Molecular Cell, Volume *33*

Supplemental Data

Cotranscriptional Recruitment of the mRNA Export Factor Yra1 by Direct Interaction with the 3′ End Processing Factor Pcf11

Sara Ann Johnson, Gabrielle Cubberley, and David L. Bentley

Table S1. Yeast strains used in this study

Strain	Genotype	Reference
DLY23	W303 MAT*a sub2::HIS3* pCM188-SUB2 (URA3)	(Libri et al., 2001)
DLY33-1	W303 MAT*a sub2::HIS3* pCM185-sub2-201 (TRP1)	(Libri et al., 2001)
DLY33-6	W303 MAT*a sub2::HIS3* pCM185-sub2-206 (TRP1)	(Libri et al., 2001)
W303-1B	MAT*a ura3-1 trp1-1 ade2-1 leu2-3,112 his3-11,15*	(Amrani et al., 1997)
rna14-1	W303-1B *rna14-1*	(Amrani et al., 1997)
DBY568	W303-1B *rna14-1 rrp6::hygro*^{r}	This study
rna15-2	W303-1B *rna15-2*	(Amrani et al., 1997)
DBY569	W303-1B *rna15-2 rrp6::hygro*^{r}	This study
NA65	W303-1B *pcf11-2*	(Amrani et al., 1997)
NA67	W303-1B *pcf11-9*	(Amrani et al., 1997)
DBY979	BY4741 *hygro*^{r}GAL1::*YLR454W*	This study
DBY980	BY4741 *hygro*^{r}GAL1::*YLR454W*::*S.c. URA3*	This study
DBY981	BY4741 *hygro*^{r}GAL1::*YLR454W*::6XBoxB	This study
DBY986	BY4741 *hygro*^{r}GAL1::*YLR454W*::6XBoxB *Pcf11*::HA-NPeptide *HIS3*	This study
DBY987	BY4741 *hygro*^{r}GAL1::*YLR454W*::6XBoxB *Pcf11*::HA *HIS3*	This study
DBY361	W303-1B *Yra1*::GFP*kan*^{r}	This study

Supplemental Experimental Procedures

To insert Box B sites into chromosomal *GAL1-YLR454W* we used a URA3 pop-in pop-out strategy. URA3 was cloned into a fragment of YLR454W (721-1671) at the BamH1 site (position 1242) in pBS (YLR454W-URA3). The excised insert was transformed into DBY979, and correct integration into the *GAL1-YLR454W* locus was confirmed in DBY980 by colony PCR. Six copies of BoxB (Wiegand et al., 2003) were cloned into the YLR454W BamHI site at +1242 in pBS (YLR454W-6XBoxB), and used to replace URA3. Integrants were selected on FOA plates, and confirmed by PCR (DBY981). Endogenous Pcf11 was tagged at its C-terminus with a 6XHA tag (non-tethered control, DBY987) or 6XHA plus the Box B binding peptide of the P22 N-protein (NAKTRRHERRRKLAIERDTI) (tethered, DBY986) (Wiegand et al., 2003). Tagging was by transformation with PCR products made using pYM15 or pYM15-N peptide templates. *RRP6* was

replaced by *hygro*^{r} in DBY568, 569 by PCR mediated gene replacement and confirmed by colony PCR.

Table S2. Plasmids used in this study

Name	Description	Reference
pGEX-Yra1	described previously	(Stutz et al., 2000)
pGEX-KG-Aly		R. Zhao
pET21b Rna14	described previously	(Kessler et al., 1996)
pET22-Rna15		M. Minet
pCMV/N	described previously	(Wiegand et al., 2003)
pCMV/CAT/B	described previously	(Wiegand et al., 2003)
pYM15	6X HA, HIS3MX6	(Janke et al., 2004)
YM15-Npeptide	N-peptide sequence was amplified from pCMV/N and inserted downstream of 6X HA at HindIII-SalI in pYM15	This study
pBS-YLR454W	fragment of YLR454W +721-1671 relative to ATG) was PCR amplified and inserted in pBlueScript KS- HindIII-SpeI	This study
pBS-YLR454W-URA3	*S.c.* URA3 was inserted into pBS-YLR454W at a unique BamHI site within the YLR454W fragment	This study
pBS-YLR454W-BoxB	6X BoxB sequence was PCR amplified from pCMV/CAT/B and inserted into pBS-YLR454W at the BamHI site at +1242	This study
pET21-Pcf11	PCR amplified full length ORF inserted into pET21a NheI-SacI	This study
pET21-Pcf11 CID	aa's 1-140 were PCR amplified and inserted into pET21a NheI-SacI	This study
pET21-Pcf11 ΔCID	aa's 141- 626 were PCR amplified and inserted into pET21a NheI-SacI	This study
pET21-Pcf11 420-608	aa's 420-608 were PCR amplified and inserted into pET21b NheI-XhoI in-frame with the 6XHis tag	This study
pET21-HuPcf11 1342-1487	aa's 1342-1487 were PCR amplified and inserted into pET21b NheI-XhoI in-frame with the 6XHis tag	This study
pET21GST-Yra1 RRM	aa's 77-167 were PCR amplified and inserted into pET21GST-TEV BamHI-EcoRI	This study
pET21GST-Yra1 N + RRM	aa's 1-167 were PCR amplified and inserted into pET21GST-TEV BamHI-EcoRI	This study
pET21GST-Yra1 RRM + C	aa's 77-226 were PCR amplified and inserted into pET21GST-TEV BamHI-XhoI	This study
pET21GST-Yra1 N	aa's 1-66 were PCR amplified and inserted into pET21GST-TEV BamHI-XhoI	This study
pET21GST-Yra1 C	aa's 124-226 were PCR amplified and inserted into pET21GST-TEV BamHI-XhoI	This study
pET21GST-Yra1N/C REF	aa's 12-210 were replaced with a KpnI site by PCR and a fragment encoding the flexible linker region (residues 92-132) of λ repressor was inserted at that site.	This study

Supplemental Figures

Fig. S1

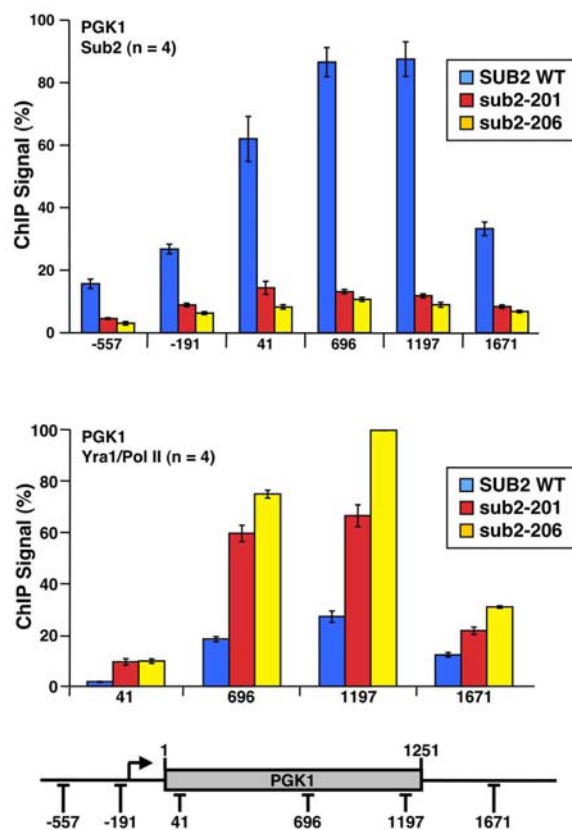

Figure S1. Yra1 recruitment to actively transcribed genes is independent of Sub2.
ChIP analysis of *PGK1* as in (Fig. 1C, D) analyzed by real-time PCR with amplicons shown in the map. Relative ChIP signals are plotted for Sub2 (top) or as the ratios of Yra1:pol II (bottom). Note, Sub2 inactivation enhances recruitment of Yra1. Mean values, normalized to the maximal signal in each data set, +/- SEM are shown.

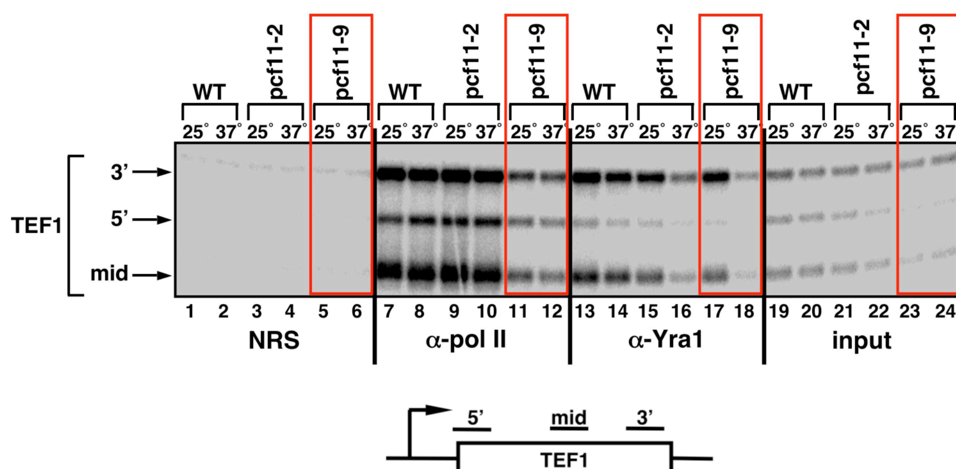

Figure S2. Co-transcriptional Recruitment of Yra1 Requires Functional Pcf11. ChIP analysis of WT, *pcf11-2*, and *pcf11-9* (marked by red boxes) cells at 25˚ and 37˚C (60 minutes) with anti-pol II, anti-Yra1 and normal rabbit serum (NRS) control as in Fig. 3A. Note *pcf11-9*, unlike *pcf11-2*, has a point mutation in the CID that prevents binding to the pol II CTD (Sadowski et al . 2003). ^{32}P-labeled PCR products correspond to the indicated amplicons of *TEF1* (see map). Upon normalization to pol II, quantification of Yra1 occupancy in the *pcf11-9* samples reveal TEF1-5' decreased 1.2 fold, TEF1-mid decreased 4.1 fold, and TEF1-3' decreased 6.0 fold; and for *pcf11-2* TEF1-5' decreased 1.7 fold, TEF1-mid decreased 2.1 fold, and TEF1-3' decreased 1.9 fold. Controls for Yra1 protein expression levels in *pcf11* mutants are shown in Fig. 2B.

Fig. S3

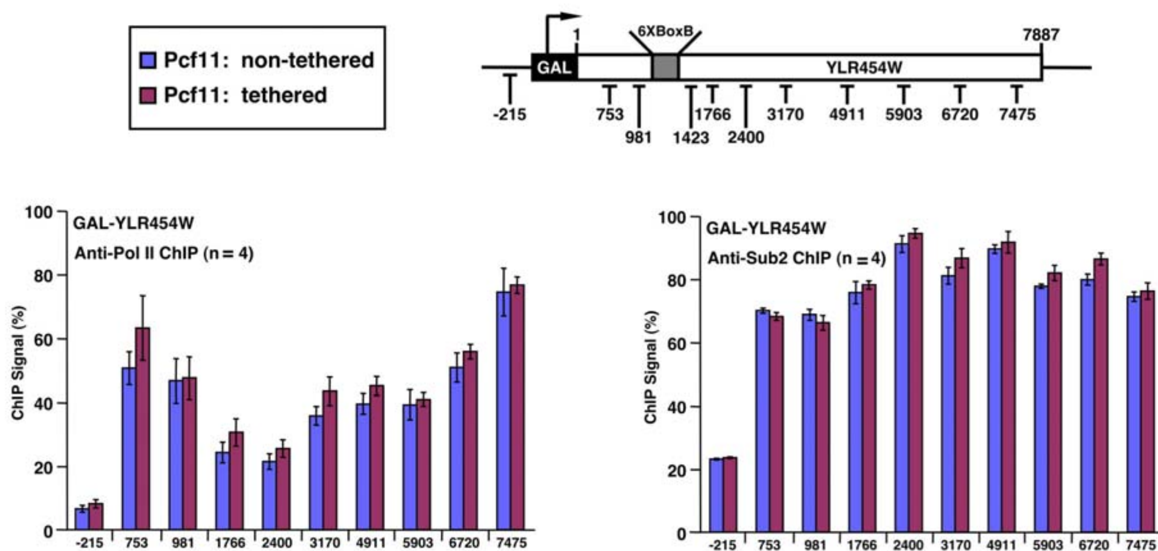

Figure S3. RNA tethering of Pcf11 does not significantly alter pol II or Sub2 recruitment to the chromosomal *GAL-YLR454W* gene. Anti-pol II (left) and anti-Sub2 (right) ChIP signals analyzed by real-time PCR for strains with tethered (purple bars) and non-tethered (blue bars) Pcf11. The same cross-linked extracts were used as in Fig. 6C. Diagram of *GAL-YLR454W-6XBoxB* and the corresponding amplicons are included above the plot. Data are represented as mean values, normalized to the maximal signal in the data set, +/- SEM.

Fig. S4

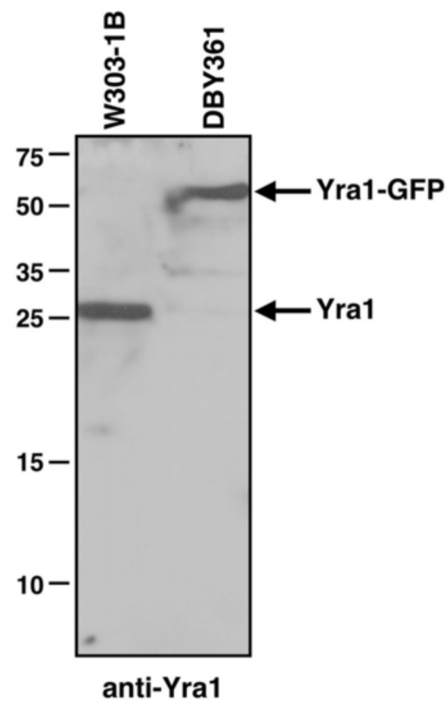

Figure S4. Rabbit polyclonal anti-Yra1 (124-226) antibody specifically recognizes endogenous yeast Yra1. Anti-Yra1 immunoblot of WT (W3031B) cells and cells in which endogenous Yra1 has been C-terminally tagged with GFP (DBY361). Addition of GFP increases the relative molecular weight of Yra1 by approximately 26 kD. Each lane loaded with 10ug of whole cell extract.

Supplemental References

Amrani, N., Minet, M., Wyers, F., Dufour, M.E., Aggerbeck, L.P., and Lacroute, F. (1997). PCF11 encodes a third protein component of yeast cleavage and polyadenylation factor I. Mol Cell Biol *17*, 1102-1109.

Janke, C., Magiera, M.M., Rathfelder, N., Taxis, C., Reber, S., Maekawa, H., Moreno-Borchart, A., Doenges, G., Schwob, E., Schiebel, E., and Knop, M. (2004). A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes. Yeast *21*, 947-962.

Kessler, M.M., Zhao, J., and Moore, C.L. (1996). Purification of the Saccharomyces cerevisiae cleavage/polyadenylation factor I. Separation into two components that are required for both cleavage and polyadenylation of mRNA 3' ends. J Biol Chem *271*, 27167-27175.

Libri, D., Graziani, N., Saguez, C., and Boulay, J. (2001). Multiple roles for the yeast SUB2/yUAP56 gene in splicing. Genes Dev *15*, 36-41.

Stutz, F., Bachi, A., Doerks, T., Braun, I.C., Seraphin, B., Wilm, M., Bork, P., and Izaurralde, E. (2000). REF, an evolutionary conserved family of hnRNP-like proteins, interacts with TAP/Mex67p and participates in mRNA nuclear export. RNA *6*, 638-650.

Wiegand, H.L., Lu, S., and Cullen, B.R. (2003). Exon junction complexes mediate the enhancing effect of splicing on mRNA expression. Proc Natl Acad Sci USA *100*, 11327-11332.