UNIVERSITY OF CALIFORNIA SAN DIEGO

Visualization of membrane-less granules in yeast and mammalian cells using modified fluorescence *in-situ* hybridization

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by

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The Thesis of Hema Mythili Kopalle is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California San Diego

2019

EPIGRAPH

You may live in the world as it is,
But you can still work to create the world as it should be.
- B. Obama

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I would like to thank Dr. Brian Zid, Vince Harjono, and Anna Guzikowski for supporting me throughout this project. These accomplishments are as much yours as my own, thank you for teaching me, supporting me, and helping me grow.

ABSTRACT OF THE THESIS

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Processing bodies (p-bodies) are transient, membrane-less constructs consisting of mRNA and RNA-binding proteins. Though constitutively expressed in mammalian cells, formation of these structures in yeast is induced by metabolic stress. While not all mRNAs localize to membrane-less granules, identifying their contents has proved difficult because of the structural nature of these droplets. It therefore becomes necessary to stabilize these granules to qualitatively observe mRNA localization. Here we present a new methodology for granule stabilization using a combination of UV cross-linking and the small dialdehyde Glyoxal. We also provide evidence suggesting Glyoxal to be an effective fixative for membrane less granule fixating in mammalian cells.

INTRODUCTION

Processing bodies (p-bodies) are transient, membrane-less constructs consisting of mRNA and RNA-binding proteins. Though constitutively expressed in mammalian cells, formation of these structures in yeast is induced by metabolic stress. While not all mRNAs localize to membrane-less granules, identifying their contents has proved difficult because of the structural nature of these droplets. It therefore becomes necessary to stabilize these granules to qualitatively observe mRNA localization. Here we present a new methodology for granule stabilization using a combination of UV cross linking and the small dialdehyde Glyoxal. We also provide evidence suggesting Glyoxal to be an effective fixative for membrane less granule fixating in mammalian cells.

Metabolic stress induces formation of two kinds of ribonucleoprotein (RNP) granules in the eukaryote *S. cervisiae.* RNPs can take many forms, but are primarily complex networks of RNA-protein interactions that may undergo liquid-liquid phase separation in the cytosol (Fig. 1A-B)[1,2].

Liquid-liquid phase separation (LLPS) refers to the phase change that occurs when otherwise dispersed particles accumulate to form a pseudo-structures liquid within an aqueous solution. As the liquid-liquid droplet gains structure and stability, it transitions to a gel-like state before becoming solid or plaque-like (Fig. 1C). This phase change is not inevitable, but has been characterized as an assembly pathway for pathogenesis in diseases like Alzheimer's which express an aggregation phenotype[1,3]. In cells, these liquid droplets are formed from proteins and fragments with intrinsically disordered regions, as well as short linear motifs and low-complexity sequences of RNA. These regions are highly dynamic, allowing for binding and complexing with other droplet components thus creating RNA-RNA-Protein interactions that sustain the phase separation[3–5].

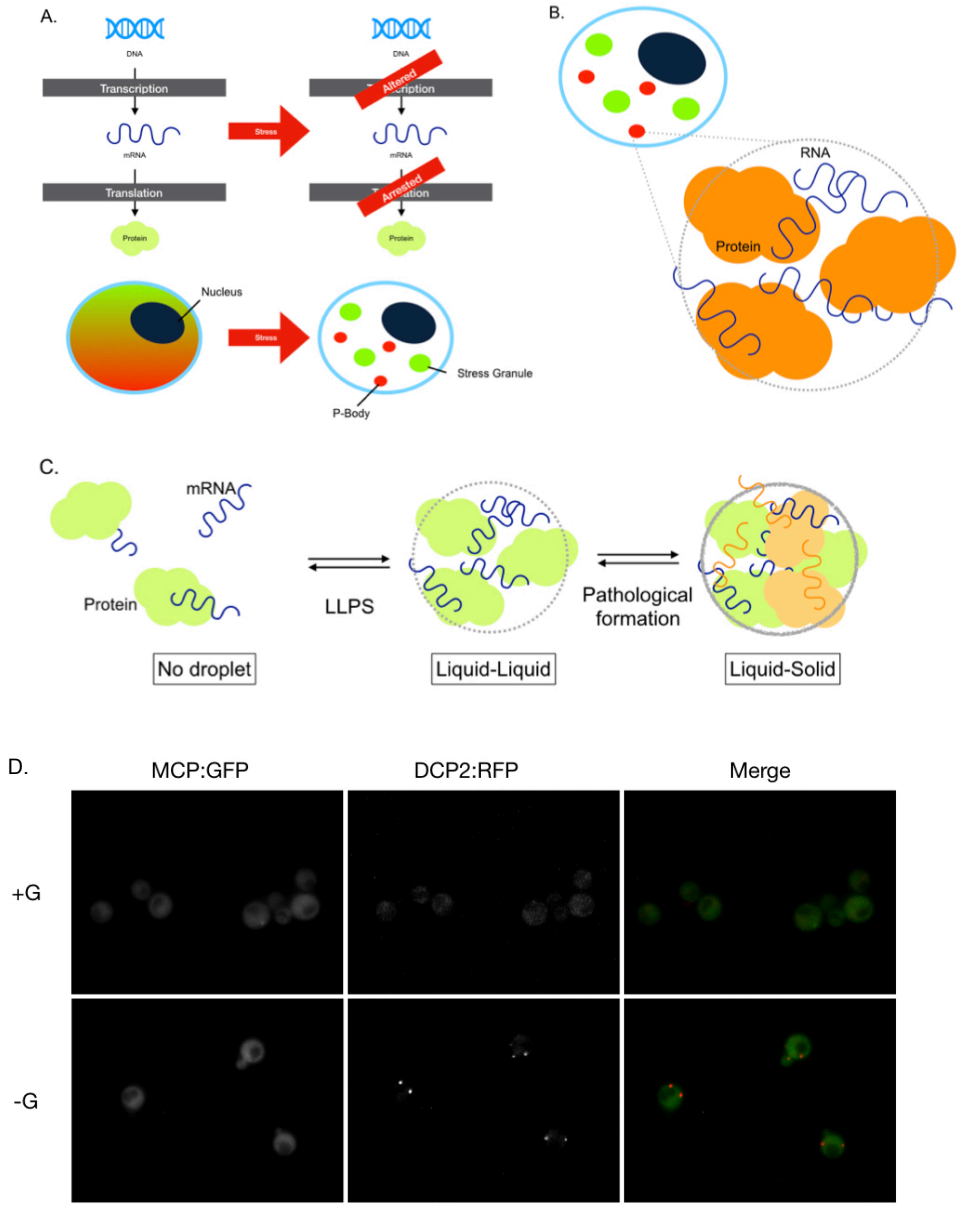

Figure 1. mRNP granules are liquid-liquid droplets. A) Extracellular stress alters transcription and arrests translation of most constructs. P-bodies and stress granules form in the cytosol after the onset of extracellular stress. B) mRNP granules consist of transiently associated RNA and RNA binding proteins. C) Liquid-liquid phase separation is reversible, as more proteins and RNA become associated, the droplet shifts to a solid phase which is indicative of pathology. D) Comparison of p-body formation after glucose starvation in yeast.

Recent fluorescence imaging of P-bodies and Stress Granules (SGs) suggest these transient foci exist as liquid-liquid droplets in the cytoplasm[1,6,7]. Single molecule tracking suggests that non-nucleating mRNAs promiscuously associate with both p-bodies and stress granules, and will travel between two foci within close proximity. This mobility is corroborated by the rapid dissolution of the granule after stress removal, indicating these RNA-protein interactions are inherently unstable (Fig. 1D)[7].

The purpose of p-bodies and stress granules is still not fully understood. Though they share many similarities, the individual components of each belong to generally different schemas. While the majority of the identified protein components of stress granules are translation initiation factors (like G3BP1), p-bodies were thought to be sites of mRNA decay[8–10]. The identified protein components of p-bodies fall broadly into three categories; translational repression, decapping proteins, and the deadenylation complex (CrR4-Not and Lsm1-7). Many of these proteins are conserved between *H. sapiens* and S. cerevisiae, such as the decapping activators DCP1/DCP2, making yeast a viable model for p-body analysis(Fig. 1B). However, mRNA decay has been shown to continue in the absence of p-body formation, suggesting alternate function for these granules[8,11,12]. Recent theories suggest p-bodies may serve as temporary storage site for translationally repressed mRNAs, the aggregation of which promotes phase separation into a liquid-liquid droplet[13]. This fluidity has served as one of the main challenges when to characterizing the mRNA components of membrane-less granules, and necessitates the development of methods to stabilize these interactions. Because of this, studies of granule composition have deviated to quantitative methods such as ribosome profiling or RNAseq. While useful for determining expression profiles of mRNA, they do not provide information about mRNA localization during stress.

MS2 Visualization of in-vivo mRNA

Real-time observation of mRNA localization can be achieved using the MS2-phage system. This requires the addition of hairpin stem-loop secondary structures on the target sequence, generally the 3' end (Fig. 2A). These stem-loops are then bound by the MS2 coat-protein, which is fused with a fluorescent protein for live-cell imaging. This binding interaction is due to the specificity of the protein binding motif, resulting in few off-target effects[14,15].

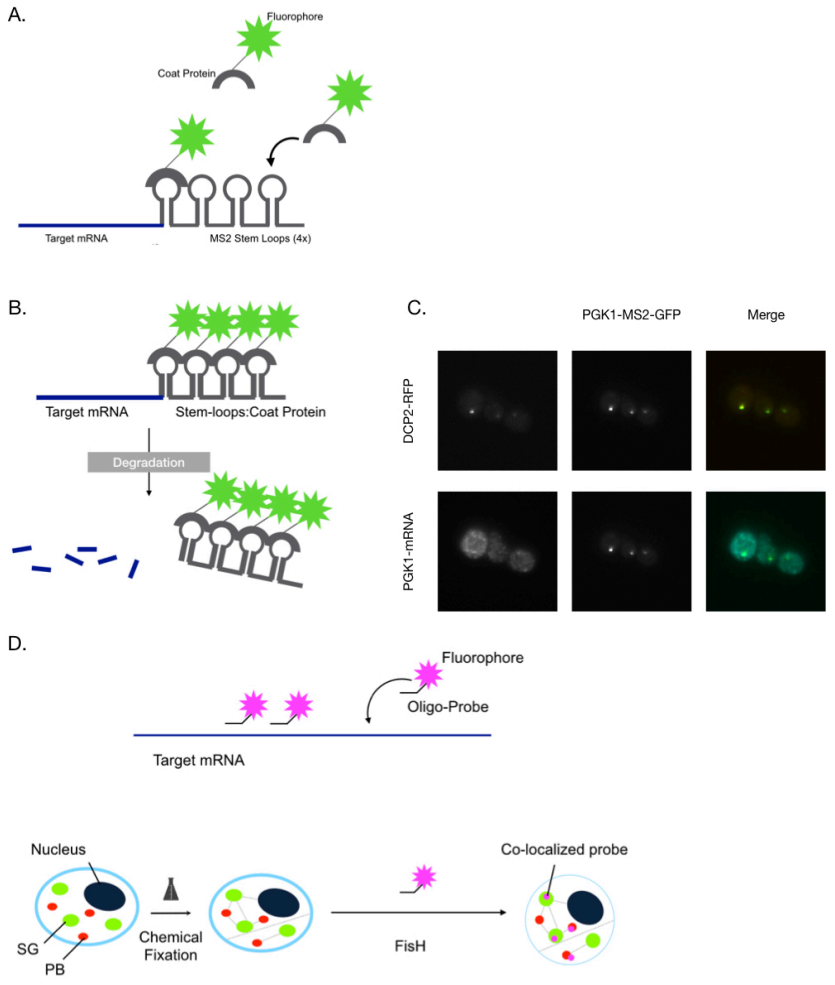

Figure 2. Mechanisms of single mRNA visualization in cells. A) The MS2 stem-loop expresses a protein binding motif specific to the MS2 Coat Protein, allowing fluorophore tagging of target mRNA in-vivo. B) incomplete degradation of the MS2-tagged target mRNA leads to MS2-Stem-loop:Coat-Protein products. C) Co-visualization of PGK1 by MS2 tagging and Fluorescence in-situ hybridization suggests conflicting localization information. D) Schematic of Fluorescence in-situ hybridization method.

When identifying putative granule localized targets, a marker such as DCP2a or G3BP1 is fused to a fluorescent protein to identify granules in the cytosol[16,17]. Co-localization of the target signal with this marker indicates the trajectory of the target as a result of extracellular stress.

However, recent publications have shown incomplete degradation of the MS2 tagged mRNA leads to an aggregation of stem-loop:coat-protein constructs and a subsequent false-positive artifact in yeast (Fig. 2B-C)[18,19]. Tutucci and colleagues suggest the coat-protein exerts a protective effect on the stem-loop structures, preventing degradation[20]. These constructs exhibit many of the hallmarks of SG localizing components, and aggregate to form foci in the cytosol[21]. This incomplete degradation seems specific to yeast, where p-bodies and SGs are often fused to the same foci. Though a different version of the MS2 system has been created, secondary verification of mRNA localization is still needed to confirm localization of endogenous mRNA[20].

Fluorescence in-situ Hybridization visualizes single mRNA

Single molecule Fluorescence in-situ Hybridization (FisH) has been used to observe individual mRNAs in fixed cells across a variety of systems[22]. The cell is chemically cross linked to prevent mRNA degradation, then hybridized with oligonucleotide probes of the anti-sense stand to the target mRNA (Fig. 2D). These probes can be exogenously conjugated to a fluorophore for visualization by microscopy. The actual probes are small, 20bp oligomers, the multiplexing of which can amplify the fluorescent signal to identify a single mRNA against the background cell. Longer and more highly expressed mRNA have a greater likelihood of visualization because of the amplification of the fluorescent signal[18,23,24].

To hybridize these constructs the cell must first be “fixed” — immobilizing the intracellular components using a fixative, typically formaldehyde[24]. The aldehyde reacts with the exposed

primary amine of a protein or nucleotide to create a Schiff base (Fig. 3B). This base then reacts with another exposed primary amine to create single-carbon adducts. Though useful for stable protein-protein crosslinking, fixation by formadehyde denatures the bound construct by creating linear adducts to the monomer[25]. This multistep fixation mechanism requires large amounts of the formaldehyde monomer, necessitating the use of the polymer Paraformaldehyde (PFA). It is highly toxic, depolymerizing in an aqueous solution to become formaldehyde (Fig. 3A). Though spontaneous and prolific, these hydration reactions are reversible in the aqueous environment of the cytosol, making them an unfavorable choice for membrane-less p-bodies.

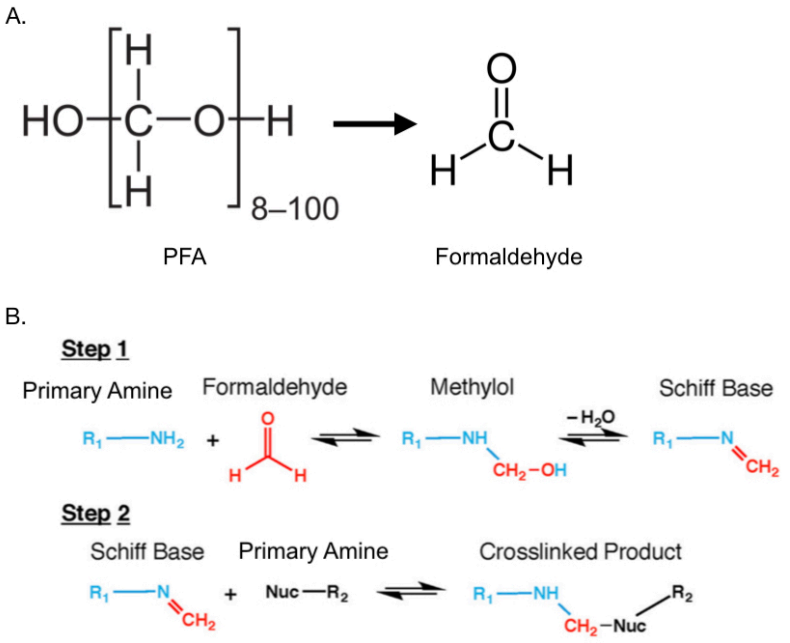

Figure 3. Formaldehyde formation and fixation mechanism. A) Paraformaldehyde depolymerizes in an aqueous environment to become formaldehyde. B) Formaldehyde crosslinking is a two-step process, forming a Schiff base that reacts with an exposed primary amine to create a linear product.

Hybridization itself is a disruptive process, requiring formamide and heat to melt RNA secondary structure, destabilizing RNA-RNA interactions within the granule[26]. Though necessary to allow annealing of the fluorescently-tagged probes, disruption of RNA further weakens the structure of the granule[24]. Without stabilization of RNA-RNA-Protein interactions

within the p-body, it becomes diffuse throughout hybridization, yielding stochastic retention of the initial granules and co-localization information. It therefore becomes necessary to develop a method to stabilize these membrane less granules for identification of their mRNA components.

Oxaldehyde (Glyoxal) is a small dialdehyde that is extensively used as a non-toxic commercial fixative[27]. At high pH, glyoxal has been shown to bind to nucleotide bases on the Watson-crick surface, creating a stable cyclic adduct[28]. The proposed mechanism requires the presence of a secondary anime in proximity to an exposed primary amine, excluding the modification of Uracil but allowing stable fixation of other base pairs. In addition to forming non-specific carbon adducts, hydrated glyoxal polymerizes and forms long oligomers that extend its fixative activity[29]. Formation of the cyclic adduct on single base pairs exposes a polymerization surface, allowing fixation without altering mRNA structure (Fig. 4A-B). Recent work by Richter and colleagues has shown glyoxal to be superior to formaldehyde in preserving mammalian cell morphology for immunohistochemistry and FisH at low pH[30]. In particular, glyoxal at pH 5 was able to retain the morphology of cells lacking strong intracellular matrices like eosinophils. This raises the possibility of glyoxal as an alternative fixative to PFA, possibly stabilizing membrane-less granules.

These aldehyde-induced carbon adducts are still reversible, necessitating additional stabilization to maintain crosslinking through hybridization. Ultra-Violet radiation (UV) was first proposed as a DNA-protein crosslinking method in the 1960s, but is not commonly used for in-situ hybridization[31]. Exposure to UVA/UVB radiation induces radical formation in the aromatic ring of purines and pyrimidines, causing dimer formation and ligation to alkaline amino acids[32]. This reaction is most common for pyrimidines, with a notable interaction between uracil and lysine[33]. Without active repair mechanisms in place, these crosslinks are irreversible in fixed cells.

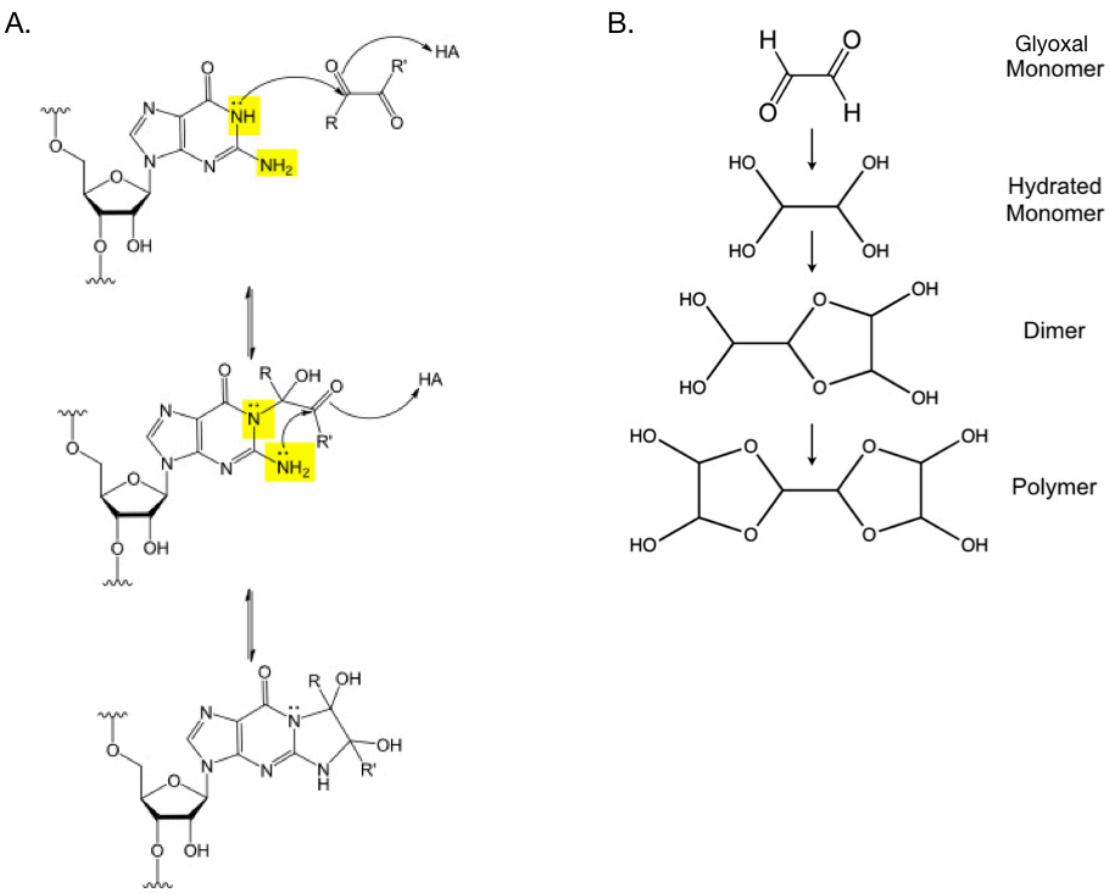

Figure 4. Glyoxal hydration and fixation. A) Glyoxal binds to both primary and secondary amines in nucleotides forming a stable ring structure with an exposed polymerization surface. B) Glyoxal forms hydrates and reacts with itself to form functionally active polymers.

RESULTS

Identification of Chemical Fixation as key stage of P-body diffusion

The current method for fluorescence in situ hybridization of membrane less granules follows the general axiom of; induce granule formation, fixation, permeabilization, hybridization, imaging. In yeast this permeabilization step requires digestion of the cell wall, inducing spheroplast formation (Fig. 5A-G). This denatures the preexisting morphology of the yeast cell to a roughly spherical shape, further straining the crosslinks made immediately after granule induction. P-body dissolution could occur at any number of steps before or after hybridization due to mechanical stress on the cell.

To identify key steps for optimization, we first visualized p-body retention at each step of our initial protocol (modified from Zid et. al). Baseline robust expression was determined as initial p-bodies present after 15 minutes glucose starvation for each experiment (Fig. 5H). P-bodies of decreasing intensity are present prior to hybridization, with noticeable decrease in retention afterwards. This suggests reversible non-specific binding is insufficient to stabilize p-bodies during RNA melting and hybridization.

Glyoxal is 9-fold more effective at p-body retention than paraformaldehyde in yeast

Glyoxal's mechanism for tissue fixation relies on the formation of carbon adducts bound to nitrogen, oxygen, or carbon. Similar to formaldehyde, it binds the primary amine of the purine or pyrimidine but without forming the Schiff base. Glyoxal forms two adducts with each nucleotide, and polymerizes with itself to form longer, functional metabolites that continue to bind species.

Direct comparison of 4%PFA to 3% Glyoxal as a fixative suggests equal efficacy of granule retention *prior* to hybridization**.** Cells treated with glyoxal preserve the nuclear space as seen in live cells, indicating preservation of morphology. Glyoxal treated cells also express higher fluorescent signal relative to PFA treated cells. After the application of the FISH method,

cells fixed with glyoxal retain an average of 0.18 p-bodies per cell compared to 0.02 when fixed with PFA, indicating that glyoxal is a more effective fixative (Fig. 6B). However, there continues to be a global diminishment of fluorescent signal, and significant reduction in total p-body retention with chemical fixation alone. This suggests the RNA and proteins within the p-bodies do not remain stably associated.

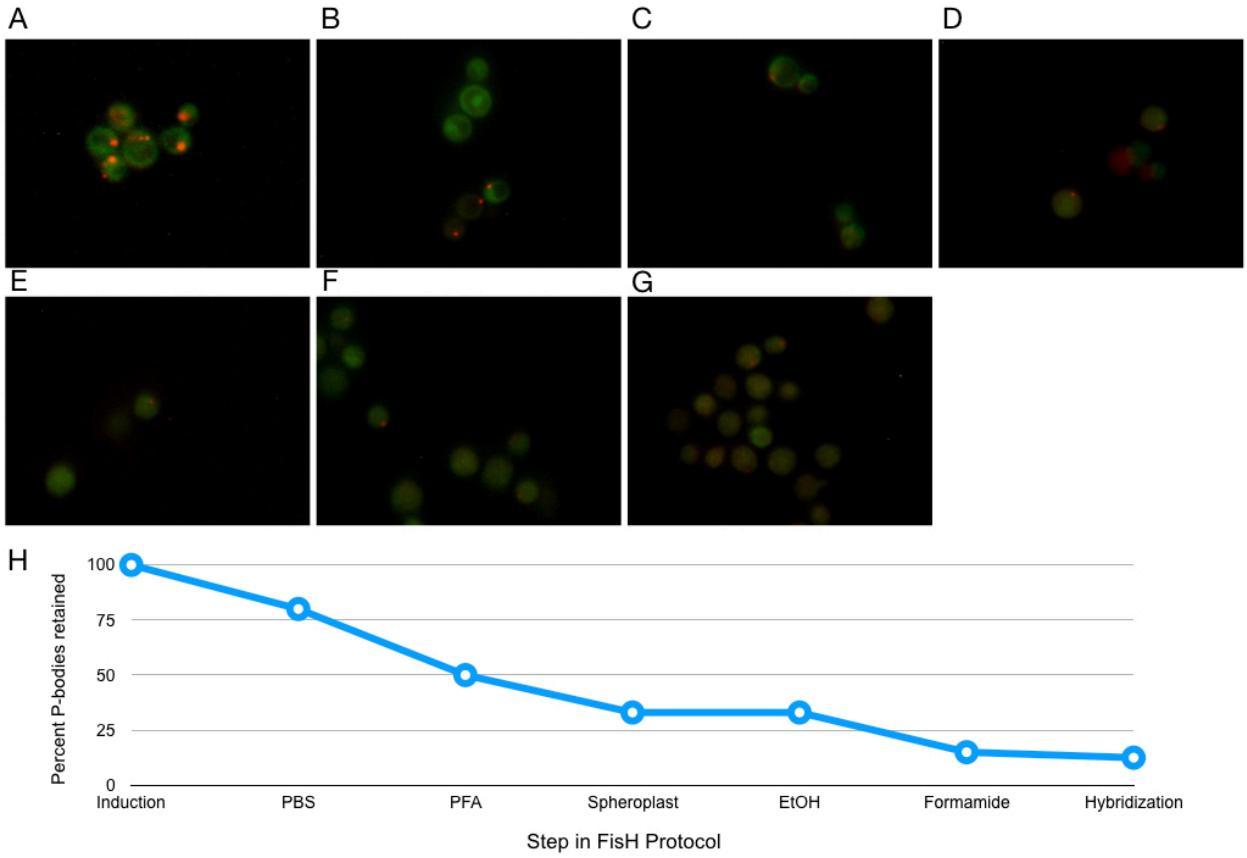

Figure 5: Longitudinal analysis of p-body retention through FisH indicates granule destabilization. A-G) Individual steps of original protocol: Induction, 1xPBS, PFA, Spheroplast formation, EtOH drying, Formamide wash, Hybridization. I) P-body retention is reduced as the cell is treated with additional steps in the hybridization protocol.

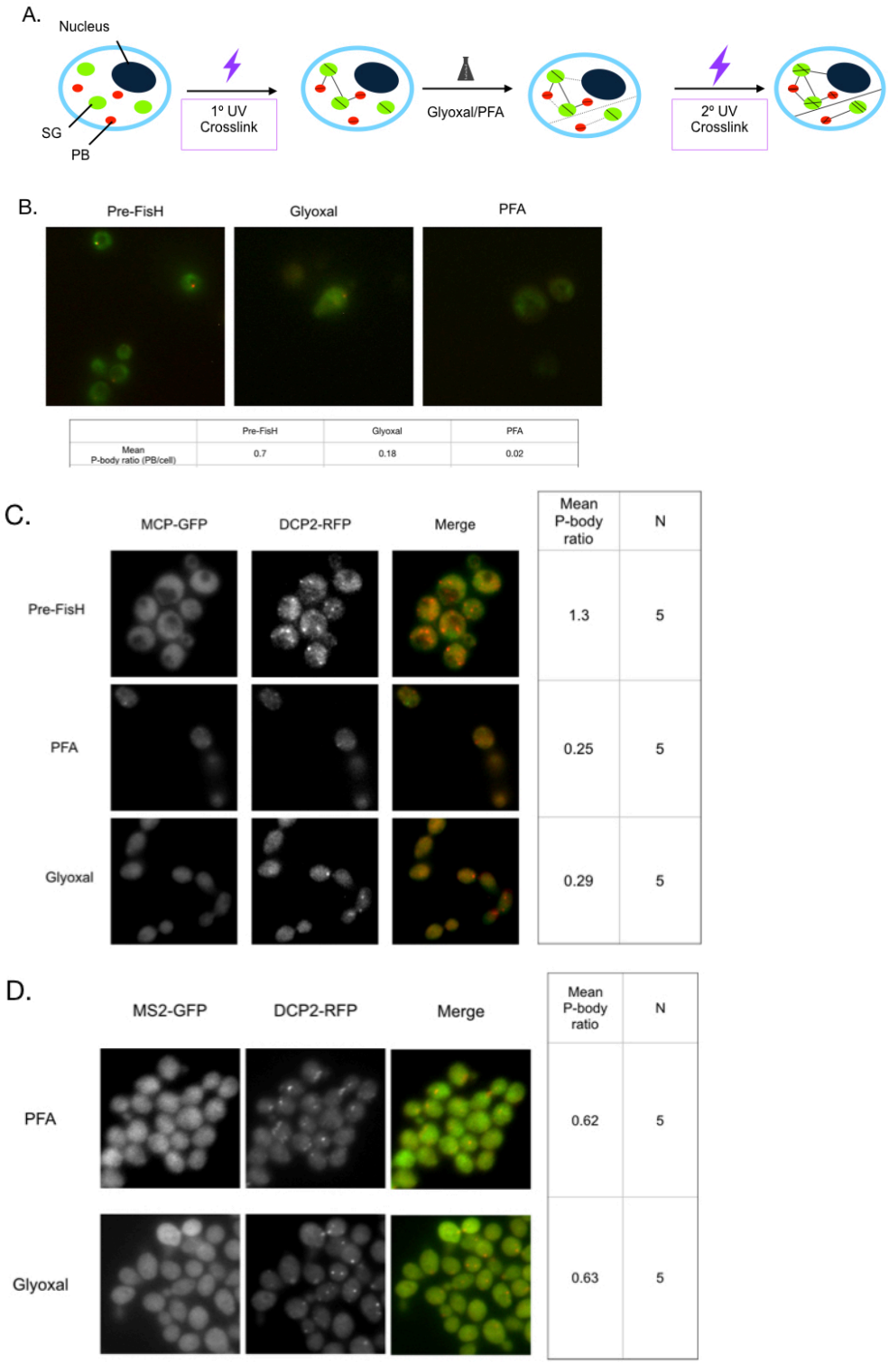

Figure 6. Dual fixation with glyoxal and UV crosslinking increases p-body retention in yeast. A) Schematic of UV crosslinking. B) Fixation and hybridization of yeast cells after glucose starvation using glyoxal and PFA. C) Visual analysis of p-body retention after the addition of

1mJ/cm^3 UV crosslinking to FisH in yeast. D) Dual UV crosslinking increases p-body ratio to 60%.

UV Cross-linking stabilizes intra-granule associations

Photooxidation is the process of breaking double bonds using UVA/UVB radiation. In live cells this induces nucleotide hydration and fusion, which activates DNA repair mechanisms to correct the double-stranded breaks. In fixed cells, this machinery is inactive and these cross-links remains stable[34].

We introduced 1mJ of UV radiation before chemical fixation to induce stable irreversible bonds (Fig. 6C). This high-energy method reduced the total sample size, but increased p-body retention prior to hybridization. With the inclusion of irreversible crosslinks, average p-bodies per cell doubles to roughly 0.25. While promising, this rate is not reflective of the robust p-body formation seen in live cells, and may indicate that diffused p-bodies are still present in the cytosol.

In the presence of a carbonyl group, UV radiation induces radical formation, initiating a chain reaction of bond reduction and hydration[35]. Because radical chain formation occurs in the presence of oxidative species, we hypothesized that a second bout of UV radiation after chemical fixation would further stabilize p-bodies (Fig. 6A). Fixation by glyoxal introduces hydrated polymers, which can react in the presence of UV radiation to create additional irreversible cross-links. Inclusion of a second bout of UV cross-linking further stabilized p-bodies, increasing average p-bodies per cell to 0.62 (Fig. 6D). By including a second UV cross-linking step immediately after chemical fixation, we induced nonspecific irreversible binding to stabilize the new bonds formed internal and external to the p-body.

Temperature attenuation prevents p-body dissociation during hybridization

Formamide is the standard hybridization solution for most in-situ hybridization. Formamide destabilizes nucleic acid strands by promoting the reduction of hydrogen bonds between base pairs. The efficacy of formamide to denature RNA secondary structure varies by

temperature, ideally around 30ºC-40ºC[24,26]. To ensure cross-linking did not interfere with probe binding and putative signal we attenuated the temperature of formamide washes to be cooler than hybridization, thus limiting p-body disruption (Fig. 7A). These modifications resulted in distinct FisH probe punch with noticeable signal-to-noise ratio (Fig. 7B).

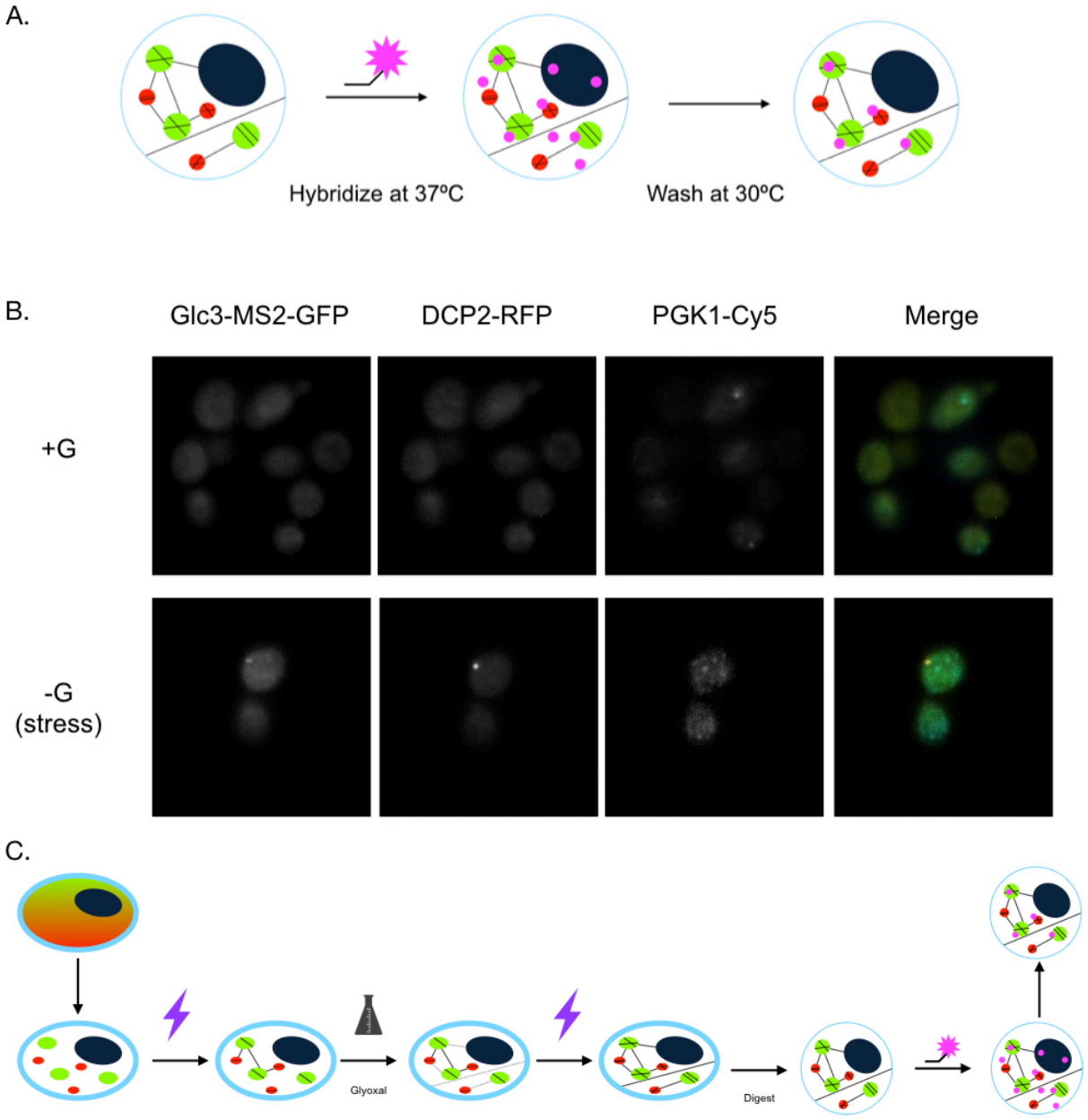

Figure 7. Attenuation of hybridization parameters promotes high signal-to-noise ratio of FISH probe signal. A) Schematic of hybridization modifications. B) UVxFISH of PGK1 mRNA in glucose starved yeast using glyoxal as a fixative. C) Diagram of full UVxFisH protocol for fixation of granules in yeast.

Efficacy of UVxFisH as a method to visualize target mRNA localization

To test the efficacy of our modified protocol we examined mRNA localization of the glycolytic factor phosphglycerate kinase 1 (PGK1) after glucose-starvation stress in yeast[19,20]. Localization PGK1-MS2 and subsequent mis-localization of the corresponding FisH signal was the preliminary indicator for stem-loop:coat-protein artifacts, and prompted the development of the MS2v6 system for use in yeast (Fig. 2C). Validation of this system did not include analysis of PGK1 localization, and as such we sought to determine if endogenous PGK1 would localize to p-bodies as suggested by live cell MS2 data.

Preliminary results via the UVxFisH method suggests PGK1 may remain cytosolic 15 minutes after the onset of glucose starvation stress (Fig. 7B). Foci formed by the stress granule marker Glc3 appear to co-localize with p-bodies, forming roughly circular shapes. As these globular structures contain fluorescent proteins, it is unclear if this stable co-localization is due to the protein-protein interactions or true co-localization[7,36,37]. PGK1 mRNA probe puncta appear to stochastically co-localize to these foci, though with equal intensity as other cytosolic puncta. Distinct puncta of the PGK1 probe are also seen in log-phase growth yeast, despite the absence of p-body formation. The high intensity of these log-phase puncta may indicate the presence of a transcription site in the genome, in close proximity to nascent PGK1 mRNA. Visualization of this single transcription site provides additional support for the validity of the UVxFisH method (Fig. 7C).

Glyoxal is sufficient to stabilize Liquid-Liquid droplets in mammalian cells

To determine the efficacy of glyoxal as a fixative for membrane less granules in a mammalian system, we sought to induce and stabilize two kind of liquid-liquid droplets. Because not all granules are induced by stress, we first sought to determine if glyoxal would be effective on light-induced granules called OptoGranules[38]. This system takes advantage of the light-inducible dimerization of the circadian protein Cryptochrome2, which is sensitive to 405nm light.

Cryptochrome2 is fused to G3BP1, a structural stress granule protein that is also used as a stress granule marker in mammalian cells (Fig. 8A). By exposing this fused construct to blue light, the Cryptochrome2 oligomerizes, creating a pseudo stress granule foci of G3BP1 scaffolding. This process is reversible, and the transient LLPS droplet will dissociate five minutes after the stimulus light is removed. We found that glyoxal rapidly stabilized and retained OptoGranule formation, with similar efficacy as PFA (Fig. 8B).

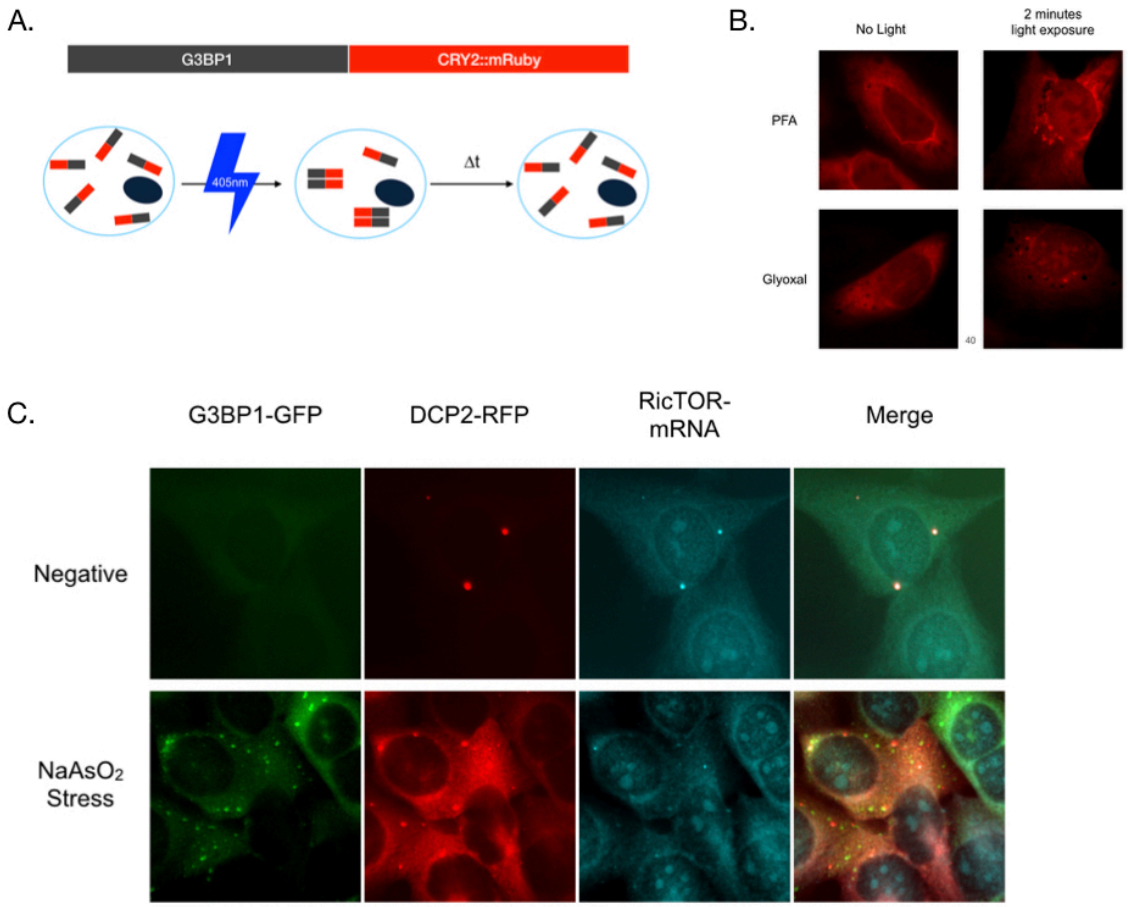

Figure 8. Glyoxal fixation of membrane-less granules in U2OS cells. A) Schematic of optogranule construct and formation. B) Comparison of glyoxal and PFA immobilization light-inducible granules in U2OS cells. C) FisH of RicTOR mRNA in U2OS after 250mM sodium arsenite stress and Glyoxal fixation.

Glyoxal is an effective fixative for FISH of stress-induced membrane less granules

Granules induced by oxidative stress (250mM $NaAsO_2$) exhibit different fixation behavior between glyoxal and PFA. Glyoxal-fixed samples retained higher fluorescence intensity and

signal-to-noise ration when compared to PFA-fixed samples prior to hybridization, suggesting glyoxal may be a more effective fixative for stress-induced granules.

To identify if glyoxal interfered with FisH probe binding, we chose RicTOR as our target mRNA. RicTOR is a long mRNA, the protein of which is involved in log-phase growth, making it a likely candidate to express metabolic localization behavior and allow single molecule FisH[21]. Forty-eight unique 20 nucleotide FisH probes were created from a 5kb stretch of the mRNA sequence. After oxidative stress, single mRNA localization of the target RicTOR suggested a variance in localization behavior between stressed and unstressed conditions in both Glyoxal and PFA treated samples. As p-bodies are constitutively expressed in mammalian cells, RicTOR signal was co-localized to p-bodies in unstressed conditions in U2OS cells. After the stress induction, co-localized RicTOR signal was diminished and puncta were observed in the cytosol.

DISCUSSION

Characterizing the mRNA components of membrane-less granules such as p-bodies and stress granules provides greater understanding of the cellular response to extracellular stress. These localization patters and the identification of translational repression in these droplets is necessary to better understand the role of mRNA in pathogenesis.

Visualization of mRNA localization thus far has been hindered by the structural nature of these granules. The current methodologies for single mRNA tracking use the MS2-phage system and fluorescence in-situ hybridization with paraformaldehyde. The MS2 system has been shown to cause false positive artifacts because of the stable association between the stem-loop modification of the target mRNA with the fluorescent coat-protein. As liquid-liquid phase separated droplets, these granules are not stable enough to maintain RNA-RNA-protein interactions through the many physically and chemically disruptive steps in FisH.

We successfully developed a protocol to stabilize and retain the majority of glucose-starvation induced p-bodies in *S. cerevisea.* By introducing irreversible UV crosslinking followed by low pH glyoxal fixation, we were able to retain significantly more p-bodies for subsequent analysis. We also have shown that glyoxal is an effective fixative for other membrane less granules in mammalian cells, including those induces by oxidative stress and non-stress induced granules as well. These methods successfully stabilize transient liquid-liquid droplets in the cytosol for qualitative data analysis of mRNA localization.

MATERIALS & METHODS

Yeast Cell Culture

All yeast strains were developed from the w303 parent strain as characterized on the Sacchromyces Genome Database. Strains were modified to express MS2 Coat Protein fused to GFP (MCP:GFP) and the p-body marker DCP2 fused to RFP (DCP2:RFP). Strains used to determine localization of PGK1 mRNA also expressed Glc3 fused to 12 version four MS2 stem-loops, to function as a stress granule marker. Cells were cultured in 50ml YPD to a final OD600 of 0.2-0.4 at 30ºC rotating.

Glucose Starvation

Yeast cells were centrifuged at 3000rpm for 5 minutes at 4ºC, then resuspended in 1ml of Synthetic Defined Media without glucose (SD-G). Cells washed twice in SD-G media before incubation in 5ml SD-G for 15minutes at 30ºC rotating.

UV Crosslinking

All cross linking occurred at 1mJ/cm3 in a UV Stratalinker 3000. Yeast cells were suspended in 4ml 1xPBS, 4ml 4%PFA in 1xPBS, or 4ml of 3% Glyoxal pH 4.95.

Fixation

The 4% Paraformalydehyde solution was prepared by diluting 32%PFA (w/v) into 1xPBS. Solution was made, at most, four weeks prior to use and stored at 4ºC in the dark. The 3%Glyoxal solution was prepared by mixing 40%(w/w) Glyoxal (SigmaAldrich) with 0.3% EtOH,

5mM Glacial Acetic Acid, and RNAse-free water. The solution was then brought to pH using 1M NaOH. The glyoxal solution was made, at most, four weeks prior to use and stored at room temperature.

Spheroplast formation

To create spheroplasts, yeast cells were resuspended in 1ml of 2.5ml/ml solution of Zymolyase in 4ºC Buffer B. Digestion was incubated for one hour at 30ºC at 300rpm rotation.

Ethanol Drying

All samples were dehydrated with 70% EtOH for one hour at 4ºC.

Hybridization

All samples were hybridized in hybridization solution with RNA probe at 1:200 dilution overnight at 37ºC in the dark.

Formamide washing

Hybridized samples were brought to room temperature before washing in 10% Formamide in 2xSSC. Mammalian cells were washed twice for 5 minutes with gentle shaking. Yeast cells were washed twice in 1ml solution for 5 minutes at 2400rpm. All samples were then incubated at 30ºC for 30 minutes before removing the 10% formamide solution.

$NaAsO_2$ stress of U2OS

Cells were seeded at $0.2x10^6$ per well in a six-well plate, using DMEM 10%FBS 1%Penicillin/Streptomycin and let culture overnight on poly-l-lysine coated coverslips. Media was replaced to 2ml one hour prior to oxidative stress. Cells were stressed with 250mM $NaAsO_2$ for 30 minutes before imaging and fixation.

OptoGranule propagation

Cells were seeded at $0.2x10^6$ per well in a six-well plate, using DMEM 10%FBS 1%Penicillin/Streptomycin and let culture overnight. Cells were then transiently co-transfected with 2.5µg OptoGranule plasmid (gift from P. Taylor) and 0.5µg linear Hygromycin B fragment using the Lipofectamine 3000 kit from Invitrogen. Cells were selected using 200µg/ml Hygromycin B for two days before experimentation.

OptoGranule Formation

Cells were seeded at $0.2x10^6$ per well in a six-well plate, using DMEM 10%FBS 1%Penicillin/Streptomycin and let culture overnight on poly-l-lysine coated coverslips. Media was replaced to 1ml 1xPBS for the duration of the experiment. Samples were exposed to two minutes of blue light and allowed to incubate in the dark for an additional three minutes before fixation.

Buffers:

Buffer B

1.2M Sorbitol
100mM $KHPO_4$ pH 7.5

Hybridization Buffer

10% Formamide
2x Saline Sodium Citrate
10mg/ml Bovine Serum Albumin
100mM Vanadyl Ribonuceoside Complex
1mg/ml E. Coli tRNA

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