, nuclear magnetic resonance spectroscopy and small-angle neutron scattering.

More information: Diana M Mitrea et al. Nucleophosmin integrates

within the nucleolus via multi-modal interactions with proteins displaying R-rich linear motifs and rRNA, *eLife* (2016). DOI: 10.7554/eLife.13571

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