

Supplemental Data

Eukaryotic Stress Granules:

The Ins and Out of Translation

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Table S1: Components of Stress Granules and P-bodies

Factor	Properties and Implicated function	Location	Organism	Ref
40S	Small subunit of ribosomes	Polysomes, SGs	Mammals, yeast	Kedersha et al, 2002; Grousl et al, 2009
Ago2	miRNA silencing; siRNA cleavage	Polysomes, SGs, PBs	Mammals	Leung et al, 2006
AKAP350	Cell signaling scaffold; centrosome/golgi associated	SGs	Mammals	Kolobova et al, 2009
APOBEC3 G	Antiviral factor	SGs, PBs	Mammals	Gallois-Montbrun et al, 2007
Ataxin-2/Pbp1	Ataxin-2: regulates SG/PB assembly; Implicated in Spinocerebellar ataxia 2 Pbp1: poly(A) tail processing; splicing; export; translational regulator; SG assembly	Polysomes, SGs, PBs (yeast)	Mammals, yeast	Nonhoff et al, 2007 Buchan et al, 2008
BRF1	mRNA decay	SGs, PBs	Mammals	Kedersha et al, 2005
Calreticulin	ER Chaperone; RNA binding protein	SGs	Mammals	Decca et al, 2007
Caprin1	Associated with cell cycle proliferation; RNA binding protein; Nucleates SGs	Polysomes, SGs	Mammals	Solomon et al, 2007;
CCAR1	Apoptosis regulator; DNA/RNA binding protein	SGs	Mammals	Kolobova et al, 2009
Ccr4	mRNA stability (Deadenylation)	PBs	Mammals, yeast	Sheth and Parker, 2003; Andrei et al, 2005
CIRP	Translational regulator; Nucleates SGs	SGs	Mammals	De Leeuw et al, 2007
CPEB	Translational regulator; Nucleates SGs	SGs, PBs	Mammals	Wilczynska et al, 2005
CUG-BP1	mRNA splicing; translational regulator; mRNA stability; Implicated in myotonic dystrophy	SGs	Mammals	Fujimura et al, 2007
Dcp1/Dcp1a	mRNA decay (decapping)	SGs, PBs	Mammals, yeast	Sheth et al, 2003; Cougot et al, 2004; Wilczynska et al, 2005

Dcp2/Dcp2	mRNA decay (decapping)	PBs	Mammals, yeast	Sheth et al, 2003; Cougot et al, 2004;
DDX1	RNA helicase; 3'end formation	SGs	Mammals	Ishiura et al, 2008
DDX3/Ded1	RNA helicase; translational regulator; mRNA export	Polysomes, SGs, PBs (yeast)	Mammals, yeast	Lai et al, 2008; Beckham et al, 2007
DIC1/DHC1	Dynein Intermediate chain/Dynein Heavy chain	SGs	Mammals	Loschi et al, 2009
DIS1	Implicated in Schizophrenia; Nucleates SGs	SGs	Mammals	Ogawa et al, 2005
Eap1	eIF4E binding protein	SGs, PBs	Yeast	Buchan et al, 2008
Ebs1	NMD factor	PBs	Yeast	Luke et al, 2007
Edc1-2	Decapping enhancer; translational regulator	PBs	Yeast	Neef and Thiele, 2009
Edc3	mRNA stability; PB assembly	PBs	Yeast	Kshirsagar and Parker, 2004
eIF2	Initiation factor; recruits initiator tRNA	Polysomes, SGs	Mammals	Kimbal et al, 2003
eIF2B	Guanine exchange factor for eIF2	Polysomes, SGs	Mammals	Kimbal et al, 2003
eIF3	Multi-subunit initiation factor	Polysomes, SGs, PBs (yeast)	Mammals, yeast	Kedersha et al, 2002; Grousl et al, 2009
eIF4A	RNA helicase; eIF4F initiation factor	Polysomes, SGs	Mammals	Kedersha et al, 2000
eIF4E	Cap binding protein; eIF4F initiation factor	Polysomes, SGs, PBs (yeast)	Mammals, yeast	Kedersha et al, 2002; Hoyle et al, 2007
eIF4E-T	eIF4E binding protein	PBs	Mammals	Andrei et al, 2005
eIF4G	eI4F scaffold; initiation factor	Polysomes, SGs, PBs (yeast)	Mammals, yeast	Kedersha et al, 2002; Hoyle et al, 2007
eRF1	Translation termination	Polysomes, PBs (yeast)	Mammals, yeast	Buchan et al, 2008
eRF3	Translation termination	Polysomes, PBs (yeast)	Mammals, yeast	Dori and Choder, 2007; Buchan et al, 2008
FAK	Kinase; phosphorylates Grb7	SGs	Mammals	Tsai et al, 2008
FAST	Kinase; splicing; anti-apoptotic regulator	SGs, PBs	Mammals	Kedersha et al, 2005
FBP/KSRP	mRNA decay	SGs	Mammals	Rothe et al, 2006
FMRP	Translational regulator; Nucleates SGs; Implicated in Fragile X syndrome	Polysomes, SGs	Mammals	Mazroui et al, 2002
FXR1P	Translational regulator	Polysomes, SGs	Mammals	Mazroui et al, 2002
FXR2P	Translational regulator	Polysomes, SGs	Mammals	Mazroui et al, 2002
G3BP	Ras signaling; SG assembly; endonuclease	SGs	Mammals	Tourriere et al, 2003

Gbp2	Export	SGs, PBs	Yeast	Buchan et al, 2008
Ge-1/Hedls	Decapping enhancer	PBs	Mammals	Yu et al, 2005
Grb7	Translational regulator; Nucleates SGs; SG disassembly role	SGs	Mammals	Tsai et al, 2008
GW182	miRNA silencing	PBs	Mammals	Eystathioy et al, 2003
hMex3A	Translation regulator	PBs	Mammals	Buchet-Poyau et al, 2007
hMex3B	Translation regulator	SGs, PBs	Mammals	Courchet et al, 2008
hnRNP A1	Splicing; export; translational regulator; mRNA stability	SGs	Mammals	Guil et al, 2006
hnRNP A3	Translational regulator; export	PBs	Mammals	Kaahira et al, 2008
hnRNP K	Chromatin remodeling; transcription; splicing; export; translational regulator; mRNA stability	SGs	Mammals	Fukuda et al, 2009
hnRNP Q	Splicing; export; translational regulator; mRNA stability	SGs, PBs	Mammals	Quaresma et al, 2009
Hrp1	3' end processing; export	SGs, PBs	Yeast	Buchan et al, 2008
Htt	Huntington protein; implicated in Huntingdon's disease	PBs	Mammals	Savas et al, 2008
Hsp27	Heat shock protein	SGs	Mammals	Kedersha et al, 1999
HuD	mRNA stability	SGs	Mammals	Burry and Smith, 2006
HuR	mRNA stability; splicing regulator; poly(A) site selection	Polysomes, SGs	Mammals	Gallouzi et al, 2000
IP5K	Generates InsP6	SGs	Mammals	Brehm et al, 2007
Importin-8	Importin	PBs, SGs	Mammals	Weinmann et al, 2009
KHC/KLC	Kinesin heavy chain/Kinesin Light Chain	SGs	Mammals	Loschi et al, 2009
Lin28	Translational regulator	Polysomes, SGs, PBs	Mammals	Balzer and Moss, 2007; Polesskaya et al, 2007
LINE1 ORF1p	Transposon protein	SGs	Mammals	Goodier et al, 2007
Lsm1	mRNA stability	PBs	Mammals, yeast	Sheth and Parker, 2003; Stoecklin et al, 2006
MBNL1	Splicing; mRNA stability; Implicated in myotonic dystrophy	SGs	Mammals	Ishiura et al, 2008
MEX67	Major mRNA export factor	SGs	Mammals	Lai et al, 2008
MLN51	Exon-Junction complex component (splicing)	SGs	Mammals	Baguet et al, 2007
Musashi	Translational regulator	SGs	Mammals	Kawahara et al, 2008
Nrp1	Putative RNA binding protein	SGs, PBs	Yeast	Buchan et al, 2008

NXF7	Cytoplasmic mRNA transport	Polysomes, SGs, PBs	Mammals	Katahira et al, 2008
p97/NAT1	eIF4G homolog; translational regulator	Polysomes, SGs	Mammals	Nousch et al, 2007
PABP/Pab1	Poly(A) binding protein; stimulates translation; mRNA stability	Polysomes, SGs, PBs (yeast)	Mammals, yeast	Kedersha et al, 1999; Hoyle et al, 2007
Pan2/3	mRNA stability (Deadenylation)	PBs	Mammals	Zheng et al, 2008
Pat1	Translational regulator; mRNA stability (decapping enhancer)	PBs	Mammals, yeast	Sheth and Parker, 2003; Scheller et al, 2007
PCBP2	Stimulates IRES translation	SGs, PBs	Mammals	Fujimura et al, 2008
Plakophilin 1/3	Cell-Cell adhesion	SGs	Mammals	Hoffman et al, 2006
PMR1	mRNA decay (endonuclease)	Polysomes, SGs, PBs	Mammals	Yang et al, 2006
Pop2/Caf1	mRNA stability (Deadenylation)	PBs	Mammals, yeast	Teixeira and Parker, 2005; Zheng et al, 2008
Prohibitin 2	Mitochondrial apoptosis regulator	SGs	Mammals	Ohn et al, 2008
PRTB	Translational regulator; Nucleates SGs	SGs	Mammals	Kim et al, 2008
Pum1	Cytoplasmic RNA binding protein; Ortholog of pumilio (<i>Drosophila</i>) and Puf (yeast) proteins	SGs	Mammals	Morris et al, 2008
Pum2	Translational regulator; nucleates SGs	SGs	Mammals	Vessey et al, 2006
RACK1	Cell signaling scaffold protein; apoptotic regulator	Polysomes, SGs	Mammals	Arimoto et al, 2008
RBM42	Nuclear mRNA binding protein	SGs	Mammals	Fukuda et al, 2009
Rap55/Scd6	PB assembly role (mammals)	SGs, PBs	Mammals, yeast	Yang et al, 2006; Barbee et al, 2006
RCK/Dhh1	RNA helicase; Translational regulator	Polysomes, SGs, PBs	Mammals, yeast	Wilczynska et al, 2005; Sheth et al, 2003
RHAU	RNA helicase; mRNA stability	SGs	Mammals	Chalupnikova et al, 2008
Roquin	mRNA stability	SGs, PBs	Mammals	Vinuesa et al; 2005; Yu et al, 2007
Rpm2	RNAse P subunit; transcriptional regulator	PBs	Yeast	Stribinskis and Ramos, 2007
RSK2	Ribosomal S6 kinase; anti-apoptotic regulator	Polysomes, SGs	Mammals	Eisinger- Mathason et al, 2008
Sam68	Translational regulator	SGs	Mammals	Henano-Mejia and He, 2009
SERBP1	mRNA stability	SGs	Mammals	Goulet et al, 2008
SGNP	Putative rRNA processing factor	SGs	Mammals	Zhu et al, 2008

Smaug 1	Translational regulator	SGs	Mammals	Baez and Boccaccio, 2005
Staufen	Translational regulator	Polysomes, SGs	Mammals	Thomas et al, 2005
SMN	snRNP assembly	Polysomes (?), SGs, U-bodies	Mammals	Bechade et al, 1999; Hua and Zhou, 2004; Liu and Gall, 2007
TDP-43	DNA/RNA binding; splicing regulator; mRNA transport	SGs	Mammals	Colombrita et al, 2009
TDRD3	Tudor/Ub-binding protein	Polysomes, SGs	Mammals	Goullet et al, 2008
TIA-1/Pub1	TIA-1: Translational regulator; nucleates SGs; SG assembly factor; splicing regulator Pub1: mRNA stability; SG assembly	SGs, PBs (yeast)	Mammals, yeast	Kedersha et al, 1999; Buchan et al, 2008
TIA-R/Ngr1	TIA-R Translational regulator; nucleates SGs; splicing regulator Ngr1: mRNA stability	SGs, PBs (yeast)	Mammals, yeast	Kedersha et al, 1999; Buchan et al, 2008
TNRC6B	miRNA repression	PBs	Mammals	Mesiter et al, 2005;
TRAF2	TNF α signaling	SGs	Mammals	Kim et al, 2005
TTP	mRNA decay	SGs, PBs	Mammals	Stoecklin et al, 2004 Kedersha et al, 2005
Upf1	RNA helicase; NMD factor	PBs	Mammals, Yeast	Sheth and Parker, 2006; Durand et al, 2007
Upf2	NMD factor	PBs	Mammals, Yeast	Sheth and Parker, 2006; Stalder and Muhlemann, 2009
Upf3	NMD factor	PBs	Mammals, Yeast	Sheth and Parker, 2006; Stalder and Muhlemann, 2009
Vts1	mRNA stability	PBs	Yeast	Rendel et al, 2008
Xrn1	5'-3' exoribonuclease	SGs, PBs	Mammals, yeast	Kedersha et al, 2005
YB-1	RNA/DNA binding protein; transcriptional regulator; splicing; translational regulator; mRNA stability	Polysomes, SGs	Mammals	Yang and Bloch, 2007 Ishiura et al, 2008
Ygr250c	Putative RNA binding protein	SGs, PBs	Yeast	Buchan et al, 2008
ZBP1	Translational repressor; mRNA stability	SGs	Mammals	Stohr et al, 2006

Table S2: Calculated exchange rates of granule components via FRAP

Factor	Granule	Granule inducing condition*	Organism	Relative Recovery rate**	Category ***	Immobile fraction****	Reference
β -gal MS2-GFP mRNA	SG	Arsenite	Human Cell lines (HeLa)	63% recovery within 30s - Estimated 1min max residency time	Fast	? (Close to a full recovery)	Mollet et al, 2008
Ago2	PB	Overexpression	Hypothalamic rat neurons (Soma and dendrites)	25% recovery within 5 mins	Very slow	80%	Cougot et al, 2008
Ago2	SG	Hippuristanol	Human Cell lines (HeLa)	55% recovery within 30s	Fast	No, full recovery	Leung and Sharp, 2006
Ago2	PB	Hippuristanol	Human Cell lines (HeLa)	15% recovery within 1 min	Slow	50%	Leung and Sharp, 2006
CPEB	SG	Overexpression	Human Cell lines (HeLa)	50% recovery within 30s	Medium	No, full recovery	Mollet et al, 2008
CPEB	SG	Arsenite	Human Cell lines (HeLa)	40% recovery within 30s	Medium	50%	Mollet et al, 2008
CUGBP1	SG	Arsenite	Human Cell lines (HeLa)	50% recovery within 60s	Medium	?	Fujimura et al, 2007
Dcp1a	PB	Overexpression	Human Cell lines (COS)	62% recovery within 30s; note slower exchange from big PBs, faster from small	Fast	? (close to full recovery)	Kedersha et al, 2005
Dcp1a	PB	Overexpression / + nocodazole / + puromycin	Human Cell lines (U2OS)	70% recovery to within 20s	Fast	? (close to a full recovery)	Aizer et al, 2008
Dcp1a	PB	Overexpression	Hypothalamic rat neurons (Soma and dendrites)	6% recovery after 5 mins	Very slow	90%	Cougot et al, 2008
Dcp1a	PB	Overexpression + synaptic stimulation	Hypothalamic rat neurons	45% recovery after 30s	Medium	?	Cougot et al, 2008
Dcp1b	PB	Overexpression / + nocodazole / + puromycin	Human Cell lines (U2OS)	50% recovery after 30s	Medium	25%	Aizer et al, 2008
Dcp2	PB	Overexpression / + nocodazole / + puromycin	Human Cell lines (U2OS)	No recovery within 50s	Near static	≤100%	Aizer et al, 2008
eIF4E	PB	Overexpression	Human Cell lines (HeLa)	Recovery of 50% in 90s	Medium	30%	Andrei et al, 2005
eIF4E-T	PB	Overexpression	Human Cell lines (HeLa)	Recovery of 25% in 90s	Slow	75%	Andrei et al, 2005
FAST	SG	Arsenite	Human Cell lines (COS)	12.5% recovery within 30s	Slow	?	Kedersha et al, 2005
G3BP	SG	Arsenite	Human Cell lines (COS)	100% recovery within 30s	Fast	No, full recovery	Kedersha et al, 2005
GW182	PB	Overexpression	Human Cell lines (COS)	18% recovery within 30s	Slow	?	Kedersha et al, 2005
hnRNP A1	SG	Arsenite	Human cell lines (HeLa)	67% recovery within 24s	Fast	? (close to full recovery)	Guil et al, 2006
Lsm6	PB	Overexpression	Human Cell lines (HeLa)	Recovery of 75% in 150s	Medium	20%	Andrei et al, 2005
PABP	SG	Arsenite	Human Cell lines (COS)	45% recovery within 30s	Medium	?	Kedersha et al, 2005
PABP	SG	Arsenite	Human cell lines (HeLa)	15% recovery within 80s	Slow	?	Guil et al, 2006
PCBP2	SG and PB	Arsenite	Human Cell lines (HeLa)	65% recovery in 30s	Fast	20%	Fujimura et al, 2008
RSK2	SG	Serum starved	Human Cell lines (MCF-7)	No recovery over 10mins	Near static	≤100%	Eisinger-Mathason et al, 2008
RSK2	SG	Serum starved + mitogen	Human Cell lines (MCF-7)	60% recovery within 20s	Fast	?	Eisinger-Mathason et al, 2008
Staufen	Neuronal granule	Overexpresson	<i>Drosophila</i> motor neurons	30% recovery within 10mins	Very slow	?	Barbee et al, 2006
TIA-1	SG	Arsenite	Human Cell lines (COS)	100% within 22-30s; 90% within 10s	Fast	No, full recovery	Kedersha et al, 2000; 2005
TIA-1	SG	Arsenite	Human cell lines (HeLa)	67% recovery within 24s	Fast	No, full recovery	Guil et al, 2006
TTP	SG and PB	Overexpression	Human Cell lines (COS)	90% recovery within 30s	Fast	? (close to a full recovery)	Kedersha et al, 2005

*For FRAP purposes, GFP/YFP tagged versions of proteins are usually overexpressed in cells, thus the possibility exists in every dataset that exchange rates may be non-physiological due to artificial levels/granule induction. Note, ‘overexpression’ used in this table essentially means no other stress condition, bar expression of the GFP/YFP protein itself, which often is sufficient to induce SGs. PBs are already visible, but may be additionally stimulated.

**Numbers are approximations of recovery relative to the amount of protein photobleached, which varies between experiments (e.g. a protein that is 50% photobleached, and which recovers to 75% of original signal, will have recovered relatively by 50%).

*** Near static = no detectable fluorescence recovery; Very slow = <10% recovery over 2 minutes; Slow = >10% but <50% recovery over 2 minutes; Medium = >50% recovery over 2 minutes <50% recovery over 30s; Fast = >50% recovery over 30s

**** ‘?’ Indicates recovery either appeared to be increasing beyond timeframe examined in experiment, thus complete, slow recovery cannot be ruled out, or that only select timepoints of recovery were presented.