Supplemental Data

A JUNCTION BRANCH POINT ADJACENT TO A DNA BACKBONE NICK DIRECTS SUBSTRATE CLEAVAGE BY *SACCHAROMYCES CEREVISIAE* MUS81-MMS4

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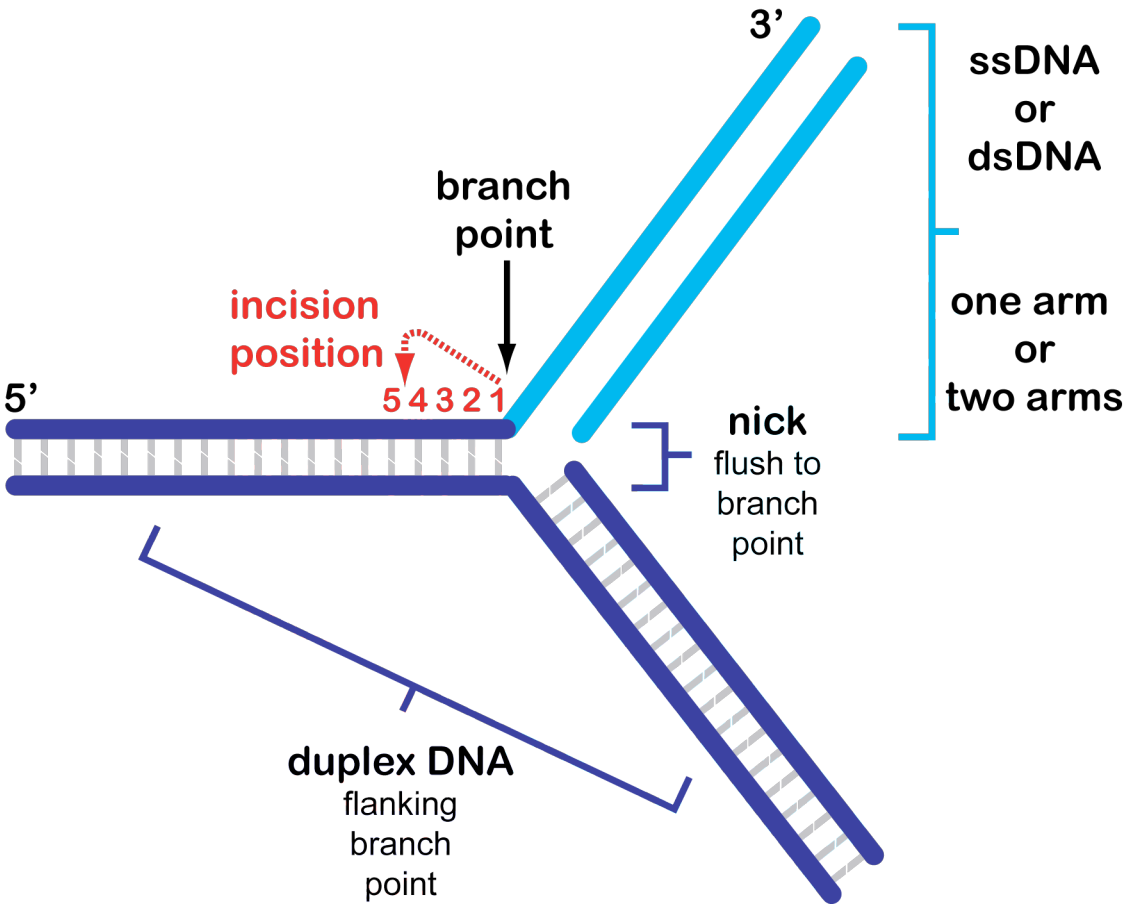
Supplemental Table 1. Mus81-Mms4 kinetic parameters on 3'-FL-related DNA joint molecules.

Substrate	V_{max} ($nM\ min^{-1}$)	K_M (nM)	k_{cat} (min^{-1})	catalytic cycle (min.)	k_{cat}/Km ($nM^{-1}min^{-1}$)
3'-FL-2 nt	3.5 ± 0.85	11.9 ± 5.6	.7	1.43	.06
3'-FL-1 nt	2.9 ± 0.48	5.3 ± 2.7	.58	1.72	.11
3'-FL[2]	4.9 ± 0.7	5.5 ± 2.6	.98	1.02	.18
3'-FL+1 nt	1.8 ± 0.33	7.4 ± 3	.36	2.78	.05
3'-FL+2 nt	0.88 ± 0.08	2.7 ± 1.1	.18	5.56	.07
3'-FL+3 nt	0.21 ± 0.05	20.9 ± 12.1	.04	25	.002
Y[1]	1.3 ± 0.04	30.4 ± 11.3	.26	3.85	.009

[1] from: Ehmsen, K.T. and Heyer, W.D. (2008) *Saccharomyces cerevisiae* Mus81-Mms4 is a catalytic structure-selective endonuclease. *Nucleic Acids Res.*, **36**, 2182-2195.



Supplemental Figure 1. Model for substrate branch point properties queried by Mus81-Mms4 during DNA joint molecule processing. Mus81-Mms4 incises a number of model joint molecule structures *in vitro*, which in principle can be described from the perspective of a branch point centered at a nick in duplex DNA. One or two arms of either single-stranded (ssDNA) or double-stranded (dsDNA) character can emerge from this branch point (depicted in *light blue*). At least two duplex arms flanking the branch point must be double-stranded DNA for optimal substrate binding (*dark blue*). For optimal substrate turnover, the 5’-end of the downstream duplex DNA must meet the substrate branch point such that it is flush to the branch point [neither retreated (leaving a gap) or advanced (presenting a short 3’-flap)]. Incision occurs 5’ to the substrate branch point, on the discontinuous strand between the fourth and fifth phosphodiester bonds as referenced from the branch point position (shown in *red*, with

phosphodiester bonds numbered right to left from the substrate branch point as depicted). The substrate recognition features discussed here are consistent with previous analyses of Mus81-Mms4/Eme1 from budding yeast (1-7), fission yeast (3,4,6,8-10) and humans (11-15), suggesting that the substrate preference might be conserved between these enzymes. As discussed more fully in the text, there is a discrepancy in the mapping of the cleavage sites between our and two previous studies (2,3) that appears to be enzyme-dependent, as identical substrates were used.

SUPPLEMENTAL REFERENCES

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