Supporting Information

Sprouse *et al*. 10.1073/pnas.0801901105

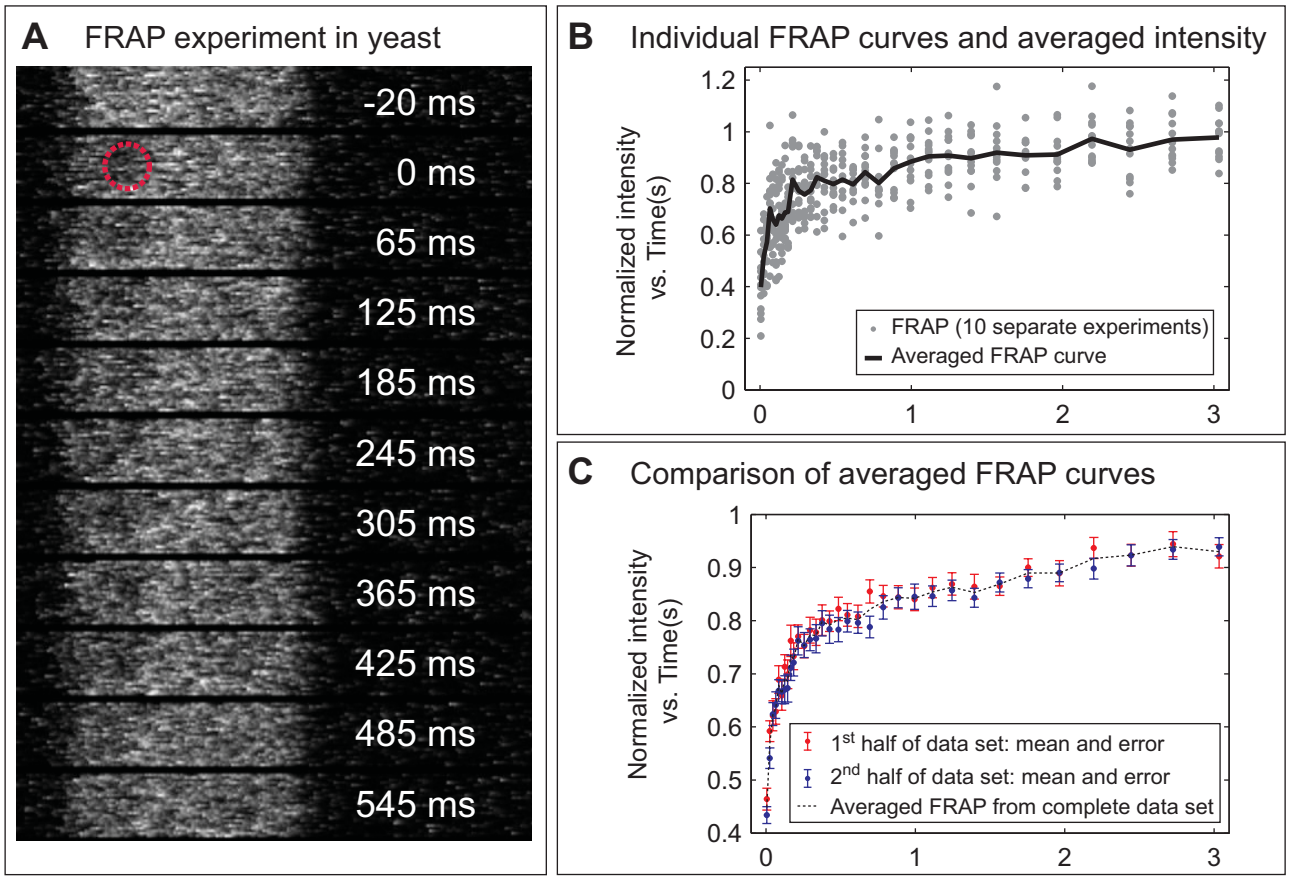
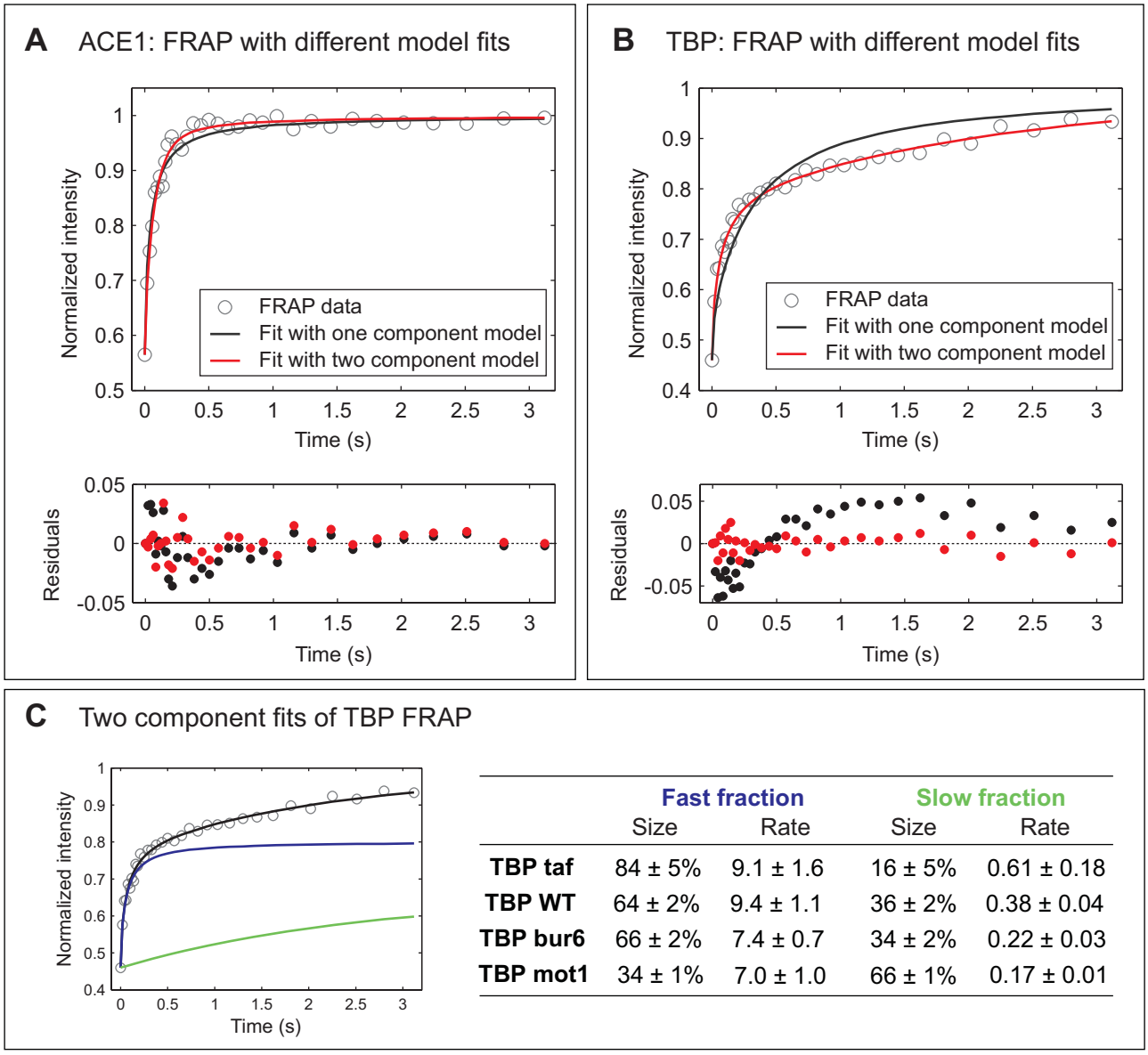


Fig. S1. Generation of FRAP data. (*A*) Images of nuclei before and after photobleaching a 0.7-μm spot, indicated by the red circle, with a single bleach pulse. Images are shown for TBP-YFP in a *mot1* cell. All FRAP data were normalized by the average intensity measured from a spot within the imaged strip that was of the same size and relative positioning as the spot where the intentional photobleach was performed. This corrects for bleaching caused by imaging and also any influx of fluorescence into the strip from regions beyond it. (*B*) Individual TBP FRAP curves are not smooth, with fluctuations in intensity caused by the low intensity levels of fluorescence and drift of the specimen in and out of focus. Points from 10 different individual FRAP curves are superimposed here to indicate the spread of data. The average FRAP curve from these 10 individual curves is shown with the solid line. (*C*) FRAP curves are the result of averaging 30–100 individual curves. These averages are robust as seen by comparing averages of subpopulations. Shown in red is the average of the first 50 TBP curves and shown in blue is the average of the remaining 50 TBP curves. Note that each of the subaverages is close to the total average curve (dotted gray line).



	Fast fraction		Slow fraction	
	Size	Rate	Size	Rate
TBP taf	84 ± 5%	9.1 ± 1.6	16 ± 5%	0.61 ± 0.18
TBP WT	64 ± 2%	9.4 ± 1.1	36 ± 2%	0.38 ± 0.04
TBP bur6	66 ± 2%	7.4 ± 0.7	34 ± 2%	0.22 ± 0.03
TBP mot1	34 ± 1%	7.0 ± 1.0	66 ± 1%	0.17 ± 0.01

Fig. S2. One- and two-component fits to FRAP data. (*A*) Most FRAP curves are well fit by a one-component model. Shown here is the FRAP for AceI fit by a one-component (black line) and a two-component model (red line). The improvement with two components is marginal, as also demonstrated by the residual plots. (*B*) Some FRAP curves are better fit by the two-component model as illustrated here for TBP. Note the improvement in the residuals plot. (*C*) When the TBP curves in WT and mutant backgrounds are fit with the two-component model, the most significant changes are seen in the fraction sizes. These change dramatically for the *taf1* and *mot1* backgrounds, but not for *bur6*. See *Materials and Methods* for the equations for the one- and two-component models, described there as frap_1(t) and frap_2(t).

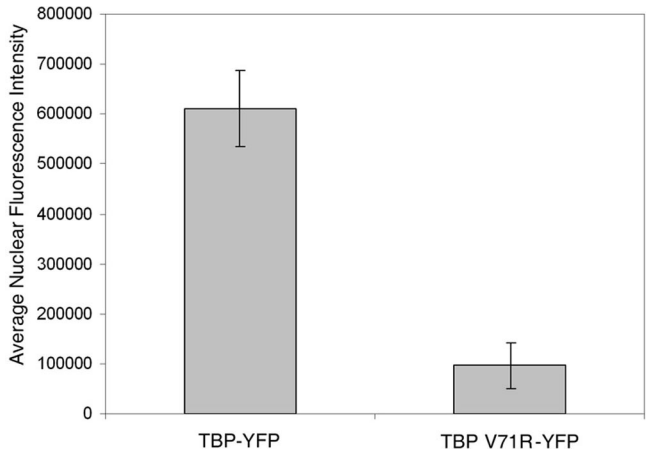
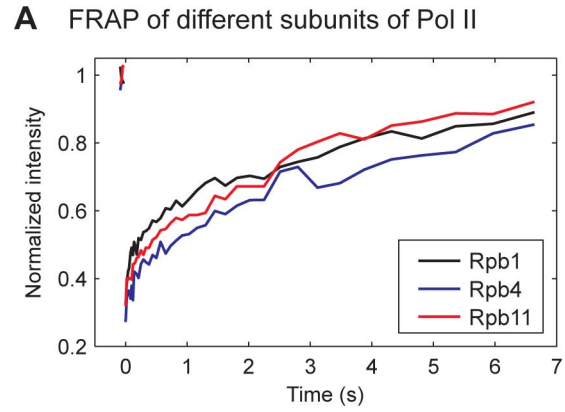
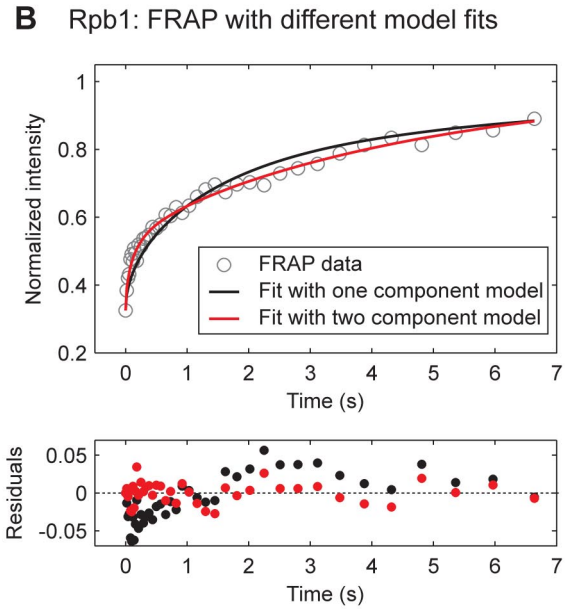


Fig. S3. Quantitation of TBP-YFP and TBP V71R-YFP by fluorescence intensity. The fluorescence intensity of the nucleus for the indicated tagged strain in WT cells was tested for ≈10 cells. Measurements were taken from the brightest focal plane using identical imaging conditions in all cases. Error bars indicate the SEM.





C Summary of two component fits

	Fast fraction		Slow fraction	
	Size	Rate	Size	Rate
Rpb1	38 ± 2%	4.9 ± 0.9	62 ± 2%	0.20 ± 0.01
Rpb4	27 ± 3%	4.2 ± 1.5	73 ± 3%	0.19 ± 0.01
Rpb11	20 ± 2%	11 ± 4.4	80 ± 2%	0.29 ± 0.01

Fig. S4. FRAP of Pol II components and model fits. (*A*) Shown are the FRAP curves for three different Pol II components. (*B*) As illustrated here for Rpb1, the one-component fits to these data consistently undershoot and then overshoot the FRAP data, as also demonstrated in the residuals plot. (*C*) The estimated parameters from these two-component fits suggest that Rpb1, Rpb4, and Rpb11 exhibit rather similar, although perhaps not identical, kinetics.

Table S1. FRAP strain list

Strain name	Construct	Genotype	Source
YTK597	YFP-NLS WT	*MAT* a/α, *mot1*Δ::*TRP1*::YFP-NLS-*TRP1-HIS3*/ *mot1*Δ::*TRP1*::YFP-NLS-*TRP1-HIS3*, pAV20 (*MOT1*+, *LEU2*)	This study
YTK598	YFP-NLS *mot1*	*MAT* a/α, *mot1*Δ::*TRP1*::YFP-NLS-*TRP1-HIS3*/ *mot1*Δ::*TRP1*::YFP-NLS-*TRP1-HIS3*, pMOT221 (*mot1–42*, *LEU2*)	This study
YTK319	Ace1-GFP WT	*MAT* a/α, *his3*Δ*1*/*his3*Δ*1*, *leu2*Δ*0*/*leu2*Δ*0*, *ura3*Δ*0*/ *ura3*Δ*0*, *met15*Δ*0*)/+, +/*lys2*Δ*0*, *ace1*Δ::*kanMX*/*ace1*Δ::*kanMX*, *TRP1*::*TRP1*-GPD-*ACE1*-GFP-*HIS3*	This study, T. Karpova
YTK610	Ace1-GFP *mot1*	*MAT* a/α, *his3*Δ*1*/*his3*Δ*1*, *leu2*Δ*0*/*leu2*Δ*0*, *ura3*Δ*0*/ *ura3*Δ*0*, *met15*Δ*0*)/+, +/*lys2*Δ*0*, *ace1*Δ::*URA3*/*ace1*Δ::*URA3*, *mot1*Δ::*kanMX*/*mot1*Δ::*kanMX*, *TRP1*::*TRP1*-GPD-*ACE1*-GFP-*HIS3*, pMOT221*(mot1–42, LEU2)*	This study, T. Karpova
YTK580	TBP-YFP WT	*MAT* a/α, *mot1*Δ::*TRP1*/mot1Δ::*kanMX*, TBP-YFP-*SpHIS5*/TBP-YFP-*SpHIS5* pAV20 (*MOT1*+, *LEU2*)	This study
YTK581	TBP-YFP *mot1*	*MAT* a/α, *mot1*Δ::*TRP1*/*mot1*Δ::*kanMX*, TBP-YFP-*SpHIS5*/TBP-YFP-*SpHIS5* pMOT221 (*mot1–42*, *LEU2*)	This study
ROSY81	TBP-YFP *bur6*	*MAT* a/α, *bur6–1*/*bur6–1*, TBP-YFP-*SpHIS5*/TBP-YFP-*SpHIS5*	This study, derived from mating with GY561*:* *MAT*a, *his4–912*d, *lys2–128*d, *suc2*Δ*uas*(*-1900*/*-390*), *ura3–52*, *leu2*D1, *bur6–1* (1)
ROSY134	TBP-YFP *taf1*	*MAT* a/α, *taf145–2*/*taf145–2*, TBP-YFP-*SpHIS5*/ TBP-YFP-*SpHIS5*	This study, derived from mating with YSW93:*MAT* a, *TRP1*, *ura3–53*, *taf145*Δ::*LEU2*, pRS313-*taf145*$^{ts-2}$ (2)
ROSY53	TBP(V71R)-YFP WT	*MAT* a, *mot1*Δ::*TRP1*, *ade5*Δ::*natMX,* pTBP-V71R-YFP-*HIS3*, pAV20 (*MOT1*+, *LEU2*)	This study, pTBP-V71R derived from pTSK274 (3)
ROSY54	TBP(V71R)-YFP *mot1*	*MAT* a, *mot1*Δ::*TRP1*, *ade5*Δ::*natMX*, pTBP-V71R-YFP-*HIS3,* pMOT221 (*mot1–42*, *LEU2*)	This study, pTBP-V71R derived from pTSK274 (3)
ROSY171	TAF1-YFP WT	*MAT* a/α, *mot1*Δ::*TRP1*/*mot1*Δ::*kanMX*, pAV20 (*MOT1*+, *LEU2*), *ade5*Δ::*natMX*/*ade5*Δ::*natMX*, TAF1-YFP-*SpHIS5*/TAF1-YFP-*SpHIS5*	This study
ROSY172	TAF1-YFP *mot1*	*MAT* a/α, *mot1*Δ::*TRP1*/*mot1*Δ::*kanMX*, pMOT221 (*mot1–42*, *LEU2*), *ade5*Δ::*natMX*/*ade5*Δ::*natMX*, TAF1-YFP-*SpHIS5*/TAF1-YFP-*SpHIS5*	This study
ROSY114	TFIIB-YFP WT	*MAT* a/α, *mot1*Δ::*TRP1*/*mot1*Δ:*kanMX*, TFIIB-YFP-*SpHIS5*/TFIIB-YFP-*SpHIS5,* pAV20 (*MOT1*+, *LEU2*)	This study
ROSY115	TFIIB-YFP *mot1*	*MAT* a/α, *mot1*Δ::*TRP1*/*mot1*Δ::*kanMX*, TFIIB-YFP-*SpHIS5*/TFIIB-YFP-*SpHIS5*, pMOT221 (*mot1–42*, *LEU2*)	This study
ROSY83	Mot1-YFP	*MAT* a/α, Mot1-YFP-*SpHIS5*/Mot1-YFP-*SpHIS5*	This study
YTK544	GFP-Rpb1	*MAT* a, *his3*Δ*1*, *leu2*Δ*0*, *met15*Δ*0*, *ura3*Δ*0*, GFP-Rpb1-*SpHIS5*	This study, T. Karpova

All strains except YTK319, YTK610, and YTK544 are derivatives of YPH499 or YPH500: MAT a or α, *ura3–52*, *lys2–801*, *ade2–101*, *leu2-*Δ*1*, *his3-*Δ*200*, *trp1-*Δ*63* (4).

1. Prelich G (1997) *Saccharomyces cerevisiae* BUR6 encodes a DRAP1/NC2α homolog that has both positive and negative roles in transcription *in vivo*. *Mol Cell Biol* 17:2057–2065.
2. Walker SS, *et al*. (1997) Yeast TAF(II) 145 required for transcription of G_1/S cyclin genes and regulated by the cellular growth state. *Cell* 90:607–614.
3. Karpova TS, *et al.* (2008) Concurrent fast and slow cycling of a transcriptional activator at an endogenous promoter. *Science* 319:466–469.
4. Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast hosts designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122:19–27.