

Double Stranded Break Repair in Aged Thymocytes  
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Cells undergoing DNA recombination, either VDJ recombination at the TCR-beta locus in CD4-CD8- thymocytes, or VJ recombination at the TCR-alpha locus in CD4+CD8+ thymocytes need to make and then repair double stranded breaks [DSB] in their DNA. Under these circumstances, phosphorylation of the histone, H2AX, would be expected, since this event is associated with DSB.

We find that DSB and gamma- $\gamma$  H2AX can be induced in linear fashion with overnight incubation with actinomycin D. This can be followed by flow cytometry. We have characterized the loss of thymocytes from aged mice [ $>16$  months of age]. These mice have only about 20% of the adult [6 month] levels of thymocytes. The aged thymocytes show a greater frequency of  $\gamma$ H2AX-bright cells than thymocytes from young mice. This suggests TCR recombination may be initiated in aged mice without resolution of the DSBs, and/or that apoptotic DSB events may be more prominent in the aged thymus.