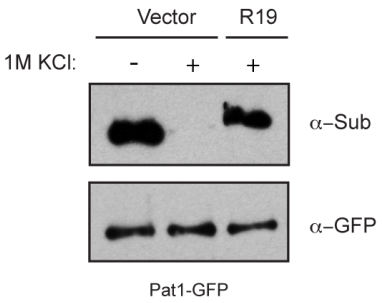

Figure S1 The presence of the *RAS2*^{val19} allele resulted in elevated levels of PKA phosphorylated Pat1. The level of PKA phosphorylation on Pat1 was assessed by Western blotting with the anti-phosphosubstrate antibody (α-Sub). The protein extracts were prepared from cells either before (-) or after a 10 min exposure to a 1M KCl salt stress (+). The cells contained either a control vector or a *RAS2*^{val19} plasmid as indicated. The bottom panel is a loading control that shows the total amount of the Pat1-GFP protein present in the cell extracts.

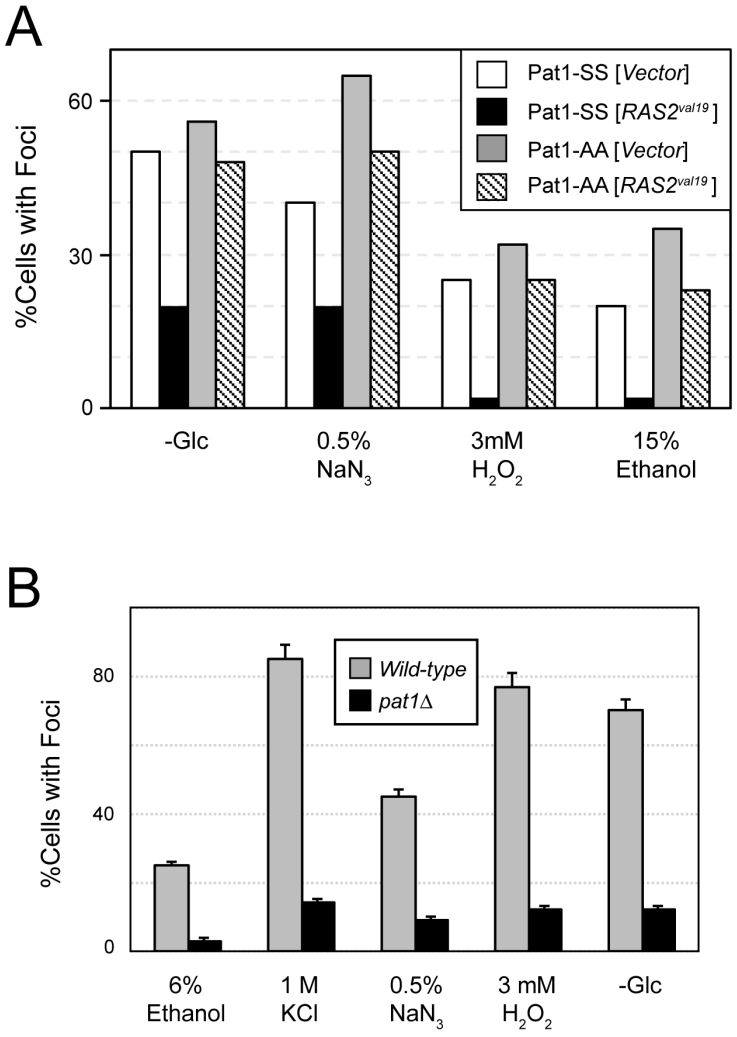

Figure S2 Quantifying the effects of *RAS2*^{val19} and the loss of Pat1 on P body foci formation in response to different stimuli. (A) Quantitation of the microscopy data shown in Figure 4C. The presence of the Pat1-AA variant suppresses the inhibitory effect that *RAS2*^{val19} has on P body foci formation. P body foci were indicated by GFP-tagged versions of either Pat1-SS or Pat1-AA, as indicated. (B) Quantitation of the microscopy data shown in Figure 4D. The fraction of cells containing an Edc3-mCh focus was assessed in wild-type and *pat1Δ* cells after a 10 min exposure to the indicated stress condition.

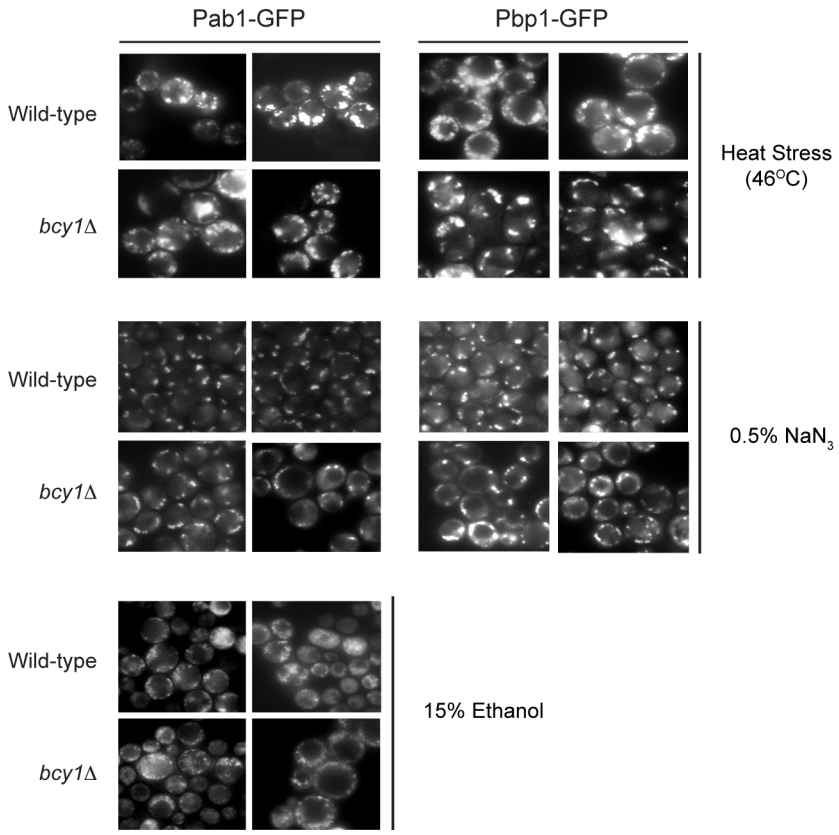

Figure S3 Stress granule foci form in normal numbers in *bcy1Δ* mutant cells. Wild-type or *bcy1Δ* cells expressing either a Pab1- or Pbp1-GFP reporter were examined by fluorescence microscopy after a 30 min exposure to the indicated stress condition.

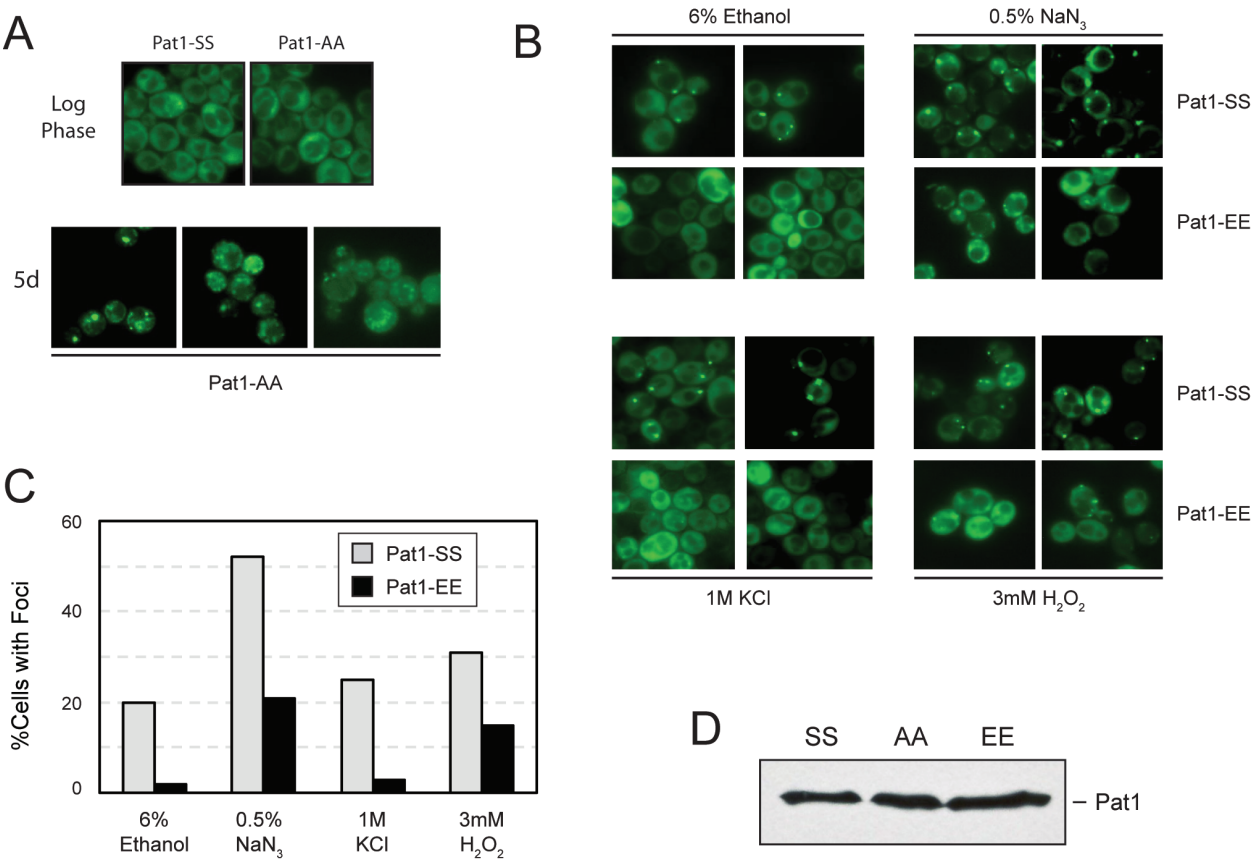

Figure S4 P body foci formation was diminished in cells expressing the phosphomimetic Pat1-EE variant. (A) P body foci formation in cells expressing the nonphosphorylatable Pat1-AA. Cells expressing the wild-type, Pat1-SS, or the Pat1-AA variant were examined by fluorescence microscopy during either log phase growth or after 5 days of incubation. (B) The presence of Pat1-EE resulted in fewer P body foci in response to all stress conditions tested. Cells expressing either Pat1-SS or Pat1-EE were examined by fluorescence microscopy after at 10 min exposure to the indicated stress conditions. (C) A graph showing the quantitation of the microscopy data presented in panel B. (D) Levels of the indicated Pat1 variants in log phase cells were determined by Western blotting with an α-GFP antibody.

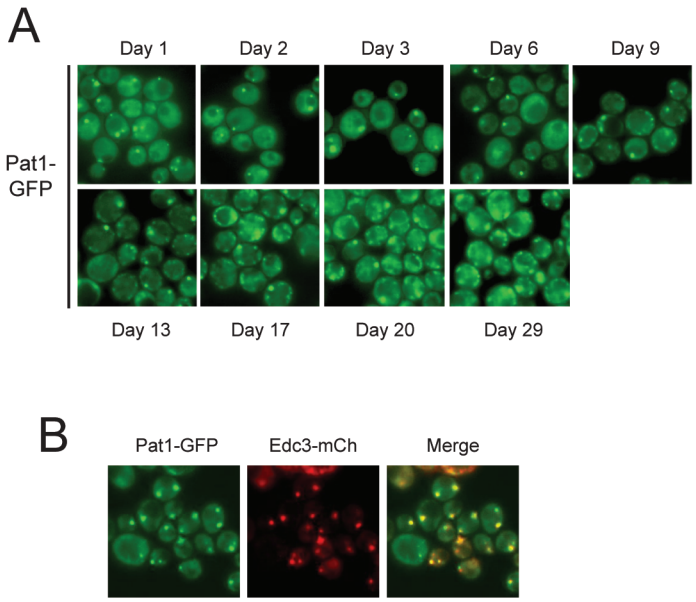

Figure S5 P body foci were present in stationary phase cells. (A) The localization of a Pat1-GFP reporter was assessed by fluorescence microscopy after the indicated days of growth. (C) The localization patterns of the Pat1-GFP and Edc3-mCh reporters is shown for cells after one day of growth in SC glucose medium.

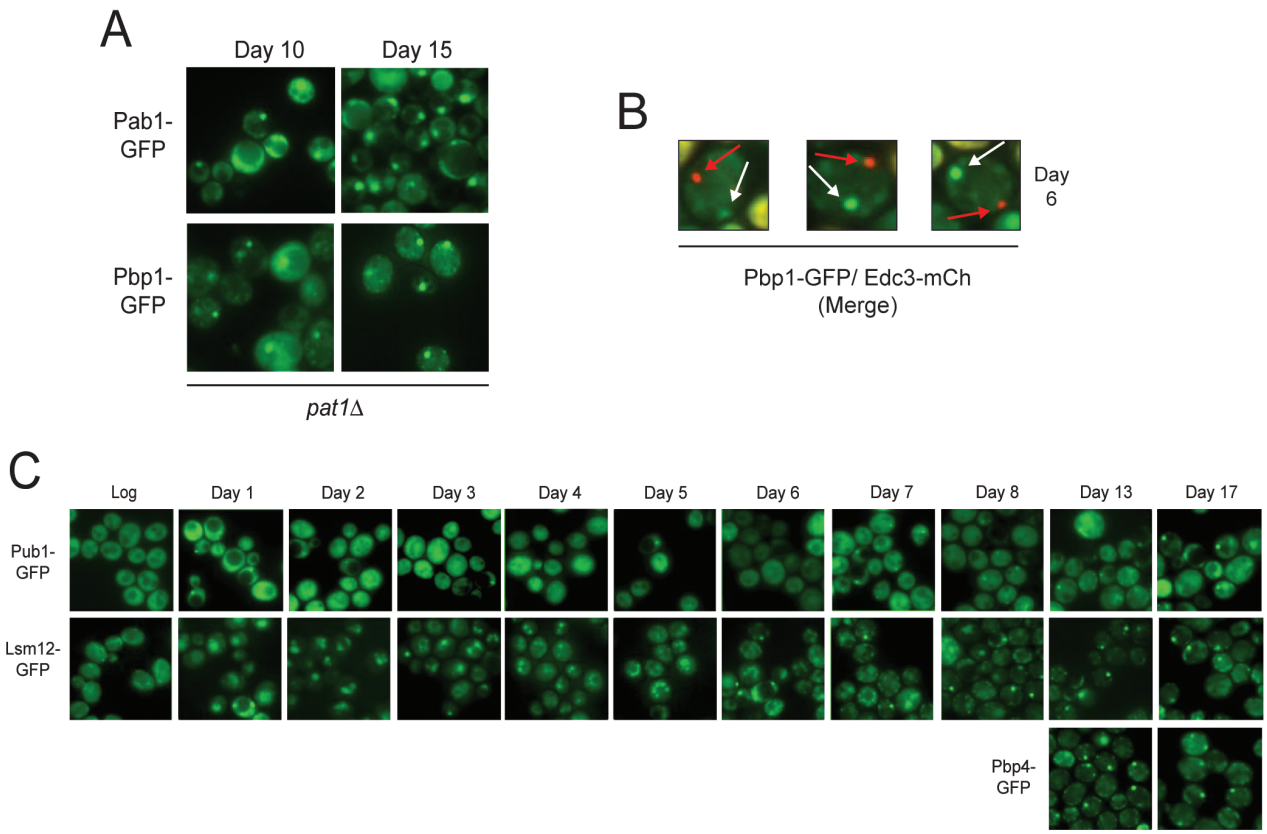

Figure S6 Stress granule foci were present in stationary phase cells. (A) The number of stress granule foci was not reduced in *pat1Δ* cells. The localization of the Pab1- and Pbp1-GFP reporters in wild-type and *pat1Δ* cells was assessed by fluorescence microscopy at the indicated times of growth. (B) P body and stress granule reporters did not co-localize in cells after 6 days of growth in an SC-glucose medium. Representative images of three cells containing both Pbp1-GFP (indicated by the white arrows) and Edc3-mCh (red arrows) foci are shown. Quantitation of the data are indicated in the text. (C) The localization of the indicated GFP-tagged stress granule reporters was assessed by fluorescence microscopy at the indicated days of growth.

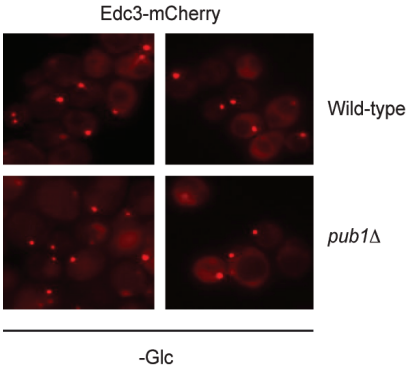

Figure S7 Similar numbers of P body foci were detected in wild-type and *pub1Δ* cells that had been exposed to a 15 min glucose starvation. Two representative images are shown for each strain background.

Table S1 Yeast strains used in this study

PHY1736 (GF260-2)	*MATa leu2 trp1 ura3 lys2*	(Valay *et al.* 1993))
PHY 1954	*MATa leu2 trp1 ura3 lys2 ras2::URA3*	This study
PHY3193 (XPY310a)	*MATa/α pep4::HIS3/pep4::HIS3 prb1_1.6R/prb1_1.6R can1/can1 ura3-52/ura3-52 his3_200/his3_200 GAL1-GST-TPK1/TPK1*	(Pan and Heitman 2002)
PHY3194 (XPY311a)	*MATa/α_pep4::HIS3/pep4::HIS3 prb1_1.6R/prb1_1.6R can1/can1 ura3-52/ura3-52 his3_200/his3_200 GAL1-GST-TPK2/TPK2*	(Pan and Heitman 2002))
PHY3362 (W303-1A)	*MATa ade2-1 can1-100 ura3-1 leu2-3,112 his3-11,15 trp1-1 TPK1 TPK2 TPK3*	(Mazon *et al.* 1993)
PHY3364 (MB23)	*PHY3362 TPK1 tpk2: :HIS3 tpk3: :URA3*	(Mazon *et al.* 1993)
PHY3367 (MB13)	*PHY3362 tpk1: :LEU2 TPK2 tpk3: :URA3*	(Mazon *et al.* 1993)
PHY3369 (MB12)	*PHY 3362 tpk1: :LEU2 tpk2: :HIS3 TPK3*	(Mazon *et al.* 1993)
PHY4231 (BY4741)	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0*	Open Biosystems
PHY 4311	*MATα his3 leu2 ura3*	(Coller and Parker 2005)
PHY4313 (yRP2067)	*MATα his3 leu2 ura3 pat1Δ::NEO*	(Coller and Parker 2005)
PHY4697 (Y3175)	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tpk2::KAN tpk3::TRP1 tpk1(M164G)*	Dr. James Broach
PHY4773	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 DCP2-GFP*	Invitrogen
PHY4774	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 XRN1-GFP*	Invitrogen
PHY4827 (yRP2162)	*MATa his4-539 leu2-3,112 trp1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG Dcp2-GFP(NEO)*	(Decker *et al.* 2007)
PHY 4833 (yRP 2183)	*MATa leu2-3,112 trp1 ura3-52 his4-539 lys2-201 cup1::LEU2/PGK1pG/MFA2pG pat1::LEU2 DCP2-GFP (NEO)*	(Teixeira and Parker 2007)
PHY 4834 (yRP1724)	*MATa2 his4-539 leu2-3,112 trp1 ura3-5 cup1::LEU2/PGK1pG/MFA2pG DHH1-GFP (NEO)*	(Decker *et al.* 2007)
PHY 4898	*MATa leu2 trp1 ura3 lys2 ras2::URA3 pat1::KAN*	This study

PHY 4899	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PAB1-GFP*	Invitrogen
PHY 4900	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PBP1-GFP*	Invitrogen
PHY 4927	*MATα his3 leu2 ura3 pat1Δ:: NEO GFP-[PAT1-SS]*	This study
PHY 4928	*MATα his3 leu2 ura3 pat1Δ:: NEO GFP-[PAT1-AA]*	This study
PHY 4929	*MATα his3 leu2 ura3 pat1Δ:: NEO GFP-[PAT1-EE]*	This study
PHY 4939	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PBP4-GFP*	Invitrogen
PHY 4940	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PUB1-GFP*	Invitrogen
PHY 4941	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LSM12-GFP*	Invitrogen
PHY 4942	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 PAT1-GFP*	Invitrogen
PHY 4859	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 pbp4::KAN*	Open Biosystems
PHY 4860	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 lsm12::KAN*	Open Biosystems
PHY 4869	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 pub1::KAN*	Open Biosystems
PHY 4870	*MATa hisΔ1 leu2Δ0 met15Δ0 ura3Δ0 pbp1::KAN*	Open Biosystems

Table S2 Plasmids used in this study

Plasmid	Description	Reference
pPHY795	*MET3*$_{pro}$*- RAS2*^{Val19} in pRS415 *(CEN, LEU2)*	(Ramachandran *et al.* 2011)
pPHY796	*MET3*$_{pro}$*- RAS2*^{Val19} in pRS416 *(CEN, URA3)*	(Ramachandran *et al.* 2011)
pPHY921	*RAS2*^{Val19} in pRS316	(Ramachandran *et al.* 2011)
pPHY2203	*CUP1*$_{pro}$*-TPK1-HA*$_3$ (*URA3*)	(Deminoff *et al.* 2006)
pPHY2659	DHH1-GFP (*CEN, URA3*)	Dr. Tien-Hsien Chang
pDHH1018		
pPHY2915	*CUP1*$_{pro}$*-TPK2-HA*$_3$ (*URA3*)	(Deminoff *et al.* 2006)
pPHY2916	*CUP1*$_{pro}$*-TPK3-HA*$_3$ (*URA3*)	(Deminoff *et al.* 2006)
pPHY3360	*PAT1*$_{pro}$*-Myc-PAT1-AA* (*CEN, HIS3*)	(Ramachandran et al, 2011)
pPHY3362	*PAT1*$_{pro}$*-Myc-PAT1-SS* (*CEN, HIS3*)	(Ramachandran *et al.* 2011)
pPHY3506	*PAT1*$_{pro}$*-GFP-PAT1-SS* (*CEN, HIS3*)	(Ramachandran *et al.* 2011)
pPHY3507	*PAT1*$_{pro}$*-GFP-PAT1-AA* (*CEN, HIS3*)	(Ramachandran *et al.* 2011)
pPHY3509	*PAT1*$_{pro}$*-GFP-PAT1-EE* (*CEN, HIS3*)	(Ramachandran *et al.* 2011)
pPHY3660	*EDC3*-mCherry (*CEN, URA3*)	(Buchan *et al.* 2011)
(pRP1574)		
pPHY3661	*PAB1*-GFP (*URA3*)	(Buchan *et al.* 2008)
(pRP1362)		
pPHY3665	*PAB1*-GFP, *EDC3*-mCherry (*CEN, URA3*)	(Buchan *et al.* 2011)
(pRP1657)		
pPHY3667	*PAB1*-GFP, *DCP2*-mCherry (*CEN, TRP1*)	(Buchan *et al.* 2011)
(pRP1660)		
pPHY3671	*EDC3*-mCherry; *PBP1*-GFP (*CEN, TRP1*)	(Swisher and Parker 2010)
(pRP1944)		
pPHY3685	*PAT1*$_{pro}$*-GFP-Pat1-SS* (*HIS3*)	This study
pPHY3691	*PAT1*$_{pro}$*-GFP-Pat1-AA* (*HIS3*)	This study
pPHY3693	*PAT1*$_{pro}$*-GFP-Pat1-EE* (*HIS3*)	This study
pPHY3702	*ADH2*$_{pro}$*-H2B*-mCherry (*CEN, LEU2*)	Dr. James Hopper
pOE79		

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