

Supplemental Information

Ultrafine anaphase bridges, broken DNA and illegitimate recombination induced by a replication fork barrier

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Supplemental Data

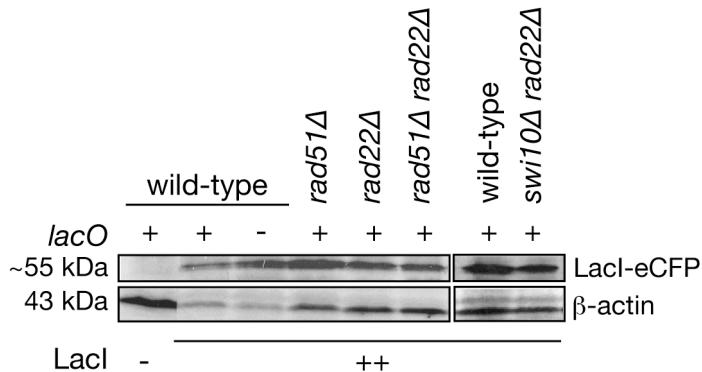


Figure S1 related to Figure 3: Example of western blot analysis showing the amount of NLS-LacI-ECFP expressed from the pREP41 *nmt* promoter (++) levels) in strains used in the recombination assays. Protein was extracted by glass bead disruption of colonies (approximately 1000 colonies per sample) taken from the same plates from which colonies for the recombination assay were picked. Blots were probed with anti-GFP (Sigma) and anti-β-actin (Abcam), the latter being used for the loading control.

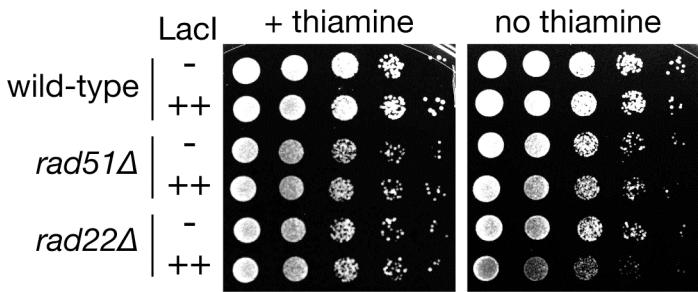


Figure S2 related to Figure 4E: The *lacO*-LacI interaction compromises the growth of a *rad22Δ* mutant. Strains containing the 115 repeat *lacO* array and either pREP41 (LacI -) or pREP41-NLS-LacI-ECFP (LacI++) were grown in EMMG without leucine and histidine, and then serially diluted and spotted onto EMMG plates lacking leucine and thiamine as indicated. Photographs of the plates were taken after four days growth at 30°C.

TABLE S1
lacO-LacI-induced mitotic recombination frequencies

Strain	thiamine ^a	<i>lacO</i> ¹¹⁵ array ^b	LacI ^c	Mean frequency of Ade ⁺ recombinants per 10 ⁴ viable cells ^d			Mean % Conversion types ^d
				Deletion type (His ⁻)	Conversion type (His ⁺)	Total (His ⁻ + His ⁺)	
Wild type (MCW1988)	-	+	-	4.05 (+/-1.79)	1.05 (+/-0.54)	5.10 (+/-1.99)	22.36 (+/-10.92)
Wild type (MCW1988)	-	+	++ (no NLS)	3.31 (+/-1.81)	1.07 (+/-0.52)	4.38 (+/-2.21)	26.0 (+/-7.56)
Wild type (MCW1988)	-	+	+	70.45 (+/-18.40)	5.60 (+/-3.98)	76.05 (+/-20.06)	7.14 (+/-4.19)
Wild type (MCW1988)	+	+	+	3.86 (+/-3.67)	0.91 (+/-0.36)	4.77 (+/-3.52)	24.60 (+/-11.41)
Wild type (MCW1988)	-	+	++	3486.26 (+/-562.09)	40.60 (+/-25.96)	3526.86 (+/-588.05)	1.17 (+/-0.78)
Wild type (MCW1988)	+	+	++	40.52 (+/-13.46)	4.48 (+/-1.34)	45.30 (+/-13.10)	10.13 (+/-2.08)
Wild type (MCW429)	-	-	-	1.52 (+/-0.69)	0.54 (+/-0.67)	2.06 (+/-0.10)	24.12 (+/-15.10)
Wild type (MCW429)	-	-	++	8.69 (+/-2.27)	4.17 (+/-3.98)	12.85 (+/-6.25)	29.56 (+/-12.79)
<i>rad51Δ</i> (MCW2087)	-	+	-	7.34 (+/-3.03)	0.03 (+/-0.03)	7.37 (+/-3.06)	0.69 (+/-0.81)

<i>rad51Δ</i> (MCW2087)	-	+	+	74.07 (+/- 20.96)	0.12 (+/- 0.28)	74.19 (+/- 20.93)	0.17 (+/- 0.41)
<i>rad51Δ</i> (MCW2087)	-	+	++	2712.66 (+/- 450.95)	2.76 (+/- 4.15)	2715.42 (+/- 455.10)	0.10 (+/- 0.14)
<i>rad22Δ</i> (MCW2088)	-	+	-	1.61 (+/- 0.06)	0.02 (+/- 0.03)	1.63 (+/- 0.62)	1.43 (+/- 2.12)
<i>rad22Δ</i> (MCW2088)	-	+	+	24.82 (+/- 11.25)	0.04 (+/- 0.10)	24.86 (+/- 11.30)	0.15 (+/- 0.27)
<i>rad22Δ</i> (MCW2088)	-	+	++	1265.38 (+/- 421.31)	6.49 (+/- 8.93)	1271.87 (+/- 430.24)	0.61 (+/- 0.84)
<i>rad51Δrad22Δ</i> (MCW2089)	-	+	-	1.24 (+/- 0.07)	0.02 (+/- 0.03)	1.26 (+/- 0.07)	1.37 (+/- 1.98)
<i>rad51Δrad22Δ</i> (MCW2089)	-	+	+	28.24 (+/- 6.49)	0.02 (+/- 0.06)	28.27 (+/- 6.50)	0.05 (+/- 0.20)
<i>rad51Δrad22Δ</i> (MCW2089)	-	+	++	1708.65 (+/- 465.85)	5.63 (+/- 11.81)	1714.28 (+/- 477.66)	0.34 (+/- 0.75)
<i>swi10Δ</i> (MCW3350)	-	+	-	8.57 (+/- 6.03)	1.85 (+/- 1.13)	10.42 (+/- 5.76)	21.95 (+/- 12.08)
<i>swi10Δ</i> (MCW3350)	-	+	+	29.77 (+/- 5.96)	8.31 (+/- 2.98)	38.08 (+/- 7.31)	21.64 (+/- 6.64)
<i>swi10Δ</i> (MCW3350)	-	+	++	2545.58 (+/- 433.85)	132.44 (+/- 57.38)	2678.02 (+/- 434.86)	5.04 (+/- 2.39)
<i>swi10Δrad22Δ</i> (MCW3409)	-	+	-	0.34 (+/- 0.22)	0.19 (+/- 0.16)	0.53 (+/- 0.28)	36.40 (+/- 18.65)
<i>swi10Δrad22Δ</i> (MCW3409)	-	+	+	0.54 (+/- 0.34)	0.46 (+/- 0.74)	1.00 (+/- 0.87)	36.55 (+/- 20.43)
<i>swi10Δrad22Δ</i> (MCW3409)	-	+	++	38.85 (+/- 20.42)	4.26 (+/- 2.57)	43.11 (+/- 20.78)	11.25 (+/- 7.16)
<i>rqh1Δ</i> (MCW2189)	-	+	-	6.90 (+/- 1.36)	1.68 (+/- 0.46)	8.58 (+/- 1.50)	19.68 (+/- 4.88)
<i>rqh1Δ</i> (MCW2189)	-	+	+	29.50 (+/- 6.24)	2.92 (+/- 1.52)	32.42 (+/- 6.65)	8.97 (+/- 3.84)
<i>rqh1Δ</i> (MCW2189)	-	+	++	2672.99 (+/- 495.70)	21.34 (+/- 10.12)	2694.33 (+/- 493.91)	0.83 (+/- 0.45)
<i>exo1Δ</i> (MCW2186)	-	+	-	1.95 (+/- 1.43)	1.14 (+/- 0.32)	3.09 (+/- 1.75)	41.19 (+/- 13.50)
<i>exo1Δ</i> (MCW2186)	-	+	+	78.82 (+/- 13.80)	5.60 (+/- 2.02)	84.42 (+/- 14.49)	6.65 (+/- 2.06)
<i>exo1Δ</i> (MCW2186)	-	+	++	2557.44 (+/- 628.17)	94.50 (+/- 27.99)	2651.94 (+/- 631.45)	3.81 (+/- 1.52)
<i>rqh1Δ exo1</i> (MCW4087)	-	+	-	8.00 (+/- 3.70)	3.66 (+/- 1.99)	11.66 (+/- 4.95)	32.08 (+/- 9.64)
<i>rqh1Δ exo1Δ</i> (MCW4087)	-	+	+	6.05 (+/- 1.83)	1.54 (+/- 1.14)	7.59 (+/- 1.76)	20.72 (+/- 12.38)
<i>rqh1Δ exo1Δ</i> (MCW4087)	-	+	++	1845.59 (+/- 892.10)	23.63 (+/- 49.89)	1869.22 (+/- 913.60)	1.19 (+/- 1.68)
<i>pfh1⁺</i> (MCW4916)	-	+	-	2.94 (+/- 1.03)	0.94 (+/- 0.39)	3.88 (+/- 1.36)	23.95 (+/- 3.81)
<i>pfh1⁺</i> (MCW4916)	-	+	+	49.31 (+/- 10.49)	2.31 (+/- 0.94)	51.63 (+/- 10.93)	4.48 (+/- 1.65)
<i>pfh1-mt[*]</i> (MCW4949)	-	+	-	4.41 (+/- 1.06)	1.06 (+/- 0.69)	5.47 (+/- 1.31)	19.01 (+/- 7.52)
<i>pfh1-mt[*]</i> (MCW4949)	-	+	+	148.45 (+/- 27.75)	7.24 (+/- 2.78)	155.69 (+/- 28.42)	4.70 (+/- 1.91)

^a Colonies were grown in the presence (+) or absence (-) of thiamine prior to testing for recombinant frequency. In the absence of thiamine the *nmt* promoter is de-repressed and drives the expression of NLS-LacI-eCFP in strains containing either pREP81-NLS-LacI-eCFP or pREP41-NLS-LacI-eCFP.

^b Strains with (+) and without (-) the 115 repeat *lacO* array.

^c Strains are transformed with either pREP1 (-), pREP81-NLS-LacI-eCFP (+), or pREP41-NLS-LacI-eCFP (++) as indicated.

^d The values in parentheses are the standard deviations about the mean.

TABLE S2
*lacO*²⁻²¹-LacI-induced mitotic recombination frequencies

Strain	<i>lacO</i> array size	LacI ^a	Mean frequency of Ade ⁺ recombinants per 10 ⁴ viable cells ^b			Mean % Conversion types ^b
			Deletion type (His ⁻)	Conversion type (His ⁺)	Total (His ⁻ + His ⁺)	
Wild type (MCW5157)	2	+	3.16 (+/-0.82)	1.05 (+/-0.50)	4.21 (+/-1.19)	24.47 (+/-6.96)
Wild type (MCW5157)	2	++	104.4 (+/-20.31)	25.24 (+/-4.53)	129.7 (+/-23.98)	19.56 (+/-2.06)
Wild type (MCW5158)	12	+	17.72 (+/-4.33)	2.17 (+/-1.02)	19.89 (+/-4.69)	10.85 (+/-4.26)
Wild type (MCW5158)	12	++	1367 (+/-179.5)	58.02 (+/-16.41)	1425 (+/-191.3)	4.04 (+/-0.77)
Wild type (MCW5159)	15	+	21.13 (+/-4.86)	2.61 (+/-1.71)	23.75 (+/-5.42)	10.86 (+/-5.08)
Wild type (MCW5159)	15	++	2103 (+/-362.5)	60.74 (+/-19.44)	2164 (+/-367.6)	2.84 (+/-0.87)
Wild type (MCW5160)	21	+	30.68 (+/-5.57)	2.28 (+/-0.48)	32.96 (+/-5.71)	7.04 (+/-1.51)
Wild type (MCW5160)	21	++	2413 (+/-452.7)	43.98 (+/-16.38)	2457 (+/-456.4)	1.82 (+/-0.75)

^a Strains are transformed with either pREP1 (-), pREP81-NLS-LacI-eCFP (+), or pREP41-NLS-LacI-eCFP (++) as indicated.

^b The values in parentheses are the standard deviations about the mean.

TABLE S3
FR-EBNA1-induced mitotic recombination frequencies

Strain	EBNA1 ^a	Mean frequency of Ade ⁺ recombinants per 10 ⁴ viable cells ^b			Mean % Conversion types ^b
		Deletion type (His ⁻)	Conversion type (His ⁺)	Total (His ⁻ + His ⁺)	
Wild type (MCW5162)	-	4.07 (+/- 0.64)	0.78 (+/- 0.12)	4.85 (+/- 0.69)	16.11 (+/- 2.13)
Wild type (MCW5162)	++	3640.92 (+/- 460.33)	70.57 (+/- 47.90)	3711.49 (+/- 455.85)	1.93 (+/- 1.30)

^a Strains are transformed with either pREP41 (-) or pREP41-NLS-EBNA1 (++) as indicated.

^b The values in parentheses are the standard deviations about the mean.