Review Article

An emerging role for stress granules in neurodegenerative disease and hearing loss

Jack L. Martin, Sally J. Dawson[1,*], Jonathan E. Gale[1,*]

UCL Ear Institute, 332 Gray's Inn Road, London WC1X 8EE, UK

ABSTRACT

Stress granules (SGs) are membrane-less cytosolic assemblies that form in response to stress (e.g., heat, oxidative stress, hypoxia, viral infection and UV). Composed of mRNA, RNA binding proteins and signalling proteins, SGs minimise stress-related damage and promote cell survival. Recent research has shown that the stress granule response is vital to the cochlea's response to stress. However, emerging evidence suggests stress granule dysfunction plays a key role in the pathophysiology of multiple neurodegenerative diseases, several of which present with hearing loss as a symptom. Hearing loss has been identified as the largest potentially modifiable risk factor for dementia. The underlying reason for the link between hearing loss and dementia remains to be established. However, several possible mechanisms have been proposed including a common pathological mechanism. Here we will review the role of SGs in the pathophysiology of neurodegenerative diseases and explore possible links and emerging evidence that they may play an important role in maintenance of hearing and may be a common mechanism underlying age-related hearing loss and dementia.

1. Introduction

The cochlea, the organ of hearing is an extremely stressful environment. Repeated exposure to noise, use of ototoxic medications to treat infections (Huth et al., 2011) or cancer (Rybak et al., 2009), viral infection (Cohen et al., 2014) and the ageing process (Keithley, 2020) can all lead to the irreversible loss of cochlear cells, particularly sensory hair cells, ultimately leading to hearing loss. Cells have evolved mechanisms to deal with and respond to these stressors including the temporary formation of stress granules (SGs). SGs are cytoplasmic aggregations of RNA binding proteins which regulate translation of specific RNAs during stress and act as cell signalling hubs in order to aid cell recovery from stress (Anderson and Kedersha, 2006). Dysregulation of SGs or mutation of SG components can lead to aberrant, persistent SGs that have been shown to play a crucial role in the pathogenesis of cancer (Anderson et al., 2015) and neurodegeneration, particularly in amy-

otrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD) (Zhang et al., 2019). Our understanding of the role of SGs in the cochlear recovery from stress is much more limited. Noise-induced hearing loss (NIHL), age-related hearing loss (ARHL) and ototoxicity result in extensive damage to cochlear cells. Determining the SG response to these insults in the cochlea could inform the development of preventative SG-based therapies. The link between hearing loss and dementia is a particularly important area of interest. ARHL is the most common sensory deficit in the elderly and has been identified as the highest modifiable risk factor for dementia (Livingston et al., 2020).The underlying reasons for the link between ARHL and dementia remain to be established. Several hypotheses have been put forward including the additional cognitive load associated with impaired hearing or the possibility that simply that the hearing loss is an early manifestation of existing cognitive decline (Peelle, 2018; Shen et al., 2018). Another possibility is that a common pathological mechanism (or mechanisms) underlies both conditions. Given the emerging central role of SGs in the pathology of several neurodegenerative diseases and the link between hearing loss in mid-life and dementia, we hypothesise that aberrant/chronic SGs may contribute to both the development of ARHL and to dementia. This review will describe the biology of SGs, will focus on emerging evidence that SGs may play an important role in maintenance of hearing and will explore the role aberrant SGs may play in the pathophysiology of neurodegenerative diseases including dementia. Additionally, the signalling pathways regulated by SGs and how their disruption by aberrant or persistent SGs may lead to hearing loss will be explored.

2. What are stress granules?

SGs are membrane-less cytosolic assemblies of messenger ribonucleoproteins (mRNPs) that form in response to the inhibition of translation initiation by environmental stress (e.g., heat, oxidative stress, hypoxia, viral infection and UV). SGs are dynamic structures that quickly form when cells are exposed to a stress stimulus, and normally disperse when the stress is resolved and normal translation conditions are restored (Kedersha et al., 1999). SGs are composed of mRNA, RNA binding proteins, 40s ribosomal subunits and mRNA associated translation initiation complexes. Multiple SG-related RNA-binding proteins (RBPs) have been identified, including: cell cycle associated protein 1 (Caprin1), T-cell intracellular antigen 1 (TIA-1), Human antigen R (HuR) and Ras GTPase-activating protein-binding protein 1 (G3BP1), (Anderson and Kedersha, 2006). SGs serve as temporary repositories for these complexes, preventing translation of bound mRNA during stress and allowing these largely pre-assembled complexes to resume gene expression when they are released after stress resolution (Anderson and Kedersha, 2009b). SGs range in size from 0.1 to 2μm and were first identified in tomato cells exposed to heat shock (Nover et al., 1983). This led to SGs originally being termed heat-stress granules, yet a characteristic of SGs is the exclusion of heat-shock proteins (HSPs) (Nover et al., 1989). Subsequent work has identified numerous proteins and mRNA that are components of SGs (see Table 1), several of which have links to hearing loss. Like other RNA granules SGs arise, at least in part, through liquid-liquid phase separation (LLPS). LLPS is a biophysical phenomenon in which RNA-protein complexes separate from the surrounding aqueous cytoplasm to create a functional cellular compartment with liquid properties (Hyman et al., 2014).

SGs interact closely with another type of RNA granule, processing bodies (PBs). PBs are cytoplasmic RNP granules that are constitutively expressed and conserved amongst eukaryotes and bear similarities to SGs (Eulalio et al., 2007). PBs are composed of translationally repressed mRNAs and proteins related to mRNA decay. The initial discovery that proteins associated with the 5' to 3'

mRNA decay pathway and decay intermediates were localised to PBs led to the hypothesis that PBs were cellular sites of mRNA decay. However, subsequent work has shown that mRNA decay can occur in the absence of PBs (Eulalio et al., 2007), and that mRNA can be recycled from PBs to translating polysomes (Brengues et al., 2005). These observations led to the emergence of an alternative model in which PBs are storage sites for translationally repressed mRNAs and inactive mRNA decay enzymes (Hubstenberger et al., 2017). PBs have been studied far less extensively than SGs and to date there is less evidence linking PB dysfunction to disease than there is for SGs. However, given the role of PBs in the storage of translationally repressed mRNAs and their interaction with SGs these have the potential to impact both hearing loss and neurodegenerative disease.

SG formation is proposed to affect biological reactions in several ways. Firstly, SGs act as an RNA triage centre, sequestering mRNA of house-keeping proteins and prioritising the continued translation of proteins that are involved with the stress response (Anderson and Kedersha, 2008). Secondly, through activation of stress-associated proteins due to their concentrating effect. For example, during viral infection SGs recruit high concentrations of anti-viral proteins, stimulating their activation and enhancing the induction of the innate immune response and restricting virus replication (Rozelle et al., 2014). Thirdly, through their modulation of signalling pathways by limiting the interactions of sequestered components of signalling pathways, such as Receptor for activated C kinase 1 (RACK1), target of rapamycin (TOR) and Tumor necrosis factor receptor associated factor 2 (TRAF2) (Takahashi et al., 2013; Arimoto et al., 2008; Kim et al., 2005). In recent years, it has become clear that the assembly and content of SGs varies depending on cell type and the stress involved.

2.1. Stress granule assembly

SGs form in response to the inhibition of translation initiation by a stress stimulus. There are two mechanisms by which this happens, the canonical and non-canonical pathways (Fig. 1). In the **canonical SG pathway**, a stress stimulus activates one or more eIF2α kinases. This leads to phosphorylation of eIF2α, a component of the ternary complex that delivers tRNA to translationally competent pre-initiation complexes assembled at the 5' ends of mRNA (Jackson et al., 2010). eIF2α phosphorylation results in the reduced ability of eIF2 to couple with GTP and consequently, to deliver initiator tRNA to ribosomes for start codon recognition, ultimately leading to a reduction in global translation initiation.

There are four known mammalian eIF2α kinases, each is activated by a distinct stimulus:

1. Heme-regulated initiation factor 2α kinase (HRI, eIF2αK1) monitors the synthesis of globin chains in order to balance them with available heme levels during erythrocyte maturation and also senses oxidative stress (Lu et al., 2001).
2. Protein kinase RNA-activated (PKR, eIF2αK2) is activated by double stranded RNA resulting from viral infection, heat shock, or UV radiation (Srivastava et al., 1998).
3. PKR-like endoplasmic reticulum kinase (PERK, eIF2αK3) is activated by disruption of protein homeostasis in the ER lumen (Harding et al., 2000).
4. General control nonderepressible 2 (GCN2, eIF2αK4) monitors amino acid levels and is activated by amino acid deprivation (Wek et al., 1995).

Phosphorylation of eIF2α by one or more of these kinases results in reduced availability of the eIF2α-GTP-tRNA$_i^{met}$ ternary complex resulting in stalled translation. Unaffected elongating ribosomes "run off" stalled polysomes, resulting in circularised mRNA still bound to the preinitiation machinery. Aggregation of

Table 1
Common stress granule components and their properties, functions and links to hearing.

Component	Properties and function	Links to hearing
Caprin1	Cell cycle proliferation; RNA binding protein; Nucleates SGs (Kedersha et al., 2016)	Deletion in mouse leads to progressive hearing loss (Nolan et al., 2022)
DDX3X	Recruitment to SG prevents assembly and activation of the NLRP3 inflammasome (Samir et al., 2019)	Characteristics of human DDX3X mutation include hearing impairments (Snijders Blok et al., 2015). NLRP3 inflammasome activation associated with several hearing losses (Nakanishi et al., 2017; Shi et al., 2017)
G3BP	Ras signaling; SG assembly (Kedersha et al., 2016)	No known role
hnRNPA1	Splicing; export; translational regulator; mRNA stability (Clarke et al., 2021)	Mislocalisation identified in MS. MS patients can present with hearing loss (Ralli et al., 2018)
HuR	mRNA stability; splicing regulator (Anderson et al., 2015)	No known link
Lin28	RNA binding protein; regulates miRNA let-7; localises to SGs (Balzer and Moss, 2007)	Controls the ability of neonatal mouse supporting cells to generate hair cells through mTOR signaling (Li and Doetzlhofer, 2020)
RACK1	Cell signaling scaffold protein; apoptotic regulator, recruitment to SGs inhibits apoptosis (Arimoto et al., 2008)	No known link
Rbm24	RNA binding. Localises to SGs under various stresses (Wang et al., 2022)	Rbm24 deficiency leads to hair cell death and hearing loss (Zhang et al., 2020). Regulated by Atoh1.
TDP-43	DNA/RNA binding; mRNA transport (Colombrita et al., 2009)	Mutations in TDP-43 associated with ALS. Madras motor neuron sub-type presents with hearing loss (Nalini et al., 2008)
TIA-1	Nucleates SGs (Gilks et al., 2004)	No known link
TRAF2	TNFα signaling, sequestered by SGs (Kim et al., 2005)	No known link
VCP/p97	Participates in SG disassembly, directing proteins to degradation (Buchan et al., 2013)	Inclusion body myopathy with Paget's disease of the bone and fronto-temporal dementia (IBMPFD) caused by VCP mutations can lead to hearing loss (Tresse et al., 2010)
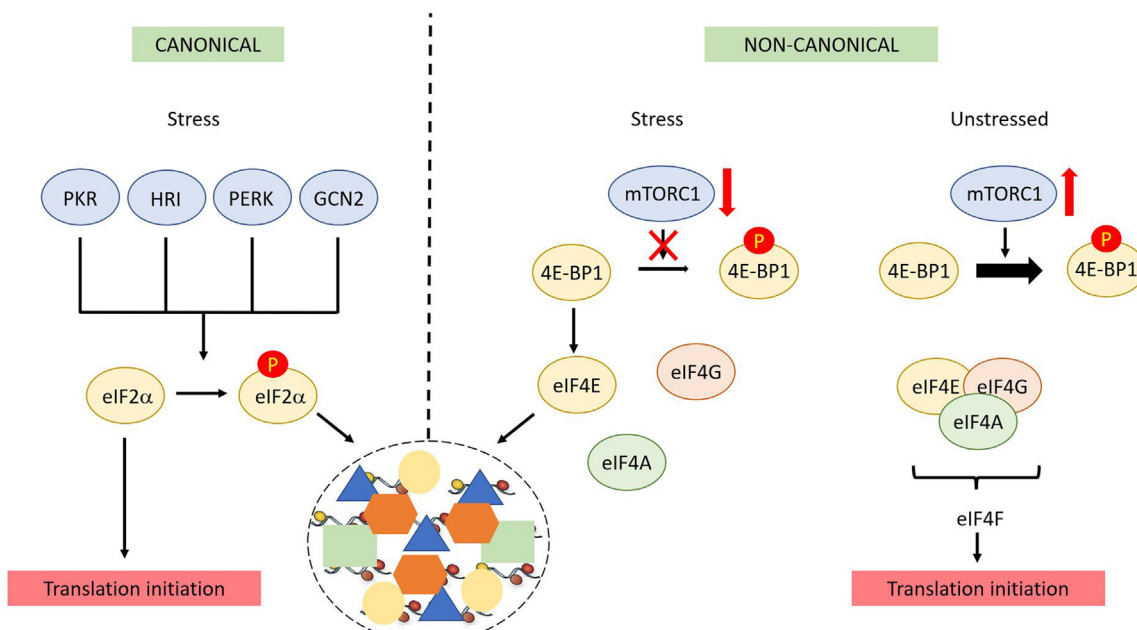
Fig. 1. Canonical and non-canonical formation of stress granules. Canonical or eIF2α dependent assembly of SGs is initiated when a stimulus activates one of the four stress kinases, leading to the phosphorylation of eIF2α and inhibition of translation initiation and subsequent SG formation. In the non-canonical or eIF2α-independent pathway, stress causes the inactivation of mTORC1, resulting in the accumulation of hypo-phosphorylated 4E-BP1. 4E-BP1 is then able to bind to eIF4E, displacing eIF4G and eIF4A from the eIF4F complex. Consequently, translation initiation is inhibited and SGs are formed.

these mRNPs results in the formation of SGs (Anderson and Kedersha, 2009a).

In the **non-canonical SG pathway**, stress induced inactivation of mammalian target of rapamycin (mTOR) leads to dephosphorylation of p-4E-BP1 and its conversion into an active form that prevents the assembly of eIF4F and inhibits translation initiation (Dang et al., 2006).

SGs are comprised of at least two phases; solid-like RNP cores that form early during SG assembly, suggesting that they may pro-

vide the specific interactions that are necessary for seeding formation of the second phase, a liquid-like SG shell (Wheeler et al., 2016). Polyribosome disassembly not only leads to the release of mRNAs and translational factors, but also of newly synthesised polypeptides. These polypeptides fold into their native structure with the assistance of chaperones. However, it is estimated that between 6% and 30% of all newly synthesised proteins are defective ribosomal products (DRiPs) that are very rapidly degraded by the ubiquitin-proteasome system (UPS) (Alberti et al., 2017). It has
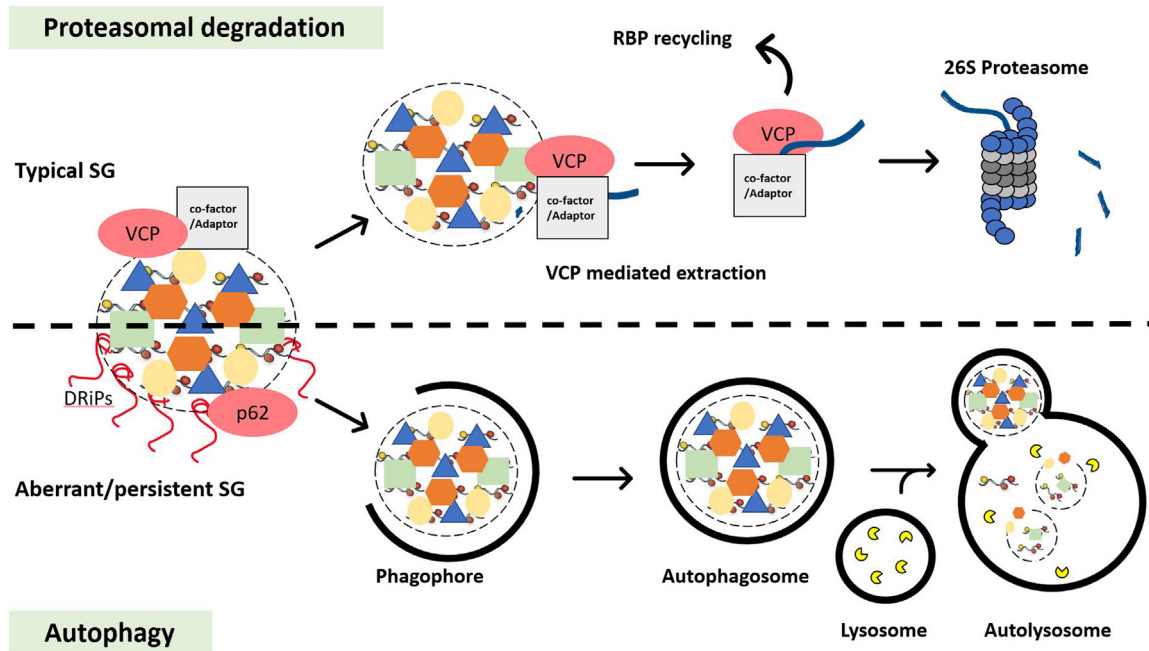
Fig. 2. Stress granule clearance by either proteasome or autophagy mediated degradation. Typical SGs are cleared by chaperone mediated proteasomal degradation. VCP in conjunction with co-factors and adaptors extract RBPs from SGs for RBP recycling or directs them to the 26S proteasome for degradation. Under conditions of VCP impairment or chronic stress, DRiP positive SGs are cleared by autophagy.

been shown previously that DRiPs and 60S are released from disassembling polysomes prior to SG assembly and are cleared with the assistance of autophagy, lysosomes, Valosin-containing protein (VCP) and the UPS. In cases of autophagy impairment or VCP depletion, DRiPs accumulate within or adjacent to SGs. Accumulation of DRiPs adjacent to or inside SGs correlates with impaired SG assembly (Seguin et al., 2014). SGs forming under conditions of VCP inhibition accumulate non-canonical components (DRiPs, 60S), which in turn can lead to impaired SG dynamics and contribute to SG persistence (Buchan et al., 2013). It has been proposed that these persistent or partially disassembled SGs, if not fully removed, may act as seeds for aggregation that can become pathogenic (Wolozin, 2012).

SGs are highly dynamic structures that exhibit liquid-like behaviour with rapid exchange rates of components. However, the exchange dynamics of SG components can vary greatly. Fluorescent recovery after photobleaching (FRAP) analysis shows that some SG proteins like TIA1 and G3BP1 rapidly shuttle between SGs and the cytoplasm, while others like HuR exchange very slowly (Kedersha et al., 2005). ATP-dependent remodelling complexes are responsible, in part, for the dynamics of SGs and impaired ATP production eliminates SG movement, fusion and fission (Jain et al., 2016). The ATP dependence of SG dynamics is consistent with a model that considers granules as active-liquids, where the energy of ATP-driven remodelling maintains the dynamic state of the assembly.

2.2. Stress granule clearance

After cell recovery from stress the subsequent disassembly and clearance of SGs is critical for the restoration of normal cellular function (Fig. 2). Chaperone-mediated disassembly is the preferred pathway of SG clearance as cells favour recycling of RBPs rather than their degradation (Mateju et al., 2017). Molecular chaperones transiently interact with and stabilise unfolded proteins to facilitate folding into their native conformations. When misfolding and aggregation cannot be prevented, chaperones can promote the tar-

geting of these misfolded proteins for degradation. An important group of molecular chaperones are the HSP family. HSPs can sense and bind to hydrophobic regions of unfolded/misfolded proteins. Small HSPs possess no ATPase activity and maintain the bound protein in a state conducive to further processing in co-operation with other chaperones. HSP70 has been shown to prevent the accumulation of misfolded proteins in SGs, maintaining their dynamic liquid-like state, in addition to promoting SG disassembly (Mateju et al., 2017).

HSP70 can bind to nucleotide exchange factors, including the Bcl-2 associated athanogene (BAG) proteins, these catalyse the dissociation of ADP from HSP70, ensuring substrate release and chaperone recycling. These factors and chaperones can also promote targeting of HSP70 bound proteins to degradation systems, such as the proteasome or autophagy. Under conditions of proteasome impairment, the HSPB8-BAG3-HSP70 chaperone complex is upregulated, which re-routes poly-ubiquitinated proteins to p62 bodies for autophagy mediated disposal. Conversely, upon inhibition of autophagy-mediated degradation, cells preferentially upregulate BAG1. Association of BAG1 with the HSP70-HSPB8 complex reroutes ubiquitinated proteins to the proteasome for degradation (Ganassi et al., 2016).

The 26S proteasome is directly involved in the clearance of arsenite-induced SGs, and impairment to the proteasome, VCP or VCP adaptors, results in the transition of SGs into an aberrant state. Persistent SGs are preferentially degraded by autophagy (Turakhiya et al., 2018). A recent study identified a novel VCP adaptor, zinc finger AN1-type containing 1 (ZFAND1), with a role in the clearance of arsenite-induced SGs, acting by recruiting VCP and the 26S proteasome (Turakhiya et al., 2018). During recovery from arsenite stress, SGs dissociate in a process requiring the HSPB8-BAG3-HSP70 chaperone complex, VCP and the 26S proteasome. In addition to the induction of SGs, arsenite stress also induces the accumulation of ubiquitylated DRiPs, which are rapidly degraded by the 26S proteasome during post-stress recovery. In the absence of ZFAND1, arsenite-induced SGs lack VCP and DRiPs accumulate in close vicinity to SGs. A significant portion of these SGs can-
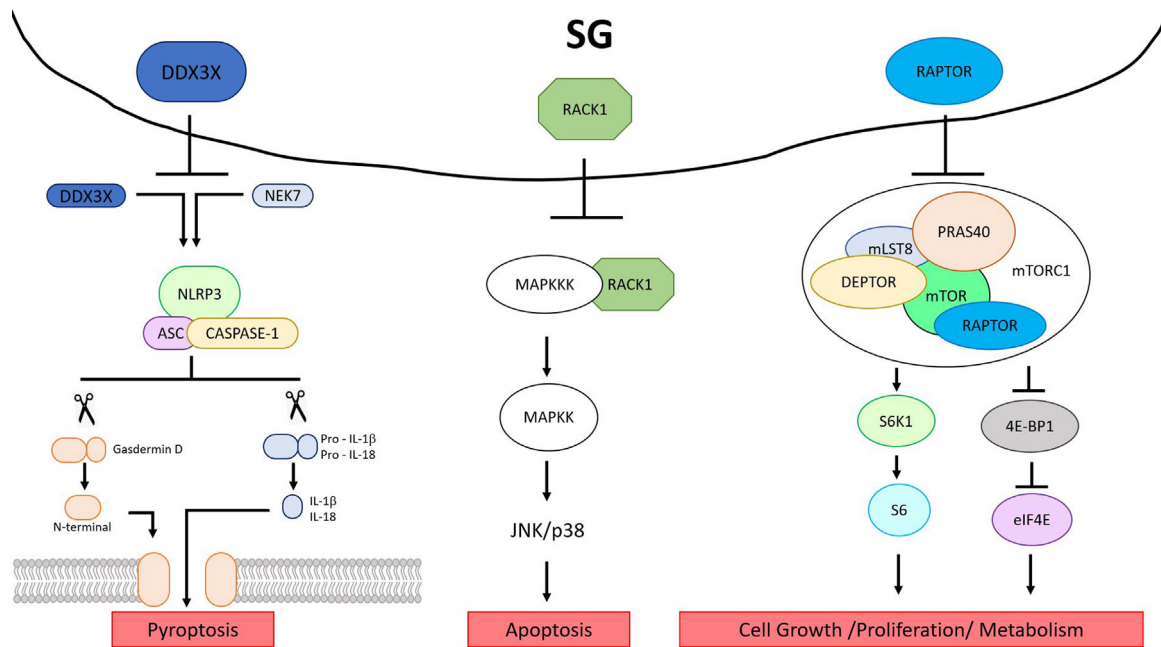
Fig. 3. Stress granules as signalling hubs. SGs can influence cell signalling by sequestering signalling proteins. Sequestration of DDX3X prevents the assembly and activation of the NLRP3 inflammasome reducing the secretion of inflammatory cytokines and pyroptosis. Sequestration of RACK1 prevents apoptosis by interrupting MAPK signalling. Sequestration of the mTORC1 component RAPTOR prevents mTORC1 formation and affects downstream signalling.

not be cleared under these conditions and are instead transformed into aberrant DRiP-positive SGs, which are then recruited by p62 into LC3 positive autophagosomes and degraded via autophagy (Buchan et al., 2013).

3. Stress granules as signalling hubs

SG formation alters multiple signalling pathways by sequestering signalling proteins, including adaptor/scaffold proteins, protein and lipid kinases, GTPases and ubiquitin modifying enzymes, many containing low-complexity regions that may regulate their interaction with SGs, summarised in Fig. 3. For example sequestration of scaffolding protein TRAF2 in SGs, plays a key role in the reduction of NFκB signalling by TNFα (Kim et al., 2005). The sequestration of RACK1 to SGs inhibits the stress-induced activation of the p38/c-jun N-terminal kinase (JNK) signalling cascade that triggers apoptosis (Arimoto et al., 2008) giving SGs a key role in regulating apoptosis as well as translation. The interaction between SGs and two other major cell signalling molecules, mTOR and DDX3X will be explored in more detail and their relevance to hearing loss will be reviewed.

3.1. mTORC1

The mTOR pathway is a central regulator of cell growth, proliferation and metabolism and has been implicated in multiple age-related diseases (Johnson et al., 2013). mTOR is a serine/threonine kinase that co-ordinates numerous cellular processes and assembles into two distinct multiprotein complexes: the rapamycin-sensitive mTORC1 and the rapamycin-insensitive mTORC2, which while differing in composition and function, are both expressed in the cochlea (Leitmeyer et al., 2015). The more extensively studied mTORC1 is a homodimer composed of mTOR, the defining sub-unit Regulatory-Associated Protein of TOR (RAPTOR) and mammalian lethal with SEC13 protein 8 (mLST8) (Kim et al., 2003). Tuberous sclerosis complex (TSC), which is composed of TSC1, TSC2 and TBC1D7 is involved in the negative regulation of mTORC1 activity

(Dibble et al., 2012). The loss or disruption of this complex results in constitutive activation of mTORC1, that is largely independent of cellular growth conditions (Zhang et al., 2003).

Emerging studies are revealing the complex crosstalk between mTORC1 and SGs, particularly in the context of ageing and age-related diseases [reviewed by (Cadena Sandoval et al., 2021)]. mTORC1 inhibition has been proposed to induce SG formation by preventing the phosphorylation of 4E-BP1 and thus the assembly of the eIF4F complex and translation initiation. However, 4E-BP1 can be targeted by several kinases and phosphatases in addition to mTORC1 (Qin et al., 2016; Kolupaeva, 2019). None of the studies examining knockdown or inhibition of 4E-BP1 and subsequent SG induction have examined whether mTORC1 is necessary (Fujimura, Sasaki, et al., 2012; Fujimura, Scharengella, et al., 2012). Furthermore, the knockdown of RAPTOR in the absence of stress is insufficient to induce SG formation (Sfakianos et al., 2018). Therefore, the question of 4E-BP1-mediated SG formation due to inactive mTORC1 remains open. Conversely, SG assembly alters mTOR signalling by sequestering both mTORC1 and downstream kinases. In human cells, oxidative or osmotic stress promotes the recruitment of RAPTOR to SGs (Kedersha et al., 2013). RAPTOR is recruited to SGs by the protein astrin, which mediates the anti-apoptotic function of SGs by preventing mTORC1 hyperactivation (Thedieck et al., 2013). During recovery from stress, reactivation of mTORC1 correlates with its release from disassembled SGs (Takahara and Maeda, 2012). The core SG component G3BP1, in the absence of stress acts as a lysosomal anchor of the TSC (Prentzell et al., 2021). This tethering suppresses signalling through mTORC1 and prevents mTORC1 hyperactivation by metabolic stimuli.

Overactive mTORC1 signalling has been identified as a significant contributor to the pathology of multiple types of hearing loss. The possibility of targeting mTOR signalling to prevent hearing loss is an emerging idea [reviewed by (Cortada et al., 2021)]. Given that SGs sequester RAPTOR (see Fig. 3) and thereby inhibit mTOR signaling, one could potentially target SGs to promote their sequestration of RAPTOR in cases where overactive mTORC1 sig-

naling is suspected to be a contributing factor. Recent work has shown that the mTORC1 inhibitor rapamycin has a protective role against aminoglycoside ototoxicity (Kim et al., 2017), cisplatin oto-toxicity (Fang and Xiao, 2014), NIHL (Yuan et al., 2015) and ARHL (Altschuler et al., 2021). Further evidence for the role of mTORC1 in ARHL was provided by a study which utilised mice with cochlear neurosensory epithelial specific deletions of the mTORC1 signalling regulators RAPTOR and TSC1. The results showed that loss of RAP-TOR led to mice developing hearing loss slower than wild-type littermates. Conversely, cKO-TSC1 mice have early onset loss of cochlear cells and accelerated hearing loss that could be rescued with rapamycin treatment (Fu et al., 2018). Interestingly, disruption of mTOR function by rapamycin in neonatal mice leads to hearing loss suggesting mTOR signalling may play a role in post-natal development and formation of cochlear hair cell synapses (Xiong et al., 2020). There is also recent evidence that mTORC1 signalling is involved in the regeneration of hair cells in the mouse cochlea (Li and Doetzlhofer, 2020; Shu et al., 2019). In support-ing cells that have been induced to proliferate by transient co-activation of Myc and Notch1, with concurrent overexpression of the pro-hair cell specification factor Atoh1, there is increased ex-pression of phosphorylated ribosomal S6 protein (Shu et al., 2019). Ribosomal protein S6 is phosphorylated through the mTORC1/S6K axis as described in Fig. 3 and when mTORC1 signalling is inhibited with rapamycin, this results in a decline in the number of prolif-erating cells and regenerated hair cell-like cells. Furthermore, loss of LIN28B, a known SG component (Balzer and Moss, 2007), atten-uates mTORC1 signalling and renders young, immature supporting cells incapable of producing hair cells (Li and Doetzlhofer, 2020). The possibility of there being a role for SGs in hair cell regen-eration is entirely unexplored. There is however recent evidence that SGs play a role in axon regeneration (Sahoo et al., 2018). Sa-hoo and colleagues found that disrupting G3BP1 activated intra-axonal mRNA translation, increased axon growth in cultured neu-rons and disassembled axonal SG-like structures. Moreover, they found that disruption of G3BP1 *in-vivo* accelerated nerve regener-ation. More recent work has identified the sequestration of RNA-binding motif protein 24 (Rbm24) within SGs (Wang et al., 2022). Rbm24 has previously been shown to be necessary for hair cell development in zebrafish (Zhang et al., 2020). Rbm24 is directly regulated by Atoh1 in a subset of hair cells (Cai et al., 2015) and Rbm24 deficiency leads to hair cell death and hearing loss in a knockout mouse model (Zheng et al., 2021). These recent discov-eries and the known propensity of SG persistence to increase with age, in combination suggest a role for SGs in auditory hair cell sur-vival and protection. It may be the case that age-related, persis-tent SGs permanently sequester signalling proteins necessary for hair cell maintenance, survival and/or recovery thereby leading to ARHL. Approaches to prevent accumulation of, or induce clearance of age-related, persistent SGs in hair cells and spiral ganglion neu-rons (SGNs) may reset the normal SG response, promote cell sur-vival and ameliorate ARHL.

3.2. DDX3X and NLRP3

DDX3X is a member of the DEAD-box helicase family, the largest helicase family in eukaryotes. DDX3X is expressed ubiqui-tously in humans and participates in multiple biological processes. Localised predominantly in the cytoplasm but also present in the nucleus, DDX3X has various roles in RNA metabolism, SG forma-tion and in NLRP3 inflammasome formation (Fig. 3). Recently, a new role for DDX3X in the stress-inflammation axis was identified. Samir and colleagues identified DDX3X as a 'live-or-die' checkpoint in cells undergoing stress (Samir et al., 2019). Under stress con-ditions, cells face a choice between survival and death. Cellular stressors cause both the formation of SGs and the formation and

activation of the NLRP3 inflammasome. DDX3X is necessary for both the formation and activation of the NLRP3 inflammasome and for the formation of SGs. The exact mechanism by which DDX3X promotes these two distinct assemblies is not fully understood. The proteins NEK7 (NIMA related kinase 7) and ASC (apoptosis-associated speck-like protein containing a CARD) are essential for NLRP3 inflammasome formation, activation and caspase-1 recruit-ment (He et al., 2016; Vajjhala et al., 2012). NEK7 acts downstream of potassium efflux and the pyrin binding domain of ASC interacts with the pyrin binding domain of NLRP3. NLRP3 inflammasome ac-tivation allows for cleavage of pro forms of inflammatory cytokines IL-1β and IL-18 into their active forms and cleavage of Gasdermin D (GSDMD). Cleavage of GSDMD produces N-terminal fragments that form plasma membrane pores, facilitating the secretion of ma-ture IL-1β and IL-18 ultimately leading to pyroptosis (Liu et al., 2016). It has been proposed that DDX3X promotes NLRP3 inflam-masome activation by interacting with the NLRP3 NACHT domain through its helicase domain (Samir et al., 2019). However, for SG formation the helicase activity of DDX3X is dispensable, but the eIF4E binding of DDX3X is necessary for SG nucleation. Knockdown or pharmacological inhibition of DDX3X inhibits the formation of SGs (Cui et al., 2020). A stress insult that is sufficient to induce SGs inhibits the formation and activation of the NLRP3 inflammasome by sequestering DDX3X, reducing NLRP3 formation and subsequent caspase-1 activation and IL-1β and IL-18 secretion (Samir et al., 2019) suggesting DDX3X sequestration is a key factor in cell fate during stress.

Inflammation has been associated with multiple types of hear-ing loss. In NIHL, noise exposure causes a robust activation of inflammatory mechanisms (Frye et al., 2019). In aminoglycoside-induced hearing loss, inflammation is thought to up-regulate the expression of aminoglycoside-permeant cation channels, increas-ing hair cell uptake of aminoglycosides (Jiang et al., 2019). In-flammation triggered by the activation of the NLRP3 inflamma-some in the ageing cochlea is a potential contributor to ARHL (Shi et al., 2017). Mutations of NLRP3 are associated with sev-eral diseases that have hearing loss as a main component. Hearing loss is one of the most common manifestations for patients with cryoporin-associated periodic syndromes (CAPS). There are multi-ple sub-types of CAPS, with the prevalence of hearing loss between sub-types ranging from 25% to 86%, with higher frequencies being affected to a greater degree than lower frequencies. Hearing loss of CAPS patients also appears to progress with age (Ahmadi et al., 2011). Furthermore, a gain-of-function mutation in NLRP3 has been shown to cause autosomal dominant non-syndromic hearing loss DFNA34 (Nakanishi et al., 2017).

Activation of the NLRP3 inflammasome has also been shown to be integral to the cognitive decline seen in dementia. APP/PS1 mice deficient in NLRP3 and caspase-1 were spared from memory deficits and LTP suppression unlike APP1/PS1 that could activate the NLRP3 inflammasome (Heneka et al., 2012). NLRP3 activation has also been shown to be essential in the Aβ-tau cascade. Tau22 mice injected with Aβ-containing APP/PS1 brain homogenates exhibited increased levels of tau hyperphosphorylation, cleaved caspase-1, IL-1β and ASC. However, when Tau22/ASC$^{-/-}$ or Tau22/Nlrp3$^{-/-}$ mice were injected, tau hyperphosphorylation did not occur. There were also lower levels of cleaved caspase-1 and IL-1β (Ising et al., 2019).

It is clear from these investigations that inflammation and specifically the NLRP3 inflammasome has a role in both ARHL and some forms of dementia but whether this is due to SG regulation of the inflammasome remains to be established. SG composition is known to change under chronic stress conditions compared to acute stress (Reineke et al., 2018). The activation of NLRP3 in ARHL (Shi et al., 2017) could be a consequence of the change in composition of SGs induced by the chronic "inflammaging"

associated with ageing (Franceschi et al., 2018). If SGs in ARHL lose the sequestration of DDX3X, there will be increased activation of the NLRP3 inflammasome. One possibility is that the SG-induced pro-inflammatory mechanism that is associated with chronic stress during ageing is common to both the cochlea and the brain and contributes to the aggregated protein pathology and cognitive decline seen in both neurodegenerative disease. The evidence behind this rationale is discussed below. If this were the case, since there is a change in composition of SGs induced by ageing-associated chronic stress, it is not clear whether targeting the SGs themselves would provide benefit or not. However, therapies using existing inhibitors targeting excess NLRP3 inflammasome activation (Chen et al., 2021) are worthy of investigation and may prove beneficial in treating ARHL and cognitive decline.

4. Stress granules and disease

SG dysfunction has been implicated in the pathogenesis or progression of a wide range of diseases, most notably in neurodegeneration and dementia (Wolozin and Ivanov, 2019). Given the link between hearing loss and dementia, and the recent evidence for a role for SGs in cochlear cell protection (Gonçalves et al., 2019; Nolan et al., 2022), which are discussed below, it is worth exploring whether SG dysfunction might be a common pathological mechanism in the two traits. Here, we describe the evidence for a role for aberrant SGs in different diseases and detail any known effects on the auditory system associated with these pathologies.

4.1. The role of stress granules in neurodegeneration

Protein aggregation is a common characteristic amongst neurodegenerative diseases that is implicated in disease pathogenesis. The aggregated proteins involved are typically specific to each neurodegenerative disease, although some like TAR DNA binding protein 43 (TDP-43) and Tau are seen in several neurodegenerative diseases. The low complexity regions found in many RBPs, important for the LLPS of SGs, can also form beta sheets that accumulate into amyloids (Wolozin and Ivanov, 2019). Disturbances in SG dynamics have been implicated as a primary driver of neurodegenerative diseases (Zhang et al., 2019). Persistence of SGs, which are normally transient structures, appears to function as a seed for the aggregation of pathological proteins. The progression of neurodegenerative diseases occurs over many years. ALS generally lasts 3-6 years (Kiernan et al., 2011) while overt clinical manifestations of Alzheimer's disease (AD) last up to 15 years, with Aβ deposition frequently preceding overt manifestations by up to a further 15 years (Scheltens et al., 2016). The chronic repeated stress associated with these diseases leads to the formation of persistent SGs that lose their anti-apoptotic phenotype and take on a "pro-death" phenotype (Reineke and Neilson, 2018).

The link between hearing loss and dementia is an area of intense interest. ARHL is the most common sensory deficit in the elderly and has been identified as the highest modifiable risk factor for dementia (Livingston et al., 2020). The underlying reasons for the link between ARHL and dementia remain to be established and several hypotheses have been put forward including the additional cognitive load associated with impaired hearing or the possibility that the hearing loss is an early manifestation of existing cognitive decline. A third possibility is that there is a common pathological mechanism or mechanisms underlying both conditions. Age is the biggest overall risk factor for dementia. Not only is hearing loss the highest modifiable risk factor, it is also highly prevalent and the risk of developing hearing impairment increases with age. Hearing loss affects more than 40% of people over 50 years of age, rising to 70% of people aged over 70 (RNID, 2018). It is hypothesised that the chronic stress of ageing, termed "inflammag-

ing" (Franceschi et al., 2000) can lead to the formation of persistent SGs, that then act as a nidus for the aggregation of disease-associated proteins. Additionally, there is evidence that as cells age SG components are more prone to aggregation, even in the absence of stress. RNA granule components including two key SG RBPs with low complexity prion-like domains poly(A)-binding protein 1 (PAB-1) and T-cell intracellular antigen related protein 2 (TIAR-2) aggregate in aged *C.elegans* in the absence of disease (Lechler and David, 2017). Given the emerging central role of SGs in the pathology of several neurodegenerative diseases and the link between hearing loss in mid-life and dementia, we hypothesise that aberrant/chronic SGs may have a common role in the development of ARHL and dementia and that this area of research warrants further investigation.

4.2. Alzheimer's disease

AD is a chronic neurodegenerative disease that is accompanied by the death of neurons and loss of synapses in the cerebral cortex and sub-cortical regions of the brain (Bloom, 2014). AD is the most common form of dementia, in the UK 1 in 14 people aged over 65 years is affected by AD, and the incidence increases with age (Alzheimer's, 2021). The two hallmarks of AD are amyloid beta (Aβ) plaque deposition and tau neurofibrillary tangles (NFTs). Tau is a microtubule-associated protein considered to be the causative factor of several neurodegenerative diseases (tauopathies) including AD and a sub-type of FTD (Orr et al., 2017). The human brain expresses six tau isoforms that are generated by alternative splicing. Under non-stressful conditions tau mainly localises to axons, where it binds to microtubules, promotes microtubule assembly and facilitates formation of the long processes that characterise axons. In response to stress tau is phosphorylated near and in its microtubule domain, which prevents tau binding to microtubules (Johnson and Stoothoff, 2004). Hyper-phosphorylated tau (p-tau) accumulates in the neuronal soma rather than the axon in response to stress, due to the stress-related phosphorylation occurring on newly synthesised tau (Lopes et al., 2016). Hyper-phosphorylation, misfolding and accumulation of p-tau protein are pathological features of AD and result in the deposition of NFTs and neuronal death. The levels of tau deposition are correlated more strongly with cognitive decline in AD than other markers such as Aβ plaques (Hanseeuw et al., 2019).

Tau undergoes LLPS, just as RBPs do and the tendency of tau to go through LLPS increases in the presence of RNA (Wegmann et al., 2018). The interaction of tau with SGs has important consequences for the pathophysiology of tauopathies because the association of tau with these structures stimulates the formation of insoluble tau aggregates. TIA-1 is an RNA binding protein with a prion-like aggregation domain that promotes SG formation under various stress conditions. TIA-1 also stimulates tau aggregation and tau toxicity both in cultured cells and in mouse models of AD by promoting tau-positive SG formation (Vanderweyde et al., 2016). Critical to the formation of Tau/TIA-1-positive SGs is the ubiquitin-specific protease 10 (USP10). Over-expression of USP10 without stress stimuli is sufficient to induce tau-positive SGs. Knockdown of USP10 attenuates tau-positive SG formation elicited by either TIA-1 over-expression or proteasome inhibition. USP10 colocalises with tau aggregates in the cell body of neurons from AD patients (Piatnitskaia et al., 2019). These findings indicate pathogenic tau aggregation is driven by SG formation and requires USP10.

The role SGs play in AD is not restricted to tau pathophysiology, for example there is evidence that SGs are contributing to the deficits seen in microglia from AD brains. Microglia from the brains of AD patients are known to be recruited to Aβ plaques where they exhibit an activated phenotype but are defective for plaque removal by phagocytosis (Ghosh and Geahlen, 2015). Microglia stressed with sodium arsenite or Aβ1-42 peptides or fibrils,

form SGs to which the tyrosine kinase SYK is recruited. This sequestration of SYK inhibits the ability of microglial cells to phagocytose Aβ fibrils. Interestingly, aged microglia are more susceptible to the formation of SGs. SGs containing SYK and phosphotyrosine are prevalent in the brains of patients with severe AD. Phagocytic activity can be restored to stressed microglia by IgG treatment (Ghosh and Geahlen, 2015). These observations suggest a mechanism by which stress compromises the function of microglial cells in AD through SG formation.

There are several links between AD and auditory pathology. In the early stage of AD, brain atrophy occurs in the central auditory cortex and related functional nuclei and the characteristic Aβ plaques and NFTs are extensively distributed throughout the relay stations of the ascending auditory pathway (Sinha et al., 1993). Aβ plaques and Tau tangles have been identified in the brainstem from the cochlear nucleus, superior olivary complex, inferior colliculus and nuclei of lateral lemniscus along the afferent auditory pathway in AD patients (Sinha et al., 1993). Additionally, mouse models of AD exhibit an early onset high frequency hearing loss that progresses to all frequencies with age. 5xFAD mice express human amyloid precursor protein (APP) and presenilin 1 (PSEN1) transgenes with five AD-linked mutations. Three of those mutations are in APP (Swedish, Florida and London mutations). The two remaining mutations are in PSEN1 (M146L and L286V). APP/PS1 mice contain transgenes for APP bearing the Swedish mutation and PSEN1 containing a L166P mutation. Both 5xFAD and APP/PS1 mice show elevated ABR thresholds. 5xFAD mice exhibit amyloid plaque deposition at 2 months of age and at 13 months exhibit large amounts of basal and apical hair cell loss, particularly outer hair cells (O'Leary et al., 2017). APP/PS1 mice have progressive early onset hearing loss, that is detectable at 2 months of age and prior to any other AD phenotypes (Liu et al., 2020). In 3xTg-AD mice that have the Swedish mutation in APP, the P301L mutation in microtubule associated protein tau (MAPT) and the M146V mutation in PSEN1 there is a reduction of SGNs at 9 months of age (Wang and Wu, 2015). These three mouse models of AD all have a C57BL genetic background. C57BL mice have an early onset hearing loss, starting as early as from 2 months (Mikaelian et al., 2009). Therefore, determining whether the hearing loss associated with these AD mouse models is due to their genetic background or AD pathology becomes difficult. Evidence of cognitive decline in C57BL mice from 6 months tends to support the theory that hearing loss precedes AD pathology, as the cognitive decline is 4 months after the onset of hearing loss (Hendrickx et al., 2022). Further evidence supporting a molecular mechanism between hearing loss and dementia is that CBA mice that have minimal ARHL also have no cognitive decline. However, when CBA mice are exposed to noise to produce moderate to severe hearing loss, several months later there are detectable deficits in spatial learning and memory (Liu et al., 2016). It may be of interest to use noise-exposed CBA mice to explore the possibility of SGs as the molecular mechanism between hearing loss and dementia.

4.3. Huntington's disease

Huntingdon's disease (HD) is a neurodegenerative disorder caused by an expansion of a polyglutamine repeat within the HD gene product huntingtin (Htt). HD manifests with midlife cognitive impairment, motor incoordination and psychiatric symptoms. Late-stage HD patients often present with hearing problems in addition to typical dysfunctions (Profant et al., 2017). The pathophysiological mechanisms underlying HD-related auditory dysfunction are poorly understood. Aggregated mutant Htt (mHtt) is the most classic cellular pathological characteristic of HD (Hoffner et al., 2007). CAG repeats in exon 1 of huntingtin causes poly glutamine extension at the N-terminal of Htt protein leading to accumulation of mHtt and neuronal loss. The average CAG repeat length in

the general population is 17-20 repeats (Kremer et al., 2010). In HD patients one copy of the gene has a CAG tract that has expanded to 36 or more repeats (Walker, 2007). Neuronal loss preferentially affects the cortico-striatal circuits which leads to characteristic chorea and as HD progresses mHtt spreads to peripheral tissue, including the inner ear (Lin et al., 2011).

Multiple studies have demonstrated the involvement of SGs in HD. Expanded Htt has been shown to re-distribute to SGs under ER stress and interact with SG components G3BP1 and Caprin1 (Ratovitski et al., 2012). HD-R6/2 transgenic mice exhibit a high density of G3BP1-positive SGs in the cortex and hippocampus. HD patients also exhibit increased numbers of SGs in the superior frontal cortex and an increase in the mislocalisation of TDP-43 in SG-positive cortical neurons (Sanchez et al., 2021).

Studies have also illustrated that hearing impairment is observed in and closely correlated with motor deficits in HD. A study from 2011 of 19 HD patients revealed HD patients had a 15dB threshold increase at high sound frequencies, with no difference in latency or inter-peak intervals in ABRs, indicating the hearing impairment in HD is associated with the peripheral auditory system rather than the central auditory system (Lin et al., 2011). However, other studies found HD patients displayed normal sound sensation but decreases in speech understanding and sound source lateralisation, indicating HD also effects the cortical and sub-cortical regions of the central auditory pathway (Profant et al., 2017). HD patients also have delayed auditory event-related potentials which are also found in those at risk of HD (Wetter et al., 2005).

4.4. Multiple sclerosis

Multiple sclerosis (MS) is a demyelinating autoimmune disorder of the CNS in which neurodegeneration plays a significant role in its pathogenesis. MS is a neuromotor disorder that can be classified as clinically isolated syndrome, relapsing-remitting, primary progressive or secondary progressive. One of the key features of MS is the development of demyelinated lesions or plaques in the CNS (Lubetzki and Stankoff, 2014). Plaques, depending on their stage can contain numerous immune cells, including T cells and macrophages (Høglund and Maghazachi, 2014). In addition to inflammation, lesions also show evidence of axonal injury. Multiple mechanisms underlying MS have been proposed, including axonal transport deficits, mitochondrial dysfunction and autoantibodies to non-myelin antigens (Campbell and Mahad, 2018).

In 2018, evidence for the involvement of SGs in MS was provided when neurons from an MS patient were shown to have high levels of mislocalisation of the RBP heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1), moving from the nucleus to the cytoplasm where it formed aggregates that co-localised with SG markers (Salapa et al., 2018). More recently, a larger study of 12 MS patients and 6 control cases found cortices from MS patients showed a reduction in the number of neurons with nuclear hnRNPA1 and TDP-43 and an increase in cytoplasmic mislocalisation compared to control brains (Salapa, Hutchinson, et al., 2020). Additionally, it has been shown that mislocalisation of TDP-43 and hnRNPA1 is a prominent feature in mouse models of MS (Salapa, Libner, et al., 2020). Interestingly, the degree of hnRNPA1 mislocalisation positively correlates with the severity of disease in experimental autoimmune encephalomyelitis models and negatively correlates with neuronal cell count. These data suggest that the severity of RBP mislocalisation may be related to neurodegeneration and disease progression. MS associated hnRNPA1 mutations (P275S and F281L) alter its function and promote SG formation. These mutations cause cytoplasmic mislocalisation, alter hnRNPA1 cluster kinetics and cause SGs to form more quickly and frequently in response to stimuli (Clarke et al., 2021). The prevalence of audio-vestibular impairments in MS is highly variable, with sudden onset sensorineural hearing loss

(SSNHL) affecting between 0.7% and 25% of patients (Ralli et al., 2018). When patients are in the remitting phase difficulty with hearing is a rare complaint. It has also been proposed that SSNHL can be an early manifestation of MS (Di Stadio et al., 2018).

4.5. Amyotrophic lateral sclerosis

ALS is a progressive neurodegenerative disease with no cure that causes both degeneration of upper and lower motor neurons and leads to atrophy of the innervating muscles and progressive paralysis. 147 genes have been identified that are associated with ALS, and the exact mechanism underlying the pathology is unknown (Dervishi et al., 2018). Several pathological processes have been associated with ALS, including protein aggregation and defects in RNA metabolism. Many mutations that cause ALS-FTD are in RNA-binding proteins associated with SGs e.g., TDP-43, fused in sarcoma (FUS), hnRNPA1, and TIA-1. These mutations largely cluster in low-complexity, intrinsically disordered regions (IDRs) and in many cases have been shown to change the dynamic properties of SGs (Ding et al., 2021). Another set of disease-causing mutations impact ubiquitin-binding proteins e.g., VCP, p62/SQSTM1 and ubiquilin-2 (UBQLN2) that facilitate the disassembly and clearance of SGs (Buchan et al., 2013).

TDP-43 itself has been shown to undergo LLPS *in-vitro* (Conicella et al., 2016) and chronic SGs contain the protein FUS which is associated with ALS (Reineke and Neilson, 2018). Chronic SGs also do not contain 40s ribosomal sub-units, which could indicate that the cell has lost the ability to translate the RNA within the SG. In the absence of external/exogenous stressors, persistent or repetitive assembly of SGs is cytotoxic and is accompanied by the evolution of SGs to cytoplasmic inclusions that recapitulate the pathology of ALS-FTD (Zhang et al., 2019).

To date there is no clear association of hearing loss with ALS, except for the rare Madras sub-type (Nalini et al., 2008). Hearing loss is however, associated with FTD and its sub-types (Johnson et al., 2021). Why the auditory system is spared in ALS is currently unknown. There are several possibilities. One possible reason for the sparing of the auditory system in ALS may be the way that TDP-43 spreads. The "dying-forward hypothesis" proposes that ALS begins in the cortical regions of the brain and spreads through connected regions via TDP-43 in a "prion-like" fashion (Lee and Kim, 2015). It has been observed that motor neurons receiving direct, monosynaptic cortical input predominantly develop TDP-43 pathology, while subcortical motor neurons do not (Eisen et al., 2017). Additionally, the fact that motor neurons without monosynaptic connections to motor neurons such as oculomotor and Onuf's nuclei are spared in ALS tends to support this hypothesis (Ragagnin et al., 2019). Another possibility for the sparing of the auditory system is the difference between motor neurons and auditory neurons. Motor neurons are unique cells, even amongst neurons. They have particularly long axons, up to 1m and have very high energetic requirements. These characteristics may render them particularly sensitive to changes in SG homeostasis compared to auditory neurons and cochlear cells. Lastly, it may simply be the case that hearing loss hasn't had the time to develop due to the shorter disease duration of ALS. The typical time from symptom onset to death in ALS is 2-3 years (Hardiman et al., 2011). Which is significantly shorter than neurodegenerative diseases that do exhibit hearing loss, such as AD, that progress over decades (Vermunt et al., 2019).

### 5. Stress granules and hearing loss

To date, the majority of our understanding of SGs in the cochlea comes from ototoxicity studies, specifically, aminoglycoside ototoxicity. In 2004, a study on the chick basilar papilla exposed to aminoglycoside treatments resulted in the first identification of SGs in the ear. The authors identified TIAR-positive granules in response to gentamicin treatment in the cytoplasm of hair cells (Mangiardi et al., 2004). The first evidence of SGs in the mammalian cochlea was published in 2011, neomycin treatment resulted in SG formation in *ex-vivo* cochlear hair cells (Towers et al., 2011). More recently, an important study demonstrated the integral role SGs play in the cochlea's response to stress. In the study, inhibition of SGs with the small molecule inhibitor ISRIB, increased hair cell death in response to aminoglycoside treatment. Conversely, inducing SG formation using the silvestrol analogue hydroxamate (-)-9 (or CR 1-31 B) protected against aminoglycoside-induced hair-cell death. Furthermore, this work provided the first evidence of SG formation in mammalian cochlear hair cells *in-vivo* (Gonçalves et al., 2019).

Recent work has shown that conditional knock-out of the key SG protein Caprin1 in the inner ear leads to an early onset and progressive hearing loss in mice (Nolan et al., 2022). These mice were also less able to recover from a temporary hearing loss after mild noise exposure. Caprin1 (also known as RNG105) is an RBP and core nucleating component of SGs, playing a key role in SG condensation by competing with USP10 for binding to G3BP1 and in recruiting specific RNAs to SGs (Kedersha et al., 2016). Interestingly, Caprin1 cKO mice are still able to form SGs. Presumably, the absence of Caprin1 will have altered the composition of these SGs, possibly reducing their auditory protective function. ABRs from Caprin1 cKO mice show reduced thresholds with a significant reduction in wave-1 amplitudes compared to wild type-mice. Reduced ABR wave 1 amplitudes in the presence of an otherwise normal audiogram are considered a characteristic feature of cochlear synaptopathy. The loss or damage to, synaptic connections between sensory hair cells and the peripheral afferent dendrites of SGNs, typically seen in after noise exposure and/or ageing. The hair cell-SGN synapses of cKO mice were shown to have abnormally large post-synaptic GluA2 puncta. The data suggest that the regulation of cochlear synaptic form and function is dependent on neuronal RNA granules and the function of Caprin1. In disease conditions, persistent SGs could impact hearing maintenance by sequestering Caprin1, preventing it from maintaining the hair cell-SGN synapse resulting in hearing loss.

6. Conclusion

Aging itself is not considered a disease, but aging and age-related diseases often share the same molecular mechanisms (Franceschi et al., 2018). The chronic stress associated with aging and age-related diseases can lead to the impaired assembly and clearance of SGs. There is compelling evidence for the role that these persistent SGs play in neurodegenerative diseases and emerging evidence of the importance of the SG response in protecting the cochlea from damage. The link between hearing loss and dementia is well-established, yet unexplained. Therefore, the possibility that age-related dysfunction/dysregulation of SGs is a common mechanism underlying ARHL and dementia becomes an interesting prospect and certainly warrants further investigation (Fig. 4). However, there remains a significant amount of work to be done to document the change in composition of persistent SGs and acute SGs within the cochlea exposed to different stresses. If this mechanism is established to play a significant role in ARHL, then manipulation of the SG pathway may be a potential target for prevention of ARHL. Prophylactic induction of SGs has already been shown to be a viable therapeutic target for the prevention of aminoglycoside ototoxicity. The induction of SGs prior to other relatively acute stressors, such as noise may afford similar protection, but remains to be tested. Given that it is the persistence of SGs that is the issue in chronic diseases such as ARHL, drugs that increase SG clearance, such as autophagy activators or specifically
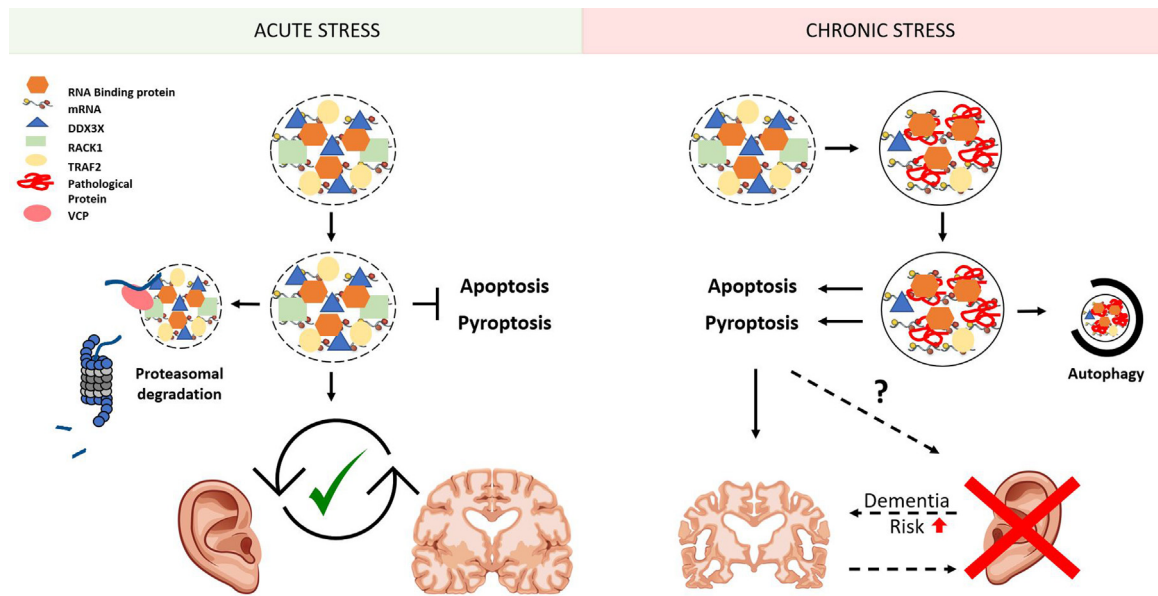
Fig. 4. Schematic illustration of the role persistent SGs play in neurodegeneration. Chronic stress or mutations in disease related proteins leads to formation of persistent SGs that have altered compositions, causing a shift from a pro-survival to a pro-death phenotype. Hearing loss is the largest, potentially modifiable risk factor for dementia, hearing loss prevention in mid-life can reduce the risk of dementia by 8%. Multiple neurodegenerative diseases that involve an aberrant SG response present with some form of hearing dysfunction. Here, we suggest that persistent SGs due to the chronic stress associated with ageing termed "inflammaging" contributes to age-related hearing loss and that persistent SG formation might be an underlying molecular mechanism between hearing loss and dementia.

targeted to persistent SGs may be beneficial in re-setting the SG response. An alternative approach would be to target the signaling pathways activated by changes in SG composition. Thus, targeting SGs or SG-related signalling pathways are potential therapeutic strategies for the treatment and/or prevention of age-related diseases including ARHL and dementia.

Data availability

No data was used for the research described in the article.

CRediT authorship contribution statement

Jack L. Martin: Writing – original draft, Writing – review & editing, Visualization. **Sally J. Dawson:** Writing – review & editing. **Jonathan E. Gale:** Writing – review & editing.

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References

Ahmadi, N., Brewer, C.C., Zalewski, C., King, K.A., Butman, J.A., Plass, N., Henderson, C., Goldbach-Mansky, R., Kim, H.J., 2011. Cryopyrin-associated periodic syndromes: otolaryngologic and audiologic manifestations. Otolaryngol. Head Neck Surg. Off. J. Am. Acad. 145 (2), 295. doi:10.1177/0194599811402296, Otolaryngology-Head and Neck Surgery.

Alberti, S., Mateju, D., Mediani, L., Carra, S., 2017. Granulostasis: protein quality control of RNP granules. Front. Mol. Neurosci. 10, 84. doi:10.3389/fnmol.2017.00084.

Altschuler, R.A., Kabara, L., Martin, C., Kanicki, A., Stewart, C.E., Kohrman, D.C., Dolan, D.F., 2021. Rapamycin added to diet in late mid-life delays age-related hearing loss in UMHET4 mice. Front. Cell. Neurosci. 15, 100. doi:10.3389/fncel.2021.658972.

Anderson, P., Kedersha, N., 2006. RNA granules. J. Cell Biol. 172 (6), 803–808. doi:10.1083/jcb.200512082, VolIssueThe Rockefeller University Press.

Anderson, P., Kedersha, N., 2008. Stress granules: the Tao of RNA triage. Trends Biochem. Sci. 33 (3), 141–150. doi:10.1016/J.TIBS.2007.12.003.

Anderson, P., Kedersha, N., 2009a. Stress granules. Curr. Biol. 19 (10), R397–R398. doi:10.1016/J.CUB.2009.03.013.

Anderson, P., Kedersha, N., 2009b. RNA granules: post-transcriptional and epigenetic modulators of gene expression. Nat. Rev. Mol. Cell Biol. 10 (6), 430–436. doi:10.1038/nrm2694, VolIssueNature Publishing Group.

Anderson, P., Kedersha, N., Ivanov, P., 2015. Stress granules, P-bodies and cancer. Biochim. Biophys. Acta (BBA) - Gene Regul. Mech. 1849 (7), 861–870. doi:10.1016/J.BBAGRM.2014.11.009.

Arimoto, K., Fukuda, H., Imajoh-Ohmi, S., Saito, H., Takekawa, M., 2008. Formation of stress granules inhibits apoptosis by suppressing stress-responsive MAPK pathways. Nat. Cell Biol. 10 (11), 1324–1332. doi:10.1038/ncb1791.

Balzer, E., Moss, E.G., 2007. Localization of the developmental timing regulator Lin28 to mRNP complexes, P-bodies and stress granules. RNA Biol. 4 (1), 16–25. doi:10.4161/rna.4.1.4364.

Bloom, G.S., 2014. Amyloid-β and Tau: the trigger and bullet in alzheimer disease pathogenesis. JAMA Neurol. 71 (4), 505–508. doi:10.1001/JAMANEUROL.2013.5847.

Brengues, M., Teixeira, D., Parker, R., 2005. Movement of eukaryotic mRNAs between polysomes and cytoplasmic processing bodies. Science 310 (5747), 486. doi:10.1126/SCIENCE.1115791, (New York, N.Y.).

Buchan, J.R., Kolaitis, R.-M., Taylor, J.P., Parker, R., 2013. Eukaryotic stress granules are cleared by autophagy and Cdc48/VCP function. Cell 153 (7), 1461–1474. doi:10.1016/j.cell.2013.05.037.

Cadena Sandoval, M., Heberle, A.M., Rehbein, U., Barile, C., Ramos Pittol, J.M., Thedieck, K., 2021. mTORC1 crosstalk with stress granules in aging and age-related diseases. Front. Aging 0, 56. doi:10.3389/FRAGI.2021.761333.

Cai, T., Jen, H.I., Kang, H., Klisch, T.J., Zoghbi, H.Y., Groves, A.K., 2015. Characterization of the transcriptome of nascent hair cells and identification of direct targets of the atoh1 transcription factor. J. Neurosci. 35 (14), 5870–5883. doi:10.1523/JNEUROSCI.5083-14.2015.

Campbell, G., Mahad, D.J., 2018. Mitochondrial dysfunction and axon degeneration in progressive multiple sclerosis. FEBS Lett. 592 (7), 1113–1121. doi:10.1002/1873-3468.13013.

Chen, Q.L., Yin, H.R., He, Q.Y., Wang, Y., 2021. Targeting the NLRP3 inflammasome as new therapeutic avenue for inflammatory bowel disease. Biomed. Pharmacother. 138, 111442. doi:10.1016/J.BIOPHA.2021.111442.

Clarke, J.P.W.E., Thibault, P.A., Salapa, H.E., Kim, D.E., Hutchinson, C., Levin, M.C., 2021. Multiple sclerosis-associated hnrnpa1 mutations alter hnrnpa1 dynamics and influence stress granule formation. Int. J. Mol. Sci. 22 (6), 1–22. doi:10.3390/ijms22062909.

Cohen, B.E., Durstenfeld, A., Roehm, P.C., 2014. Viral causes of hearing loss: a review for hearing health professionals. Trends Hear. 18. doi:10.1177/2331216514541361.

Colombrita, C., Zennaro, E., Fallini, C., Weber, M., Sommacal, A., Buratti, E., Silani, V., Ratti, A., 2009. TDP-43 is recruited to stress granules in conditions of oxidative insult. J. Neurochem. 111 (4), 1051–1061. doi:10.1111/J.1471-4159.2009.06383.X.

Conicella, A.E., Zerze, G.H., Mittal, J., Fawzi, N.L., 2016. ALS mutations disrupt phase separation mediated by α-helical structure in the TDP-43 low-complexity C-terminal domain. Structure, 24 (9), 1537–1549. doi:10.1016/J.STR.2016.07.007/ATTACHMENT/A6F07B8D-7FF4-42F5-80D2-1C10A4FC407E/MMC2.MP4.

Cortada, M., Levano, S., Bodmer, D., 2021. mTOR signaling in the inner ear as potential target to treat hearing loss. Int. J. Mol. Sci. 22 (12). doi:10.3390/IJMS22126368.

Cui, B.C., Sikirzhytski, V., Aksenova, M., Lucius, M.D., Levon, G.H., Mack, Z.T., Pollack, C., Odhiambo, D., Broude, E., Lizarraga, S.B., Wyatt, M.D., Shtutman, M., 2020. Pharmacological inhibition of DEAD-Box RNA Helicase 3 attenuates stress granule assembly. Biochem. Pharmacol. 182, 114280. doi:10.1016/J.BCP.2020.114280.

Dang, Y., Kedersha, N., Low, W.K., Romo, D., Gorospe, M., Kaufman, R., Anderson, P., Liu, J.O., 2006. Eukaryotic Initiation factor 2α-independent pathway of stress granule induction by the natural product pateamine A. J. Biol. Chem. 281 (43), 32870–32878. doi:10.1074/JBC.M606149200.

Dementia UK report | Alzheimer's Society. (n.d.). Retrieved November 2, 2021, https://www.alzheimers.org.uk/about-us/policy-and-influencing/dementia-uk-report?downloadID=1491.

Dervishi, I., Gozutok, O., Murnan, K., Gautam, M., Heller, D., Bigio, E., Ozdinler, P.H., 2018. Protein-protein interactions reveal key canonical pathways, upstream regulators, interactome domains, and novel targets in ALS. Sci. Rep. 8 (1), 1–19. doi:10.1038/s41598-018-32902-4, 2018 8:1.

Di Stadio, A., Dipietro, L., Ralli, M., Meneghello, F., Minni, A., Greco, A., Stabile, M.R., Bernitsas, E., 2018. Sudden hearing loss as an early detector of multiple sclerosis: a systematic review. Eur. Rev. Med. Pharmacol. Sci. 22 (14), 4611–4624. doi:10.26355/EURREV_201807_15520.

Dibble, C.C., Elis, W., Menon, S., Qin, W., Klekota, J., Asara, J.M., Finan, P.M., Kwiatkowski, D.J., Murphy, L.O., Manning, B.D., 2012. TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of mTORC1. Mol. Cell 47 (4), 535. doi:10.1016/J.MOLCEL.2012.06.009.

Ding, Q., Chaplin, J., Morris, M.J., Hilliard, M.A., Wolvetang, E., Ng, D.C.H., Noakes, P.G., 2021. TDP-43 mutation affects stress granule dynamics in differentiated NSC-34 motoneuron-like cells. Front. Cell Dev. Biol. 9. doi:10.3389/FCELL.2021.611601.

Eisen, A., Braak, H., Del Tredici, K., Lemon, R., Ludolph, A.C., Kiernan, M.C., 2017. Cortical influences drive amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 88 (11), 917–924. doi:10.1136/JNNP-2017-315573.

Eulalio, A., Behm-Ansmant, I., Izaurralde, E., 2007. P bodies: at the crossroads of post-transcriptional pathways. Nat. Rev. Mol. Cell Biol. 8 (1), 9–22. doi:10.1038/nrm2080, 2007 8:1.

Eulalio, A., Behm-Ansmant, I., Schweizer, D., Izaurralde, E., 2007. P-Body formation is a consequence, not the cause, of RNA-mediated gene silencing. Mol. Cell. Biol. 27 (11), 3970–3981. doi:10.1128/MCB.00128-07.

Fang, B., Xiao, H., 2014. Rapamycin alleviates cisplatin-induced ototoxicity in vivo. Biochem. Biophys. Res. Commun. 448 (4), 443–447. doi:10.1016/j.bbrc.2014.04.123.

Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000. Inflamm-aging: an evolutionary perspective on immunosenescence. Ann. N.Y. Acad. Sci. 908 (1), 244–254. doi:10.1111/J.1749-6632.2000.TB06651.X.

Franceschi, C., Garagnani, P., Morsiani, C., Conte, M., Santoro, A., Grignolio, A., Monti, D., Capri, M., Salvioli, S., 2018. The continuum of aging and age-related diseases: Common mechanisms but different rates. Front. Med. doi:10.3389/fmed.2018.00061, (Vol. 5, Issue MAR). Frontiers Media S.A.

Frye, M.D., Ryan, A.F., Kurabi, A., 2019. Inflammation associated with noise-induced hearing loss. J. Acoust. Soc. Am. 146 (5), 4020. doi:10.1121/1.5132545.

Fu, X., Sun, X., Zhang, L., Jin, Y., Chai, R., Yang, L., Zhang, A., Liu, X., Bai, X., Li, J., Wang, H., Gao, J., 2018. Tuberous sclerosis complex–mediated mTORC1 overactivation promotes age-related hearing loss. J. Clin. Invest. 128 (11), 4938. doi:10.1172/JCI98058.

Fujimura, K., Sasaki, A.T., Anderson, P., 2012. Selenite targets eIF4E-binding protein-1 to inhibit translation initiation and induce the assembly of non-canonical stress granules. Nucleic Acids Res. 40 (16), 8099–8110. doi:10.1093/NAR/GKS566.

Fujimura, K., Scharengella, D., Ivanova, V., Ivanov, P., Anderson, P., & Emara, M. (2012). Hydrogen peroxide induces stress granule formation independent of eukaryotic initiation factor 2α phosphorylation. 2012 (1), BMP57. 10.5339/QFARF.2012.BMP57.

Ganassi, M., Mateju, D., Bigi, I., Mediani, L., Poser, I., Lee, H.O., Seguin, S.J., Morelli, F.F., Vinet, J., Leo, G., Pansarasa, O., Cereda, C., Poletti, A., Alberti, S., Carra, S., 2016. A surveillance function of the HSPB8-BAG3-HSP70 chaperone complex ensures stress granule integrity and dynamism. Mol. Cell 63 (5), 796–810. doi:10.1016/J.MOLCEL.2016.07.021.

Ghosh, S., Geahlen, R.L., 2015. Stress Granules modulate SYK to cause microglial cell dysfunction in alzheimer's. Disease. EBioMedicine 2 (11), 1785–1798. doi:10.1016/j.ebiom.2015.09.053.

Gilks, N., Kedersha, N., Ayodele, M., Shen, L., Stoecklin, G., Dember, L.M., Anderson, P., 2004. Stress granule assembly is mediated by prion-like aggregation of TIA-1. Mol. Biol. Cell 15 (12), 5383–5398. doi:10.1091/mbc.E04-08-0715.

Gonçalves, A.C., Towers, E.R., Haq, N., Porco, J.A., Pelletier, J., Dawson, S.J., Gale, J.E., 2019. Drug-induced stress granule formation protects sensory hair cells in mouse cochlear explants during ototoxicity. Sci. Rep. 9 (1), 1–9. doi:10.1038/s41598-019-48393-w.

Hanseeuw, B.J., Betensky, R.A., Jacobs, H.I.L., Schultz, A.P., Sepulcre, J., Becker, J.A., Cosio, D.M.O., Farrell, M., Quiroz, Y.T., Mormino, E.C., Buckley, R.F., Papp, K.V., Amariglio, R.A., Dewachter, I., Ivanoiu, A., Huijbers, W., Hedden, T., Marshall, G.A., Chhatwal, J.P., …, Johnson, K., 2019. Association of amyloid and tau with cognition in preclinical alzheimer disease: a longitudinal study. JAMA Neurol. 76 (8), 915–924. doi:10.1001/JAMANEUROL.2019.1424.

Hardiman, O., Van Den Berg, L.H., Kiernan, M.C., 2011. Clinical diagnosis and management of amyotrophic lateral sclerosis. Nat. Rev. Neurol. 7 (11), 639–649. doi:10.1038/nrneurol.2011.153, 2011 7:11.

Harding, H.P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., Schapira, M., Ron, D., 2000. Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol. Cell 6 (5), 1099–1108. doi:10.1016/S1097-2765(00)00108-8.

He, Y., Zeng, M.Y., Yang, D., Motro, B., Núñez, G., 2016. Nek7 is an essential mediator of NLRP3 activation downstream of potassium efflux. Nature 530 (7590), 354. doi:10.1038/NATURE16959.

Hendrickx, J.O., De Moudt, S., Calus, E., De Deyn, P.P., Van Dam, D., De Meyer, G.R.Y., 2022. Age-related cognitive decline in spatial learning and memory of C57BL/6J mice. Behav. Brain Res. 418, 113649. doi:10.1016/J.BBR.2021.113649.

Heneka, M.T., Kummer, M.P., Stutz, A., Delekate, A., Schwartz, S., Vieira-Saecker, A., Griep, A., Axt, D., Remus, A., Tzeng, T.C., Gelpi, E., Halle, A., Korte, M., Latz, E., Golenbock, D.T., 2012. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature 493 (7434), 674–678. doi:10.1038/nature11729, 2012 493:7434.

Hoffner, G., Souès, S., Djian, P., 2007. Aggregation of expanded huntingtin in the brains of patients with huntington disease. Prion 1 (1), 26. doi:10.4161/PRI.1.1.4056.

Høglund, R.A., Maghazachi, A.A., 2014. Multiple sclerosis and the role of immune cells. World J. Exp. Med. 4 (3), 27. doi:10.5493/WJEM.V4.I3.27.

Hubstenberger, A., Courel, M., Bénard, M., Souquere, S., Ernoult-Lange, M., Chouaib, R., Yi, Z., Morlot, J.B., Munier, A., Fradet, M., Daunesse, M., Bertrand, E., Pierron, G., Mozziconacci, J., Kress, M., Weil, D., 2017. P-body purification reveals the condensation of repressed mRNA regulons. Mol. Cell 68 (1), 144–157. doi:10.1016/J.MOLCEL.2017.09.003, e5.

Huth, M.E., Ricci, A.J., Cheng, A.G., 2011. Mechanisms of aminoglycoside ototoxicity and targets of hair cell protection. Int. J. Otolaryngol. 1–19. doi:10.1155/2011/937861, 2011.

Hyman, A.A., Weber, C.A., & Jülicher, F. (2014). Liquid-liquid phase separation in biology. 30, 39–58. 10.1146/ANNUREV-CELLBIO-100913-013325.

Ising, C., Venegas, C., Zhang, S., Scheiblich, H., Schmidt, S.V., Vieira-Saecker, A., Schwartz, S., Albasset, S., McManus, R.M., Tejera, D., Griep, A., Santarelli, F., Brosseron, F., Opitz, S., Stunden, J., Merten, M., Kayed, R., Golenbock, D.T., Blum, D., …, Heneka, M.T., 2019. NLRP3 inflammasome activation drives tau pathology. Nature 575 (7784), 669–673. doi:10.1038/s41586-019-1769-z, 2019 575:7784.

Jackson, R.J., Hellen, C.U.T., Pestova, T.V., 2010. The mechanism Of eukaryotic translation initiation and principles of its regulation. Nat. Rev. Mol. Cell Biol. 11 (2), 113. doi:10.1038/NRM2838.

Jain, S., Wheeler, J.R., Walters, R.W., Agrawal, A., Barsic, A., Parker, R., 2016. ATPase-modulated stress granules contain a diverse proteome and substructure. Cell 164 (3), 487–498. doi:10.1016/j.cell.2015.12.038.

Jiang, M., Li, H., Johnson, A., Karasawa, T., Zhang, Y., Meier, W.B., Taghizadeh, F., Kachelmeier, A., Steyger, P.S., 2019. Inflammation up-regulates cochlear expression of TRPV1 to potentiate drug-induced hearing loss. Sci. Adv. 5 (7). doi:10.1126/sciadv.aaw1836, eaaw1836.

Johnson, G.V.W., Stoothoff, W.H., 2004. Tau phosphorylation in neuronal cell function and dysfunction. J. Cell Sci. 117 (24), 5721–5729. doi:10.1242/JCS.01558.

Johnson, J.C.S., Marshall, C.R., Weil, R.S., Bamiou, D.E., Hardy, C.J.D., Warren, J.D., 2021. Hearing and dementia: from ears to brain. Brain 144 (2), 391–401. doi:10.1093/BRAIN/AWAA429.

Johnson, S.C., Rabinovitch, P.S., Kaeberlein, M., 2013. MTOR is a key modulator of ageing and age-related disease. Nature 493, 338–345. doi:10.1038/nature11861, VolIssue 7432NIH Public Access.

Kedersha, N., Ivanov, P., Anderson, P., 2013. Stress granules and cell signaling: more than just a passing phase? Trends Biochem. Sci 38 (10), 494–506. doi:10.1016/J.TIBS.2013.07.004.

Kedersha, N.L., Gupta, M., Li, W., Miller, I., Anderson, P., 1999. RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2α to the assembly of mammalian stress granules. J. Cell Biol. 147 (7), 1431–1441. doi:10.1083/jcb.147.7.1431.

Kedersha, N., Panas, M.D., Achorn, C.A., Lyons, S., Tisdale, S., Hickman, T., Thomas, M., Lieberman, J., McInerney, G.M., Ivanov, P., Anderson, P., 2016. G3BP-Caprin1-USP10 complexes mediate stress granule condensation and associate with 40S subunits. J. Cell Biol. 212 (7), 845–860. doi:10.1083/JCB.201508028/VIDEO-2.

Kedersha, N., Stoecklin, G., Ayodele, M., Yacono, P., Lykke-Andersen, J., Fitzler, M.J., Scheuner, D., Kaufman, R.J., Golan, D.E., Anderson, P., 2005. Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. J. Cell Biol. 169 (6), 871–884. doi:10.1083/jcb.200502088.

Keithley, E.M., 2020. Pathology and mechanisms of cochlear aging. J. Neurosci. Res. 98 (9), 1674–1684. doi:10.1002/JNR.24439.

Kiernan, M.C., Vucic, S., Cheah, B.C., Turner, M.R., Eisen, A., Hardiman, O., Burrell, J.R., Zoing, M.C., 2011. Amyotrophic lateral sclerosis. Lancet North Am. Ed. 377 (9769), 942–955. doi:10.1016/S0140-6736(10)61156-7.

Kim, D.H., Sarbassov, D.D., Ali, S.M., Latek, R.R., Guntur, K.V.P., Erdjument-Bromage, H., Tempst, P., Sabatini, D.M., 2003. GβL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. Mol. Cell 11 (4), 895–904. doi:10.1016/S1097-2765(03)00114-X.

Kim, W.J., Back, S.H., Kim, V., Ryu, I., Jang, S.K., 2005. Sequestration of TRAF2 into stress granules interrupts tumor necrosis factor signaling under stress conditions. Mol. Cell. Biol. 25 (6), 2450–2462. doi:10.1128/mcb.25.6.2450-2462.2005.

Kim, Y.J., Tian, C., Kim, J., Shin, B., Choo, O.S., Kim, Y.S., Choung, Y.H., 2017. Autophagic flux, a possible mechanism for delayed gentamicin-induced ototoxicity. Sci. Rep. 7 (1), 1–11. doi:10.1038/srep41356.

Kolupaeva, V., 2019. Serine-threonine protein phosphatases: Lost in translation. Biochim. Biophys. Acta (BBA) Mol. Cell Res. 1866 (1), 83–89. doi:10.1016/J.BBAMCR.2018.08.006.

Kremer, B., Goldberg, P., Andrew, S.E., Theilmann, J., Telenius, H., Zeisler, J., Squitieri, F., Lin, B., Bassett, A., Almqvist, E., Bird, T.D., & Hayden, M.R. (2010). A worldwide study of the huntington's disease mutation: the sensitivity and specificity of measuring CAG repeats. 330 (20), 1401–1406. doi:10.1056/NEJM199405193302001.

Lechler, M.C., David, D.C., 2017. More stressed out with age? Check your RNA granule aggregation. Prion 11 (5), 313–322. doi:10.1080/19336896.2017.1356559.

Lee, S., Kim, H.-J., 2015. Prion-like mechanism in amyotrophic lateral sclerosis: are protein aggregates the key? Exp. Neurobiol. 24 (1), 1. doi:10.5607/EN.2015.24.1.1.

Leitmeyer, K., Glutz, A., Radojevic, V., Setz, C., Huerzeler, N., Bumann, H., Bodmer, D., Brand, Y., 2015. Inhibition of mTOR by rapamycin results in auditory hair cell damage and decreased spiral ganglion neuron outgrowth and neurite formation in vitro. Biomed. Res. Int. 2015/2015/925890, 2015.

Li, X.J., Doetzlhofer, A., 2020. LIN28B/let-7 control the ability of neonatal murine auditory supporting cells to generate hair cells through mTOR signaling. Proc. Nat. Acad. Sci. U.S.A. 117 (36), 22225–22236. doi:10.1073/pnas.2000417117.

Lin, Y.S., Chen, C.M., Soong, B.W., Wu, Y.R., Chen, H.M., Yeh, W.Y., Wu, D.R., Lin, Y.J., Poon, P.W.F., Cheng, M.L., Wang, C.H., Chern, Y., 2011. Dysregulated brain creatine kinase is associated with hearing impairment in mouse models of Huntington disease. J. Clin. Invest. 121 (4), 1519–1523. doi:10.1172/JCI43220.

Liu, L., Shen, P., He, T., Chang, Y., Shi, L., Tao, S., Li, X., Xun, Q., Guo, X., Yu, Z., Wang, J., 2016. Noise induced hearing loss impairs spatial learning/memory and hippocampal neurogenesis in mice. Sci. Rep. 6 (1), 1–9. doi:10.1038/srep20374, 2016 6:1.

Liu, X., Zhang, Z., Ruan, J., Pan, Y., Magupalli, V.G., Wu, H., Lieberman, J., 2016. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. Nature 535 (7610), 153–158. doi:10.1038/nature18629, 2016 535:7610.

Liu, Y., Fang, S., Liu, L.M., Zhu, Y., Li, C.R., Chen, K., Zhao, H.B., 2020. Hearing loss is an early biomarker in APP/PS1 Alzheimer's disease mice. Neurosci. Lett. 717, 134705. doi:10.1016/j.neulet.2019.134705.

Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., Brayne, C., Burns, A., Cohen-Mansfield, J., Cooper, C., Costafreda, S.G., Dias, A., Fox, N., Gitlin, L.N., Howard, R., Kales, H.C., Kivimäki, M., Larson, E.B., Ogunniyi, A., …, Mukadam, N., 2020. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet North Am. Ed. 396 (10248), 413–446. doi:10.1016/S0140-6736(20)30367-6.

Lopes, S., Vaz-Silva, J., Pinto, V., Dalla, C., Kokras, N., Bedenk, B., Mack, N., Czisch, M., Almeida, O.F.X., Sousa, N., Sotiropoulos, I., 2016. Tau protein is essential for stress-induced brain pathology. Proc. Natl Acad. Sci. 113 (26), E3755–E3763. doi:10.1073/PNAS.1600953113.

Lu, L., Han, A.-P., Chen, J.-J., 2001. Translation initiation control by heme-regulated eukaryotic initiation factor 2α kinase in erythroid cells under cytoplasmic stresses. Mol. Cell. Biol. 21 (23), 7971. doi:10.1128/MCB.21.23.7971-7980.2001.

Lubetzki, C., Stankoff, B., 2014. Demyelination in multiple sclerosis. Handb. Clin. Neurol. 122, 89. doi:10.1016/B978-0-444-52001-2.00004-2.

Mangiardi, D.A., McLaughlin-Williamson, K., May, K.E., Messana, E.P., Mountain, D.C., Cotanche, D.A., 2004. Progression of hair cell ejection and molecular markers of apoptosis in the avian cochlea following gentamicin treatment. J. Comp. Neurol. 475 (1), 1–18. doi:10.1002/CNE.20129.

Mateju, D., Franzmann, T.M., Patel, A., Kopach, A., Boczek, E.E., Maharana, S., Lee, H.O., Carra, S., Hyman, A.A., Alberti, S., 2017. An aberrant phase transition of stress granules triggered by misfolded protein and prevented by chaperone function. EMBO J. 36, 1669–1687. doi:10.15252/embj.201695957.

Mikaelian, D.O., Warfield, D., & Norris, O. (2009). Genetic progressive hearing loss in the C57/M6 mouse: relation of behaviorial responses to cochlear anatomy. 77 (1–6), 327–334. doi:10.3109/00016487409124632.

Nakanishi, H., Kawashima, Y., Kurima, K., Chae, J.J., Ross, A.M., Pinto-Patarroyo, G., Patel, S.K., Muskett, J.A., Ratay, J.S., Chattaraj, P., Park, Y.H., Grevich, S., Brewer, C.C., Hoa, M., Kim, H.J., Butman, J.A., Broderick, L., Hoffman, H.M., Aksentijevich, I., …, Griffith, A.J., 2017. NLRP3 mutation and cochlear autoinflammation cause syndromic and nonsyndromic hearing loss DFNA34 responsive to anakinra therapy. Proc. Nat. Acad. Sci. U.S.A. 114 (37), E7766–E7775. doi:10.1073/pnas.1702946114.

Nalini, A., Thennarasu, K., Yamini, B.K., Shivashankar, D., Krishna, N., 2008. Madras motor neuron disease (MMND): clinical description and survival pattern of 116 patients from Southern India seen over 36 years (1971–2007). J. Neurol. Sci. 269 (1–2), 65–73. doi:10.1016/J.JNS.2007.12.026.

Nolan, L.S., Chen, J., Gonçalves, A.-C., Bullen, A., Towers, E.R., Steel, K.P., Dawson, S.J., Gale, J.E., 2022. Targeted deletion of the RNA-binding protein Caprin1 leads to progressive hearing loss and impairs recovery from noise exposure in mice. Sci. Rep. 12 (1), 1–16. doi:10.1038/s41598-022-05657-2, 2022 12:1.

Nover, L., Scharf, K.D., Neumann, D., 1983. Formation of cytoplasmic heat shock granules in tomato cell cultures and leaves. Mol. Cell. Biol. 3 (9), 1648. doi:10.1128/MCB.3.9.1648.

Nover, L., Scharf, K.D., Neumann, D., 1989. Cytoplasmic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. Mol. Cell. Biol. 9 (3), 1298–1308. doi:10.1128/mcb.9.3.1298-1308.1989.

O'Leary, T.P., Shin, S., Fertan, E., Dingle, R.N., Almuklass, A., Gunn, R.K., Yu, Z., Wang, J., Brown, R.E., 2017. Reduced acoustic startle response and peripheral hearing loss in the 5xFAD mouse model of Alzheimer's disease. Genes Brain Behav. 16 (5), 554–563. doi:10.1111/gbb.12370.

Orr, M.E., Sullivan, A.C., Frost, B., 2017. A brief overview of tauopathy: causes, consequences, and therapeutic strategies. Trends Pharmacol. Sci. 38 (7), 637. doi:10.1016/J.TIPS.2017.03.011.

Peelle, J.E., 2018. Listening effort: how the cognitive consequences of acoustic challenge are reflected in brain and behavior. Ear Hear. 39 (2), 204. doi:10.1097/AUD.0000000000000494.

Piatnitskaia, S., Takahashi, M., Kitaura, H., Katsuragi, Y., Kakihana, T., Zhang, L., Kakita, A., Iwakura, Y., Nawa, H., Miura, T., Ikeuchi, T., Hara, T., Fujii, M., 2019. USP10 is a critical factor for Tau-positive stress granule formation in neuronal cells. Sci. Rep. 9 (1), 1–15. doi:10.1038/s41598-019-47033-7.

Prentzell, M.T., Rehbein, U., Sandoval, M.C., Meulemeester, A.-S.De, Baumeister, R., Brohée, L., Berdel, B., Bockwoldt, M., Carroll, B., Chowdhury, S.R., Deimling, A., Demetriades, C., Figlia, G., Consortium, G.E.R., Araujo, M.E.G., Heberle, A.M., Heiland, I., Holzwarth, B., Huber, L.A., Thedieck, K., 2021. G3BPs tether the TSC complex to lysosomes and suppress mTORC1 signaling. Cell 184 (3), 655. doi:10.1016/J.CELL.2020.12.024.

Profant, O., Roth, J., Bureš, Z., Balogová, Z., Lišková, I., Betka, J., Syka, J., 2017. Auditory dysfunction in patients with Huntington's disease. Clin. Neurophysiol. 128 (10), 1946–1953. doi:10.1016/J.CLINPH.2017.07.403.

Qin, X., Jiang, B., & Zhang, Y. (2016). 4E-BP1, a multifactor regulated multifunctional protein. 15 (6), 781–786. doi:10.1080/15384101.2016.1151581.

Ragagnin, A.M.G., Shadfar, S., Vidal, M., Jamali, M.S., Atkin, J.D., 2019. Motor neuron susceptibility in ALS/FTD. Front. Neurosci. 13 (JUN), 532. doi:10.3389/FNINS.2019.00532/BIBTEX.

Ralli, M., Stadio, A.Di, Visconti, I.C., Russo, F.Y., Orl, M.P.o., Balla, M.P., Greco, A., Vincentiis, M., 2018. Otolaryngologic symptoms in multiple sclerosis: a review. Int. Tinnitus J. 22 (2), 160–169. doi:10.5935/0946-5448.20180027.

Ratovitski, T., Chighladze, E., Arbez, N., Boronina, T., Herbrich, S., Cole, R.N., Ross, C.A., 2012. Huntingtin protein interactions altered by polyglutamine expansion as determined by quantitative proteomic analysis. Cell Cycle 11 (10). doi:10.4161/CC.20423, 2006.

Reineke, L.C., Cheema, S.A., Dubrulle, J., Neilson, J.R., 2018. Chronic starvation induces noncanonical pro-death stress granules. J. Cell Sci. 131 (19). doi:10.1242/JCS.220244/265837/AM/CHRONIC-STARVATION-INDUCES-NON-CANONICAL-PRO-DEATH.

Reineke, L.C., Neilson, J.R., 2018. Differences between acute and chronic stress granules, and how these differences may impact function in human disease. Biochem. Pharmacol. doi:10.1016/J.BCP.2018.10.009.

Rozelle, D.K., Filone, C.M., Kedersha, N., Connor, J.H., 2014. Activation of stress response pathways promotes formation of antiviral granules and restricts virus replication. Mol. Cell. Biol. 34 (11), 2003–2016. doi:10.1128/mcb.01630-13.

Rybak, L.P., Mukherjea, D., Jajoo, S., Ramkumar, V., 2009. Cisplatin ototoxicity and protection: clinical and experimental studies. Tohoku J. Exp. Med. 219 (3), 177 /pmc/articles/PMC2927105/.

Sahoo, P.K., Lee, S.J., Jaiswal, P.B., Alber, S., Kar, A.N., Miller-Randolph, S., Taylor, E.E., Smith, T., Singh, B., Ho, T.S.Y., Urisman, A., Chand, S., Pena, E.A., Burlingame, A.L., Woolf, C.J., Fainzilber, M., English, A.W., Twiss, J.L., 2018. Axonal G3BP1 stress granule protein limits axonal mRNA translation and nerve regeneration. Nat. Commun. 9 (1). doi:10.1038/s41467-018-05647-x.

Salapa, H.E., Hutchinson, C., Popescu, B.F., Levin, M.C., 2020. Neuronal RNA-binding protein dysfunction in multiple sclerosis cortex. Ann. Clin. Trans. Neurol. 7 (7), 1214–1224. doi:10.1002/acn3.51103.

Salapa, H.E., Johnson, C., Hutchinson, C., Popescu, B.F., Levin, M.C., 2018. Dysfunctional RNA binding proteins and stress granules in multiple sclerosis. J. Neuroimmunol. 324, 149–156. doi:10.1016/j.jneuroim.2018.08.015.

Salapa, H.E., Libner, C.D., Levin, M.C., 2020. Dysfunctional RNA-binding protein biology and neurodegeneration in experimental autoimmune encephalomyelitis in female mice. J. Neurosci. Res. 98 (4), 704–717. doi:10.1002/jnr.24554.

Samir, P., Kesavardhana, S., Patmore, D.M., Gingras, S., Malireddi, R.K.S., Karki, R., Guy, C.S., Briard, B., Place, D.E., Bhattacharya, A., Sharma, B.R., Nourse, A., King, S.V., Pitre, A., Burton, A.R., Pelletier, S., Gilbertson, R.J., Kanneganti, T.D., 2019. DDX3X acts as a live-or-die checkpoint in stressed cells by regulating NLRP3 inflammasome. Nature 573 (7775), 590–594. doi:10.1038/s41586-019-1551-2.

Sanchez, I.I., Nguyen, T.B., England, W.E., Lim, R.G., Vu, A.Q., Miramontes, R., Byrne, L.M., Markmiller, S., Lau, A.L., Orellana, I., Curtis, M.A., Maxwell Faull, R.L., Yeo, G.W., Fowler, C.D., Reidling, J.C., Wild, E.J., Spitale, R.C., Thompson, L.M., 2021. Huntington's disease mice and human brain tissue exhibit increased G3BP1 granules and TDP43 mislocalization. J. Clin. Invest. 131 (12). doi:10.1172/JCI140723.

Scheltens, P., Blennow, K., Breteler, M.M.B., de Strooper, B., Frisoni, G.B., Salloway, S., Van der Flier, W.M., 2016. Alzheimer's disease. Lancet doi:10.1016/S0140-6736(15)01124-1, (Vol. 388, Issue 10043, pp. 505–517). Lancet Publishing Group.

Seguin, S.J., Morelli, F.F., Vinet, J., Amore, D., De Biasi, S., Poletti, A., Rubinsztein, D.C., Carra, S., 2014. Inhibition of autophagy, lysosome and VCP function impairs stress granule assembly. Cell Death Differ. 21 (12), 1838–1851. doi:10.1038/cdd.2014.103.

Sfakianos, A.P., Mellor, L.E., Pang, Y.F., Kritsiligkou, P., Needs, H., Abou-Hamdan, H., Désaubry, L., Poulin, G.B., Ashe, M.P., Whitmarsh, A.J., 2018. The mTOR-S6 kinase

pathway promotes stress granule assembly. Cell Death Differ. 25 (10), 1766–1780. doi:10.1038/s41418-018-0076-9, 2018 25:10.

Shen, Y., Ye, B., Chen, P., Wang, Q., Fan, C., Shu, Y., & Xiang, M. (2018). Cognitive decline, dementia, alzheimer's disease and presbycusis: examination of the possible molecular mechanism. Front. Neurosci., 12 (JUN). doi:10.3389/FNINS.2018.00394.

Shi, X., Qiu, S., Zhuang, W., Yuan, N., Wang, C., Zhang, S., Sun, T., Guo, W., Gao, F., Yang, S., Qiao, Y., 2017. NLRP3-inflammasomes are triggered by age-related hearing loss in the inner ear of mice. Am. J. Transl. Res. 9 (12), 5611–5618.

Shu, Y., Li, W., Huang, M., Quan, Y.Z., Scheffer, D., Tian, C., Tao, Y., Liu, X., Hochedlinger, K., Indzhykulian, A.A., Wang, Z., Li, H., Chen, Z.Y., 2019. Renewed proliferation in adult mouse cochlea and regeneration of hair cells. Nat. Commun. 10 (1). doi:10.1038/S41467-019-13157-7.

Sinha, U.K., Hollen, K.M., Rodriguez, R., Miller, C.A., 1993. Auditory system degeneration in alzheimer's disease. Neurology 43 (4), 779–785. doi:10.1212/wnl.43.4.779.

Snijders Blok, L., Madsen, E., Juusola, J., Gilissen, C., Baralle, D., Reijnders, M.R.F., Venselaar, H., Helsmoortel, C., Cho, M.T., Hoischen, A., Vissers, L.E.L.M., Koemans, T.S., Wissink-Lindhout, W., Eichler, E.E., Romano, C., Van Esch, H., Stumpel, C., Vreeburg, M., Smeets, E., …, Kleefstra, T., 2015. Mutations in DDX3X are a common cause of unexplained intellectual disability with gender-specific effects on WNT signaling. Am. J. Hum. Genet. 97 (2), 343. doi:10.1016/J.AJHG.2015.07.004.

Srivastava, S.P., Kumar, K.U., Kaufman, R.J., 1998. Phosphorylation of Eukaryotic translation initiation factor 2 mediates apoptosis in response to activation of the double-stranded RNA-dependent protein kinase. J. Biol. Chem. 273 (4), 2416–2423. doi:10.1074/JBC.273.4.2416.

Takahara, T., Maeda, T., 2012. Transient sequestration of TORC1 into stress granules during heat stress. Mol. Cell 47 (2), 242–252. doi:10.1016/j.molcel.2012.05.019.

Takahashi, M., Higuchi, M., Matsuki, H., Yoshita, M., Ohsawa, T., Oie, M., Fujii, M., 2013. Stress granules inhibit apoptosis by reducing reactive oxygen species production. Mol. Cell. Biol. 33 (4), 815–829. doi:10.1128/mcb.00763-12.

Thedieck, K., Holzwarth, B., Prentzell, M.T., Boehlke, C., Kläsener, K., Ruf, S., Sonntag, A.G., Maerz, L., Grellscheid, S.-N., Kremmer, E., Nitschke, R., Kuehn, E.W., Jonker, J.W., Groen, A.K., Reth, M., Hall, M.N., Baumeister, R., 2013. Inhibition of mTORC1 by astrin and stress granules prevents apoptosis in cancer cells. Cell 154 (4), 859–874. doi:10.1016/J.CELL.2013.07.031.

Towers, E.R., Kelly, J.J., Sud, R., Gale, J.E., Dawson, S.J., 2011. Caprin-1 is a target of the deafness gene Pou4f3 and is recruited to stress granules in cochlear hair cells in response to ototoxic damage. J. Cell Sci. 124 (7), 1145. doi:10.1242/JCS.076141.

Tresse, E., Salomons, F.A., Vesa, J., Bott, L.C., Kimonis, V., Yao, T.P., Dantuma, N.P., Taylor, J.P., 2010. VCP/p97 is essential for maturation of ubiquitin-containing autophagosomes and this function is impaired by mutations that cause IBMPFD. Autophagy 6 (2), 217–227. doi:10.4161/auto.6.2.11014.

Turakhiya, A., Meyer, S.R., Marincola, G., Böhm, S., Vanselow, J.T., Schlosser, A., Hofmann, K., Buchberger, A., 2018. ZFAND1 Recruits p97 and the 26S proteasome to promote the clearance of arsenite-induced stress granules. Mol. Cell 70 (5), 906–919. doi:10.1016/J.MOLCEL.2018.04.021, e7.

Vajjhala, P.R., Mirams, R.E., Hill, J.M., 2012. Multiple binding sites on the pyrin domain of ASC Protein allow self-association and Interaction with NLRP3 protein. J. Biol. Chem. 287 (50), 41732. doi:10.1074/JBC.M112.381228.

Vanderweyde, T., Apicco, D.J., Youmans-Kidder, K., Ash, P.E.A., Cook, C., Lummertz da Rocha, E., Jansen-West, K., Frame, A.A., Citro, A., Leszyk, J.D., Ivanov, P., Abisambra, J.F., Steffen, M., Li, H., Petrucelli, L., Wolozin, B., 2016. Interaction of tau with the RNA-binding protein TIA1 regulates tau pathophysiology and toxicity. Cell Rep. 15 (7), 1455–1466. doi:10.1016/j.celrep.2016.04.045.

Vermunt, L., Sikkes, S.A.M., van den Hout, A., Handels, R., Bos, I., van der Flier, W.M., Kern, S., Ousset, P.J., Maruff, P., Skoog, I., Verhey, F.R.J., Freund-Levi, Y., Tsolaki, M., Wallin, Å.K., Olde Rikkert, M., Soininen, H., Spiru, L., Zetterberg, H., Blennow, K., …, Coley, N., 2019. Duration of preclinical, prodromal and dementia alzheimer disease stages in relation to age, sex, and APOE genotype. Alzheimer's Dement. J. Alzheimer's Assoc. 15 (7), 888. doi:10.1016/J.JALZ.2019.04.001.

Walker, F.O., 2007. Huntington's disease. Lancet North Am. Ed. 369 (9557), 218–228. doi:10.1016/S0140-6736(07)60111-1.

Wang, S.-E., Wu, C.-H., 2015. Physiological and histological evaluations of the cochlea between 3xTg-AD mouse model of alzheimer's diseases and R6/2 mouse model of huntington's diseases. Chin. J. Physiol. 58 (6), 359–366. doi:10.4077/CJP.2015.BAD334.

Wang, Y., Li, W., Zhang, C., Peng, W., Xu, Z., 2022. RBM24 is localized to stress granules in cells under various stress conditions. Biochem. Biophys. Res. Commun. 608, 96–101. doi:10.1016/j.bbrc.2022.03.160.

Wegmann, S., Eftekharzadeh, B., Tepper, K., Zoltowska, K.M., Bennett, R.E., Dujardin, S., Laskowski, P.R., MacKenzie, D., Kamath, T., Commins, C., Vanderburg, C., Roe, A.D., Fan, Z., Molliex, A.M., Hernandez-Vega, A., Muller, D., Hyman, A.A., Mandelkow, E., Taylor, J.P., Hyman, B.T., 2018. Tau protein liquid–liquid phase separation can initiate tau aggregation. EMBO J. 37 (7). doi:10.15252/embj.201798049.

Wek, S.A., Zhu, S., Wek, R.C., 1995. The histidyl-tRNA synthetase-related sequence in the eIF-2 alpha protein kinase GCN2 interacts with tRNA and is required for activation in response to starvation for different amino acids. Mol. Cell. Biol. 15 (8), 4497. doi:10.1128/MCB.15.8.4497.

Wetter, S., Jacobson, M., Peavy, G., Hamilton, J., Salmon, D., Murphy, C., 2005. Olfactory and auditory event-related potentials in Huntington's disease. Neuropsychology 19 (4), 428–436. doi:10.1037/0894-4105.19.4.428.

Wheeler, J.R., Matheny, T., Jain, S., Abrisch, R., Parker, R., 2016. Distinct stages in stress granule assembly and disassembly. ELife 5. doi:10.7554/eLife.18413.

Wolozin, B., 2012. Regulated protein aggregation: stress granules and neurodegeneration. Mol. Neurodegener. 7 (1), 56. doi:10.1186/1750-1326-7-56.

Wolozin, B., Ivanov, P., 2019. Stress granules and neurodegeneration. Nat. Rev. Neurosci. 20 (11), 649. doi:10.1038/S41583-019-0222-5.

Xiong, W., Wei, W., Qi, Y., Du, Z., Qu, T., Liu, K., Gong, S., 2020. Autophagy is required for remodeling in postnatal developing ribbon synapses of cochlear inner hair cells. Neuroscience 431, 1–16. doi:10.1016/j.neuroscience.2020.01.032.

Yuan, H., Wang, X., Hill, K., Chen, J., Lemasters, J., Yang, S.M., Sha, S.H., 2015. Autophagy attenuates noise-induced hearing loss by reducing oxidative stress. Antioxid. Redox Signal. 22 (15), 1308–1324. doi:10.1089/ars.2014.6004.

Zhang, H., Cicchetti, G., Onda, H., Koon, H.B., Asrican, K., Bajraszewski, N., Vazquez, F., Carpenter, C.L., Kwiatkowski, D.J., 2003. Loss of Tsc1/Tsc2 activates mTOR and disrupts PI3K-Akt signaling through downregulation of PDGFR. J. Clin. Invest. 112 (8), 1223. doi:10.1172/JCI17222.

Zhang, P., Fan, B., Yang, P., Temirov, J., Messing, J., Kim, H.J., Taylor, J.P., 2019. Chronic optogenetic induction of stress granules is cytotoxic and reveals the evolution of ALS-FTD pathology. ELife doi:10.7554/eLife.39578, 8.

Zhang, Y., Wang, Y., Yao, X., Wang, C., Chen, F., Liu, D., Shao, M., Xu, Z., 2020. Rbm24a is necessary for hair cell development through regulating mRNA stability in Zebrafish. Front. Cell Dev. Biol. 8. doi:10.3389/fcell.2020.604026.

Zheng, L., Yuan, H., Zhang, M., Wang, C., Cai, X., Liu, J., Xu, X.Q., 2021. Rbm24 regulates inner-ear-specific alternative splicing and is essential for maintaining auditory and motor coordination. RNA Biol. 18 (4), 468–480. doi:10.1080/15476286.2020.1817265.