Supplemental Materials for

Single RNA counting reveals alternative modes of gene expression in yeast

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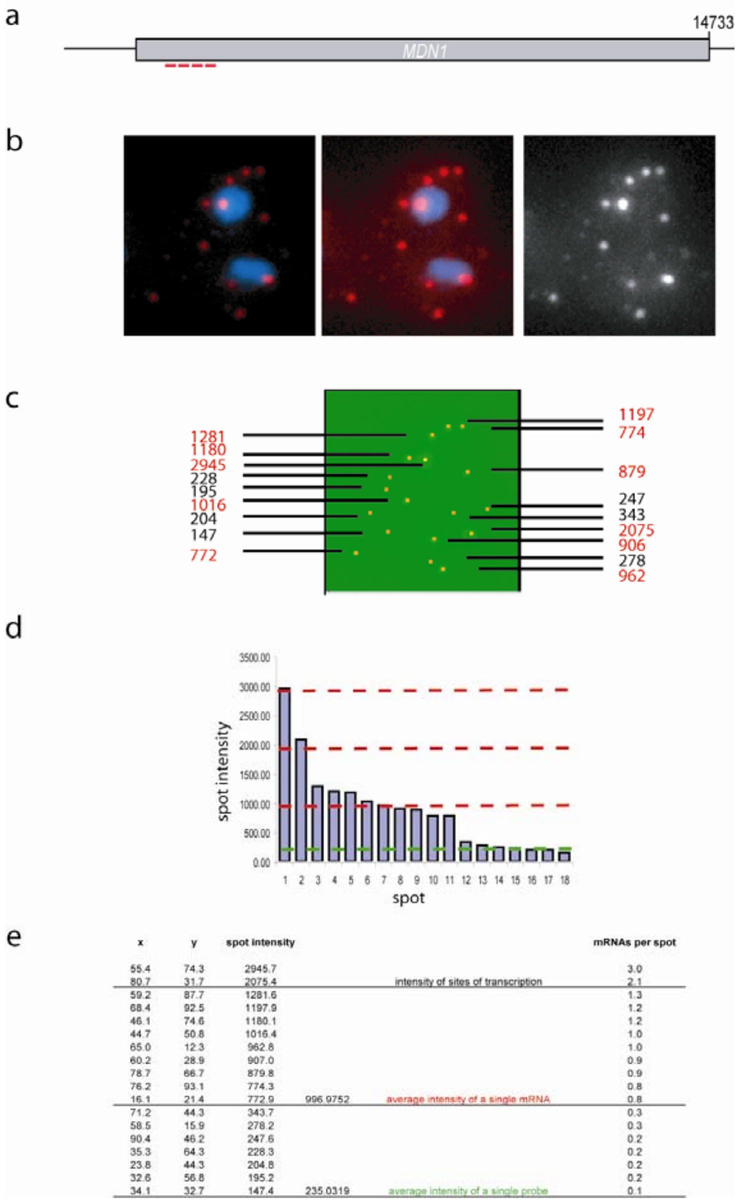

x	y	spot intensity			mRNAs per spot
55.4	74.3	2945.7			3.0
80.7	31.7	2075.4		intensity of sites of transcription	2.1
59.2	87.7	1281.6			1.3
68.4	92.5	1197.9			1.2
46.1	74.6	1180.1			1.2
44.7	50.8	1016.4			1.0
65.0	12.3	962.8			1.0
60.2	28.9	907.0			0.9
78.7	66.7	879.8			0.9
76.2	93.1	774.3			0.8
16.1	21.4	772.9	996.9752	average intensity of a single mRNA	0.8
71.2	44.3	343.7			0.3
58.5	15.9	278.2			0.3
90.4	46.2	247.6			0.2
35.3	64.3	228.3			0.2
23.8	44.3	204.8			0.2
32.6	56.8	195.2			0.2
34.1	32.7	147.4	235.0319	average intensity of a single probe	0.1

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)
MDN1 sc 1	0.90	2.52	0.60	0.24	0.26
MDN1 sc 2	0.80	0.20	0.20	1.00	0.80
MDN1 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.

						Figure 3,6		
gene	length (bases)	nascent chains critical X^2_m (P=10%)*	mRNA abundance critical X^2_N (P=10%)†	global fit critical X^2_m (P=10%)*	a (min^{-1})	b (min^{-1})	c (min^{-1})	τ (min)
MDN1	13474	4.61	19.81	25.99	0.70	0.12	0.19	16.75
KAP104	1645	2.71	15.99	21.06	0.70	0.12	0.27	3.50
DOA1	1701	2.71	14.68	19.81	0.70	0.12	0.14	2.90
POl1	2575	4.61	24.77	30.81	0.07	0.68	2.00	3.75
PDR5	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
MDN1	0.80	0.20	0.20	1.94	2.14
POL1	0.06	0.08	0.32	4.42	24.25
PDR5	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
MDN1	0.30	2.28	1.30	16.13	6.28
POL1	0.07	0.68	2.00	21.04	9.50
PDR5	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].

	gene	***MDN1***	***DOA1***	***KAP104***	***POL1***	***PDR5***
in situ	*average*	***6.12*** +/- 0.42	***2.59*** +/- 0.53	***4.93*** +/- 0.59	***3.13*** +/- 0.59	**13.4** +/- 1.2
	stdev	2.80 +/- 0.21	1.61 +/- 0.35	2.36 +/- 0.35	3.56 +/- 0.64	10.16 +/- 0.97
Holstege et al.	*mRNAs per cell*	1.20	1.10	0.80	0.70	12
Ghaemmaghami et al.	*proteins per cell*	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	TT**T** GTC GTG GAT AG**T** GTG GAC CT**T** AGG GAC GA**T** AAC GCC ACA GAT **T**GA CG
MDN1 860	C**T**C CCG AGT **T**GA CGA AGA GAG GAA ACC G**T**T TTA TGA G**T**A GGG ACA AAG G**T**T
MDN1 1104	C**T**A TAA GTA CCC A**T**C TCC CTT CTT **T**GA CCG CGG **T**AG CGA GAA CAC CAG C**T**C
MDN1 1210	TT**T** GCA GCC TTT ACA GTC **T**CT CCT CTG GA**T** GGA ATG GT**T** AGT TCG CGC **T**T
MDN1 region 2	
MDN1 4350	CAC CT**T** TCT GCA AGA AGC A**T**A TAG CCA C**T**G GCA GCA AGC **T**GC TCA TA**T** CC
MDN1 4511	C**T**A AGA CGG ATA G**T**C GGC GCA TTC C**T**T TTG TCC AAG **T**AA CAG AGC CAA **T**G
MDN1 5028	AGG C**T**A TCG GAT GAA CCC **T**GT TCT GC**T** AAC AAC AAA C**T**C CTT TCT GGC **T**C
MDN1 5286	A**T**G ACA CCA CTT GT**T** GCA TTT CCG CC**T** CCC AGC TTC **T**TG CCA AAC CA**T** TC
MDN1 region 3	
MDN1 8912	G**TA** GGT TAG G**T**G AAC ACT TGA TAG **T**GG AGG AAA GTA GCA AT**T** CTG CGT CAC **T**A
MDN1 9393	ATA GAC ATG CC**T** GTA CGA TAT ACC C**T**A GGG GAT TGA GG**T** GCG TCG TCA **T**C
MDN1 9204	AT**T** GCC GGA TCA **T**AG GGA GAA TC**T** GGG ACG AA**T** AGC AGA AGC AAA CC**T** GT
MDN1 10551	GTG TTA T**T**C AGG GAT ACT **T**CC GAG GAA GCG TAT T**T**T TCC CAT TCA GC**T** AG
MDN1 region 4	
MDN1 13668	ATC C**T**G ACT TTT TGC TG**T** CAA CGT CAT TG**T** TAG CGT CA**T** GCG CGT CGA **T**A
MDN1 13576	TCG GCG **T**CT TCT ACT AAC **T**CT TTC TGG **T**CC ACT TCT TGC **T**CC TCC CTA **T**C
MDN1 13232	AT**T** CTG AGT CGT TCC **T**GA ACC TCC AAC A**T**C GTC CTG T**T**C TTG TGT G**T**C CG
MDN1 13111	A**T**C AGC CTT TTC C**T**C AAC GCC ATC AAG ACC **T**TC TGT AT**T** CTG CTC ACC **T**CC
KAP104	
KAP104 99	CTA G**T**T GCA ACA CAT AG**T** CTT CGG CGG GC**T** TCC ATG TCG A**T**G CCA TCT **T**T
KAP104 425	A**T**A CCG GCG GTA GC**T** CTA TTG TTC **T**GA AGA TCC TG**T** AGG GAG TAG TGT **T**G
KAP104 1421	CCT GCC **T**CT TTC TTT T**T**C ACA ATA CGG GG**T** GCA ATG GGC **T**TG ATG TCC **T**C
KAP104 2870	AGT CC**T** TCT CAG GCA C**T**A CTA TTG **T**CG GGT CTT GAT G**T**G ATT TGG CT**T** CC
DOA1	
DOA1 555	G**T**T CCA TCC C**T**C GAA ACA C**T**A GCA ACC TTT GAA **T**CA TCC ACA GC**T** ACC AC
DOA1 614	A**T**C CTT GTC CTG **T**AT AAA CTA CCG **T**AC CGA GCC AT**T** GGT CAT CTT **T**AG ACC AC
DOA1 899	C**T**G AGA AGG ATA CGA C**T**T TGG CAT CCC A**T**A CAG AGG CA**T** TGT GTG CT**T** GC
POL1	
POL1 1856	CGC **T**AG ATA AAT CCG A**T**G GAA TGG TGT C**T**C GAC TGG AC**T** TCG GTG TT**T** GG
POL1 2034	G**T**A GGC GTG ATA TTC **T**GA GGC TTA TC**T** ACC GAA ACT **T**CA ACC GCA CAG **T**G
POL1 2283	C**T**G ACT CTA CCG GG**T** AGT TTC TGT TT**T** GCC AGT GCA GC**T** AAA CCT AA**T** GG
PDR5	
PDR5 111	**T**CT GTG CGG **T**CA GAG TCC TTG CCA G**T**T TTT GGA T**T**C GAG CTT CTG TA**T** GC
PDR5 219	C**T**G GAG CTT CAG GA**T** CAG AGA ATA TCG GG**T** TTA CAC CT**T** CCA CAC CGC **T**A
PDR5 797	AGA AC**T** TAA GTG CT**T** CTG GTG C**T**T CCG CAG ATG **T**CG CCT ATC AG**T** CAA CT
PDR5 1417	T**T**G GAC TGC TTA GCA A**T**G TGG GCT TCC **T**TA ATA GCT **T**CA CGG CTT GCT **T**C
CCW12	
CCW12-59	GG**T** GAC CAA AGT GG**T** AGA TTC TTG GC**T** GAC AGT AGC AG**T** GGT AAC GT**T** AG
CCW12-140	G**T**C ATC GAC GG**T** GAC GGT AGC GG**T** GGA AAC CAA AGC **T**GG GGA GAC AGT **T**T
CCW12-191	C**T**T TGG GGC T**T**C AGT GGT CAA TGG GCA CCA GG**T** GGT GTA TTG AGT GA**T** AA
CCW12-245	GG**T** GTT CTT TGG AGC **T**TC AGT AGA GG**T** AAC TGG AGC AGC AG**T** AGA AGT AC
rRNA-ITS2	
ITS2-1	A**T**A GGC CAG CAA TTT CAA GT**T** AAC TCC AAA GAG TAT CAC **T**C

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