Review

Stress granules: the Tao of RNA triage

Paul Anderson and Nancy Kedersha

Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital and Harvard Medical School, Smith 652, One Jimmy Fund Way, Boston, MA 02115, USA

Cytoplasmic RNA structures such as stress granules (SGs) and processing bodies (PBs) are functional byproducts of mRNA metabolism, sharing substrate mRNA, dynamic properties and many proteins, but also housing separate components and performing independent functions. Each can exist independently, but when coordinately induced they are often tethered together in a cytosolic dance. Although both self-assemble in response to stress-induced perturbations in translation, several recent reports reveal novel proteins and RNAs that are components of these structures but also perform other cellular functions. Proteins that mediate splicing, transcription, adhesion, signaling and development are all integrated with SG and PB assembly. Thus, these ephemeral bodies represent more than just the dynamic sorting of mRNA between translation and decay.

mRNA triage: reprogramming translation during stress

Post-transcriptional regulation of gene expression is crucial for development, differentiation, immune signaling and neuronal plasticity [1]. mRNA biogenesis and function require the concerted efforts of RNA-binding proteins that shepherd the mRNA transcript through its capping, splicing, polyadenylation, nuclear export, association with ribosomes and ultimate decay [2]. Stresses (see Glossary), such as heat shock, oxidative stress, ischemia or viral infection, trigger a sudden translational arrest, leading to rapid polysome disassembly [3]. This event causes many proteins involved in normal mRNA processing events to assume ancillary 'emergency' functions, activating a process of molecular triage in which mRNA from disassembling polysomes is sorted and the fate of individual transcripts is determined. Cytoplasmic stress granules (SGs) are the morphological consequence of this triage process [3,4]. Recent reports indicate that SGs also recruit proteins involved in metabolic signaling pathways, enabling the assembly of SGs to influence cell metabolism and survival [5,6]. The surprising finding that SGs and processing bodies (PBs) also contain RNA-induced silencing complexes (RISCs) suggests that these RNA granules are integrated with microRNA (miRNA)-induced translational silencing pathways thus potentially influencing diverse cellular pathways and cell fate decisions [7–9]. A recent report links inosine-containing double-stranded RNA to SGs [10]. These RNAs are derived from noncoding RNAs possessing inverted repeat sequences or from viral RNA in virus infected cells. Inosine-modified RNAs bind strongly to SG components and inhibit translation initiation, suggesting that they also nucleate SG assembly. Previously dismissed as nonspecific aggregates, SGs have moved from artifact to 'matter of fact' in less than eight years.

SGs and PBs: kissing cousins

SGs are closely related to a second class of RNA granule known as the PB or GW182-containing body (GW body)

Glossary

ARE: AU-rich element; an RNA domain, found at the 3′ end of many mRNAs, that promotes silencing or decay.
Argonaute (Ago): a family of proteins associated with microRNAs, containing both a PIWI domain and a PAZ (Piwi Argonaute Zwille) domain. A subset of Argonaute proteins possess endonuclease 'slicer' activity and cleave mRNA, whereas others only silence translation.
eIF2α: a regulatory subunit of the eukaryotic translation initiation factor 2 (eIF2) complex, which is part of a larger ternary complex (eIF2–GTP–$tRNA_i^{Met}$) that positions the initiator methionine at the first codon of an mRNA and enables ribosome joining to commence protein translation. Phosphorylation of eIF2α on Ser51 is a requisite signal for the assembly of SGs in cells exposed to environmental stresses.
FMRP, FXR1: fragile X mental retardation protein; fragile X mental retardation-related protein 1; related proteins that regulate protein translation. The absence of FMRP causes a syndrome of mental retardation due to abnormal brain development.
G3BP: Ras-GTPase-activating protein SH3-domain-binding protein; a multidomain, multifunctional protein that is quantitatively concentrated at SGs in cells subjected to environmental stress. During poliovirus infection, the 3C proteinase cleaves G3BP to prevent SG assembly.
GW182: a large multidomain protein containing glycine-tryptophan (GW) repeats, associated with miRNAs. GW182 is required for miRNA-induced gene silencing whereas knockdown of GW182 inhibits PB assembly.
GW bodies: cytoplasmic foci containing the protein GW182, which are usually identical to processing bodies. It is not yet known whether all metazoan processing bodies contain GW182.
MicroRNA: small (~21-nt) RNAs that regulate mRNA expression and stability in metazoan organisms. miRNAs are assembled into RNP structures that contain at least one Argonaute protein and other proteins such as GW182 and FXR1.
Poly(A)-binding protein 1 (PABP-1): a protein that binds to poly(A) tails of mRNA and regulates mRNA stability and protein translation. It is a prominent component of SGs but not PBs.
Polysomes: mRNAs bound to translating ribosomes.
Prion-related domain: a protein domain rich in hydrophibic amino acids that is capable of assuming two stable conformations, one soluble and one insoluble. The insoluble conformers self-aggregate and are associated with various pathologies.
Processing bodies: cytoplasmic foci containing components of the 5′ to 3′ mRNA decay machinery, including DCP1a, DCP2, RCK (p54), hedls (GE-1) and edc3 (enhancer of decapping 3).
RNP: ribonucleoprotein; an RNA–protein complex, often containing multiple proteins bound to the same RNA. mRNPs contain mRNA bound to proteins but not to ribosomes.
Stress: a rapid change in environmental conditions. In this review, we refer to stresses such as heat shock, exposure to oxidants, unfolded proteins or double-stranded RNA, that activate an eIF2α kinase.
Stress granules: transient, dynamic cytoplasmic sites containing aggregates of mRNA bound to 48S preinitiation factors.
TIA-1, TIAR: T cell internal antigen-1, TIA-1-related; two related mRNA-binding proteins that contain three RNA-recognition motifs and a C-terminal prion-related domain, which recognize specific mRNAs and promote their silencing and decay.
TTP: tristetraprolin; a zinc-finger-containing protein that promotes the decay of ARE-containing mRNAs at PBs.

Corresponding authors: Anderson, P. (panderson@rics.bwh.harvard.edu); Kedersha, N. (nkedersha@rics.bwh.harvard.edu).

0968-0004/$ – see front matter doi:10.1016/j.tibs.2007.12.003

[11,12]. Both PBs and SGs are simultaneously assembled in cells subjected to environmental stress [13,14], both are assembled on untranslated mRNA derived from disassembled polysomes, and both contain a subset of shared proteins including FAST (Fas-activted serine/threonine kinase), XRN1 (5′–3′ exoribonuclease 1), eukaryotic translation initiation factor 4E (eIF4E), tristetraprolin (TTP), BRF1 (butyrate response factor 1) and BRF2 (butyrate response factor 2) [14]. In metazoans, both SGs and PBs have been linked to miRNA-mediated silencing [7,8]. However, SGs and PBs differ in several ways: (i) only PBs are observed in actively growing, unstressed cells; (ii) SG assembly, but not PB assembly, usually requires the stress-induced phosphorylation of eIF2α [14]; and (iii) SGs are defined by the translation initiation factors comprising the noncanonical 48S preinitiation complex – e.g. eIF3, eIF4A, eIF4G, poly(A)-binding protein 1 (PABP-1) and small ribosomal subunits – whereas PBs are defined by components of the mRNA decay machinery, for example the decapping enzymes DCP1a (decapping protein 1a), DCP2 and hedls (human enhancer of decapping, large subunit)/GE-1. The recent discovery of a possible minimal SG-like body in *Saccharomyces cerevisiae* [15,16], the organism in which PB functions were first elucidated [17], suggests that SG-like bodies exist in all eukaryotes (Box 1).

Box 1. Ontogeny of SGs

In fission yeast (*Schizosaccharomyces pombe*), environmental stress (e.g. thermal or osmotic stress) induces the assembly of cytoplasmic granules that contain RNA, the eIF3i, eIF3e and eIF3b subunits of the eIF3 complex, and eIF4E concurrent with polysome disassembly. These granules seem to be likely orthologs of SGs [88], despite their original description as possible sites of preferred translation during stress. Although eIF3-positive SGs are absent from the budding yeast *Saccharomyces cerevisiae*, recent results indicate that granules containing eIF4E, eIF4G and PABP-1 (EGP-bodies) are assembled in response to glucose starvation [15,16]. The lack of eIF3 or 40S ribosomal subunits in EGP-bodies and their nonreliance on phospho-eIF2α distinguishes them from SGs observed in other eukaryotes [15,16]. Nevertheless, these EGP-bodies might share some functions with SGs, for example housing mRNAs that are temporarily silenced during stress. Differences between the EGP-bodies assembled in *S. cerevisiae* and the eIF3-positive SGs in *S. pombe* might arise from differences between the core translational machineries found in these organisms: *S. pombe* possesses numerous 'non-core' eIF3 components that are absent in *S. cerevisiae*, among them eIF3d, eIF3e, eIF3f, eIF3h, eIF3j, eIF3k and eIF3m. Moreover, *S. pombe* has at least two different eIF3 complexes, both containing essential core subunits but differing in eIF3e and eIF3m, leading Zhou *et al.* [89] to propose that these different eIF3 complexes are involved in the translation of different sets of mRNA. Thus the absence of 'complete' SGs in *S. cerevisiae* could indicate a lack of specialized eIF3 complexes required for *bona fide* SG assembly. Also, several SG proteins are associated with splicing – either as regulators of alternative splicing (e.g. TIA-1, TIAR and HuR) or as part of the protein complex deposited on splice junctions after splicing occurs (MLN51). *S. cerevisiae* contains few spliced genes relative to *S. pombe*, and has virtually no dependence on alternative splicing – thus, splicing-related proteins important for SG assembly could be absent in *S. cerevisiae*. Finally, many SG components are associated with RNAi [7], notably microRNAs, Argonaute and FMRP or FXR1. *S. pombe* contains genes encoding Argonaute and Dicer; *S. cerevisiae* does not, possibly contributing to the lack of SGs in budding yeast.

SGs and PBs display distinctive types of movement in the cytoplasm and exhibit complex interactions with each other [14]. SGs are relatively fixed in the cytoplasm, yet they constantly change shape, fuse and divide, as revealed by time-lapse video microscopy [14]. By contrast, PBs move rapidly without changing their size or spherical shape. PBs intermittently and transiently dock at SGs, enabling the possible transfer of selected messenger ribonucleoproteins (mRNPs) to occur.

The eIF2α kinases: cellular stress sensors

The integrated stress response comprises a series of changes in cellular metabolism that enable the cell to repair stress-induced damage and survive adverse environmental conditions. Noxious conditions (e.g. excess heat, oxidation, UV irradiation, viral infection) induce eukaryotic cells to halt protein synthesis in a stereotypic response that conserves anabolic energy for the repair of molecular damage. The translational arrest that accompanies environmental stress is potentially selective: one study shows that the translation of ~25% of mRNAs is significantly reduced, whereas the translation of another 25% of mRNAs (including transcripts encoding heat-shock proteins) is significantly enhanced [18]. Stress-induced reprogramming of protein expression also entails stabilizing or destabilizing selected groups of mRNAs [18]. Thus, post-transcriptional reprogramming of mRNA translation and decay reconfigures the proteome during adverse environmental conditions.

In metazoans, five eIF2α kinases monitor environmental stress and directly modulate the translation machinery. These include: (i) PKR (protein kinase R), a double-stranded RNA-dependent kinase that is activated by viral infection, heat and UV irradiation [19]; (ii) PERK (PKR-like endoplasmic reticulum kinase; also known as PEK, or pancreatic eIF2α kinase), a resident endoplasmic reticulum (ER) protein that is activated when unfolded proteins accumulate in the ER lumen [20,21]; (iii) GCN2 (general control nonderepressible 2), a protein that monitors amino acid levels in the cell and responds to amino acid deprivation [22]; (iv) HRI (heme-regulated initiation factor 2α kinase), a protein that ensures the balanced synthesis of globin chains and heme during erythrocyte maturation and senses oxidative stress produced by arsenite [23]; and (v) Z-DNA kinase, an enzyme involved in the host antiviral response [22]. Stress-induced phosphorylation of eIF2α on Ser51 inhibits global protein translation by reducing levels of the eIF2–GTP–$tRNA_i^{Met}$ ternary complex that is required for cap-dependent translation initiation [3]. Because cap-independent translation initiated at IRES (internal ribosome entry site) elements is less inhibited by phosphorylation of eIF2α, some IRES-containing mRNAs are selectively translated in stressed cells [24]. Thus, the eIF2α kinases collectively monitor different types of cellular stress and regulate cap-dependent translation initiation rates through their common substrate, Ser51 of eIF2α.

Inhibition of translation initiation enables elongating ribosomes to 'run off' translating mRNA, a process that results in polysome disassembly. Much of the mRNA derived from disassembled polysomes assembles into

SGs [3,4]. The protein and RNA composition of SGs is dynamic: their core components are in equilibrium with polysomes. Drugs that inhibit translation elongation (e.g. cycloheximide) prevent SG assembly, whereas drugs that promote premature termination (e.g. puromycin) promote SG assembly [25]. Remarkably, the requirement for phospho-eIF2α in SG assembly can be bypassed using drugs that inhibit translation initiation by targeting the helicase eIF4A. These drugs (pateamine A and hippuristanol) induce SGs even in mutant cells devoid of phospho-eIF2α [26–29]. The anti-inflammatory lipid mediator 15d-PGJ2 also targets eIF4A, blocking its interactions with eIF4G and inducing SG assembly in the absence of phospho-eIF2α [30]. Thus, blocking initiation downstream of 48S assembly causes SG formation, indicating that the accumulation of free mRNPs is crucial for SG assembly. Conversely, mitotic cells are unable to assemble SGs or PBs as a result of elongational stalling, which stabilizes polysomes, similar to the effects of cycloheximide treatment [31].

SG protein composition and classification: who's in and who's out

SG components include a diverse group of mRNAs and proteins, some with no previously known links to RNA metabolism (Table 1). The first and defining class of SG components consists of stalled initiation complexes, still bound to mRNA and recruited to SGs from disassembling polysomes. This class includes mRNA transcripts, eIF3, eIF4F (comprising eIF4E, eIF4A and eIF4G), eIF4B, small ribosomal subunits and PABP-1 [3,32]. These core SG components are universal markers for all SGs.

A second class of SG components consists of mRNA-binding proteins linked to translational silencing or mRNA stability, which are reliable SG markers but might not be universal to all SGs. Translational silencing members of this group include TIA-1 (T cell internal antigen-1) and TIAR (TIA-1-related) [33], fragile X mental retardation protein (FMRP) and fragile X mental retardation-related protein 1 (FXR1) [61], FAST [14], Argonaute [8], CPEB (cytoplasmic polyadenylation element-binding protein) [13], pumillio [35], smaug [36], ataxin-2 [37] and Rap55 (RNA-associated protein 55, also called Lsm14) [38] (Table 1). RNA decay-associated SG components include the Argonaute proteins, tristetraprolin (TTP) and BRF1 [39], the RNA helicase RCK (also termed p54) [13], the endonuclease PMR1 (polysome-associated RNAse 1) [40], and zipcode binding protein 1 (ZBP1) [41]. Some of these proteins have been observed in polysomes (e.g. FXRP1 [42], RCK, PMR1 [40]) and RISCs [43], whereas others are excluded from polysomes (e.g. TIA-1, TIAR) [32]. The latter are probably constitutively active translational silencers: when associated with an mRNA, translation is suppressed and SG assembly is promoted. By contrast, translational silencing by polysome-associated RCK, FXR1 and Argonaute (possibly as part of RISC) must be under regulatory control because they associate with actively translating

Table 1. Selected and newly identified SG-associated proteins

Protein	Cellular location or state	Relevant binding partners	Refs	Nucleates SGs?	Known functions
Ago2	PBs, SGs, polysomes	FXR1, RISC	[8]	Yes	RNAi slicer
APOBEC3G	SGs and PBs	?	[49,84]	No	Antiviral response
Ataxin-2	SGs	PABP-1	[37]	No	Translation
Caprin-1	SGs	G3BP	[45]	Yes	Cell growth
CPEB	SGs and PBs	RCK, eIF4E, FXR1	[13]	Yes	mRNA silencing
DIS1	SGs	eIF3h	[55]	Yes	Unknown
eIF3	SGs, polysomes	40S, eIF4G	[14]	?	Translation
eIF4E	SGs, PBs, polysomes	CPEB, smaug, eIF4G, 4ET	[14]	No	Translation
eIF4G	SGs, polysomes	eIF4E, eIF3, PABP-1	[14]	?	Translation
FAST	PBs and SGs	TIA-1	[14]	Yes	Translation
FMRP and FXR1	SGs, PBs, polysomes	Ago2, RISC	[34]	Yes	Translation
FBP and KSRP	SGs	TIA-1	[53]	No	RNA decay
G3BP	SGs	Caprin	[44]	Yes	Ras signaling
HuR	SGs or PBs	?	[62]	No	RNA stability
IP5K	SGs	?	[48]	Yes	Signaling
Lin28	SGs, PBs, polysomes	?	[85]	?	Development
LINE 1 ORF1p	SGs	?	[47]	Yes	Transposon
MLN51	SGs	Exon junction	[51]	No	Splicing
PABP-1	Polysomes, SGs	eIF4G, eIF3, ataxin-2	[33]	No	Translation, stability
RCK (p54)	SGs, PBs, polysomes	Hedls (GE-1), TTP	[13]	Yes	mRNA decay
Plakophilin	SGs	G3BP, FXR1	[54]	No	Adhesion
PMR1	SGs, PBs, polysomes	TIA-1	[40]	No	mRNA decay
Pumilio 2	SGs	?	[35]	Yes	mRNA silencing
Rap55	SGs and PBs	?	[38]	?	mRNA silencing
Rpb4	SGs and PBs	?	[86]	?	Transcription
SRC3	SGs	TIA-1	[52]	No	Transcription
Staufen	SGs	?	[50]	No	mRNA silencing
SMN	SGs	SMN complex	[46]	Yes	RNP assembly
TIA-1 and TIAR	SGs or PBs	FAST, SRC3, PMR1, FBP	[32]	Yes	mRNA silencing
TRAF2	SGs	eIF4G	[5]	No	Signaling
TTP and BRF-1	SGs and PBs	RCK (p54)	[39]	Yes	mRNA decay
YB-1	SGs, PBs, polysomes	?	[87]	?	Cold-shock RNA binding protein
ZBP1	SGs	?	[41]	No	Localization

4E-T, eIF4E transporter; Lin, lineage; Rpb4, RNA polymerase II subunit B32; YB-1, Y-box protein 1.

polysomes. In an inactive state, these proteins associate with translating mRNAs, but their activation or overexpression promotes translational silencing, polysome disassembly and SG assembly.

A third class of SG-associated proteins includes RNA-binding proteins that regulate aspects of RNA metabolism other than mRNA translation or decay (e.g. splicing, RNA editing and RNA localization). When overexpressed, many of these proteins nucleate SG assembly – e.g. G3BP (Ras-GTPase-activating protein SH3-domain-binding protein), [44], caprin [45], FAST [14], SMN (survival of motor neurons) [46], the long interspersed nuclear element 1 (LINE 1) transposon ORF1p (open reading frame 1) [47] and 1,3,4,5,6-pentakisphosphate 2-kinase (IP5K) [48] – whereas others do not – e.g. APOBEC3G (apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G) [49], Staufen [50], MLN51 [51] and ZBP1 [41]). Some SG-associated proteins probably serve as molecular scaffolds that define the SG domain, which remains relatively constant, despite the fact that most SG proteins thus far examined (e.g. TIA-1, TIAR, G3BP, PABP-1 and TTP) shuttle through SGs much more rapidly than changes in SG morphology would suggest [14,25]. One candidate scaffolding protein is FAST, a TIA-1-binding protein that nucleates both SGs and PBs and seems to be stably associated with both [14].

An important newly discovered class of SG components is recruited to SGs by interacting with core SG components through so-called 'piggyback' interactions. For example, SRC3 (steroid coactivator 3) [52], FAST [6], FBP/KSRP (FUSE-binding protein, or KH-type splicing regulatory protein) [53] and PMR1 [40] all bind to TIA-1, an interaction that promotes their recruitment to SGs. An 'orphan' class of SG-associated proteins includes those not clearly linked to RNA metabolism, including TRAF2 (tumor necrosis factor receptor-associated factor 2), plakophilin 1 and plakophilin 3, IP5K [48], and Disrupted-in-Schizophrenia (DIS1). SG targeting of these proteins is also probably mediated by 'piggyback' interactions with core SG components; for example, TRAF binds to eIF4G [5], plakophilin 3 interacts with G3BP and PABP-1 [54], and DIS1 binds to eIF3 [55]. These proteins might have as yet unrecognized roles in RNA metabolism, or they might integrate SG formation with other cellular signaling pathways [5].

SG-associated mRNAs

Less is known about which specific mRNA transcripts are included in or exempt from SG recruitment. Although only 50% of cytoplasmic poly(A) RNA and poly(A)-binding protein-1 is recruited to SGs, nearly 90% of TIA-1 is recruited to SGs; this indicates that the mRNA content of SGs is selective [3]. Endogenous cellular mRNAs encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-actin, c-MYC, insulin-like growth factor II (IGF-II) and H19 are quantitatively recruited to SGs [41], whereas mRNAs encoding heat-shock protein 70 (HSP70) [56] and heat-shock protein 90 (HSP90) [41] are largely excluded. HSP90 and HSP70 mRNAs are transcriptionally activated by heat shock, concurrent with SG assembly, and both are translated preferentially during this stress condition whereas other mRNAs are not. Thus, their exclusion from SGs parallels their preferential retention in polysomes. Precisely how this occurs is not clear, although several unusual aspects of HSP70 mRNA structure might be involved. First, HSP70 mRNA lacks introns, presumably to ensure rapid protein expression. MLN51, one of four proteins deposited at splicing junctions, is recruited to SGs [51]; by avoiding splicing and thus MLN51 binding, mRNAs lacking introns could be less prone to SG recruitment. Second, HSP70 mRNA possesses a long and structured 5′ untranslated region (UTR) that ensures its translation by a 'shunting' mechanism [57] that does not require eIF4A-dependent mRNA scanning. Because eIF4A inactivation promotes SG assembly [26,27], mRNAs dependent on 5′-UTR scanning are probably preferred candidates for SG incorporation. Moreover, certain virally derived 5′ leader sequences encode 'SG-resistant' mRNAs ([58], Box 2). A single species of green fluorescent protein (GFP)-tethered reporter mRNA localizes to both SGs and PBs [14], indicating that recruitment to SGs versus PBs is not intrinsically sequence-specific, but rather is determined by the protein-coding component of the individual transcript.

Stages of SG assembly

SG assembly links stalled initiation, polysome disassembly and mRNP aggregation in a series of reversible stages (Figure 1). Even when fully formed, SGs continue to sort their mRNP contents, routing them to other cellular sites and fates.

Box 2. SGs and virus infection

SG assembly has been widely noted to occur during viral reprogramming of the host translational machinery. Several different classes of virus interact with TIA-1, TIAR or SGs in ways that affect viral replication, each using a different approach to manipulate SG assembly. West Nile virus (WNV) minus-strand RNA contains a stem-loop structure that binds to TIAR [67]. During infection, TIAR is sequestered at viral replication foci and SG assembly is inhibited [68]. TIAR binding is crucial for the infection because WNV replication is severely compromised in fibroblasts lacking TIAR [67]. Like WNV, Sendai virus encodes an RNA that sequesters TIAR and inhibits SG assembly. These results support a crucial role for TIAR in the assembly of SGs.

Recent results reveal that poliovirus targets a different SG protein to facilitate viral infection. Poliovirus infection results in the assembly of short-lived SGs. The disassembly of SGs is linked to the cleavage of G3BP by poliovirus 3C protease. Expression of a cleavage-resistant G3BP enables SGs to persist, resulting in inhibition of viral replication [69]. SGs are similarly assembled, then disassembled, in cells infected with Semliki Forest virus (SFV) [59]. In MEFs (mouse embryo fibroblasts) lacking TIA-1, fewer SGs are induced during early SFV infection, and the onset of host protein inhibition is delayed [59]. Thus, SFV seems to use SGs to regulate the shutdown of host protein synthesis.

Several other viruses have less-established links to TIA-1, TIAR or SGs. For example, herpes simplex virus 1 (HSV-1) replication is enhanced in MEFs lacking either TIA-1 or TIAR [67]. During HSV-1 infection, TIA-1 and TIAR accumulate in the cytoplasm 6 h postinfection, where they can modulate viral replication or cell survival [70]. In cells infected with HSV-1 lacking UL41, a viral protein required for the inhibition of host protein synthesis, TIA-1 and TIAR are found in cytoplasmic SGs. Because UL41 normally promotes the degradation of host mRNA, SGs might contribute to the reduction in host protein synthesis. Finally, the replication of vesicular stomatitis virus, sindbis virus and vaccinia virus also is enhanced in MEFs lacking TIA-1 [67].

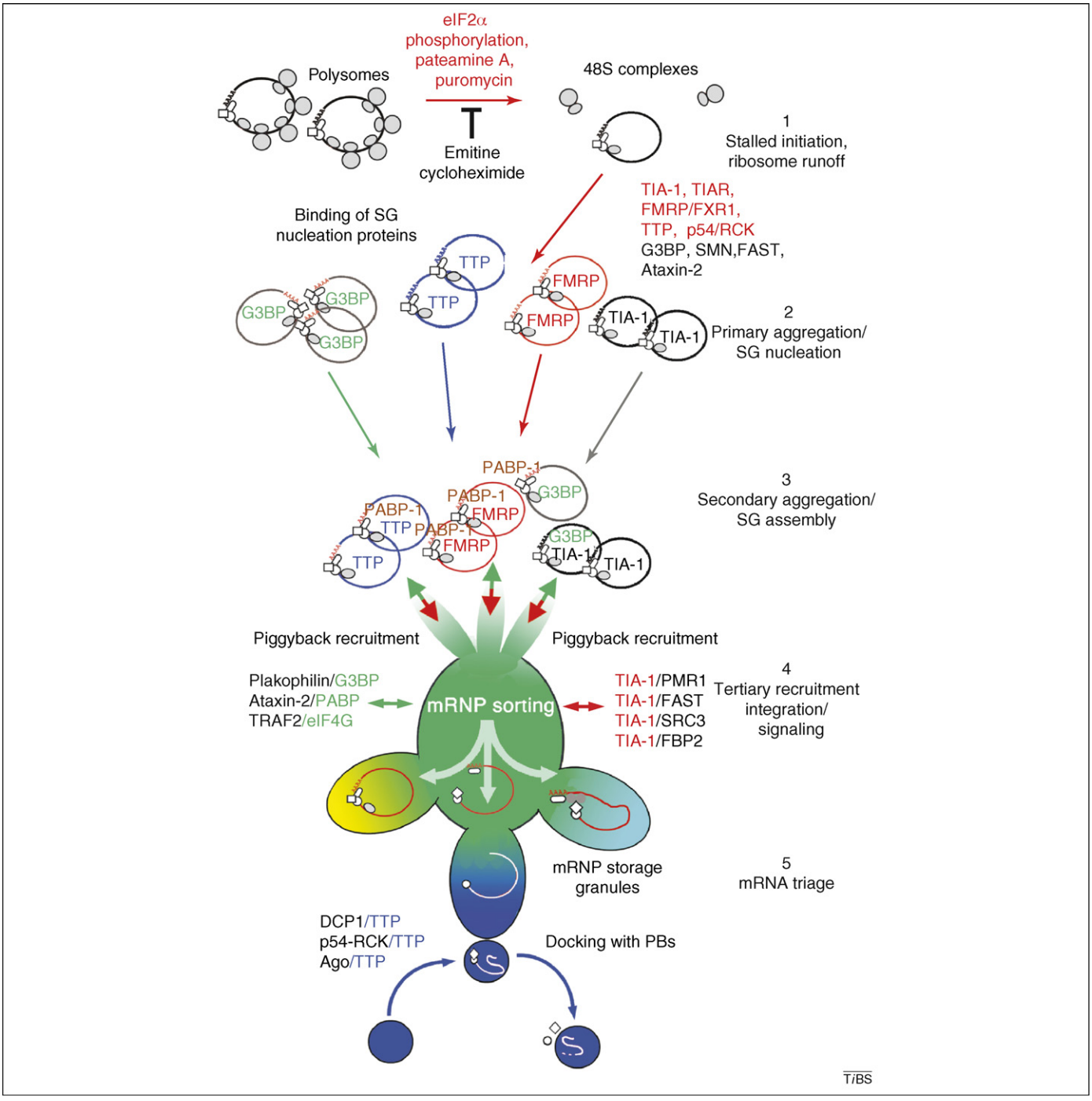

Figure 1. Model of stress granule assembly. The process of SG assembly can be divided into discrete stages that are marked by the specific composition and localization of mRNPs subject to translational arrest. Stage 1: SG assembly begins with stalled initiation that permits ribosomes to run off polysomes, converting them into mRNPs from which SGs are assembled. Primary aggregation and SG nucleation (stage 2) occurs when heterogeneous 48S-bound transcripts are bound by RNA-binding proteins that possess homotypic aggregation properties, such as G3BP (green), TIA-1 (black), TTP (blue), and FMRP (red), each of which binds preferentially to specific transcripts (those of matched colors). Secondary aggregation and crosslinking (stage 3) occur when PABP-1 (brown), bound to all poly(A)-containing transcripts, crosslinks smaller oligomers to assemble microscopically visible aggregates. Some transcripts are bound to multiple SG nucleating proteins, which enhances the crosslinking process to form progressively larger SGs, which can then recruit non-RNA-binding proteins (e.g. TRAF2, plakophilins, SRC3, FAST) through piggyback recruitment and integrate SG assembly with other cellular signaling pathways (stage 4). Within SGs, transcripts are subjected to mRNA triage, as the aggregation properties of specific proteins are modulated by HSP70 (e.g. TIA-1, CPEB), phosphorylation (e.g. G3BP, TTP) or interactions with other proteins (e.g. TTP–14-3-3). Finally (stage 5), specific transcripts are sorted out of SGs by translation initiation, assembly into other RNP granules, or by transfer into PBs by PB-targeting proteins such as TTP. An important feature of the model is that the process is reversible: that is, mRNAs that enter the SGs after polysome disassembly can be reinitiated and returned to the polysome fraction.

Stage 1: Stalled initiation and ribosome runoff

Phosphorylation of eIF2α [33], or drug-induced inactivation of eIF4A [26,27], result in abortive initation complexes; transcripts thus affected are converted into 48S mRNPs as their ribosomes 'run-off'. Conditions preventing ribosome elongation and run-off (e.g. cycloheximide, mitosis, certain viral mRNAs) do not permit SG assembly [25,31,59].

Stage 2: Primary aggregation and SG nucleation

Free 48S mRNPs are essential substrates for SG assembly. Numerous SG-associated proteins promote SG assembly

when overexpressed (e.g. a phosphomimetic mutant eIF2α [33], TIA-1 or TIAR [60], TTP or BRF1 [39], G3BP [44], RCK [13], caprin-1 [45], CPEB [13], FAST [14], FXR1 and FRMP [61], Argonaute-2 [8], LINE 1 ORF1p [47], SMN [46], smaug [36], DIS1 [55]), whereas other proteins or mutants can inhibit SG assembly – e.g. S51A mutant eIF2α [33], ataxin-2 [37], the TIA-1 prion-related domain (PRD) [60] and mutant G3BP [44]. This finding indicates that many proteins independently promote SG assembly. 'Primary aggregation' is mediated by protein(s) that initiate mRNP aggregation and thus physically nucleate SG assembly. TIA-1, TIAR, TTP, BRF1, FMRP, FXR1, CPEB, G3BP and SMN constitute examples of SG nucleators: they induce SGs when overexpressed; they become part of the SGs they nucleate; and their ability to induce 'spontaneous' SGs requires nonpolysomal 48S mRNPs [23,27]. Once nucleation is initiated, SGs assemble with fairly uniform protein composition, that is, containing the core SG components eIF3, eIF4F, PABP-1 and small ribosomal subunits. SG nucleators shift the equilibrium between mRNPs and polysomes, in effect competing for 48S complexes before initiation is completed by ribosomal joining.

Stage 3: Secondary aggregation

Each mRNA transcript binds to multiple proteins that are capable of homotypic (and in some cases heterotypic) interactions. These protein–protein interactions promote secondary aggregation of mRNPs, and thus assemble microscopically visible SGs. Time-lapse photomicroscopy studies show SG assembly beginning with the simultaneous formation of numerous small SGs, which progressively fuse into larger and fewer structures [25]. Many SG components (e.g. TIA-1 and TIAR [33], HuR (Hu antigen R) [62], FAST [6], SRC3 [52]) are predominantly nuclear shuttling proteins, whose contribution to SG assembly requires nuclear export. Thus, regulated shuttling of SG-associated proteins might contribute to the rate and extent of SG assembly.

Stage 4: Integration and signaling

Certain proteins that lack mRNA-binding properties are recruited to SGs through protein–protein interactions with the SG nucleating proteins or with components of SG-associated 48S mRNPs. Several TIA-1-binding proteins (e.g. SRC3, FAST, PMR1) are recruited to SGs in this 'piggyback' manner [40,52]. Additional examples of piggyback recruitment include TRAF2 (bound to eIF4G), a protein that regulates NF-κB-dependent cell survival [5], and plakophilin 3 (bound to G3BP), a protein that promotes cellular adhesion [54]. Thus, the cadre of proteins recruited to SGs can integrate other aspects of cellular metabolism with the translational response to stress.

Stage 5: mRNA triage

Initially, SGs were thought to be passive repositories of the untranslated mRNAs that accumulate during stress. By sequestering untranslated mRNA from the degradation machinery, SGs could protect most cellular mRNAs, enabling translation reinitiation when environmental conditions improved. This logical hypothesis was thoroughly debunked in studies using fluorescence recovery after photobleaching (FRAP) to quantify the residence time of proteins in SGs. FRAP analysis revealed that GFP–TIA-1 and GFP–PABP-1 rapidly shuttle in and out of SGs with a residence half-life of 2 and 8 s, respectively [25]. These studies helped to shape the mRNA triage model [3,4], which describes SGs as self-organized compartments within which specific transcripts are selected for decay by destabilizing proteins (e.g. TTP, BRF1), whereas other transcripts are bound by stabilizing proteins (e.g. HuR) for export or storage, within the SG or elsewhere. In addition, some transcripts can be reinitiated and reconverted into polysomes – either actively by recruiting the necessary factors, or passively, by eluding recruitment to PBs or to exosome-mediated decay machinery. In addition to RNA-binding proteins, specific miRNAs might also facilitate the triage process in SGs, although experimental proof for such a mechanism is lacking.

Several RNA-binding proteins can escort specific mRNAs between SGs and PBs. The destabilizing factors TTP and BRF1 preferentially bind to transcripts with AU-rich elements (AREs) in their 3′-UTRs and promote their decay [63,64]. Both TTP and BRF1 concentrate in SGs while their associated mRNAs undergo translational arrest [39]; surprisingly their recruitment to SGs, but not to PBs, is regulated. Phosphorylation of TTP at Ser52 and Ser178 promotes binding of 14-3-3 proteins, which reduces the mRNA destabilizing activity of TTP and simultaneously prevents TTP association with SGs [39]. Overexpression of TTP or BRF1, unlike other proteins shared by SGs and PBs, strongly stabilizes interactions between SGs and PBs, tethering them together [14]. Paradoxically, FRAP analysis indicates that overexpressed TTP is only fleetingly present (half-life of 2 to 5 s) at both SGs or PBs, yet it promotes much longer (60 min) physical tethering of SGs with PBs [14]. Given the affinity of TTP and BRF1 for specific transcripts and their decay-promoting properties, the TTP- or BRF1-induced tethering of SGs to PBs probably indicates increased transfer of TTP- or BRF1-bound mRNAs to PBs for decay (Figure 2). Protein–protein interactions between TTP or BRF1 and components of the decapping machinery [65] are likely to facilitate this directed movement.

ZBP1 is another RNA-binding protein that regulates the movement of selected mRNAs into or out of SGs [41]. ZBP1 binds to a *cis* element found in the 3′-UTR of target transcripts. In stressed cells, ZBP1 is concentrated at SGs bound to its associated mRNAs. Overexpression of ZBP1 neither induces SG assembly nor promotes SG–PB interactions. Rather, ZBP1 retains its associated transcripts within SGs while enhancing their stability. These examples illustrate how protein–mRNA interactions specify different mRNA fates through their spatial regulation into and out of SGs.

SG disassembly

Disassembly of SGs in cells recovering from stress occurs rapidly, within minutes. Before disassembly, SGs are relatively few and large (microns in diameter). Multiple SGs within a single cell synchronously disperse, appearing to dissolve rather than fragment [14,25]. However, visualiz-

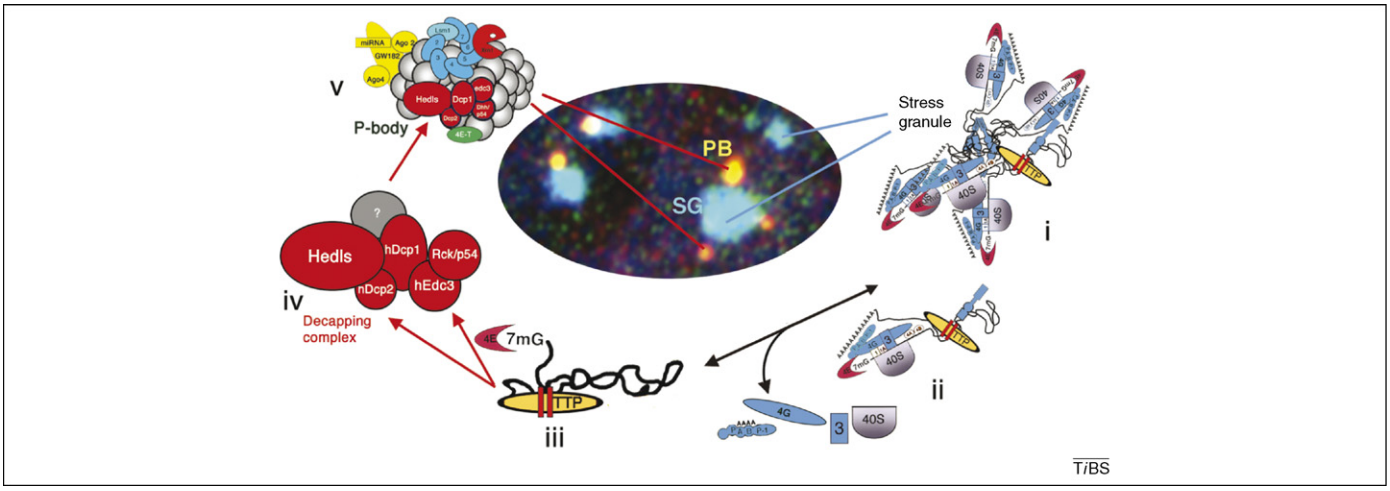

Figure 2. TTP dynamically links SGs with PBs during mRNA triage. TTP (yellow) binds to ARE-containing mRNA transcripts while they are still bound to the translational machinery and accompanies them as they accumulate at SGs (i). Normally (ii, iii), ribosomes and initiation factors are removed from the mRNA transcripts before TTP escorts the latter to the decapping machinery (depicted in red) for decay (iv). When overexpressed, the flood of TTP-bound transcripts released from polysomes is directed to PBs before or concurrent with removal of initiation machinery (v), possibly because the flux of mRNA transcripts cannot be deadenylated rapidly enough to enable removal of PABP-1 and eIF4F. By recruiting mRNA transcripts still bound to the translational machinery to PBs, TTP links SGs to PBs with a dynamic flux of mRNA.

ing SGs in real time requires the selection of one protein marker, typically a nucleating RNA-binding protein such as TIA-1 or G3BP. Because SGs seem to be composed of multiple semi-independent mRNPs, data obtained using any one marker are probably subject to bias. Studies using cell lines that stably express different SG components will be required to provide a global view of SG assembly and disassembly.

Regulated aggregation events in SG assembly and dynamics

Most proteins that nucleate SG assembly contain domains that bind to RNA directly, in addition to distinct domains that mediate homotypic aggregation. TIA-1 and TIAR possess glutamine-rich PRDs at their carboxyl termini [60], which are essential for SG assembly. When expressed in COS-7 cells, full-length recombinant TIA-1 nucleates the assembly of *bona fide* SGs, whereas recombinant TIA-1 lacking the PRD does not [60]. The PRD from the well-characterized yeast translation termination factor Sup35p can substitute for the PRD of TIA-1 to promote SG assembly, indicating that prion-mediated self-aggregation is required for TIA-1-mediated SG assembly [60]. Several other SG-nucleating proteins possess glutamine-rich motifs (e.g. RCK, CPEB, G3BP) that might promote SG assembly by a similar mechanism. Like their prion relatives, the aggregation of TIA-1 or TIAR is regulated by molecular chaperones [60] and is blocked by HSP70 overexpression. This finding suggests that HSP70 levels are involved in a feedback loop that promotes SG disassembly when HSP70 levels return to normal. In this model, minimal constitutive levels of HSP70 are continuously required to prevent TIA-1 aggregation. Stress-induced denaturation of other cytoplasmic proteins recruits both HSP70 and ATP for protein renaturation, thus diverting HSP70 away from TIA-1, promoting TIA-1 aggregation and consequent SG nucleation. The successful refolding of denatured proteins releases HSP70 and the free HSP70 then solubilizes TIA-1; the subsequent TIA-1 disaggregation promotes SG disassembly. HSP70 was recently reported to disassemble SGs induced in response to proteasome inhibition [66]. Similarly, other modes of regulated aggregation contribute to SG assembly: self-aggregation of G3BP, an important nucleator of SGs, is regulated by its phosphorylation at Ser149 [44].

SGs in infection and disease

SGs might participate in life-or-death decisions in stressed cells by selectively regulating the expression of proteins involved in cell survival. The duration of SG-mediated reprogramming of mRNA translation and decay beyond a critical threshold can activate apoptosis. Indeed, many viruses regulate the assembly or disassembly of SGs [59,67–70] (Box 2), suggesting their importance in balancing the translation of host- and virus-encoded mRNAs. SGs have also been implicated in disease pathogenesis [34,71–81] (Box 3), providing further evidence for a role in the integration of life-and-death decisions.

Concluding remarks and future perspectives

There is an emerging consensus that translation initiation is in dynamic equilibrium with an active process of translational silencing. In growing somatic cells, the rate of translation initiation exceeds the rate of translation silencing and most, but not all, cytoplasmic mRNA is located in polysomes [32]. Cellular stress shifts this equilibrium such that the silencing rate exceeds the initiation rate. Many SG components mediate the translational silencing of virgin mRNA transcripts during development and differentiation (e.g. CPEB [1], pumilio [82], smaug [36] and staufen [83]); the recruitment of these proteins to SGs suggests that they can also silence polysome-derived transcripts, but precisely how these proteins are activated during stress remains unknown. Recent findings indicate that miRNAs and the Argonaute proteins, also involved in development and differentiation, are found in SGs in addition to PBs [7]. Perhaps some cases of regulated SG–PB tethering involve miRNA loading or RISC assembly (or both), using proteins stored or recycled in PBs. It will be interesting to determine if molecular modifications of the Argonaute proteins

Box 3. SGs in disease

SGs have been proposed to contribute to the pathogenesis of several different diseases.

Cancer

A major determinant of tumor radiotherapy efficacy is endothelial cell damage. Irradiation-induced hypoxia (severe oxygen deprivation) induces tumor cells to express hypoxia-inducible factor 1 (HIF-1), a transcription factor that induces the expression of mRNAs encoding the endothelial survival factors vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). SGs inhibit the translation of select HIF-1-induced transcripts, including VEGF and bFGF during hypoxia to regulate tumor cell survival after irradiation [71].

Fragile X syndrome

Mutations in the gene encoding the fragile X mental retardation protein (FMRP) cause fragile X syndrome [72]. FMRP is an RNA-binding protein that regulates synaptic protein synthesis, and fragile X patients have immature dendritic spines, probably the result of aberrant protein translation. FMRP interacts with RISC components, thus implicating miRNA-mediated translational silencing in fragile X etiology. Like other RISC components, FMRP is found in both SGs and PBs. The ability of FMRP to regulate the assembly of neuronal RNA granules suggests that this process has a role in disease pathogenesis [34,73].

Spinal motor atrophy

Mutations in the *SMN1* gene are associated with an autosomal recessive neuromuscular disease known as spinal motor atrophy [74]. In motor neurons, SMN1 is found in cytoplasmic RNA granules that might be involved in mRNA transport or regulation (or both) [75], and SMN1 nucleates SGs when overexpressed in cultured cells [46]. Thus, SMN1-mediated assembly or function of RNA granules in motor neurons might influence disease progression through the disruption of protein translation.

Ischemia–reperfusion

Ischemia–reperfusion (I–R) injury is a major determinant of neural toxicity following cardiac arrest or stroke. Cerebral hypoxia induces PERK-dependent eIF2α phosphorylation and the subsequent assembly of neuronal SGs [76–79]. Thus, SG assembly and disassembly might influence the degree of ischemia-dependent neuronal damage.

Gentamicin-induced ototoxicity

Aminoglycoside antibiotics induce hearing loss by damaging sensory hair cells in the cochlea. Gentamicin inhibits protein synthesis by interfering with translation termination or ribosome recycling (or both) [79]. In chickens exposed to toxic doses of gentamicin, SGs are assembled in cochlear cells before the apoptotic death of the cells, demonstrating that SGs assemble under clinically relevant conditions [80].

Immunity

When naive T cells first encounter their antigen, they initiate the transcription of cytokines appropriate to their functional class. However, antigen-primed T cells expressing abundant cytokine mRNA do not secrete cytokines until restimulated with antigen. Polysome profiles of primed T cells reveal that cytokine mRNA is excluded from polysomes. T-cell priming induces eIF2α phosphorylation and SG assembly [81]. Although antigen restimulation does not immediately dissolve SGs, the expression of a TIA-1 truncation mutant that prevents SG formation promotes cytokine secretion from primed T cells. Thus T-cell activation induces a stress response that delays cytokine translation.

mediate SG–PB interactions, similar to the observed effect of TTP phosphorylation.

Although mRNP composition clearly determines whether a given transcript is subject to SG recruitment, the relative importance of the mRNA and its protein coat are unknown. Why is the ARE-binding protein AUF1 (AU-rich element-binding protein 1) exempt from SG recruitment, in contrast to other ARE-binding proteins such as HuR (whose molecular architecture is similar to that of AUF1) and TTP (which promotes decay of similar AU-rich targets)? What is the role of G3BP in SGs, and why does it nucleate such abnormally large SGs? How do translation termination and ribosome release fit into the picture? Does polyadenylation influence the movement of mRNA between RNA granules? Does the RISC function in SGs, in PBs or in the cytosol? The lack of ready answers to these and other questions will motivate research in this emerging field for some time to come.

SGs and PBs spatially integrate molecular, morphological and functional aspects of translation and decay. We now have a framework that describes how RNA and protein interactions at the molecular level create these dynamic cytoplasmic domains. Some connections with other cellular pathways and compartments are emerging, as are links to nuclear transcription and splicing. We have further to travel, and triage lies ahead – but we begin to know the Way.

Acknowledgements

We thank Dan Schoenberg and Georg Stoecklin for critical review of the manuscript. Work in our laboratories is supported by grants from the National Institutes of Health and the American College of Rheumatology. We regret that we were unable to cite many relevant papers due to size limitations.

References

1 Richter, J.D. (2007) CPEB: a life in translation. *Trends Biochem. Sci.* 32, 279–285
2 Moore, M.J. (2005) From birth to death: the complex lives of eukaryotic mRNAs. *Science* 309, 1514–1518
3 Anderson, P. and Kedersha, N. (2002) Stressful initiations. *J. Cell Sci.* 115, 3227–3234
4 Anderson, P. and Kedersha, N. (2006) RNA granules. *J. Cell Biol.* 172, 803–808
5 Kim, W.J. *et al.* (2005) Sequestration of TRAF2 into stress granules interrupts tumor necrosis factor signaling under stress conditions. *Mol. Cell. Biol.* 25, 2450–2462
6 Li, W. *et al.* (2004) FAST is a survival protein that senses mitochondrial stress and modulates TIA-1-regulated changes in protein expression. *Mol. Cell. Biol.* 24, 10718–10732
7 Leung, A.K. and Sharp, P.A. (2007) microRNAs: a safeguard against turmoil? *Cell* 130, 581–585
8 Leung, A.K. *et al.* (2006) Quantitative analysis of Argonaute protein reveals microRNA-dependent localization to stress granules. *Proc. Natl. Acad. Sci. U. S. A.* 103, 18125–18130
9 Liu, J. *et al.* (2005) MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. *Nat. Cell Biol.* 7, 719–723
10 Scadden, A.D. (2007) Inosine-containing dsRNA binds a stress-granule-like complex and downregulates gene expression *in trans*. *Mol. Cell* 28, 491–500
11 Parker, R. and Sheth, U. (2007) P bodies and the control of mRNA translation and degradation. *Mol. Cell* 25, 635–646
12 Eulalio, A. *et al.* (2007) P bodies: at the crossroads of post-transcriptional pathways. *Nat. Rev. Mol. Cell Biol.* 8, 9–22
13 Wilczynska, A. *et al.* (2005) The translational regulator CPEB1 provides a link between dcp1 bodies and stress granules. *J. Cell Sci.* 118, 981–992
14 Kedersha, N. *et al.* (2005) Stress granules and processing bodies are dynamically liked sites of mRNP remodeling. *J. Cell Biol.* 169, 871–884
15 Brengues, M. and Parker, R. (2007) Accumulation of polyadenylated mRNA, Pab1p, eIF4E, and eIF4G with P-bodies in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 18, 2592–2602

16 Hoyle, N.P. *et al.* (2007) Stress-dependent relocalization of translationally primed mRNPs to cytoplasmic granules that are kinetically and spatially distinct from P-bodies. *J. Cell Biol.* 179, 65–74
17 Sheth, U. and Parker, R. (2003) Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. *Science* 300, 805–808
18 Kawai, T. *et al.* (2004) Global mRNA stabilization preferentially linked to translational repression during the endoplasmic reticulum stress response. *Mol. Cell. Biol.* 24, 6773–6787
19 Srivastava, S.P. *et al.* (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, 2416–2423
20 Harding, H.P. *et al.* (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol. Cell* 6, 1099–1108
21 Harding, H.P. *et al.* (2000) Perk is essential for translational regulation and cell survival during the unfolded protein response. *Mol. Cell* 5, 897–904
22 Wek, S.A. *et al.* (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, 4497–4506
23 McEwen, E. *et al.* (2005) Heme-regulated inhibitor (HRI) kinase-mediated phosphorylation of eukaryotic translation initiation factor 2 (eIF2) inhibits translation, induces stress granule formation, and mediates survival upon arsenite exposure. *J. Biol. Chem.* 280, 16925–16933
24 Robert, F. *et al.* (2006) Initiation of protein synthesis by hepatitis C virus is refractory to reduced eIF2.GTP.Met-tRNA(i)(Met) ternary complex availability. *Mol. Biol. Cell* 17, 4632–4644
25 Kedersha, N. *et al.* (2000) Dynamic shuttling of TIA-1 accompanies the recruitment of mRNA to mammalian stress granules. *J. Cell Biol.* 151, 1257–1268
26 Mazroui, R. *et al.* (2006) Inhibition of ribosome recruitment induces stress granule formation independently of eukaryotic initiation factor 2alpha phosphorylation. *Mol. Biol. Cell* 17, 4212–4219
27 Dang, Y. *et al.* (2006) Eukaryotic initiation factor 2alpha-independent pathway of stress granule induction by the natural product pateamine A. *J. Biol. Chem.* 281, 32870–32878
28 Bordeleau, M.E. *et al.* (2005) Stimulation of mammalian translation initiation factor eIF4A activity by a small molecule inhibitor of eukaryotic translation. *Proc. Natl. Acad. Sci. U. S. A.* 102, 10460–10465
29 Low, W.K. *et al.* (2005) Inhibition of eukaryotic translation initiation by the marine natural product pateamine A. *Mol. Cell* 20, 709–722
30 Kim, W.J. *et al.* (2007) Anti-inflammatory lipid mediator 15d-PGJ2 inhibits translation through inactivation of eIF4A. *EMBO J.* 26, 5020–5032
31 Sivan, G. *et al.* (2007) Ribosomal slowdown mediates translational arrest during cellular division. *Mol. Cell. Biol.* 27, 6639–6646
32 Kedersha, N. *et al.* (2002) Evidence that ternary complex (eIF2-GTP-tRNA(i)(Met))-deficient preinitiation complexes are core constituents of mammalian stress granules. *Mol. Biol. Cell* 13, 195–210
33 Kedersha, N.L. *et al.* (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, 1431–1441
34 Antar, L.N. *et al.* (2005) Localization of FMRP-associated mRNA granules and requirement of microtubules for activity-dependent trafficking in hippocampal neurons. *Genes Brain Behav.* 4, 350–359
35 Vessey, J.P. *et al.* (2006) Dendritic localization of the translational repressor Pumilio 2 and its contribution to dendritic stress granules. *J. Neurosci.* 26, 6496–6508
36 Baez, M.V. and Boccaccio, G.L. (2005) Mammalian Smaug is a translational repressor that forms cytoplasmic foci similar to stress granules. *J. Biol. Chem.* 280, 43131–43140
37 Nonhoff, U. *et al.* (2007) Ataxin-2 interacts with the DEAD/H-box RNA helicase DDX6 and interferes with P-bodies and stress granules. *Mol. Biol. Cell* 18, 1385–1396
38 Yang, W.H. *et al.* (2006) RNA-associated protein 55 (RAP55) localizes to mRNA processing bodies and stress granules. *RNA* 12, 547–554
39 Stoecklin, G. *et al.* (2004) MK2-induced tristetraprolin:14-3-3 complexes prevent stress granule association and ARE-mRNA decay. *EMBO J.* 23, 1313–1324
40 Yang, F. *et al.* (2006) Polysome-bound endonuclease PMR1 is targeted to stress granules via stress-specific binding to TIA-1. *Mol. Cell. Biol.* 26, 8803–8813
41 Stohr, N. *et al.* (2006) ZBP1 regulates mRNA stability during cellular stress. *J. Cell Biol.* 175, 527–534
42 Kim, S.H. *et al.* (2006) Fragile X mental retardation protein shifts between polyribosomes and stress granules after neuronal injury by arsenite stress or *in vivo* hippocampal electrode insertion. *J. Neurosci.* 26, 2413–2418
43 Maroney, P.A. *et al.* (2006) Evidence that microRNAs are associated with translating messenger RNAs in human cells. *Nat. Struct. Mol. Biol.* 13, 1102–1107
44 Tourriere, H. *et al.* (2003) The RasGAP-associated endoribonuclease G3BP assembles stress granules. *J. Cell Biol.* 160, 823–831
45 Solomon, S. *et al.* (2007) Distinct structural features of caprin-1 mediate its interaction with G3BP-1 and its induction of phosphorylation of eukaryotic translation initiation factor 2alpha, entry to cytoplasmic stress granules, and selective interaction with a subset of mRNAs. *Mol. Cell. Biol.* 27, 2324–2342
46 Hua, Y. and Zhou, J. (2004) Survival motor neuron protein facilitates assembly of stress granules. *FEBS Lett.* 572, 69–74
47 Goodier, J.L. *et al.* (2007) LINE-1 ORF1 protein localizes in stress granules with other RNA-binding proteins, including components of RNA interference RNA-induced silencing complex. *Mol. Cell. Biol.* 27, 6469–6483
48 Brehm, M.A. *et al.* (2007) Intracellular localization of human Ins(1,3,4,5,6)P5 2-kinase. *Biochem J.* 408, 335–345
49 Kozak, S.L. *et al.* (2006) The anti-HIV-1 editing enzyme APOBEC3G binds HIV-1 RNA and messenger RNAs that shuttle between polysomes and stress granules. *J. Biol. Chem.* 281, 29105–29119
50 Thomas, M.G. *et al.* (2005) Staufen recruitment into stress granules does not affect early mRNA transport in oligodendrocytes. *Mol. Biol. Cell* 16, 405–420
51 Baguet, A. *et al.* (2007) The exon-junction-complex-component metastatic lymph node 51 functions in stress-granule assembly. *J. Cell Sci.* 120, 2774–2784
52 Yu, C. *et al.* (2007) An essential function of the SRC-3 coactivator in suppression of cytokine mRNA translation and inflammatory response. *Mol. Cell* 25, 765–778
53 Rothe, F. *et al.* (2006) Identification of FUSE-binding proteins as interacting partners of TIA proteins. *Biochem. Biophys. Res. Commun.* 343, 57–68
54 Hofmann, I. *et al.* (2006) Identification of the junctional plaque protein plakophilin 3 in cytoplasmic particles containing RNA-binding proteins and the recruitment of plakophilins 1 and 3 to stress granules. *Mol. Biol. Cell* 17, 1388–1398
55 Ogawa, F. *et al.* (2005) A functional link between Disrupted-In-Schizophrenia 1 and the eukaryotic translation initiation factor 3. *Biochem. Biophys. Res. Commun.* 338, 771–776
56 Kedersha, N. and Anderson, P. (2002) Stress granules: sites of mRNA triage that regulate mRNA stability and translatability. *Biochem. Soc. Trans.* 30, 963–969
57 Rubtsova, M.P. *et al.* (2003) Distinctive properties of the 5′-untranslated region of human hsp70 mRNA. *J. Biol. Chem.* 278, 22350–22356
58 Kedersha, N. and Anderson, P. (2007) Mammalian stress granules and processing bodies. *Methods Enzymol.* 431, 61–81
59 McInerney, G.M. *et al.* (2005) Importance of eIF2alpha phosphorylation and stress granule assembly in alphavirus translation regulation. *Mol. Biol. Cell* 16, 3753–3763
60 Gilks, N. *et al.* (2004) Stress granule assembly is mediated by prion-like aggregation of TIA-1. *Mol. Biol. Cell* 15, 5383–5398
61 Mazroui, R. *et al.* (2002) Trapping of messenger RNA by fragile X mental retardation protein into cytoplasmic granules induces translation repression. *Hum. Mol. Genet.* 11, 3007–3017
62 Gallouzi, I.E. *et al.* (2000) HuR binding to cytoplasmic mRNA is perturbed by heat shock. *Proc. Natl. Acad. Sci. U. S. A.* 97, 3073–3078
63 Carballo, E. *et al.* (1998) Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin. *Science* 281, 1001–1005
64 Stoecklin, G. *et al.* (2002) Functional cloning of BRF1, a regulator of ARE-dependent mRNA turnover. *EMBO J.* 21, 4709–4718

65 Fenger-Gron, M. *et al.* (2005) Multiple processing body factors and the ARE binding protein TTP activate mRNA decapping. *Mol. Cell* 20, 905–915
66 Mazroui, R. *et al.* (2007) Inhibition of the ubiquitin-proteasome system induces stress granule formation. *Mol. Biol. Cell* 18, 2603–2618
67 Li, W. *et al.* (2002) Cell proteins TIA-1 and TIAR interact with the 3′ stem-loop of the West Nile virus complementary minus-strand RNA and facilitate virus replication. *J. Virol.* 76, 11989–12000
68 Emara, M.M. and Brinton, M.A. (2007) Interaction of TIA-1/TIAR with West Nile and dengue virus products in infected cells interferes with stress granule formation and processing body assembly. *Proc. Natl. Acad. Sci. U. S. A.* 104, 9041–9046
69 White, J.P. *et al.* (2007) Inhibition of cytoplasmic mRNA stress granule formation by a viral proteinase. *Cell Host Microbe* 2, 295–305
70 Esclatine, A. *et al.* (2004) Herpes simplex virus 1 induces cytoplasmic accumulation of TIA-1/TIAR and both synthesis and cytoplasmic accumulation of tristetraprolin, two cellular proteins that bind and destabilize AU-rich RNAs. *J. Virol.* 78, 8582–8592
71 Moeller, B.J. *et al.* (2004) Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: role of reoxygenation, free radicals, and stress granules. *Cancer Cell* 5, 429–441
72 Garber, K. *et al.* (2006) Transcription, translation and fragile X syndrome. *Curr. Opin. Genet. Dev.* 16, 270–275
73 Khandjian, E.W. *et al.* (2004) Biochemical evidence for the association of fragile X mental retardation protein with brain polyribosomal ribonucleoparticles. *Proc. Natl. Acad. Sci. U. S. A.* 101, 13357–13362
74 Monani, U.R. (2005) Spinal muscular atrophy: a deficiency in a ubiquitous protein; a motor neuron-specific disease. *Neuron* 48, 885–896
75 Rossoll, W. *et al.* (2003) Smn, the spinal muscular atrophy-determining gene product, modulates axon growth and localization of beta-actin mRNA in growth cones of motoneurons. *J. Cell Biol.* 163, 801–812
76 Kayali, F. *et al.* (2005) Prolonged translation arrest in reperfused hippocampal cornu Ammonis 1 is mediated by stress granules. *Neuroscience* 134, 1223–1245
77 Montie, H.L. *et al.* (2005) PERK is activated differentially in peripheral organs following cardiac arrest and resuscitation. *Resuscitation* 66, 379–389
78 DeGracia, D.J. *et al.* (2006) Immunohistochemical mapping of total and phosphorylated eukaryotic initiation factor 4G in rat hippocampus following global brain ischemia and reperfusion. *Neuroscience* 139, 1235–1248
79 Murphy, G.J. *et al.* (2006) Exogenous control of mammalian gene expression via modulation of translational termination. *Nat. Med.* 12, 1093–1099
80 Mangiardi, D.A. *et al.* (2004) Progression of hair cell ejection and molecular markers of apoptosis in the avian cochlea following gentamicin treatment. *J. Comp. Neurol.* 475, 1–18
81 Scheu, S. *et al.* (2006) Activation of the integrated stress response during T helper cell differentiation. *Nat. Immunol.* 7, 644–651
82 Wickens, M. *et al.* (2002) A PUF family portrait: 3′UTR regulation as a way of life. *Trends Genet.* 18, 150–157
83 Kim, Y.K. *et al.* (2007) Staufen1 regulates diverse classes of mammalian transcripts. *EMBO J.* 26, 2670–2681
84 Gallois-Montbrun, S. *et al.* (2007) Antiviral protein APOBEC3G localizes to ribonucleoprotein complexes found in P bodies and stress granules. *J. Virol.* 81, 2165–2178
85 Balzer, E. and Moss, E.G. (2007) Localization of the developmental timing regulator Lin28 to mRNP complexes, P-bodies and stress granules. *RNA Biol.* 4, 16–25
86 Lotan, R. *et al.* (2005) The RNA polymerase II subunit Rpb4p mediates decay of a specific class of mRNAs. *Genes Dev.* 19, 3004–3016
87 Yang, W.H. and Bloch, D.B. (2007) Probing the mRNA processing body using protein macroarrays and "autoantigenomics". *RNA* 13, 704–712
88 Dunand-Sauthier, I. *et al.* (2002) Sum1, a component of the fission yeast eIF3 translation initiation complex, is rapidly relocalized during environmental stress and interacts with components of the 26S proteasome. *Mol. Biol. Cell* 13, 1626–1640
89 Zhou, C. *et al.* (2005) PCI proteins eIF3e and eIF3m define distinct translation initiation factor 3 complexes. *BMC Biol.* 3, 14