RNA impacts formation of biomolecular condensates in the nucleus

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ABSTRACT
Biomolecular condensates are membrane-less compartments that are formed through an assembly of proteins and nucleic acids in the cell. Dysregulation of biological condensates has been implicated in diseases such as neurodegeneration and cancer. Ribonucleic acid (RNA) is known to affect the assembly of proteins in vitro, if and how RNA is involved in regulating biomolecular condensates in cells is not well investigated. Here we examined two nuclear proteins, FUS and HP1α, in which RNA was found to have an opposite contribution for the assembly of these proteins. Reduction of nuclear RNA, by inhibiting the transcription, triggered assembly of FUS that had been distributed in the nucleoplasm, whereas it dispersed spontaneously formed HP1α assembly. Notably, the cell cycle-dependent phosphorylation-mimicking substitutions in HP1α promoted its assembly formation. These transcription inhibitor experiments are versatile to examine diverse roles of nuclear RNA in regulating biomolecular condensates, in both physiological and pathological conditions.

INTRODUCTION
Cells organize compartments in their interior to differentiate and facilitate biological processes. The nucleus and mitochondria contain a lipid bilayer to physically separate inside and outside and maintain their internal biochemical environment. Another type of cellular compartment including nucleoli, promyelocytic leukaemia (PML) bodies involved in genome maintenance, Cajal bodies and P granules in RNA metabolism, and stress granules are formed without a physical barrier. The principle that underlie this compartmentalization is explained by the phenomenon called liquid-liquid phase separation, or LLPS (Banani et al. 2017; Shin and Brangwynne 2017).

LLPS is a physical property in which a single liq-
including FUS (fused in sarcoma) have been observed in the neurons of ALS patients, which is thought to causally relate to their degeneration (Kwiatkowski et al. 2009; Vance et al. 2009). FUS primarily localizes in the nucleus and forms biomolecular condensates to promote transcription and DNA damage repair (Altmeyer et al. 2015; Wei et al. 2020). However, the cytoplasmic mis-localized FUS, caused by its mutations or other conditions, triggers an aberrant transformation into the FUS aggregates.

What is the difference in environment between the nucleus and the cytoplasm? One apparent difference is the concentration of RNA. After transcription, only a small fraction is exported to the cytoplasm for translation and a large amount of RNA remains in the nucleus (Nozawa and Gilbert 2019). It is estimated that the concentration of RNA in the nucleus is approximately 36 times higher than that in the cytoplasm (Maharana et al. 2018). Besides being fundamental elements for transcription and translation, another role of nuclear RNA is to regulate global chromatin structures by forming a mesh-like structure with scaffold attachment factor A (SAF-A) (also called heterogeneous nuclear ribonucleoprotein (hnRNP U) (Nozawa et al. 2017; Nozawa and Gilbert 2019). Nuclear RNA is also involved in protein assembly through electrostatic interactions by virtue of its negatively charged phosphate backbone (Zhang et al. 2017; Maharana et al. 2018). These observations exemplify the role of RNA in interfering with the formation of condensates, while less is known whether RNA serves as a seed to attract RNA binding proteins to nucleate assemblies in the nucleus.

Here we examined if decreased levels of nuclear RNA affect protein assembly activities of FUS and heterochromatin protein 1α (HP1α) in the nucleus. Our observations indicate the contribution of nuclear RNA in preventing the self-assembly of FUS and in promoting the formation of the HP1α-assembly. Interestingly, the cell cycle-dependent phosphorylation of HP1α revealed a significant effect on their nuclear assembly. We propose that transcription inhibitor experiment is a versatile method to examine the involvement of nuclear RNA in the formation of biomolecular condensate.

MATERIAL AND METHODS

Cell culture. HEK293T cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FCS and 10 mM HEPES-NaOH (pH 7.5) at 37°C in a 5% CO₂ environment. Transcription was blocked by adding α-amanitin (50 mg/mL) and actinomycin D (50 ng/mL) for 5 h.

Plasmids and plasmid transfection. cDNA for human FUS is a gift from Takuya Yoshizawa (Ritsumeikan University). cDNA for human HP1α is generated from hTERT-RPE1 cell mRNA by reverse transcription PCR. Point mutations of HP1α were constructed by a standard PCR cloning strategy. All cDNAs were inserted into a modified pcDNA5/FRT expression vector (ThermoFisher) with an N-terminal mEGFP tag. mEGFP is a monomeric enhanced GFP (EGFP) variant having Ala substitution at Lys 206. HP1α E-E has seven Glu substitutions at Ser 11, Ser 12, Ser 13, Ser 14, Ser 92, Ser 95 and Ser 97. HP1α E-A has four Glu substitutions at Ser 11, Ser 12, Ser 13, and Ser 14, and three Ala substitutions Ser 92, Ser 95 and Ser 97. HP1α A-A has seven Ala substitutions at Ser 11, Ser 12, Ser 13, Ser 14, Ser 92, Ser 95 and Ser 97. All plasmids were verified by sequencing. Plasmid transfection was carried out by incubating cells in a 6 well plate with 2 μg plasmids with 4 μL FuGENE HD (Promega) for 24 h.

Imaging analysis. Cells were fixed with 2% paraformaldehyde in PBS for 10 min at room temperature (rt), followed by incubation in PBS containing 0.2% Triton X-100 for 10 min at rt. DNA was then stained with 0.1 μg/mL DAPI. After staining, cells were mounted with ProLong Gold anti-fade mounting reagent (Invitrogen). Images were acquired on an LSM 880 confocal laser scanning microscope (Zeiss) using 63× objective lens (1.4. NA Plan Apochromat Oil DIC M27) with immersion oil (Zeiss Immersol 518F; refractive index 1.518). Scale of x, y was set at 0.053, 0.053 μm/pixel, respectively, and scan speed was typically 1 μs/pixel. Seven images per cell were acquired at 0.5-μm intervals and were Z-stacked with maximum intensity projection (Image J, version 2.1.0/1.53c). The area of the HP1α assembly was quantified for sizes larger than 0.05 μm² with Image J. For the analysis of HP1α disassembly with transcription inhibition, 3 images per cell were captured every 5 min for 5 h with a 60× objective lens, at 0.5-μm intervals, 1% of 488 laser excitation, 50 msec exposure time on CellVoyager CV1000 (Yokogawa) and Z-stacked with maximum intensity projection (Image J, version 2.1.0/1.53c).

RESULTS

FUS forms assembly in response to transcription inhibition

FUS is an RNA binding protein containing RGG
RNA regulates protein assembly

RNA regulates protein assembly and is known to have a self-assembly property through the LLPS mechanism (Wang et al. 2018). When we expressed mEGFP-fused human FUS in HEK293T cells, mEGFP-FUS revealed diffusible distribution throughout the entire nucleus, but excluded from DAPI low-density nucleoli (Fig. 1A). To examine whether nuclear RNA affects the FUS behavior, we treated these cells with α-amanitin, an RNA polymerase II (pol II) inhibitor, expecting to reduce the level of cellular RNA. The α-amanitin treatment resulted in an accumulation of mEGFP-FUS in nucleoli, which coincided with its apparent decrease from the nucleoplasm (Fig. 1A). The control, mEGFP alone was also distributed in the nucleus, but its distribution was not affected with α-amanitin treatment. As ribosomal RNA (rRNA) is enriched in nucleoli, we assumed that mEGFP-FUS was attracted to ribosomal RNA upon decrease of pol II-transcribed RNA and was released from the nucleoplasm.

To test this possibility, we next treated cells with a combination of α-amanitin and low-dose actinomycin D to inhibit not only RNA pol II but also RNA polymerase I (pol I), which purely transcribes rRNA. As rRNA comprises more than 80 to 90% of cellular RNA (Palazzo and Lee 2015), cellular RNA was expected to significantly decrease by actinomycin D treatment. In a majority of mEGFP-FUS expressing cells, the inhibition of both RNA polymerases led to the formation of multiple round-shaped assemblies of mEGFP-FUS in the nucleus, while mEGFP alone was not affected (Fig. 1B). We reasoned that decreased levels of cellular RNA caused a deprivation of RNA from FUS and thereby promoted the FUS-FUS self-association. Supporting this interpretation, the disruption of nuclear RNA by introduction of RNase A, by microinjection, into the nucleus has been shown to trigger the FUS assembly (Maharana et al. 2018). These observations suggest that nuclear RNA contributes to preventing spontaneous FUS self-assembly.

**HP1α spontaneously forms nuclear assembly that is enhanced by phosphorylation-mimicking substitutions**

To further investigate the role of nuclear RNA in protein assembly, we next focused on HP1. HP1 is an evolutionally conserved protein from fission yeast to human and is involved in the formation of heterochromatin, the chromosomal domain that is highly condensed throughout the cell cycle and is kept transcriptionally inactive (Janssen et al. 2018). Human HP1α, one of the three subtypes, and Drosophila HP1α are shown to undergo LLPS in vitro (Larson et al. 2017; Strom et al. 2017). In early stages of Drosophila embryo, HP1a behaves like liquid-droplet during the first stage of heterochromatin formation (Strom et al. 2017). These observations led to the notion that the HP1α self-assembly contributed through the LLPS mechanism contributes the heterochromatin formation.

HP1α contains a chromodomain that binds to the tri-methylated histone H3 tail and a chromoshadow domain that dimerizes and provides an interface for binding partners; the rest of regions are structurally disordered (Fig. 2A) (Brasher et al. 2000; Lachner...
et al. 2003). The N-terminal disordered region is known to be constitutively phosphorylated throughout the cell cycle and the central disordered region, called hinge domain, is additionally phosphorylated during mitosis (Fig. 2A) (Kettenbach et al. 2011; Nishibuchi et al. 2014, 2019; Abe et al. 2016). The phosphorylation of the N-terminal region promotes the HP1α self-assembly via LLPS in vitro (Larson et al. 2017; Wang et al. 2019), indicating that negatively charged phosphate enhances multivalent electrostatic interactions among HP1α.

Therefore, we first examined whether phosphorylation statuses affect the efficiency of HP1α assembly in the cellular context. To do this, we prepared series of mEGFP fused HP1α mutants, including the interphase-like mutant carrying phospho-mimicking
glutamates (E) at N-terminal region (E-A mutant), the M phase-like mutant carrying glutamates additionally at the hinge domain (E-E mutant), and the non-phosphorylatable mutant having alanine substitutions at those phosphosites (A-A mutant) (Fig. 2A). When expressed in HEK293T cells, all three mutants of mEGFP-HP1α formed multiple round-shaped nuclear assemblies (Fig. 2B). Interestingly, we found that mEGFP-HP1α A-A, E-A, and E-E mutants increasingly, in that order, formed larger assemblies (Fig. 2C; the average area of 0.28, 0.80, and 1.99 μm², respectively). Consistent with the previous observation in vitro (Larson et al. 2017; Wang et al. 2019), these results suggest that the phosphorylation of the N-terminus promotes the HP1α assembly in the nucleus. Moreover, the mitotic phosphorylation of the hinge region further promotes the formation of nuclear assemblies.

Formation of HP1α nuclear assembly involves RNA

Because HP1α is known to have an affinity for RNA (Muchardt et al. 2002; Maison et al. 2011; Roach et al. 2020), it was possible that the assemblies of HP1α mutants involve nuclear RNA. Supporting this possibility, the addition of DNA has shown to promote the LLPS-driven HP1α droplet formation in vitro (Larson et al. 2017; Wang et al. 2019; Erdel et al. 2020). As HP1α is highly enriched with positively charged amino acids such as arginines and lysines, we could assume that negatively charged nucleic acids assist multivalent electrostatic interactions among HP1α molecules.

To test this idea, we inhibited transcription with α-amanitin and actinomycin D treatments. The live cell imaging demonstrated that the size of mEGFP-HP1α assembly gradually decreased and dispersed during the 5-hour treatment (Fig. 2C). The proportion of cells that have nuclear assemblies, in all three HP1α mutants, decreased significantly by the transcription inhibitor treatments (Fig. 2D). These results indicate that nuclear RNA facilitates the formation of HP1α assembly throughout the cell cycle.

DISCUSSION

Decreased levels of cellular RNA by transcription inhibition led to an emergence of nuclear assembly in one case and a decline in the other (Fig. 3). Implication from these observations is that nuclear RNA contributes to the formation of biomolecular condensates by regulating protein assembly or disassembly. While the nature of RNA requires further investigations, RNAs transcribed by pol II, namely messenger RNAs of coding sequence as well as a spectrum of non-coding RNAs (ncRNAs), are the candidates.

Diffusive nucleoplasmic distribution of FUS required physiological levels of pol II-transcribed
RNA (Fig. 1), FUS has multiple RGG RNA-binding motifs providing a preferential association with nuclear RNA. In the presence of sufficient density of nuclear RNA, the RNA binding affinity of FUS seems to be competitive, and antagonizing the self-assembly activity of FUS. In line with this notion, an excess amount of RNA inhibited the self-assembly of FUS in vitro (Maharana et al. 2018). This particular condition may recapitulate the RNA enriched nucleus.

The turnover of nuclear RNA is highly dynamic and the half-life of global nuclear RNA is estimated to be about 1 hour (Nozawa et al. 2017). Therefore, inhibiting the transcription for 5 hours must have had a significant impact on the amount and proportion of nuclear RNA, which provoked the formation of FUS assembly (Fig. 1). This outcome would be analogous to the FUS aggregation in the cytoplasm of ALS patient neurons, as the concentration of RNA in the cytoplasm is considerably lower than that in the nucleus. Additionally, liquid compartments of FUS convert to an aggregated state with time in vitro (Patel et al. 2015). Thus, cytoplasmic environment is presumed to induce the self-assembly of FUS that mis-localized in the cytoplasm and cause its aggregation in the long term.

Does nuclear RNA always have an inhibitory effect on the self-assembly of FUS? Unexpectedly, in vitro analysis showed that a small amount of specific RNA rather promotes the nucleation of FUS assembly (Maharana et al. 2018). This contradictory observation suggests that FUS can assemble with RNA in a specific situation in the nucleus. It is worth noting that FUS is known to assemble at high RNA concentrations, as RNA acts as a nucleation-competent RNA or RNA-like molecules, which leads to the FUS assembly to create a compartment. In our experimental settings, we were unable to recognize such FUS assembly, which could be attributed to the overexpression of FUS. Thus, nuclear RNA seems to be involved in both assembly and disassembly of FUS.

In contrast, the nuclear assemblies of HP1α were vanished with transcription inhibition, indicating that HP1α assembles in an RNA dependent manner (Fig. 2). We envisage two possible explanations for the different behavior of FUS and HP1α, namely, the affinity to RNA and the propensity to self-assemble. Binding to RNA seems to be mediated through different mechanisms: whereas FUS has multiple RNA-binding motifs, HP1α has no obvious RNA-binding motif (Muchardt et al. 2002; Maison et al. 2011; Roach et al. 2020). Although HP1α has been shown to associate with RNA, the affinity of HP1α to RNA is presumably lower than that of FUS. There seems to be a remarkable difference in the self-assembly propensities: FUS undergoes LLPS at around 10 μM, whereas HP1α does so at over 190 μM in vitro (Patel et al. 2015; Larson et al. 2017). This indicated that the self-assembly activity of HP1α is much lower than that of FUS. Based on these different properties, we predict that HP1α forms assembly in a limited compartment where HP1α is concentrated involving RNA. HP1α has been proposed to target pericentromeric heterochromatin through locally derived RNAs (Maison et al. 2011). It is therefore tempting to speculate that HP1α facilitates the formation of heterochromatin through its both activities, i.e., RNA binding and self-assembly.

The hinge region of HP1α is proposed to be its RNA binding region (Muchardt et al. 2002; Maison et al. 2011). Notably, the hinge region shows a preference for RNA rather than DNA and three lysine residues at 104–106 are essential for the binding (Muchardt et al. 2002). Because lysine residue is positively charged and RNA is negatively charged, electrostatic interactions between them are predicted to occur. The observation that the glutamic acid substitutions mimicking phosphorylation at the hinge region promoted the transcription-dependent HP1α assembly indicates that negatively charged glutamic acids contributed to, rather than counteracted to, the RNA binding. We speculate that the substitution leads to structurally reorientation of the hinge region such that lysine residues are exposed to RNA.

The phosphorylation of the HP1α’s hinge region occurs during mitosis (Kettenbach et al. 2011; Abe et al. 2016; Nishibuchi et al. 2019). Our data indicate that the mitotic configuration of HP1α, HP1α E-E mutant, has a higher self-assembly activity than the interphase version, HP1α E-A mutant. This may imply that the higher self-assembly activity of HP1α is achieved in mitosis. Of note, the chromosome passenger complex (CPC), containing Aurora B kinase, has been found to form an LLPS-driven condensate at the inner centromere to maintain for the faithful chromosome segregation (Trivedi et al. 2019). Because HP1α is also concentrated with the CPC in the inner centromere and is required for the full Aurora B activity (Abe et al. 2016), an appeal-
ing hypothesis would be that the self-assembly activity of HP1α contributes to the formation or regulation of the Aurora B condensate. Remarkably, HP1α is absent at the condensate widely in cancer cell lines with chromosomal instability (CIN) (Abe et al. 2016). Further studies to elucidate the role and the mechanism of HP1α assembly at the Aurora B condensate provide a clue to understanding the basis of CIN in cancers.

Not only the Aurora B condensate, but also other biomolecular condensates are altered in cancers (Nozawa et al. 2020). In particular, alterations in the composition, number, and size of nucleoli are one of the pathological indicators of malignant potential (Montanaro et al. 2008; Orsolic et al. 2016). Research has begun to investigate whether small-molecule compounds for cancer treatment are selectively enriched in nuclear assemblies and whether the activity of drugs is altered by their enrichment within them (Klein et al. 2020). The design of small-molecule compounds is also explored to control protein assemblies associated with ALS (Fang et al. 2019; Wheeler et al. 2019; Babinchak et al. 2020).

In conclusion, RNA in the nucleus seems to entail an essential role in regulating biomolecular condensates. We propose that the involvement of RNA can be readily examined by transcription inhibitor treatment experiments. Based on the findings on FUS and HP1α, identification of the nuclear RNA-mediated biomolecular condensates provides a clue to understanding and manipulating specific cellular compartments having physiological or pathological relevance.

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CONFLICTS OF INTEREST

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REFFERENCES

Abe Y, Sako K, Takagaki K, Hirayama Y, Uchida K S, et al. (2016) HP1-assisted Aurora B kinase activity prevents chromosome segregation errors. Dev Cell 36, 487–497.

Aguzzi A and Altmeyer M (2016) Phase separation: Linking cellular compartmentalization to disease. Trends Cell Biol 26, 547–558.

Alberti S and Dormann D (2019) Liquid-liquid phase separation in disease. Annu Rev Genet 53, 171–194.

Altmeyer M, Neelsen K J, Teloni F, Pozdnyakova I, Pellegrino S, et al. (2015) Liquid demixing of intrinsically disordered proteins is seeded by poly(ADP-ribose). Nat Commun 6, 8088.

Babinchak WM, Dumim BK, Venus S, Boyko S, Putnam AA, et al. (2020) Small molecules as potent biphasic modulators of protein liquid-liquid phase separation. Nat Commun 11, 5574.

Banani SF, Lee HO, Hyman AA and Rosen MK (2017) Biomolecular condensates: organizers of cellular biochemistry. Nat Rev Mol Cell Biol 18, 285–298.

Brasher SV, Smith BO, Fogh RH, Nietlispach D, Thiru A, et al. (2000) The structure of mouse HP1 suggests a unique mode of single peptide recognition by the shadow chromo domain dimer. EMBO J 19, 1587–1597.

Erdel F, Rademacher A, Vlijn R, Tünnemann J, Frank L, et al. (2020) Mouse heterochromatin adopts digital compaction states without showing hallmarks of HP1-driven liquid-liquid phase separation. Mol Cell 78, 236–249.

Fang MY, Markmiller S, Vu AQ, Javaherian A, Dowdle WE, et al. (2019) Small-molecule modulation of TDP-43 recruitment to stress granules prevents persistent TDP-43 accumulation in ALS/FTD. Neuron 103, 802–819.

Janssen A, Colmenares SU and Karpen GH (2018) Heterochromatin: Guardian of the genome. Annu Rev Cell Dev Biol 34, 265–288.

Kettenbach NA, Schwepe KD, Faherty KB, Pechenick D, Pletnek AA, et al. (2011) Quantitative phosphoproteomics identifies substrates and functional modules of Aurora and polo-like kinase activities in mitotic cells. Sci Signal 4, rs5.

Klein IA, Boija A, Afeyan LK, Hawken SW, Fan M, et al. (2020) Partitioning of cancer therapeutics in nuclear condensates. Science 368, 1386–1392.

Kwiatkowski TJ, Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, et al. (2009) Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 323, 1205–1208.

Lachner M, OCarroll D, Rea S, Mechtler K and Jenuwein T (2001) Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 410, 116–120.

Larson AG, Elnatan D, Keenen MM, Trinka MJ, Johnston JB, et al. (2017) Liquid droplet formation by HP1α suggests a role for phase separation in heterochromatin. Nature 547, 236–240.

Maharana S, Wang J, Papadopoulos DK, Richter D, Pozniakovsky A, et al. (2018) RNA buffers the phase separation behavior of prion-like RNA binding proteins. Science 360, 918–921.

Maison C, Bailly D, Roche D, Montes de Oca R, Probst AV, et al. (2011) SUMOylation promotes de novo targeting of
HP1α to pericentric heterochromatin. *Nat Genet* **43**, 220–227.

Montanaro L, Treré D and Derenzini M (2008) Nucleolus, ribosomes, and cancer. *Am J Pathol* **173**, 301–310.

Muchardt C, Guilleme M, Seeler JS, Trouche D, Dejean A, et al. (2002) Coordinated methyl and RNA binding is required for heterochromatin localization of mammalian HP1alpha. *EMBO Rep* **3**, 975–981.

Nishibuchi G, Machida S, Osakabe A, Murakoshi H, Hiragami-Hamada K, et al. (2014) N-terminal phosphorylation of HP1α increases its nucleosome-binding specificity. *Nucleic Acids Res* **42**, 975–981.

Nishibuchi G, Machida S, Nakagawa R, Yoshimura Y, Hiragami-Hamada K, et al. (2019) Mitotic phosphorylation of HP1α regulates its cell cycle-dependent chromatin binding. *J Biol Chem* **165**, 433–446.

Nozawa RS, Boteva L, Soares CD, Naughton C, Dun RA et al. (2017). SAF-A regulates interphase chromosome structure through oligomerization with chromatin-associated RNAs. *Cell* **169**, 1214–1227.

Nozawa RS and Gilbert N (2019) RNA: Nuclear glue for folding the genome. *Trends Cell Biol* **29**, 201–211.

Strom AR, Emelyanov AV, Mir M, Fyodorov DV, Darzacq X, et al. (2017) Phase separation drives heterochromatin domain formation. *Nature* **547**, 241–245.

Shin Y and Brangwynne CP (2017) Liquid phase condensation in cell physiology and disease. *Science* **357**, eaaf4382.

Singatulina AS, Hamon L, Sukhanova MV, Desforges B, Joshi V, et al. (2019) PARP-1 activation directs FUS to DNA damage sites to form PARG-reversible compartments enriched in damaged DNA. *Cell Rep* **27**, 1809–1821.

Wang J, Choi JM, Holehouse AS, Lee HO, Zhang X, et al. (2018) A molecular grammar governing the driving forces for phase separation of prion-like RNA binding proteins. *Cell* **174**, 688–699.

Wang L, Gao Y, Zheng X, Liu C, Dong S, et al. (2019) Histone modifications regulate chromatin compartmentalization by contributing to a phase separation mechanism. *Mol Cell* **76**, 646–659.

Wei MT, Chang YC, Shimobayashi SF, Shin Y, Strom AR, et al. (2020) Nucleated transcriptional condensates amplify gene expression. *Nat Cell Biol* **22**, 1187–1196.

Wheeler RJ, Lee HO, Poser I, Pal A, Doeleman T, et al. (2019) Small molecules for modulating protein driven liquid-liquid phase separation in treating neurodegenerative disease. *bioRxiv* doi: 10.1101/721001.

Zhang H, Elbaum-Garfinkle S, Langdon EM, Taylor N, Occhipinti P, et al. (2015) RNA controls polyQ protein phase transitions. *Mol Cell* **60**, 220–230.