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How Arsenic, an Inorganic Pollutant, is Involved in the Physiology of Biomolecular Condensates in the Cell

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The existence of membrane-less organelles in the cells has been known for a relatively long time. Of the membrane-less organelles, stress granules, processing bodies, and PML-NBs have been intensively investigated in relation to arsenic. The membrane-less organelles, which concentrate biomolecules (proteins, nucleic acids), have recently been shown to self-organize by means of phase separation/transition. These biomolecular condensates (membrane-less organelles) can provide local enhancement of the efficiency of specific reactions. The biomolecular condensates have attracted dramatic attention over the last decade because highly organized biochemical complexes in the cell have long been understood by the membrane-dependent compartmentalization. In this mini review, we highlight the initiation of phase separation for each biomolecular condensate in which arsenic could be involved. We further reflect on the adequacy of the arsenic-dependent ROS levels for the formation of biomolecular condensates. These perspectives led us to re-evaluate the biological action of arsenic from a biophysical and bio-rheological point of view.

Keywords: phase separation, biomolecular condensates, stress granules, PML-NBs, arsenic

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INTRODUCTION

Liquid-liquid phase separation (LLPS) is a physiologic cellular process during which some biomolecules such as proteins and RNA are highly concentrated in liquid droplet-like structures that are not surrounded by a lipid bilayer membrane. These droplet-like cellular structures such as bodies, granules, and speckles are known as biomolecular condensates. They are formed either in the cytoplasm or nucleus in response to changes in the intracellular environment (Banani et al., 2017) (Riback et al., 2020). The membrane-less property at the interface of each biomolecular condensate facilitates flow of water and solutes. Therefore, biomolecular condensates can be distinguished from aggregates with no fluidity. The aggregates can be formed irreversibly when the cell experiences the prolonged supersaturation of biomolecules (Shin et al., 2017). The features of interactions between proteins and biopolymers (e.g., stalled mRNA in stress granule formation) required to constitute biomolecular condensates are weak, multivalent, and dynamic. The protein domains that promote the formation of biomolecular condensates involve intrinsically disordered regions (IDRs), repeated modular domains, oligomerization domains, and/or substrate-binding (e.g., RNA-binding) domains (Hofmann et al., 2020) (Sabari et al., 2020). IDRs are regions that are often computationally predicted not to form fixed 3-D structures but rather are conformationally heterogeneous, and thus can engage in dynamic flexible multivalent interactions with partner molecules (Mittag and Parker, 2018). The valence as well as patterning of a collection of amino acid side chain interactions such as π-π or cation-π within

TABLE 1 | Examples of biomolecular condensates in the cell.

Name	Location	Representative components	Arsenic involved	Other notes	Representative references
Nucleolus	Nucleus	Ribosomal proteins, rRNA, snoRNA, Npm1, fibrillarin	not found at present study	disrupted upon transcriptional inhibition	Andersen et al. (2005); Feric et al. (2016)
Cajal body		Coilin, Gemin2, scaRNA, Smn		disrupted upon transcriptional inhibition	Gall, (2000); Enwerem et al. (2014)
Nuclear speckle		Neat2 or Malat1 (Poly-A(+) RNA), SC-35		increase in size upon transcriptional inhibition	Spector and Lamond, (2011); Nakagawa et al. (2012)
Paraspeckle		Neat1or MENε/β (Poly-A(+) RNA).Sfpq, p54nrb, FUS, PSP1		disrupted upon transcriptional (RNA polymerase II) inhibition arginine uptake by mCAT2 translation: in response to stress (IFN and LPS)	Prasanth et al. (2005); Sasaki et al. (2009); Fox and Lamond, (2010); Nakagawa et al. (2011); West et al. (2016)
PML-NB		PML, SUMO, DAXX, p53	**arsenite**: directly oligomerize peptides via -S-As-S- crosslinking oxidative stress (including **arsenite**): oligomerize peptides via -S-S- formation **arsenite**: directly alter conformation to fewer secondary structure **arsenite**, ? Sb3+ : directly alter conformation favorable (Ubc9 recruitment) to SUMO-SIM interactome formation	IFN: important for protein level threshold for LLPS disrupted upon virus infection disrupted upon APL pathology	Chelbi-Alix et al. (1998); Muller et al. (1998); Zhang et al. (2010); Jeanne et al. (2010)
P-body	Cytoplasm	untranslated mRNA, Dcp1a, Edc3, Rck/p54	yeast: oxidative stress (**arsenite**): details unknown mammal: oxidative stress (**arsenite**) triggers enlargement: details unknown	induced by osmotic stress	Yang et al. (2004); Souquere et al. (2009); van Leeuwen and Rabouille (2019); Riggs et al., (2020)
Stress granule		untranslated mRNA, G3BP1, UBAP2L, TIA1	oxidative stress (**arsenite**, selenite, H_2O_2, etc.): influences the translation sensor far upstream of LLPS	cycloheximide (locking mRNA on ribosome thus inhibited) puromycin (striping mRNA from ribosome thus promoted) induced by ER stress (thapsigargin, heat shock) induced by osmotic stress or UV	McEwen et al. (2005); Fujimura et al. (2012); Kedersha et al. (2016)

Abbreviations: P-body; Processing body, IFN; interferon, LPS; lipopolysaccharide, ER; endoplasmic reticulum, mCAT2; mouse cationic amino acid transporter 2, Sb; Antimony, APL; acute promyelocytic leukemia, SUMO; small ubiquitin-like modifier, SIM; SUMO interaction motif

IDRs contributes to LLPS in cells (Brangwynne et al., 2015) (Sabari et al., 2020). Mixing of proteins that each contain a different type of repeated modular domains results in liquid droplet formation (Li et al., 2012). Li *et al.* used two proteins that contain tandemly arranged Src homology 3 domain and a proline-rich motif, respectively. The finding extended the idea that increased valence promotes LLPS.

The necessity of biomolecular condensates in cells is enigmatic if one simply considers the studies of mutant mice. For example, coilin (Cajal body resident) knockout mice are semi-lethal and shows reduced fecundity (Walker et al., 2009); plants with a mutation in coilin gene homozygously or flies of null mutants for coilin are fully viable (Nizami et al., 2010). Another example is Neat1 non-coding RNA (Paraspeckle resident); *Neat1*$^{-/-}$ mice are normal (Nakagawa et al., 2011). Also, PML (promyelocytic leukemia-nuclear bodies (PML-NBs) resident) knockout mice develop normally (Niwa-Kawakita et al., 2017). However, biomolecular condensates may benefit various cellular processes by locally enhancing the reaction kinetics and specificities or by limiting reactions through temporal sequestration of biomolecules (Banani et al., 2017).

Explicit evidence indicates that arsenic is both a multi-organ carcinogen (IPCS, 2001) (Salnikow and Zhitkovich, 2008) (IARC Monogr Eval Carcinog Risks Hum, 2012) and a chemotherapeutic agent for certain cancers, such as acute promyelocytic leukemia (APL) (de The et al., 1991) (Kakizuka et al., 1991). This led to the notion that binding to cysteine residues is the initial step in a variety of arsenic-induced toxicological and pharmacological processes, because arsenite (As^{3+}) has a high affinity for thiol groups (Spuches et al., 2005) (Mizumura et al., 2010). Studies of arsenic trioxide (As_2O_3) as a chemotherapeutic agent for the treatment of APL revealed that arsenite can particularly react with promyelocytic leukemia (PML) protein, a scaffold protein of PML-NBs. Now, PML-NBs are recognized to be one of the biomolecular condensates formed by LLPS. Since arsenic has not been well documented as a player for the LLPS, we discuss the involvement of arsenic in the formation of biomolecular condensates, considering stress granules and PML-NBs as examples.

In this manuscript, we use the word "Nucleation" to describe the initiation step of LLPS.

STRESS GRANULES

Physiology of Stress-Induced Biomolecular Condensates

Stress granules are one of the most widely investigated biomolecular condensates in the cytoplasm (**Table 1**). Stress granule formation is considered to be an adaptive response to various acute stresses, acting to "triage" nascent mRNA from degradation and subsequent fitness upon stress relief (van Leeuwen and Rabouille, 2019). Actually, translational stalling triggered by stresses fine-tunes the cellular proteome by sorting housekeeping mRNA into stress granules, while excluding mRNA transcribed from stress-responsive genes (e.g., *Hsp70*, a molecular chaperone) from stress granules (Kedersha and Anderson, 2002). An example of an unfavorable cellular condition is viral infection. Stalling of viral mRNAs upon infection followed by stress granule formation plays a role in the cellular defense strategy against viral replication. However, if stress granules chronically persist in non-dividing cells (e.g., neurons) in particular, stress granules often contribute to the pathology accompanying protein aggregates (Riggs et al., 2020).

Nucleation of Stress Granules

Non-translating mRNA and abortive translation initiation complexes, which are released upon stress-triggered translational stalling, are both bound by RNA-binding proteins. Ras GTPase-activating protein-binding protein 1 (G3BP1) is an example of such an RNA-binding protein and is also called as stress granule nucleator (SG nucleator), because *bona fide* stress granules can be formed by G3BP1 overexpression even in the absence of stresses (Tourriere et al., 2003). G3BP1 centrally mediates the condensation of stalled mRNA and translation initiation complexes by locally enhancing their concentrations at foci (Kedersha et al., 2016) (Hofmann et al., 2020). G3BP1 not only has an RNA-binding domain, but also has IDRs and an oligomerization domain. Although IDRs are considered universal drivers for LLPS, IDRs in G3BP1 are dispensable for stress granule formation. Rather, an oligomerization domain at the N-terminus of G3BP1 highly contributes to interactive networks that increase the valence of G3BP1-RNA and drive LLPS (Sanders et al., 2020).

Arsenite is not Directly Involved in Nucleation, but Influences the Translation Sensor far Upstream of Nucleation

Two complementary regulatory mechanisms are largely responsible for stress-induced stalling of translational initiation: One is the preparation of ribosome-tRNA, and the other is the preparation of mRNA. Regarding the first mechanism, eukaryotic initiation factor 2 subunit alpha (eIF2α) is essential for 43S preinitiation complex (40S ribosomal subunit-eIF2α-GTP-tRNA^{Met}) assembly. Because the phosphorylated form of eIF2α (eIF2α-*P*) firmly binds its guanine nucleotide exchange factor, eIF2B, which can exchange GDP only when eIF2α is not phosphorylated, phosphorylation of eIF2α inhibits reformation of eIF2α-GTP-tRNA^{Met} (Hinnebusch, 2014). Arsenite-induced (200 μM, 1 h, HAP1 cells, human myeloid line) stress granule formation is mediated by heme-regulated inhibitor kinase (HRI) (Aulas et al., 2017), which is one of four kinases targeting eIF2α: HRI, the mammalian homologue of yeast general control nonderepressible 2 (Gcn2p), protein kinase R (PKR), and PKR-like endoplasmic reticulum kinase (PERK) (McEwen et al., 2005). Mouse reticulocytes treated with arsenite (200 μM, <1 h) show autophosphorylation (i.e., activation) of HRI, which can be inhibited by pretreatment with the reactive oxygen species (ROS) scavenger, *N*-acetyl-L-cysteine. However, adding arsenite to lysates *in vitro* does not activate HRI, suggesting that arsenite does not directly act on HRI but that ROS contribute (Lu et al., 2001). Consistent with this observation, arsenite has little effect on the activity of reconstituted HRI *in vitro* (Martinkova et al., 2007). Fetal liver cells obtained from *Hri*$^{-/-}$ mice fail to recover from increased ROS levels induced by arsenite treatment, suggesting that HRI is required to mitigate acute oxidative stress (Suragani et al., 2012).

PML-NBS

Physiology of Dynamically Modifiable Biomolecular Condensates

Typically, approximately 5–20 PML-NBs are present per cell, with the size ranging from 0.2 to 1 μm. Although PML protein is an eponymous component of PML-NBs, more than 100 proteins are included inside PML-NBs. PML-NBs are ubiquitously distributed in adult organisms (Bernardi and Pandolfi, 2003) (Udagawa et al., 2021) and are involved in apoptosis, cell proliferation, senescence, tumor suppression, antiviral resistance, etc. (Salomoni and Pandolfi, 2002) (Maroui et al., 2012). Seven isoforms of human PML protein are known, of which PMLI-PMLVI have a nuclear localization signal and form PML-NBs (Nisole et al., 2013). All PML protein isoforms are tripartite motif (TRIM) family members that have an RBCC motif: Really Interesting New Gene (RING), two b-Boxes, and a coiled-coil domain. Interferons (α, β, and γ) drastically induce PML expression, leading to an increased number of swollen PML-NBs (Chelbi-Alix et al., 1995). The discovery of interplay between PML-NBs and innate immunity pushed the field forward. Producing proteins that colocalize with PML and have an ability to disrupt PML-NBs is one of the counter-adaptations for many viruses to deal with the intrinsic immunity of the host (Scherer and Stamminger, 2016) (van Gent et al., 2018). One example can be seen from a crystallographic analysis of the immediate early protein IE1 of primate cytomegalovirus. The analysis demonstrated the structural resemblance of core domain of IE1 to the coiled-coil domain of TRIM proteins. The similarity potentially endows IE1 to recognize PML/TRIM19 and subsequently, to antagonize the PML-mediated intrinsic

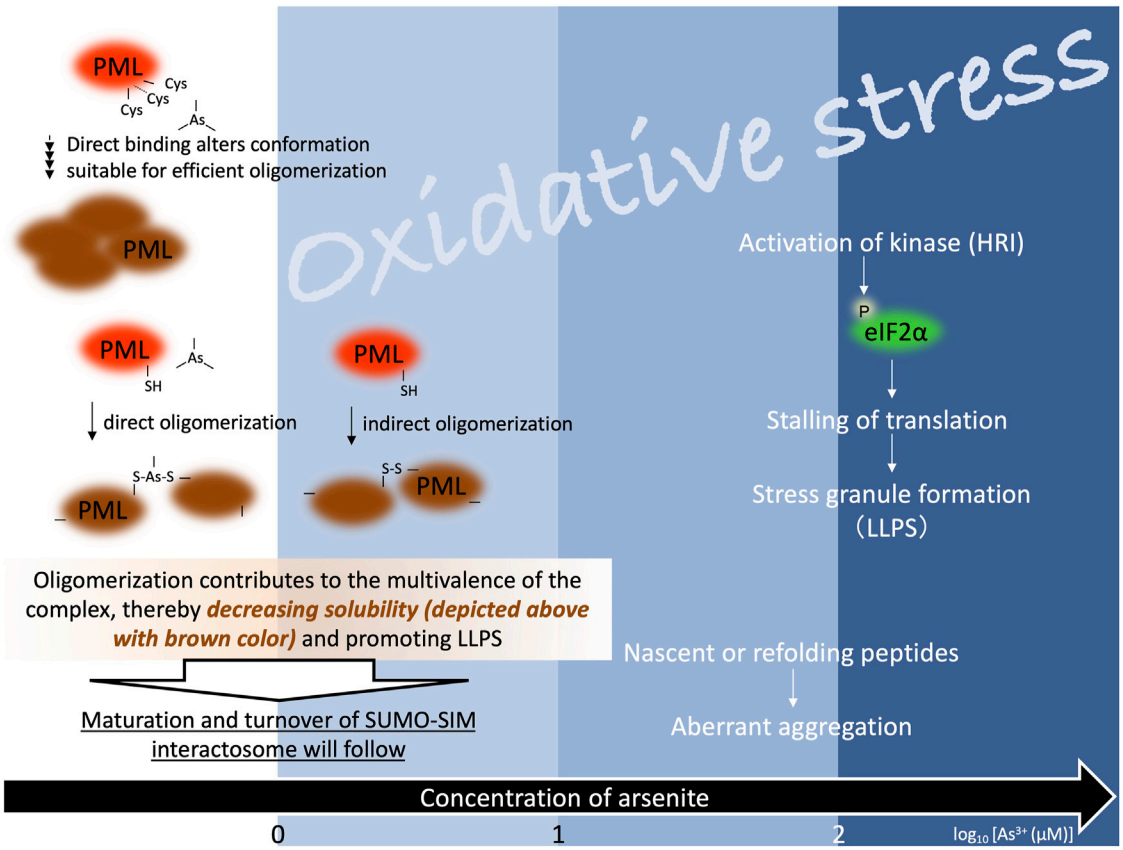

FIGURE 1 | Arsenite is involved in the formation of biomolecular condensates. The blue gradient colors represent different levels of oxidative stress at increasing concentration of arsenite. The numbers at the bottom indicate the logarithm of the arsenite concentration. Stress granule formation induced by higher concentration of arsenite is mediated by heme-regulated inhibitor kinase (HRI), which is one of four kinases targeting eIF2α (eIF2α-P: phosphorylated form of eukaryotic initiation factor 2 subunit alpha) (dark blue panel, the right side of the figure). Arsenite-dependent solubility changes of PML protein can occur below the level of oxidative stress induction (white panel, the left side of the figure). Tetramerization of PML protein can occur in the absence of arsenite. Although how intra-molecular arsenic binding facilitates inter-molecular interactions is unknown, arsenite promotes oligomerization of PML protein. Further, regarding the promotion of inter-molecular interactions, arsenite acts as an indirect (i.e., oxidation-dependent -S-S- formation via oxidative stress) as well as direct (i.e., arsenic crosslinks PML polypeptides) oligomerizer. Like PML protein, client proteins in PML-NBs often possess small ubiquitin-like modifier (SUMO) conjugation sites and SUMO interaction motif (SIM). Arsenite-dependent solubility change of PML protein is followed by SUMOylation. SUMO-SIM-rich environment of PML-NBs is likely to be advantageous for multiple biological functions. SUMOylated PML protein is further ubiquitinated by the SUMO-dependent ubiquitin E3-ligase and adequately degraded via the ubiquitin-proteasome system.

immunity (Scherer et al., 2014). It is important to note that the antiviral activity of PML protein depends on the coiled-coil domain (Chelbi-Alix et al., 1998).

In the pathology of APL, PML-retinoic acid receptor *a* fusion protein (PML-RARα, the t(15; 17) gene translocation product) homo-dimerization via the coiled-coil domain of PML moiety promotes transcriptional repression of differentiation genes (Occhionorelli et al., 2011). At the same time, hetero-dimerization of PML-RARα with wild-type PML protein impedes PML protein oligomerization and adequate nucleation (see below), resulting in a micro-speckled appearance. Importantly, some retinoids capable of differentiating hematopoietic progenitor cells but not of degrading PML-RARα fail to induce remission of APL (Ablain et al., 2013). Retinoic acid can degrade PML-RARα and partly liberate wild-type PML protein to reform PML-NBs. Synergistic efficacy of arsenite and retinoic acid not only depends on the ability of arsenic to degrade PML-RARα but also to actively reform PML-NBs. Both PML-RARα degradation and PML-NB reformation induce p53-dependent senescence of APL-initiating cells, thereby inhibiting their self-renewal (Ablain et al., 2014) (de The, 2018).

Nucleation of PML-NBs

LLPS is dictated by a threshold concentration of the protein (Lee et al., 2020). Interferon-dependent augmentation of the PML protein level in the nucleoplasm (Chelbi-Alix et al., 1995), therefore serves as an important factor for nucleation of PML-NBs.

Given that PML protein is a sensitive ROS sensor (Sahin et al., 2014) (Niwa-Kawakita et al., 2017) and that prolonged treatment with *N*-acetyl-L-cysteine can deplete PML-NBs (Jeanne et al., 2010), oxidation-dependent -S-S- formation can stochastically oligomerize PML protein, which drives the nucleation of PML-NBs.

A recent crystallographic study on the RING domain of PML protein revealed that three residues (F52Q53F54) in a loop as well

as L73 in the classic C3HC4-type RING finger motif constitute a PML/TRIM19-specific sequence among TRIM family proteins. The unique sequence is required for tetramerization of PML protein and for PML-NB nucleation (particularly L73) (Wang et al., 2018). A subsequent study showed the mode of RBCC oligomerization which is vital for PML-NB nucleation (Li et al., 2019). Li *et al.* reported the crystal structure of the B1 domain of PML protein, showing that two bulky residues (W157 and F158), which are also unique to PML/TRIM19 among TRIM family proteins, are positioned at the oligomerization interface.

Arsenite can Directly Promote Nucleation of PML-NBs

Regarding the direct effect of arsenite on PML-NBs, we should look at two pieces of evidence on the direct binding mode of arsenic to PML protein. First lines of evidence are from Zhang *et al.*, as follows. X-ray absorption spectroscopy data showed that arsenic coordinates with three sulfur atoms from conserved cysteines (C60/77/80 or C72/88/91). Circular dichroism analysis showed that the recombinant RING domain of PML protein titrated with arsenite showed fewer secondary structures than when stabilized by zinc. Two-dimensional nuclear magnetic resonance (NMR) spectra revealed that arsenite-bound PML protein tends to oligomerize, which is clearly different from that of well-folded zinc-bound PML protein. Further, a gel filtration assay demonstrated biochemically that arsenite-bound PML oligomers are primarily octamers that are largely sensitive to 6 M urea; however, some remained as dimers. Authors hypothesized that these urea-resistant dimers would be maintained via arsenic-mediated crosslinking even in the denatured condition (Zhang et al., 2010). Although how intra-molecular arsenic binding activates inter-molecular interactions is unknown, these observations suggest that arsenite acts as an indirect as well as direct oligomerizer.

The latter evidence of the binding mode of arsenic on PML protein revealed that besides the RING domain, the B2 domain appears to be required. Biochemical analysis showed that arsenic binds to the C212/C213 di-cysteines of the B2 domain. Data using the fluorescent diarsenical probe FIAsH, which cooperatively binds two CC motifs (the fluorescence is dependent on C212/C213), as well as stoichiometric analysis using atomic absorption suggested that arsenic crosslinks PML polypeptides. Also, the arsenic-binding defective C212A or C213A mutants exhibited strongly diffuse PML protein appearance in the nucleoplasm with only one or two abnormally enlarged PML structures (Jeanne et al., 2010), suggesting that arsenite acts as oligomerizer for the nucleation of PML-NBs.

How Arsenite-Binding Alter PML/TRIM19-Specific Region to Trigger LLPS?: A Potential Mechanism

C3HC4-type RING conformation of PML determines higher selectivity to arsenite (Zhou et al., 2011) (Zhou et al., 2014) (Kaiming et al., 2018). Given that an *in vitro* experiment demonstrates that the perturbation of secondary structure of protein upon arsenite binding leads to oligomerization (Varma et al., 2018), partial unfolding of the secondary structure of PML protein upon arsenite-binding could trigger oligomerization of PML protein and subsequent nucleation of PML-NBs. Although much remains to be determined, the terminal of the helix in which C77/80, corresponding to i/i+3 arrangements of vicinal cysteines (Cline et al., 2003), locates can be destabilized by arsenite. The binding of arsenic will form disordered new conformations of PML protein around the tetramerization interface.

Arsenite-Binding Subsequently Facilitates Maturation and Turnover of PML-NBs

PML protein has the longest residence time (several minutes to 1 h) compared to that of client proteins such as Sp100 and Daxx (several seconds to 1 min) (Brand et al., 2010), reflecting that PML protein is the only scaffold protein of PML-NBs. Most human isoforms of PML protein have a small ubiquitin-like modifier (SUMO) interaction motif (SIM) that non-covalently interacts with SUMO or SUMOylated proteins. SUMOylation is a post-translational modification process regulating multiple cellular events such as gene expression and is mediated by SUMO conjugating enzyme, Ubc9. Like PML protein, client proteins including Ubc9 often possess SUMO conjugation sites and SIMs (Sahin et al., 2014). Importantly, the PML-3KRΔSIM mutant, which is devoid of three lysine residues important for SUMOylation and SIM, can nucleate, suggesting that the nucleation of PML-NBs does not rely on SUMO-SIM (Lallemand-Breitenbach et al., 2001) (Sahin et al., 2014). However, given that poly-SUMO and poly-SIM synthetic peptides are used as a model of multivalent interactions between repeated modular domains (Banani et al., 2016), the SUMO-SIM-rich environment of PML-NBs is advantageous for maturation and integrity of this biomolecular condensate. The PML-3KR mutant indeed fails to recruit clients such as Sp100, SUMO1, and Daxx (Zhong et al., 2000). The recruitment of Ubc9 to PML-NBs was observed in $Pml^{-/-}$ MEF cells stably co-expressed with PML protein and Ubc9, which were exposed to 1 μM arsenite for 1 h (Wang et al., 2018). These results were consistent with the findings in *in vitro* assays in which arsenic binding altered the conformation of the RING domain of PML protein and enhanced RING-mediated interactions with Ubc9 (Zhang et al., 2010).

Although Ubc9 recruitment into PML-NBs favors hyper-SUMOylation of multiple clients (Sahin et al., 2014), and would thus be beneficial for PML-NBs as an interactosome (Li et al., 2019), SUMOylated PML protein is further ubiquitinated by the SUMO-dependent ubiquitin E3-ligase, RNF4, and degraded via the ubiquitin-proteasome system (Lallemand-Breitenbach and de The, 2010); this is common to an elimination mechanism for PML-RARα fusion protein in APL pathology. Because prolonged supersaturation of biomolecular condensates can be pathologic as described, the SUMO-dependent ubiquitin E3-ligase system may adequately prevent the aging of the gel-like state of PML-NBs.

DISCUSSION

Arsenite induces the formation of stress granules at a very high, some hundreds of μM, concentration. As in the case of stress granule induction, relatively lower concentration of arsenite produces ROS (Flora, 2011), and thus can at least in part indirectly contribute to the ROS-induced disulfides that crosslink PML protein (Jeanne et al., 2010). Some caution should be paid to this idea because theoretically upon exposure to excessive oxidants, critical protein thiols can be oxidized to transiently form protein sulfenic acid (PSOH) or protein sulfinic acid (PSO_2H), or be fully oxidized into protein sulfonic acid (PSO_3H) (i.e., inhibitory to oligomerization) (Jeanne et al., 2010). In this scenario, arsenite potentially serves as a reductant against protein sulfenic acid (PSOH), reverting the protein to the thiol form (Saurin et al., 2004).

Oligomerization contributes to the multivalence of the complex, thereby decreasing solubility and promoting LLPS (Banani et al., 2017). Immunoblot analysis showed that most PML protein is detected in the detergent-soluble fraction, whereas in cells exposed to 0.1–1 μM arsenite for 1–2 h, almost all PML protein is detected in the detergent-insoluble fraction, which corresponds to the fraction associated with the nuclear matrix (Lallemand-Breitenbach et al., 2001) (Hirano et al., 2013) (Hirano et al., 2015). Examination of CHO cells stably transfected with an antioxidant responsive element demonstrated that promotor activation requires 1 μM arsenite (with 6 h arsenite exposure), or requires at least 2 h (with 3 μM arsenite exposure). Both are above the required levels for insolubilization (i.e., 0.1–1 μM arsenite for 1–2 h), suggesting that arsenite-dependent solubility changes of PML protein can occur below the level of oxidative stress induction (Hirano et al., 2013). For the illustration of concentration range, the plasma arsenic level in APL patients treated: ca. 0.5 μM (Au et al., 2008); the provisional WHO guideline value of arsenic in drinking-water: ca. 0.13 μM (Ravenscroft et al., 2009).

It has been reported that many kinds of proteins before folding or during refolding are especially sensitive to higher concentration (i.e., comparable to that used in the induction of stress granule formation) of arsenite and heavy metals *in vitro* (Sharma et al., 2008) (Jacobson et al., 2012). If nascent protein in the cytosol is exposed to arsenite before being properly folded, it is expected that the protein has more chance to hyper-oligomerize resulting in aberrant aggregation because unfolded protein is conformationally mobile (Ramadan et al., 2009). Of note, the solubility changes of PML protein are not observed at all in HEK293 cells exposed to Cd^{2+} for 2 h even at an equivalent to the concentration of arsenite that efficiently promotes LLPS (Hirano et al., 2015). These suggest that arsenite-dependent solubility shift of PML protein is distinct from protein aggregation (**Figure 1**).

Finally, biomolecular condensates are related to many aspects of cellular physiology and communicate with neighboring membrane-bound organelles. Further investigations of spatio-temporal dynamics of biomolecular condensates should shed new light on biological activities of arsenic.

AUTHOR CONTRIBUTIONS

OU: Conceptualization, Writing. SH: Supervision, Writing.

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