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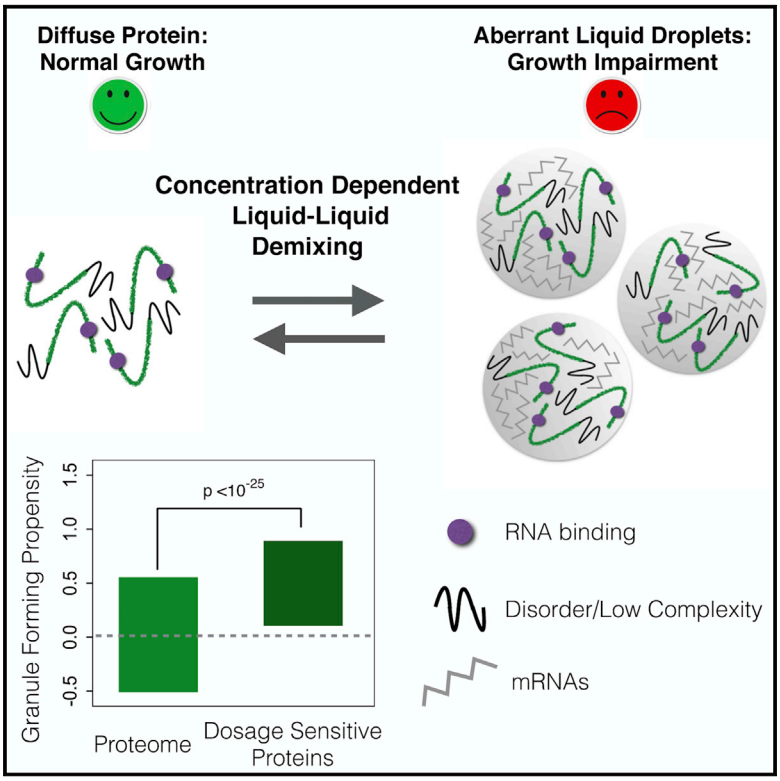
A Concentration-Dependent Liquid Phase Separation Can Cause Toxicity upon Increased Protein Expression

Graphical Abstract



Highlights

- Dosage-sensitive proteins in yeast have a high propensity for liquid-liquid demixing
- Increased protein concentration can force a liquid phase separation, titrating proteins and RNAs from the cytoplasm
- Preventing liquid-liquid demixing averts dosage sensitivity
- Inappropriate liquid phase separation may be a determinant of human genetic disease

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In Brief

Bolognesi et al. report that proteins that are harmful when overexpressed have properties associated with liquid-liquid demixing and that increased protein concentration can force a liquid phase transition causing cellular toxicity.

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A Concentration-Dependent Liquid Phase Separation Can Cause Toxicity upon Increased Protein Expression

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SUMMARY

Multiple human diseases are associated with a liquid-to-solid phase transition resulting in the formation of amyloid fibers or protein aggregates. Here, we present an alternative mechanism for cellular toxicity based on a concentration-dependent liquid-liquid demixing. Analyzing proteins that are toxic when their concentration is increased in yeast reveals that they share physicochemical properties with proteins that participate in physiological liquid-liquid demixing in the cell. Increasing the concentration of one of these proteins indeed results in the formation of cytoplasmic foci with liquid properties. Demixing occurs at the onset of toxicity and titrates proteins and mRNAs from the cytoplasm. Focus formation is reversible, and resumption of growth occurs as the foci dissolve as protein concentration falls. Preventing demixing abolishes the dosage sensitivity of the protein. We propose that triggering inappropriate liquid phase separation may be an important cause of dosage sensitivity and a determinant of human disease.

INTRODUCTION

A subset of the proteins encoded in any genome is toxic when their expression level is increased (Gelperin et al., 2005; Sopko et al., 2006). Even small increases in dosage can be detrimental (Tomala et al., 2014) with dosage sensitivity causing a large number of human diseases, ranging from developmental defects to psychiatric disorders (Girirajan et al., 2011; Veitia and Birchler, 2010).

Whether an individual protein is toxic or not when overexpressed may depend both on its specific functions and on its intrinsic physicochemical properties. For example, imbalance in regulatory networks or in the assembly of protein complexes (Papp et al., 2003; Veitia, 2003), aggregation (Geiler-Samerotte et al., 2011; Tartaglia et al., 2007), and mass-action driven promiscuous molecular interactions (Vavouri et al., 2009) have all been suggested to cause dosage sensitivity. However, the precise molecular mechanism by which each individual protein becomes harmful when overexpressed is normally unknown.

The cytoplasm and nuclei of cells are crowded environments containing very high concentrations of macromolecules. One principle that is becoming increasingly appreciated as a means for how cells organize and compartmentalize their contents is liquid-liquid phase separation (Brangwynne et al., 2009; Hyman et al., 2014; Jain et al., 2016). Liquid demixing creates non-membrane bound organelles that rapidly exchange molecules with the surrounding cytoplasm and increases the concentration of particular macromolecules within the separated phase. Examples include germ granules (Brangwynne et al., 2009), the nucleolus (Brangwynne et al., 2011; Weber and Brangwynne, 2015), and other ribonucleoprotein (RNP) assemblies (Lin et al., 2015; Mitchell et al., 2013). Although the precise molecular details about how liquid-liquid demixing is initiated are still unknown, the process is tightly controlled (Wippich et al., 2013).

Several proteins involved in physiological liquid-liquid demixing are prone to form protein aggregates or amyloids when they carry disease-causing mutations (Hyman et al., 2014; Kim et al., 2013; Patel et al., 2015) and indeed these mutations can promote a transition from a liquid droplet to a solid phase in vitro (Patel et al., 2015). This has led to the proposal that a liquid-to-solid phase transition is a mechanism of cellular toxicity (Patel et al., 2015).

Here, based on a proteome-wide analysis in yeast, we report that dosage-sensitive proteins share characteristics with proteins known to undergo physiological liquid-liquid demixing. Overexpressing one of these proteins revealed that it is indeed the induction of a liquid-liquid phase separation that correlates with toxicity. The condensation of this concentration-dependent liquid phase requires the RNA-binding domains of the protein and decondensation occurs as protein concentrations drop, reversing the growth impairment. Genetically preventing the