Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# ATP enhances at low concentrations but dissolves at high concentrations liquid-liquid phase separation (LLPS) of ALS/FTD-causing FUS

Jian Kang, Liangzhong Lim, Jianxing Song*

*Department of Biological Sciences, Faculty of Science, National University of Singapore, 10 Kent Ridge Crescent, 119260, Singapore*

ARTICLE INFO

*Article history:*
Received 19 August 2018
Accepted 3 September 2018
Available online xxx

*Keywords:*
Liquid-liquid phase separation (LLPS)
Fused in sarcoma (FUS)
Amyotrophic lateral sclerosis (ALS)
Frontotemporal dementia (FTD)
Adenosine triphosphate (ATP)
NMR spectroscopy

ABSTRACT

ATP is the universal energy currency but mysteriously its cellular concentration is much higher than that needed for providing energy. Recently ATP was decoded to act as a hydrotrope to dissolve liquid-liquid phase separation (LLPS) of FUS whose aggregation leads to ALS/FTD. By DIC microscopy and NMR, here we characterized the effect of ATP on LLPS of FUS and its N-/C-terminal domains. Very unexpectedly, we found that like nucleic acids, ATP enhances LLPS of FUS at low but dissolves at high concentrations. Intriguingly, ATP monotonically dissolves LLPS of NTD, while it induces LLPS of CTD at low but dissolves at high concentrations. Our study reveals for the first time that ATP can enhance LLPS most likely by behaving as a bivalent binder. Most importantly, our results imply that age-dependent reduction of ATP concentrations may not only result in decreasing its capacity in preventing protein aggregation, but also in enhancing aggregation.



## 1. Introduction

Living cells need subcompartments to achieve spatiotemporal regulation of various biological reactions. It has been increasingly found that in addition to classic membrane-bound organelles, there exist many membrane-less organelles capable of compartmentalizing and concentrating specific sets of molecules, which include nucleolus, Cajal bodies and nuclear speckles in the nucleoplasm, as well as stress granules, P-bodies and germ granules in the cytoplasm [1,2]. Recently it has been revealed that these membrane-less organelles are not structurally defined complexes such as the ribosome, but are dynamic macromolecular assemblies formed by weak and multivalent interactions among components. Amazingly, these structures behave as liquid droplets, which are round, dynamic and able to coalesce into a larger assembly upon contacting with one another [1,2]. Very recently, the formation of these liquid-like droplets of biomolecules within the cytoplasm or nucleoplasm has been characterized to arise from the self-assembly through a physicochemical process known as liquid-liquid phase separation (LLPS), which has been now recognized to represent a general mechanism for forming membrane-less intracellular organelles [1,2].

One group of proteins involved in driving LLPS is constituted by RNA-binding proteins (RBPs) composed of RNA-binding motif (RRM) and low-complexity (LC) domains, which include FUS and TDP-43 [1–4]. FUS is involved in forming cellular granules in cytoplasm including stress granules (SGs) composed of both RBPs and nucleic acids in response to environmental stresses. Intriguingly, these dynamic liquid droplets can further become “aged” or exaggerated into amyloid fibrils or inclusions that lead to a large spectrum of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) [1–10]. FUS consists of 526-residues which is composed of three major domains (Fig. 1A): the N-terminal domain (NTD) over residues 1–267 composed of the QGSY-rich prion-like domain (PLD) and an RG-rich region (RGG1), an RRM and C-terminal domain (CTD) over 371–526 containing RGG2, a zinc finger (ZnF) and RGG3.

Recently, cellular factors mediating LLPS are beginning to be identified. These factors critically mediate LLPS and also protein aggregation. Consequently they represent key targets for developing therapeutic strategies/molecules to treat a large variety of human diseases. Very strikingly, ATP, the universal energy currency in the cell, has been decoded to dissolve LLPS and aggregates of several RBPs including FUS [11]. It was proposed that ATP dissolves LLPS and aggregated proteins by acting as a hydrotropic molecule (Fig. 1B): while its aromatic adenine ring is clustered over the

* Corresponding author.
*E-mail address:* dbssjx@nus.edu.sg (J. Song).

https://doi.org/10.1016/j.bbrc.2018.09.014
0006-291X/

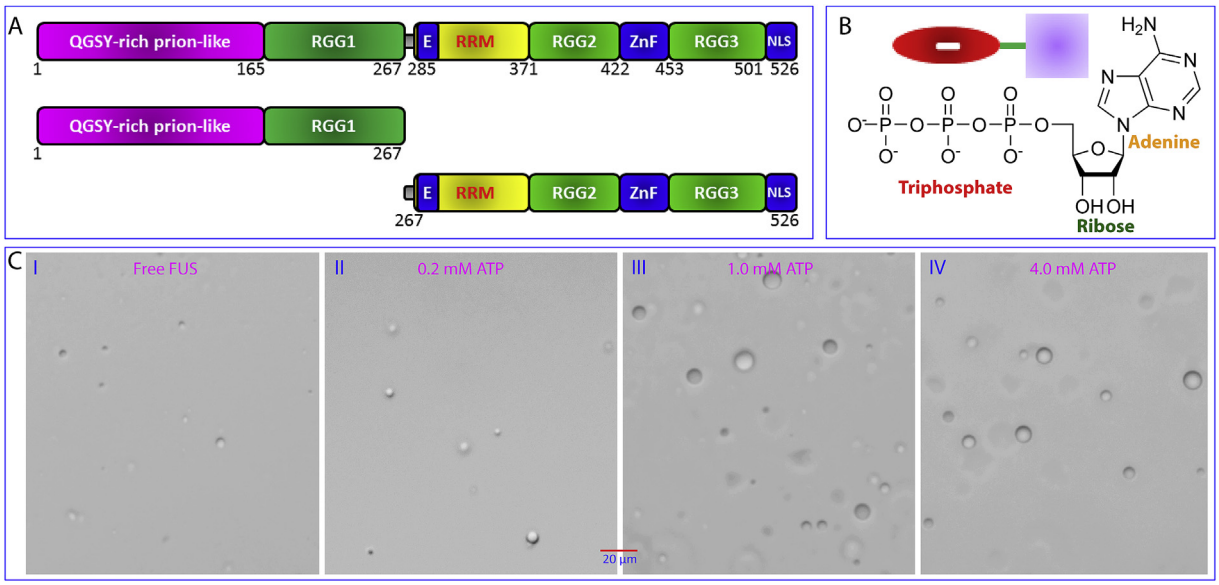


**Fig. 1. Two-stage effect of ATP on LLPS of FUS.** (A) 526-residue FUS is composed of: N-terminal low-sequence complexity (LC) region (1–267) including a QGSY-rich prion-like domain (PLD) and an RG/RGG-rich region (RGG1); RNA-recognition motif (RRM: 285–370); and C-terminal domain CTD (371–526) which contains an RG/RGG-rich region (RGG2), a zinc finger (ZnF), another RG/RGG-rich region (RGG3) carrying a nuclear localization signal (NLS). (B) Chemical structure of ATP. Inlet: a cartoon model of ATP showing its triphosphate chain, which is highly polar and negatively-charged, and relatively hydrophobic aromatic ring of adenine base linked by a ribose. (C) DIC microscopy images of liquid droplets formed by FUS in the presence of ATP at different concentrations. The videos used to output these images are provided in Supplementary Data.

aromatic or hydrophobic patches of proteins by π-π or hydrophobic interactions, the polar triphosphate chain strongly interacts with water [12,13]. Very recently, nucleic acids including RNA [14] and single-stranded DNA (ssDNA) [15] have been also found to have a two-stage effect on LLPS of several RBPs including FUS and TDP-43: enhancement of LLPS at low concentrations but dissolution at high concentrations.

So far, no biophysical characterization of the effect of ATP on LLPS has been reported. Here, we attempted to understand the biophysical basis of the effect of ATP on LLPS of FUS as monitored by differential interference contrast (DIC) microscopy and NMR spectroscopy. Very unexpectedly, in addition to the dissolution of LLPS of FUS at high ATP concentrations which was previously reported [11], we found for the first time that like nucleic acids, ATP could in fact enhance LLPS of the full-length FUS at low concentrations. To understand this observation, we further assessed the effect of ATP on LLPS of the dissected FUS NTD and CTD. Remarkably, ATP monotonically dissolves LLPS of the FUS NTD. On the other hand, ATP is able to induce LLPS of the FUS CTD at low concentrations but dissolves at high concentrations. The results together allow the proposal of a speculative model for ATP to mediate LLPS of FUS, in which ATP acts more than just as a hydrotrope, but also behaves as a bivalent binder. Most importantly, our results imply that age-dependent reduction of ATP concentrations may not only just result in decreasing its capacity in preventing protein aggregation, but also in enhancing aggregation at low ATP concentrations. This thus holds immediate implications in understanding the molecular mechanisms as well as further design of therapeutic molecules for various human diseases caused by protein aggregation such as neurodegenerative diseases and cardiovascular diseases [13].

## 2. Materials and methods

### 2.1. Preparation of recombinant FUS proteins

Previously, we have cloned DNA fragments encoding FUS and its differentially-dissected fragments into a modified vector pET28a with a C-terminal His-tag [16]. However, although the presence of 6xHis-tag had no detectable effect on their solution conformations, we found that the His-tag has in general weakened the capacity in LLPS, consistent with the recent report [18]. Therefore, in the present study, we removed the His-tag by adding a stop codon immediately after the DNA sequences encoding the full-length FUS, FUS NTD over 1–267 and FUS CTD over 371–526 (Fig. 1A). The expression and purification of these recombinant FUS proteins followed the previous protocols [16]. The purity of the recombinant proteins was checked by SDS-PAGE gels, and the molecular weights were verified by a Voyager STR matrix-assisted laser desorption ionization time-of-flight-mass spectrometer (Applied Biosystems).

The same procedures were used to generate isotope-labeled proteins for NMR studies except that the bacteria were grown in M9 medium with addition of $(^{15}NH_4)_2SO_4$ for $^{15}N$-labeling. The protein concentration was determined by the UV spectroscopic method in the presence of 8 M urea, under which the extinct coefficient at 280 nm of a protein can be calculated by adding up the contribution of Trp, Tyr, and Cys residues [10,15,16].

ATP was purchased from SigmaAldrich with the same catalog number as previously reported [11]. $MgCl_2$ was added into ATP for stabilization by forming the ATP-Mg complex [11]. The protein and ATP samples were all prepared in 5 mM sodium phosphate buffer with the final solution pH at 6.0. For all samples studied here by DIC and NMR, $ZnCl_2$ was also added to the samples of the full-length FUS and CTD containing the zinc finger (ZnF) to reach a final concentration of 4 mM.

### 2.2. Differential interference contrast (DIC) microscopy

The formation of liquid droplets was imaged at 25 °C on 50 μl of different FUS samples at a protein concentration of 20 μM in 5 mM sodium phosphate buffer at pH 6.0 in the presence of ATP at different molar concentrations: 0, 0.2, 0.6, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mM by DIC microscopy (OLYMPUS IX73 Inverted Microscope System with OLYMPUS DP74 Color Camera) as previously described [15].

Please cite this article in press as: J. Kang, et al., ATP enhances at low concentrations but dissolves at high concentrations liquid-liquid phase separation (LLPS) of ALS/FTD-causing FUS, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.09.014