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Cancer Mutations in SPOP Put a Stop to Its Inter-compartmental Hops

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In this issue of *Molecular Cell*, Bouchard et al. (2018) identify liquid-liquid phase separation as a mechanism for substrate-triggered localization of SPOP and ubiquitination machinery to different nuclear bodies and describe how cancer mutations disrupt this process.

In addition to well-characterized membrane-bound organelles, a diverse population of membraneless organelles also exist. The latter category encompasses cytoplasmic and nuclear bodies, which have been proposed to form via liquid-liquid phase separation (LLPS) of macromolecules. Although the functions and compositions of these biomolecular condensates are not fully known, it is well documented that disease mutations in their phase separating components promote their dysfunction by disrupting disassembly and/or driving liquid-to-solid phase transitions (Boeynaems et al., 2018). Interestingly, many of these systems are associated with neurological disorders such as amyotrophic lateral sclerosis (ALS) and Alzheimer's and Parkinson's diseases. Here, Mittag and coworkers present a new mechanism through which disruption of LLPS could lead to cancer (Bouchard et al., 2018). The authors show that SPOP (speckle-type POZ protein), a substrate adaptor protein for cullin3-RING ubiquitin E3 ligase (CRL3), functions by phase separating with ubiquitination substrates to form distinct membraneless organelles. Cancer mutations in SPOP, which are found in up to 11% of prostate cancers (Barbieri et al., 2012), negatively regulate the LLPS process between SPOP and substrates and prevent ubiquitination of substrates, leading to upregulated substrate levels and disrupted proteostasis.

Contrary to its namesake, SPOP is found in various nuclear bodies including speckles, PML bodies, and DNA-damage loci (Marzahn et al., 2016; Nagai et al., 1997). Although underlying mechanisms of SPOP's localization to these different bodies were not known, the presence of substrates could be a contributing factor, as demonstrated by the formation of distinct membraneless bodies containing SPOP and one of its substrates, Gli3 (Marzahn et al., 2016). Since the cellular signals for the relocalization of SPOP to different bodies are largely unknown, Bouchard et al. (2018) cleverly used transient overexpression of SPOP and substrates to trigger such an event. The authors showed that when transiently co-expressed in HeLa or prostate cancer cell lines, both SPOP and substrate moved away from their respective compartments to newly formed SPOP:substrate compartments. Thorough characterizations of wild-type and mutant SPOP and substrates revealed that SPOP oligomerization and SPOP:substrate interactions together promote the LLPS process that drives the assembly of SPOP-containing nuclear bodies (Figure 1).

Oligomerization is closely associated with the ability to form the multivalent interactions that drive biomolecular condensation into liquid-like compartments (Dao et al., 2018; Mitrea et al., 2018). While SPOP's oligomerization is required for its localization into membraneless organelles, SPOP does not independently undergo LLPS (Marzahn et al., 2016). Rather, Bouchard et al. (2018) show that LLPS occurs when SPOP interacts with substrates such as death-domain-associated protein (DAXX) and androgen receptor (AR). Their data suggest that SPOP's oligomerization primes SPOP to phase separate with substrate targets. Convincingly, they show that oligomerization-incompetent SPOP mutants fail to phase separate with substrate *in vitro* and fail to colocalize with substrate in cells (Figure 1).

Weak, multivalent interactions between biomolecules are necessary for phase separation under physiologically relevant conditions (Mittag and Parker, 2018). Therefore, Bouchard et al. (2018) hypothesized that SPOP:substrate multivalent interactions promoted LLPS and, correspondingly, the formation of distinct SPOP:substrate membraneless nuclear compartments. The authors demonstrated that DAXX and many other SPOP substrates contain multiple SPOP-binding (SB) motifs comprising short Ser/Thr polar sequences. Through meticulous biophysical characterization, they determined that the intermolecular interactions between SB-motif peptides and the MATH domain of SPOP were weak, on the order of 40–500 μM. Together, these collective weak interactions are likely the "stickers" that promote SPOP:substrate LLPS. For confirmation, the authors scrambled the sequence of SB-motifs and found that both SPOP:substrate binding and LLPS propensity were significantly reduced. Furthermore, transient expression studies of binding-incompetent SPOP or substrate mutants failed to produce SPOP:substrate nuclear bodies. Interestingly, overexpression of disease-linked mutant SPOP and substrate partially rescues the formation of SPOP:substrate compartments. These findings are consistent with a model whereby increasing protein concentrations recover the transient interactions necessary to promote SPOP:substrate LLPS. In other words, SPOP cancer mutations not only reduce SPOP:substrate binding, but also increase the

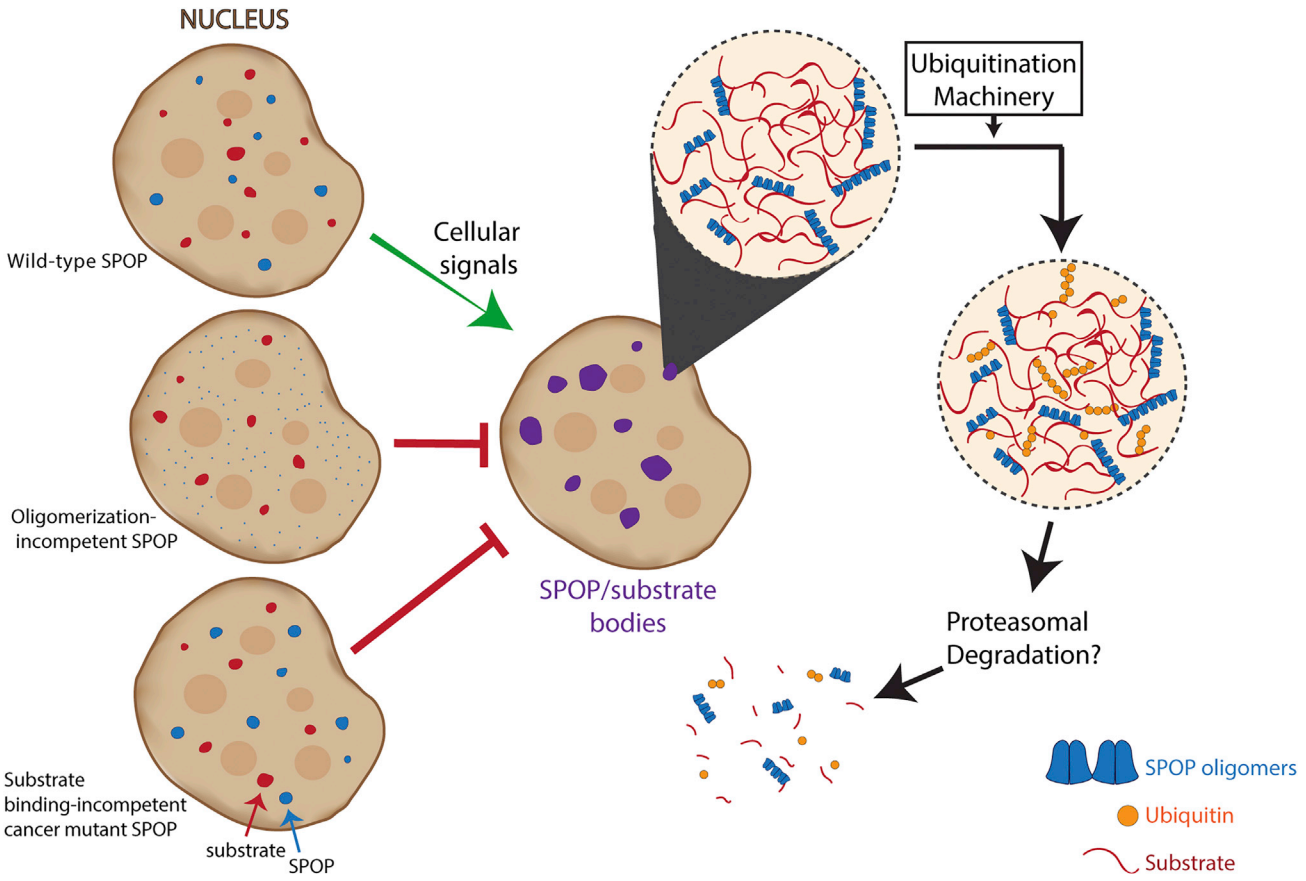

Figure 1. Mutations Disrupt LLPS-Mediated Colocalization of SPOP and Substrate to Ubiquitination Compartments
In nuclei, wild-type SPOP (part of the cullin3-RING ubiquitin ligase) colocalizes with substrates to form SPOP/substrate membraneless nuclear bodies where substrate ubiquitination likely occurs. However, mutations that hinder the ability of SPOP to self-associate (middle) or interact with substrates (bottom) prevent the formation of SPOP/substrate membraneless compartments, and inhibit substrate ubiquitination and downstream processing. SPOP cancer mutations (bottom) disrupt multivalent interactions with substrate that are necessary to promote LLPS-mediated colocalization of SPOP and substrate.

protein concentration threshold required for SPOP:substrate LLPS.

As SPOP is a substrate adaptor protein for the Cullin3 ubiquitin (Ub) ligase, the authors tested whether SPOP:substrate liquid bodies are active ubiquitination centers. They elegantly constructed an *in vitro* ubiquitination phase-separating system containing Ub-charged E2, neddylated cullin E3 ligase, SPOP, and DAXX substrate. Using fluorescently labeled Ub, they demonstrated that Ub colocalized inside SPOP membraneless bodies only in the presence of functional ubiquitinating enzymes, and that DAXX was ubiquitinated. Ubiquitination occurred in either filamentous or liquid assemblies of SPOP/DAXX. Surprisingly, ubiquitination kinetics appeared to be the same in the presence or absence of mesoscale assemblies *in vitro*. However, it is important to note that the dynamics in SPOP/DAXX bodies are significantly reduced compared to the dilute phase, as shown by fluorescence recovery after photobleaching (FRAP) experiments. This decrease in dynamics in mesoscale assemblies might offset the increase in local protein concentration, accounting for lack of improvement in enzymatic activity.

Bouchard et al. (2018) provide convincing evidence that phase-separated droplets are not just sequestration centers, but active compartments. Their research provokes a myriad of questions. Can different SPOP:substrate membraneless compartments exist concurrently in cell nuclei? What are the cellular signals (including post-translational modifications) that promote endogenous SPOP recruitment to these different substrate bodies? Once substrates are ubiquitinated in SPOP phase-separated bodies, how are they degraded? Is proteasome machinery recruited into these SPOP bodies, as recently demonstrated in stress granules (Turakhiya et al., 2018)? Are ubiquitinated substrates able to leave on their own, or are they directed out of membraneless bodies to proteasome or degradation machinery elsewhere?

This work is both highly relevant to the pathology of SPOP-related diseases and far-reaching in implications. Currently, the membraneless organelles predominantly associated with protein quality control are stress granules. The recognition that ubiquitination occurs inside SPOP mesoscale assemblies, the formation of which might be vital to the correct localization of some E3 ubiquitin ligases to their substrates, is an intriguing twist in the current narrative. SPOP:substrate

LLPS-directed ubiquitination is an interesting new example of protein quality control of phase-separated proteins. This work provides a possible mechanism for the recruitment of ubiquitination machinery to other membraneless compartments, including stress granules, in which ubiquitin-like post-translational modifications (e.g., SUMOylation and NEDDylation) have been shown to occur. Interestingly, disruption to phase separating components of stress granules could also be disease-causing, possibly leading to protein-containing ubiquitinated inclusions characteristic of neurological disorders such as ALS.

Recently, Schuster et al. (2018) show that LLPS systems can be robustly engineered to recruit and release cargo. Membraneless organelles can be programmed to compartmentalize proteins, much in the same way SPOP is compartmentalized to substrate-dependent membraneless bodies. These works provide a potential foundation for developing therapeutic avenues for SPOP-mediated cancers once we understand the molecular determinants and cellular signals for localization to different membraneless organelles.

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Entering the Pocket: Crystal Structure of a Prostaglandin D2 Receptor

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In this issue of *Molecular Cell*, crystal structures of a prostaglandin D2 receptor determined by Wang et al. (2018) reveal novel insights into differential ligand recognition among the members of lipid-binding GPCRs, and provide a structural framework for the identification of novel therapeutics in inflammatory disorders.

G protein-coupled receptors (GPCRs) bind an incredibly diverse spectrum of ligands including small molecules, peptides, hormones, proteins, and lipids. There is a large number of GPCRs that specifically recognize various types of lipids such as phospholipids, lysophospholipids, fatty acids, and eicosanoids as their cognate ligands, and initiate a wide range of downstream signaling responses (van Jaarsveld et al., 2016). Considering the widespread role of lipids in different aspects of cellular signaling and physiology, structural understanding of lipid-binding GPCRs has emerged as a major focus area in recent years. Indeed, in the past few years alone crystal structures of sphingosine-1-phosphate receptor (S1P1R), lysophosphatidic acid receptor (LPA1R), free fatty acid receptor (FFAR1), leukotriene B4 receptor (BLT1R), and cannabinoid receptor (CB1R) have all been determined. In this issue, Wang et al. now present the crystal structures of a prostaglandin D2 receptor, referred to as CRTH2 (chemoattractant receptor-homologous molecule expressed on