Commentary

Melatonin and phase separation: potential interactions and significance

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ABSTRACT

This commentary explores the leading edge of current understanding of the potential interactions associated with melatonin and the regulation of membraneless organelles (MLOs) formed via liquid-liquid phase separation (LLPS) presented in recently published hypothetical reviews. As the scientific community increasingly recognizes the relevance of biomolecular condensates as fundamental organizers and propellers of cellular biochemistry, and that LLPS may be the quintessential process that provides insight into elusive physiological and pathological cellular conditions, the ancient role of melatonin in this new and exciting framework of cellular biology must be fully realized to its maximum potential.

Key words: liquid-liquid phase separation, biomolecular condensates, ATP, RNA, stress granules, cardiolipin, lipid raft, membrane fluidity, m^6A modification.

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1. INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is a ubiquitous, mitochondria-targeted molecule present in all tested archaea, eukarya, and bacteria (1, 2). Since its discovery in the bovine pineal gland in 1958, there are close to 30,000 scientific publications that represent extensive and intensive work on melatonin archived in the U.S. National Institutes of Health (NIH) National Library of Medicine. The study of melatonin reveals an impressive and seemingly inexhaustible array of functions and features, leading to its description in literature as a hormone, an antioxidant, an anticancer agent, an antiviral, an autocoid, a chronobiotic, a hypnotic, an anxiolytic, a glycolytic, a sleep aid, a universal panacea, a biological modifier, and a Higgs boson, just to name a few. The recently published hypothetical reviews presenting melatonin as a broad-based metabolic buffer that regulates LLPS of biomolecular condensates via antioxidant-dependent and -independent means (3, 4) offer an unconventional glimpse into the complex inner-workings of the master key that may finally unlock the mystery behind this versatile, pleiotropic, and sometimes contradictory ancient molecule that has protected living organisms for more than three billion years. LLPS is a

rapid, reversible, energy-efficient thermodynamic process fueled by the reduction or a negative change in global free energy (5). To offset energetic costs for LLPS, which is entropically unfavorable, cells must rely upon adenosine triphosphate (ATP) to capture and transfer free energy, as well as other energetically favorable, multivalent protein-protein interactions including post translational modifications (PTMs) such as phosphorylation, ubiquitination, and SUMOylation to regulate condensate nucleation, composition, and growth (4). During early evolution, membraneless compartments rich in ribonucleic acids (RNAs) with intrinsically disordered regions prone to phase separation likely formed the requisite "bioreactors" in prebiotic chemistries that contributed to the origins of life (6). Increased cellular complexity in eukaryotes is correlated with a significantly higher level of protein disorder compared to less complex prokaryotes implying that increased complexity in cellular signaling and communication requires support from additional flexibility in protein-protein interactions (7). Both ATP and RNA regulate biomolecular condensates in a biphasic manner where lower levels stimulate LLPS to assemble MLOs and higher levels disassemble condensates (8, 9). Lower levels of physiological ATP may provide energy requisite for fueling PTMs where cells use the external energy input to drive chemical reactions out of equilibrium in order to control the size and number of MLOs, whereas higher levels of physiological ATP act as biological hydrotropes to disassemble MLOs (8). Similarly, the feedback mechanisms associated with RNA electrostatic interactions either promote the formation or dissolution of condensates at low or high levels of RNA concentration, respectively (9).

Increasing evidence shows that not only animals, but plants also rely upon phase separation in response to abiotic stress. When exposed to excess heat, *Arabidopsis* utilize RNA-binding proteins to form stress granules (SGs) via LLPS to improve heat resistance (10). The rapid formation of MLOs is an adaptive, evolutionarily tuned response in all eukaryotes in response to a variety of exogenous and endogenous stresses. The assembly of SGs that contain hundreds of proteins and mRNAs allows the rapid transcription and encoding of stress-response proteins, and the temporary stalling of translation in a large number of mRNAs (11). The ability of a living organism to control ATP production levels and fine-tune RNA multivalency via PTMs, which are ATP-dependent processes, and RNA modification by N^6-methyladenosine (m^6A) can ultimately affect its ability to respond to stress by adjusting the rheological properties of MLOs formed by LLPS; this alters viscoelastic properties and controls the temporal and spatial distribution of ribonucleoprotein (RNP) granules enriched with RNA and RNA-binding proteins (RBPs) (12). Aberrant LLPS that alters the efficiency of MLO formation and dissolution can result in pathological protein aggregations that induce and exacerbate diseases including neurodegenerative disorders (4) and cancer (3). The fact that melatonin is synthesized by living organisms in all three domains in the cellular empire since the beginning of life, and that plants, animals, and humans increase melatonin under stress (13) may imply its unique and vital position in the regulation of LLPS in living organisms (4).

2. POTENTIAL INTERACTIONS

The ability of melatonin to increase mitochondrial ATP production was first reported by Martin et al. in 2001 and 2002 (14). Since then, melatonin has been extensively reported as a potent glycolytic that inhibits aerobic glycolysis (the "Warburg effect"), redirecting pyruvate metabolism away from aerobic fermentation of glucose by glycolysis towards the citric acid (tricarboxylic acid, Krebs) cycle and oxidative phosphorylation (OXPHOS) in mitochondria (15). Melatonin is increased under endogenous and exogenous stress in order to ensure appropriate levels of ATP are

produced for the regulation of LLPS in the formation of SGs. SGs are MLOs that cannot be formed without the presence of ATP, requisite for maintaining their liquid-like and dynamic behaviors (16). More importantly, increased melatonin ensures the timely dissolution of MLOs upon resolution of stress factors, some of which may also be attenuated by melatonin. The maintenance of adequate production of intracellular and extracellular ATP that can both stimulate and dissolve SGs, in addition to providing phosphoryl groups to PTM processes such as phosphorylation that can tune SG dynamics, therefore, preserves protein homeostasis to prevent over accumulation of SGs that could result in excess pathological, insoluble aggregates often implicated in neurological diseases and cancer (3, 4).

Melatonin exerts a wide array of antioxidant-dependent and -independent effects to optimize the production of intracellular/extracellular ATP and balance the intricate, interdependent reactions between membranes and MLOs. Even though the melatonin molecule is nonpolar and uncharged in the entire pH range, its ability to form strong H-bonds with hydrophilic lipid headgroups at hydrophilic/hydrophobic membrane interfaces turns it into a robust scavenger of both aqueous and lipophilic free radicals. The presence of both hydrophilic and lipophilic moieties allows melatonin and its metabolites to rapidly neutralize dominant reactive oxygen species (ROS) including the hydroxyl radical ($^{\bullet}$OH) and the hydroperoxyl radical ($^{\bullet}$OOH) that can cause sustained peroxidation chain reactions that damage unsaturated phospholipids including cardiolipin (CL) in plasma and mitochondrial membranes, respectively (4, 17). The maintenance of intact CL in mitochondrial inner membrane (IMM) cristae invaginations under oxidative attack could translate into a one-fold increase in ATP production via OXPHOS. The unique cone-shaped molecular geometry of CL increases bending elasticity of the IMM to support the intense negative curvatures at the apex of cristae invaginations that perfectly host dimerized ATP synthases that are seven-fold more active than ATP monomers. The intrinsic nature of CL to "trap" protons not only enhances ATP production, but also elevates the production of ROS due to the accumulation of H^+ that readily form $^{\bullet}$OOH. As a potent antioxidant, melatonin and its metabolites protect CL from lipid peroxidation cascades to maintain requisite ATP production even under elevated oxidative stress conditions (4).

Similarly, non-mitochondrial, extracellular ATP synthase and ATPase are mostly localized in membrane lipid rafts with negative curvatures. Upon cellular stress, mitochondrial ATP synthases are rapidly translocated to lipid rafts (18). Therefore, preserving optimal nanoscopic lipid raft domains becomes a high priority when an organism is under oxidative stress. At room temperature, lipid peroxidation from oxidative stress increases the non-raft liquid disordered phase, thus inhibiting the formation of lipid rafts. Melatonin stabilizes lipid liquid ordered (L_o)–liquid disordered (L_d) phase separation over a range of temperatures, preventing the transition to non-raft L_d phase potentially via reduction of line tension and increasing membrane fluidity (4, 19). The ability to regulate membrane lipid composition, reduce line tension, stabilize nanoscopic lipid raft domain under oxidative stress presents melatonin as indispensable for the proper formation of SGs which nucleate at plasma membranes (20). Under stress conditions, nanoscopic lipid raft domains maintained by the presence of melatonin serve as vital signaling hotspots that can modulate their composition and the concentration of signal transduction proteins that are recruited to SGs to enhance cell survival. Without melatonin to maintain negative membrane curvature, line tension, proper lipid raft composition, and size, lipid rafts may fail to form or become enlarged to aggregate pro–apoptotic signaling molecules including NLRP3 inflammasome. Furthermore, without adequate melatonin to maintain proper lipid composition and curvature of membranes under oxidative stress conditions, lipid peroxidation can induce pathological alterations to lipid

membranes that impact the ability of ATP synthases to produce requisite energy to support optimal LLPS for SG assembly and disassembly (4). Additionally, nuclear RNA export to cytosol is also modulated by the composition and structure of lipid domains in nuclear membranes.

During evolution, for perhaps more than three billion years, living organisms utilized melatonin as the master key to adjust and control the tight relationship between ATP and RNA that fine-tunes LLPS and MLO formation and dissolution in response to stress. Melatonin regulates not only ATP production, but also RNA export and modification. RNA can be extremely effective in regulating condensate formation and dissolution due to the high negative charge densities buried in their phosphate backbones, providing powerful electrostatic interactions that promote condensate formation at low levels. Whereas, higher levels of negatively charged RNA molecules repel proteins with positive charges to dissolve condensates (4, 9). Therefore, the control of RNA export into cytosol from the nucleus becomes a major regulatory rheostat in condensate formation and dissolution.

Nuclear membrane fluidity is positively correlated with nuclear RNA release (21). Lipid peroxidation in the nuclear envelope reduces membrane fluidity to negatively impact biogenesis of the nuclear pore complex (NPC) which not only mediates RNA export into the cytosol, but also bidirectional protein transport that affects MLO nucleation (22). Melatonin can ensure appropriate RNA release even under high stress conditions via antioxidant-dependent and -independent means. The stabilization of membrane curvature and fluidity in the nuclear envelope is dependent upon the formation of high curvature lipid raft domains that can attract specific proteins that modulate lipid bilayer composition to adjust membrane tension and support spontaneous curvature of the NPCs. The presence of adequate melatonin under high stress conditions not only prevents lipid peroxidation chain reactions, but also preserves requisite membrane morphology to optimize the regulation of RNA nuclear export (4). RNA exported into cytosol is subsequently regulated by various RNA modifications that can enhance or inhibit LLPS (23).

The reversible chemical modification of RNA occurs mostly via N^6-methyladenosine (m^6A) which involves the transfer of a methyl group to the sixth position of the purine ring in RNA adenosine by m^6A methyltransferases ("writers"). These dynamic, reversible installations by m^6A "writers" such as METTL3 and METTL14 enhance LLPS and the formation of SGs by attracting "readers" from the YTH domain proteins that are not only elevated by oxidative stress conditions, but also are recruited into SGs to facilitate the triaging of selected mRNA transcripts into SGs (24). However, m^6A modifications can also inhibit LLPS, as the modifications can be "erased" by demethylases including fat mass and obesity-associated protein (FTO), and alpha-ketoglutarate-dependent dioxygenase alkB homolog 5 (ALKBH5). Therefore, the ability of melatonin to modulate the expression of "writers", "readers", and "erasers" in a "smart", pleiotropic manner gives melatonin the ultimate flexibility in the regulation of LLPS and the formation/dissolution of biomolecular condensates under both physiological and pathological contexts (3,4).

3. CONCLUSION

The ability to use melatonin to harness LLPS, the formation, and the timely dissolution of biomolecular condensates confers enhanced survival and resilience in plants and animals challenged by exogenous and endogenous stresses. Further clarification on potential anti-tumor mechanisms as well as drug synergies associated with the regulation of LLPS by melatonin that may unlock the maximum potential of melatonin in health and disease should steer the direction of future research on melatonin.

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