Lesson 14

Mechanisms of control of mRNA processing & degradation in neural cells: P-bodies and stress granules

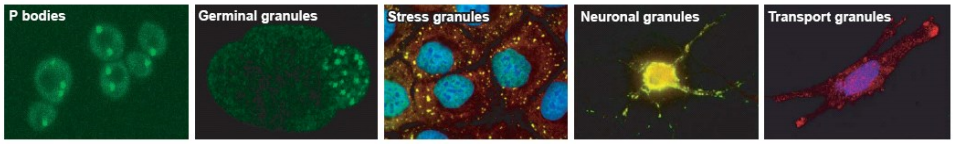
The different mRNA granules

Polysomes - translating mRNA

Stress Granules - mRNA stopped in translation initiation
(= "lost in translation")

P-bodies – mRNA for degradation + translation repression
(= "a place to die, a place to sleep")

mRNP granules (byproducts of mRNA metabolism)



S.cerevisiae (yeast,mammals) | *C.elegans* - germ cells (also Drosophila, amphibians) | human cell culture (mammals) | rat hippocampal neurons (mammal neurons) | chicken fibroblast

(Wickens, Science 2003)

RNA granules could be ancient

RNA granules may provide an early form of epigenetic regulation

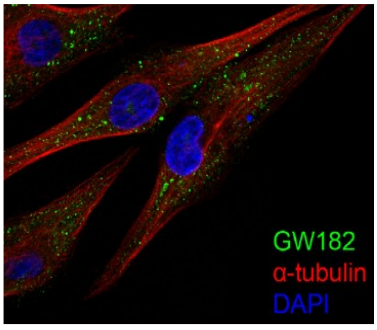
Sequester mRNAs until they reached a certain cytoplasmic region. (transport, neuronal granules)

Control which mRNAs are expressed under severe stress (SG)

Cytoplasmic heredity in germ cells (germinal granules)

What are P-bodies?

human autoimmune serum produced a unique cytoplasmic discrete speckled staining pattern on human HeLa cells (Eystathioy et al., 2002)



- mammalian processing (P) bodies

also known as:

- GW bodies (glycine- and tryptophan-rich cytoplasmic bodies;
- or Dcp-containing bodies

J.J. Moser, M.J. Fritzler / The International Journal of Biochemistry & Cell Biology 42 (2010) 828–843

Autoantibodies against PB correlated with autoimmune diseases

GW182/TNRC6A,
GW2/TNRC6B,
GW3/TNRC6C,
Ge-1/Hedls/RCD8,
LSm1-7,
Ago2/EIF2C2,
RAP55/LSm14A,
diacyl-phosphatidylethanolamine

Idiopathic ataxia,
Motor and sensory neuropathy,
Sjögren Syndrome,
Sistemic Lupus Erythematosus,
Rheumatoid arthritis
Primary biliary cirrhosis

What are P-bodies?

cytoplasmic foci: aggregates of translationally repressed mRNPs

Translation repression & RNA interference (RNAi) or RNA silencing pathways, non-sense 5'→3' degradation, mRNA transport and stabilization

conserved core proteins:

- **mRNA decapping machinery**
- **deadenylase complex**

} **general repression / decay machinery**

additional proteins:

species/condition specific:

- **mi/siRNA repression factors (RISC)**
- **RNA binding prot-s + translation repressors**
- **nonsense-mediated decay (NMD) proteins**

= degradation of improperly processed mRNA (premature stop-codons)

C.J. Decker and R. Parker

DEGRADATION OF EUKARYOTIC mRNA

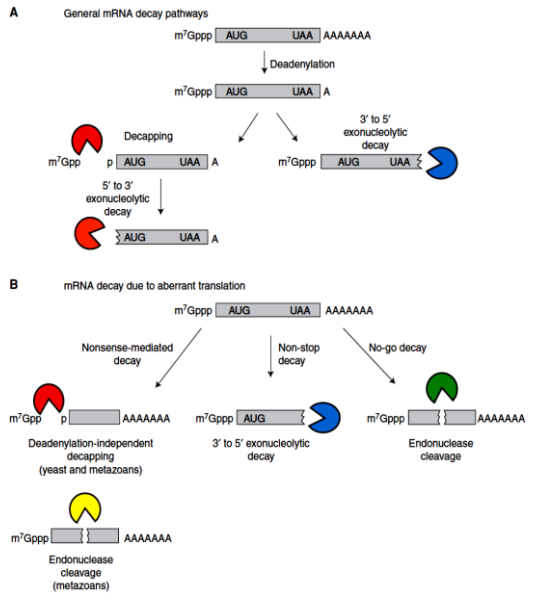
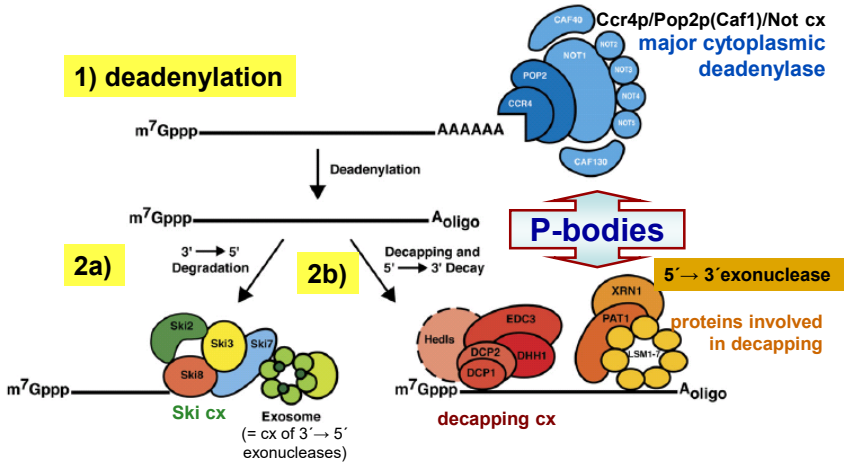


Figure 1. Eukaryotic mRNA decay pathways. (*A*) General mRNA decay pathways. (*B*) Specialized decay pathways that degrade translationally aberrant mRNAs.

DEGRADATION OF EUKARYOTIC mRNA

deadenylation-dependent pathways



3´→5´ degradation

predominant in yeast

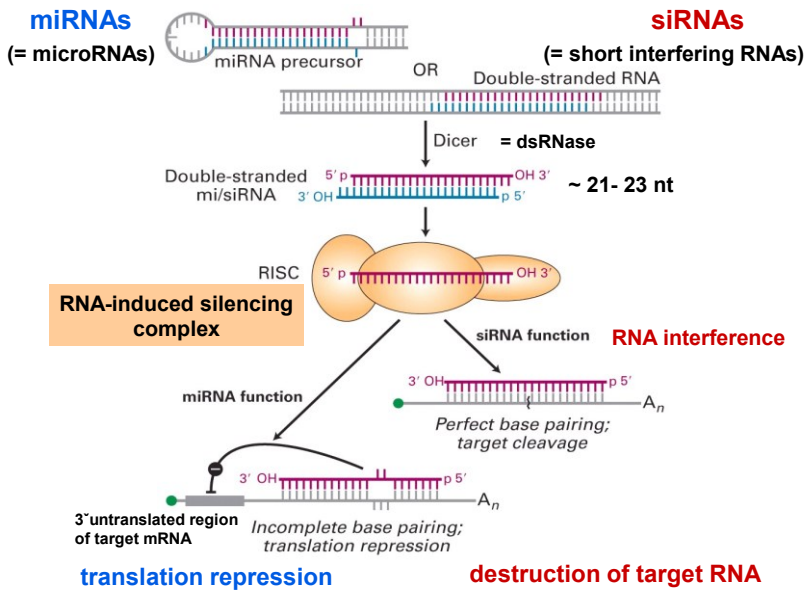
decapping + 5´→ 3´ decay

predominant in mammals

http://www.ibiology.org/ibioseminars/genetics-gene-regulation/roy-parker-part-1.html

(adapted from Parker&Sheth, MolCell 2007)

GENE SILENCING



(Lodish et al.,5th ed., adapted from Hutvágner& Zamore 2002)

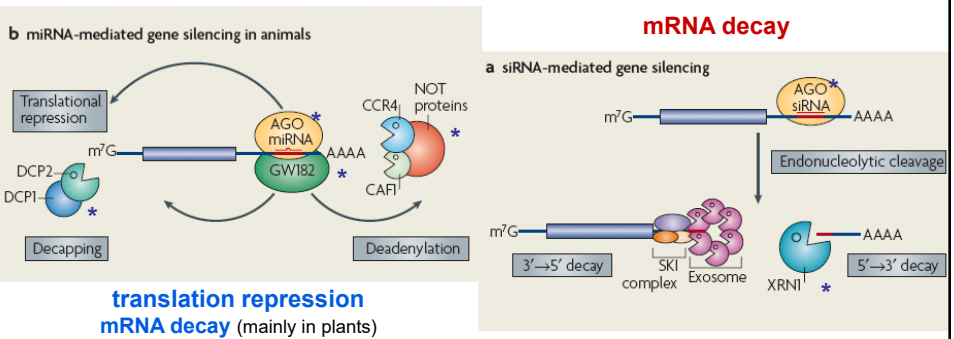
GENE SILENCING & P-bodies

miRNAs

may target mRNAs into P-bodies

siRNAs

mRNA decay



translation repression

mRNA decay (mainly in plants)

*** = P-body components**

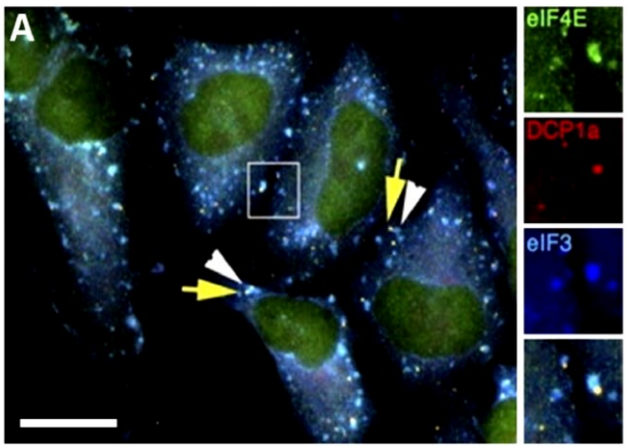
AGO* = Argonaute proteins – essential components of RISC

characterictic domains: PAZ & PIWI (similar to RNase-H domain)

(Eulalio, Nat Rev Mol Cell Biol 2007)

What is a Stress Granule?

J.J. Moser, M.J. Fritzler / The International Journal of Biochemistry & Cell Biology 42 (2010) 828–843



Stress Granules are non-membranous mRNPs (Found in Yeast,Protozoa,Metazoa,Plants)

Range in size from 0.1 to 2.0 µm (Virus= 100 nm, Bacteria=1.5 µm, Eucaryotic Cell 15-60 µm

Stress Granule

100–2000 nm non-membranous transient cytoplasmic bodies induced upon environmental stress

>**response to arrest of translation initiation** induced by treatment with drugs (sodium arsenite, cycloheximide), heat shock, osmotic shock, oxygen deprivation, glucose deprivation, UV irradiation or viral infection

contain aggregates of mRNA + translation initiation factors

48S preinitiation cx: eIF4 subunits, 40S ribosomal subunits, poly(A)binding protein 1 (PABP-1)

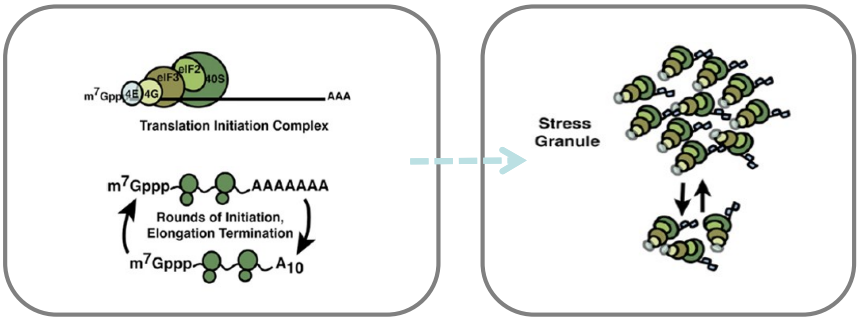
RNA binding proteins with self-interaction domains
(TIA proteins)

often associated with P-bodies

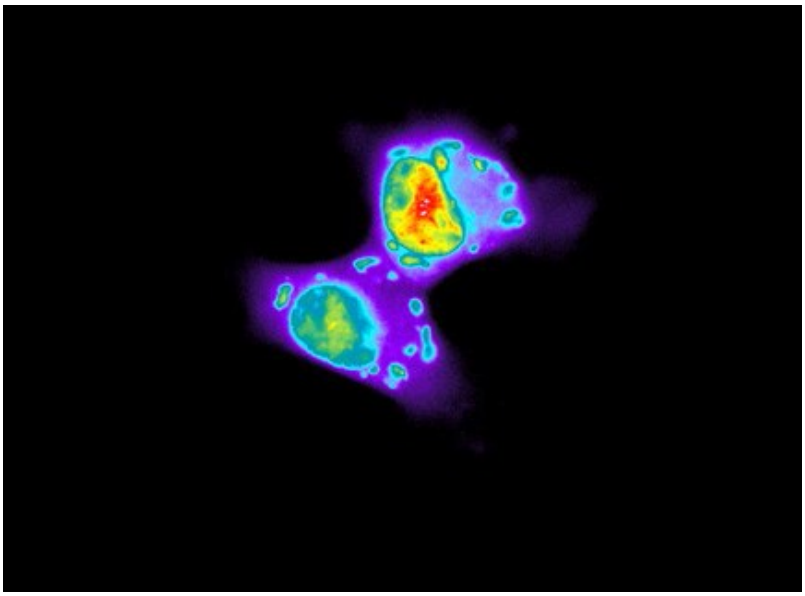
? mRNA moving between the compartments

Stress Granule Formation

- eIF2alpha phosphorylation induces the formation of Stress Granules
- Thare are many eIF2alpha kinases that can phosphorylate in response to various stresses



Stress Granule Formation

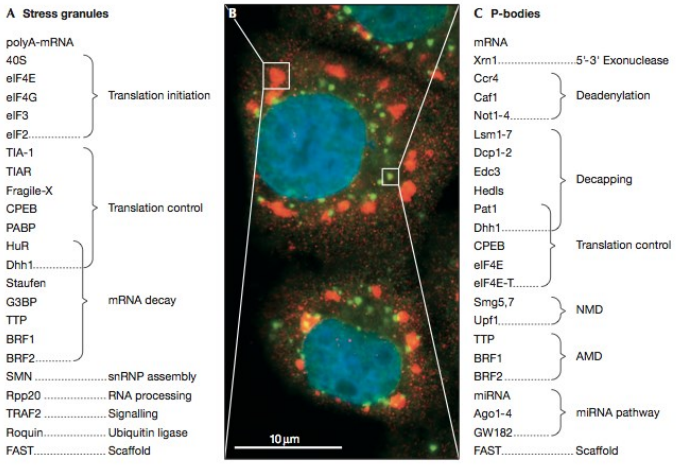


Kedersha et al. 2000 JCB

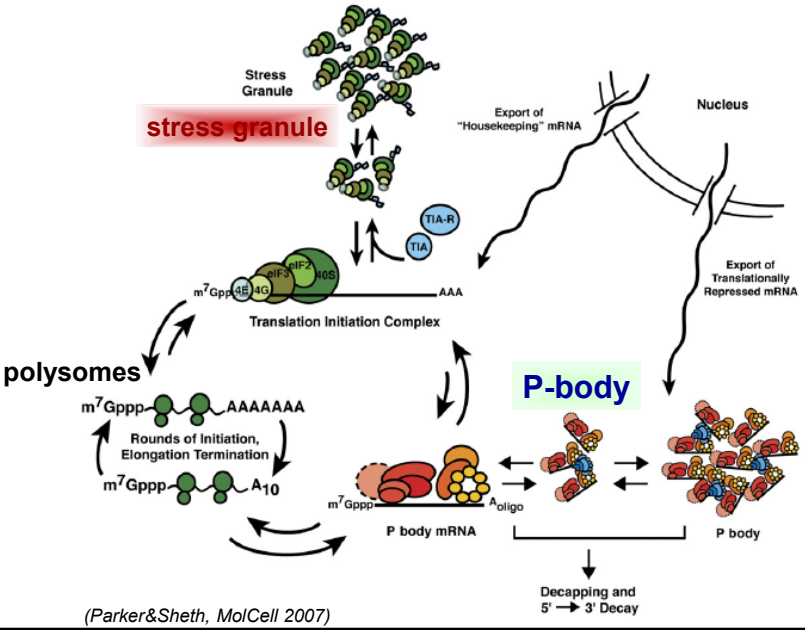
Distinguishing between SG and PB

•Composition. There are lots of shared components but many are unique to either the Stress Granule or the P-body.

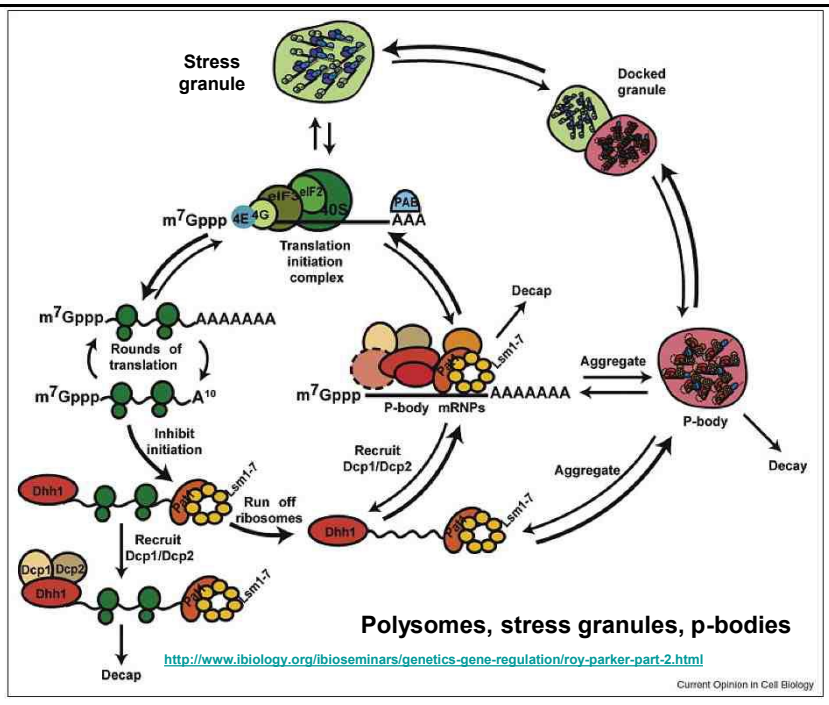
•SG markers include PABP, eIF3, 4A, and 4G whereas PB markers include DCP2, XRN4 etc.



CYCLING OF EUKARYOTIC mRNA



(Parker&Sheth, MolCell 2007)



Molecular Cell
Review

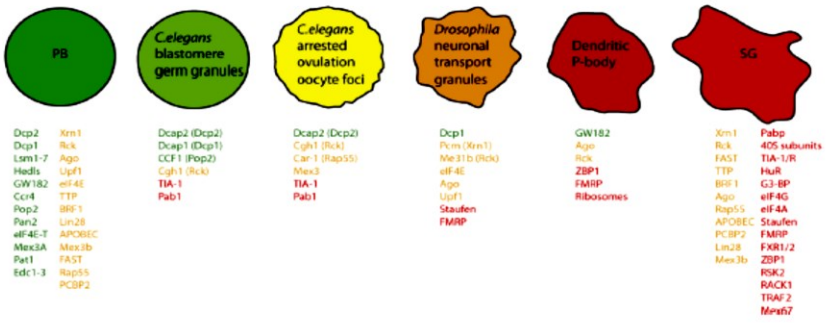
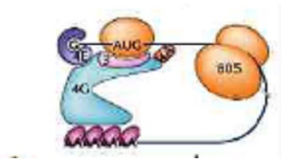


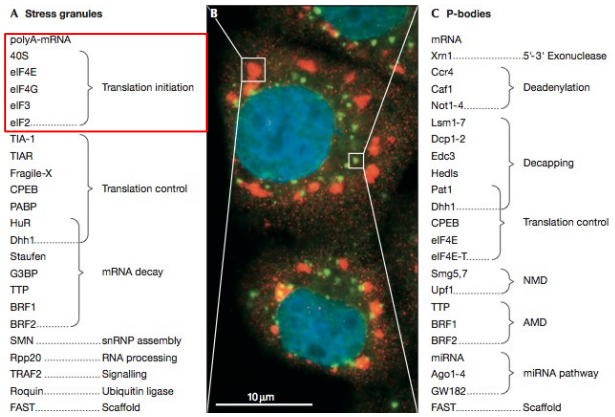
Figure 1. A Continuum of mRNP Granules
Select examples of mRNP granules with compositional similarities to both stress granules and P-bodies: *C. elegans* blastomere germ granules (Gallo et al., 2008), *C. elegans*-arrested ovulation oocyte foci (Jud et al., 2008), *Drosophila* neuronal transport granules (Barbee et al., 2006), dendritic P-body (Cougot et al., 2008). Components observed solely in stress granules are highlighted in red, those solely in P-bodies in green, and those seen in both foci in yellow. Lists are not exhaustive, and with specific experimental manipulation, some P-body/stress granule "distinct" components have been observed in both structures.

1. Stalled initiation complexes

mRNA transcripts
eIF3
eIF4F (eIF4E, eIF4A and eIF4G)
eIF4B
small ribosomal subunits
PABP-1



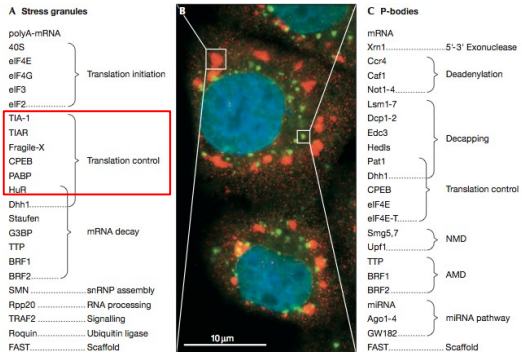
Polysome



2. mRNA binding proteins linked to translational silencing (mRNA stability)

A. Translational silencing members; TIA-1, TIAR, FMRP, FAST, Argonaute, CPEB (cytoplasmic polyadenylation element binding protein), FXR1, Lsm14

TIA-1-(T cell internal antigen 1) -binds 3' UTR (ARE) of COX-2 (Cyclooxygenase-2) conversion of free arachidonic acid to prostaglandins
-Translational silencer(Dixon et al., 2003)
-Nucleates SGs
-Interacts with other proteins (FAST, SRC3, PMR1,FBP)



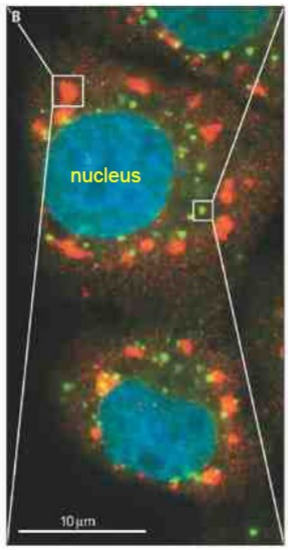
Stress granules

polyA-mRNA
40S
eIF4E
eIF4G
eIF3
eIF2
— Translation initiation

TIA-1
TIAR
Fragile-X
CPEB
PABP
HuR
Dhh1
— Translation control

Staufen
G3BH
TTP
BRF1
BRF2
— mRNA decay

SMN — snRNP assembly
Rpp20 — RNA processing
TRAF2 — Signaling
Roquin — Ubiquitin ligase
FAST — Scaffold



EMBO reports 7:143

P-bodies

mRNA
Xrn1 — 5'-3' Exonuclease
Ccr4
Caf1
Not1-4
— Deadenylation

Lsm1-7
Dcp1-2
Edc3
Hedls
Pat1
— Decapping

Dhh1
CPEB
eIF4E
eIF4E-T
— Translational control

Smg5,7
Upf1
— Nonsense mediated Decay (NMD)

TTP
BRF1
BRF2
— ARE mediated Decay (AMD)

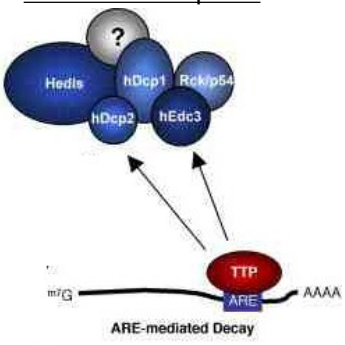
miRNA
Ago1-4
GW182
— miRNA pathway

FAST — Scaffold

B. RNA decay-associated SG components;

–Argonaute proteins, TTP, BRF1, PMR1, RNA helicase RCK

TTP- Tris-tetraprolin



Some of them in polysomes;
FXRP1, RCK, PMR1, Argonaute

TIA-1 and TIAR- constitutively active translational silencers

Present in both SG and PB

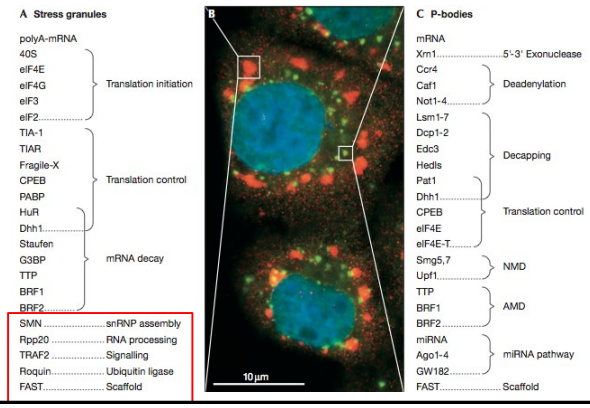
3. RNA binding proteins that regulate aspects of RNA metabolism

(splicing, RNA editing and RNA localization)
SMN, FAST, transposon ORF1, Rpp20, LINE1, IP5K

SMN – assembly of snRNPs

-Survival of Motor Neurons protein (product of the spinal muscular atrophy-determining gene

-Part of a large protein complex that functions in the assembly of spliceosomal snRNPs (ATP dependent) (Pellizzoni et al., 2002).



FAST – fas-activated serine-threonine phosphoprotein

-Survival protein that is tethered – mitochondrial membrane
-Environmental conditions – FAST moves to SG
-A regulator of alternative splicing
eg. Promotes the inclusion of FGFR2 (Fibroblast growth factor receptor -2) exon IIIb (recruited by IAS1- a U-rich intronic splicing enhancer) (Simarro et al., 2007)
- Acts as scaffolding protein – bind to TIA-1 and nucleates both SG and PBs (associated with both SG & PB)

<u>Rpp20- RNA processing</u>

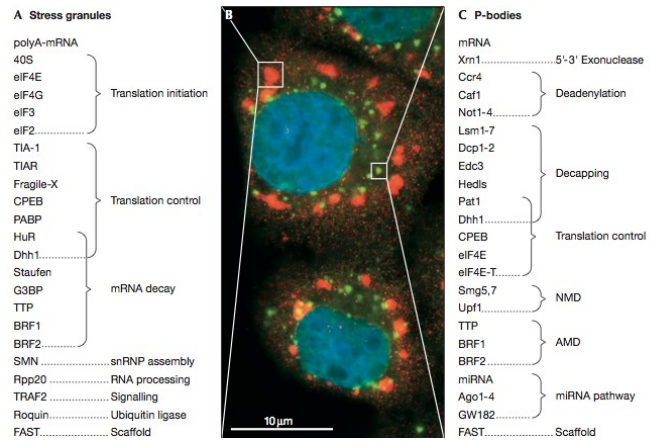
-Subunit of t-RNA processing enzyme ribonulease P (RNaseP)
-Ribonuclease P required for the processing of 5' of precursor t-RNA
(Jarrous et al., 1999).

4. Newly discovered SG components;

recruited to SG by interacting with core SG components ("piggyback" interactions)
eg. FAST, FBP/KSRP, PMR1 all bind to TIA-1

5. 'Orphan' class of SG associated proteins

eg. TRAF binds to eIF4G; DIS1 binds to eIF3



Their mRNA composition - selective

-contain transcripts encoding housekeeping genes
-Endogenous cellular mRNAs encoding glyceradehyde-3-phospate dehydrogenase (GAPDH), β-actin, IGF2 (Insuline-like growth factor II

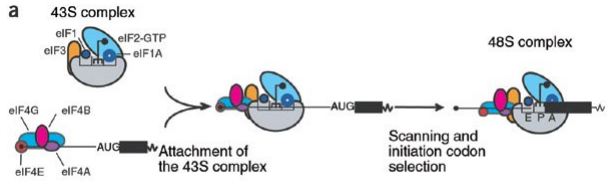
-exclude those encoding stress-induced genes
- Heat shock protein 70 and 90 (*HSP70, HSP90*)
(Kedersha and Anderson, 2009)

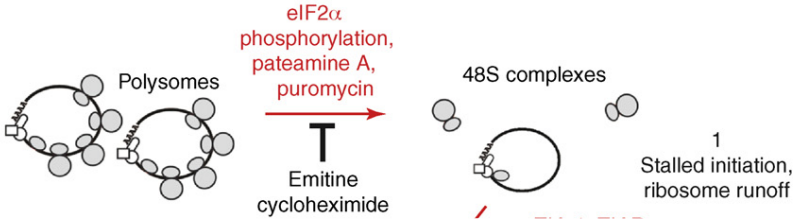
SGs are selective and turnover quickly

- Under stress only some mRNAs are found in SGs.
- Only ~25% of transcripts can be found in SGs
- Of note, under heat stress, the transcripts for HSPs are excluded from SG assembly.
- SG markers vary. Some are always with the SG, others under only some conditions.
- SG components have a short half life. Seconds to Minutes. (mRNA Triage and sorting?)

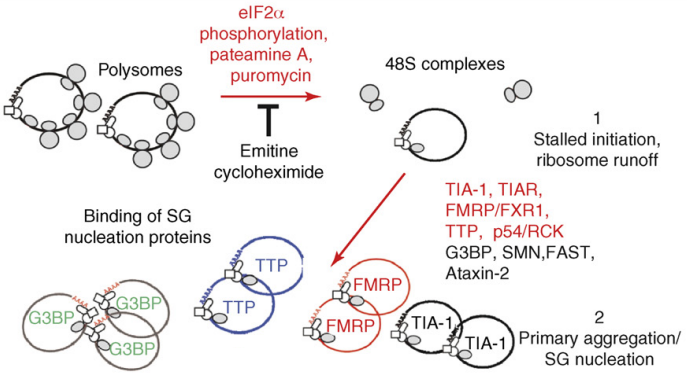
Stages of SG assembly

Stage 1: Stalled initiation and ribosome runoff



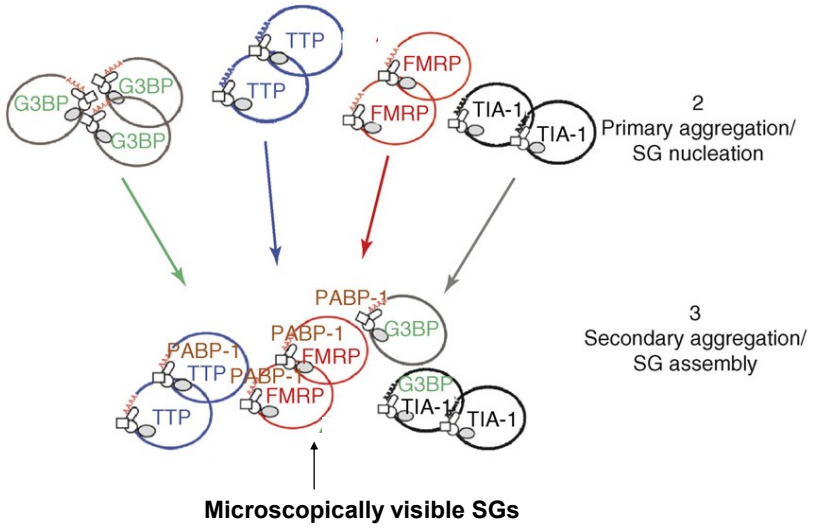


Stage 2: Primary aggregation/SG nucleation



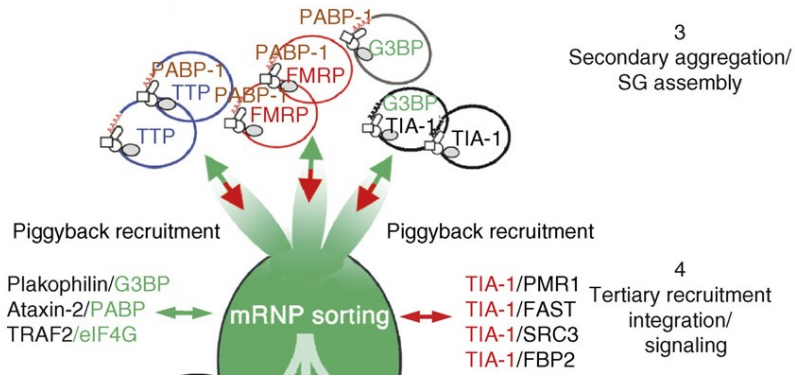
Stress granule nucleators: they induce SGs when over-expressed;
they become part of the SGs they nucleate;
their ability to induce SGs requires nonpolysomal 48S mRNPs

Stage 3: Secondary aggregation

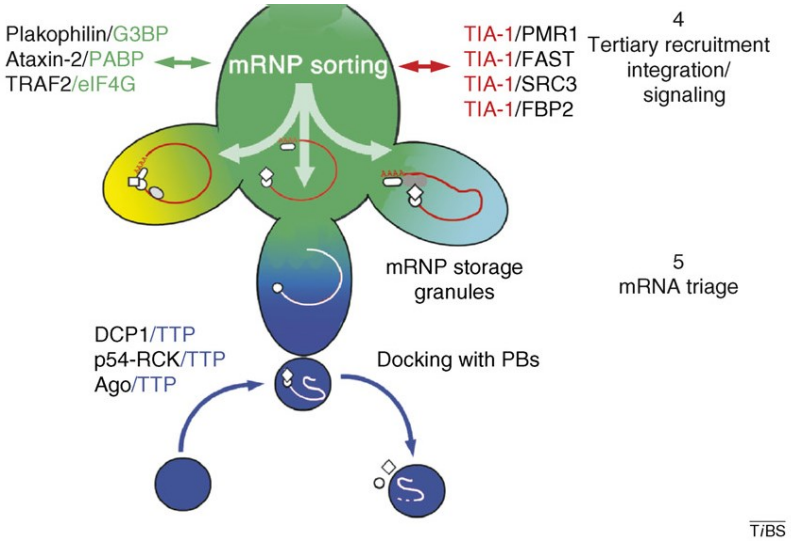


Microscopically visible SGs

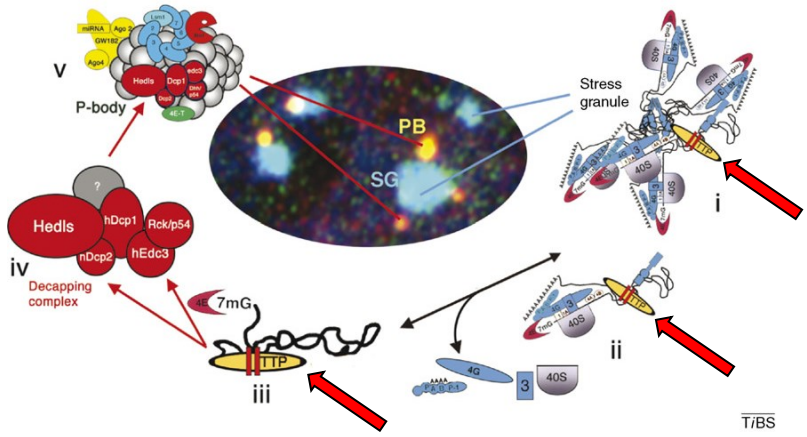
Stage 4: Integration and signaling



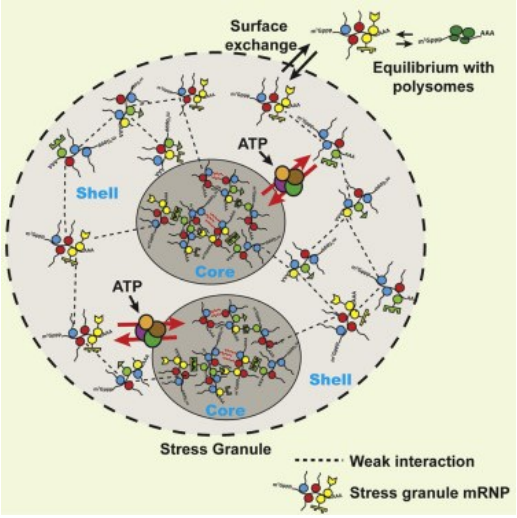
Stage 5: mRNA triage



TTP dynamically links SGs with PBs during mRNA triage

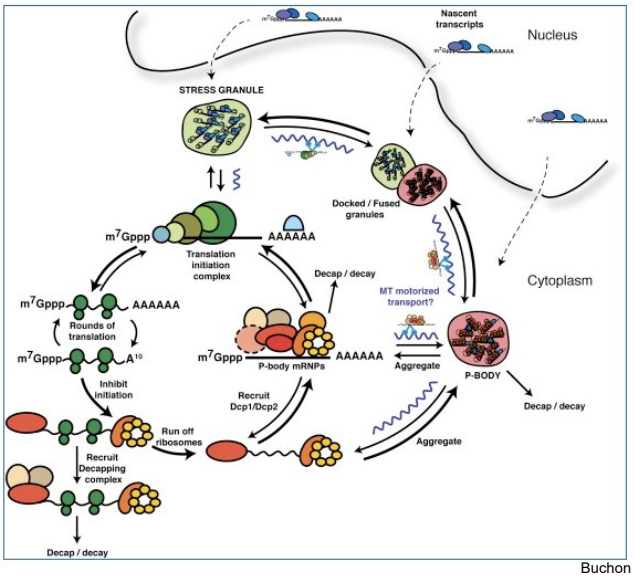


ATP modulates structure and protein content of Stress Granules and its equilibrium with polysomes



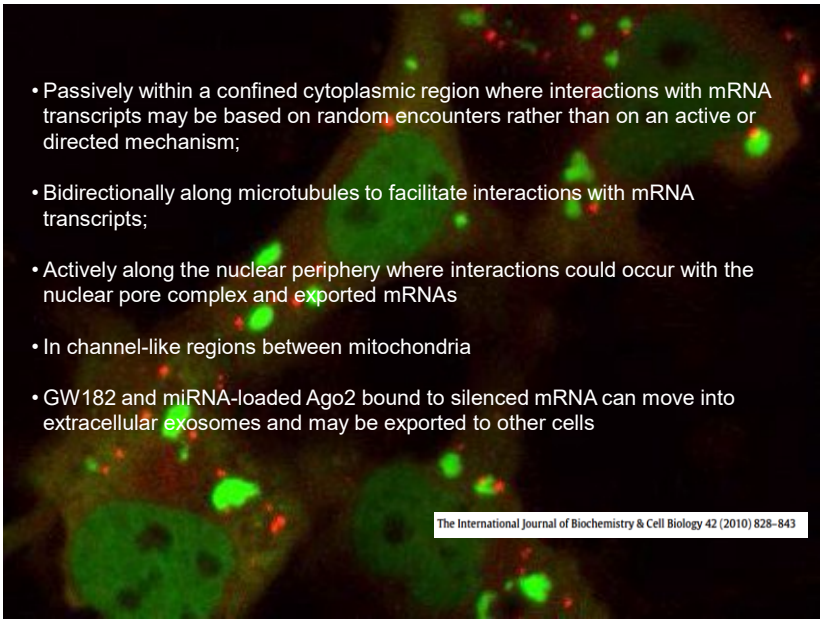
Jain et al., Cell 2016

Cellular transport ofSG and PB



Buchon & Parker (2010) Mol Cell

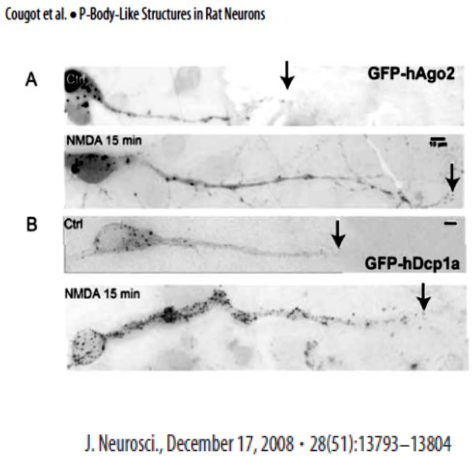
P-bodies move dynamically in a number of ways

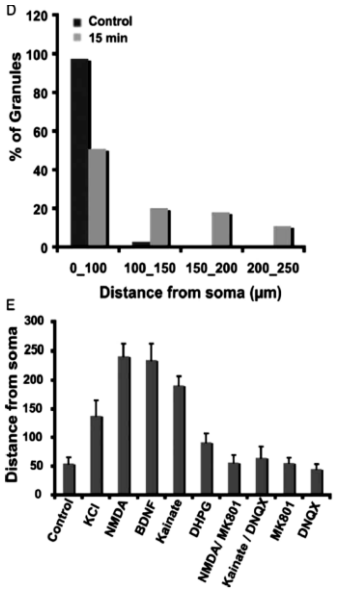


- Passively within a confined cytoplasmic region where interactions with mRNA transcripts may be based on random encounters rather than on an active or directed mechanism;
- Bidirectionally along microtubules to facilitate interactions with mRNA transcripts;
- Actively along the nuclear periphery where interactions could occur with the nuclear pore complex and exported mRNAs
- In channel-like regions between mitochondria
- GW182 and miRNA-loaded Ago2 bound to silenced mRNA can move into extracellular exosomes and may be exported to other cells

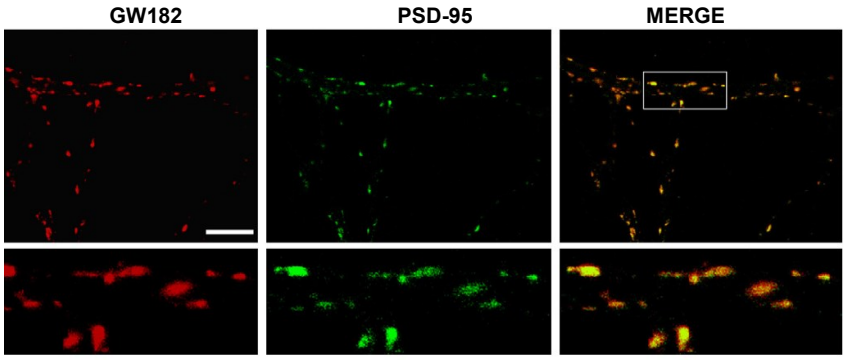
The International Journal of Biochemistry & Cell Biology 42 (2010) 828–843

P-bodies move dynamically in stimulated neurons





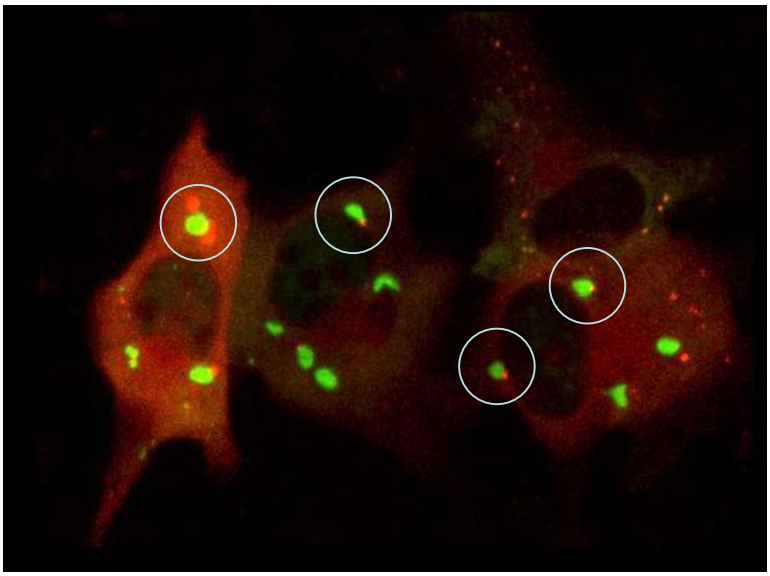
P-bodies are located within Post-Synaptic densities



The International Journal of Biochemistry & Cell Biology 42 (2010) 828–843

High stability of neuronal P-bodies suggest that unlike P-bodies in other cell types, they represent stable structures, consistent with the idea that they are involved in mRNP storage rather than degradation

SG component echange (PB and SG docking)



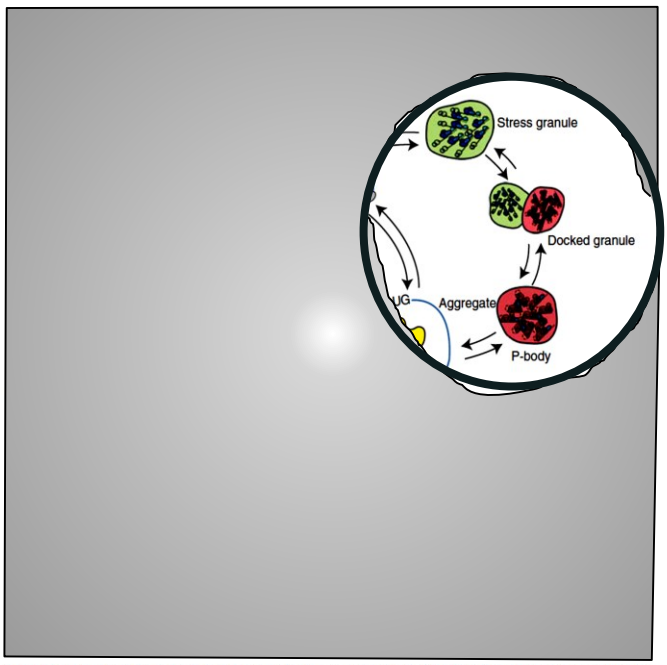


Figure 3. Model of the "mRNA cycle." Showing the dynamic movement of mRNA between polysomes, P-bodies, and stress granules, and the possible mRNP transitions between the different states of the mRNA.

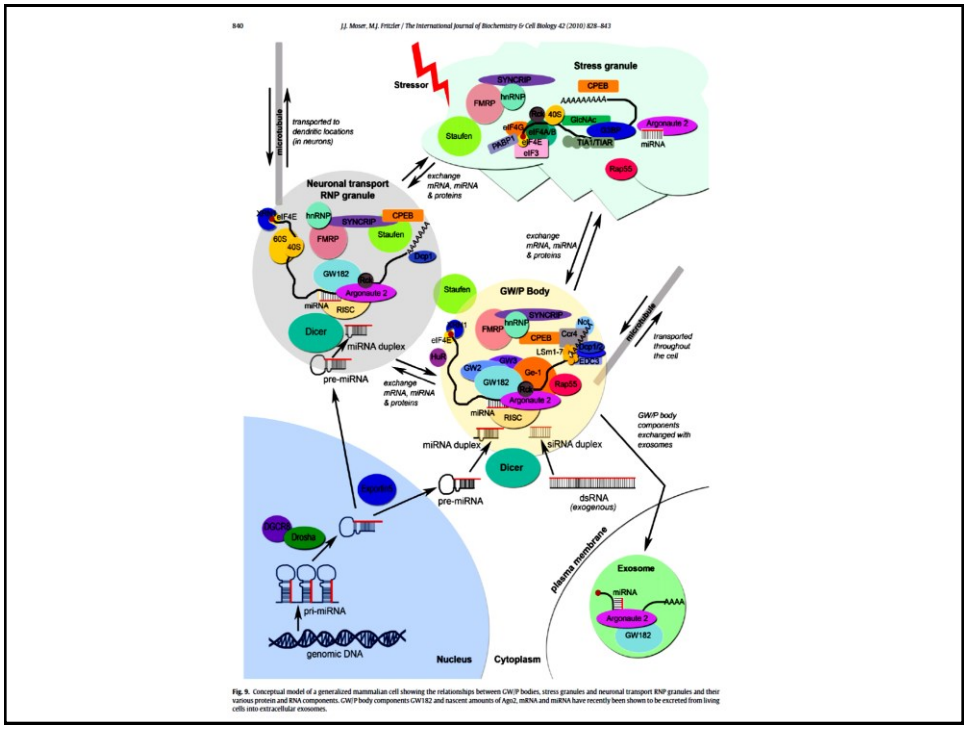
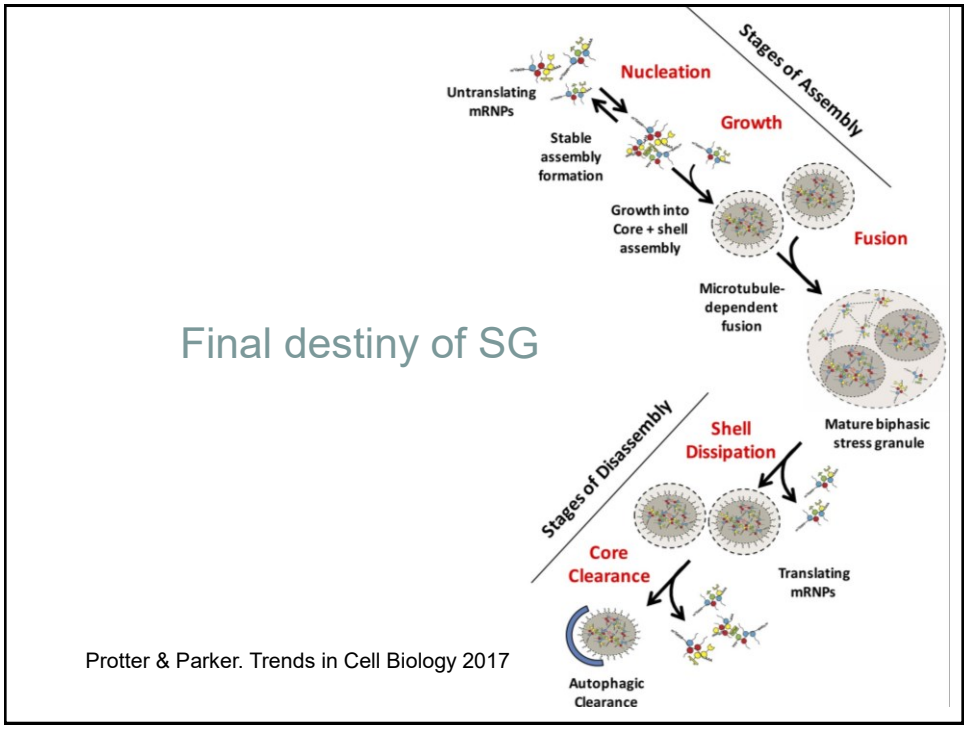
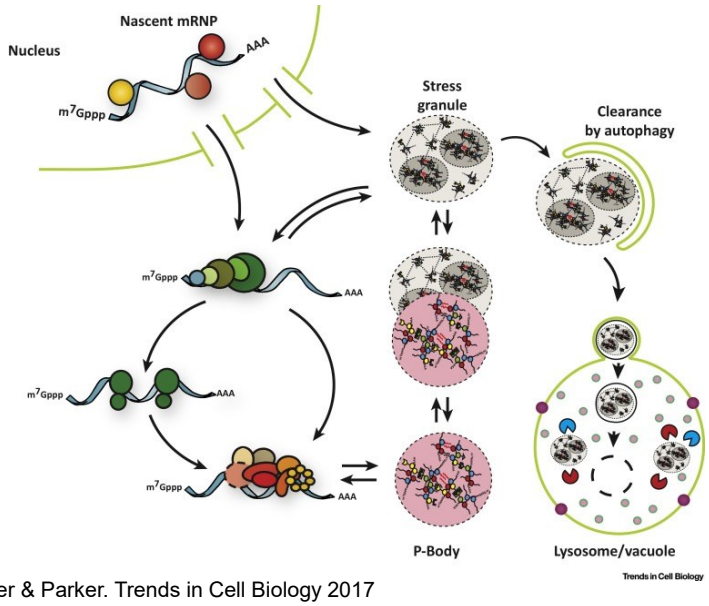


Fig. 9. Conceptual model of a generalized mammalian cell showing the relationships between GW/P bodies, stress granules and neuronal transport RNP granules and their various protein and RNA components. GW/P body components GW182 and nascent amounts of Ago2, miRNA and miRNA have recently been shown to be excreted from living cells into extracellular exosomes.



Final destiny of SG

Protter & Parker. Trends in Cell Biology 2017



Protter & Parker. Trends in Cell Biology 2017

Sequestration of TRAF2 into stress granules interrupts tumor necrosis factor signaling under stress conditions (Mol. Cel. Biol. 2005, 2450-2462)