Single Molecule Tracking Of Ace1p In *Saccharomyces cerevisiae* Defines A Characteristic Residence Time For Non-specific Interactions Of Transcription Factors With Chromatin

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SUPPLEMENTARY MATERIALS AND METHODS

Strains and plasmids

Diploids homozygous for *in situ* HaloTag fusions and *pdr5Δ* were constructed by mating the appropriate haploids. To construct pTSK405, *TRP1* gene was replaced in pTSK241 (1) with *URA3* SacII/SacI fragment, amplified from yeast genomic DNA by PCR, using oligoes T682 and T683 (see Supplementary Table S2 for all primer sequences). For C-terminal *in situ* fusion to *ACE1,* 3X-GFP was amplified from pTSK405 using primers T695 and T697. To construct pTSK561, NotI-SacII fragment with HaloTag was PCR amplified from pFC15K HaloTag CMVd1 Flexi Vector (Cat. No.: G1601, Promega, USA) using T900 and T901 and inserted into pTSK405, replacing 3x GFP. For C-terminal *in situ* fusions with *URA3* selection, HaloTag was amplified from pTSK561 using T886 and T887 for *HHT1*-HaloTag, or T961 and T962 for *HSF1*-HaloTag, or T695 and T697 for *ACE1*-HaloTag fusions. All the fusions were verified by diagnostic PCR. For *PDR5* deletion, *LoxP-KanMX-LoxP* cassette was amplified from pUG6 (2) using T518 and T519 and transformed into wild-type yeast strain. Transformants were verified by diagnostic PCR.

Media and growth conditions

Yeast cultures were grown at 30^{o}C unless stated otherwise in YPD or CSM, as described. For SMT, CSM+SC media (CSM+6.5 mg/ml sodium citrate) was used. 200 µg/ml of G418 were added to YPD for *KanMX* marker selection. For growth curves, cells were grown overnight to saturation and then diluted into 50 ml of fresh YPD in 500 ml flask at OD_{600} 0.1 and grown with aeration. OD_{600} was measured every two hours. For copper sensitivity assay, cells were grown overnight to saturation in CSM, then diluted into fresh CSM, and grown for 6 hrs (until OD_{600} reached 0.8 to 1). Ten-fold serial dilutions were made in standard 96 well plate, from which diluted cultures were spotted onto the CSM plates with different concentrations of the $CuSO_4$ (0 µM, 100 µM, 250 µM, 500 µM) using a Replica Plater (Sigma, USA, Cat. No.: R-2383-1EA). Plates were incubated at 30ºC for 48 hrs and images were captured using a standard gel-documentation system. For HS experiments, cells were grown at 25ºC. After labeling the log phase cells with HaloTag ligand, cells were concentrated by centrifugation, and resuspended in 25 µl of CSM+SC media in a thin-walled glass tubes (Thermo Scientific, Cat no.: 14-961-26). The tubes were incubated in a 39ºC water bath for 15 min before imaging.

RNA extraction and quantification by qPCR:

RNA preparation was performed as described (1). cDNA was prepared using the iScript cDNA synthesis kit (BioRad, Inc. Cat no.: 1708891) starting with 1 mg of total RNA. Quantitative real-time PCR (Q-PCR) was performed as described (1). Primers used for the quantification of *CUP1* are T531 and T532. For normalization of *CUP1* cDNA, the following *TUB1* gene specific primers were used: TUB1F and TUB1R. To confirm the absence of contaminating genomic DNA in cDNA preparations, reverse transcriptase negative (-RT) samples were used as a control, which produced the Ct value difference of ≥15 cycles between "-RT" and "+RT" samples, indicating negligible amount of genomic DNA contamination in cDNA samples. This experiment was repeated thrice and qPCR was performed in duplicates.

HILO imaging system used in these experiments is based on an Olympus IX81 inverted microscope (Olympus Scientific Solutions, Waltham, MA) with a 150x 1.45 NA oil immersion objective lens (UAPON150XOTIRF) and Evolve 512 EMCCD camera (Photometrics, Tucson, AZ). The system has four laser lines: 405 nm (iFLEX-2000, Excelitas Technologies Corp., Waltham, MA), 473 nm (FB-473-500, RGBLase, LLC, Fremont, CA), 561 nm (iFLEX-Mustang, Excelitas Technologies Corp., Waltham, MA), and 647 nm (OBIS 647 LX, Coherent, Inc., Santa Clara, CA). An acousto-optic tunable filter (AOTFnC-400.650, AA Optoelectronic, Orsay, France) is used to select the laser line and control laser power. Microscope and image acquisition are controlled with Micro-manager Open Source Microscopy Software (Open Imaging, Inc, San Francisco, CA).

SUPPLEMENTARY REFERENCES

1. Karpova, T.S., Kim, M. J., Spriet, C., Nalley, K., Stasevich, T. J., Kherrouche, Z., Heliot, L., and McNally, J. G. (2008) Concurrent fast and slow cycling of a transcriptional activator at an endogenous promoter. Science, **319**, 466-469.
2. Guldener, U., Heck, S., Fielder, T., Beinhauer, J., and Hegemann, J. H. (1996) A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res., **24**, 2519-2524.

SUPPLEMENTARY TABLES

Supplementary Table 1: List of yeast strains used in this study

Sr. no.	Strain ID	Genotype
1	YTK54	his3-d1 leu2-d0 met15-d0 ura3-d0 cup1-d::LEU2
2	YTK82	his3-d1 leu2-d0 ura3-d0 met15-d0 his3-d1 leu2-d0 ura3-d0 lys2-d0
3	YTK1404	his3-d1 leu2-d0 met15-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP ACE1-3xGFP::URA3 his3-d1 leu2-d0 lys2-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP ACE1-3xGFP::URA3
4	YTK1414	his3-d1 leu2-d0 met15-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP his3-d1 leu2-d0 lys2-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP
5	YTK1440	his3-d1 leu2-d0 met15-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP HHT1-HaloTag::URA3 his3-d1 leu2-d0 lys2-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP HHT1-HaloTag::URA3
6	YTK1446	his3-d1 leu2-d0 lys2-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP ACE1-HaloTag::URA3 his3-d1 leu2-d0 met15-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP ACE1-HaloTag::URA3
7	YTK1454	his3-d1 leu2-d0 met15-d0 ura3-d0 HHT1-HaloTag::URA3 his3-d1 leu2-d0 lys2-d0 ura3-d0 HHT1-HaloTag::URA3
8	YTK1479	his3-d1 leu2-d0 met15-d0 ura3-d0 ACE1-3xGFP::URA3 his3-d1 leu2-d0 lys2-d0 ura3-d0 ACE1-3xGFP::URA3
9	YTK1486	his3-d1 leu2-d0 met15-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP HSF1-HaloTag::URA3 his3-d1 leu2-d0 lys2-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP HSF1-HaloTag::URA3
10	YTK1490	his3-d1 leu2-d0 met15-d0 ura3-d0 ACE1-3xGFP::URA3 his3-d1 leu2-d0 lys2-d0 ura3-d0 HHT1-HaloTag::URA3
11	YTK1491	his3-d1 leu2-d0 met15-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP HHT1-HaloTag::URA3 his3-d1 leu2-d0 lys2-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP ACE1-3xGFP::URA3
12	YTK1492	his3-d1 leu2-d0 lys2-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP ACE1-3xGFP::URA3 his3-d1 leu2-d0 met15-d0 ura3-d0 pdr5-d::LoxP-KAN-LoxP

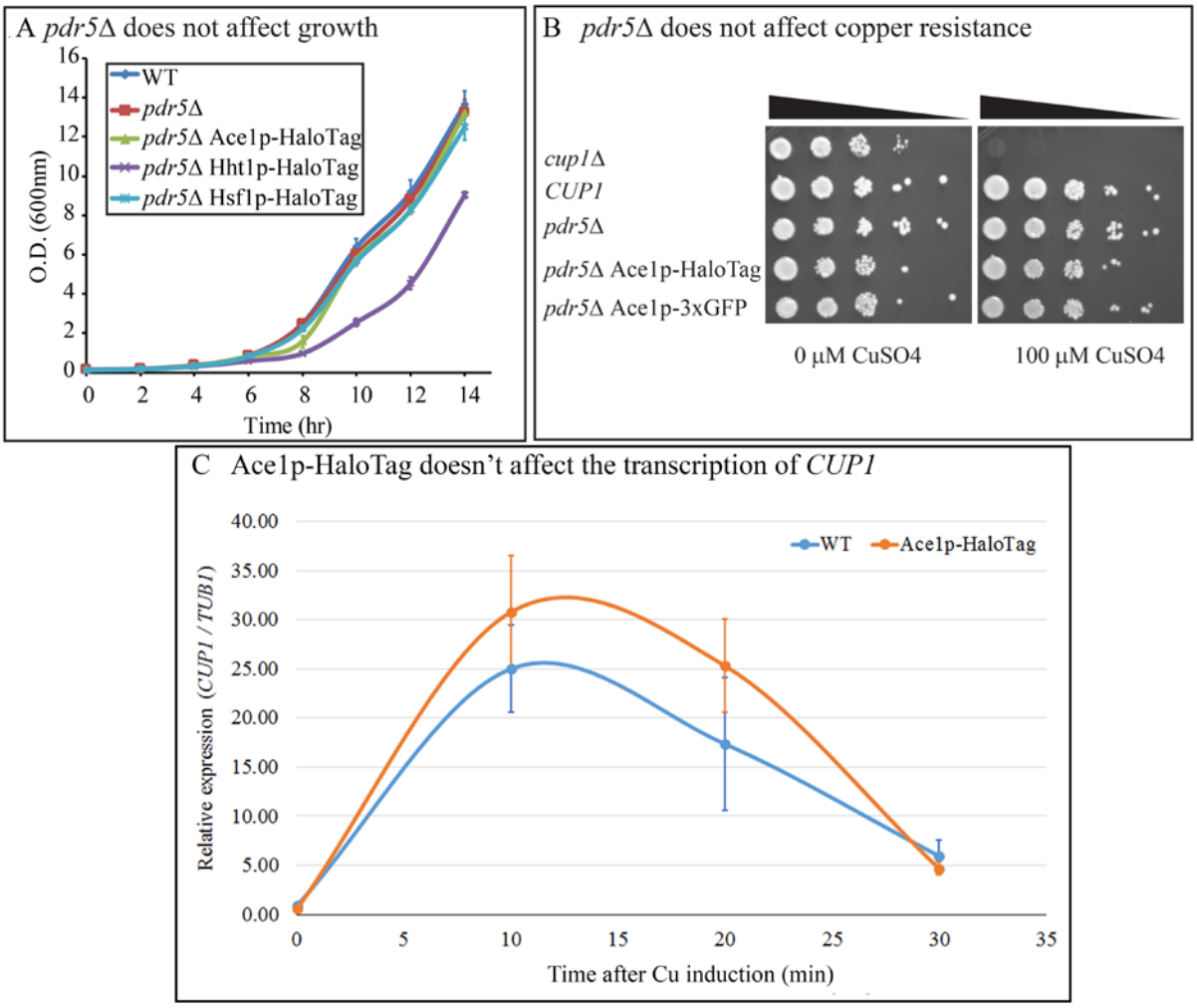
Supplementary Table 2: Primer sequences

Sr. No.	Primer ID	Description	Sequence
1	T682	For constructing pTSK405	catgccgcggggttaatgtggctgtggt
2	T683		ccgagagctcgcgtccatctttacagtcct
3	T900	For amplifying *HALO* tag from pTSK557 and inserting it between NotI and SacII sites of pTSK405	catggcggccgccgctgctgccgctgcagctgctgcagctgcgatgggttccgaaatcggtactggctttcca
4	T901		ccgaccgcggttaaccggaaatctccagagtag
5	T886	For C-terminal *HALO* tagging of *HHT1*	tccaaaagaaggatatcaagttggctagaagattaagaggtgaaagatca caagcggccgccgctgctgc
6	T887		attgtgtttttgttcgtttttttactaaaactgatgacaatcaacaaacta gcgtccatctttacagtcct
7	T888	Diagnostic primers for checking *HHT1-HALO* fusion	ctgttgatcagaaagttgcc
8	T889		gcctggctttttttcgaggg
9	T961	For C-terminal *HALO* tagging of *HSF1*	aggacccgacagagtacaacgatcaccgcctgccaaacgagctaagaaa caagcggccgccgctgctgc
10	T962		ctatattaaatgattatatacgctatttaatgaccttgccctgtgtacta gcgtccatctttacagtcct
11	T798	Diagnostic primers for checking *HSF1-HALO* fusion	acctgccaagctttaatgac
12	T847		atactatattaaatgattatatacgctatttaatgaccttgccctgtgta gcgtccatctttacagtcct
13	T695	For C-terminal *HALO* tagging of *ACE1*	gttggcagcaaggaagatga
14	T697		ggcccgactgccagcttgccgggagaacaaacaaccgccaata gcgtccatctttacagtcct
15	T695	Diagnostic primers for checking *ACE1-HALO* fusion	gttggcagcaaggaagatga
16	T701		aatttatccgaatctcggcc
17	T518	For *PDR5* deletion using pUG6	agacaaaaatgcccgaggccaagcttaacaataacgtcaacgacgtcagctgaagcttcgtacgc
18	T519		tcttggagagtttaccgttctttttaggcactcttgctaaccagtagacataggccactagtggatctg
19	T523	Diagnostic primers for checking deletion of *PDR5*	aggccgaaacaagacgtatt
20	T524		ggcacagttaagaaataatg
21	T531	For quantification of *CUP1* transcription by qPCR	catttcccagagcagcatga
22	T532		gtcatgagtgccaatgccaa
23	TUB1F	For quantification of *TUB1* (reference gene) transcription by qPCR	cgttccaagggctatttacg
24	TUB1R		tacggcaaattccagcttgg

Supplementary Table 3: Concentrations of JF_{646} and TMR used in this study

Protein	**Concentration of JF_{646} used for SM tracking**	**Concentration of TMR used for counter staining of the nuclei**
Hht1p-HaloTag	0.005 nM	100 nM
Ace1p-HaloTag	0.25 nM	1000 nM
Hsf1p-HaloTag	1 nM	1000 nM

SUPPLEMENTARY FIGURES



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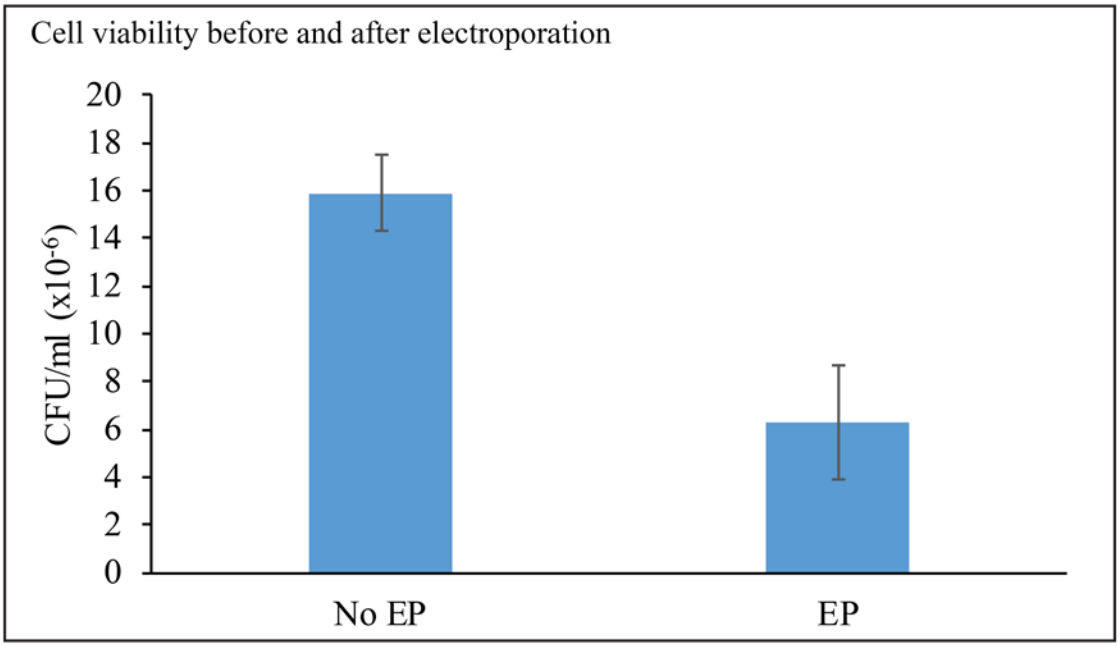
Supplementary Figure S1: TMR staining, pdr5Δ disruption and HaloTag fusions do not affect growth rate and expression of CUP1. (A) Growth curves of WT *PDR5* (YTK82), *pdr5*Δ (YTK1414), *pdr5*Δ Ace1p-HaloTag (YTK1446), *pdr5*Δ Hht1p-HaloTag (YTK1440) and *pdr5*Δ Hsf1p-HaloTag (YTK1486). C-terminal HaloTag fusions of Ace1p and Hsf1p and deletion of *PDR5* had no effect on the growth rate, whereas the growth rate of Hht1p-HaloTag is slightly impeded. Experiment was repeated twice; the error bars represent the standard deviation (s.d.) (B) Growth of progressive dilutions of cell cultures on plates with $CuSO_4$. *pdr5*Δ (YTK 1414), *pdr5*Δ Ace1p-HaloTag (YTK1446), and *pdr5*Δ Ace1p-3xGFP (YTK1404) are as resistant to Cu^{2+} as *PDR5* (YTK82) at the concentration used for induction of transcription in our tests (100 mM $CuSO_4$). Copper-sensitive *cup1*Δ (YTK54) serves as a negative control. (C) Relative quantification of gene expression by RT-PCR shows that Halo tagging of *ACE1* (YTK1446) doesn't affect the transcriptional output from *CUP1* gene; slow cycle of *CUP1* transcription is similar to the wild-type strain (YTK82). Data for *CUP1* are normalized to the expression level of the *TUB1* gene, which does not change in response to copper. Error bars represent standard error of the means (s.e.m) for three independent mRNA extractions.



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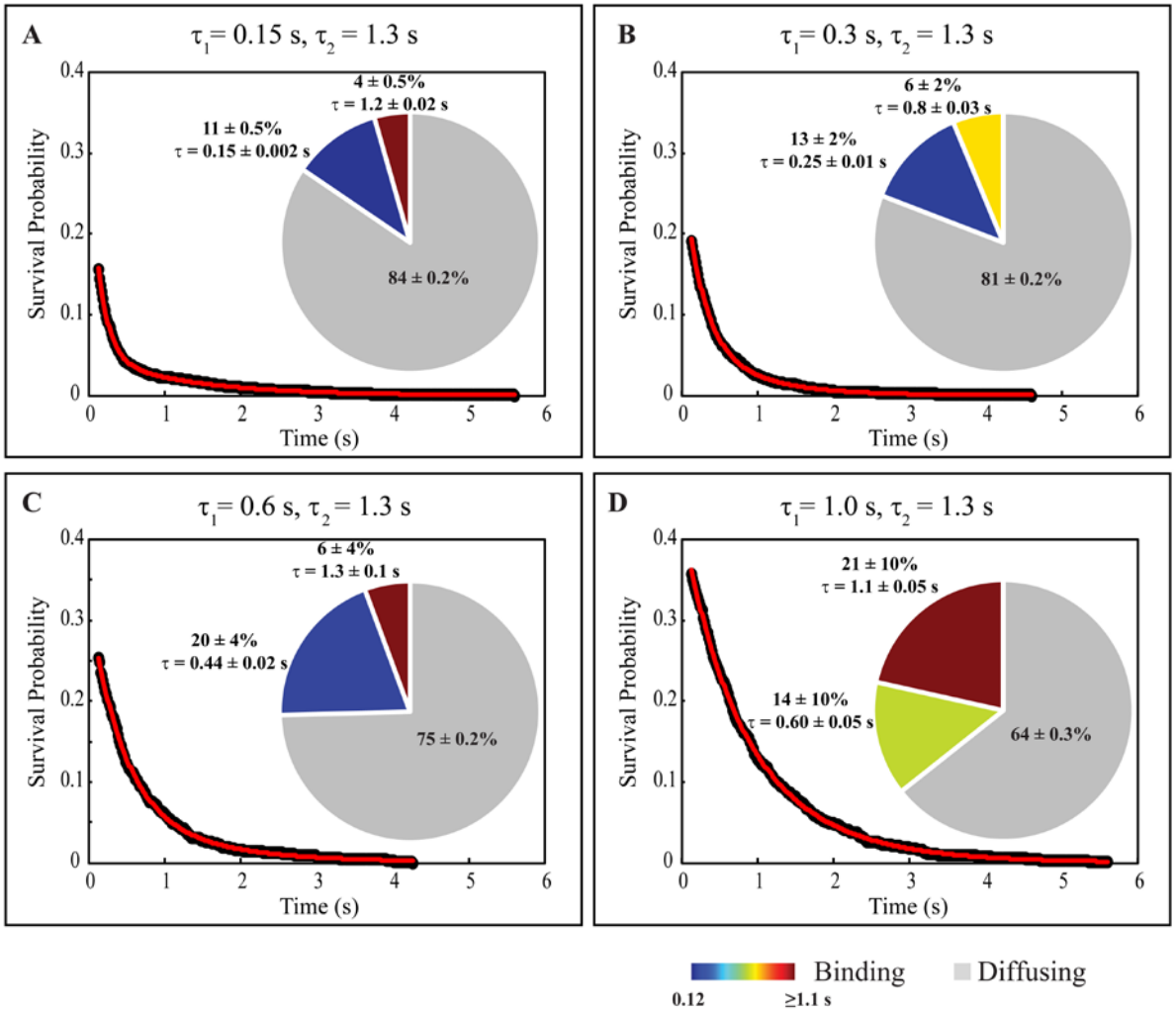
Supplementary Figure S2: Cell viability was quantified before and after electroporation using plate assay. 10 times serial dilutions of the cell suspension were spread on YPD agar plates and plates were incubated at 30 °C for 48 hours. Colonies were counted manually and Colony Forming Units (CFU) were calculated per ml of the cell suspension used for electroporation. Experiment was repeated twice and the error bars indicate s.e.m.



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Supplementary Figure S3: Sensitivity of curve fitting to residence time values. Simulations were conducted of 2-component binding to evaluate the performance of the curve fitting by setting the fast binding component's residence time to (A) 0.15 s, (B) 0.3 s, (C) 0.6 s, and (D) 1 s, while keeping the slow binding component's residence time fixed at 1.3 s, and the fraction of the fast component at 85%. Graphs show the survival plot from the simulations (black dots), and the best-fitting two-component exponential (red line). Pie charts show the extracted populations, with the extracted residence times next to the corresponding slices.