

BOX 1 - Mus81 and Mms4/Eme1 -

Mus81 is a structure-specific endonuclease within the same family as XPF (157) and discovered due to its interaction with Rad54 (170) and replication checkpoint kinase Cds1 (36). Analogous to XPF, it only functions via its C-terminus (120) with its partner MMS4 (*S. cerevisiae*) or Eme1 (*S. pombe*) (64, 67). Mms4 function was revealed due to sporulation defects in the mutant (91). Biochemically, the *Sp*Mus81-Eme1 heterodimer was characterized as a Holliday junction resolvase, able to cleave HJs and the meiotic defect of the *Sp*Mus81 mutant could be rescued by expression of the bacterial HJ resolvase RusA (35). The human MUS81 also nicked HJs and protein abundance increased after cells were challenged with agents that block replication (64). However, HJ cleavage is inefficient, asymmetric and reveals both nicks and gaps, while RusA cleaves perfectly symmetric. In addition, mammalian cell extracts reveal two fractions with HJ cleavage activity, one with efficient symmetric cleavage and associated with ATP dependent branch migration and a second, containing MUS81, revealing asymmetric cleavage (72). Also it was shown that the protein was much more (75x) active on replication fork and 3' flap structures (67, 72, 325, 481) leaving 3-6nt gaps (16, 481). This led to the conclusion that Mus81-Eme1 is a flap or replication fork specific endonuclease rather than a HJ specific endonuclease, even though the cleavage of HJs could be a 6-fold increased by including a single nick in the HJ substrate (67, 126).

In support with this is the observation that the Mms4 mutant is hypersensitive to agents interfering with replication, such as camptothecin and UV light. However, not by IR, which induces DSBs that should be repaired by recombination leading to HJs (16, 170). Mouse *Eme1KO* embryonic stem cells are hypersensitive to DNA cross-linking agents (MMC, cis-Platinum), which impose huge problems for replication forks and only mildly sensitive to IR, UV. *Eme1KO* cells display increased spontaneous genomic instability (2). However, the KO mutation has no effect on recombination processes as gene targeting, gene conversion or SCE. Increased SCE was only detected upon induced DNA damage.

Investigation of UV induced damage in human S-phase cells revealed that MUS81 is a nuclear protein accumulating in the nucleoli, recruited to regions with UV damage, but not in cells that were blocked from replication, suggesting that the enzyme is recruited to stalled replication forks (129). Due to its role in replication forks rather than HJs based on both biochemical and genetic studies it has been proposed that Mus81 resolves stalled replication forks by either nicking flapped ends, D-loops and/or half junctions arising before the HJ is formed (157, 161). Recently, the RAD51 paralogues RAD51C and XRCC3 were proposed to be the mammalian HJ endonuclease (see RAD51 paralogues, (257)).