

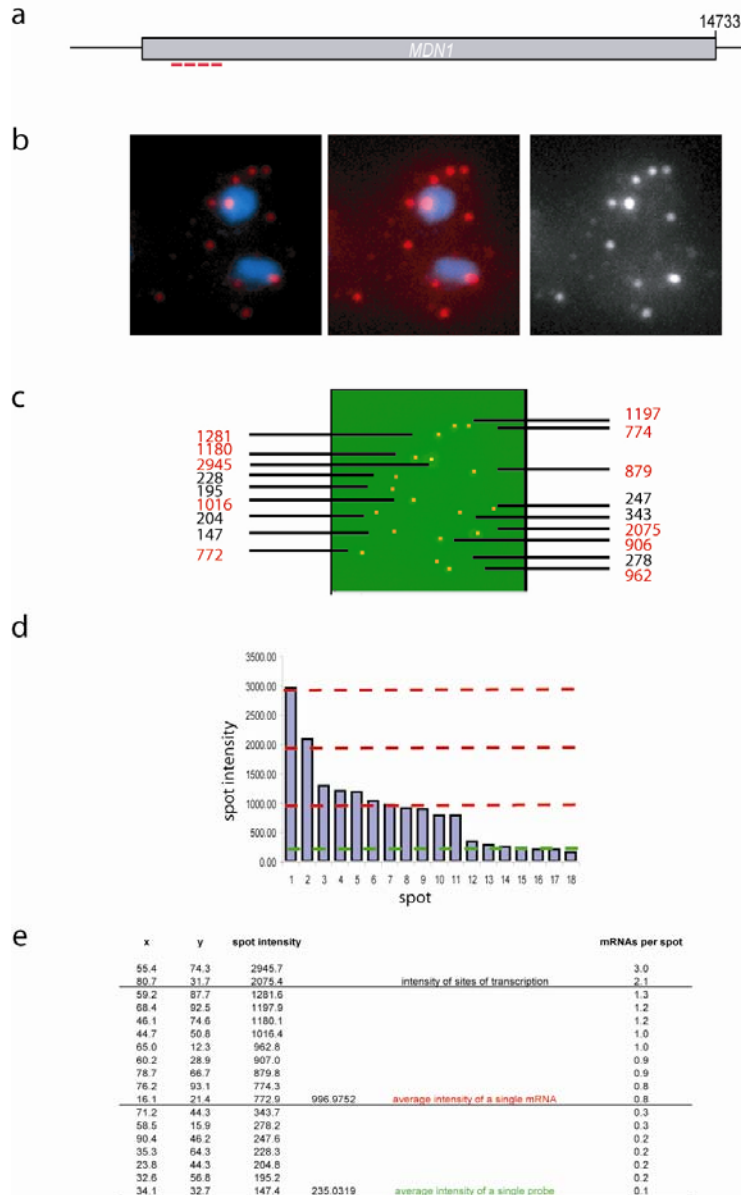
Supplemental Materials for

Single RNA counting reveals alternative modes of gene expression in yeast

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Supplementary Figure 1: Intensity of a single mRNA can be calculated by determining the fluorescence intensity emitted from a single probe. (a) FISH probes hybridizing to the 5' end of *MDN1* mRNA we used for hybridization shown in (b). A small number of single probes tend to hybridize unspecifically to the cells what can be visualized by changing the contrast levels (b, compare left and middle panel). The intensity is determined using the same spot detection algorithm as used in Figure 2, but by changing the settings to allow the detection of lower intensity spots (c). Signal intensity of each spot corresponding to a single DNA probe is shown in black, signal intensities of single mRNA and sites of transcription are shown in red. Consistent with the 4 probes used in the hybridization (a), intensity of single mRNA signals in the cytoplasm is 4 times the intensity of a single probe (d, e). Nascent mRNAs at the site of transcription are 2 resp. 3 times the intensity of a single mRNA in the cytoplasm, as also shown in Figure 2d.

Supplementary Tables

Supplemental Table 1: Parameters for the three scenarios of *MDN1* transcription shown in Figure 5.

	Figure 5				
	a (min ⁻¹)	b (min ⁻¹)	c (min ⁻¹)	c/b	a/(a+b)
<i>MDN1</i> sc 1	0.90	2.52	0.60	0.24	0.26
<i>MDN1</i> sc 2	0.80	0.20	0.20	1.00	0.80
<i>MDN1</i> sc 3	0.30	0.03	0.17	6.80	0.92

Supplemental Table 2: Parameters used in the numerical simulations. Length is the distance from the most 3' probe to the end of the gene. The critical χ^2 values are those used in model discrimination. The curves in Figure 3 and 6 are calculated from the tabulated parameters and the numerical model described in Materials and Methods. τ is the value which minimizes the nascent chain distribution χ^2 .

gene	length (bases)	nascent chains critical χ^2_m (P=10%)*	mRNA abundance critical χ^2_N (P=10%)†	global fit critical χ^2_m (P=10%)*	a (min ⁻¹)	Figure 3,6		τ (min)
					b (min ⁻¹)	c (min ⁻¹)		
<i>MDN1</i>	13474	4.61	19.81	25.99	0.70	0.12	0.19	16.75
<i>KAP104</i>	1645	2.71	15.99	21.06	0.70	0.12	0.27	3.50
<i>DOA1</i>	1701	2.71	14.68	19.81	0.70	0.12	0.14	2.90
<i>POI1</i>	2575	4.61	24.77	30.81	0.07	0.68	2.00	3.75
<i>PDR5</i>	3070	17.27	54.10	74.40	0.44	4.70	6.70	4.50

* 4 free parameters: *a*, *b*, *c*, τ

† 3 free parameters: *a*, *b*, *c*

Supplemental Table 3: Parameters for the three genes in Figure 7.

	Figure 7 black circles				
	a (min ⁻¹)	b (min ⁻¹)	c (min ⁻¹)	X ² _m	X ² _N
<i>MDN1</i>	0.80	0.20	0.20	1.94	2.14
<i>POL1</i>	0.06	0.08	0.32	4.42	24.25
<i>PDR5</i>	0.44	4.70	6.70	10.60	50.80

	Figure 7 blue circles				
	a (min ⁻¹)	b (min ⁻¹)	c (min ⁻¹)	X ² _m	X ² _N
<i>MDN1</i>	0.30	2.28	1.30	16.13	6.28
<i>POL1</i>	0.07	0.68	2.00	21.04	9.50
<i>PDR5</i>	0.30	5.30	11.30	36.19	32.32

Supplemental Table 4: Summary of the expression state of genes measured in this study and compared to Holstege et al.¹ and Ghaemmaghami et al.².

	<i>gene</i>	<i>MDN1</i>	<i>DOA1</i>	<i>KAP104</i>	<i>POL1</i>	<i>PDR5</i>
<i>in situ</i>	<i>average</i>	6.12 +/- 0.42	2.59 +/- 0.53	4.93 +/- 0.59	3.13 +/- 0.59	13.4 +/- 1.2
	<i>stdev</i>	2.80 +/- 0.21	1.61 +/- 0.35	2.36 +/- 0.35	3.56 +/- 0.64	10.16 +/- 0.97
	<hr/>					
<i>Holstege et al.</i>	<i>mRNAs per cell</i>	1.20	1.10	0.80	0.70	12
<i>Ghaemmaghami et al.</i>	<i>proteins per cell</i>	538	6800	2130	1050	42000

Supplemental Table 5: FISH probes used in this study.

Probes used in this study are listed below. Bold letters show modified bases labeled with either cy3 cy3.5 or cy5. A schematic view of the position of the probes on the corresponding mRNAs is shown in Figures 3, 4 and 6.

MDN1 region 1	
MDN1 794	TTT GTC GTG GAT AGT GTG GAC CTT AGG GAC GAT AAC GCC ACA GAT TGA CG
MDN1 860	CTC CCG AGT TGA CGA AGA GAG GAA ACC GTT TTA TGA GTA GGG ACA AAG GTT
MDN1 1104	CTA TAA GTA CCC ATC TCC CTT CTT TGA CCG CGG TAG CGA GAA CAC CAG CTC
MDN1 1210	TTT GCA GCC TTT ACA GTC TCT CCT CTG GAT GGA ATG GTT AGT TCG CGC TT
MDN1 region 2	
MDN1 4350	CAC CTT TCT GCA AGA AGC ATA TAG CCA CTG GCA GCA AGC TGC TCA TAT CC
MDN1 4511	CTA AGA CGG ATA GTC GGC GCA TTC CTT TTG TCC AAG TAA CAG AGC CAA TG
MDN1 5028	AGG CTA TCG GAT GAA CCC TGT TCT GCT AAC AAC AAA CTC CTT TCT GGC TC
MDN1 5286	ATG ACA CCA CTT GTT GCA TTT CCG CCT CCC AGC TTC TTG CCA AAC CAT TC
MDN1 region 3	
MDN1 8912	GTA GGT TAG GTG AAC ACT TGA TAG TGG AGG AAA GTA GCA ATT CTG CGT CAC TA
MDN1 9393	ATA GAC ATG CCT GTA CGA TAT ACC CTA GGG GAT TGA GGT GCG TCG TCA TC
MDN1 9204	ATT GCC GGA TCA TAG GGA GAA TCT GGG ACG AAT AGC AGA AGC AAA CCT GT
MDN1 10551	GTG TTA TTC AGG GAT ACT TCC GAG GAA GCG TAT TTT TCC CAT TCA GCT AG
MDN1 region 4	
MDN1 13668	ATC CTG ACT TTT TGC TGT CAA CGT CAT TGT TAG CGT CAT GCG CGT CGA TA
MDN1 13576	TCG GCG TCT TCT ACT AAC TCT TTC TGG TCC ACT TCT TGC TCC TCC CTA TC
MDN1 13232	ATT CTG AGT CGT TCC TGA ACC TCC AAC ATC GTC CTG TTC TTG TGT GTC CG
MDN1 13111	ATC AGC CTT TTC CTC AAC GCC ATC AAG ACC TTC TGT ATT CTG CTC ACC TCC
KAP104	
KAP104 99	CTA GTT GCA ACA CAT AGT CTT CGG CGG GCT TCC ATG TCG ATG CCA TCT TT
KAP104 425	ATA CCG GCG GTA GCT CTA TTG TTC TGA AGA TCC TGT AGG GAG TAG TGT TG
KAP104 1421	CCT GCC TCT TTC TTT TTC ACA ATA CGG GGT GCA ATG GGC TTG ATG TCC TC
KAP104 2870	AGT CCT TCT CAG GCA CTA CTA TTG TCG GGT CTT GAT GTG ATT TGG CTT CC
DOA1	
DOA1 555	GTT CCA TCC CTC GAA ACA CTA GCA ACC TTT GAA TCA TCC ACA GCT ACC AC
DOA1 614	ATC CTT GTC CTG TAT AAA CTA CCG TAC CGA GCC ATT GGT CAT CTT TAG ACC AC
DOA1 899	CTG AGA AGG ATA CGA CTT TGG CAT CCC ATA CAG AGG CAT TGT GTG CTT GC
POL1	
POL1 1856	CGC TAG ATA AAT CCG ATG GAA TGG TGT CTC GAC TGG ACT TCG GTG TTT GG
POL1 2034	GTA GGC GTG ATA TTC TGA GGC TTA TCT ACC GAA ACT TCA ACC GCA CAG TG
POL1 2283	CTG ACT CTA CCG GGT AGT TTC TGT TTT GCC AGT GCA GCT AAA CCT AAT GG
PDR5	
PDR5 111	TCT GTG CGG TCA GAG TCC TTG CCA GTT TTT GGA TTC GAG CTT CTG TAT GC
PDR5 219	CTG GAG CTT CAG GAT CAG AGA ATA TCG GGT TTA CAC CTT CCA CAC CGC TA
PDR5 797	AGA ACT TAA GTG CTT CTG GTG CTT CCG CAG ATG TCG CCT ATC AGT CAA CT
PDR5 1417	TTG GAC TGC TTA GCA ATG TGG GCT TCC TTA ATA GCT TCA CGG CTT GCT TC
CCW12	
CCW12-59	GGT GAC CAA AGT GGT AGA TTC TTG GCT GAC AGT AGC AGT GGT AAC GTT AG
CCW12-140	GTC ATC GAC GGT GAC GGT AGC GGT GGA AAC CAA AGC TGG GGA GAC AGT TT
CCW12-191	CTT TGG GGC TTC AGT GGT CAA TGG GCA CCA GGT GGT GTA TTG AGT GAT AA
CCW12-245	GGT GTT CTT TGG AGC TTC AGT AGA GGT AAC TGG AGC AGC AGT AGA AGT AC
rRNA-ITS2	
ITS2-1	ATA GGC CAG CAA TTT CAA GTT AAC TCC AAA GAG TAT CAC TC

1. Holstege, F.C. et al. Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* **95**, 717-28 (1998).
2. Ghaemmaghani, S. et al. Global analysis of protein expression in yeast. *Nature* **425**, 737-41 (2003).