

Fates of mRNAs and proteins under abiotic stress

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Plant Stress Seminar, January 27, 2009

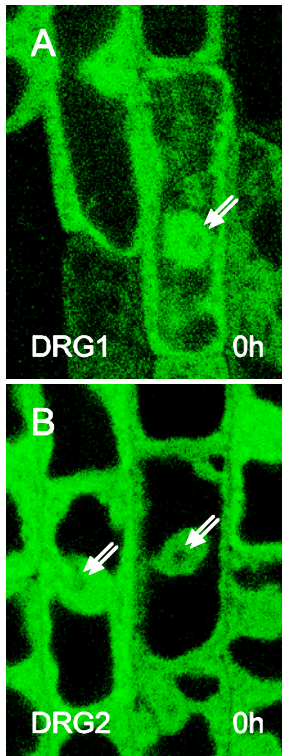
C. Weber, L. Novar and M. Fauth (2008)

Plant stress granules and mRNA processing
bodies are distinct from heat stress granules

The Plant Journal 56:517-530

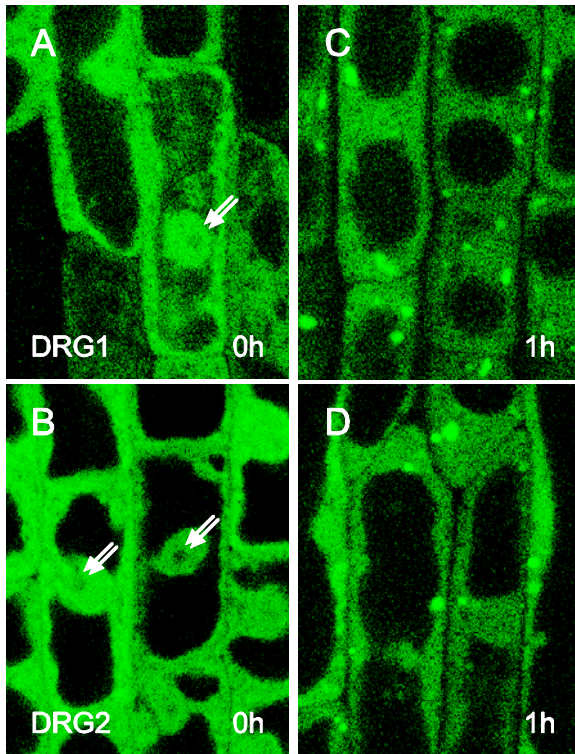
DRG1-GFP and DRG2-GFP localization

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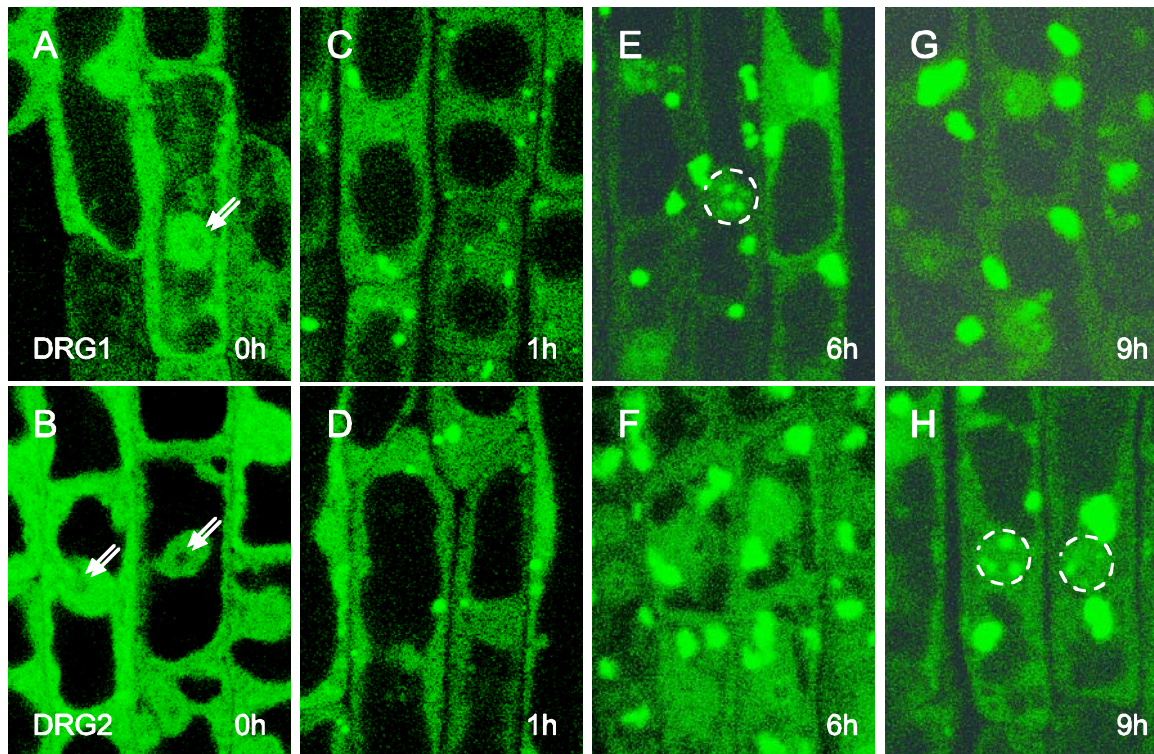
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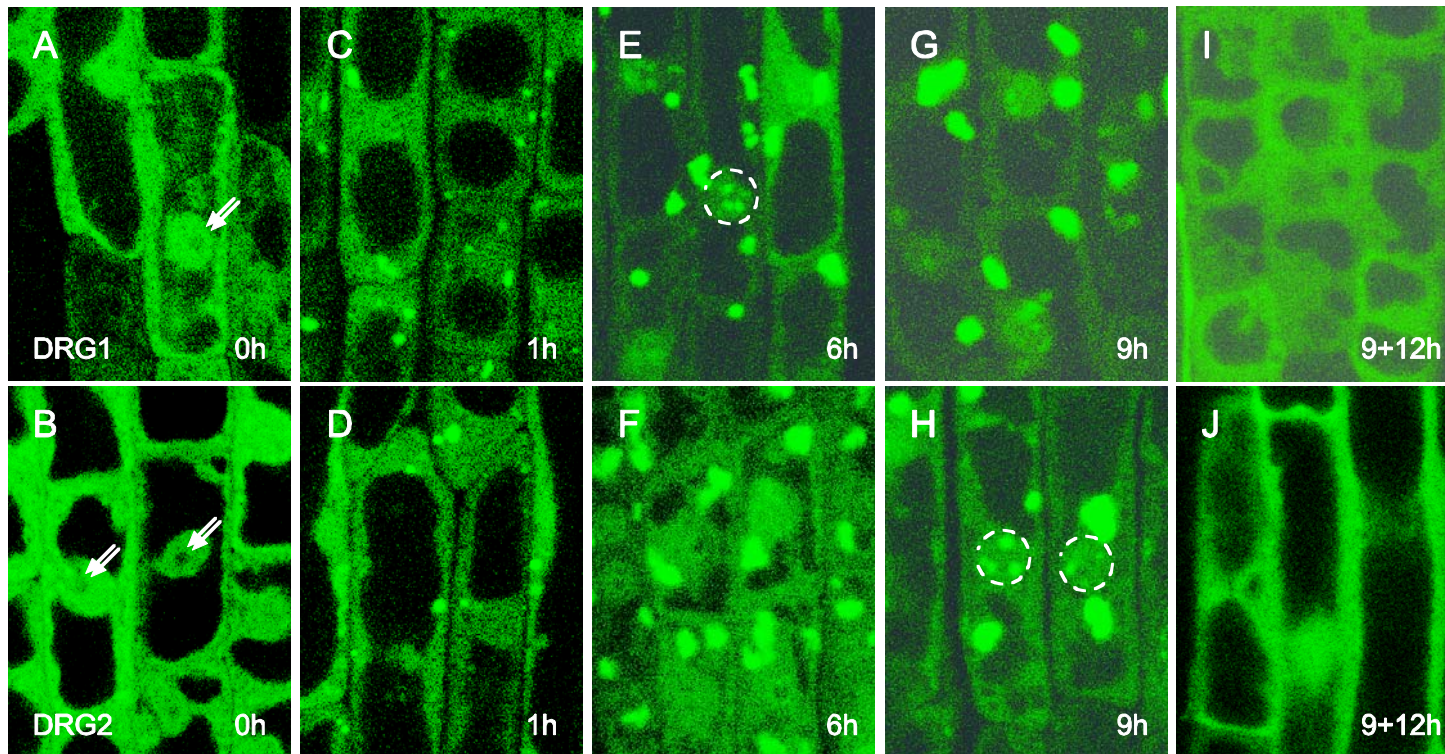
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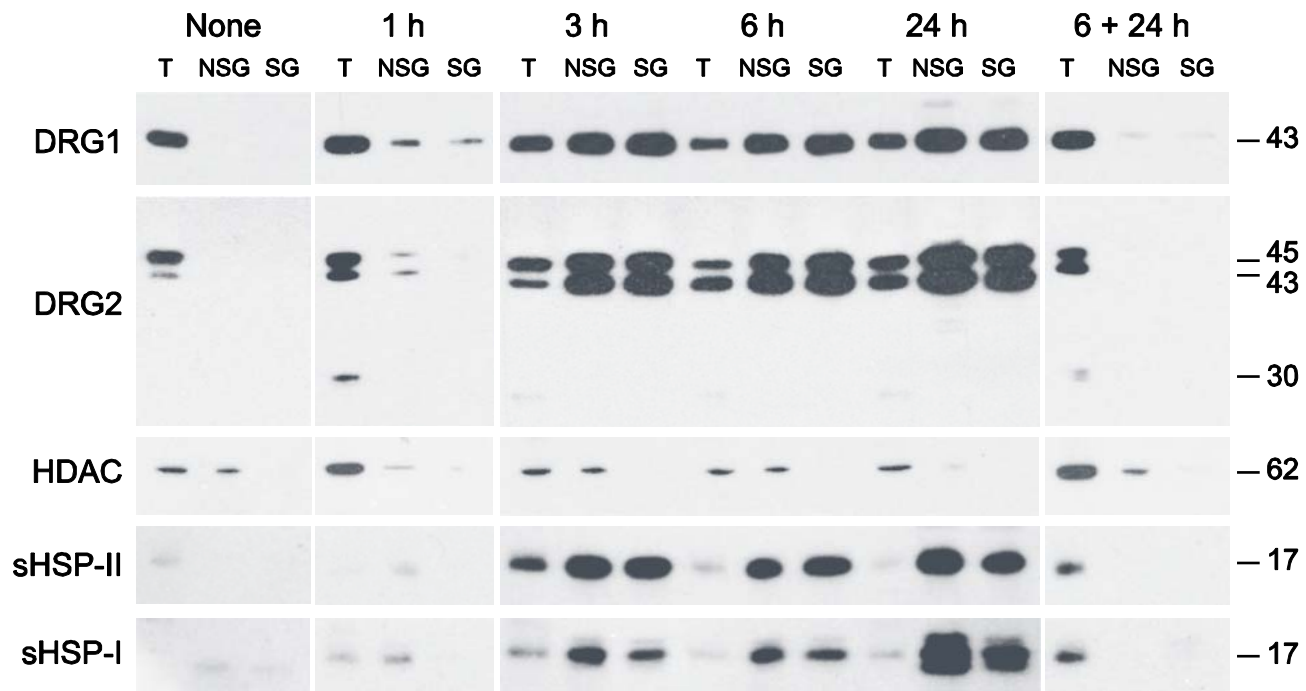
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- 4) Granules disperse during recovery from heat stress (also rather slowly).

What are these granules?



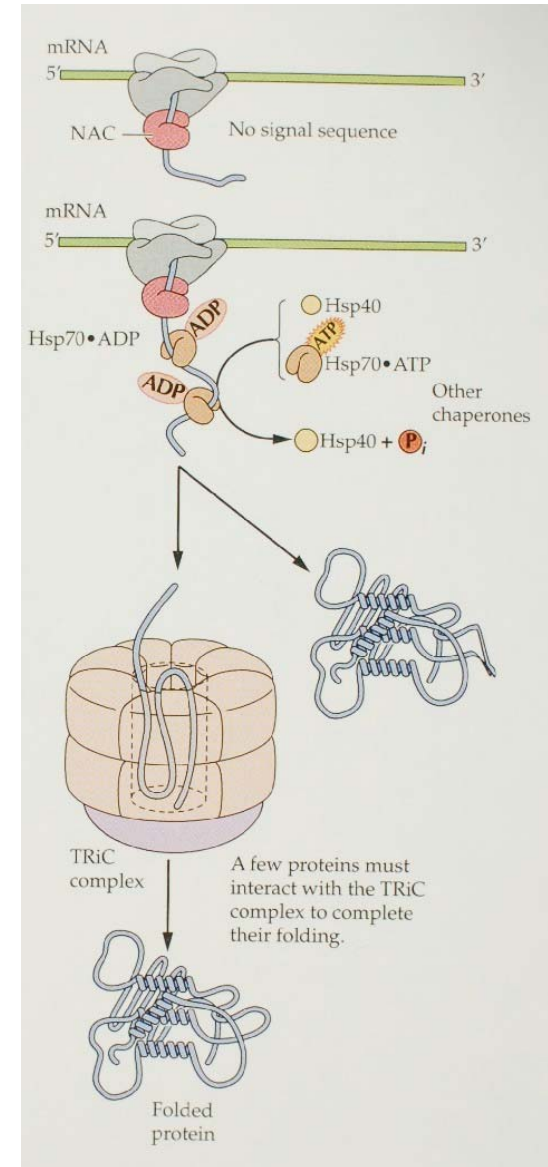
Analysis of cell fractions after heat stress.

Fractions: **T**, Total; **NSG**, nuclei + granules; **SG**, granules only
DRG1 and DRG2 co-purify in SGs with two sHSPs (class I and class II).
sHSPs are known components of HSGs, Heat Stress Granules



Chaperones, HSPs and protein folding

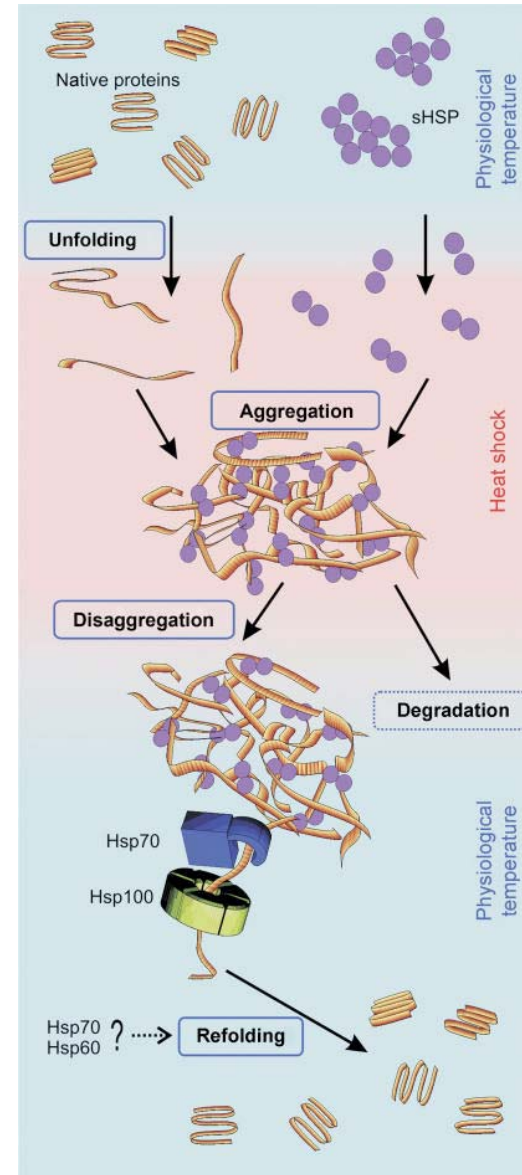
- “foldases”, “holdases” and “disaggregases”
- Major families (all are highly conserved):
 - HSP100
 - HSP90
 - HSP70/HSP40 (co-translational; many proteins)
 - HSP60/chaperonin (post-translational; some proteins)
 - sHSP (small HSPs)
- Most are ATPases; essential for normal protein folding
- Versions in cytosol, ER, mitochondria, and chloroplasts



Some HSPs are induced and all are up-regulated by heat and other stresses

Major families:

- HSP100s
- HSP90s
- HSP70s
- HSP60s/chaperonins
- **sHSPs (small HSPs)**

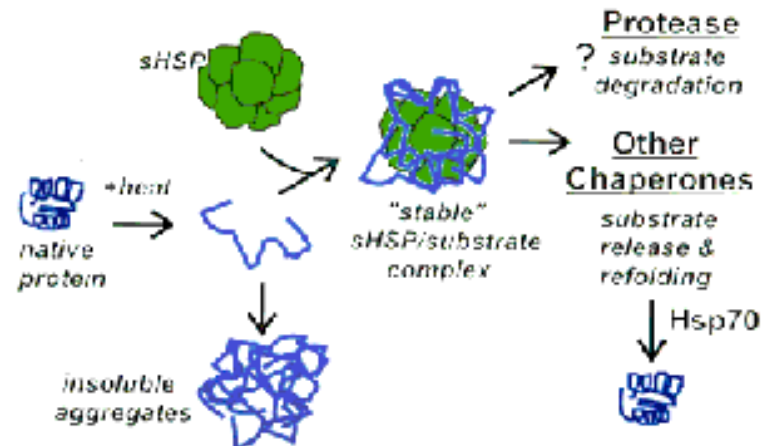
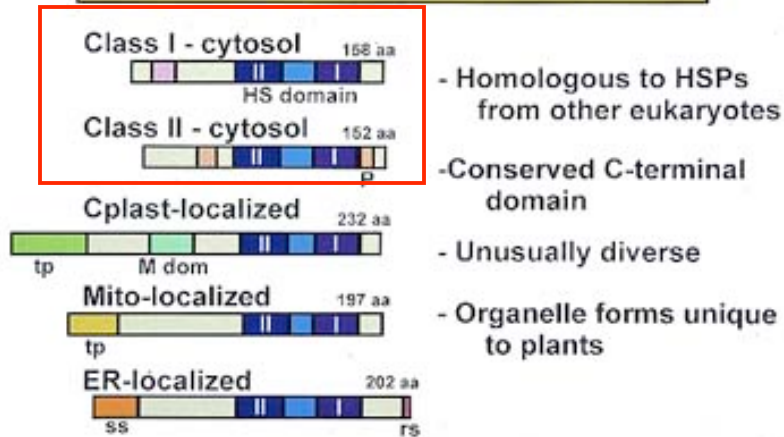


Graphics stolen from...



University of Arizona

Plant Small HSP Gene Families



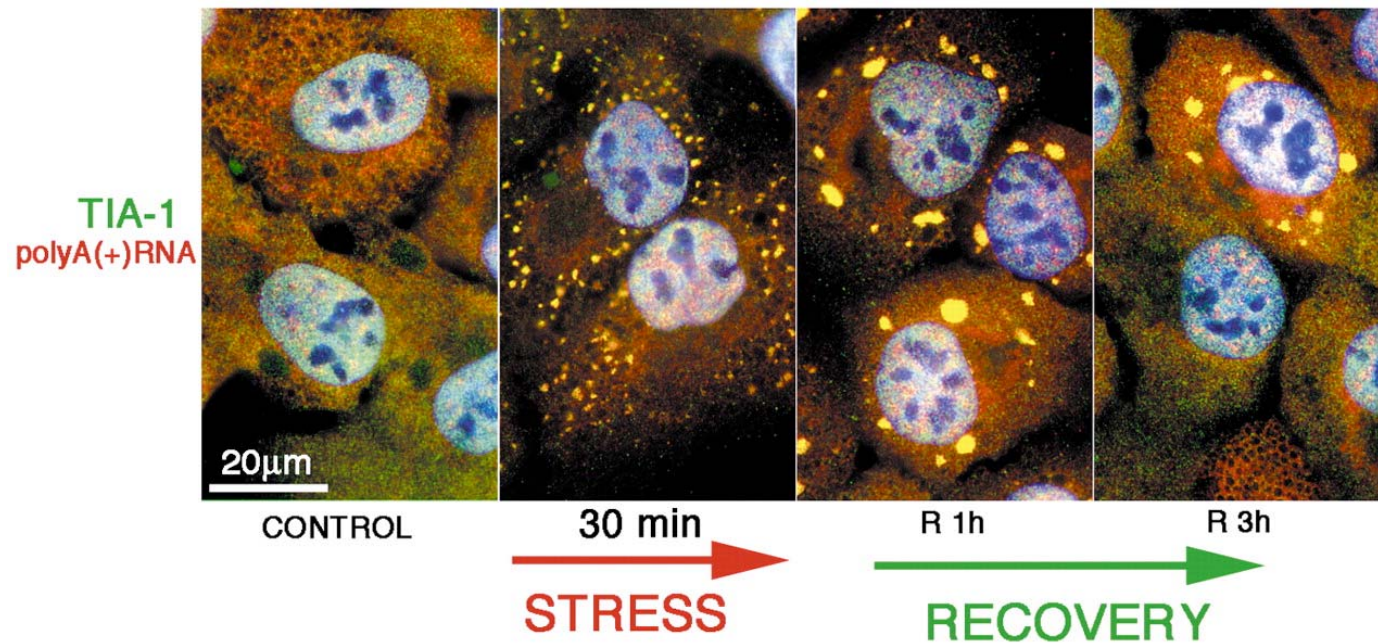
Chaperones facilitate a range of processes including:

- 1) protein folding
- 2) transport of proteins across membranes
- 3) modulation of protein activity
- 4) regulation of protein degradation
- 5) prevention of irreversible protein aggregation

RNPs: mRNA-protein particles

- Polysomes: actively translated messages
- Processing Bodies (**PBs**): mRNA degradation
- Stress Granules (**SGs**): 48S* pre-initiation complexes; storage of housekeeping mRNAs during stress?
- Heat Stress Granules (**HSGs**): plant-specific; HSP70, sHSPs; storage of storage of housekeeping mRNAs during stress???

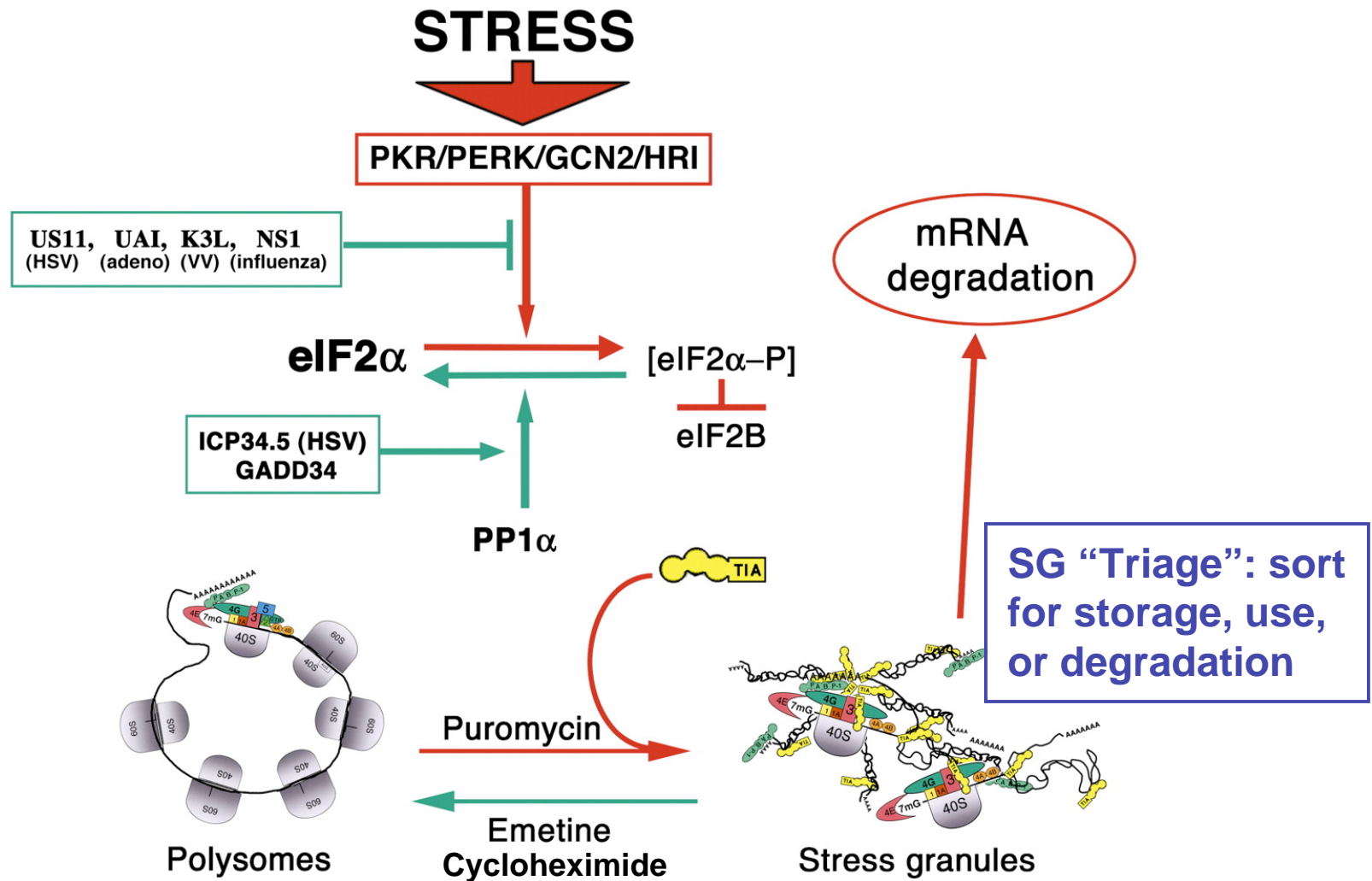
1. Assembly and disassembly of arsenite-induced stress granules



Anderson, P. et al. *J Cell Sci* 2002;115:3227-3234



3. The dynamic equilibrium between polysomes and stress granules is regulated by the availability of eIF2-GTP-tRNA^{Met} and TIA proteins



Anderson, P. et al. J Cell Sci 2002;115:3227-3234

And finally...Weber et al., 2008. Plant stress granules and mRNA processing bodies are distinct from heat stress granules

Concept: Loss of homeostatis (stress) → some mRNAs into PBs and SGs

Plant systems: tomato, tobacco and Arabidopsis protoplasts; Arabidopsis tissues

Techniques: transient expression of fluorescent-tagged proteins; BIFC; Ab localization of markers

RNP markers

- 1) PBs: DCP1 and DCP2: decapping
XRN4: exonuclease
- 2) SGs: eIF4E: initiation factor assoc. w/ capping
RBP47 and UBP1: TIA1 homologues; RRM domains (RNA binding);
prion-like domains (aggregation)
- 3) HSGs: sHSPs; do NOT contain A+RNA

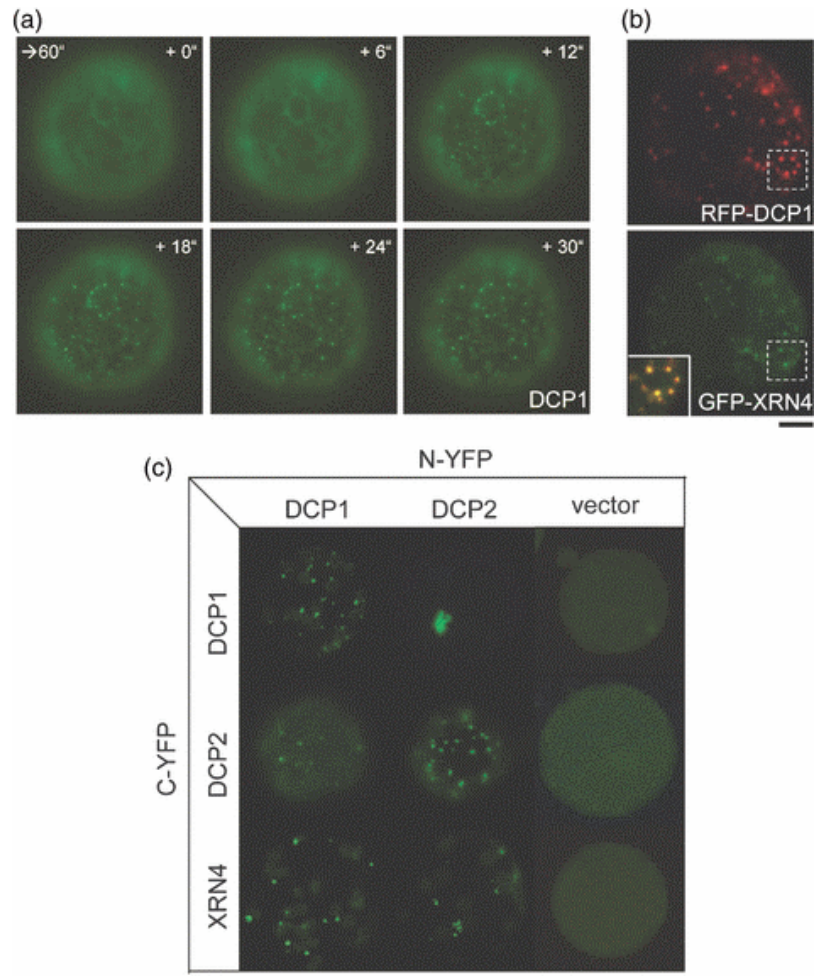


Fig. 1 Processing Bodies

PBs markers tagged with GFP, RFP or YFP; transient expression in tobacco mesophyll protoplasts

a) PBs form within seconds of adding coverslip → anoxia

b) DCP1 and XRN4 co-localize

c) BIFC (bimolecular fluorescence complementation) also indicates close association of 3 markers

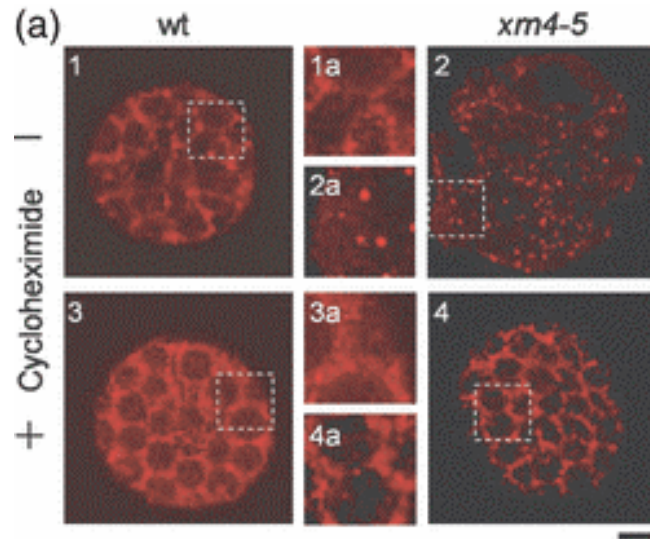


Figure 3a. DCP1 in Arabidopsis wild-type (wt) and *xrn4-5* plants (protoplasts)

- mutant has more PBs (mRNAs not degraded, therefore it accumulates)
- CHX arrests translation → less free mRNA → fewer PBs

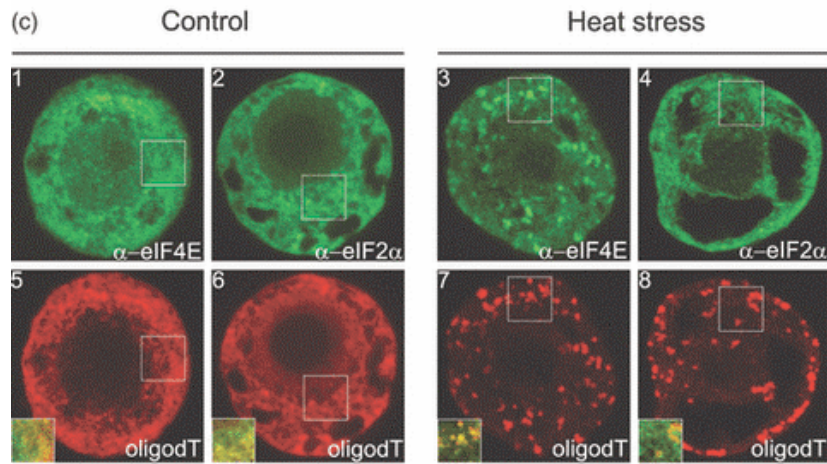
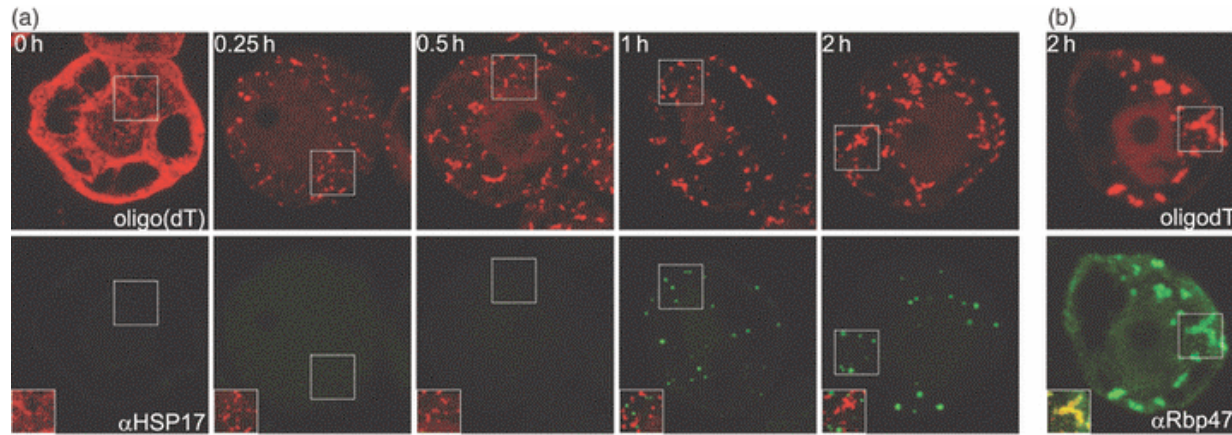


Fig. 4 PolyA+ mRNA, eIF4E and RB47 occur in granules of tomato cells that are NOT HSGs.

a) mRNA does NOT co-localize with sHSP17 (HSG marker)

b) mRNA DOES co-localize with Rpb47 (SG marker; no heat)

c) mRNA DOES co-localize with eIF4E diffusely before HS and in granules after HS. eIF2 α is NOT in SGs after HS.

Weber et al., 2008. Plant stress granules and mRNA processing bodies are distinct from heat stress granules

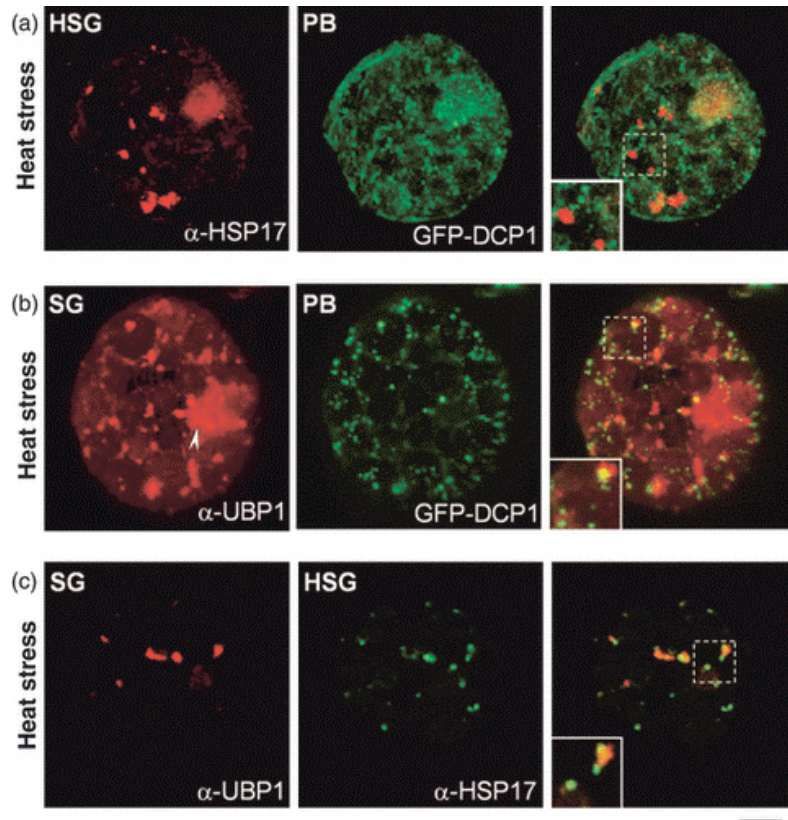


Fig. 7 Summary (tobacco protoplasts)

a) HSGs \neq PBs

b) SGs \neq PBs, but they may interact (exchange mRNAs?)

c) SGs \neq HSGs, but they may interact (exchange HSPs and client proteins?)

DRGs interact with HSPs and ribosomes: Are they chaperones?