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Biogenesis of stress granules and their role in the regulation of stress-induced male reproduction disorders

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Abstract

Stress granules (SGs) are conserved messenger ribonucleoprotein (mRNP) granules that form through rapid coalescence in the cytoplasm of eukaryotic cells under stressful environments. These dynamic membrane-free organelles can respond to a variety of both intracellular and extracellular stressors. Studies have shown that stress conditions such as heat stress, arsenite exposure, and hypoxic stress can induce SGs formation. The formation of SGs helps mitigates the effects of environmental stimuli on cells, protects them from damage, and promotes cell survival. This paper focuses on the biogenesis of SGs and summarizes the role in regulating environmental stress-induced male reproductive disorders, with the aim of exploring SGs as a potential means of mitigating male reproduction disorders. Numerous studies have demonstrated that the detrimental effects of environmental stress on germ cells can be effectively suppressed by regulating the formation and timely disassembly of SGs. Therefore, regulating the phosphorylation of eIF2α and the assembly and disassembly of SGs could offer a promising therapeutic strategy to alleviate the impacts of environmental stress on male reproduction health.

Introduction

Stress granules (SGs) are a highly conserved cytoplasmic ribonucleoprotein (RNP) granules [1]. These unique mRNP granules form rapidly form within the cytoplasm of eukaryotic cells in response to stressful conditions such as heat shock, endoplasmic reticulum stress, and hypoxia. SGs varies in size, ranging from approximately 200 nm to over 5 μm [2], and SGs are dynamic, membrane-free organelles that respond to a range of intracellular and extracellular stressors, dissolving once the stress is alleviated [3]. RNP granules are membrane-free subcellular organelles formed by the condensation of RNA and proteins in eukaryotic cells. These structures, known as mRNP granules, consist of mRNA and proteins, and they influence the function and localization of mRNA [4] (Fig. 1). SGs play a crucial role in regulating stress responses, including hypoxia, heat shock, viral infections, and signaling pathway transduction. Proper formation of SGs is essential for cell survival under stress conditions. Improper SG formation can increase cellular sensitivity to stress, ultimately leading to cell death. Conversely, abnormal accumulation of SGs has been linked to neurodegenerative diseases and certain cancers.

During mammalian development, oocytes and sperm are derived from primordial germ cells (PGC). In

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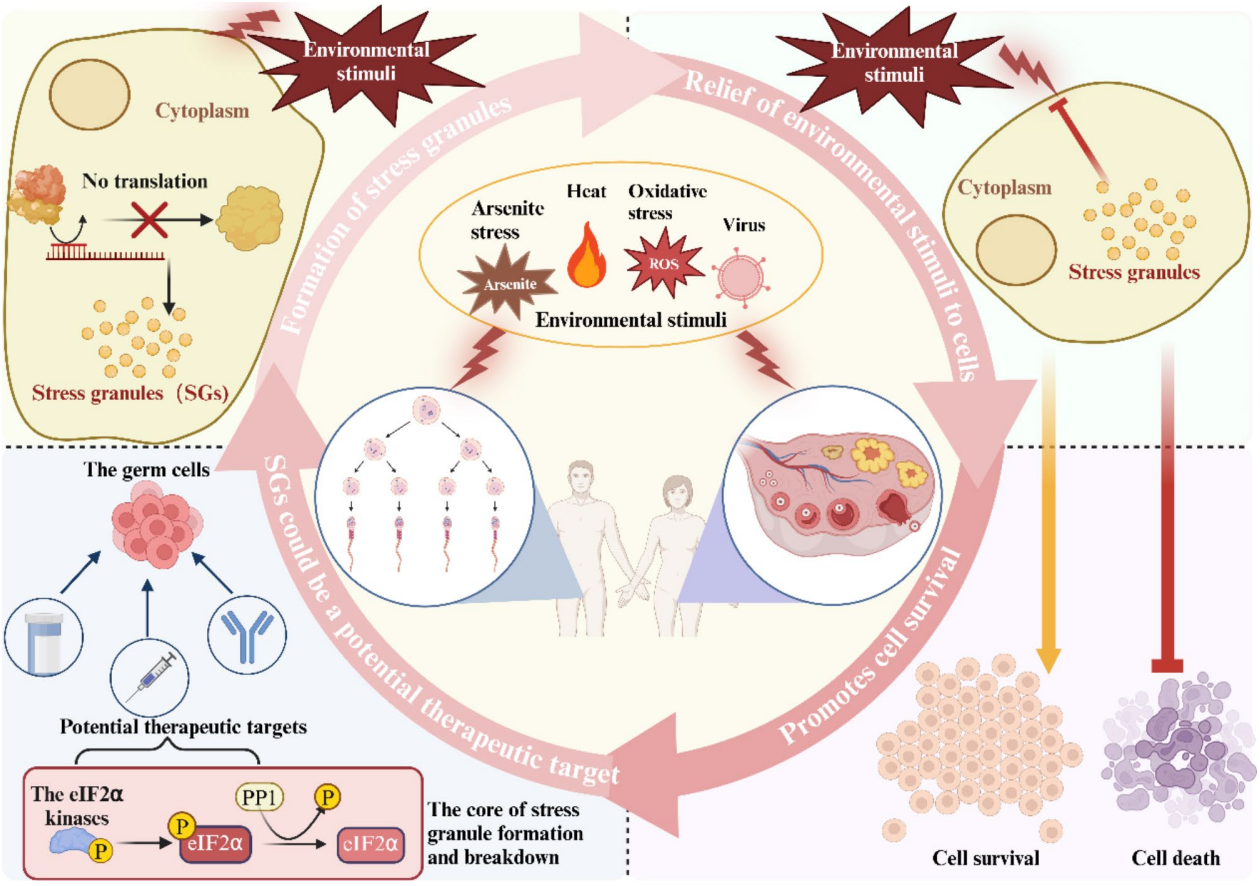
Graphical abstract



Keywords Stress granules, Environmental stress, Reproductive health

contrast to somatic cells, germ cells are responsible for transmitting genetic information and undergo a precise and unique form of cell proliferation and division—meiosis—critical for their development. In contrast, somatic cells complete their growth through mitosis alone. The development of germ cells is a delicate and prolonged process. In most mammals, a complete spermatogenic cycle lasts approximately 40–90 days [5, 6], while human follicles take around 290 days to develop from primary follicles to mature secondary follicles [7]. Germ cells are highly sensitive to environmental stimuli, forming SGs in response, which will be the focus of this review.

Environmental factors pose numerous challenges to reproductive health, including infertility [8], menstrual irregularities [9], sexual dysfunction [10], and prostate issues [11]. Approximately 17.5% of the global adult population—about one-sixth of the global population—suffers from infertility [12]. These conditions not only reduce quality of life but also increase the risk of other chronic diseases. In addition, infertility, menstrual irregularities, and sexual dysfunction frequently lead to significant psychological stress, contributing to mental health issues such as anxiety and depression. Environmental factors exert a profound impact on reproductive health, either directly affecting the reproductive system or indirectly by disrupting the endocrine system and altering immune function. Therefore, effectively managing stress is essential for maintaining reproductive health.

The formation of SGs halts the synthesis of non-essential proteins, helping protect mRNA from damage and promoting cell survival. SGs can impact reproductive health both directly and indirectly. For example, during spermatogenesis, heat stress induces the formation of SGs, which protect normal spermatogenesis from germ cell apoptosis by phosphorylating eIF2α and recruiting RNA-binding proteins such as DAZL, BOULE, RACK [13, 14]. Although the specific role of SGs in reproductive health remains under investigation, their potential to maintain the normal function of germ cells and reproductive organs, as well as their response to various

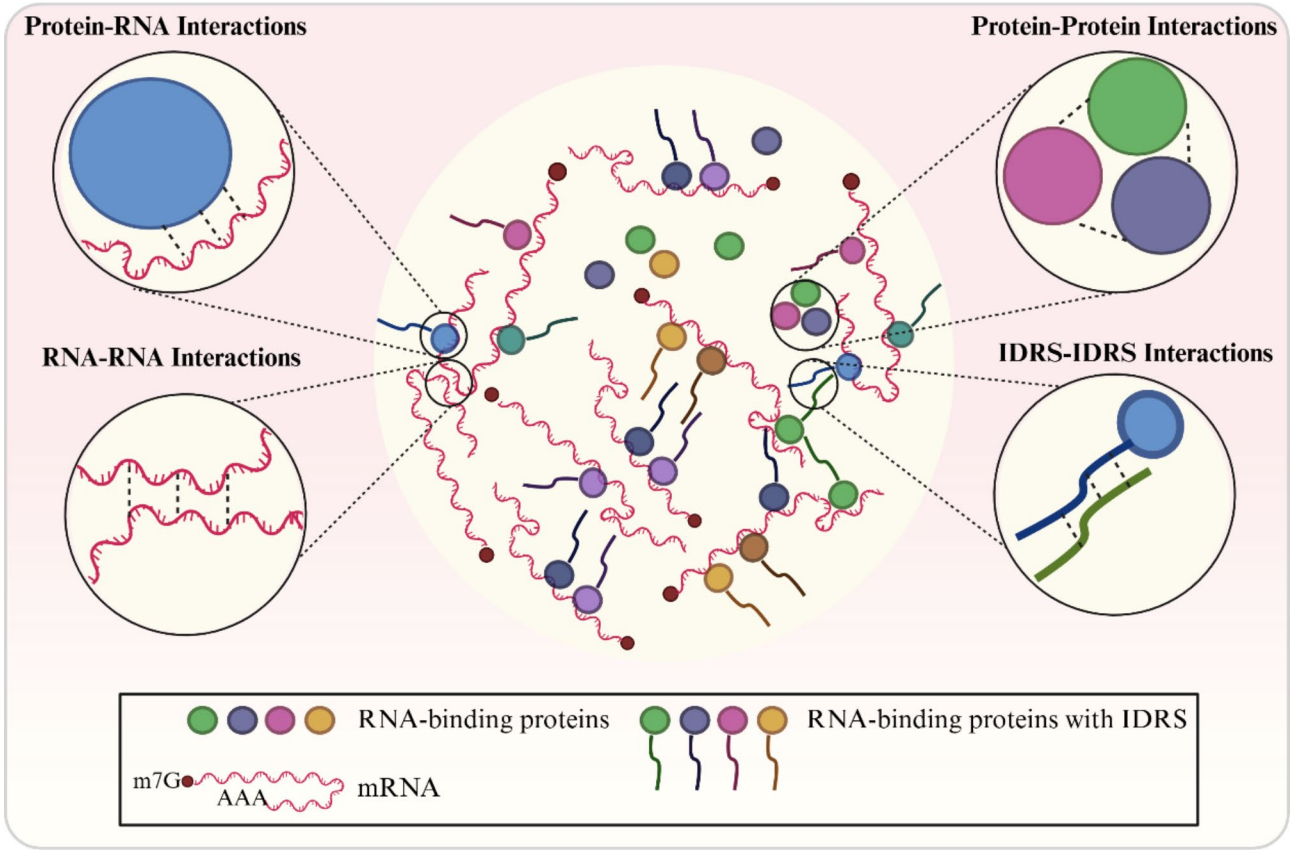


Fig. 1 Diagram of stress granules patterns

stressors, underscores their significance in reproductive medicine research. Data suggest that SGs formation can inhibit germ cell apoptosis and mitigate the effects of stressors on germ cells, thereby positively influencing reproductive health. These findings indicate that SGs play a crucial role in the reproductive systems' response to environmental challenges and can effectively reduce the adverse effects of these stimuli on germ cells.

This review focuses on the impact of environmental stress conditions on male reproductive health and summarizes and discusses the role of SGs in the regulating male reproductive disorders induced by environmental stimuli.

Formation, characterization, and role of SGs in cellular stress

The discovery of SGs

SGs were first observed in the cytoplasm of heat-shocked suspension cultures of Peruvian tomato (*Lycopersicon peruvianum*) in 1983. Similar granular aggregates were subsequently observed in the cytoplasm of heat-shocked tomato leaves, maize germ sheaths, and other plant cytoplasm. At that time, these transient structures were not yet named stress granules but were referred to as heat-shock granules, as most of the major small heat shock proteins were structural components of these newly identified granular aggregates [15]. In 1986, such similar granular aggregates were shown to be formed in chick embryo fibroblasts under heat stress [16], showing that the phenomena is conserved. Interestingly, in 1988, it was found that chicken embryo fibroblasts were able to form granular aggregates containing heat shock proteins when induced by a variety of stressors. Therefore their name was revised to stress granules (SG) [17]. In 1999, phosphorylation of eIF2α was linked to the assembly of mammalian SGs by the RNA-binding proteins TIA-1, TIAR [18]. In 2002, the accumulation of SGs was first observed in fission yeast under environmental stress conditions [19]. Since then, much of the research on the environmental induction of SG formation has been conducted using the yeast model [20].

Characterization of SGs and their comparison with other subcellular organelles

SGs contain numerous untranslated mRNAs, various translation initiation factors, and both RNA-binding and non-RNA-binding proteins. Analysis of the protein fractions within SGs, particularly the "core," reveals that approximately 50% of the components are RNA-binding proteins [3]. This suggests that SGs are dynamic,

heterogeneous structures, with a stable core rich in proteins and mRNAs, surrounded by a dynamic shell. The assembly, disassembly, and interaction between the core and shell are driven by weak multivalent interactions among the various protein and RNA components, which are rapidly and dynamically organized through phase separation and percolation [21]. The composition of SGs varies under different stress conditions, further emphasizing their complexity and variability [22] (Fig. 2A).

Exosomes are formed within multivesicular bodies (MVBs) through endocytosis, resulting in small vesicles with a diameter of approximately 30–150 nm. These vesicles are eventually released into the extracellular matrix when MVBs fuse with the plasma membrane. The membrane structure of exosomes, similar to that of the plasma membrane, comprises a lipid bilayer and encapsulates a diverse array of biomolecules such as proteins, lipids, mRNA, miRNA, DNA, and other small molecules [28]. Particularly, exosomes secreted by the parietal plasma of epididymal epithelial cells can fuse with spermatozoa, promoting spermatid maturation by delivering paternally-derived sncRNAs [29]. These sncRNAs are then transferred to the oocyte, where they participate in regulating embryo development and zygote health [30] (Fig. 2B).

Inflammasomes were first described in 2002 as multiprotein complexes with a molecular weight of approximately 700 kDa [31]. They form in the cytoplasm when pattern-recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs) or host-derived danger-signaling molecules (DAMPs). Capable of sensing various stimuli, inflammasomes trigger inflammatory and immune responses. These complexes are primarily composed of sensor protein, adapter protein, and inflammatory caspases, which facilitate cytokine maturation and secretion, mediate inflammatory responses and induce cellular pyroptosis [32] (Fig. 2C).

Autophagosomes are essential intermediate structures in cellular autophagy, responsible for encapsulating damaged organelles, misfolded proteins, and other cellular debris, and ultimately transporting these materials to lysosomes for degradation and recycling. Their formation and function are critical for maintaining intracellular homeostasis, responding to stress, and regulating cell fate [33] (Fig. 2D).

Cajal bodies are complex, membrane-free spherical structures formed by Liquid-liquid phase separation (LLPS) in the nuclei of higher eukaryotic cells, with diameters ranging from 0.2 to 2 μm. Their molecular composition is intricate, including various components of the nucleic acid-protein complexes of snRNPs necessary for mRNA splicing, as well as snoRNAs, scaRNAs, and other associated proteins. Cajal bodies play crucial roles in RNA metabolism, the formation of transcribed

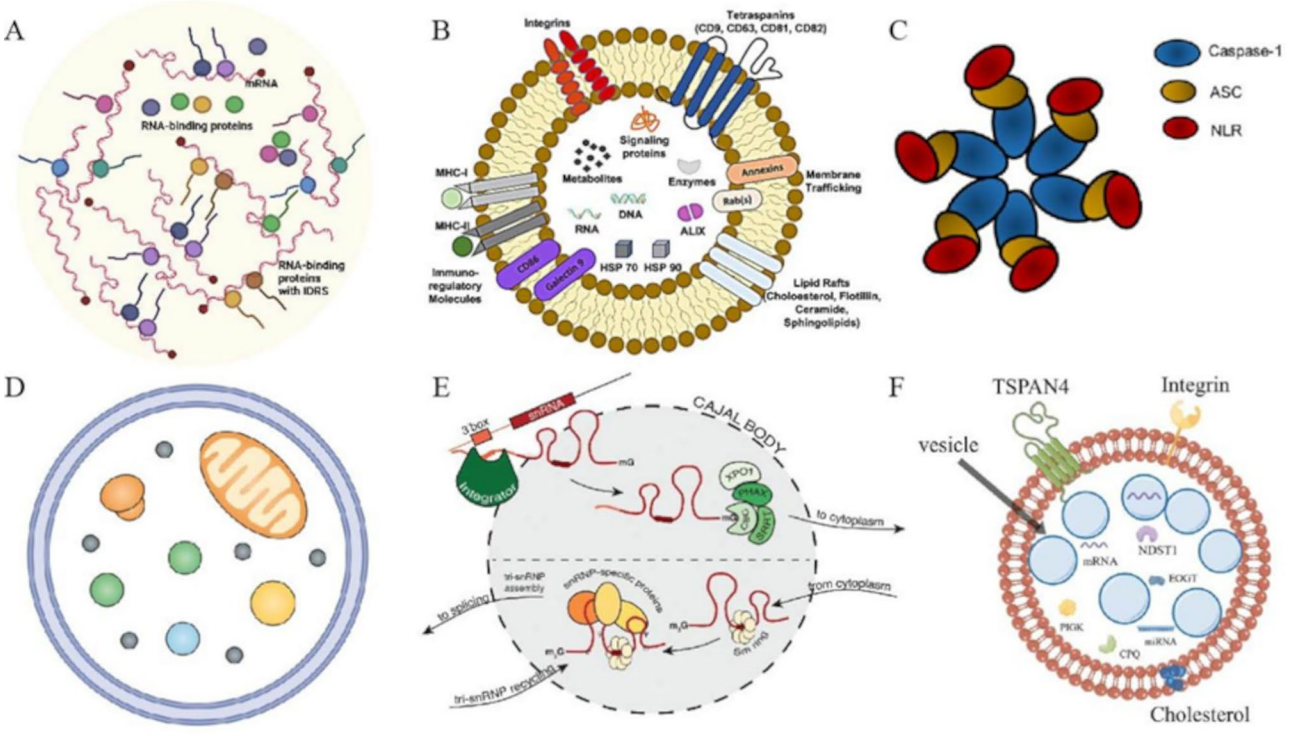


Fig. 2 Comparison of composition of stress granules with other subcellular bodies. **A**: Stress granule; **B**: Exosome [23]; **C**: Inflammasome [24]; **D**: Autophagosome [25]; **E**: Cajal body [26]; **F**: Migrasome [27]

ribonucleoproteins, RNA splicing, and telomere maintenance [34] (Fig. 2E).

Migrasomes, a newly discovered subcellular structure first identified in 2014, form during cell migration when cells leave behind retraction fibers. Vesicles measuring 1–3 μm in diameter develop at the tips or intersections of these fibers and are referred to as "migrasomes" [35]. These structures contain mRNAs and proteins that accumulate damaged mitochondria, playing crucial roles in mediating signaling, regulating cellular homeostasis, and facilitating material transport [36–39] (Fig. 2F).

Unlike exosomes, inflammasomes, autophagosomes, Cajal bodies, and migrasomes, which are also subcellular structures, SGs are membrane-free organelles formed in the cytoplasm through liquid-liquid phase separation (LLPS) during cellular stress. These organelles regulate mRNA stability, translation, and degradation within the cell. Similarly to inflammasomes, SGs are multiprotein complexes, but are different because the protein components (translation factors, RNA-binding proteins, and other stress-related proteins) are primarily associated with untranslated mRNA. In contrast to autophagosomes, SGs lack a bilayer membrane and do not degrade damaged cellular components. The main characteristics and differences between SGs and other subcellular structures are listed in Table 1.

Spermatogenesis in the male

The process of spermatogenesis in the testis

Spermatogenesis is a complex process in the male reproductive system that involves the self-renewal and differentiation of spermatogonial stem cells into mature spermatozoa. In mammalian testis, the process of spermatogenesis consists of three stages: mitosis, meiosis and spermatogenesis [40]. First the spermatogonial stem cells (SSCs) undergo mitosis to produce new spermatogonia. One of the daughter cells becomes a new spermatogonial stem cell and remains in the stem cell pool, retaining stem cell characteristics and responsible for self-renewal to maintain the stability of the spermatogonial cell pool. The other one becomes an A1 spermatogonial cell and continues to differentiate and divides once to produce two A2 spermatogonial cells, which then undergoes successive mitotic divisions to produce four A3 spermatogonial cells, and eigh A4 spermatogonial cells. These A spermatogonial cells will divide, and with each division they will accumulate differentiation characteristics in preparation for transformation into type B spermatogonia. After the formation of type A4 spermatogonia, the type A4 spermatogonia will further divide to form sixteen intermediate spermatogonia. The intermediate spermatogonia further proliferate and finally differentiate into thirty two B spermatogonia, which marks the shift from A to B cell differentiation, and the B spermatogonia will undergo a final mitotic division and form sixty four primary spermatocytes, which will enter the next stage of spermatogenesis [41, 42]. Subsequently, type B spermatogonia will enter the prophase stage in preparation for meiosis initiated by retinoic acid (RA) and STRA8 [43]. Once the spermatogonia begin their first meiotic division they become primary spermatocytes, which complete their first meiotic division to become two secondary spermatocytes, which then undergo a second meiotic division, in which the chromosome number is halved to form four haploid spermatocytes [44, 45]. Therefore, according to the process of spermatogenesis, one type A1 spermatogonia is theoretically capable of producing two hundred and fifty-six spermatocytes. Moreover, the spermatogonia undergo a series of morphological changes while being nourished by the supporting cells, the spermatogonia further develop into spermatozoa in a process called spermatogenesis, followed by complex morphological changes in the round spermatogonia as well as the transformation and modification of the nucleoproteins,

Table 1 Differences between stress granules and other subcellular structures

	Membrane construction	Contain	Functionality	Reference
Stress granules	membrane-free structure	Consisting of untranslated mRNA, translation factors, RNA-binding proteins, and other proteins associated with stress	Involved in mRNA protection, translational regulation and cellular stress response	[1, 2, 3, 21]
Exosomes	membrane-bound structure	Proteins, lipids, mRNA, miRNA, DNA, and various other small molecules	Involved in intercellular communication and material transfer	[15–17]
Inflammasomes	membrane-free structure	Multiple proteins	Involved in immune response and inflammatory regulation	[31, 32]
Autophagosomes	membrane-bound structure	Damaged organelles, misfolded proteins and other cellular wastes	Maintenance of intracellular homeostasis, response to stress and regulation of cell fate	[33]
Cajal bodies	membrane-free structure	Various components of snRNP involved in mRNA splicing, as well as snoRNAs, scaRNAs and other related proteins	Involved in RNA metabolism, transcription ribonucleoprotein formation, RNA splicing and telomere maintenance	[21]
Migrasomes	membrane-bound structure	mRNA and protein and accumulate damaged mitochondria	Mediates signal transduction, regulates cellular homeostasis, mediates substance transport	[35–38]

resulting in the formation of mature spermatozoa. However, in reality, 70–90% of spermatogonia are lost during spermatogenesis and only 10–30% of the cells are able to form spermatozoa [46, 47]. This suggests that the process of spermatogenesis is susceptible to environmental factors that reduce sperm quality.

The spermatogenic cycle of different animals

Studies have shown that under stress conditions SGs are distributed at specific stages of spermatogenesis, primarily occurring prior to meiosis rather than throughout the entire process. This suggests that mitigating the effects during critical stages of spermatogenesis can benefit overall spermatogenesis. The duration of one spermatogenic epithelial cycle and a complete spermatogenic cycle in different animal species are listed in Table 2.

SGs biogenesis and mechanism of action

Formation and decomposition of SGs

The Integrated Stress Response (ISR) is a complex signaling pathway in eukaryotic cells, activated in response to various physiological changes and pathological conditions, including extracellular factors such as hypoxia, amino acid deprivation, glucose deprivation, and viral infection [54–56]. Typically, cellular stress leads to translational arrest via ISR activation. This activation is mediated by the phosphorylation of eIF2α (eukaryotic translation initiation factor 2α) by one or more of four specific kinases: HRI (heme-regulated eIF2 kinase), PKR (viral infection-regulated eIF2 kinase), PERK (endoplasmic reticulum stress-regulated eIF2 kinase), and GCN2 (amino acid deprivation-regulated eIF2 kinase). Phosphorylation of eIF2α is a critical step in SGs formation, as it converts eIF2α from a substrate of eIF2B into an inhibitor, thus blocking the formation of the eIF2-GTP-Met-tRNA ternary complex and halting the initiation of mRNA translation initiation [57]. An alternative pathway for SGs formation, independent of eIF2α phosphorylation, involves the disruption of the eIF4 complex. This can be achieved through pharmacological inhibition of eIF4A or eIF4E [58], leading to disassembly of the multimer and preventing the assembly of the translation initiation complex. This disruption marks the first step in SG formation: translation initiation arrest.

Liquid-liquid phase separation (LLPS) is a density transition characterized by the segregation of molecules in solution into distinct light and dense liquid fractions [59]. Weak multivalent interactions between proteins, protein-RNA, RNA-RNA, and protein intrinsically disordered regions (IDRs) promote the formation of phase-separated states in SGs [60, 61]. Research indicates that the UBAP2L core forms first, upstream of the G3BP1 core, and facilitates its formation [62]. SGs are RNP granules that dynamically incorporate translationally stalled mRNAs, releasing significant amounts of unbound mRNA into the cytosol, which is essential for SG formation and stability. G3BP1 acts as a central node and molecular switch, triggering RNA-dependent LLPS in response to increased intracellular free RNA concentration, leading to SG assembly [60]. Caprin1 binds to G3BP, promoting SG formation [63], while stalled translational complexes associate with core SG proteins such as TIA-1 [64], TIAR [65], G3BP [63], and PABPC1 [66]. These proteins contribute to the aggregate of mRNPs and form the SG core, storing translation initiation factors, RNA-binding proteins, and signaling molecules [67]. This aggregation process constitutes the second step in SG formation: the nucleation and assembly of mRNPs into oligomers.

When SG formation is initiated, small granules appear simultaneously. These small SGs subsequently fuse to create mature clustered SGs in a microtubule-dependent manner [2]. This fusion and maturation process constitutes the third step in SG formation: involves further aggregation, leading to the development of fully mature SGs. These series of events are crucial for the proper formation and function of SGs.

SGs formed under stressful conditions do not dissolve rapidly; the dissolution of intact SGs typically takes several hours [68]. During disassembly, larger SGs break down into smaller SGs, which then dissolve individually, without further fragmentation [69]. This process likely reflects the progressive reversal of multivalent interactions involving proteins and RNAs. Interestingly, translation is restored before the complete dissolution of SGs, with nearly half of the transcripts being actively translated during stress recovery while SGs are still present [70]. Dephosphorylation also contributes to SG clearance; ATF4, a transcription factor, induces the expression of GADD34, a phosphatase regulatory subunit that recruits protein phosphatase 1 (PP1) to eIF2α, triggering its dephosphorylation and thus alleviating translation arrest [71]. Studies have shown that autophagy or molecular chaperones, such as the HSPB8-BAG3-HSP70 complex, can mediate SG clearance [72]. The autophagy pathway specifically removes persistent or aberrant SGs

Table 2 A complete spermatogenic cycle and seminiferous epithelium cycle in different animals

Species	Spermatogenic epithelial cycle (days)	One complete spermatogenic cycle (days)	Reference
Homo sapiens	16	74	[48]
Bovine	13.5	61	[49]
Mus musculus	8.6	38.7	[6]
Sus scrofa	12.3	55	[50]
Ovis aries	10.6	48	[51]
Canis lupus familiaris	13	62	[52]
Felis silvestris catus	12.5	56.3	[53]

[73]. The solubilization of SGs is influenced by the duration and severity of stress conditions: SGs formed from acute heat stress are not dependent on autophagy for catabolism, whereas those induced by prolonged heat stress rely on autophagy for clearance [74, 75]. Additionally, several deaggregation enzymes, such as VCP [76] and the endoplasmic reticulum-associated protein FAF2 [77], are essential for SG clearance. Damage to these components significantly delays SG dissolution and may lead to the formation of abnormal SGs (Fig. 3).

Functions and mechanisms of action of SGs

SGs in cells help mitigate responses to stressors such as viral invasion, chemical damage, and oxidative stress. Research indicates that SGs can inhibit viral replication independently of the RLR pathway, thereby acting outside immune control. This highlight their role as multifunctional cellular "shock absorbers" that protect homeostasis by curbing toxic immune responses and viral replication [77]. APE1 promotes phosphorylation of YBX1 at the S174 and S176 sites, enhancing SG formation and promoting cell survival [78]. In KRAS-mutated cells, SGs significantly increase in response to various stress stimuli, controlling prostaglandin metabolism and providing cytoprotection through both cell-autonomous and non-autonomous mechanisms [79]. SGs can inhibit apoptosis by suppressing reactive oxygen species (ROS) levels, a function regulated by two SG components: G3BP1 and USP10. G3BP1 increases ROS levels by inhibiting the antioxidant activity of USP10. When SGs are formed under stress conditions, G3BP1 and USP10 are recruited into SGs, and this inhibition is inactivated. Therefore, SGs formation weakens the ability of G3BP1 to inhibit the antioxidant activity of USP10, which in turn reduces ROS levels and inhibits apoptosis [80]. Additionally, SGs can alleviate endoplasmic reticulum stress (ERS)-mediated apoptosis, protecting hepatocytes from hypoxia-induced injury during acute liver failure (ALF) [81].

Numerous studies have demonstrated that the anti-apoptosis effects in cells are associated with the formation of SGs. Exposure to various stress conditions can result in either cell recovery or apoptosis, depending on the type and intensity of the stress. Under conditions such as nitrite exposure, hypoxia, and heat stress, the formation of SGs is promoted. These SGs inhibit

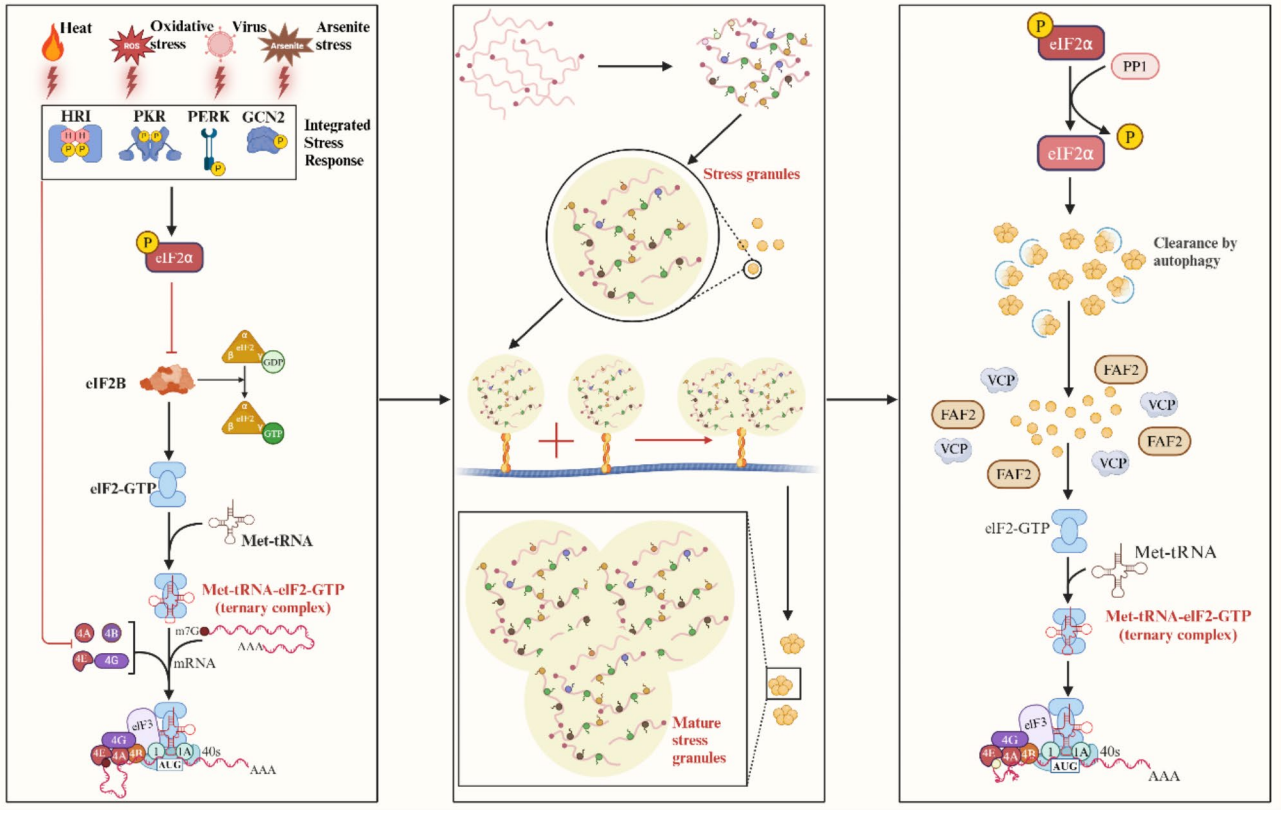


Fig. 3 Formation and decomposition of stress granules. In cells under stress conditions translation initiation is halted and RNA-dependent LLPS occurs in the cytoplasm in response to increased intracellular free RNA concentration, which in turn leads to the assembly of SGs. mRNPs are assembled into oligomers, at which point the SGs are initially formed, and then through further aggregation, fusion, and maturation ultimately mature SGs are formed. After the stress conditions subside, the larger SGs break down into smaller SGs, which then dissolve individually

stress-induced apoptosis by recruiting key proteins in the relevant signaling pathways. For instance, SGs regulate cellular signaling by recruiting proteins such as TOR, RACK1, and TRAF2. When DYRK3 is inactivated, it prevents the disassembly of SGs and the release of mTORC1, which is then recruited into SGs, inhibiting mTOR signaling [82, 83]. RACK1, a pro- apoptosis protein, is known to activate apoptosis pathways, but SGs inhibit caspase-3 activation by recruiting RACK1, thereby promoting cell survival [84]. Additionally, stressed cells recruit translation factors and TRAF2 into SGs through protein-protein interactions, blocking tumor necrosis factor signaling and thus the pro-inflammatory response [85]. Previous research has shown that TM4SF1-AS1 associates with various SG-associated proteins, including G3BP2, RACK1, and DDX3. TM4SF1-AS1 promotes SGs formation and inhibits apoptosis in gastric cancer cells by sequestering RACK1 within SGs [86] (Fig. 4).

Effects of SGs produced by environmental stimuli on germ cells

Environmental stimuli are first detected by cell surface receptors, which then activate a series of cascading signaling pathways, with the phosphorylation of eIF2α being central. Under stress conditions such as heat shock, oxidative stress, nutrient deprivation, or viral infection, the phosphorylation level of eIF2α increases, leading to a global inhibition of protein synthesis. This triggers the aggregation of mRNAs with RNA-binding proteins to form SGs, temporarily sequestering mRNA until the stress is alleviated, thereby allowing normal translation to resume. This process ensures that cells maintain basic

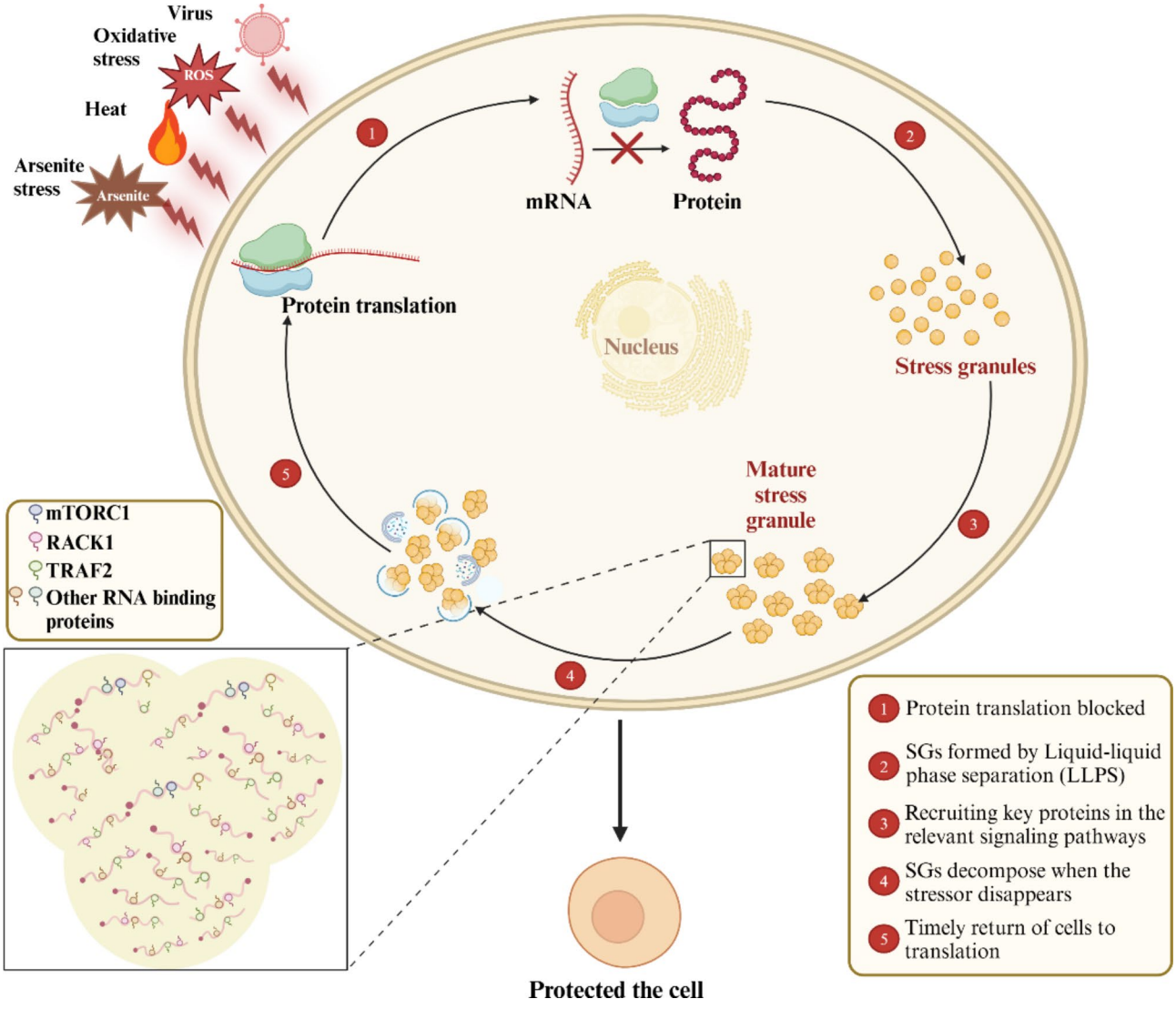


Fig. 4 Role of stress granules in cellular stress response. SGs protect cells from stress by recruiting proteins such as TOR, RACK1, or TRAF2 that affect signaling in the apoptosis pathway under stress conditions

functions and viability under adverse conditions. Arsenite induces oxidative stress in cells, promoting eIF2α phosphorylation and SG assembly [87], which in turn reduces cellular damage caused by arsenite. Physical stressors, such as extreme temperatures, also impact cellular physiology; high temperatures trigger a heat shock response, increasing the expression of heat shock proteins (HSPs) and promoting SG formation [88].

Research has shown that SGs are developmental stage-specific. After heat stress, SGs are primarily found in the testicular lumen during stages I-VIII, with the highest distribution in stages IV-VI, and are almost absent in stages IX-XII. SGs are widely distributed in spermatogonial cells, early spermatocytes, and late spermatocytes but are not formed in round or elongated spermatids [13]. Because germ cells carry the function of gene transmission, it has been shown that the effects caused by environmental factors may be transmitted epigenetically through germ cells to offspring [89, 90], so we need to pay extra attention to the effects of environmental factors on germ cells. This mechanism is crucial for protecting germ cells from heat-induced DNA damage and protein misfolding. In reproductive health, high temperature exposure affects spermatogenesis and oocyte maturation, with SGs potentially acting as a buffer, reducing direct damage to germ cells [13, 14, 91].

SGs participate in the regulation of reproductive disorders under environmental stimulation

Role of SGs in reproductive dysfunction following chemical exposure

Endocrine-disrupting chemicals (EDCs) are widespread and can accumulate in the environment as well as in everyday household products, including cosmetics, plastic food packaging, and pharmaceuticals. In the male reproductive system, EDCs are associated with various health issues, such as cryptorchidism [92], testicular cancer [93], and reduced semen quality [94]. Heavy metals are introduced into the environment through natural sources like rock weathering andvolcanic eruptions, as well as through human activities, including industrial emissions, mineral extraction, and automobile exhaust. These metals disrupt multiple aspects of reproductive functions in both women and men, including hormonal imbalance [95], sperm count, viability and spermatogenesis [96].

Arsenic (As) is the 20th most abundant element in the Earths' crust and is widely distributed in the environment. It predominantly exists in the terrestrial environment in inorganic forms, including arsenate (AsIII) and arsenite (AsV), both of which are toxic to humans and animals, Inorganic arsenic is water-soluble, leading to its accumulation in biological cells [97]. Exposure to and accumulation of arsenic predisposes individuals to cancerous and non-cancerous diseases. Chronic arsenic exposure can also impact reproductive health and alter responses to environmental stressors by affecting estrogenic and androgenic signaling processes [98, 99]. Arsenite, in particular, acts as an endocrine disruptor, inducing the formation of SGs. Studies have shown that heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNPA2B1) is involved in the formation of SGs under arsenite-induced stress. Deletion of hnRNPA2B1 promotes the disassembly of SGs, leading to support-cell-only syndrome (SCOS) and complete male sterility [100]. Additionally, arsenite induces the formation of SGs containing SERPINE1 mRNA-binding protein 1 (SERBP1), which inhibits apoptosis caused by arsenite stress and promotes the clearance of SGs to prevent their abnormal accumulation [101]. These findings suggest that many ribonucleoproteins may be present in SGs formed under arsenite-induced stress conditions, and some may regulate the assembly and disassembly of SGs, thereby supporting the survival of germ cells.

Role of SGs in reproductive dysfunction in heat stress exposure

The reproductive system of male mammals is more susceptible to the adverse effects of elevated ambient temperatures due to a lower physiological temperature than a constant body temperature resulting from its distance from the body cavity. The normal spermatogenesis process in male mammals requires temperatures that are 2–7 °C lower than the core temperature, and sperm quality decreases significantly as scrotal temperature increases [102]. The main effects of heat stress on cells include increased membrane fluidity, changes in cellular morphology including rounding and shrinkage due to cytoskeletal modifications and reorganization of microfilaments [103, 104] and heat stress causes significant morphological changes in many intracellular materials such as mitochondrial swelling, increase in visible size and enlargement of cristae space, fragmentation of the endoplasmic reticulum and the Golgi apparatus, and under conditions of heat stress leads to the nucleolus disassembly and consequently the release of a variety of proteins, resulting in damage to the ultrastructure of the nucleus [105]. Spermatogonia, on the other hand, are more susceptible to heat stress than other testicular cells due to their delicate and prolonged development, during which there are multiple divisions [106]. The results of existing studies indicate that heat stress induces germ cell apoptosis and spermatogenic disorders in male mammals, and the scrotum can be affected by heat stress to produce damage such as apoptosis, autophagy, lipid peroxidation, oxidative stress, DNA fragmentation, and damage to the blood-testis barrier which can result in the destruction of testicular structure, cell death, and gamete development

abnormalities, which ultimately leads to decreased fertility in males and male animals and even infertility [107, 108].

SGs can form in germ cells under heat stress conditions. Studies have shown that SGs are widely distributed in spermatogonia, early spermatocytes, and late spermatocytes under heat stress, whereas they are absent in round and elongated spermatids, SGs formation inhibits heat stress-induced apoptosis [13] (Fig. 5). It has been previously demonstrated that SGs in male germ cells contain the spermatogonial cell-specific marker DAZL, which protects against heat-induced apoptosis by sequestering specific signaling molecules like RACK1 to block apoptosis MAPK pathways [14]. The testis-specific protein MAGE-B2 regulates the translation of the SGs core protein G3BP by displacing DDX5, enhancing cellular tolerance to heat stress [109]. Under heat stress, NEDD4 controls spermatogonial stem cell homeostasis by regulating the involvement of messenger ribonucleoprotein complexes (mRNPs) in SG synthesis and promoting SG disassembly during recovery to prevent pathological accumulation [110]. The TIAR-1 protein facilitates SG formation in *Caenorhabditis elegans* under heat stress, protecting female germ cells from heat shock [91]. These findings suggest that heat stress induces SGs formation by phosphorylating eIF2α and recruiting RNA-binding proteins, such as DAZL, BOULE, RACK1, ultimately protecting normal spermatogenesis and inhibiting germ cell apoptosis. Additionally, heat-induced SGs can protect female germ cell formation and prevent apoptosis.

Role of SGs in reproductive dysfunction influenced by other factors

Traumatic brain injury (TBI) can significantly affect male reproductive function, which relies on the brain as well as the hypothalamic and pituitary centers. The intact hypothalamic-pituitary-gonadal (HPG) axis is crucial for male reproduction. The hypothalamus releases gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These hormones stimulate the interstitial and supporting cells in the testis respectively. The interstitial cells produce most of the body's testosterone, while the supporting cells release androgen-binding proteins. These process is essential for spermatogenesis [111]. Studies have shown that TBI can induce the formation of SGs in vivo, with the extent of SG formation directly related to the severity of the trauma [112]. For example, a 20-year-old taekwondo athlete developed reproductive dysfunction following a competition [113]. Other studies have documented various changes in hypopituitarism caused by exercise-associated TBI, such as growth hormone deficiency and central dysuria [114–116]. Sports commonly associated with head trauma and consequently with TBI risk to the HPG axis include soccer, cycling, boxing, and skiing [111]. These findings suggest that various environmental factors in life, such as TBI, may induce the formation of SGs, which protect the cells to mitigate the effects of stimuli in life, and thus enhance the stress tolerance of the cells to protect germ cells from stress damage. The role of stress granules-associated proteins in reproductive dysfunction under environmental stimulation are listed in Table 3.

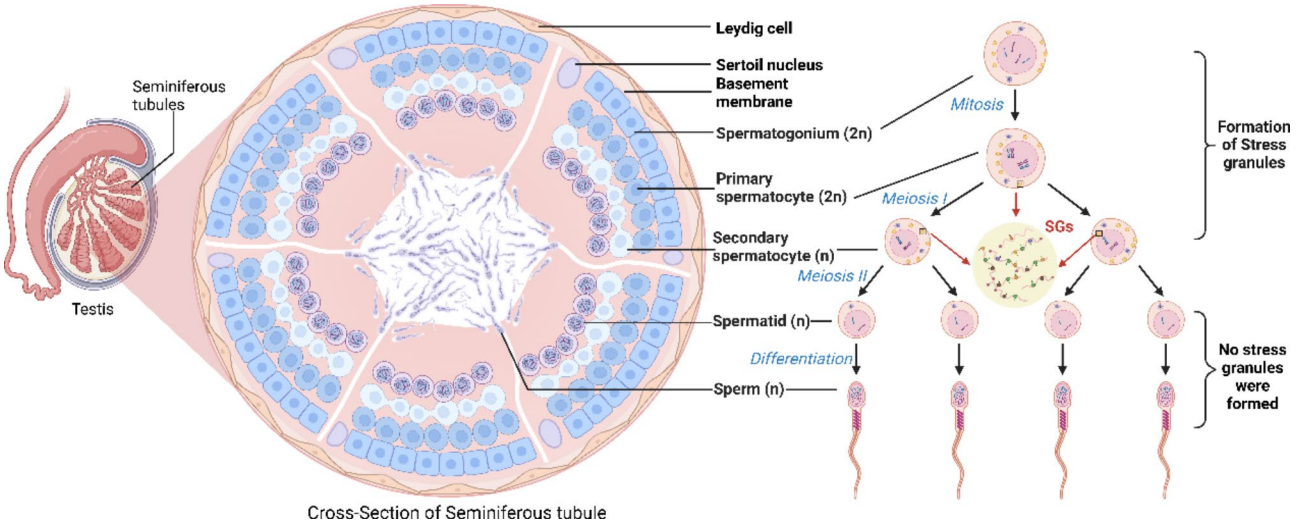


Fig. 5 Spermatogenesis and the stages of distribution of SGs under stress conditions. Cross-sections of the seminiferous tubules and specific periods of formation of SGs during spermatogenesis

Table 3 The role of stress granules-associated proteins in reproductive dysfunction under environmental stimulation

Stress granule-associated protein	Functionalities	Reference
hnRNPA2B1	Involved in the assembly of SGs, promoting their breakdown, and inhibiting Support Cell Only Syndrome (SCOS) triggered by stress conditions, thereby addressing male infertility.	[100]
SERBP1	Involved in the formation of SGs, it inhibits arsenite stress-induced apoptosis, promotes the clearance of SGs to prevent abnormal accumulation, and suppresses germ cell apoptosis induced by thermal stimulation.	[101]
DAZL	A SGs marker specific to spermatogenic cells, which shields against apoptosis triggered by heat stress.	[14]
BOULE	BOULE plays a role similar to DAZL and is a conserved protein found in male germ cells.	[13]
NEDD4	The regulation of spermatogonial stem cell homeostasis can facilitate SGs dissolution during recovery, thereby preventing the accumulation of pathological SGs.	[110]
MAGE-B2	Regulation of the formation of SGs core protein G3BP1 increases cellular heat stress tolerance	[109]
TIAR-1	Promoting SGs formation in *C. elegans* under heat stress protects the female germ cells from heat shock.	[91]

SGs could be a potential therapeutic target

Phosphorylation of eIF2α is a crucial step in the formation of SGs, positioning its upstream kinases and downstream phosphatases as potential therapeutic targets. Modulating these proteins using small molecule inhibitors or activators can regulate SGs formation, thereby influencing disease processes. Studies have shown that antioxidants, such as cyanidin-3-O-glucoside (C3G) and its metabolites, PCA and vitamin C, can mitigate the effects of environmental stress on testicular tissues by modulating eIF2α phosphorylation. This reduces the translation-blocking process, thereby decreasing SG formation and enhancing the stress tolerance of male germ cells [117]. Consequently, exploring small molecule inhibitors or activators that regulate eIF2α phosphorylation, both upstream and downstream, could bolster stress tolerance and mitigate germ cell damage caused by environmental stressors by modulating SGs formation.

Research has demonstrated that numerous stress granule-associated proteins play pivotal roles in SGs formation and mitigating cellular damage. Proteins such as hnRNPA2B1, SERBP1, and NEDD4 are crucial for the timely clearance of SGs, thus protecting germ cells from damage, preventing Support Cell Only Syndrome (SCOS), and reducing male infertility and aberrant protein accumulation [100, 101, 110] (Fig. 6). Key RNA-binding proteins like G3BP1 and TIA-1/TIAR are essential in SGs assembly. Investigating molecules that interfere with protein-protein interactions or target specific abnormally accumulating RNA-binding proteins could offer new therapeutic avenues. This approach would promote the timely disassembly of SGs and restore normal protein synthesis. Future research should focus on proteins that regulate SGs formation and timely disassembly, as well as the interactions among these proteins, to dynamically manage SGs and improve reproductive health. In cancer cells, SGs formation is accelerated in response to stress from radiotherapy and chemotherapy, significantly reducing the efficacy of these treatments. APE1 has been shown to promote SGs formation through YBX1 phosphorylation [78], suggesting that inhibiting YBX1 phosphorylation could suppress SGs formation in ovarian cancer, thereby enhancing the effects of chemotherapy and radiotherapy. Studies have shown that PKI-402, a dual PI3K/mTOR inhibitor, promotes the formation of SGs in ovarian cancer cells, which play a protective role by intercepting the signaling factor ATF5, inducing changes in localization and modulating mitochondrial unfolded proteins, activating adaptive stress responses and increasing drug resistance [118]. Moreover, an abnormal increase in SGs has been observed in SPOP-mutant prostate cancers cells, where SG assembly enhances cancer cell resistance to chemotherapeutic agents [119]. These findings underscore the importance of exploring molecular targeted drugs that affect SGs in cancer cells. Identifying proteins and factors that promote SG clearance could lead to the development of drugs that enhance the effectiveness of radiotherapy and chemotherapy in ovarian cancer, prostate cancer, and other cancers.

While SGs hold significant promise as therapeutic targets, several challenges persist. The function of SGs varies across different cell types and stress conditions, requiring a precise understanding of their roles in specific diseases. Furthermore, regulating SGs without disrupting normal cellular functions or inducing unwanted side effects is crucial when investigating their therapeutic potential.

Summary

SGs are being studied in full swing as membrane-free organelles that sense and resist changes in the external environment. Their main feature and function is that as a dynamic membrane-free cytoplasmic organelle they can respond to a variety of stress factors both inside and outside the cell and dissolve after the stress is relieved. SGs can promote cell survival, by blocking signaling and inhibiting apoptosis. Current research on SGs primarily focuses on neurodegenerative diseases, cancer and inflammatory diseases. The unique division process and

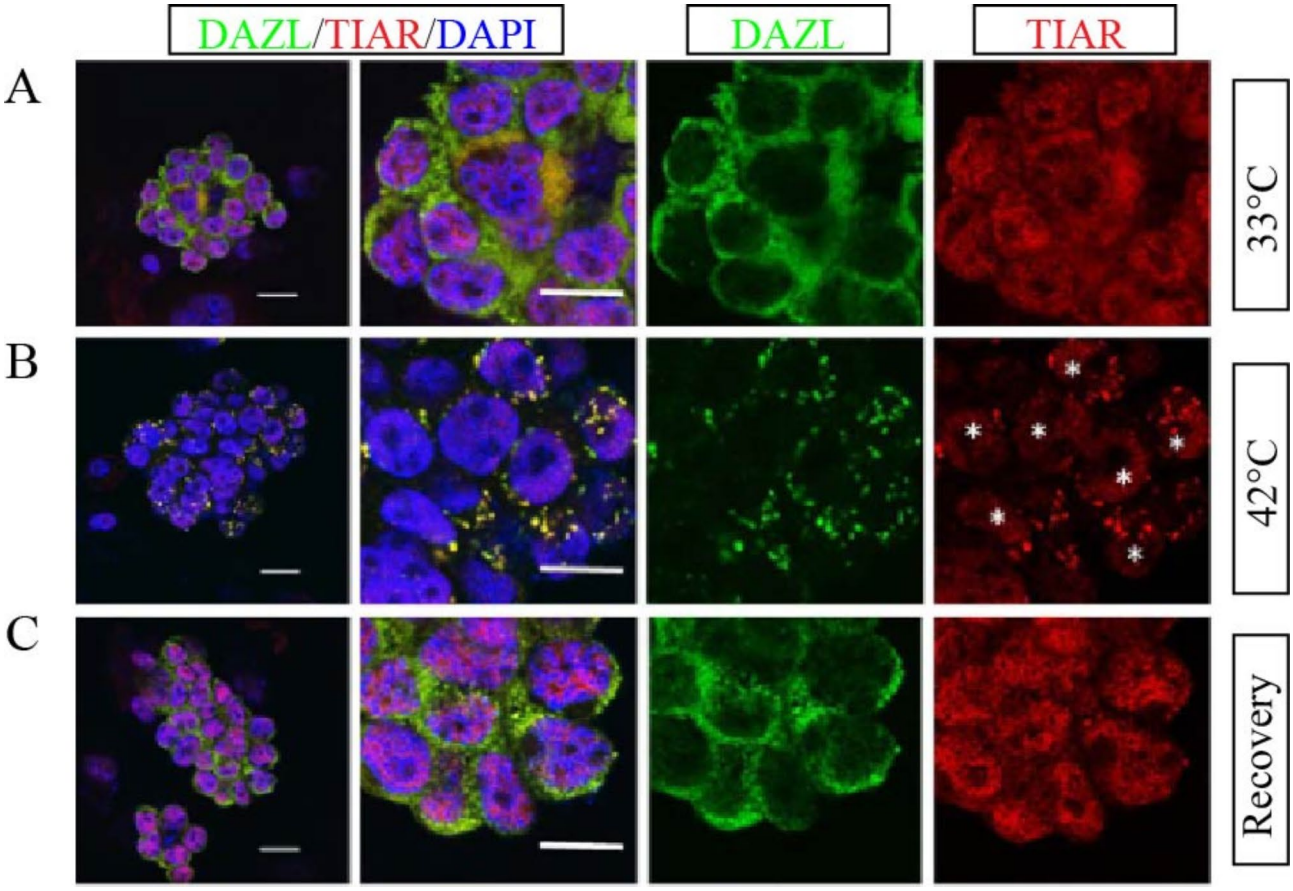


Fig. 6 Formation of stress granules in germline stem cells (GSCs) induced by heat stress (42 °C, 20 min) and in the recovery group (33 °C, 3 h) after heat stress induction [110]. **A-B**: Scale bar, 10 μm. **C**: *GSCs with SGs

prolonged development timeline of germ cells make them particularly vulnerable to environmental factors, which can be harmful. Environmental stimuli can trigger the formation of SGs during gametogenesis, affecting the process of gametogenesis itself. Since gametogenesis is essential for intergenerational transmission, changes induced by environmental stressors may be passed to the next generation via the gametes, leading to potential epigenetic inheritance. Therefore, SGs hold significant importance in the study of reproductive disorders and fertility regulation.

Author contributions

M.G. and L.Z. conceptualized the review and revised manuscript. J.L. and L.S. completed the literature search, diagrams and figures preparation, original and revised manuscript writing. K.W. and S.W. contributed to partial revised manuscript writing. Y.W.、Y.P.、S.C. and T.Z. contributed to partial original manuscript writing. Y.Z.、L.L.、L.S and S.Z. provided advices and critical review in the original and revised manuscript. All authors reviewed and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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References

1. Kedersha N, Anderson PS, Granules. Sites of mRNA triage that regulate mRNA stability and translatability. Biochem Soc Trans. 2002;30.

2. Nadezhdina ES, Lomakin AJ, Shpilman AA, Chudinova EM, Ivanov PA. Microtubules govern stress granule mobility and dynamics. Biochim Biophys Acta. 2010;1803(3):361–71. https://doi.org/10.1016/j.bbamcr.2009.12.004
3. Jain S, Wheeler JR, Walters RW, Agrawal A, Barsic A, Parker R. ATPase-modulated stress granules contain a diverse proteome and substructure. Cell. 2016;164(3):487–98. https://doi.org/10.1016/j.cell.2015.12.038
4. Buchan JR, mRNP, Granules. Assembly, function, and connections with disease. RNA Biol. 2014;11(8):1019–30. https://doi.org/10.4161/15476286.2014.972208
5. Staub C, Johnson L. Review: spermatogenesis in the bull. Animal. 2018;12(s1):s27–35. https://doi.org/10.1017/S1751731118000435
6. Griswold MD, Spermatogenesis. The commitment to meiosis. Physiol Rev. 2016;96(1):1–17. https://doi.org/10.1152/physrev.00013.2015
7. Orsi NM, Baskind NE, Cummings M. Anatomy, development, histology and normal function of the ovary. In: Wilkinson N, editor. Pathology of the Ovary, Fallopian Tube and Peritoneum. Springer International Publishing: Cham,; 2023. pp. 1–33. https://doi.org/10.1007/978-3-031-39659-5_1
8. Yu J, Zhou Y, Luo H, Su X, Gan T, Wang J, Ye Z, Deng Z, He J. Mycoplasma genitalium infection in the female reproductive system: diseases and treatment. Front Microbiol. 2023;14:1098276. https://doi.org/10.3389/fmicb.2023.1098276
9. Makiyan Z. Systematization for female genital anatomic variations. Clin Anat. 2021;34(3):420–30. https://doi.org/10.1002/ca.23668
10. Lotti F, Maggi MS. Dysfunction and male infertility. Nat Rev Urol. 2018;15(5):287–307. https://doi.org/10.1038/nrurol.2018.20
11. Wu F, Yang H, Liu Y, Yang X, Xu B, Liu W, Xu Z, Deng Y. Manganese exposure caused reproductive toxicity of male mice involving activation of GnRH secretion in the hypothalamus by prostaglandin E2 receptors EP1 and EP2. Ecotoxicol Environ Saf. 2020;201:110712. https://doi.org/10.1016/j.ecoenv.2020.110712
12. Cox CM, Thoma ME, Tchangalova N, Mburu G, Bornstein MJ, Johnson CL, Kiarie J. Infertility prevalence and the methods of estimation from 1990 to 2021: a systematic review and meta-analysis. Hum Reprod Open. 2022;2022(4):hoac051. https://doi.org/10.1093/hropen/hoac051
13. Kim B, Rhee K. BOULE, a deleted in azoospermia homolog, is recruited to stress granules in the mouse male germ cells. PLoS ONE. 2016;11(9):e0163015. https://doi.org/10.1371/journal.pone.0163015
14. Kim B, Cooke HJ, Rhee K. DAZL is essential for stress granule formation implicated in germ cell survival upon heat stress. Development. 2012;139(3):568–78. https://doi.org/10.1242/dev.075846
15. Nover L, Scharf KD, Neumann D. Formation of cytoplasmic heat shock granules in tomato cell cultures and leaves. Mol Cell Biol. 1983;3(9):1648–55. https://doi.org/10.1128/mcb.3.9.1648-1655.1983
16. Collier NC, Schlesinger MJ. The dynamic state of heat shock proteins in chicken embryo fibroblasts. J Cell Biol. 1986;103(4):1495–507. https://doi.org/10.1083/jcb.103.4.1495
17. Collier NC, Heuser J, Levy MA, Schlesinger MJ. Ultrastructural and biochemical analysis of the stress granule in chicken embryo fibroblasts. J Cell Biol. 1988;106(4):1131–9. https://doi.org/10.1083/jcb.106.4.1131
18. Kedersha NL, Gupta M, Li W, Miller I, Anderson P. RNA-binding proteins Tia-1 and Tiar link the phosphorylation of Eif-2α to the assembly of mammalian stress granules. J Cell Biol. 1999;147(7):1431–42. https://doi.org/10.1083/jcb.147.7.1431
19. Dunand-Sauthier I, Walker C, Wilkinson C, Gordon C, Crane R, Norbury C, Humphrey T. Sum1, a component of the fission yeast eIF3 translation initiation complex, is rapidly relocalized during environmental stress and interacts with components of the 26S proteasome. MBoC. 2002;13(5):1626–40. https://doi.org/10.1091/mbc.01-06-0301
20. Grousl T, Vojtova J, Hasek J, Vomastek T. Yeast stress granules at a glance. Yeast. 2022;39(4):247–61. https://doi.org/10.1002/yea.3681
21. Mittag T, Parker R. Multiple modes of protein–protein interactions promote RNP granule assembly. J Mol Biol. 2018;430(23):4636–49. https://doi.org/10.1016/j.jmb.2018.08.005
22. Cherkasov V, Grousl T, Theer P, Vainshtein Y, Glässer C, Mongis C, Kramer G, Stoecklin G, Knop M, Mogk A, Bukau B. Systemic control of protein synthesis through sequestration of translation and ribosome biogenesis factors during severe heat stress. FEBS Lett. 2015;589(23):3654–64. https://doi.org/10.1016/j.febslet.2015.10.010
23. Kimiz-Gebologlu I, Oncel SS, Exosomes. Large-scale production, isolation, drug loading efficiency, and biodistribution and uptake. J Controlled Release. 2022;347:533–43. https://doi.org/10.1016/j.jconrel.2022.05.027
24. Poli G, Fabi C, Bellet MM, Costantini C, Nunziangeli L, Romani L, Brancorsini S. Epigenetic mechanisms of inflammasome regulation. Int J Mol Sci. 2020;21(16):5758. https://doi.org/10.3390/ijms21165758
25. Nakatogawa H. Mechanisms governing autophagosome biogenesis. Nat Rev Mol Cell Biol. 2020;21(8):439–58. https://doi.org/10.1038/s41580-020-0241-0
26. Staněk D. Cajal bodies and snRNPs - friends with benefits. RNA Biol. 2017;14(6):671–9. https://doi.org/10.1080/15476286.2016.1231359
27. Xu X, Wu T, Lin R, Zhu S, Ji J, Jin D, Huang M, Zheng W, Ni W, Jiang F, Xuan S, Xiao M. Differences between migrasome, a 'new organelle', and exosome. J Cell Mol Med. 2023;27(23):3672–80. https://doi.org/10.1111/jcmm.17942
28. Rezaie J, Ajezi S, Avci ÇB, Karimipour M, Geranmayeh MH, Nourazarian A, Sokullu E, Rezabakhsh A, Rahbarghazi R. Exosomes and their application in biomedical field: difficulties and advantages. Mol Neurobiol. 2018;55(4):3372–93. https://doi.org/10.1007/s12035-017-0582-7
29. Rowlison T, Comizzoli P. The knowns and unknowns about epididymal extracellular vesicles in different animal species. Adv Biol (Weinh). 2022;6(1):e2101066. https://doi.org/10.1002/adbi.202101066
30. Luo J, Tan X, Li H, Ding X. sncRNAs in epididymosomes: the contribution to embryonic development and offspring health. Int J Mol Sci. 2022;23(18):10851. https://doi.org/10.3390/ijms231810851
31. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell. 2002;10(2):417–26. https://doi.org/10.1016/s1097-2765(02)00599-3
32. Christgen S, Place DE, Kanneganti T-D. Toward targeting inflammasomes: insights into their regulation and activation. Cell Res. 2020;30(4):315–27. https://doi.org/10.1038/s41422-020-0295-8
33. Zhao YG, Zhang H. Autophagosome maturation: an epic journey from the ER to lysosomes. J Cell Biol. 2018;218(3):757–70. https://doi.org/10.1083/jcb.201810099
34. Taliansky ME, Love AJ, Kołowerzo-Lubnau A, Smoliński DJ. Cajal bodies: evolutionarily conserved nuclear biomolecular condensates with properties unique to plants. Plant Cell. 2023;35(9):3214–35. https://doi.org/10.1093/plcell/koad140
35. Ma L, Li Y, Peng J, Wu D, Zhao X, Cui Y, Chen L, Yan X, Du Y, Yu L. Discovery of the migrasome, an organelle mediating release of cytoplasmic contents during cell migration. Cell Res. 2015;25(1):24–38. https://doi.org/10.1038/cr.2014.135
36. Jiao H, Jiang D, Hu X, Du W, Ji L, Yang Y, Li X, Sho T, Wang X, Li Y, Wu Y-T, Wei Y-H, Hu X, Yu L. Mitocytosis, a migrasome-mediated mitochondrial quality-control process. Cell. 2021;184(11):2896–e291013. https://doi.org/10.1016/j.cell.2021.04.027
37. Zhu M, Zou Q, Huang R, Li Y, Xing X, Fang J, Ma L, Li L, Yang X, Yu L. Lateral transfer of mRNA and protein by migrasomes modifies the recipient cells. Cell Res. 2021;31(2):237–40. https://doi.org/10.1038/s41422-020-00415-3
38. Jiang D, Jiang Z, Lu D, Wang X, Liang H, Zhang J, Meng Y, Li Y, Wu D, Huang Y, Chen Y, Deng H, Wu Q, Xiong J, Meng A, Yu L. Migrasomes provide regional cues for organ morphogenesis during zebrafish gastrulation. Nat Cell Biol. 2019;21(8):966–77. https://doi.org/10.1038/s41556-019-0358-6
39. Zhang X, Yao L, Meng Y, Li B, Yang Y, Gao F, Migrasome. A new functional extracellular vesicle. Cell Death Discov. 2023;9(1):1–7. https://doi.org/10.1038/s41420-023-01673-x
40. Oatley JM, Brinster RL. Regulation of spermatogonial stem cell self-renewal in mammals. Annu Rev Cell Dev Biol. 2008;24:263–286. https://doi.org/10.1146/annurev.cellbio.24.110707.175355
41. Oakberg EF. Spermatogonial stem-cell renewal in the mouse. Anat Rec. 1971;169(3):515–31. https://doi.org/10.1002/ar.1091690305
42. Chan F, Oatley MJ, Kaucher AV, Yang Q-E, Bieberich CJ, Shashikant CS, Oatley JM. Functional and molecular features of the Id4 + germline stem cell population in mouse testes. Genes Dev. 2014;28(12):1351–62. https://doi.org/10.1101/gad.240465.114
43. Hogarth CA, Griswold MD. The key role of vitamin A in spermatogenesis. J Clin Invest. 2010;120(4):956–62. https://doi.org/10.1172/JCI41303
44. Wang Z, Xu X, Li J-L, Palmer C, Maric D, Dean J. Sertoli cell-only phenotype and scRNA-Seq define PRAMEF12 as a factor essential for spermatogenesis in mice. Nat Commun. 2019;10(1):5196. https://doi.org/10.1038/s41467-019-13193-3
45. Anderson EL, Baltus AE, Roepers-Gajadien HL, Hassold TJ, de Rooij DG, van Pelt AMM, Page DC. Stra8 and its inducer, retinoic acid, regulate meiotic initiation in both spermatogenesis and oogenesis in mice. Proc Natl Acad Sci U S A. 2008;105(39):14976–80. https://doi.org/10.1073/pnas.0807297105

46. Costa DS, Faria FJC, Fernandes CAC, Silva JCB, Auharek SA. Testis morphometry and kinetics of spermatogenesis in the feral pig (Sus Scrofa). Anim Reprod Sci. 2013;142(1–2):63–70. https://doi.org/10.1016/j.anireprosci.2013.09.007
47. França LR, Avelar GF, Almeida FFL. Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. Theriogenology. 2005;63(2):300–18. https://doi.org/10.1016/j.theriogenology.2004.09.014
48. Muciaccia B, Boitani C, Berloco BP, Nudo F, Spadetta G, Stefanini M, de Rooij DG, Vicini E. Novel stage classification of human spermatogenesis based on acrosome Development1. Biol Reprod. 2013;89(3):60, 1–10. https://doi.org/10.1095/biolreprod.113.111682
49. Staub C, Johnson L. Review: spermatogenesis in the bull. Animal. 2018;12(s27–s35). https://doi.org/10.1017/S1751731118000435
50. Costa GMJ, Leal MC, Silva JV, Cássia A, Ferreira S, Guimarães DA, França LR. Spermatogenic cycle length and sperm production in a feral pig species (Collared Peccary, Tayassu Tajacu). J Androl. 2010;31(2):221–30. https://doi.org/10.2164/jandrol.109.008524
51. Zeng W, Avelar GF, Rathi R, Franca LR, Dobrinski I. The length of the spermatogenic cycle is conserved in porcine and ovine testis xenografts. J Androl. 2006;27(4):527–33. https://doi.org/10.2164/jandrol.05143
52. Soares JM, Avelar GF, França LR. The seminiferous epithelium cycle and its duration in different breeds of dog (Canis Familiaris). J Anat. 2009;215(4):462–71. https://doi.org/10.1111/j.1469-7580.2009.01122.x
53. Silva RC, Costa GMJ, Andrade LM, França LR. Testis stereology, seminiferous epithelium cycle length, and daily sperm production in the ocelot (Leopardus Pardalis). Theriogenology. 2010;73(2):157–67. https://doi.org/10.1016/j.theriogenology.2009.08.009
54. Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM, Ron D. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell. 2003;11(3):619–33. https://doi.org/10.1016/s1097-2765(03)00105-9
55. Ron D. Translational control in the endoplasmic reticulum stress response. J Clin Invest. 2002;110(10):1383–8. https://doi.org/10.1172/JCI16784
56. Wek RC, Jiang H-Y, Anthony TG. Coping with stress: eIF2 kinases and translational control. Biochem Soc Trans. 2006;34(Pt 1):7–11. https://doi.org/10.1042/BST20060007
57. Perkins DJ, Barber GN. Defects in translational regulation mediated by the α subunit of eukaryotic initiation factor 2 inhibit antiviral activity and facilitate the malignant transformation of human fibroblasts. Mol Cell Biol. 2004;24(5):2025–40. https://doi.org/10.1128/MCB.24.5.2025-2040.2004
58. Mazroui R, Sukarieh R, Bordeleau M-E, Kaufman RJ, Northcote P, Tanaka J, Gallouzi I, Pelletier J. Inhibition of ribosome recruitment induces stress granule formation independently of eukaryotic initiation factor 2α phosphorylation. MBoC. 2006;17(10):4212–9. https://doi.org/10.1091/mbc.e06-04-0318
59. Banani SF, Lee HO, Hyman AA, Rosen MK. Biomolecular condensates: organizers of cellular biochemistry. Nat Rev Mol Cell Biol. 2017;18(5):285–98. https://doi.org/10.1038/nrm.2017.7
60. Yang P, Mathieu C, Kolaitis R-M, Zhang P, Messing J, Yurtsever U, Yang Z, Wu J, Li Y, Pan Q, Yu J, Martin EW, Mittag T, Kim HJ, Taylor JP. G3BP1 is a tunable switch that triggers phase separation to assemble stress granules. Cell. 2020;181(2):325–e34528. https://doi.org/10.1016/j.cell.2020.03.046
61. Millar SR, Huang JQ, Schreiber KJ, Tsai Y-C, Won J, Zhang J, Moses AM, Youn J-Y. A new phase of networking: the molecular composition and regulatory dynamics of mammalian stress granules. Chem Rev. 2023;123(14):9036–64. https://doi.org/10.1021/acs.chemrev.2c00608
62. Cirillo L, Cieren A, Barbieri S, Khong A, Schwager F, Parker R, Gotta M. UBAP2L forms distinct cores that act in nucleating stress granules upstream of G3BP1. Curr Biol. 2020;30(4):698–e7076. https://doi.org/10.1016/j.cub.2019.12.020
63. Kedersha N, Panas MD, Achorn CA, Lyons S, Tisdale S, Hickman T, Thomas M, Lieberman J, McInerney GM, Ivanov P, Anderson P. G3BP-Caprin1-USP10 complexes mediate stress granule condensation and associate with 40S subunits. J Cell Biol. 2016;212(7):845–60. https://doi.org/10.1083/jcb.201508028
64. Gilks N, Kedersha N, Ayodele M, Shen L, Stoecklin G, Dember LM, Anderson P. Stress granule assembly is mediated by prion-like aggregation of TIA-1. MBoC. 2004;15(12):5383–98. https://doi.org/10.1091/mbc.e04-08-0715
65. Dolliver SM, Kleer M, Bui-Marinos MP, Ying S, Corcoran JA, Khaperskyy DA. Nsp1 proteins of human coronaviruses HCoV-OC43 and SARS-CoV2 inhibit stress granule formation. PLoS Pathog. 2022;18(12):e1011041. https://doi.org/10.1371/journal.ppat.1011041
66. Amen T, Kaganovich D. Quantitative photoconversion analysis of internal molecular dynamics in stress granules and other membraneless organelles in live cells. STAR Protoc. 2020;1(3):100217. https://doi.org/10.1016/j.xpro.2020.100217
67. Protter DSW, Parker R. Principles and properties of stress granules. Trends Cell Biol. 2016;26(9):668–79. https://doi.org/10.1016/j.tcb.2016.05.004
68. Cherkasov V, Hofmann S, Druffel-Augustin S, Mogk A, Tyedmers J, Stoecklin G, Bukau B. Coordination of translational control and protein homeostasis during severe heat stress. Curr Biol. 2013;23(24):2452–62. https://doi.org/10.1016/j.cub.2013.09.058
69. Wheeler JR, Matheny T, Jain S, Abrisch R, Parker R. Distinct stages in stress granule assembly and disassembly. Elife. 2016;5:e18413. https://doi.org/10.7554/eLife.18413
70. Wilbertz JH, Voigt F, Horvathova I, Roth G, Zhan Y, Chao JA. Single-molecule imaging of mRNA localization and regulation during the integrated stress response. Mol Cell. 2019;73(5):946–e9587. https://doi.org/10.1016/j.molcel.2018.12.006
71. Jackson RJ, Hellen CUT, Pestova TV. The mechanism of eukaryotic translation initiation and principles of its regulation. Nat Rev Mol Cell Biol. 2010;11(2):113–27. https://doi.org/10.1038/nrm2838
72. Ganassi M, Mateju D, Bigi I, Mediani L, Poser I, Lee HO, Seguin SJ, Morelli FF, Vinet J, Leo G, Pansarasa O, Cereda C, Poletti A, Alberti S, Carra S. A surveillance function of the HSPB8-BAG3-HSP70 chaperone complex ensures stress granule integrity and dynamism. Mol Cell. 2016;63(5):796–810. https://doi.org/10.1016/j.molcel.2016.07.021
73. Turakhiya A, Meyer SR, Marincola G, Böhm S, Vanselow JT, Schlosser A, Hofmann K, Buchberger A. ZFAND1 recruits P97 and the 26S proteasome to promote the clearance of arsenite-induced stress granules. Mol Cell. 2018;70(5):906–e9197. https://doi.org/10.1016/j.molcel.2018.04.021
74. Maxwell BA, Gwon Y, Mishra A, Peng J, Nakamura H, Zhang K, Kim HJ, Taylor JP. Ubiquitination is essential for recovery of cellular activities after heat shock. Science. 2021;372(6549):eabc3593. https://doi.org/10.1126/science.abc3593
75. Gwon Y, Maxwell BA, Kolaitis R-M, Zhang P, Kim HJ, Taylor JP. Ubiquitination of G3BP1 mediates stress granule disassembly in a context-specific manner. Science. 2021;372(6549):eabf6548. https://doi.org/10.1126/science.abf6548
76. Buchan JR, Kolaitis R-M, Taylor JP, Parker R. Eukaryotic stress granules are cleared by autophagy and Cdc48/VCP function. Cell. 2013;153(7):1461–74. https://doi.org/10.1016/j.cell.2013.05.037
77. Paget M, Cadena C, Ahmad S, Wang H-T, Jordan TX, Kim E, Koo B, Lyons SM, Ivanov P, tenOever B, Mu X, Hur S. Stress granules are shock absorbers that prevent excessive innate immune responses to dsRNA. Mol Cell. 2023;83(7):1180–e11968. https://doi.org/10.1016/j.molcel.2023.03.010
78. Mao S, Xie C, Liu Y, Zhao Y, Li M, Gao H, Xiao Y, Zou Y, Zheng Z, Gao Y, Xie J, Tian B, Wang L, Hua Y, Xu H. Apurinic/Apyrimidinic Endodeoxyribonuclease 1 (APE1) promotes stress granule formation via YBX1 phosphorylation in ovarian cancer. Cell Mol Life Sci. 2024;81(1):113. https://doi.org/10.1007/s00018-023-05086-y
79. Grabocka E, Bar-Sagi D, Mutant KRAS. Enhances tumor cell fitness by upregulating stress granules. Cell. 2016;167(7):1803–e181312. https://doi.org/10.1016/j.cell.2016.11.035
80. Takahashi M, Higuchi M, Matsuki H, Yoshita M, Ohsawa T, Oie M, Fujii M. Stress granules inhibit apoptosis by reducing reactive oxygen species production. Mol Cell Biol. 2013;33(4):815–29. https://doi.org/10.1128/MCB.00763-12
81. Li W-Y, Yang F, Li X, Wang L-W, Wang Y. Stress granules inhibit endoplasmic reticulum stress-mediated apoptosis during hypoxia-induced injury in acute liver failure. World J Gastroenterol. 2023;29(8):1315–29. https://doi.org/10.3748/wjg.v29.i8.1315
82. Wippich F, Bodenmiller B, Trajkovska MG, Wanka S, Aebersold R, Pelkmans L. Dual specificity kinase DYRK3 couples stress granule condensation/dissolution to mTORC1 signaling. Cell. 2013;152(4):791–805. https://doi.org/10.1016/j.cell.2013.01.033
83. Takahara T, Maeda T. Transient sequestration of TORC1 into stress granules during heat stress. Mol Cell. 2012;47(2):242–52. https://doi.org/10.1016/j.molcel.2012.05.019
84. Park Y-J, Choi DW, Cho SW, Han J, Yang S, Choi CY. Stress granule formation attenuates RACK1-mediated apoptotic cell death induced by morusin. Int J Mol Sci. 2020;21(15):5360. https://doi.org/10.3390/ijms21155360
85. Kim WJ, Back SH, Kim V, Ryu I, Jang SK. Sequestration of TRAF2 into stress granules interrupts tumor necrosis factor signaling under stress conditions. Mol Cell Biol. 2005;25(6):2450–62. https://doi.org/10.1128/MCB.25.6.2450-2462.2005
86. Kitajima H, Maruyama R, Niinuma T, Yamamoto E, Takasawa A, Takasawa K, Ishiguro K, Tsuyada A, Suzuki R, Sudo G, Kubo T, Mitsuhashi K, Idogawa M,

Tange S, Toyota M, Yoshido A, Kumegawa K, Kai M, Yanagihara K, Tokino T, Osanai M, Nakase H, Suzuki H. TM4SF1-AS1 inhibits apoptosis by promoting stress granule formation in cancer cells. Cell Death Dis. 2023;14(7):1–14. https://doi.org/10.1038/s41419-023-05953-3
87. Rayman JB, Karl KA, Kandel ER. TIA-1 self-multimerization, phase separation, and recruitment into stress granules are dynamically regulated by Zn2. Cell Rep. 2018;22(1):59–71. https://doi.org/10.1016/j.celrep.2017.12.036
88. Verma A, Sumi S, Seervi M. Heat shock proteins-driven stress granule dynamics: yet another avenue for cell survival. Apoptosis. 2021;26(7–8):371–84. https://doi.org/10.1007/s10495-021-01678-w
89. Lismer A, Kimmins S. Emerging evidence that the mammalian sperm epigenome serves as a template for embryo development. Nat Commun. 2023;14(1):2142. https://doi.org/10.1038/s41467-023-37820-2
90. Xavier MJ, Roman SD, Aitken RJ, Nixon B. Transgenerational inheritance: how impacts to the epigenetic and genetic information of parents affect offspring health. Hum Reprod Update. 2019;25(5):518–40. https://doi.org/10.1093/humupd/dmz017
91. Huelgas-Morales G, Silva-García CG, Salinas LS, Greenstein D, Navarro RE. The stress granule RNA-binding protein TIAR-1 protects female germ cells from heat shock in caenorhabditis elegans. G3 (Bethesda). 2016;6(4):1031–47. https://doi.org/10.1534/g3.115.026815
92. Fénichel P, Chevalier N, Lahlou N, Coquillard P, Wagner-Mahler K, Pugeat M, Panaïa-Ferrari P, Brucker-Davis F. Endocrine disrupting chemicals interfere with leydig cell hormone pathways during testicular descent in idiopathic cryptorchidism. Front Endocrinol (Lausanne). 2018;9:786. https://doi.org/10.3389/fendo.2018.00786
93. Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, Toppari J, Andersson A-M, Eisenberg ML, Jensen TK, Jørgensen N, Swan SH, Sapra KJ, Ziebe S, Priskorn L, Juul A. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. Physiol Rev. 2016;96(1):55–97. https://doi.org/10.1152/physrev.00017.2015
94. Jurewicz J, Hanke W, Radwan M, Bonde JP. Environmental factors and semen quality. Int J Occup Med Environ Health. 2009;22(4):305–29. https://doi.org/10.2478/v10001-009-0036-1
95. Bhardwaj JK, Bikal P, Sachdeva SN. Cadmium as an ovarian toxicant: a review. J Appl Toxicol. 2024;44(1):129–47. https://doi.org/10.1002/jat.4526
96. Bhardwaj JK, Paliwal A, Saraf P. Effects of heavy metals on reproduction owing to infertility. J Biochem Mol Toxicol. 2021;35(8):e22823. https://doi.org/10.1002/jbt.22823
97. Mandal BK, Suzuki KT. Arsenic round the world: a review. Talanta. 2002;58(1):201–35. https://doi.org/10.1016/S0039-9140(02)00268-0
98. Rosenblatt AE, Burnstein KL. Inhibition of androgen receptor transcriptional activity as a novel mechanism of action of arsenic. Mol Endocrinol. 2009;23(3):412–21. https://doi.org/10.1210/me.2008-0235
99. Celino-Brady FT, Lerner DT, Seale AP. Experimental approaches for characterizing the endocrine-disrupting effects of environmental chemicals in fish. Front Endocrinol (Lausanne). 2020;11:619361. https://doi.org/10.3389/fendo.2020.619361
100. Wang X, Fan X, Zhang J, Wang F, Chen J, Wen Y, Wang L, Li T, Li H, Gu H, Zhang Y, Yuan S. hnRNPA2B1 represses the disassembly of arsenite-induced stress granules and is essential for male fertility. Cell Rep. 2024;43(2):113769. https://doi.org/10.1016/j.celrep.2024.113769
101. Wang F, Wang L, Gan S, Feng S, Ouyang S, Wang X, Yuan S. SERBP1 promotes stress granule clearance by regulating 26S proteasome activity and G3BP1 ubiquitination and protects male germ cells from Thermostimuli damage. Research. 2023;6:0091. https://doi.org/10.34133/research.0091
102. Mínguez-Alarcón L, Gaskins AJ, Chiu Y-H, Messerlian C, Williams PL, Ford JB, Souter I, Hauser R, Chavarro JE. Type of underwear worn and markers of testicular function among men attending a fertility center. Hum Reprod. 2018;33(9):1749–56. https://doi.org/10.1093/humrep/dey259
103. Luchetti F, Mannello F, Canonico B, Battistelli M, Burattini S, Falcieri E, Papa S. Integrin and cytoskeleton behaviour in human neuroblastoma cells during hyperthermia-related apoptosis. Apoptosis. 2004;9(5):635–48. https://doi.org/10.1023/B:APPT.0000038043.03799.6f
104. Pawlik A, Nowak JM, Grzanka D, Gackowska L, Michalkiewicz J, Grzanka A. Hyperthermia induces cytoskeletal alterations and mitotic catastrophe in P53-deficient H1299 lung cancer cells. Acta Histochem. 2013;115(1):8–15. https://doi.org/10.1016/j.acthis.2012.02.006
105. Wang Y, Guan J, Wang H, Wang Y, Leeper D, Iliakis G. Regulation of Dna replication after heat shock by replication protein A-Nucleolin interactions. J Biol Chem. 2001;276(23):20579–88. https://doi.org/10.1074/jbc.M100874200
106. Widlak W, Vydra N. The role of heat shock factors in mammalian spermatogenesis. Adv Anat Embryol Cell Biol. 2017;222:45–65. https://doi.org/10.1007/978-3-319-51409-3_3
107. Zhang P, Zheng Y, Lv Y, Li F, Su L, Qin Y, Zeng W. Melatonin protects the mouse testis against heat-induced damage. Mol Hum Reprod. 2020;26(2):65–79. https://doi.org/10.1093/molehr/gaaa002
108. Alves MBR, Arruda RP. Batissaco, L.; Garcia-Oliveros, L. N.; Gonzaga, V. H. G.; Nogueira, V. J. M.; Almeida, F. dos S.; Pinto, S. C. C.; Andrade, G. M.; Perecin, F.; da Silveira, J. C.; Celeghini, E. C. C. Changes in miRNA levels of sperm and small extracellular vesicles of seminal plasma are associated with transient scrotal heat stress in bulls. Theriogenology. 2021;161:26–40. https://doi.org/10.1016/j.theriogenology.2020.11.015
109. Lee AK, Klein J, Tacer KF, Lord T, Oatley MJ, Oatley JM, Porter SN, Pruett-Miller SM, Tikhonova EB, Karamyshev AL, Wang Y-D, Yang P, Korff A, Kim HJ, Taylor JP, Potts PR. Translational repression of G3BP in cancer and germ cells suppresses stress granules and enhances stress tolerance. Mol Cell. 2020;79(4):645–e6599. https://doi.org/10.1016/j.molcel.2020.06.037
110. Zhou Z, Kawabe H, Suzuki A, Shinmyozu K, Saga Y. NEDD4 controls spermatogonial stem cell homeostasis and stress response by regulating messenger ribonucleoprotein complexes. Nat Commun. 2017;8(1):15662. https://doi.org/10.1038/ncomms15662
111. Javed Z, Qamar U, Sathyapalan T. Pituitary and/or hypothalamic dysfunction following moderate to severe traumatic brain injury: current perspectives. Indian J Endocrinol Metab. 2015;19(6):753–63. https://doi.org/10.4103/2230-8210.167561
112. Anderson EN, Gochenaur L, Singh A, Grant R, Patel K, Watkins S, Wu JY, Pandey UB. Traumatic injury induces stress granule formation and enhances motor dysfunctions in ALS/FTD models. Hum Mol Genet. 2018;27(8):1366–81. https://doi.org/10.1093/hmg/ddy047
113. Tanriverdi F, Unluhizarci K, Selcuklu A, Casanueva FF, Kelestimur F. Transient hypogonadotropic hypogonadism in an amateur kickboxer after head trauma. J Endocrinol Invest. 2007;30(2):150–2. https://doi.org/10.1007/BF03347414
114. Foley CM, Wang DH. Central diabetes insipidus following a sports-related concussion: a case report. Sports Health. 2012;4(2):139–41. https://doi.org/10.1177/1941738111434275
115. Tanriverdi F, Unluhizarci K, Coksevim B, Selcuklu A, Casanueva FF, Kelestimur F. Kickboxing sport as a new cause of traumatic brain injury-mediated hypopituitarism. Clin Endocrinol (Oxf). 2007;66(3):360–6. https://doi.org/10.1111/j.1365-2265.2006.02737.x
116. Ives JC, Alderman M, Stred SE. Hypopituitarism after multiple concussions: a retrospective case study in an adolescent male. J Athl Train. 2007;42(3):431–9.
117. Cai D, Li X, Xu Q, Li H, Liu R, Chen J, Jiang X, Sun J, Lai C, Bai W. Cyanidin-3-O-glucoside and protocatechuic acid alleviate heat stress-induced testicular damage. Food Funct. 2023;14(4):2200–11. https://doi.org/10.1039/d2fo03423a
118. Lin N, Sun L, Chai J, Qi H, Zhao Y, Ma J, Xia M, Hu X. Stress granules affect the dual PI3K/mTOR inhibitor response by regulating the mitochondrial unfolded protein response. Cancer Cell Int. 2024;24(1):38. https://doi.org/10.1186/s12935-024-03210-x
119. Shi Q, Zhu Y, Ma J, Chang K, Ding D, Bai Y, Gao K, Zhang P, Mo R, Feng K, Zhao X, Zhang L, Sun H, Jiao D, Chen Y, Sun Y, Zhao S, Huang H, Li Y, Ren S, Wang C. Prostate cancer-associated SPOP mutations enhance cancer cell survival and docetaxel resistance by upregulating caprin1-dependent stress granule assembly. Mol Cancer. 2019;18(1):170. https://doi.org/10.1186/s12943-019-1096-x

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