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Double-strand breaks and novel DNA intermediates derived from partial processing of alkylation damage in G2 cells of yeast

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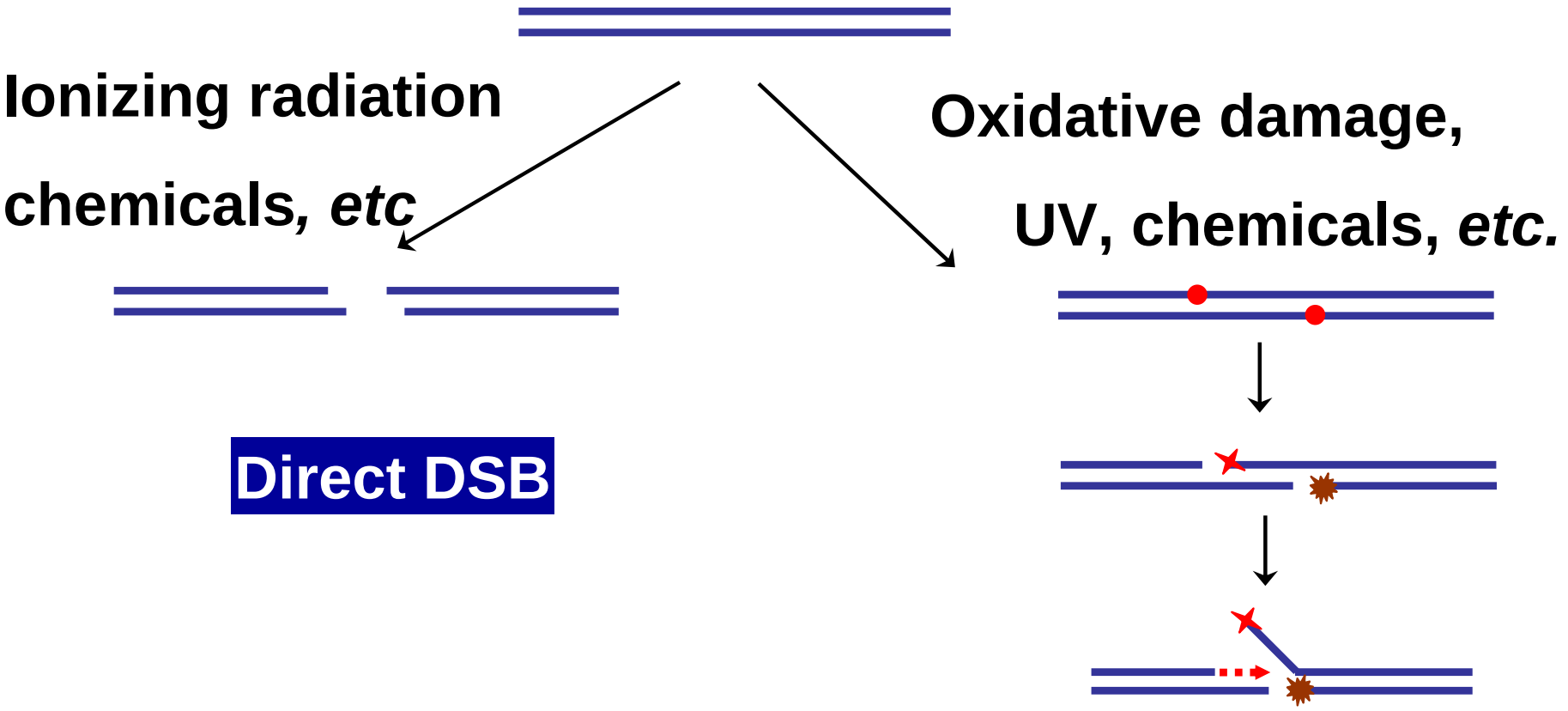
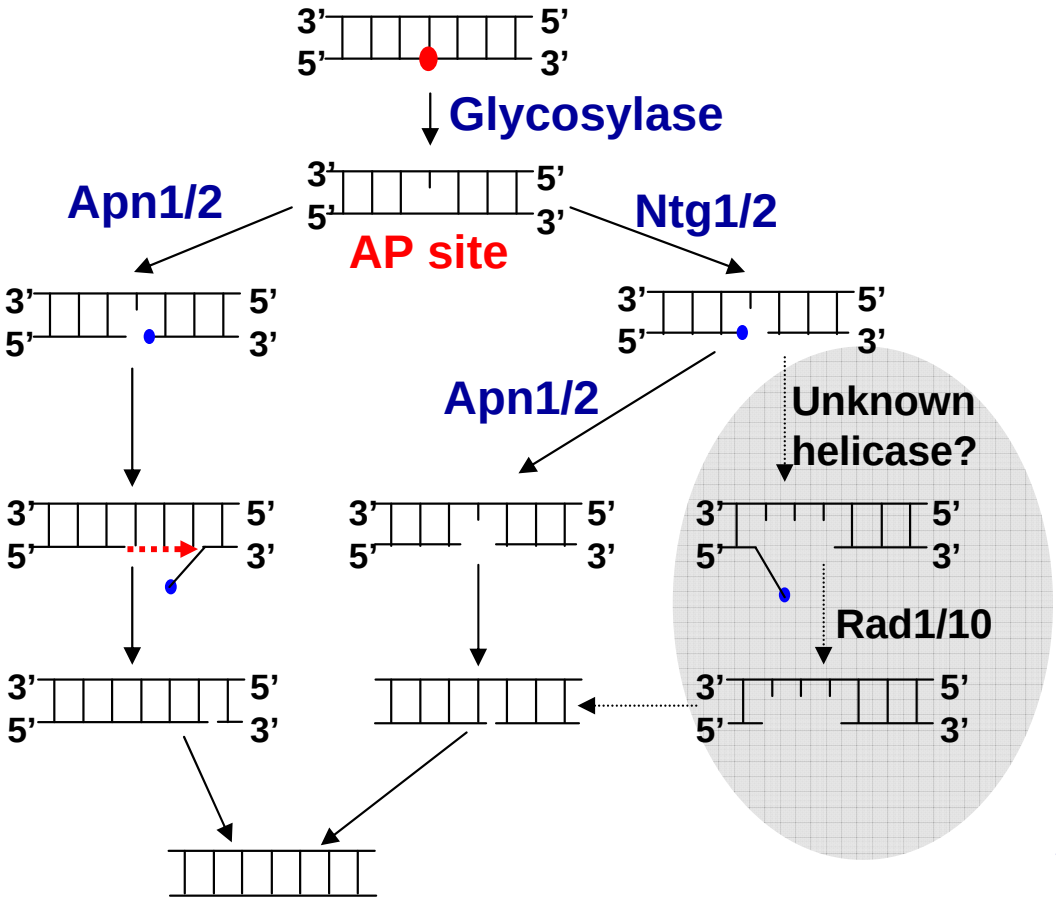
‘Derived’ DSBs and ‘dirty’ break ends



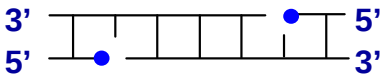
- Inefficient removal of blocking groups at strand breaks (‘dirty’ ends) might lead to derived DSBs

Yeast Base Excision Repair (BER), AP endonucleases and 3'-dirty ends



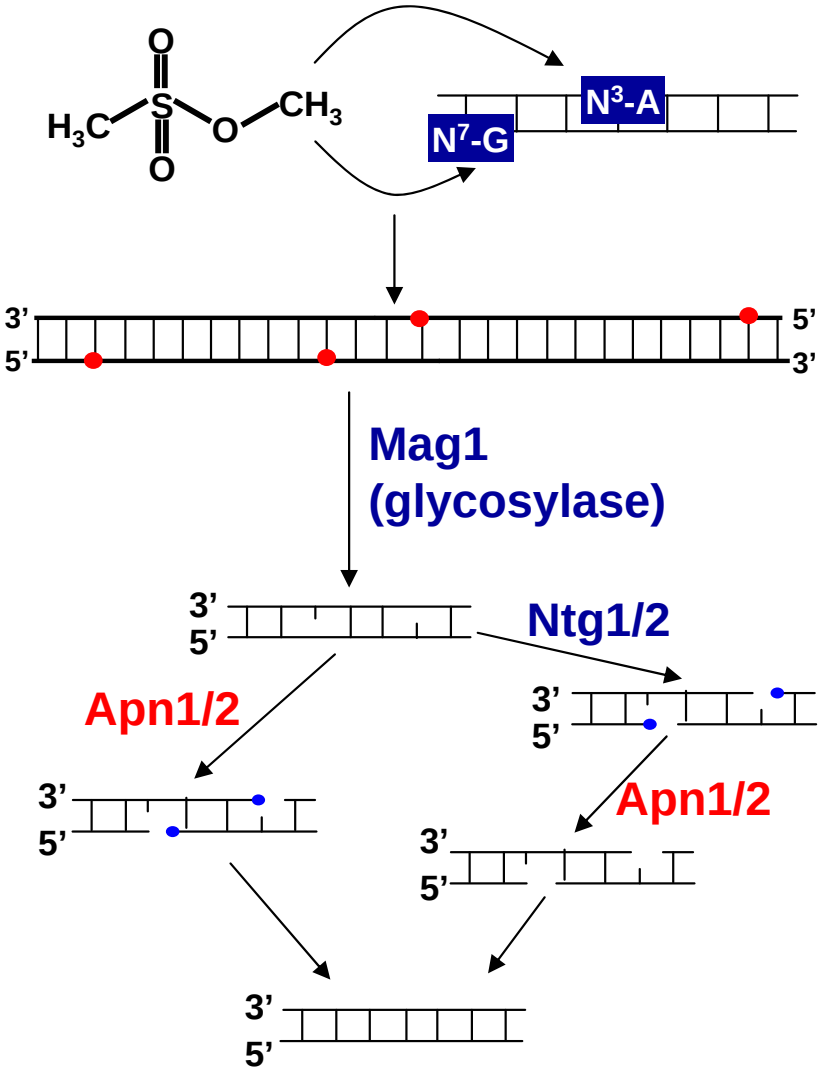
Modified from Boiteux
DNA Repair (2004)

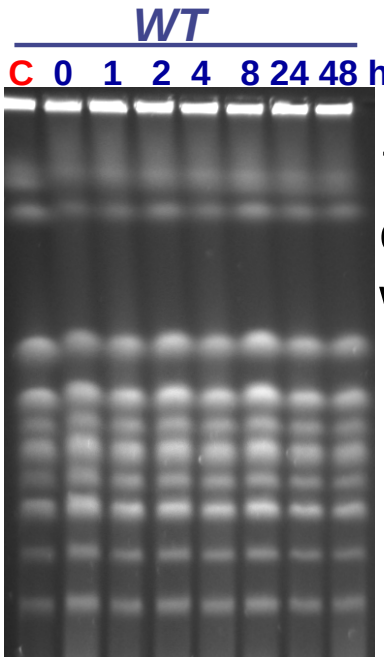
1. Can base damage generate DSBs outside of S-phase?
2. Can repair generated SSBs be converted into DSBs within the cell?



3. What are the consequences of 3'-dirty ends?

Does MMS generate DSBs in wild type G1 or G2 cells?



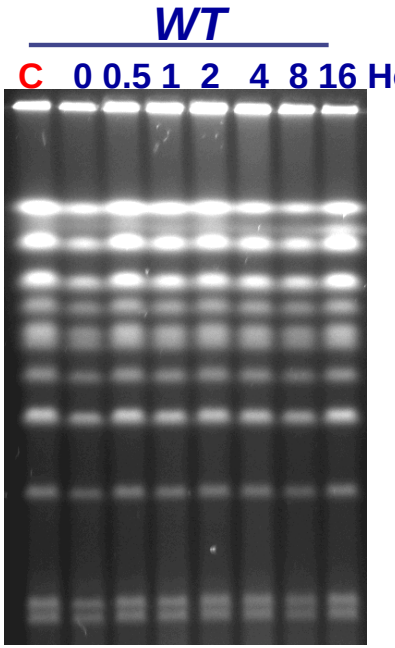


1% MMS for 15min;

chromosomes(200 - 2200kb) were separated by PFGE.

G1

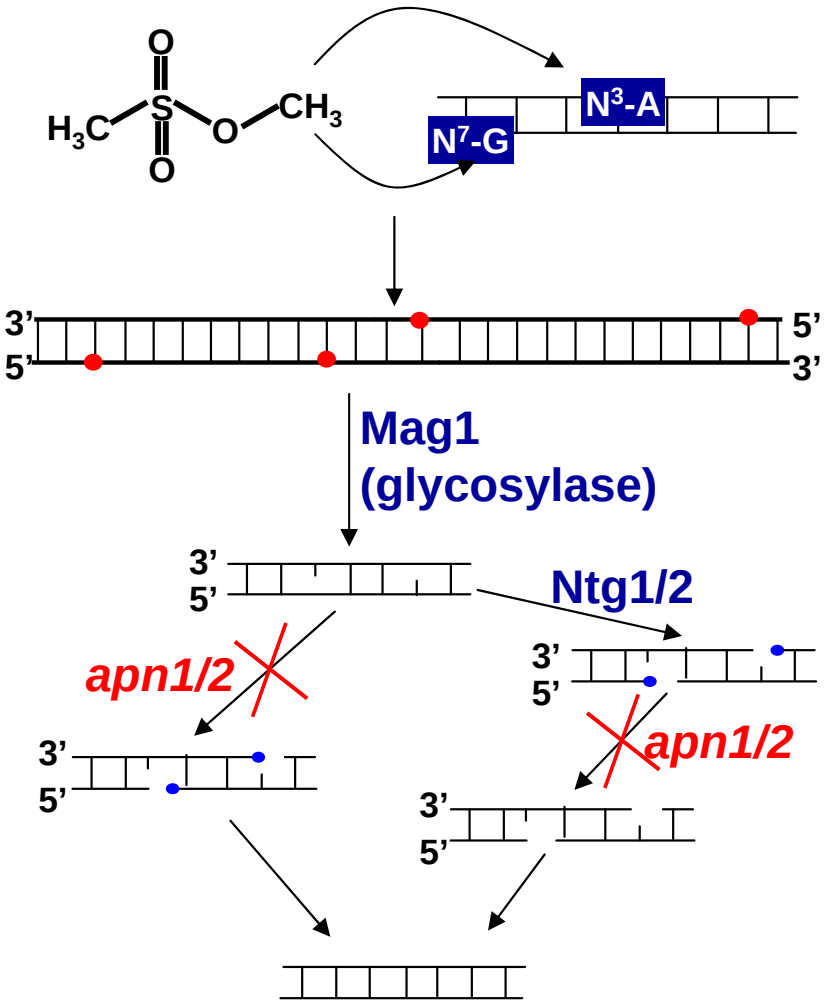
Liquid holding repair in PBS

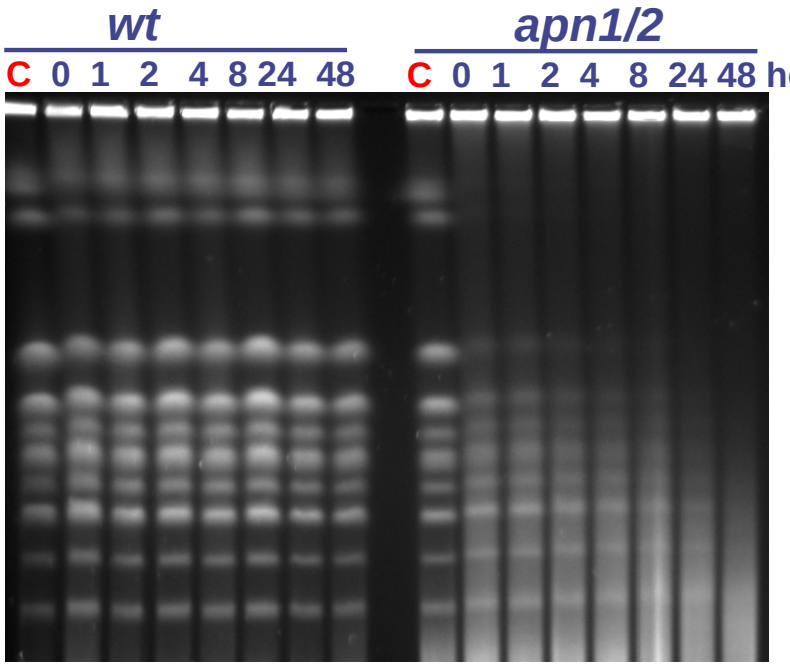


G2

Nocadazole arrested, repair in YPD media

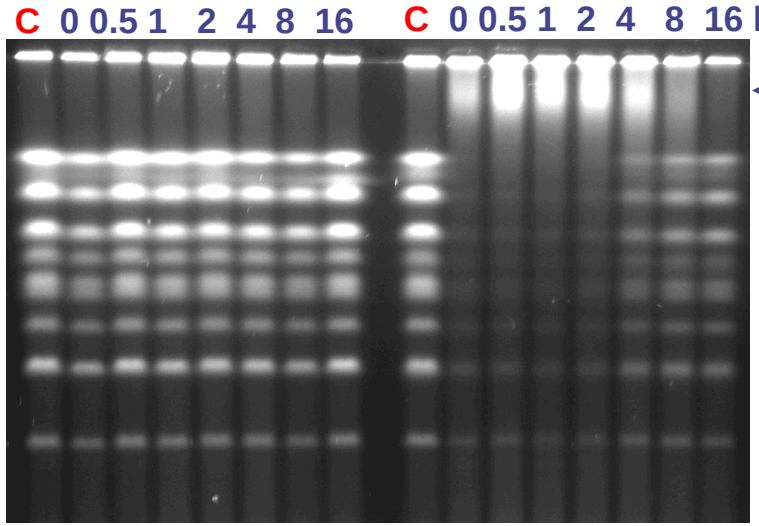
Does partial processing of alkylation damage (apn1⁻, apn2⁻) lead to "DSBs" in G1 and/or in G2





G1

Increase in "DSBs" with time



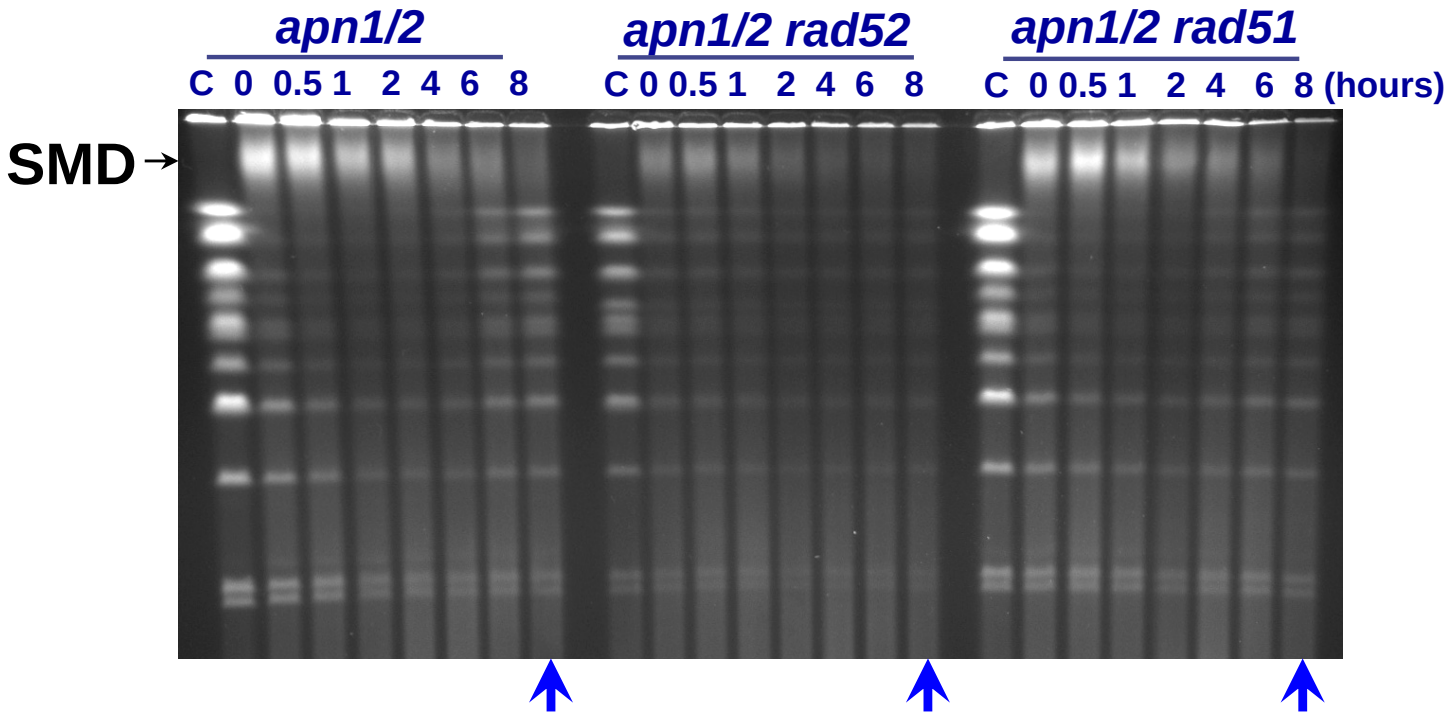
← **Slow Moving DNA (SMD)**

G2

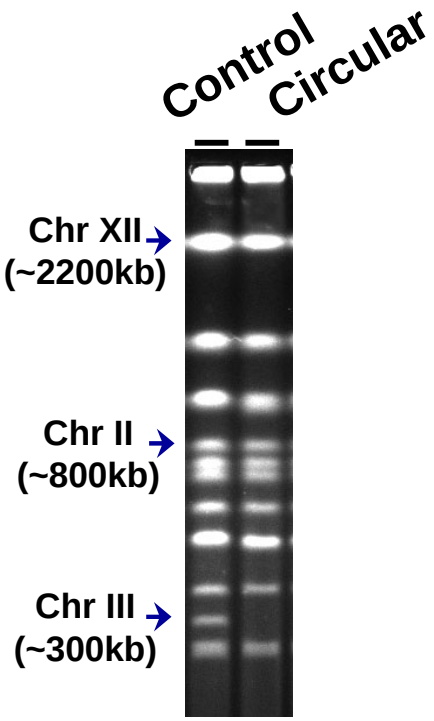
Chromosome restitution

G1 cells, Ma et al., NAR, 2008; G2 cells, Ma et al., PLoS Genetics, in press

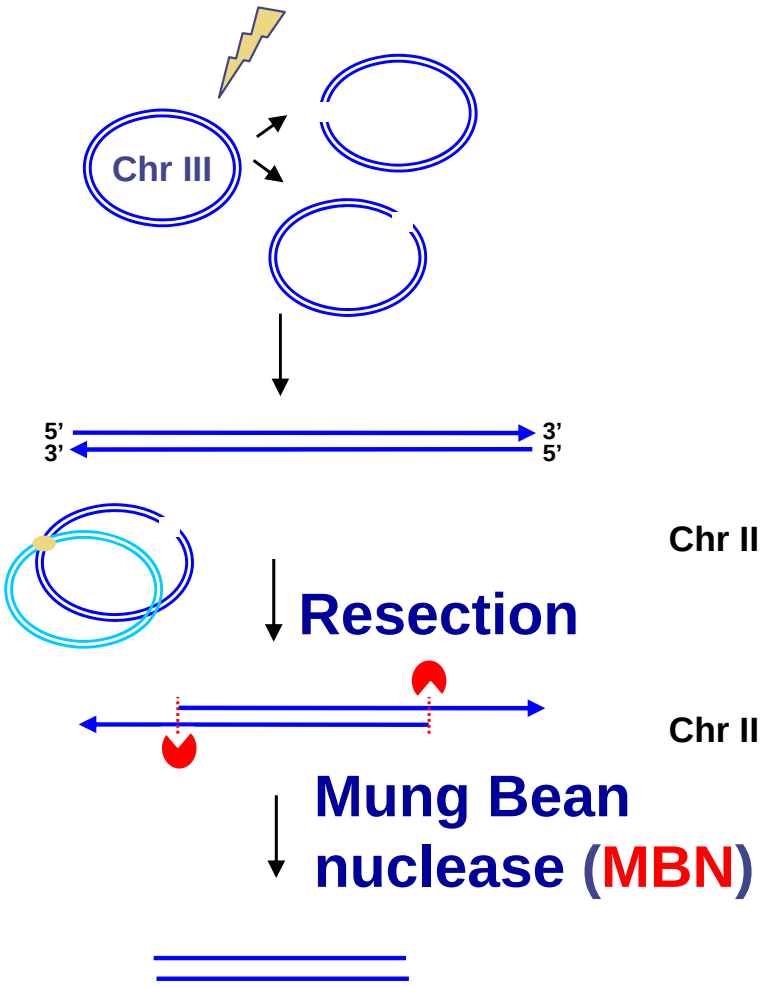
Chromosome restitution--unlike SMD--occurs through DSB recombinational repair in G2

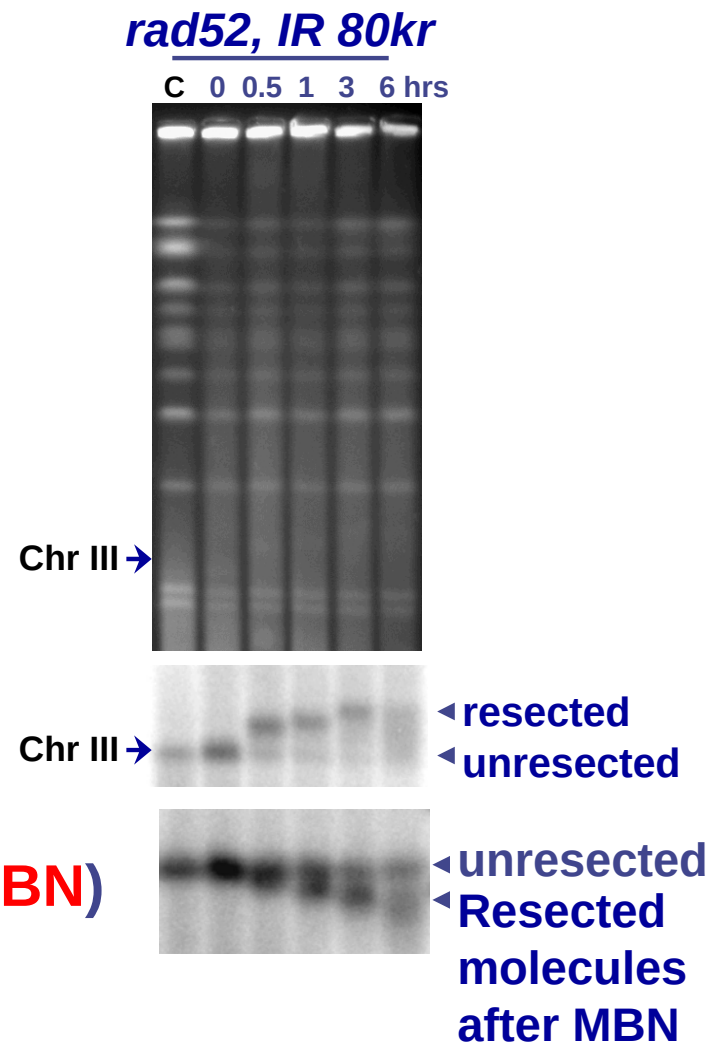


Are the random DSBs generated by MMS in G2 cells subject to resection, an early step in DSB repair



Circular chromosome does not enter the gel

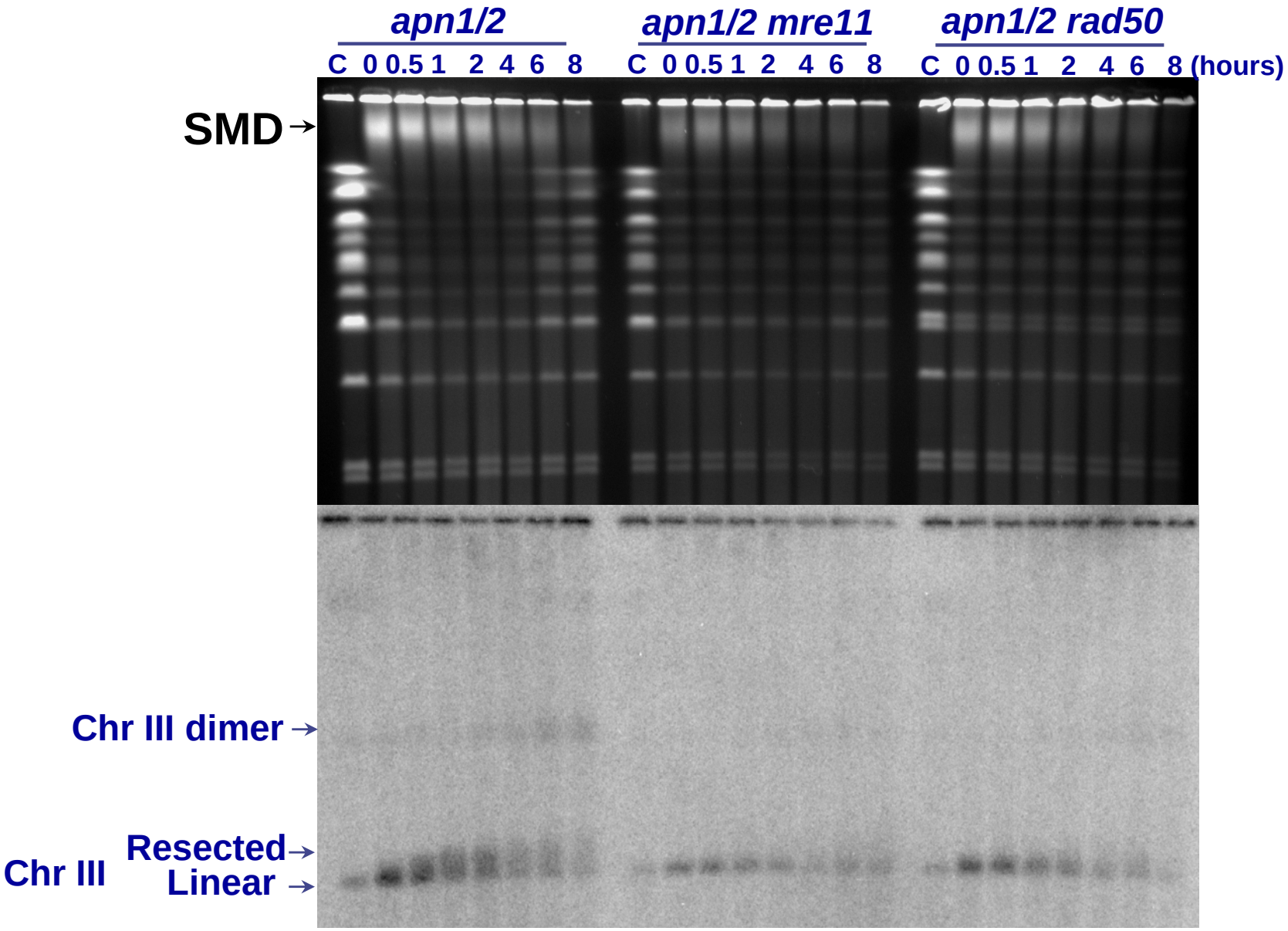




Westmoreland, Ma, Malkova, Resnick PLoS Genetics, 2009

MMS-derived DSBs in G2 cells are resected;
Resection depends on Rad50/Mre11 (MRX)

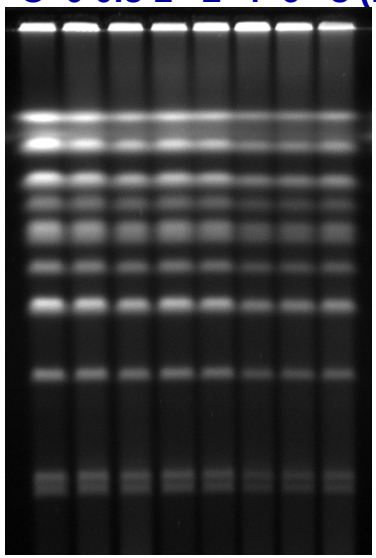
Resection does not depend on Rad51 or Rad52 (not shown)



Deletion of *MAG1* or stabilization of AP sites prevents SMD as well as DSBs

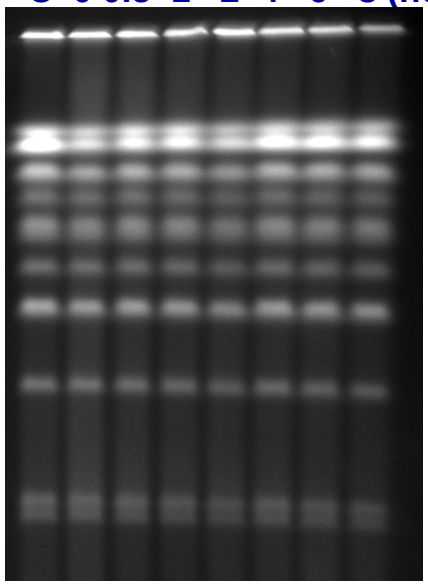
apn1/2 mag1

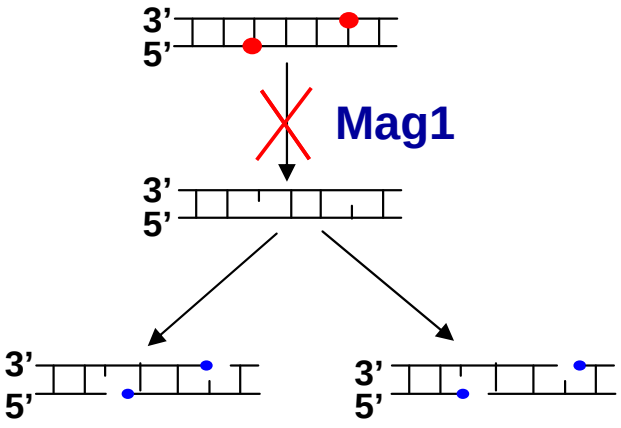
C 0 0.5 1 2 4 6 8 (hours)

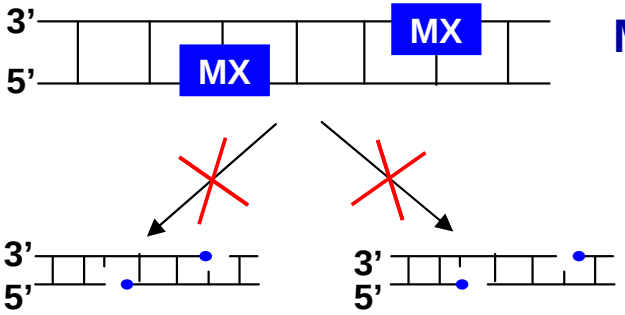


apn1/2 + MX

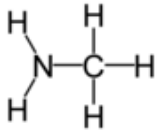
C 0 0.5 1 2 4 6 8 (hours)



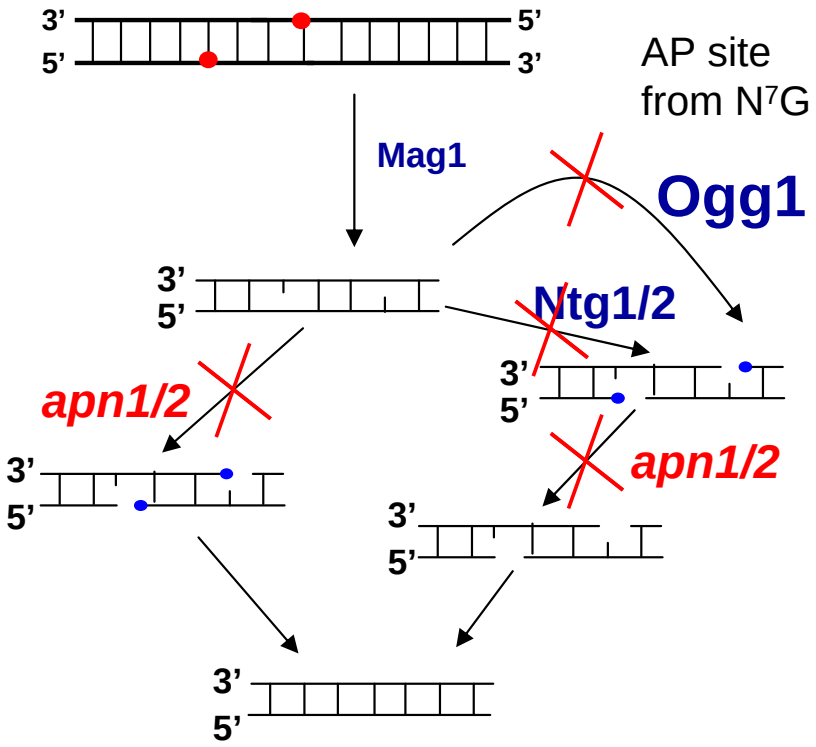


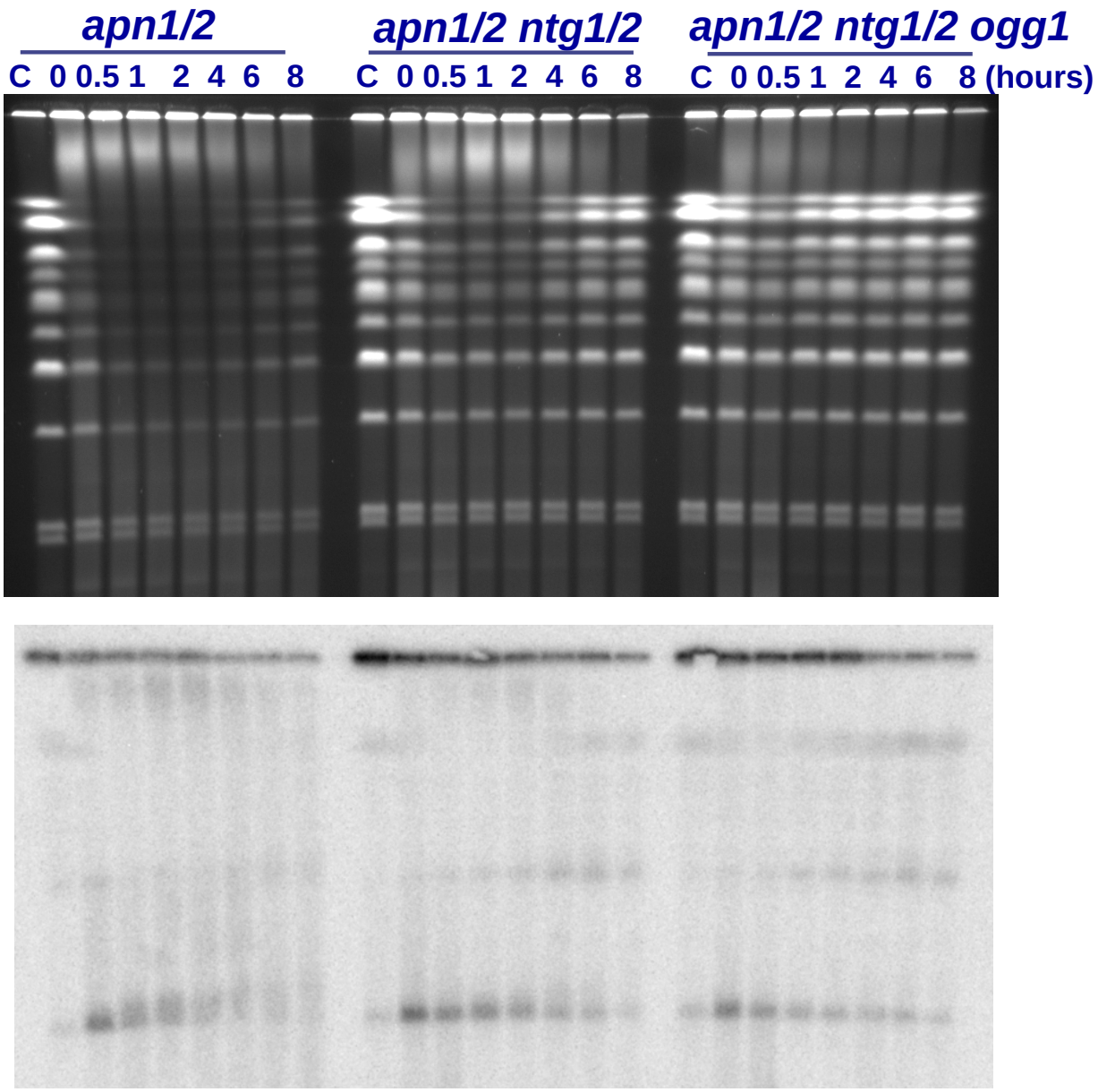


Methoxyamine (MX) binds to AP sites

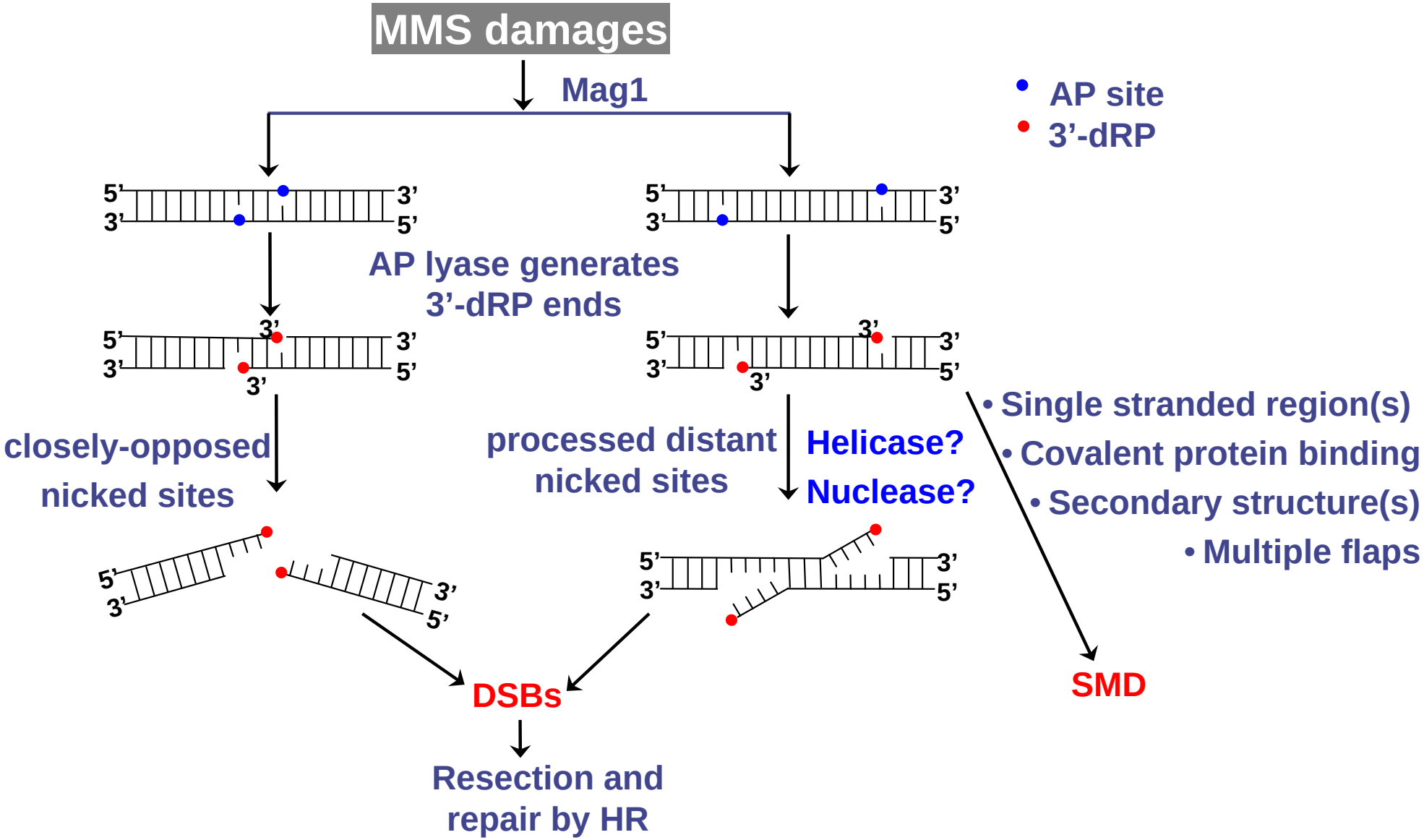


Resectable DSBs and SMD in the *apn1 apn2* mutant are due to a combination of Ntg1/2 and Ogg1





Derived DSBs and SMD intermdiates resulting from partial processing of MMS lesions



Ma, Westmoreland, Gordenin, Resnick, et al., *PLoS Genetics*, in press