RNA gets in phase

Shambaditya Saha and Anthony A. Hyman
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Several neurological disorders are linked to tandem nucleotide repeat expansion in the mutated gene. Jain and Vale (2017. Nature. https://doi.org/10.1038/nature22386) show that, above a pathological threshold repeat number, base pairing interactions drive phase separation of RNA into membrane-less gels, suggesting that RNA can scaffold the assembly of phase-separated compartments that sequester proteins/RNAs causing toxicity.

Many compartments in cells carry out important biological functions but lack surrounding membranes. Prominent examples are the nucleolus, stress granules, and P granules. Recently, there has been increasing interest in understanding how these compartments assemble. Studies suggest that liquid–liquid phase separation is at the heart of this problem (Brangwynne et al., 2009; Banani et al., 2017): Like the demixing of oil and water, certain proteins and RNAs phase separate from the surrounding cytoplasm or nucleoplasm to form liquid-like compartments. Considerable research has gone into understanding which proteins drive phase separation. It has been suggested that certain “scaffold” proteins have the ability to phase separate into minimal compartments. Other “client” proteins and RNAs target into the minimal compartments to modulate biological function (Banani et al., 2017). Interestingly, after phase separation, liquid-like compartments can change their material properties to a more solid-like gel state (Patel et al., 2015; Banani et al., 2017). The obvious difference between a liquid and a gel is that the resident proteins in gels rearrange in space much more slowly. Because of their varying material properties, these compartments have been called “bimolecular condensates.” It has been suggested that changes in material properties of phase-separated compartments could contribute to the onset of neurodegeneration (Banani et al., 2017). The idea stems from the observation that mutations associated with neurodegenerative diseases promote a considerably faster liquid-to-gel transition in phase-separated compartments in vitro (Patel et al., 2015; Banani et al., 2017). Gelation of intracellular compartments may result in neurotoxicity by sequestering key proteins and inhibiting their normal function.

A common question one asks students is: Which is bigger, the protein or the mRNA from which it is translated? The answer of course is that the mRNA is usually much larger. However, there has been much less work on the role of RNA in phase separation and physiology of non-membrane–bound compartments. By binding to specific scaffold proteins, RNA lowers the critical concentration of protein required for phase separation (Saha et al., 2016; Banani et al., 2017). Interestingly, RNA can also modulate the material properties of phase-separated compartments in vitro (Zhang et al., 2015; Banani et al., 2017). In their paper, Jain and Vale (2017) show that RNA could have a more profound role in organization of phase-separated compartments. This is because RNA alone can phase separate without the help of proteins, taking on either liquid- or gel-like states. This suggests that RNA itself could scaffold the formation of compartments, and that the material properties of the RNA scaffolds could influence the material properties of the compartments themselves (Jain and Vale, 2017).

Jain and Vale (2017) studied RNA molecules that have been implicated in neurodegenerative repeat expansion disorders. These disorders are associated with the incorporation of long tandem repeats of 3–12 nucleotides within the protein-coding (exon) or noncoding regions of the genome (La Spada and Taylor, 2010). Many diseases have been associated with repeat expansion. For instance, healthy individuals contain fewer than 20 repeats of the trinucleotide CAG in exon 1 of the huntingtin (HTT) gene. However, in patients with Huntington’s disease, the number increases to more than 35 repeats. Similarly, the number of GGGGCC repeats in intron 1 of the C9orf72 gene increases from 2–23 in healthy individuals to 700–1,600 in patients with amyotrophic lateral sclerosis (ALS) or frontotemporal dementia (FTD). Significant effort has gone into understanding how these nucleotide repeats could lead to disease pathologies, and certain common themes have emerged. It turns out that both the RNA and protein products of these repeat-containing regions interfere with cellular homeostasis (La Spada and Taylor, 2010). Problems could stem from loss of function of the gene associated with repeat expansions. Alternatively, in a gain-of-function scenario, the RNA and protein products could sequester key cellular proteins that result in disruption of normal cellular processes and eventual toxicity. Manifestation of disease pathology is generally observed only when repeat expansions exceed a minimum threshold length. The role of repeat expansions in disease has been a mystery. Why are repeat-containing RNA molecules more potent disease-causing agents compared with non-repeat–containing ones?

Jain and Vale (2017) explored the contribution of RNA in repeat expansion disorders by looking at repeats of CAG found in Huntington’s disease and spinocerebellar ataxsis patients, repeats of CUG found in myotonic dystrophy patients, and repeats of GGGGCC found in individuals with familial ALS and FTD associated with the C9orf72 gene. The authors focused on formation of disease-associated foci in the nucleus. These

Correspondence to Anthony A. Hyman: hyman@mpi-cbg.de

© 2017 Saha and Hyman This article is distributed under the terms of an Attribution-Noncommercial-Share Alike-No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms/). After six months it is available under a Creative Commons License (Attribution-Noncommercial-Share Alike 4.0 International license, as described at https://creativecommons.org/licenses/by-nc-sa/4.0/).
foci are thought to sequester some key RNA-binding proteins (e.g., splicing factors) and therefore disrupt their normal function. Jain and Vale (2017) began by studying repeat-containing RNAs in vitro and found that interactions among CAG/CUG/GGG GCC repeat-containing regions of RNA are sufficient to generate micrometer-scale structures that are reminiscent of disease-associated nuclear foci (Fig. 1). Importantly, control RNA of equivalent length but with the sequence scrambled lost the ability to phase separate. Interactions that support phase separation include Watson–Crick base pairing among repeats of CAG or CUG or formation of G-quadruplexes via noncanonical Hoogsteen base pairing among GGG GCC repeats. Also, interactions among a minimum number of tri- or hexa-nucleotide repeats are required, underscoring the importance of multivalent interactions in driving phase separation (Banani et al., 2017). Although it is clear from this work that repeat-containing RNA molecules can phase separate in vitro, more work is required to understand the role of RNA in scaffolding the assembly of nuclear foci in the complex environment of the nucleus. For instance, treatment with doxorubicin, a nucleic acid intercalator, interfered with phase separation of exogenously expressed repeat-containing RNA in the nucleus but did not completely disrupt nuclear speckles (Jain and Vale, 2017). Nevertheless, it is remarkable that determinants of phase separation in terms of RNA sequence correlate well with repeat expansion disorders.

The repeat-driven phase-separated RNA condensates form gels in vitro. Therefore, a testable hypothesis that emerges from the study by Jain and Vale (2017) is that toxicity ensues when phase-separated RNA gels sequester key enzymes in vivo and thus interfere with their normal function (Fig. 1). Evidence for this idea comes from several observations. For instance, poly-GGGGCC localizes to nuclear speckles, a common site of mRNA processing, in U2OS cells and potentially converts the liquid-like compartment into a solid-like gel state. Sequestration of key proteins and RNAs in gel-like nuclear foci could disrupt cellular homeostasis and result in toxicity.

Figure 1. Contribution of repeat-containing RNA in neurodegenerative disease. (A) Poly-CAG–, poly-CUG–, or poly-GGGGCC–containing RNA (blue) can phase separate into solid-like gels in vitro. (B) In healthy cells, RNA (orange) and proteins (green) phase separate into nuclear foci that are liquid-like. (C) In patients, repeat-containing RNAs enhance phase separation resulting in more nuclear foci compared with healthy cells. Further, repeat-containing RNAs can transform liquid-like nuclear foci–containing proteins (green) and RNA (blue) into a solid-like gel state. Sequestration of key proteins and RNAs in gel-like nuclear foci could disrupt cellular homeostasis and result in toxicity.

An exciting part of this study is that Jain and Vale (2017) mapped the biophysical data to disease phenotype. As observed in myotonic dystrophy patients, nuclear speckles sequestered a majority of the repeat-containing RNA molecules and the endogenous splicing factor muscleblind-like-1 (MBNL-1) protein (La Spada and Taylor, 2010). However, unlike in patients, these cells did not die but appeared to grow at a normal pace. One possibility is that the precise mechanism of poly-GGGGCC–dependent toxicity in patients may vary because in C9orf72-dependent ALS and FTD patients poly-GGGGCC forms nuclear foci that are distinct from nuclear speckles (Lee et al., 2013). Also, it has been estimated that the amount of poly-GGGGCC–containing RNA in C9orf72-dependent ALS and FTD patients is limited to 1–10 transcripts per cell (Liu et al., 2017). It remains to be established if such a limited pool of transcripts could contribute significantly to neurotoxicity merely via phase separation. Some studies have shown that translation of poly-GGGGCC–containing RNA is required for toxicity (Mizielinska et al., 2014). Being located in the intronic region of the C9orf72 gene, poly-GGGGCC generates toxic dipeptide repeat-containing proteins via a noncanonical translation pathway (Zu et al., 2011). Future research will reveal the
relative contributions of RNAs and proteins toward toxicity in neurodegenerative repeat expansion disorders.

The work by Jain and Vale (2017) opens up exciting avenues of future investigation relevant to both fundamental biology of intracellular organization and translational research into the mechanisms of repeat expansion disorders. For instance, the finding that RNA can phase separate without the help of proteins inspires future investigations into the role of long noncoding RNA in assembly of non-membrane-bound paraspeckles in the nucleus (Clemson et al., 2009). Jain and Vale (2017) found that, in vitro, all of poly-CAG, poly-CUG, and poly-GGGGCC RNAs phase separate into solid-like gels. However, when expressed in cells, only poly-GGGGCC RNA was able to maintain a solid-like gel state in nuclear speckles. Poly-CAG and poly-CUG RNAs also localized in nuclear speckles but existed in a liquid-like state. Could this difference be attributed to the strength of interactions among the repeats of RNA or specific structural features (e.g., hairpins of poly-CAG or poly-CUG vs. G-quadruplexes of poly-GGGGCC)?

In conclusion, the work of Jain and Vale (2017) provides strong support for the idea that the material state of a membrane-less compartment emerges from the interaction among its resident proteins and RNAs. Interactions that favor a liquid-like state compete with the ones that promote a solid-like state, and the material state of the compartment is a result of all these interactions. Identification and characterization of macromolecules that prevent gelation of poly-CAG or poly-CUG in vivo will be of significant interest because these may be critical determinants of disease onset in repeat expansion disorders.

Acknowledgments

We thank Simon Alberti and Ankur Jain for valuable comments on the manuscript and Franziska Friedrich for help with preparation of the illustrations in Fig. 1. We are unable to cite original work of many colleagues because of space limitations.

The authors declare no competing financial interests.

References

Banani, S.F., H.O. Lee, A.A. Hyman, and M.K. Rosen. 2017. Biomolecular condensates: Organizers of cellular biochemistry. Nat. Rev. Mol. Cell Biol. 18:285–298. http://dx.doi.org/10.1038/nrm.2017.7

Brangwynne, C.P., C.R. Eckmann, D.S. Courson, A. Rybarska, C. Hoege, J. Gharaikhani, F. Jülicher, and A.A. Hyman. 2009. Germ line P granules are liquid droplets that localize by controlled dissolution/condensation. Science. 324:1729–1732. http://dx.doi.org/10.1126/science.1172046

Clemson, C.M., J.N. Hutchinson, S.A. Sara, A.W. Ensminger, A.H. Fox, A. Chess, and J.B. Lawrence. 2009. An architectural role for a nuclear noncoding RNA: NEAT1. RNA is essential for the structure of paraspeckles. Mol. Cell. 33:717–726. http://dx.doi.org/10.1016/j.molcel.2009.01.026

Jain, A., and R.D. Vale. 2017. RNA phase transitions in repeat expansion disorders. Nature. 546:243–247. http://dx.doi.org/10.1038/nature22386

La Spada, A.R., and J.P. Taylor. 2010. Repeat expansion disease: Progress and puzzles in disease pathogenesis. Nat. Rev. Genet. 11:247–258. http://dx.doi.org/10.1038/nrg2748

Lee, Y.-B., H.-J. Chen, J.N. Peres, J. Gomez-Deza, J. Attig, M. Štalekar, C. Troakes, A.L. Nishimura, L. Scottor, C. Vance, et al. 2013. Hexanucleotide repeats in ALS/FTD form length-dependent RNA foci, sequester RNA binding proteins, and are neurotoxic. Cell Reports. 5:1178–1186. http://dx.doi.org/10.1016/j.celrep.2013.10.049

Liu, J., J. Hu, A.T. Ludlow, J.T. Pham, J.W. Shay, J.D. Rothstein, and D.R. Corey. 2017. c9orf72 disease-related foci are each composed of one mutant expanded repeat RNA. Cell Chem. Biol. 24:141–148. http://dx.doi.org/10.1016/j.chembiol.2016.12.018

Mizielinska, S., S. Grönke, T. Niccolis, C.E. Ridler, E.L. Clayton, A. Devoy, T. Moens, F.E. Norona, I.O.C. Woollacott, J. Pietrzyk, et al. 2014. c9orf72 repeat expansions cause neurodegeneration in Drosophila through arginine-rich proteins. Science. 345:1192–1194. http://dx.doi.org/10.1126/science.1256800

Patel, A., H.O. Lee, J. Jawerth, S. Maharana, M. Jahnel, M.Y. Hein, S. Stoynov, J. Mahamid, S. Saha, T.M. Franzmann, et al. 2015. A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. Cell. 162:1066–1077. http://dx.doi.org/10.1016/j.cell.2015.07.047

Saha, S., C.A. Weber, M. Nousch, O. Adame-Aranza, C. Hoege, M.Y. Hein, E. Osborne-Nishimura, J. Mahamid, M. Jahnel, J. Jawerth, et al. 2016. Polar positioning of phase-separated liquid compartments in cells regulated by an mRNA competition mechanism. Cell. 166:1572–1584. e16. http://dx.doi.org/10.1016/j.cell.2016.08.006

Zhang, H., S. Elbaum-Garfinkle, E.M. Langdon, N. Taylor, P. Occhipinti, A.A. Bridges, C.P. Brangwynne, and A.S. Gladfelter. 2015. RNA controls PolyQ protein phase transitions. Mol. Cell. 60:220–230. http://dx.doi.org/10.1016/j.molcel.2015.09.017

Zu, T., B. Gibbens, N.S. Doty, M. Gomes-Pereira, A. Huguet, M.D. Stone, J. Margolis, M. Peterson, T.W. Markowski, M.A.C. Ingram, et al. 2011. Non-ATG-initiated translation directed by microsatellite expansions. Proc. Natl. Acad. Sci. USA. 108:260–265. http://dx.doi.org/10.1073/pnas.1013343108