Supporting Information

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SI Text

Strains. All cloning was carried out in the *E. coli* SURE 2 strain: *e*14-, (*mcr*A-), Δ(*mcr*CB-*hsd*SMR-*mrr*)171, *end*A1, *sup*E44, *thi*-1, *gyr*A96, *rel*A1, *lac*, *rec*B, *rec*J, *sbc*C, *umu*C::Tn5(*kan*^{r}), *uvr*C, [F', *pro*AB, *lac*I^{q}ZΔM15::Tn10(*tet*^{r})]. Replication studies were performed in *E. coli* SURE 2, or DH5α (φ80d*lac*ZΔM15, *rec*A1, *end*A1, *gyr*AB, *thi*-1, *hsd*R17(r_{K-}, m_{K+}), *sup*E44, *rel*A1, *deo*R, Δ(*lac*ZYA-*arg*F) U169, *pho*A) strains, *S. cerevisiae* CH1585 strain (*MATa leu2-Δ1, trp1-Δ63, ura3–52, his3–200*), and *COS-1* fibroblasts (ATCC CRL-1650).

The *tof1*Δ and *mrc1*Δ *S. cerevisiae* strains were obtained by one-step gene disruption using a kanamycin cassette PCR-amplified from the pFA6A-KanMX4 plasmid (1) using the following hybrid primers: 5′-ATGAGTATTAAGGATTTTTCAAATGAGATATGGTTCGTAA GTCGCCTCACATATGATAATACCAGATCTGTTTAGCTTGCCTC-3′; Tof1-R, 5′-GTGAAGTAGTTCGTCACACTATGCAACAAGATGTTAAAGCCGGAATCTATAAAGTTGTTGATGAATTCGAGCTCGTTTTCGAC-3′; Mrc1-F, 5′-GCATTTCAAACACATTATGTTGGAAAAAAACCAAGAACAGACAAACAACTAAGGAAGTTCGTTATTCGCCAGATCTGTTTAGCTTGCCT-3′; Mrc1-R, 5′-CGCCTTTGATAACTGAGAGATTGGCAAAACGCTTTCATCTTTGGATAATTTAATGCTATCCATATCATCGATGAATTCGAGCTCGTT-3′.

Plasmids. The blunt-ended BamHI, XhoI fragments of plasmids pHS-D, pHS-I, p36-I, p39-I, and p14-I (2), containing various *Alu* repeats, were cloned into the SmaI site of the pSV2neo (Invitrogen), generating plasmids pSV-D100, pSV-I100, pSV-I94, pSV-I86, and pSV-I75 as well as between the BamHI and XhoI fragment of the pYES2 (Invitrogen), generating plasmids pYES-D100 and pYES-I100.

To generate an inverted *Alu* repeat with a 52-bp-long spacer (pSV-I100-s52), the following oligonucleotides were annealed and ligated into the SalI site of pSV-I100: 5′-TCGAACACTTAAGCTTCAATGTGATCTGATGCGGCCGCTC-3′ and 5′-TCGAGAGCGGCCGCATCAGATCACATTGAAGCTTAAGTGT-3′. To obtain an inverted *Alu* repeat without a spacer sequence (pSV-I100-s0), the two halves of the inverted repeat were PCR-amplified from the pSV-I100 by using the following primers: P1, 5′-GCCGCGGGCGCCGCGGATAGCCGCTGCTGGTTTCCTGGAT-3′, which anneals before the repeat upstream of the Eco81I site; P2, 5′-*TATACTTAAGGGGCCC*CGCGGCGGCCGGGCGCGGTGGCTCA-3′, which anneals to the 5′ end of the *Alu* sequence and contains a flap with the ApaI site (flap sequence italicized; ApaI site underlined), and P3, 5′- GGCGCCCGCGGCGGACGCCGGCTGGATGATCCTCCA-3′, which anneals after the *Alu* repeat downstream from the BshTI site.

The Eco81I/BshTI fragment of pSV2neo was ligated with the P1-P2 PCR product cleaved with Eco81I and ApaI and the P2-P3 PCR product cleaved with ApaI and *Bsh*TI. The resulting spacer-less construct was confirmed by DNA sequencing. To generate plasmids pYES-I100-s52 and pYES-I100-s0, the BamHI-XhoI fragments of pSV-I100-s52 and pSV-I100-s0 were ligated with the BamHI-XhoI fragment of the pYES2.

Isolation of Replication Intermediates. Replication intermediates from *E. coli* were isolated as described (3). To isolate replication intermediates from yeast cells, 200 ml of culture was grown in complete synthetic medium without uracil to OD_{600} = 2.0. The growth was stopped by the addition of NaN_3 to the final concentration of 0.1%. Cells were quickly chilled on ice and then centrifuged at 2,500 × *g* for 5 min. The pellet was resuspended in 4 ml of NIB buffer (17% glycerol, 50 mM morpholinepropanesulfonic acid, 150 mM NaOAc, 2 mM $MgCl_2$, 0.5 mM spermidine, 0.15 mM spermine, pH 7.2); cells were disrupted with an equal volume of glass beads by vortexing for 15 min with chilling on ice every 30 s. The cell lysate was centrifuged at 13,000 × *g* for 25 min; the pellet was resuspended in 5 ml of buffer G2 (Qiagen), incubated at 37°C for 30 min and then treated with 50 μl proteinase K (20 mg/ml) for 1 h. After centrifugation at 12,000 × *g* for 10 min, the supernatant was mixed with an equal volume of buffer QBT (Qiagen) and loaded on a Qiagen Genomic-tip 100/G column. From this step, the protocol for DNA isolation on Qiagen Genomic-tip 100/G was followed.

COS-1 cells were grown in DMEM (Invitrogen) with 10% calf serum (Biologos). Cells were transfected at 40–50% confluency with 1 μg of plasmid DNA by using Lipofectin reagent (Invitrogen). Episomal DNA was extracted at 24 h posttransfection by using Hirt's protocol (4). Briefly, cells were lysed in 10 mM Tris·HCl (pH 8), 75 mM EDTA, 10 mM NaCl, 0.6% SDS, 200 μg/ml proteinase K; upon lysis, the NaCl concentration was adjusted to 1 M, the cell lysate was incubated at 4°C overnight and then centrifuged at 17,000 × *g* for 45 min. The plasmid DNA in the supernatant was phenol-chlorophorm-extracted and precipitated with isopropanol.

2D Electrophoresis. Bacterial replication intermediates were digested with EcoRI/NcoI or EcoRI /CpoI (pSVD and pSVI100-s52), generating a 3-kb fragment that was analyzed by 2D electrophoresis. Replication intermediates isolated from *COS-1* cells were digested with DpnI for 2 h and then cleaved with EcoR*I*/NcoI. Yeast plasmid replication intermediates were digested with BglI/BseRI or BglI /*BsaBI* (pYESI100-s0 and pYESI100-s52).

After restriction digest, DNA was precipitated with isopropanol and loaded on a 0.4% agarose gel to run for 13 h at 1 V/cm. The second dimension was run at 4°C in 1% agarose with 0.3 μg/ml of ethidium bromide at 5 V/cm for 6 h. The gel was transferred on a Hybond XL nylon membrane and hybridized with a ^{32}P-labeled probe. Our probe was the distal 1 kb of the restriction fragment analyzed by electrophoresis.

Quantitative analysis of 2D gels was performed on either a Storm 860 PhosphorImager using Imagequant software or Bio-Rad Pharos FX PhosphorImager using Quantity One software. The severity of replication fork slow-down was calculated as the ratio between the maximum radioactive count of the bulge (Fig. 4*A*) and an average between the radioactive counts of two points on the adjacent arc (Fig. 4*A*). The values for each construct are averages of at least three independent experiments with corresponding standard deviations. The comparison of replication slowing in WT and mutant yeast strains were carried out by *t* test. Differences were considered significant for $P < 0.05$.

1. Wach A, Brachat A, Pohlmann R, Philippsen P (1994) New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast* 10:1793–1808.
2. Lobachev KS, *et al.* (2000) Inverted Alu repeats unstable in yeast are excluded from the human genome. *EMBO J* 19:3822–3830.
3. Krasilnikova MM, Samadashwily GM, Krasilnikov AS, Mirkin SM (1998) Transcription through a simple DNA repeat blocks replication elongation. *EMBO J* 17:5095–5102.
4. Hirt B (1967) Selective extraction of polyoma DNA from infected mouse cell cultures. *J Mol Biol* 26:365–369.

Table S1. Primary quantification data

	Construct	Exp.	Peak	Baseline1	Baseline 2	Ratio	Average	SD
E. coli								
	I-100	1	14,116	2169	2167	6.51107011	5.97965739	0.46781583
		2	13,298	2495	2229	5.6299746		
		3	1119	207	179	5.79792746		
	I-94	1	1719	541	682	2.8111202	3.31307026	0.47565149
		2	418	134	114	3.37096774		
		3	4022	1040	1101	3.75712284		
	I-86	1	1293	794	602	1.85243553	2.31300968	0.46761164
		2	6193	2504	2883	2.29923891		
		3	4673	1616	1737	2.78735461		
	I-75	1	124	65	66	1.89312977	2.1235609	0.27946862
		2	379	190	181	2.04312668		
		3	297	109	135	2.43442623		
	I-100-s52	1	5637	947	1011	5.75791624	6.10768642	0.60151728
		2	5435	907	691	6.80225282		
		3	8215	1502	1349	5.76289021		
COS-1 cells								
	I-100	1	266	100	96	2.71428571	2.66672863	0.1369734
		2	255	105	98	2.51231527		
		3	147	54	52	2.77358491		
	I-94	1	495	245	270	1.9223301	1.71983442	0.16158169
		2	57	39	25	1.78125		
		3	72	50	40	1.6		
		4	104	80	52	1.57575758		
	I-86	1	458	301	316	1.48460292	1.35425042	0.1231708
		2	2049	1531	1531	1.33834095		
		3	1931	1557.5	1557.5	1.23980738		
	I-100-s52	1	1886	1492	1486	1.26662189	1.25463245	0.0808777
		2	2983	2553	2553	1.1684293		
		3	1382	1152	928	1.32884615		
	I-100-s0	1	1175	602	611	1.93734542	2.32583025	0.35647047
		2	639	256	276	2.40225564		
		3	550	207	210	2.63788969		
Yeast strain								
WT	I-100	1	100	56	48	1.92307692	1.58748767	0.35881155
		2	123	93	81	1.4137931		
		3	537	404	424	1.29710145		
		4	3691	3065	2712	1.27782586		
		5	790	415	365	2.02564103		
WT	I-100-s0	1	2964	1535	1429	2	1.66282165	0.29244148
		2	85	56	59	1.47826087		
		3	37	24	25	1.51020408		
tof1Δ	I-100-s0	1	100	50	39	2.24719101	2.2776832	0.07547728
		2	26	10	12	2.36363636		
		3	30	15	12	2.22222222		
mrc1Δ	I-100-s0	1	170	79	70	2.28187919	2.46373887	0.15950743
		2	43	17	17	2.52941176		
		3	347	150	119	2.57992565		