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Role of Assemblysomes in Cellular Stress Responses

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ABSTRACT

Assemblysomes are recently discovered intracellular RNA–protein complexes that play important roles in cellular stress response, regulation of gene expression, and also in co-translational protein assembly. In this review, a wide spectrum overview of assemblysomes is provided, including their discovery, mechanism of action, characteristics, and potential applications in several fields. Assemblysomes are distinct liquid–liquid phase-separated condensates; they have certain unique properties differentiating them from other cellular granules. They are composed of ribosome-nascent protein chain complexes and are resistant to cycloheximide and EDTA. The discovery and observation of intracellular condensates, like assemblysomes, have further expanded our knowledge of cellular stress response mechanisms, particularly in DNA repair processes and defense against proteotoxicity. Ribosome profiling experiments and next-generation sequencing of cDNA libraries extracted from EDTA-resistant pellets—of ultracentrifuged cell lysates—have shed light on the composition and dynamics of assemblysomes, revealing their role as repositories for pre-made stress-responsive ribosome-nascent chain complexes. This review gives an exploration of assemblysomes' potential clinical applications from multiple aspects, including their usefulness as diagnostic biomarkers for chemotherapy resistance and their implications in cancer therapy. In addition, in this overview, we raise some theoretical ideas of industrial and agricultural applications connected to these membraneless organelles. However, we see several challenges. On one hand, we need to understand the complexity of assemblysomes' multiple functions and regulations; on the other hand, it is essential to bridge the gap between fundamental research and practical applications. Overall, assemblysome research can be perceived as a promising upcomer in the improvement of biomedical settings as well as those connected to agricultural and industrial aspects.

1 | Introduction

In recent years, our understanding of intracellular organization and function has been revolutionized due to the discovery and growing data on intracellular membraneless cell organelles. As dynamic members of cytoplasmic membraneless organelles, assemblysomes are composed of translationally paused ribosome-nascent chain complexes (RNC), that are

Abbreviations: BLM, bloom syndrome helicase; CCR4, carbon catabolite repression 4; CHX, cycloheximide; CNOT1, CCR4-NOT transcription complex subunit 1; DmBLM, *Drosophila melanogaster* bloom syndrome helicase; DSBs, double-strand breaks; dSTORM, direct stochastic optical reconstruction microscopy; EDTA, ethylenediaminetetraacetic acid; HEX, 1,6-hexanediol; HR, homologous recombination; LLPS, liquid–liquid phase separation; *mwh*, *multiple wing hairs*; *flr*, *flare*; NCAs, Not1-containing assemblysomes; Not1, negative on TATA-less 1; NST, no special type; P-bodies, processing bodies; qPCR, quantitative polymerase chain reaction; RAD10, radiation sensitive 10; RAD14, radiation sensitive 14; RNAPII, RNA polymerase II; RNAi, ribonucleic acid interference; RNCs, ribosome-nascent chain complexes; Rpb1, RNA polymerase B (II) subunit 1; Rpt1, proteasome regulatory particle base subunit 1; Rpt2, proteasome regulatory particle base subunit 2; RQC, ribosome-associated quality control; RT, reverse transcription; SGs, stress granules; Sgs1, slow growth suppressor 1; SMART, somatic mutation and recombination test; SMC5, structural maintenance of chromosomes protein 5; SMC6, structural maintenance of chromosomes protein 6; TEM, transmission electron microscopy; UAS, upstream activation sequence; UV, ultraviolet.

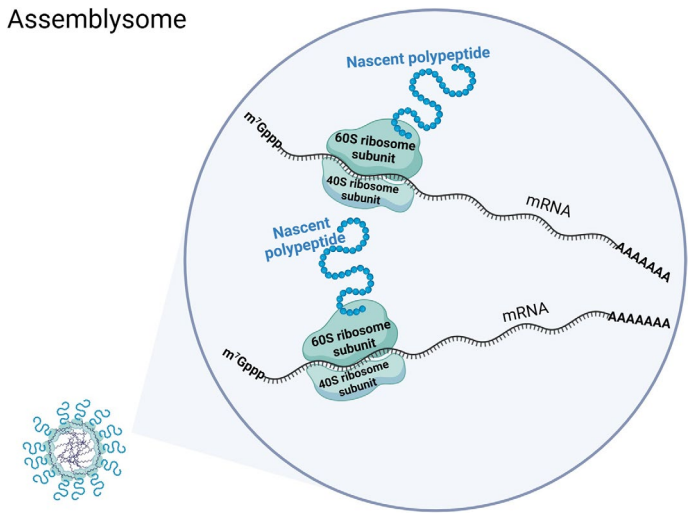

FIGURE 1 | Composition of assemblysomes. Assemblysomes are cytoplasmic granules composed of translationally inactive RNCs (Panasenko et al. 2019; Németh-Szatmári et al. 2023). Created in BioRender: Gombás, B. 2024. https://BioRender.com/n63i390.

likely to be formed by liquid–liquid phase separation (LLPS) and play crucial roles in the development of effective stress responses (Figure 1) (Panasenko et al. 2019; Németh-Szatmári et al. 2023). Not1-containing assemblysomes (NCAs) were identified in brewer's yeast (*Saccharomyces cerevisiae*) as a platform for co-translational assembly of proteasome subunits, later as a result of further bioinformatic analysis to find other potentially paused RNCs forming phase-separated assemblysomes, Sgs1-RNCs were identified (Panasenko et al. 2019; Németh-Szatmári et al. 2023). Sgs1 is a DNA helicase, which is part of the Top3–Rmi1–Sgs1 complex and plays a crucial role in the repair of DNA double-strand breaks (DSBs) in yeast (Gravel et al. 2008; Mimitou and Symington 2008; Zhu et al. 2008). It turned out that as NCAs, Sgs1-RNCs are composed of ribosomes paused in translation, containing nascent polypeptide chains and are only sedimentable via high-speed ultracentrifugation, exhibiting resistance to ethylenediaminetetraacetic acid (EDTA; disconnecting ribosome subunits) and cycloheximide (CHX; a translation elongation inhibitor), which prevents stress granule (SG) formation. CHX has no effect on assemblysomes as in their case translation is already stalled (Panasenko et al. 2019; Németh-Szatmári et al. 2023). In addition, it is relatively quick to finish translation of disordered nascent chains of RNCs essential for the formation of assemblysomes (Siegel and Sisler 1963; Panasenko et al. 2019; Németh-Szatmári et al. 2023). dSTORM and transmission electron microscopy (TEM) investigations suggested that multiple Sgs1-RNCs form a ring-like shape in which eight to nine ribosomes and the attached mRNAs, as well as the associated nascent protein chains, are closely packed (Németh-Szatmári et al. 2023). Assemblysomes contain RNCs with single ribosomes—instead of mRNAs loaded with multiple ribosomes giving rise to nascent chains of various lengths—suggesting that the translation initiation of consecutive ribosomes is blocked when assemblysomes are forming (Figure 2) (Panasenko et al. 2019). They store important gene products including DNA repair enzymes in the form of RNCs, capable of completing remaining translation in case of DNA damage (e.g., Németh-Szatmári et al. 2023; Gombás and Villányi 2024). Thus, these distinctive cellular machineries not only ensure efficient protein production but also play pivotal roles in maintaining cellular homeostasis and adapting to environmental stressors.

Assemblysomes can be understood as hubs for the coordinated protein assembly and/or maturation through the reversible binding of partially translated nascent gene products, ensuring their proper folding and functionality in a timely manner (Panasenko et al. 2019; Németh-Szatmári et al. 2023). Moreover, assemblysomes serve as platforms for the recruitment of chaperones, translation factors, and quality control machinery, thereby optimizing the efficiency and fidelity of protein synthesis (Panasenko et al. 2019; Németh-Szatmári et al. 2023; Nagy-Mikó et al. 2023).

Despite the importance of assemblysomes, their discovery is relatively recent; therefore, our understanding of their function, structure, and composition is still constantly evolving. In this paper, our goal is to present a broad overview from the discovery of assemblysomes to their potential future perspectives, not only in biomedical research and therapeutics but also in theoretically possible agricultural and industrial applications. In this article, we present what we know so far about the mechanisms underlying assemblysome formation, function, and regulation, and we also aim to highlight their possible importance and relevance in contributing to all colors of biotechnology.

2 | Assemblysomes Are Distinct Cytoplasmic Entities

It can be really challenging for cells to organize complex biochemical reactions properly in space and time. This problem is solved by different compartments that have been created through evolution, which provide unique chemical environments and suitable control options for different reactions (Hyman et al. 2014). Two main types of these organelles can be distinguished: most of them are bordered by a membrane (secretory vesicles, endoplasmic reticulum, mitochondria, lysosomes); however, in many cases, the surrounding

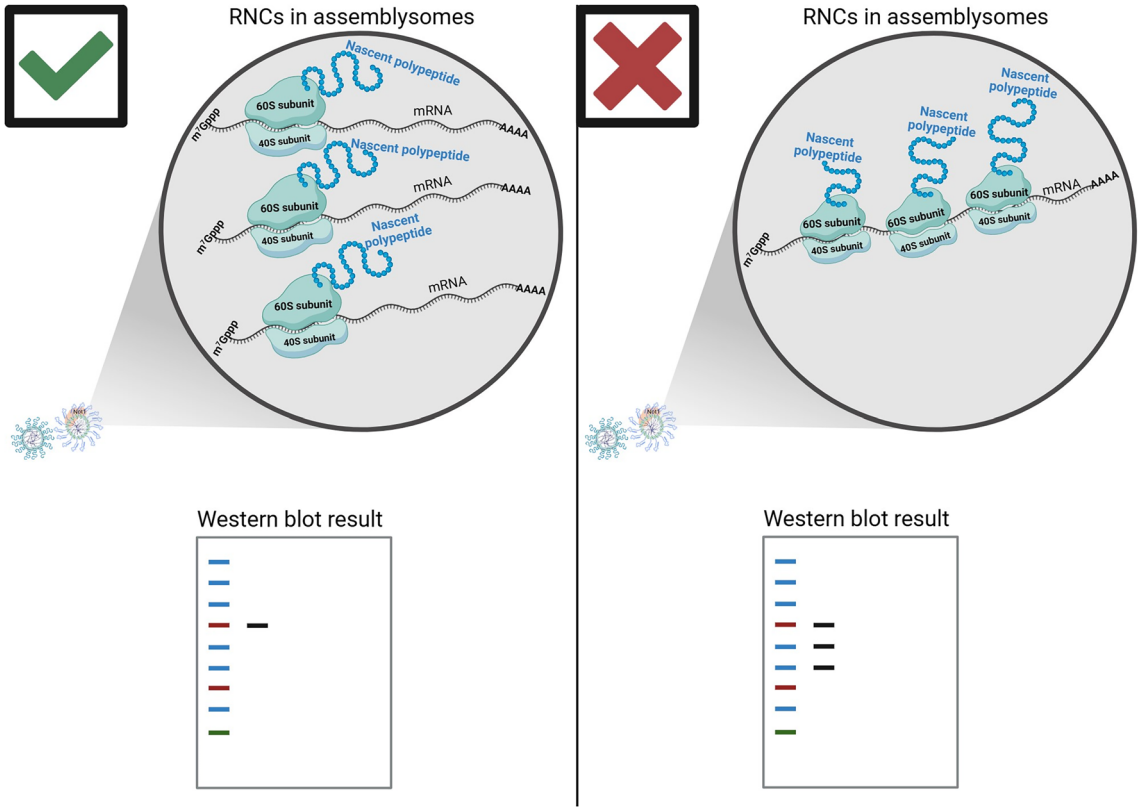

FIGURE 2 | Assemblysomes consist of single translationally paused ribosome-nascent chain complexes. Left: The real situation where assemblysomes contain only a single paused ribosome on every mRNA, which phase separates with the entire ribosome-nascent chain complex. This scenario leads to only one visible band on a Western blot image. Right: An imaginary scenario where assemblysomes contain bumped ribosomes on each mRNA, giving rise to fragments of different lengths. In this theoretic case, there are multiple translationally active ribosomes on mRNAs. (Panasenko et al. 2019; Németh-Szatmári et al. 2023). Created in BioRender: Gombás, B. 2024. https://BioRender.com/p74m056.

membrane is missing (e.g., nucleolus, centrosomes, Cajal bodies, SGs and processing bodies [P-bodies]) (Gall 2003; Boisvert et al. 2007; Luzio et al. 2007; Decker and Parker 2012; Mahen and Venkitaraman 2012; Friedman and Nunnari 2014; Shin and Brangwynne 2017). Membraneless compartments are dynamic structures. Like liquid drops, they can fuse, exchange components with the cytoplasm, and can be easily deformed under pressure (Hyman and Brangwynne 2011; Hyman and Simons 2012; Weber and Brangwynne 2012; Brangwynne 2013). Due to these properties, membraneless organelles are able to quickly react to environmental changes. However, the whole picture is more complicated. In some cases, they show multilayered structures with solid-like characters (Kroschwald et al. 2017). For instance, mammalian SGs contain a more stable, solid-like core, which is surrounded by a less concentrated shell (Jain et al. 2016; Wheeler et al. 2016).

More than likely, these liquid-phase condensates are formed by a well-regulated process called LLPS. During LLPS, proteins are usually separated from their environment by nucleic acids and create liquid-like drops in which they will be present in higher concentrations compared to their environment (Hyman et al. 2014). Many membraneless organelles have been shown to require a concentration threshold for their formation (Shin and Brangwynne 2017). Phase separation is driven by weak transient interactions between multivalent domains or intrinsically disordered regions (IDRs) (Shin and Brangwynne 2017). These regions are rich in polar and charged amino acids, while they are lacking hydrophobic amino acids that drive high-order folding (Dunker et al. 2001; Vucetic et al. 2003; Shin and Brangwynne 2017). Due to this structural composition, IDRs are unable to adopt a well-defined secondary or tertiary structure, but they are able to change their conformation by interacting with proteins or by post-translational modifications (Dyson and Wright 2005; Shin and Brangwynne 2017). This may explain why ribosomes arrested in translation in assemblysomes are able to be hidden from the ribosome quality control (RQC) mechanism (Inada 2013; Panasenko et al. 2019).

SGs form around translation initiation factors and non-translated mRNAs under conditions of limited translation initiation, while P-bodies aggregate translationally stalled mRNAs and contain components of the mRNA decay machinery (Figure 3) (Kedersha and Anderson 2002; Fenger-Grøn et al. 2005; Kedersha et al. 2005, 2008, 2013; Anderson and Kedersha 2006, 2008, 2009; Moser and Fritzler 2010; Khong et al. 2017). Both SGs and P-bodies contain proteins with IDRs, which are the leaders of phase separation (Nissan and Parker 2008).

The discovery and analysis of assemblysomes revealed a unique category of granules formed by LLPS, differentiated from other well-characterized condensates by several notable characteristics (Panasenko et al. 2019; Németh-Szatmári et al. 2023). Unlike previously discovered P-bodies and SGs,

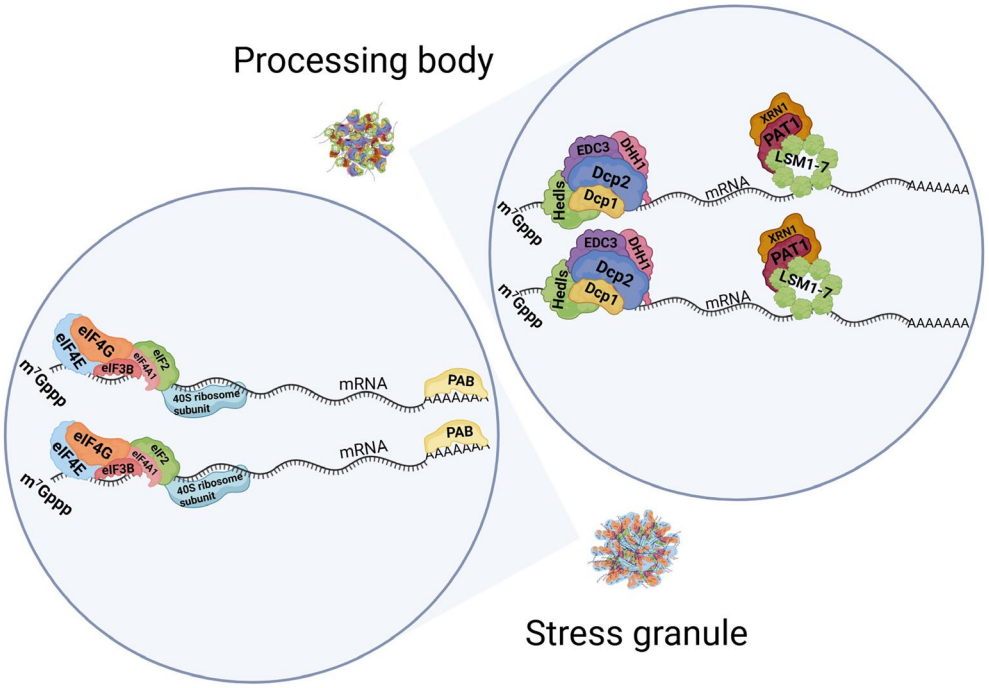

FIGURE 3 | The composition of P-bodies and stress granules. P-bodies and SGs are both phase-separated, membraneless organelles with some important protein elements indicated (Kedersha and Anderson 2002; Fenger-Grøn et al. 2005; Kedersha et al. 2005, 2008, 2013; Anderson and Kedersha 2006, 2008, 2009; Parker and Sheth 2007; Moser and Fritzler 2010). Created in BioRender: Gombás, B. 2024. https://BioRender.com/s34f213.

assemblysomes exhibit a lighter composition consisting mainly of different types of nascent polypeptide chains associated with ribosomes and mRNAs (Panasenko et al. 2019; Németh-Szatmári et al. 2023). In addition, Sgs1-containing assemblysomes, observed using dSTORM microscopy by following a tag on the N-terminal (N-term) sticking out of the ribosome, are comparatively smaller than both P-bodies and SGs (Gilks et al. 2004; Eulalio et al. 2007; Nissan and Parker 2008; Németh-Szatmári et al. 2023).

Second, assemblysomes display resistance to CHX, a trait not shared by P-bodies and SGs (more than likely it is a consequence of the paused state of translation and quick translation of the disordered N-term of the nascent protein protruding from the ribosome which drives their phase separation) and EDTA (assumed to be due to the compact spatial structure caused by LLPS) (Sheth and Parker 2003; Mollet et al. 2008; Panasenko et al. 2019). Conversely, P-bodies are sensitive to RNase, unlike SGs and assemblysomes (Teixeira et al. 2005; Jain et al. 2016; Khong et al. 2017; Panasenko et al. 2019).

Thirdly, according to our knowledge so far, all kinds of assemblysomes contain large ribosomal subunits, which are not found in SGs (Jain et al. 2016; Panasenko et al. 2019; Németh-Szatmári et al. 2023). Finally, assemblysomes undergo dismantling instead of formation upon UV treatment, a characteristic of particular interest (Kwon et al. 2007; Németh-Szatmári et al. 2023). It is plausible that there may be transitions from assemblysomes to SGs, similar to the communication between other cytoplasmic phase-separated granules, P-bodies, and SGs (Buchan et al. 2008). In summary, assemblysomes emerge as a distinct type of granule with unique responses to stressors, indicating a distinct functional role within cellular processes. Table 1 summarizes the main characteristics of assemblysomes, SGs, and P-bodies.

3 | About the Discovery of Assemblysomes

The discovery of assemblysomes, specifically NCAs, was a result of the investigation into the co-translational assembly mechanism of proteasome subunits Rpt1 and Rpt2 (Panasenko et al. 2019). Ribosome profiling experiments in yeast were performed to find mRNAs that are translated with frequent ribosome stalls, suggesting that protein synthesis is coupled with co-translational events in their case. An accumulation of ribosome footprints at specific codons of *RPT1* and *RPT2* mRNAs indicated ribosome pausing. This was particularly interesting because Rpt1 and Rpt2 proteins are adjacent proteins in the regulatory particle of the 26S proteasome. The ribosome profiling data revealed that ribosomes were pausing at specific codons within the *RPT1* and *RPT2* mRNAs, particularly near the sequences encoding the AAA-ATPase domains of both proteins, making it possible that interacting helices stick out of the ribosome exit tunnel when ribosomes reached the stalling codons. Based on the observation of ribosome pausing, it was hypothesized that co-translational assembly of Rpt1 and Rpt2 might occur. This would allow the nascent peptides to interact as they are being synthesized by ribosomes (see also the graphical abstract). To test this hypothesis, plasmids were created expressing nascent chains of Rpt1 and Rpt2, which were designed to mimic the stalled ribosome state during translation. These nascent chains were expressed with stretches of lysine codons at their C-term to induce ribosome stalling.

TABLE 1 | Main features of stress granules, P-bodies and assemblysomes. Modified from Németh-Szatmári et al. (2023).

	Stress granules	P-bodies	Assemblysomes
Liquid–liquid phase separation is involved in their formation	Yes (Wheeler et al. 2016)	Yes (Luo et al. 2018)	Yes (Németh-Szatmári et al. 2023)
Triggers in their formation	Stress induced (Kederhsa et al. 1999; Kedersha and Anderson 2002; Wheeler et al. 2016)	Constitutive in some cell lines, but increased in size and number in response to stress (Kedersha et al. 2005; Ohn et al. 2008; Wheeler et al. 2016)	Constitutive, but reportedly increased in size and number upon proteotoxic stress (Panasenko et al. 2019; Németh-Szatmári et al. 2023)
Size	100–2000 nm (Gilks et al. 2004; Anderson and Kedersha 2008, 2009; Kedersha and Anderson 2009; Moser and Fritzler 2010; Kedersha et al. 2013)	400–500 nm (Ayache et al. 2015)	100–200 nm (Németh-Szatmári et al. 2023)
Cycloheximide sensitivity	Yes (Mollet et al. 2008)	Yes (Sheth and Parker 2003)	No (Panasenko et al. 2019)
RNase sensitivity	No (Jain et al. 2016)	Yes (Sheth and Parker 2003)	No (Panasenko et al. 2019)
Velocity used for their sedimentation	18,000 × *g* (Jain et al. 2016)	10,000 × *g* (Hubstenberger et al. 2017)	163,000 × *g* (Németh-Szatmári et al. 2023.)
mRNAs can return to translation	Yes (Das et al. 2022)	Yes (Brengues et al. 2005)	Yes (Németh-Szatmári et al. 2023; Gombás and Villányi 2024)
Presence of Ribosome subunits	40S (Kedersha et al. 2002)	No ribosomal subunits (Sheth and Parker 2003; Parker and Sheth 2007)	40S and 60S (Panasenko et al. 2019)

Lysines are positively charged, and due to the negative charge of the ribosome exit tunnel, translation of multiple lysine residues results in ribosome stalling (Lu et al. 2007; Charneski and Hurst 2013; Panasenko et al. 2019). Assemblysome formation was observed when expression was induced from the plasmid, even when CHX was added in parallel with induction (Panasenko et al. 2019). The nascent chains of Rpt1 and Rpt2 were found to be stable and associated with ribosomes, despite the expectation that RQC mechanisms would degrade them (Inada 2013). This stability suggested a potential role in co-translational assembly, namely in protecting the RNC from RQC until the partner RNC arrives to be able to translate the two subunits into one protein complex providing stable and soluble assembly by co-translation. Further analysis using sucrose gradient sedimentation revealed the presence of stable Rpt1 and Rpt2 nascent chains in heavy fractions of the gradient, indicating their association with ribosomes in dense bodies. The heavy bodies containing stable Rpt1 and Rpt2 nascent chains were found to also contain the scaffold protein Not1 of the Ccr4-Not complex (Lau et al. 2009; Panasenko et al. 2019). Deletion and amino acid exchange in Rpt1-RNC revealed the crucial role of disordered N-terminal and ubiquitination of lysine residues in assemblysome formation. It was hypothesized that the role of these is to induce LLPS of RNCs that form assemblysomes; this way, protect the mRNAs and halfway produced nascent chains from RQC (Inada 2013; Panasenko et al. 2019).

4 | Limitations of Ribosome Profiling Approach in Identifying Assemblysome Components

Although ribosome profiling led to their discovery, it is challenging to identify assemblysome regulated gene products solely through ribosome profiling for several reasons. Ribosome profiling provides information only about the location of ribosomes on mRNA transcripts, indicating where translation occurs and where ribosomes might be paused at a certain timepoint (Ingolia et al. 2012). Assemblysomes, including NCAs, are dynamic complexes that may transiently form during specific stages of protein synthesis or assembly (Panasenko et al. 2019; Németh-Szatmári et al. 2023). Ribosome profiling provides static snapshots of translation at a particular moment and may not capture the transient interactions involved in assemblysome formation (Ingolia et al. 2012). However, authors encountered challenges, since identifying assemblysome components is the major limitation of ribosome profiling due to assemblysomes' resistance

to RNase digestion evidenced before (Panasenko et al. 2019). Ribosome profiling, which involves sequencing of mRNA fragments protected by ribosomes from RNase treatment, is unable to capture assemblysome-associated mRNAs due to their RNase-resistant nature (Ingolia et al. 2012).

This limitation necessitated the development of an innovative computational method to predict unidentified candidates of assemblysomes based on assumed stall sites and N-terminal disorder propensity of assemblysome components as described in Section 5 (Németh-Szatmári et al. 2023). Interestingly, a comparison of metagene analysis of ribosome profiling data between wild-type and *not5Δ* cells reveals that in the absence of Not5, there is a greater coverage of ribosomes on the first 100 codons compared to wild-type cells. This observation suggests a role for Not5 in condensate formation, potentially through the regulation of Not1 mRNA imprinting (Gupta et al. 2016; Allen et al. 2021). The higher ribosome coverage on the initial 100 codons at the 5′ end of mRNAs in *not5Δ* indicates an increased initiation rate in the mutant. This may be a result from a failure in NCA formation, which in turn reduces new initiation (Figure 2). This finding highlights the significance of widespread Not1-dependent assemblysome formation, as evidenced by metagene analysis of ribosome profiling data comparing wild-type and *not5Δ* yeast cells (Panasenko et al. 2019; Allen et al. 2021).

5 | Assemblysomes Contain Halfway Translated DNA Repair Components

Using a systematic in silico approach, candidate genes were predicted based on specific criteria, which were: amino-terminal disorder propensity, post-translational modification sites, and the presence of rare codon pairs, which lead to stalling and subsequent protrusion of the disordered N-terminal region from the ribosome (Matsuda et al. 2014; Shin and Brangwynne 2017; Panasenko et al. 2019; Németh-Szatmári et al. 2023). Computational analysis coupled with experimental validation facilitated the discovery of novel genes associated with assemblysomes; thus, their roles in stress response processes—especially in DNA repair—were illuminated (Németh-Szatmári et al. 2023). The significance of DNA repair components residing within partially translated assemblysomes lies in their ability to rapidly respond to DNA damage. In the event of DNA damage, transcription is halted; therefore, the presence of pre-existing mRNA encoding repair components is necessary that can swiftly complete translation upon damage occurrence (Sirbu and Cortez 2013). During stress, translation initiation as well is often halted, but having DNA repair proteins already present is not ideal due to their toxic nature (Holcik and Sonenberg 2005; Lavigne et al. 2017; Birkbak et al. 2018). Resuming translation from assemblysomes not only enables rapid response to DNA damage, crucial for cell and multicellular organism survival, but also prevents the potentially harmful expression of DNA repair proteins in the absence of damage (Birkbak et al. 2018). Thus, retaining these proteins in a partially synthesized state within assemblysomes serves a dual purpose: enabling prompt activation when needed and preventing detrimental effects from premature expression (Németh-Szatmári et al. 2023).

Later, for the purpose of revealing transcripts that are possibly regulated by assemblysomes in higher eukaryotes, ultracentrifuged lysates of the MCF7 breast tumor cell line were used to sequence mRNA libraries obtained from ribosome pellets and EDTA-pellets (Németh-Szatmári et al. 2023). The ranking of mRNAs based on their pellet and EDTA-pellet read ratio revealed distinct patterns. For each individual transcript, the lower this ratio is (closer to 1), the more likely it is that the mRNA is in assemblysomes, while the top mRNAs of the list (called EDTA-sensitive pellets) are associated with actively translating polysomes. Top-ranking mRNAs are enriched in mitochondrial and ribosomal protein transcripts, where translation is facilitated by Not1 mRNA imprinting instead of propagation of NCA formation (Gupta et al. 2016). Conversely, mRNAs enriched in stress response and DNA repair processes were prevalent in the bottom-ranking EDTA-pellet-associated transcripts, suggesting a segregation of transcripts based on their translational activity and functional relevance. This segregation highlights the dynamic nature of assemblysomes in regulating mRNA translation in response to cellular demands. A large number of identical gene ontology (GO) categories were observed between the top-ranking amino-terminal disordered human complex subunits and EDTA-pellet-associated mRNAs, particularly those related to stress response and DNA repair (Table 2).

Furthermore, analysis of the distribution of disordered amino acids among the top and bottom hits of the mRNA ranking from sequencing confirms that EDTA-pellet-associated mRNAs encode more disordered proteins compared to translated mRNAs, which agrees with previous results. These findings validate the in silico prediction that amino-terminal disorder propensity indicates the presence of gene products in EDTA-pellets. Thus, it suggests a role for the disordered N-terminal sequence of the proteins protruding from the exit tunnel of the 60S ribosome in the formation of assemblysomes to regulate translation, particularly in processes related to stress response and DNA repair. Moreover, the proportion of mRNAs encoding proteins with amino-terminal disordered domains in EDTA-pellets was enriched compared to randomly selected mRNAs, which is an indirect confirmation of observations made earlier.

Thus, the above summarized key findings confirm the role of assemblysomes in the DNA damage response. Németh-Szatmári et al. propose that, in some sense, assemblysomes can be understood as multifaceted intracellular repositories for several different pre-made stress-responsive gene products, allowing for rapid protein synthesis without the need for de novo transcription and translational initiation (Németh-Szatmári et al. 2023).

These findings indicate that assemblysomes could make it possible to respond promptly in case of stress-induced gene expression, particularly in eukaryotic DNA damage response. This mechanism competes with stress-induced gene expression in prokaryotes, where transcription occurs simultaneously with the rate-limiting translation process (Proshkin et al. 2010; Watson 1970). This holds significance in terms of competition, especially considering that in prokaryotes, transcription and translation occur concurrently, thereby making the rate-limiting

TABLE 2 | GO term analysis on ultracentrifugated pellets (±EDTA) associated mRNAs.

	Reflist	Input	Expected	(Under/over)	Fold enrichment
Overrepresented GOs in EDTA-pellet					
Regulation of microtubule cytoskeleton organization (GO:0070507)	157	36	15.88	+	2.27
Actin cytoskeleton organization (GO:0030036)	544	111	55.01	+	2.02
Actin filament-based process (GO:0030029)	610	120	61.68	+	1.95
Regulation of cytoskeleton organization (GO:0051493)	535	103	54.10	+	1.90
Microtubule cytoskeleton organization (GO:0000226)	532	102	53.80	+	1.90
Cytoskeleton organization (GO:0007010)	1217	231	123.07	+	1.88
Cellular response to alcohol (GO:0097306)	93	22	9.40	+	2.34
Cellular response to endogenous stimulus (GO:0071495)	1078	170	109.01	+	1.56
Regulation of cellular response to stress (GO:0080135)	709	111	71.70	+	1.55
Cellular response to DNA damage stimulus (GO:0006974)	734	131	74.22	+	1.76
DNA repair (GO:0006281)	490	86	49.55	+	1.74
Regulation of response to DNA damage stimulus (GO:2001020)	310	59	31.35	+	1.88
Regulation of DNA repair (GO:0006282)	210	41	21.24	+	1.93
Double-strand break repair (GO:0006302)	201	41	20.33	+	2.02
Signal transduction in response to DNA damage (GO:0042770)	136	32	13.75	+	2.33
Regulation of double-strand break repair (GO:2000779)	134	29	13.55	+	2.14
Regulation of protein-containing complex assembly (GO:0043254)	410	65	41.46	+	1.57
Plasma membrane bounded cell projection assembly (GO:0120031)	405	64	40.95	+	1.56
Regulation of protein-containing complex disassembly (GO:0043244)	129	32	13.04	+	2.45
Negative regulation of protein-containing complex disassembly (GO:0043242)	80	21	8.09	+	2.60
Positive regulation of autophagy (GO:0010508)	140	28	14.16	+	1.98
Regulation of autophagy (GO:0010506)	340	57	34.38	+	1.66
Overrepresented GOs in pellet					
Translation (GO:0006412)	377	134	26.35	+	5.09
Cytoplasmic translation (GO:0002181)	124	73	8.67	+	8.42
Ribosome biogenesis (GO:0042254)	296	64	20.69	+	3.09
Mitochondrial translation (GO:0032543)	108	47	7.55	+	6.23

(Continues)

TABLE 2 | (Continued)

	Reflist	Input	Expected	(Under/over)	Fold enrichment
Ribosomal small subunit biogenesis (GO:0042274)	74	24	5.17	+	4.64
Ribosomal large subunit biogenesis (GO:0042273)	72	24	5.03	+	4.77
Mitochondrial gene expression (GO:0140053)	139	49	9.71	+	5.04
Mitochondrial ATP synthesis coupled electron transport (GO:0042775)	90	46	6.29	+	7.31
ATP synthesis coupled electron transport (GO:0042773)	90	46	6.29	+	7.31
ATP biosynthetic process (GO:0006754)	85	46	5.94	+	7.74
Mitochondrial respiratory chain complex assembly (GO:0033108)	95	43	6.64	+	6.48
Proton motive force-driven ATP synthesis (GO:0015986)	73	43	5.10	+	8.43
NADH dehydrogenase complex assembly (GO:0010257)	60	31	4.19	+	7.39
Mitochondrial respiratory chain complex I assembly (GO:0032981)	60	31	4.19	+	7.39
Mitochondrial transport (GO:0006839)	153	27	10.69	+	2.52
Mitochondrial membrane organization (GO:0007006)	123	21	8.60	+	2.44
Underrepresented GOs in pellet					
Response to stimulus (GO:0050896)	8096	472	565.84	−	0.83
Cellular response to stimulus (GO:0051716)	6479	356	452.83	−	0.79
Response to chemical (GO:0042221)	4035	229	282.01	−	0.81
Regulation of response to stimulus (GO:0048583)	4001	216	279.64	−	0.77
Regulation of signaling (GO:0023051)	3355	186	234.49	−	0.79
Detection of stimulus (GO:0051606)	685	14	47.88	−	0.29
Detection of chemical stimulus (GO:0009593)	526	7	36.76	−	0.19

Note: The top 3000 pellet-associated mRNAs and the bottom 3000 EDTA-pellet-associated mRNAs (of a pellet/EDTA-pellet read count ratio ranked mRNA list) were used for GO term analysis. Gene ontology categories' color codes stand according to the followings: yellow: cytoskeletal; blue: stress response; pink: DNA repair; gray: complex assembly; violet: autophagy; dark green: translation; red: mitochondrial. Modified from Németh-Szatmári et al. (2023).

step of a gene expression response solely reliant on translation speed. It is improbable that evolution allowed eukaryotic cells to conduct the two primary gene expression processes sequentially in response to stress.

6 | Assemblysomes Are Formed Upon Proteotoxic but Dismantled Upon Genotoxic Stress

During proteotoxic stress, cells are basically dependent on several different pathways to cope with the accumulation of misfolded and aggregated proteins (Hipp et al. 2014). Increased 26S proteasome assembly is one of the major responses to proteotoxic stress, which provides the degradation of inoperable proteins (Panasenko et al. 2019). A prerequisite for a quick and efficient proteasome assembly is the rapid appearance of assemblysomes containing two proteasome regulatory particle subunits: Rpt1 and Rpt2 (Panasenko et al. 2019). NCAs are particles in which *RPT1* or *RPT2* mRNAs co-localize with Not1 (Panasenko et al. 2019). NCAs not only facilitate the co-translational assembly of Rpt1 and Rpt2, but also act as crucial regulators of protein homeostasis under proteotoxic stress. Assemblysomes appear to regulate translation initiation on paused RNCs, thereby preventing ribosome collision and counteracting RQC (Figure 2) (Inada 2013; Németh-Szatmári et al. 2023). By limiting new translation initiation, assemblysomes provide an environment conducive for the association of partner subunits. In addition, they concentrate factors necessary for folding, pausing, and subsequent translation elongation. Critically, proteins released from NCAs without their partners are rendered unstable, potentially

preventing their aggregation and maintaining cytoplasmic integrity; this is especially important under proteotoxic stress conditions.

From a different perspective, the localization of CNot1 was investigated in human LNCaP prostate cancer cells under proteotoxic stress induced by arsenite treatment. Immunofluorescence analysis revealed the formation of distinct punctate CNot1 particles in the cytoplasm before arsenite stress, which increased in number and brightness post-stress. These particles were distinct from classical SGs marked by G3BP1 and were also induced by other stressors and proteasome inhibition. Notably, CNot1 particles were unaffected by CHX treatment, which inhibits SG formation (Panasenko et al. 2019). Further investigation using RNA fluorescence in situ hybridization demonstrated colocalization of CNot1 with Rpt1- and Rpt2-encoding mRNAs, which encode proteasome subunits. Importantly, the knockdown of CNot1 disrupted the colocalization of *RPT1* and *RPT2* mRNAs under arsenite stress, highlighting the necessity of CNot1 for their association. These observations revealed that the granules, which cannot be formed without CNot1—NCAs—play a prominent role in the proteotoxic stress response in higher eukaryotes (Panasenko et al. 2019).

In contrast, genotoxic effects, such as UV (in *Saccharomyces cerevisiae*) and X-ray (in *Drosophila melanogaster*), have been found to reduce the amount of DNA repair protein encoding mRNAs stored in assemblysomes while increasing the quantity of them in polysomes (Németh-Szatmári et al. 2023; Gombás and Villányi 2024). In silico analysis revealed assemblysome targets: *SGS1*, *RAD10*, and *RAD14* mRNAs—encoding proteins involved in DNA repair—were confirmed to be present in EDTA-resistant ribosome pellets. UV irradiation of yeast cultures—mimicking acute DNA damage—caused an increase in pellet/EDTA-pellet ratios. The mRNA content of these fractions was analyzed using RT-qPCR to quantify actively translating mRNAs of specific genes. These increased ratios suggest a shift of assemblysome-associated mRNAs toward translating polysomes in response to genotoxic stress, without significant de novo mRNA synthesis. The observed increase in mRNA ratios upon UV treatment suggests a reactivation of translation within assemblysomes to meet the demands of DNA damage response processes (see also the graphical abstract) (Németh-Szatmári et al. 2023).

The above-mentioned ultracentrifugation-based method was employed to separate different ribonucleoprotein entities, including assemblysomes, P-bodies, SGs, and polysomes, based on the different sucrose sedimentation properties and EDTA sensitivity of these entities (Jain et al. 2016; Hubstenberger et al. 2017; Panasenko et al. 2019; Németh-Szatmári et al. 2023). EDTA was used to dissociate polysomes, allowing the isolation of assemblysomes. The mRNA content of these fractions was analyzed using RT-qPCR to quantify translating (actively in polysomes or stalled in assemblysomes) forms of specific mRNAs (e.g., *SGS1*, *RAD10*, *RAD14*).

The RT-qPCR analysis revealed distinct mRNA profiles in pellets obtained with and without EDTA treatment. Pellets can be considered as samples containing both polysomes and assemblysomes, while the EDTA-pellet contains only assemblysomes. *ACT1* mRNA, encoding actin, served as a control and showed a high pellet/EDTA-pellet ratio, indicating association with polysomes rather than assemblysomes. In the case of *SGS1* mRNA, a ratio close to 1 was exhibited, suggesting enrichment in the EDTA-resistant fraction, characteristic of assemblysomes. *RAD10* and *RAD14* mRNAs showed higher ratios than *SGS1*, indicating partial association with assemblysomes (Figure 4).

The observed increase in mRNA ratios upon UV treatment indicates translational reactivation of RNCs in assemblysomes; that way, they can meet the demands of DNA damage response processes (see also the graphical abstract) (Németh-Szatmári et al. 2023).

To give an example of how the pellet/EDTA-pellet ratio helps to identify how individual mRNAs shift between assemblysomes and ribosome-bound states, we provide Figure 4 summarizing the results of the above qPCR analysis of EDTA-pellet and pellet fractions of sucrose cushion ultracentrifuge experiments and total extracts modified from Németh-Szatmári et al. (2022, 2023). Figure 4 shows how a couple of yeast (*S. cerevisiae*) gene products involved in genotoxic (*SGS1*, *RAD10*, *RAD14*) and proteotoxic (*RPT1*, *RPT2*) stress responses shift between assemblysome and ribosome-associated forms in response to these stresses, and how their concentrations change in total cellular extracts. A general trend is that genotoxic stress (UV in this example) leads to decreased mRNA concentrations in EDTA-pellets, whereas proteotoxic stress (metal exposure in this case) has the opposite effect. It is important to notice that according to these results, assemblysome dynamics are regulated in unison, regardless of which gene products they contain. Proteotoxic stress enhances their formation, but genotoxic stress leads to their transformation into polysomes. The assemblysome-polysome shift can be better visualized if we follow the pellet/EDTA-pellet ratios (Figure 4).

7 | 1,6-Hexanediol, a Compound That Transforms Assemblysomes Into Polysomes

As stated above, the ranking of yeast and human complex subunits based on their N-terminal disorder propensity revealed that stress-responsive genes, particularly those involved in DNA repair, were among the highest-ranking ones (Németh-Szatmári et al. 2023). Ribosomes themselves contain disordered protein structures on their surface (Peng et al. 2014; Vandelli et al. 2022). At the same time, the N-terminal disordered amino acid sequence played a crucial role in the formation of assemblysomes, as reported in yeast, suggesting the significance of disordered structures in assemblysome formation from RNCs (Panasenko et al. 2019). Given that disordered proteins have been shown to be capable of undergoing LLPS, it seemed plausible that assemblysomes form through a similar mechanism as SGs and P-bodies, which are also composed of disordered proteins (Vandelli et al. 2022). The hypothesis proposed that when ribosomes with disordered surfaces express proteins with disordered N-terminals upon protrusion from the exit tunnel of the ribosome, a biophysical reaction occurs, causing them to undergo reversible phase separation into a gel-like structure through LLPS. This mechanism was suggested to safeguard these RNCs from RQC (Inada 2013; Panasenko et al. 2019). To test this hypothesis, 1,6-hexanediol

(HEX) was employed to disrupt the alleged phase separation. HEX is an aliphatic alcohol that is commonly used to test membraneless organelles—formed by LLPS (P-bodies, SGs)—due to its ability to interfere with weak hydrophobic protein–protein/RNA–protein interactions (Kroschwald et al. 2017). Literature data suggest that the effect of HEX is concentration-dependent because of its pleiotropic abilities and that this aliphatic alcohol can effectively disrupt weak, hydrophobic interactions, while electrostatic interactions are insensitive to treatment (Düster et al. 2021; Muzzopappa et al. 2022). Experiments in mammalian and yeast cells have shown that pre-existing SGs reappeared after 10 min following

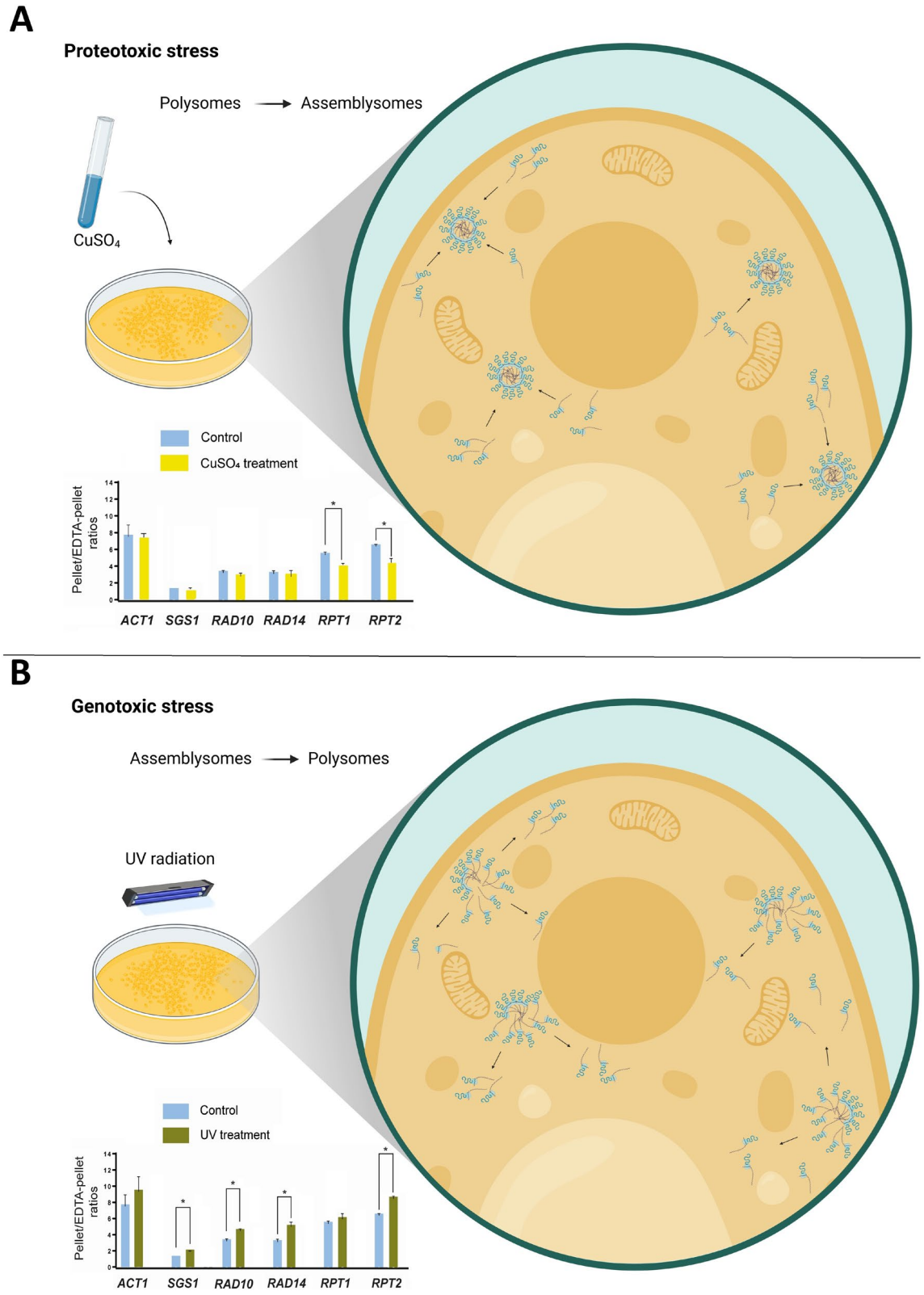

FIGURE 4 | Legend on next page.

FIGURE 4 | Results of qPCR analysis following mRNAs encoding factors involved in genotoxic and proteotoxic stresses. Schematic representation of applied treatments of yeast cells, with an enlarged cell depicting assemblysome and polysome responses to the different treatments. The data presented on each inlet is from Németh-Szatmári et al. (2022, 2023). Previously introduced factors involved in genotoxic (*SGS1*, *RAD10*, *RAD14*) and in proteotoxic (*RPT1* and *RPT2*) stresses were followed in ultracentrifuge pellets in the presence or absence of EDTA with qPCR. Pellet/EDTA-pellet ratios were calculated based on C_t values of qPCR experiments. *ACT1*: actin was used as a control. Significant differences are highlighted by *. (Németh-Szatmári et al. 2022, 2023). (A) Yeast cells treated with $CuSO_4$ as a proteotoxic agent, which leads to polysome to assemblysome formation of all indicated RNCs, even those that counteract genotoxic stress. Created in BioRender: Gombás, B. 2025. https://BioRender.com/o06e159. (B) Yeast cells treated with UV as a genotoxic agent, which leads to the assembly of polysome formation of all indicated RNCs, even those that counteract proteotoxic stress. Created in BioRender: Gombás, B. 2025. https://BioRender.com/z23a636.

the initial reduction (Wheeler et al. 2016). In the case of yeasts, 10% HEX treatment disrupted the nuclear pore and cytoskeletal network, while in mammalian cells, 3.5% HEX treatment affected cell viability and morphology (Wheeler et al. 2016). Although some questions still remain about its ability to interfere with LLPS, results demonstrate that HEX is an effective tool for testing the physical properties of membraneless compartments, with proper care (e.g., different growth and HEX treatment conditions) (Wheeler et al. 2016; Kroschwald et al. 2017; Liu et al. 2021; Muzzopappa et al. 2022; Németh-Szatmári et al. 2023). Therefore, the concentration of HEX was carefully optimized to selectively dismantle the presumably phase-separated ribosomes (assemblysomes) in the cytoplasm while preserving structures formed by phase separation, such as those in the nucleus, thus allowing for targeted investigation (Itoh et al. 2021; Lafontaine et al. 2021). Another challenge arose from reports indicating that HEX interferes with the function of kinases and phosphatases, compounding its pleiotropic effects (Düster et al. 2021). However, the strategy of using low concentrations of HEX to selectively target cytoplasmic phase separation has proven to be effective (Németh-Szatmári et al. 2023).

7.1 | Experiments on Human Cell Lines

When applied at concentrations that did not adversely affect kinases and phosphatases, HEX did not impair the DNA damage response of the radiation-resistant human breast carcinoma cell line A549 (Németh-Szatmári et al. 2023). This demonstrated that nuclear phase separation events involved in the DNA damage response remained unaffected by low-concentration HEX treatment. Interestingly, consecutive X-ray treatment—in combination with HEX—resulted in significantly reduced survival of A549 cells. This phenomenon was attributed to the dissolution of phase-separated ribosomes by HEX, which could then transition into polysomes and complete the translation of DNA damage response elements—a beneficial process in the event of DNA damage. However, once these ribosomes were dismantled, the pool of partially synthesized genotoxic stress response proteins was depleted. Consequently, upon the second irradiation, the cells lacked the necessary protection, and the survival rate dropped significantly compared to the non-HEX-treated control (Németh-Szatmári et al. 2023). Assemblysome-polysome transforming potential of HEX was also confirmed on A549 cells using electron microscopy by following the significant reduction of ring-oriented ribosomes upon treatment (Németh-Szatmári et al. 2023).

7.2 | Experiments on *D. melanogaster*

Although HEX has been proved to be effective in dissolving assemblysomes and turning them into translating ribosomes in single cell systems, new data was published recently about its similar impact in a metazoan organism (Amankwaa et al. 2022; Németh-Szatmári et al. 2023; Gombás and Villányi 2024). Not only the toxicity but also the mutagenic activity of HEX were tested in vivo in *D. melanogaster* using the sophisticated SMART wingspot method, which provides information about the genotoxic capacity of any chosen effect on fruit fly's wing disc cells (Szabad et al. 1983; Würgler et al. 1985; Graf et al. 1984; Szabad 2021). This system is based on *mwh*/*flr* (recessive alleles determining wing hair type) transheterozygote animals, that genotype enables the creation of mosaic spots (easily distinguishable from wild hair phenotype) resulted by homologous recombination (HR) events in imaginal disc cells. First of all third instar larvae were exposed to the chosen effect. In the second step looking for genetic mosaic spots on the wings of the adult animals (emerged from the larvae) took place. Mosaic spots on wings are induced by earlier mitotic recombination events in the wing disc cells, which resulted *mwh* homozygote daughter cells. The last step was calculation of mutational frequency using the formula: $f = \frac{n \times m}{N \times C}$, (where "$n$" is the number of mosaic spots, "m" is the average size of the spots, "N" is the number of wings checked, and "C" is the number of cells comprising a wing blade [about 30 000]). Already knowing that in other organisms assemblysomes contain certain helicases (orthologues of *D. melanogaster*'s DmBLM: Sgs1, BLM) involved in genotoxic stress response known for creating damage in DNA when overexpressed, our main goal was to determine the indirect genotoxic effect of this chemical (Ellis et al. 1995; Kusano et al. 1999; Hickson 2003; Birkbak et al. 2018; Németh-Szatmári et al. 2023). According to the starting theory (at specific concentrations) HEX can cause chromosome rearrangements indirectly by dissolving LLPS assemblysomes that way liberating certain DNA repair proteins (e.g., DmBLM) that can modify the intact DNA by inducing HR. In the first step HEX was mixed into standard Drosophila medium that made it possible to execute a systematic treatment due to ingestion of the compound. The results of this study strongly support the original idea as we found HEX can induce HR in Drosophila wing disc cells by a systematic exposure (Figure 5). The compound 1,2-hexanediol, which is structurally similar to HEX but has not been shown to interfere with LLPS was used as control molecule. The effect of HEX was mitigated successfully with X-ray treatment (unlike 1,2-hexanediol's), and it

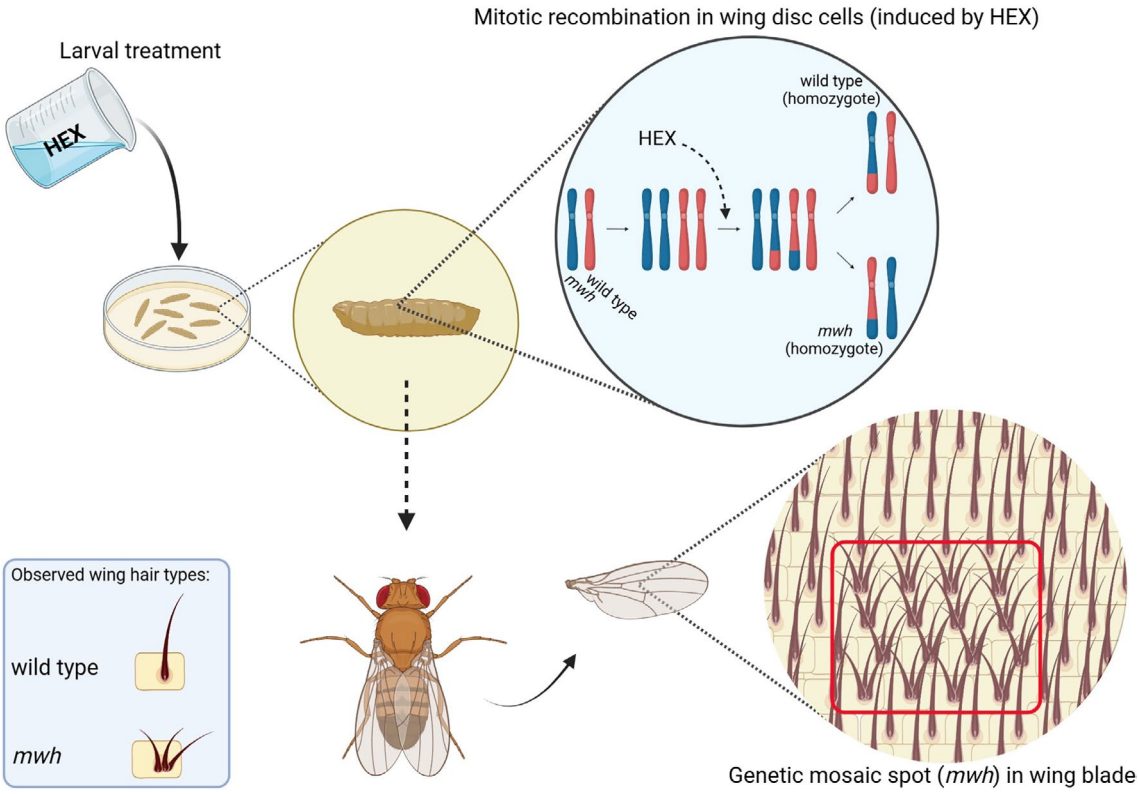

FIGURE 5 | SMART wingspot method indicated that HEX is inducing HR in *Drosophila melanogaster*'s wing disc cells. *mwh* mosaic spots on wing blades of fruit flies emerged from HEX-treated larvae are indicators of previous mitotic recombination events in larval wing disc cells resulting from the treatment (Gombás and Villányi 2024). Created in BioRender. Gombás, B. 2024. https://BioRender.com/h28q411.

turned out it was a consequence of its ability to induce DNA DSBs (Bradley and Kohn 1979). Knowing SMC5/6 complex's assumed mediator role in recruiting RecQ helicases, like BLM to DNA DSBs in eukaryotes, the question about its impact in HEX's mechanism of action arose (Harvey et al. 2004; Eladad et al. 2005; Lehmann 2005; Bermúdez-López et al. 2016; Rossi et al. 2020). As interpreted, the mitigating effect of X-ray was a result of the following phenomenon. DSBs caused by X-ray irradiation (when it was added to HEX treatment) made SMC5/6 complex to recruit DmBLM to the sites of DNA damage—as it was presumed previously—unlike treatment with HEX alone which led to several recombination events in random places throughout the chromosomes (Gombás and Villányi 2024). It was supported by RNAi silencing in UAS Gal4 system that SMC5 recruits DmBLM to the sites of DSBs, as it was found that the effect of X-ray was additive to HEX's in SMC5-silenced fruit flies (Gombás and Villányi 2024). This study reveals also that HEX was less toxic to a balancer chromosome carrier fruit fly strain, which is another indirect sign that strengthen the starting theory, knowing that balancer chromosomes inhibit HR (Sturtevan 1926; Ashburner 1989; Greenspan 1997; Lindsley and Zimm 2012; Crown et al. 2018; Gombás and Villányi 2024). Another result that supports the hypothesis about the connection between HEX and HR is that the rescue effect of X-ray did not occur in case of UV treatment which is unlikely to induce this type of modification in DNA (Sinha and Häder 2002).

Also, the changes in *DmBLM* mRNA between polysomes and assemblysomes were followed by RT-qPCR. HEX dramatically depleted the mRNA content of assemblysomes (even more than X-ray irradiation did), while it increased its presence in translating polysomes (Gombás and Villányi 2024). These findings not only support the previous data about HEX's effect on assemblysomes and strengthens the assumption of HEX's presumed mechanism of action, but also highlight the important roles of these granules in the genotoxic stress response and even open up perspectives for future applications of this chemical too.

8 | Signs of NCAs Discovered in Clinical Samples may Predict Failure of Chemotherapy

The CNot1 protein, the largest subunit of the Ccr4-Not complex and the most important, essential subunit of NCAs, potentially contributes to tumor cell resistance to transcription inhibitors like epirubicin (Villanyi and Collart 2015; Nagy-Mikó et al. 2023). A widely administered drug in neoadjuvant chemotherapy, epirubicin is an anthracycline topoisomerase II inhibitor—that acts as a DNA-intercalating agent—blocking both the transcription and replication processes (Robert 1994). The formation of condensates containing mRNA—that can act as reservoirs for gene expression—can facilitate resistance to this compound. Rpb1, a subunit of RNA polymerase II (RNAPII), is prone to aggregate when RNAPII assembly is compromised, and the Ccr4-Not complex, involving Not1, is crucial for RNAPII assembly, particularly for RPB1 folding (Villanyi et al. 2014). Invasive breast carcinoma of no special type (NST), especially non-regressive tumors, might be able to exhibit resistance to chemotherapy drugs like epirubicin by compensating for transcription loss via

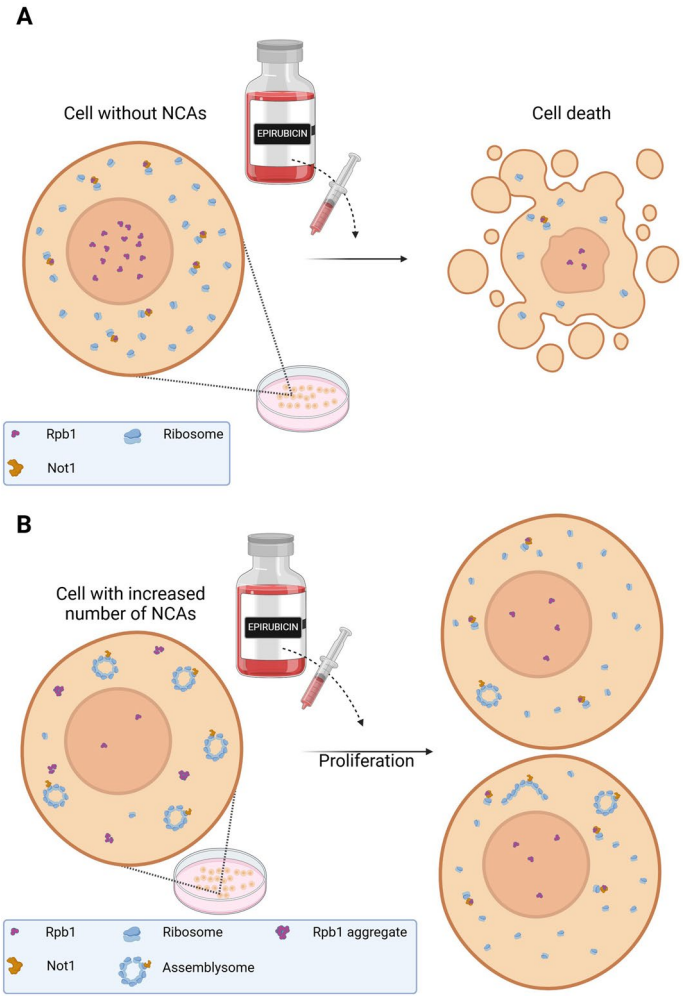

FIGURE 6 | Cytoplasmic Rpb1 aggregation is a sign of elevated assemblysome presence and predicts resistance to neoadjuvant chemotherapy. (A) Tumor cell clones where Not1 is soluble exhibit normal Rpb1 folding and rely on transcription as a main gene expression step in maintaining cellular homeostasis, are sensitive to the transcription blocker epirubicin. In this case, transcription blockers can completely prevent gene expression, leading to cell death (Nagy-Mikó et al. 2023). Created in BioRender. Gombás, B. 2024. https://BioRender.com/p18s738. (B) Tumor cell clones where Not1 is associated with phase separated entities exhibit Rpb1 aggregation due to poor folding and rely on translation as a main gene expression step in maintaining cellular homeostasis, are resistant to the transcription blocker epirubicin. In this case, phase-separated granules can serve as reservoirs of half-translated gene products; therefore, cells can survive even in the presence of transcription blockers (Nagy-Mikó et al. 2023). Created in BioRender. Gombás, B. 2024. https://BioRender.com/j43h899.

mRNA reservoirs—such as assemblysomes—potentially leading to Not1 depletion or limitation and impaired protein complex assembly including RNAPII, which serves as a biomarker (Figure 6) (Nagy-Mikó et al. 2023). Therefore, Rpb1 aggregation present in tumor biopsy samples could be an indicator of resistance to transcription blockers (suggesting a potential predictive marker for chemotherapy resistance in breast cancer) (Nagy-Mikó et al. 2023). Protein-mediated LLPS was hypothesized to contribute to carcinogenesis, which indirectly supports the original idea (Ming et al. 2019). Cytoplasmic aggregation of Rpb1 is associated with resistance to epirubicin, suggesting potential treatment implications. Patients exhibiting cytoplasmic Rpb1 foci in biopsy samples may be at high risk of ineffective treatment with transcription blockers and could consider alternative therapies like surgery or administration of proteasome inhibitors instead of nucleoside analogs. The aggregation of Rpb1 is observed in various cancer types but not in healthy tissues, indicating a potential oncogenic mutation rather than a pre-existing condition in healthy tissues. This screening methodology and the link to NCAs (which are responsible for chemotherapy resistance) may assist in identifying and addressing non-regressive cases, but it still requires further validation and data collection for clinical implementation (Nagy-Mikó et al. 2023).

9 | Future Perspectives Regarding the Role of Assemblysomes

Assemblysomes, as condensates composed of RNAs, ribosomes, and proteins, are implicated in the cellular stress response. We assume their composition, dynamics, and regulatory mechanisms could suggest exciting prospects for exploiting their potential in clinical, agricultural, and industrial settings; furthermore, they can provide information in the context of environmental protection (Figure 7). Despite the very promising potential of assemblysomes in various areas, which will be

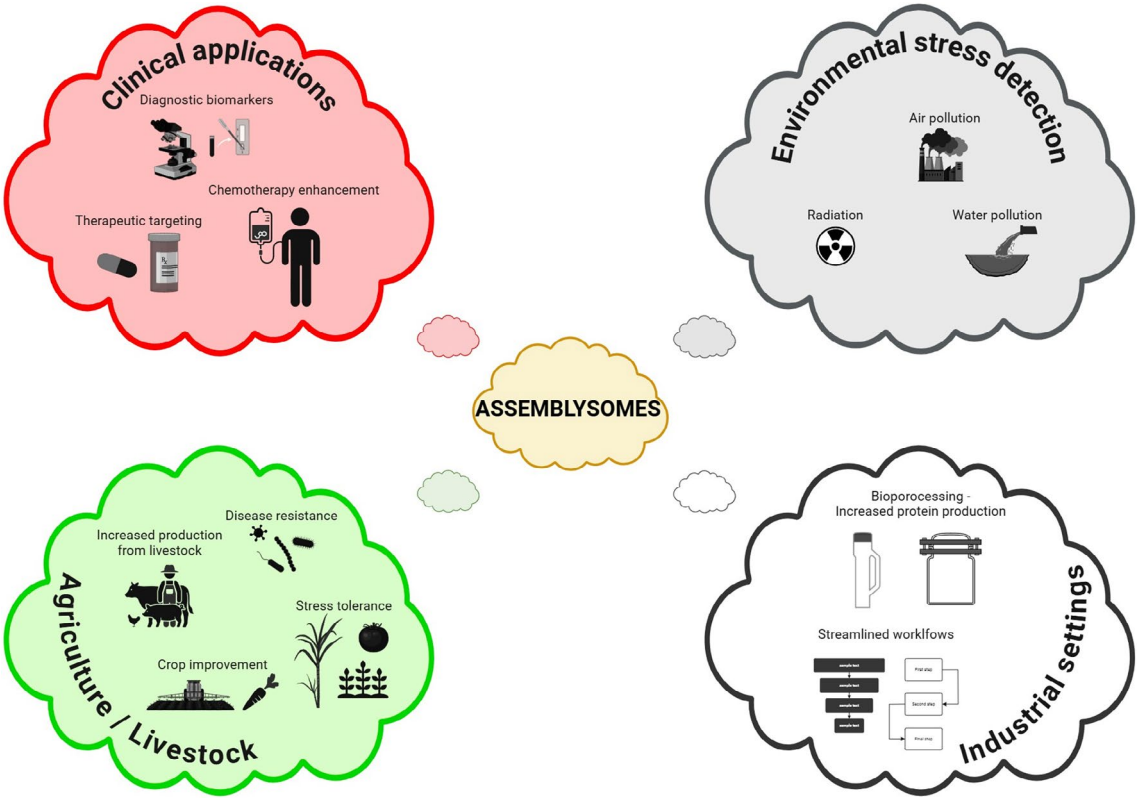

FIGURE 7 | Suggested application fields of assemblysomes. Assemblysomes could be applied in various fields due to their complex roles in the cellular physiology of eukaryotes. Even four "colors" of biotechnology may be affected by the utilization of the data available on assemblysomes. Created in BioRender. Gombás, B. 2024. https://BioRender.com/z53n137.

described in the following paragraphs, most of these ideas still need to be confirmed and supported by scientific data.

9.1 | Clinical Applications

9.1.1 | Therapeutic Targeting

In addition to offering indirect intracellular indicators of certain oncological treatments, assemblysomes could serve as targets for developing new cancer therapies, similarly to SGs, which are known to be already involved in tumor treatment strategies (Li et al. 2023; Jia et al. 2024). SGs regulate gene expression, which correlates with proliferation, invasion, metastasis, survival, metabolism reprogramming, and immune evasion of cancer (Riggs et al. 2020; Li et al. 2023). Therefore, monitoring SG components in cancer has oncotherapeutic relevance because these membraneless organelles act on several pathways to fight against antitumor therapies: influence cell cycle inhibition; enable the translation of components of signaling pathways involved in survival; and reduce therapy-induced protein degradation (Zhou et al. 2023; Jia et al. 2024). It is also essential to take into consideration that many chemotherapeutic agents (like cisplatin, bortezomib) induce the formation of SGs, thereby helping the development of resistance against the applied therapy, similarly to the observations in neoadjuvant therapy (Fournier et al. 2010; de Vilas-Boas et al. 2016; Nagy-Mikó et al. 2023; Jia et al. 2024). Although targeting these components in anticancer therapies promises many advantages, these are broad-spectrum agents that are unable to distinguish between pathological and normal stress response SGs (Jia et al. 2024). It is important to underline that assemblysomes might have a similar impact, as their presence was connected with poor response to neoadjuvant therapy in breast cancer (Li et al. 2023; Nagy-Mikó et al. 2023). Having complementary or parallel importance during stress as SGs, it is important to follow assemblysomes as well in clinical samples to be able to fully predict potential stress response capacities of malignant cells. By modulating their formation or function, it may be possible to disrupt tumor cell survival mechanisms or sensitize them to already existing treatments (Németh-Szatmári et al. 2023; Nagy-Mikó et al. 2023). Recent discoveries on the topic of toxicity associated with HEX reveal the role of the reservoir of assemblysomes within the cellular milieu (Gombás and Villányi 2024). Cells subjected to significant doses of genotoxic agents, such as those present in tobacco smoke, may exhibit a heightened presence of DNA repair proteins halted in synthesis within assemblysomes compared to unstressed cells (Kier et al. 1974). Given the propensity of tumor cells to originate from stressed cellular environments undergoing oncogenic alterations, in addition, it arose that phase separation targeting can be beneficial in cancer therapy (as it is possible that protein-mediated LLPS can lead to carcinogenesis), HEX emerges as a promising candidate worthy of investigation (Ming et al. 2019). Ming et al. concluded by examining pancreatic cancer cells that HEX effectively inhibited cell proliferation and induced cell death (Ming et al. 2019). Other researchers also believe that HEX is a potential therapeutic agent, as it is capable of disrupting and reducing neovascularization (Jiang et al. 2023). The capability of this chemical to increase the frequency of recombination events in malignant cells is connected to the

elevated abundance of assemblysomes' gene products encoding DNA repair proteins, which makes it a promising extension to complement existing therapies (Németh-Szatmári et al. 2023; Gombás and Villányi 2024). As a consequence, HEX may be more harmful to these types of cancer cells than to healthy cells due to the DNA damaging effect of certain helicases liberated from assemblysomes (Birkbak et al. 2018; Gombás and Villányi 2024). Furthermore, there are indirect and also direct signs of the phenomenon that neoadjuvant therapy-resistant and radioresistant tumors demonstrate insensitivity to conventional therapies owing to the heightened accumulation of DNA repair constituents (or even other beneficial proteins) sequestered within assemblysomes, which implicates that decreasing the assemblysome number within them by HEX (that way sensitizing them to treatments) could be advantageous (Németh-Szatmári et al. 2023; Nagy-Mikó et al. 2023).

We propose that HEX can hold potential as a robust chemotherapeutic agent for addressing cancers stemming from the malignant conversion of healthy cells, particularly those influenced by exposure to deleterious environmental stimuli. In types of tumors, where cells' ability to survive is (at least partly) based on phase-separated entities, HEX could be a useful part of therapeutic strategies. The intricate interplay among HEX, assemblysomes and genomic instability presents novel avenues for further exploration within the realms of cancer investigation and therapeutic innovation.

9.1.2 | Diagnostic Biomarkers

Alterations in assemblysome composition, number, or activity may serve as diagnostic biomarkers for various diseases, such as in the case of Rpb1 in invasive carcinoma of NST (Nagy-Mikó et al. 2023). An indirect consequence of data published by the Villányi laboratory is that elevated assemblysome abundance could be an indicator of ineffective treatments in the case of chemotherapy or radiotherapy; however, further confirmation is needed (Nagy-Mikó et al. 2023; Németh-Szatmári et al. 2023). To give an example, in the case of an increased number of NCAs containing gene products involved in the proteotoxic stress response, this contraindicates the administration of proteasome inhibitors in chemotherapy regimens. On the contrary, in case it turns out that assemblysomes of tumor cells are composed of RNCs involved in the DNA damage response, treatment with nucleoside analogs will likely be inefficient (Nagy-Mikó et al. 2023; Németh-Szatmári et al. 2023). Thus, monitoring assemblysome number and dynamics in patient samples could provide valuable insights into disease progression and treatment response, and it may contribute to choosing the most effective therapy in each individual case.

9.2 | Hypothetical Agricultural Applications

9.2.1 | Crop Improvement

Understanding all the stress responses mediated by assemblysomes in plants could be very helpful in developing stress-tolerant crop varieties. Manipulating assemblysomes' activity may enhance plant resilience to environmental stressors, thereby improving crop yield and quality. In agriculture, weed plants may have a greater capacity for phase separation, contributing to their tolerance of dry conditions. Breeding crops with elevated assemblysome levels—or altered dynamics of them—could enhance their resistance to biological stresses like pests and fungi. In animal husbandry and breeding, selection traits toward the enrichment of assemblysomes can be beneficial for breeding stress-tolerant lineages, if these organelles are able to provide elevated tolerance.

9.2.2 | Disease Resistance

Assemblysomes may possibly play a role in plant and animal defense mechanisms against pathogens. It can be possible to reduce the need for chemical pesticides or antibiotics, respectively, by targeting assemblysome components. This could enhance disease resistance in agricultural crops and livestock. On the other hand, pests may develop resistance to pesticides and antibiotics through the enrichment of certain types of assemblysomes in their cytoplasm. This resistance can manifest in two scenarios: first, genotoxic pesticides can be counteracted by assemblysomes containing DNA repair proteins, allowing cells to respond effectively to exposure, as assemblysomes provide an immediate response mechanism. Second, proteotoxic agents can be neutralized by assemblysomes containing proteasome subunit RNCs. Therefore, it could be a considerable idea to use HEX in combination with pesticides and antibiotics due to its ability to deplete assemblysomes, making pests more susceptible to treatments. In addition, HEX may lower the effective dose of certain pesticides by removing assemblysomes from the cytoplasm. We hypothesize that enriching assemblysomes with necessary gene products in a halfway synthesized state could help pests evade antibiotics and antifungals that inhibit transcription or translation initiation, with HEX potentially offering assistance in such cases.

9.3 | Environment Protection

We can consider assemblysomes as molecular memories. Various types of cellular stresses in the past could induce the formation of assemblysomes containing gene products that can mitigate these detrimental effects. In response to specific stressors, certain mRNAs are shielded from degradation mechanisms and stored within assemblysomes. These structures retain the ability to resume translation when the same stressors reoccur. As a result, the mRNAs stored in assemblysomes within organisms inhabiting soil or natural water bodies serve as indicators of previous stress events, including those triggered by chemicals or radiation. Qualitative and quantitative observation of assemblysome mRNA content of microbes will provide valuable data for monitoring purposes in clarifying the consequences of pollution on natural habitats.

9.4 | Possible Industrial Applications

Presumably, there are cases when workflows of bioprocessing can be streamlined and made more efficient. This could be executed by using—and taking advantage

of—assemblysome-mediated protein complex assembly. By optimizing assemblysome function, it may be theoretically possible to enhance the efficiency of recombinant protein production and biopharmaceutical manufacturing. In well-controlled environments such as fermenters, sophisticated stress responses are much less necessary as the conditions are closely monitored. It is more crucial for cells used as biofactories to possess translation-competent ribosomes rather than phase-separated ones, especially in the absence of environmental perturbations, when all conditions are precisely regulated artificially. Consequently, depleting assemblysomes would increase productivity substantially in industrial fields.

9.4.1 | Biofuel Production

Assemblysomes play a role in regulating gene expression in microorganisms used for industrial biofuel production. Manipulating cytoplasmic assemblysome density, dynamics, or activity could enhance metabolic pathways involved in biofuel synthesis, leading to increased yields and cost-effectiveness.

10 | Conclusions

In the future, many challenges will appear in the field of assemblysome research. First of all, an understanding of assemblysomes' complexity is needed. Further research and observation are required to clarify the regulation and function of assemblysomes underlying mechanisms in different organisms in systematic and cellular contexts. Still, assemblysomes are already considered an easy-to-follow cell organelle that reveals the stress response capacity of a given cell. This emerges as a molecular memory of past stresses and provides a quick gene expression regulatory response for the cells once they have to face the stress repetitively. Translation to practice: Translational efforts and interdisciplinary collaboration are needed to bring closer fundamental research on LLPS granules and applications in agricultural, industrial, and medical areas. Finally, as an emerging field of research and technology, it is extremely important to carefully and responsibly address ethical considerations connected to the use of assemblysomes.

Overall, assemblysomes seem to be a promising area in several different fields. Biomedical, agricultural, and industrial research could benefit from the expanding data on assemblysomes. Knowing their various roles in cellular physiology, different applications can offer exciting opportunities for developing innovative solutions to pressing challenges in human health, agriculture, and biotechnology. Continued exploration of assemblysome biology and its applications holds the potential to revolutionize diverse fields and improve lives worldwide.

Author Contributions

Bence György Gombás: visualization (equal), writing – original draft (equal), writing – review and editing (equal). **Orsolya Németh-Szatmári:** writing – review and editing (supporting). **Bence Nagy-Mikó:** writing – review and editing (supporting). **Zoltán Villányi:** conceptualization (lead), resources (lead), supervision (lead), writing – original draft (lead), writing – review and editing (lead).

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Related WIREs Articles

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