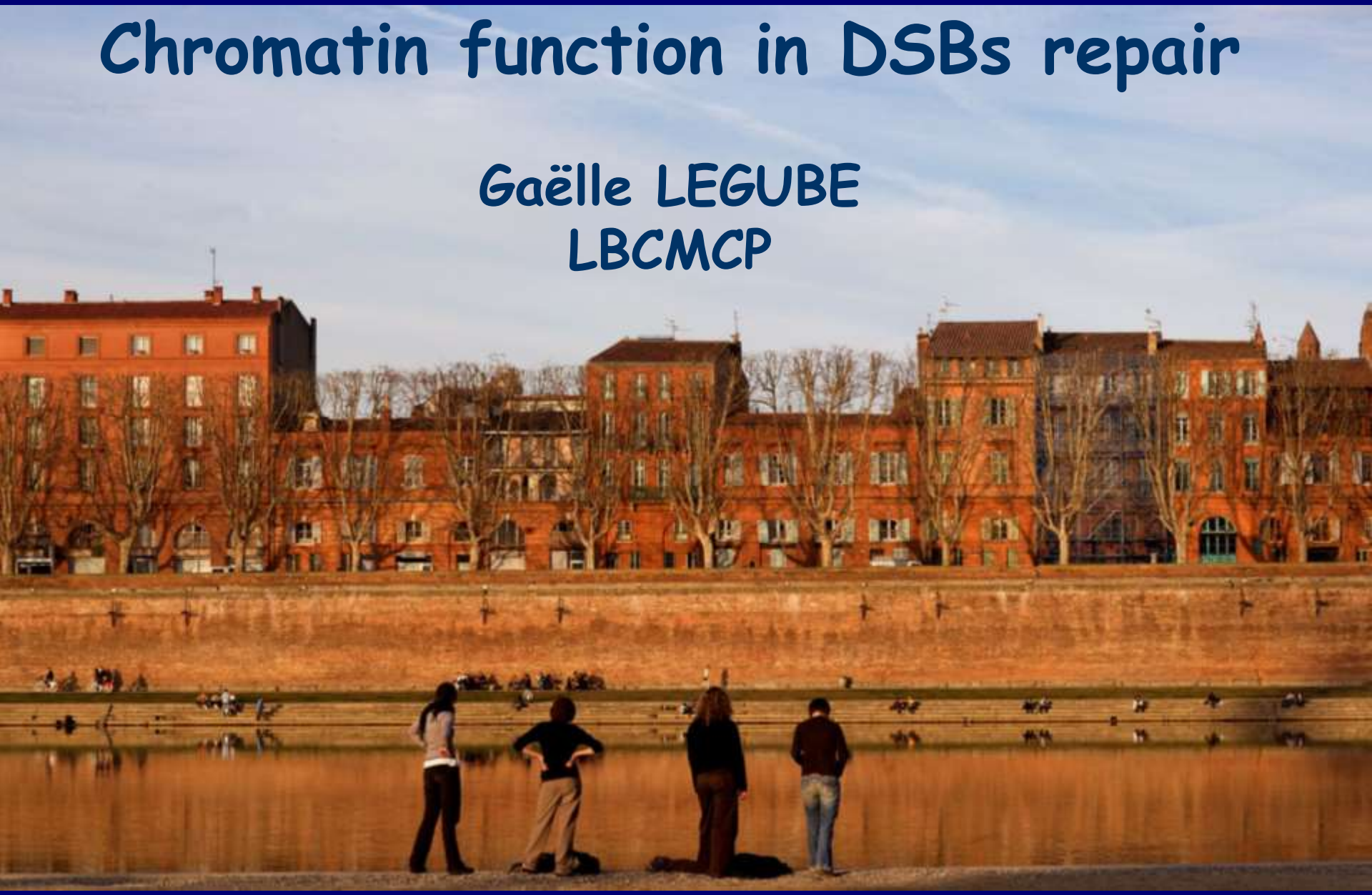
Chromatin function in DSBs repair

Gaëlle LEGUBE
LBCMCP

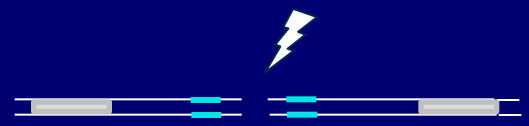


GSO, Toulouse October 2014

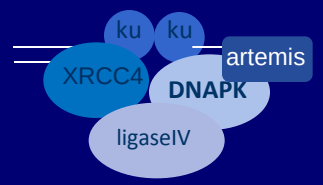
DSBs repair

UV, Cigarette smoke,…

VDJ recombinaison, meiosis,..

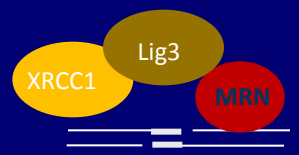


C-NHEJ



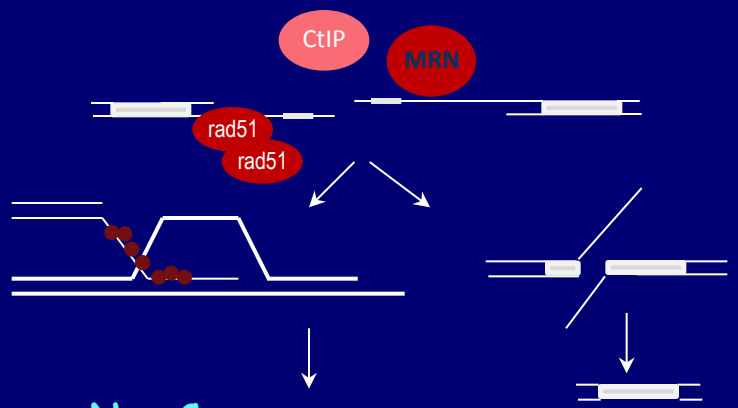
Sequence restored
Possible mutation

Alt-NHEJ



Deletion

Homology dependant Repair



Non Cross-over

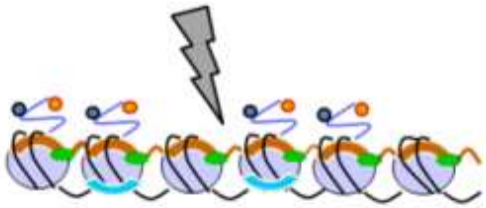
Cross-over (possible LOH)

Large deletion

?

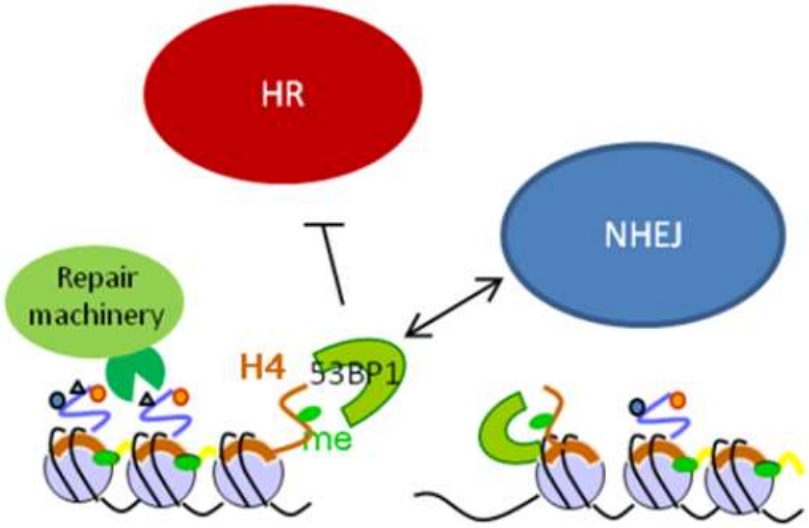
Cell Cycle/ Cell Type……

DSBs repair on chromatin



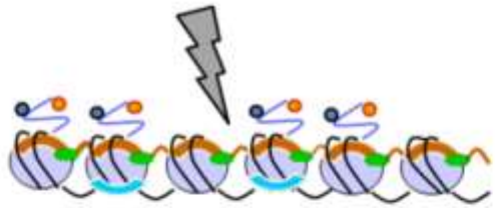
Influence of **preexisting** chromatin structure on repair

PRIME

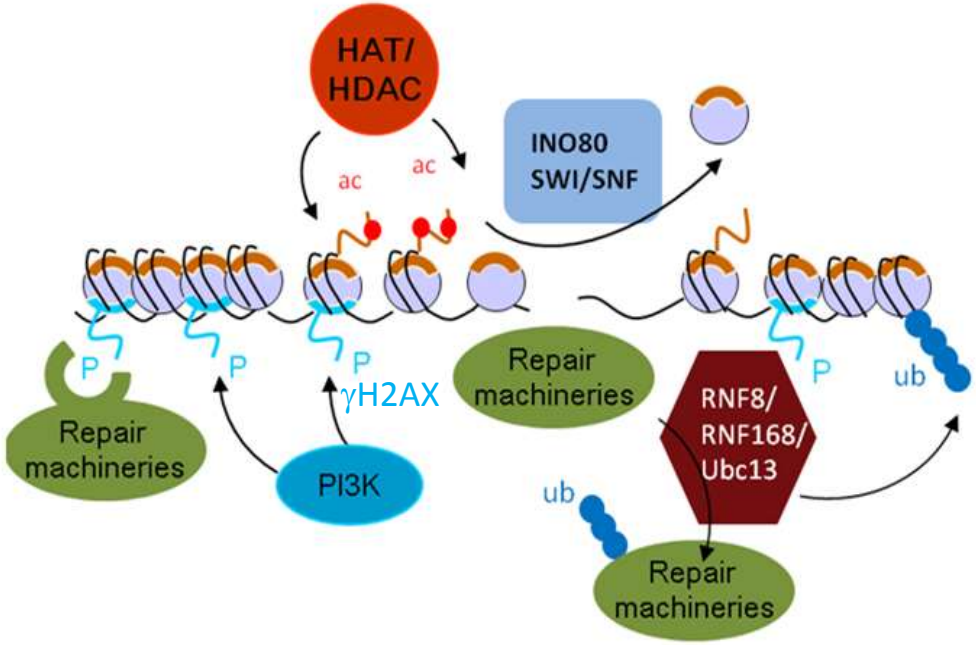


DSBs repair on chromatin

REPAIR

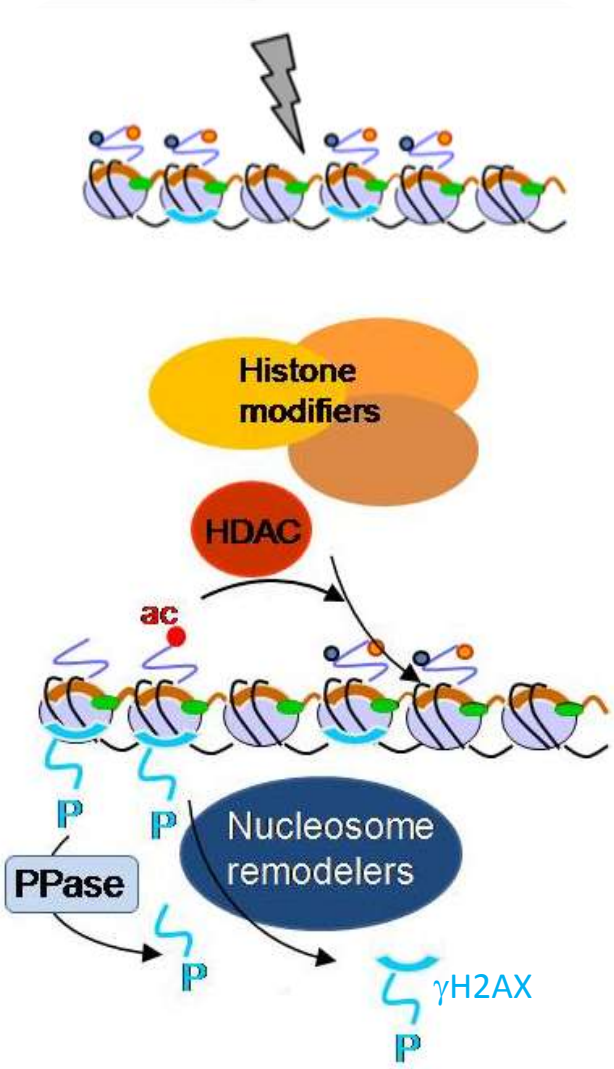


DSB-induced chromatin changes that helps repair

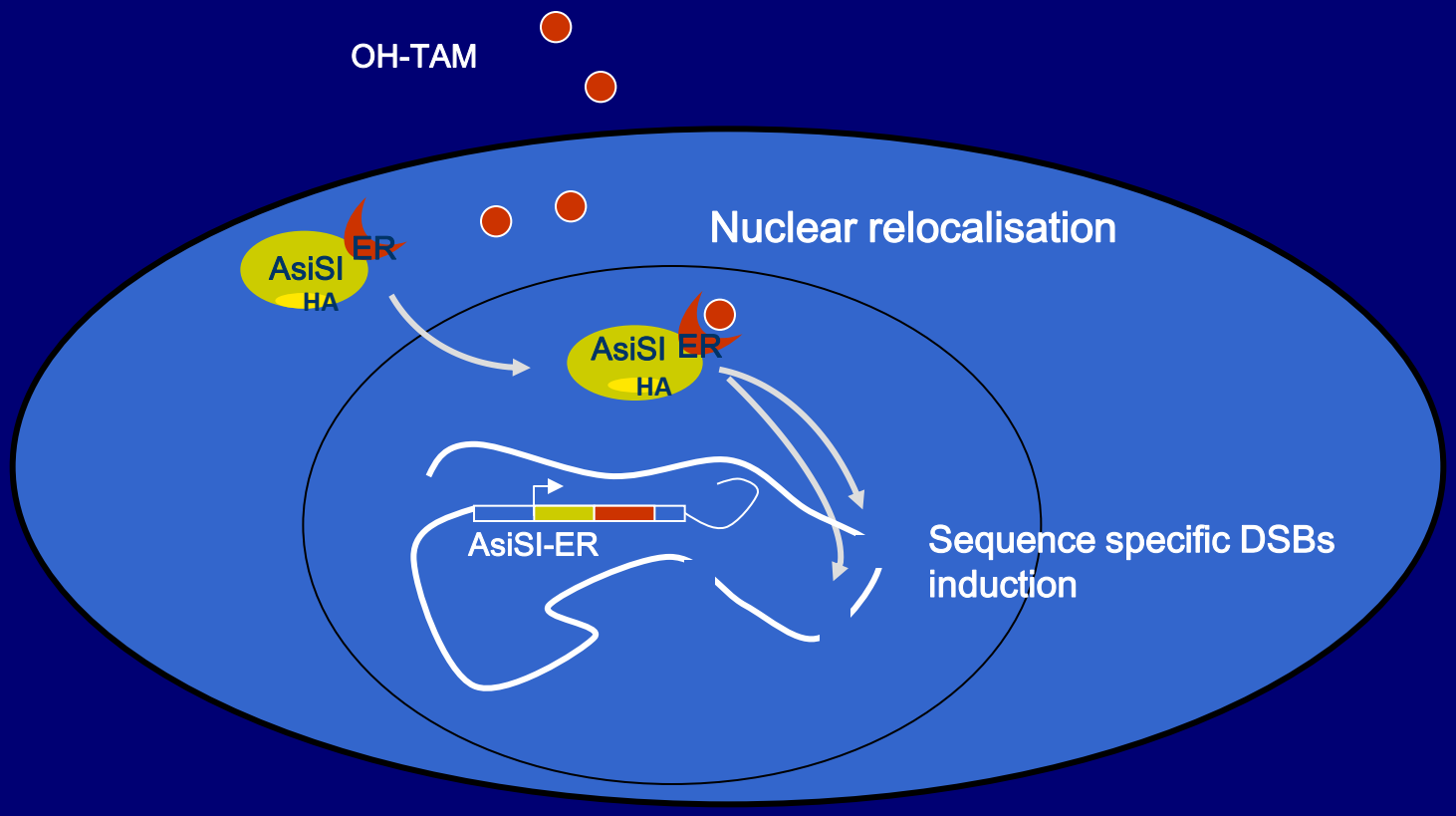


DSBs repair on chromatin

RESTORE



DIvA, a sequence specific DSB inducible cell line

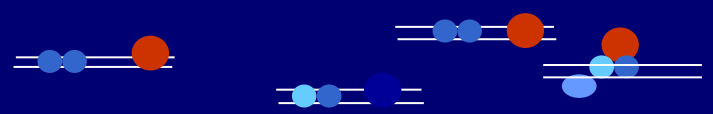


ChIP-seq to caracterize chromatin structure and repair events at AsiSI induced breaks

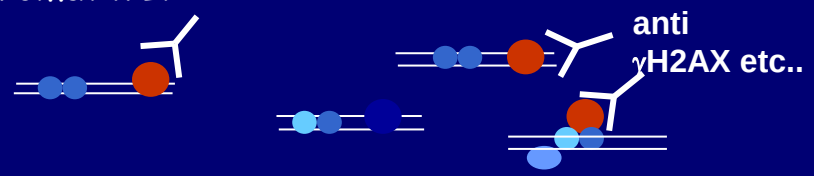
Chromatin IP

Crosslink

Sonication (~500pb)

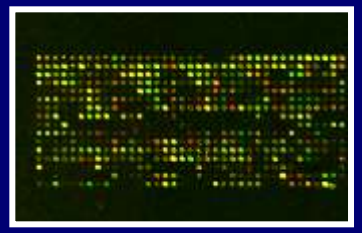


Chromatin IP



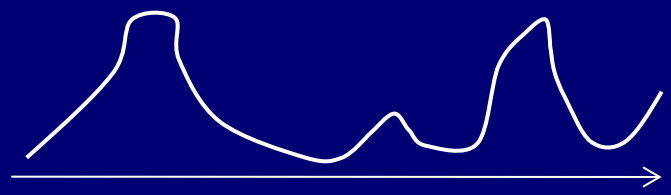
Reverse crosslink, DNA purification

ChIP-chip



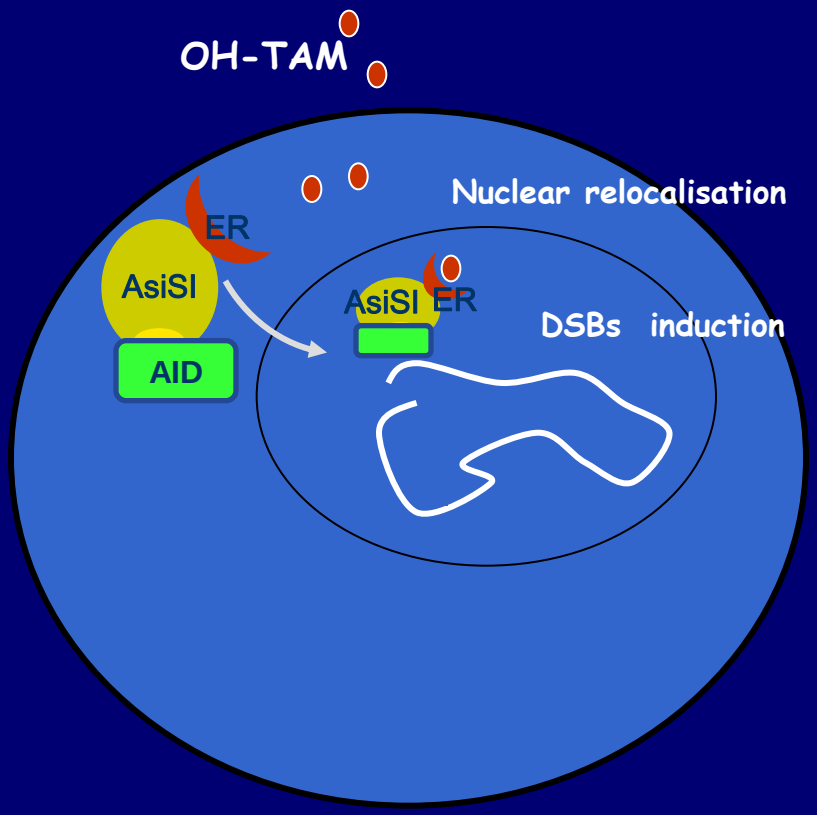
ChIP-seq

Studying DSB repair on multiples breaks simultaneously, with a high resolution



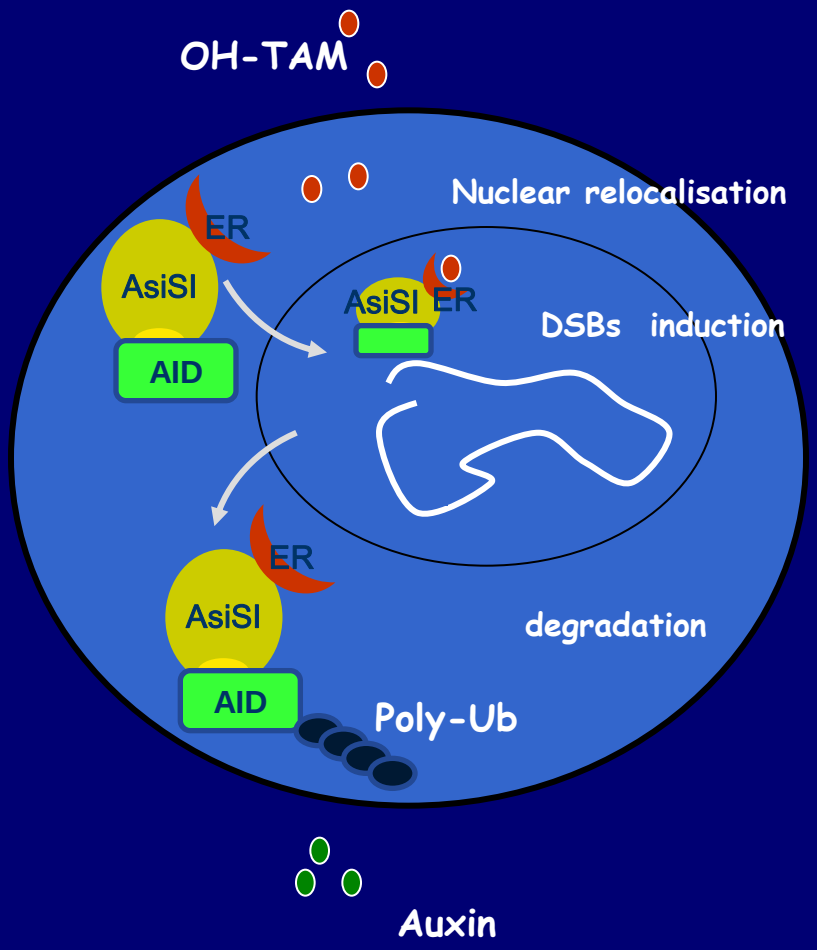
chromosome

DIvA, a sequence specific DSB inducible cell line



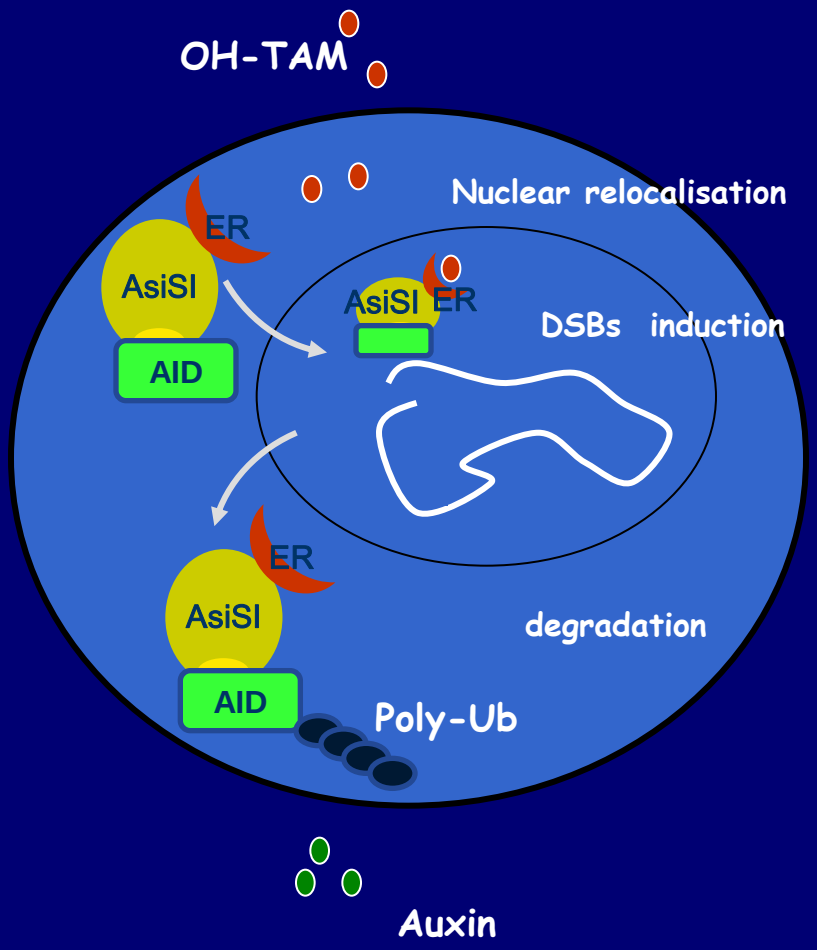
Aymard et al, 2014

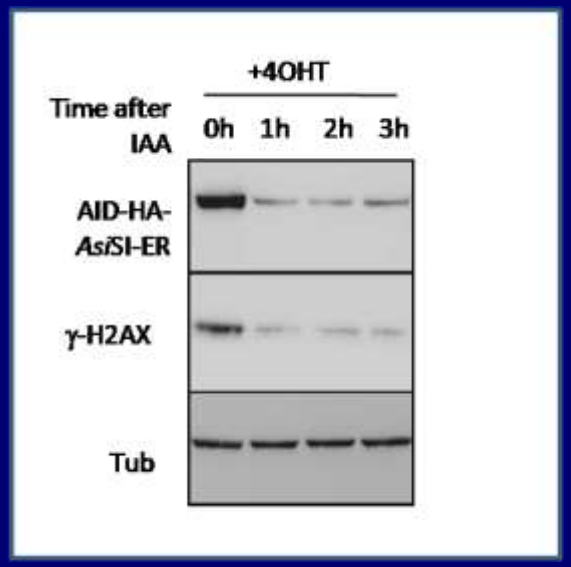
DIvA, a sequence specific DSB inducible cell line



Aymard et al, 2014

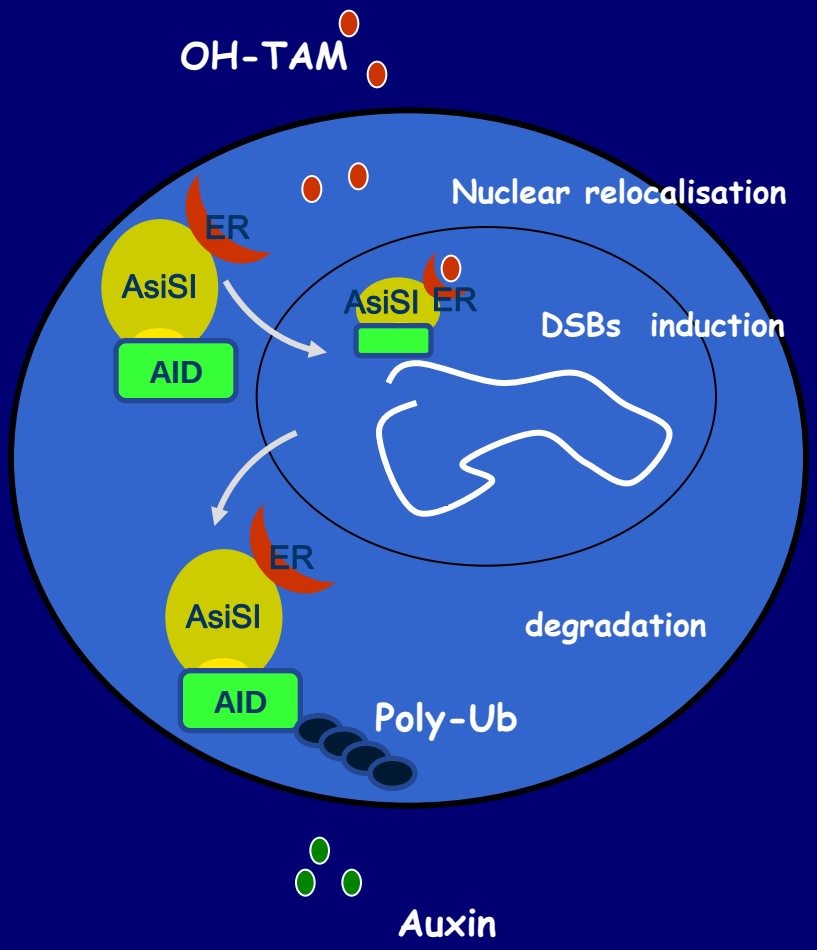
DIvA, a sequence specific DSB inducible cell line

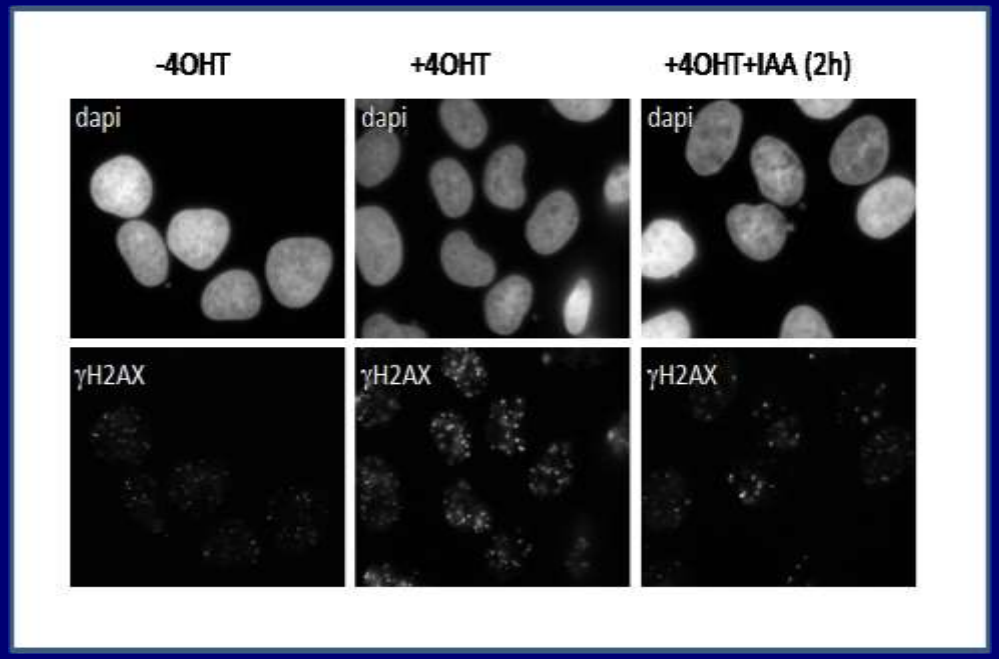




Aymard et al, 2014

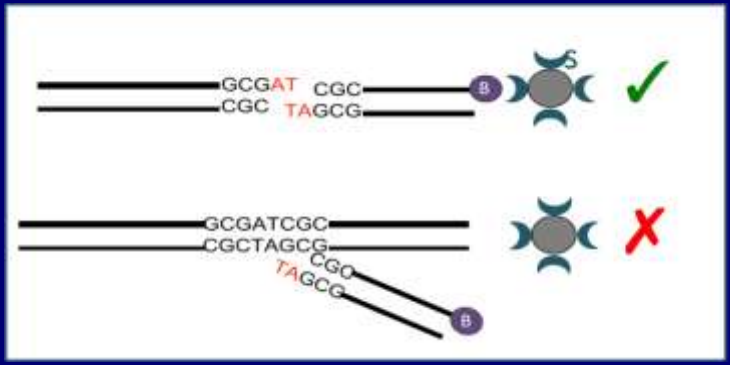
DIvA, a sequence specific DSB inducible cell line

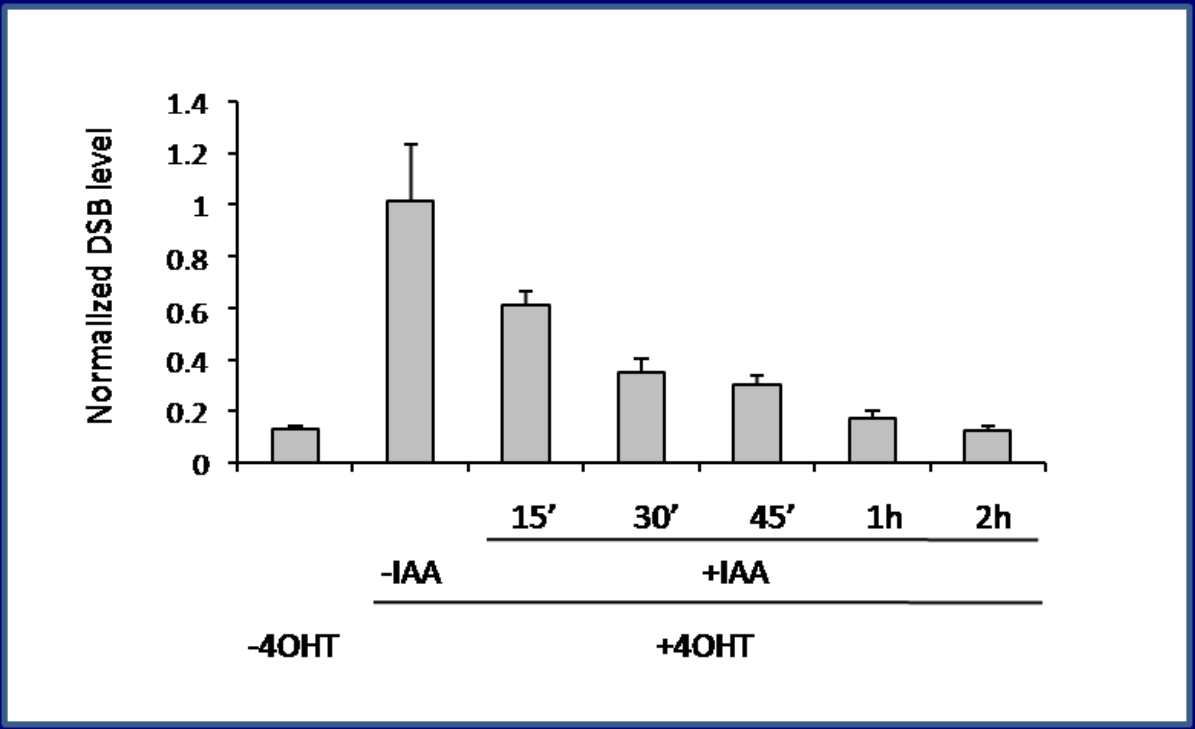




Aymard et al, 2014

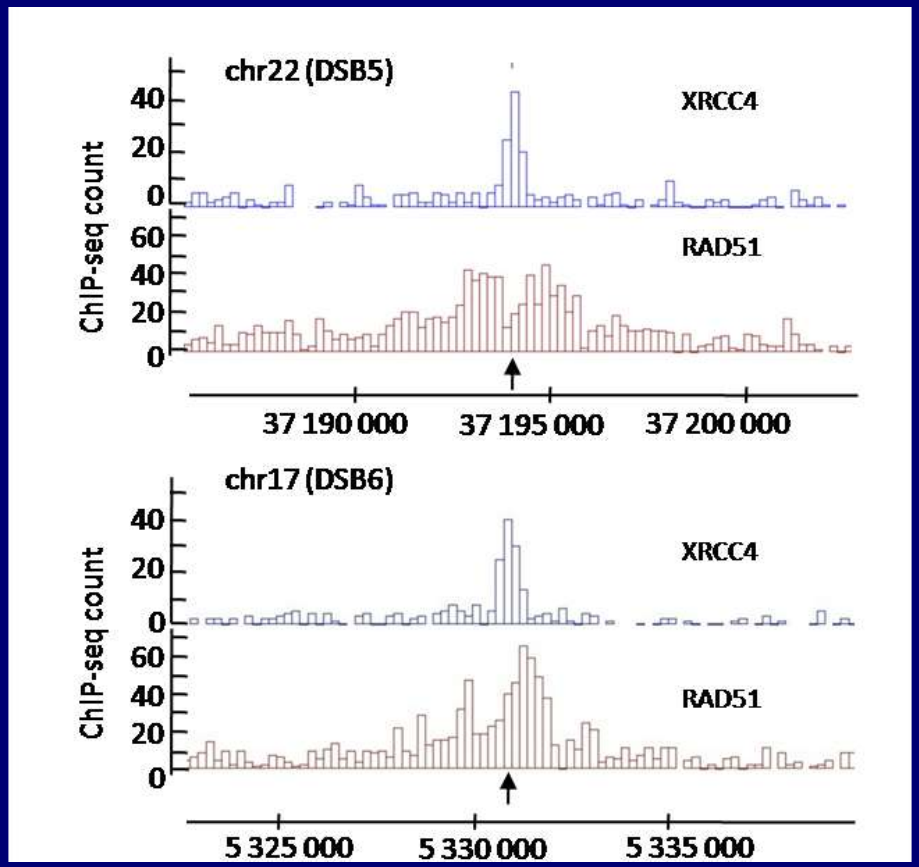
A protocol to measure repair kinetics at AsiSI induced breaks

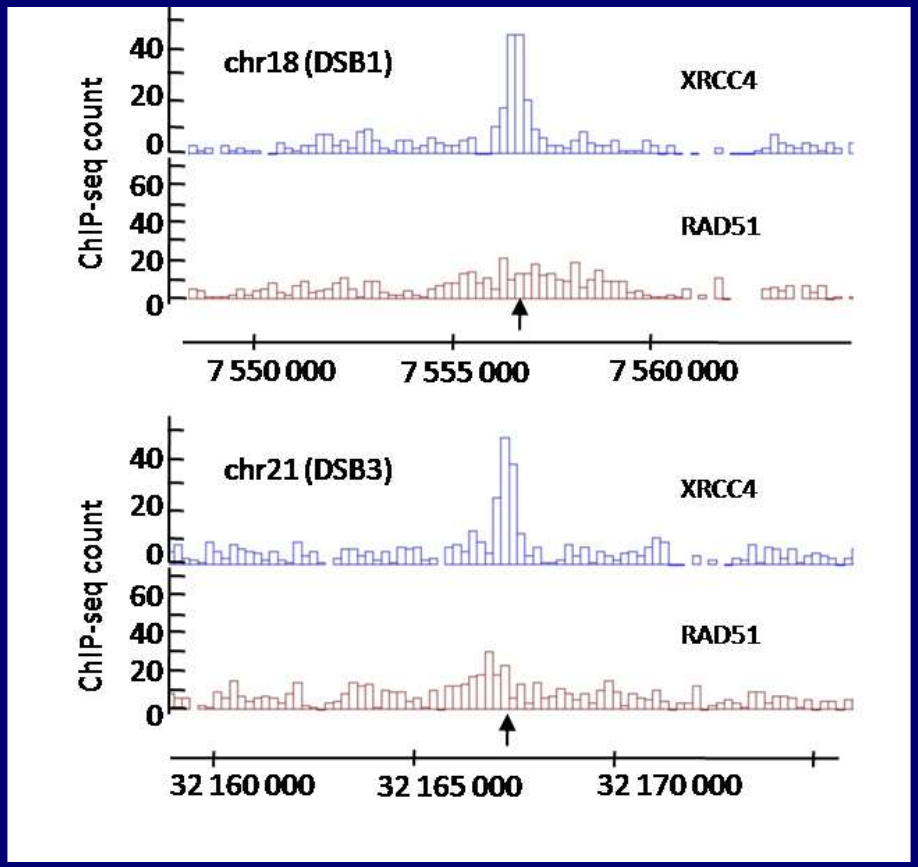




Chailleux et al, 2014

ChIP-seq XRCC4/RAD51



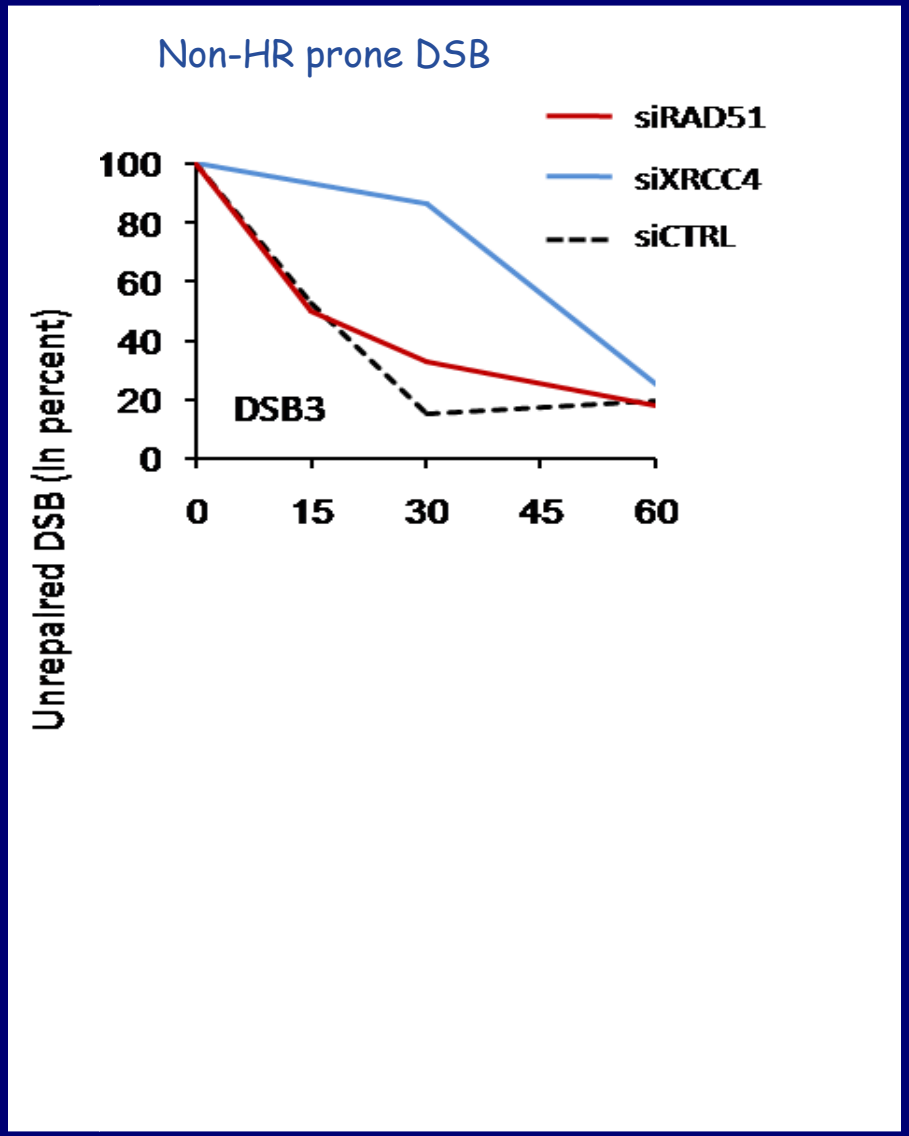


HR-prone

Non HR-prone

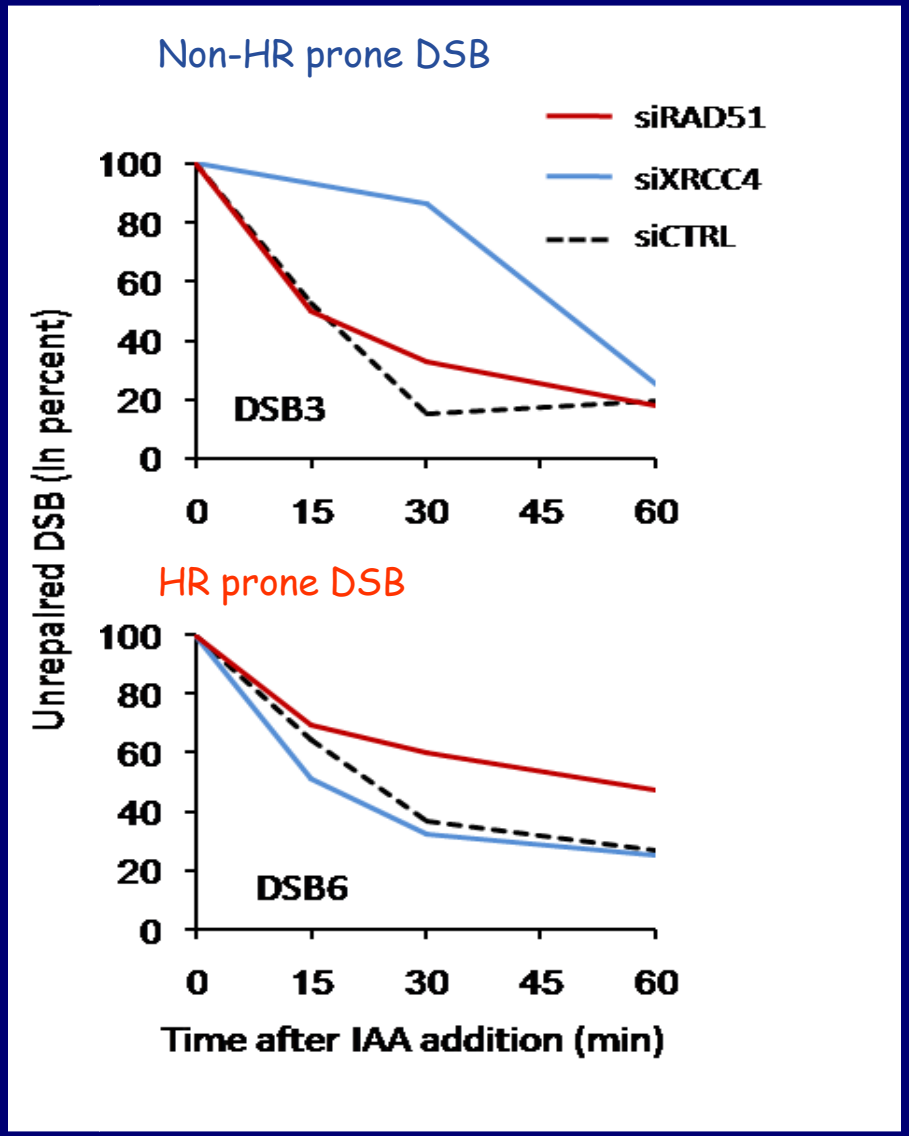
Aymard et al, 2014

HR-prone sites rely on RAD51 for repair



Aymard et al, 2014

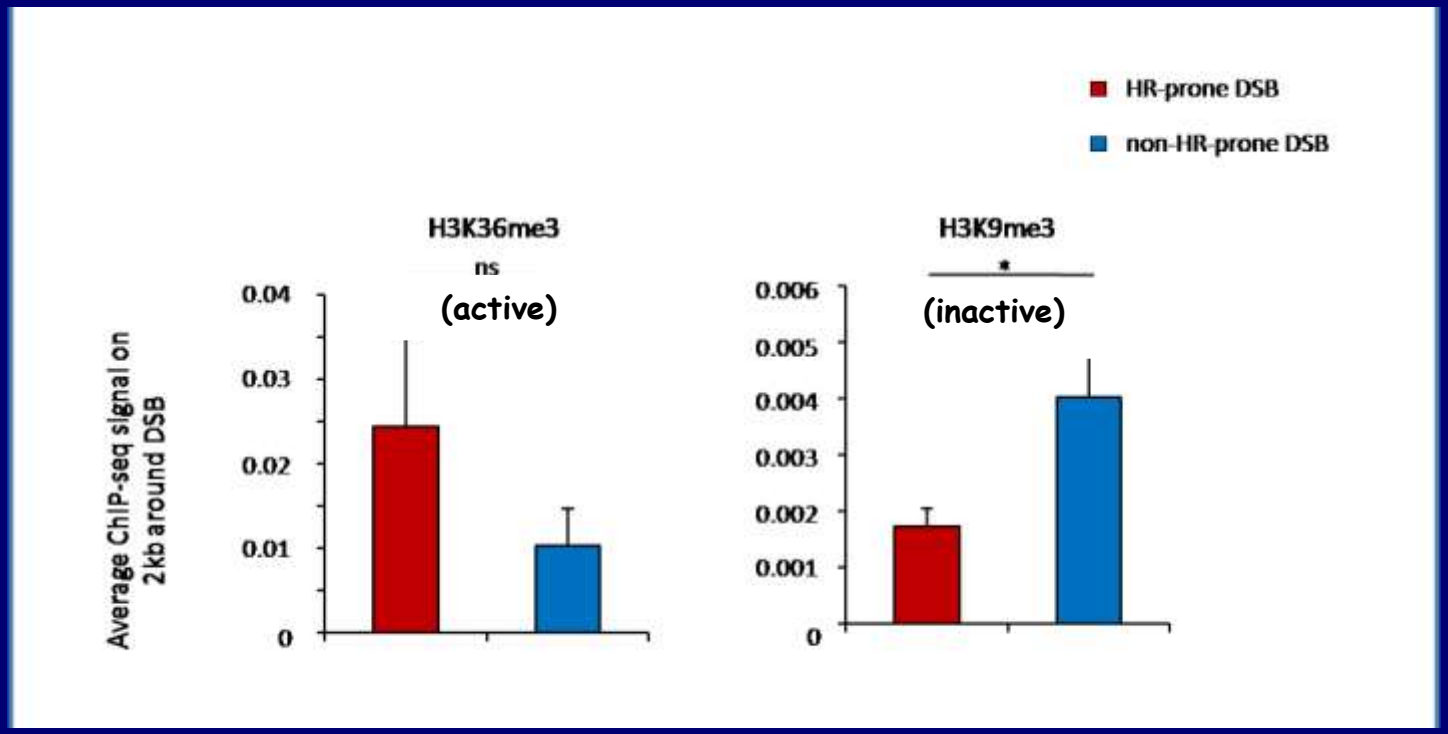
HR-prone sites rely on RAD51 for repair



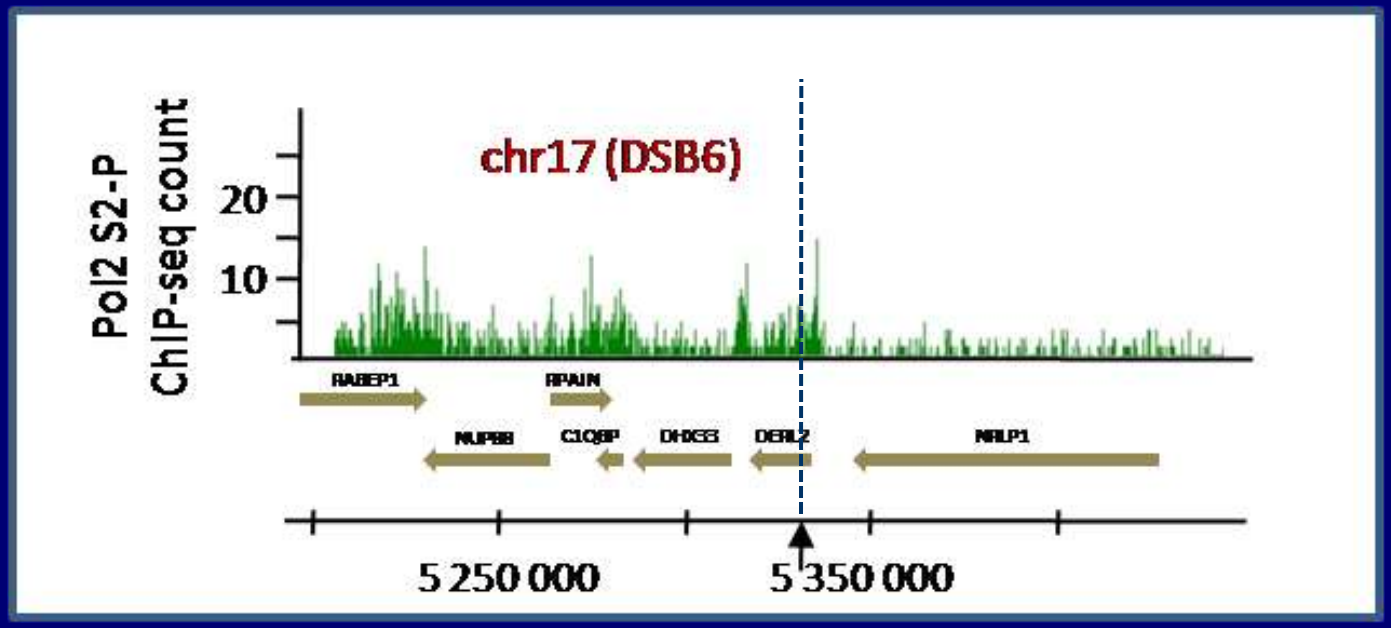
Aymard et al, NSMB, 2014

HR-prone sites are enriched in active marks.

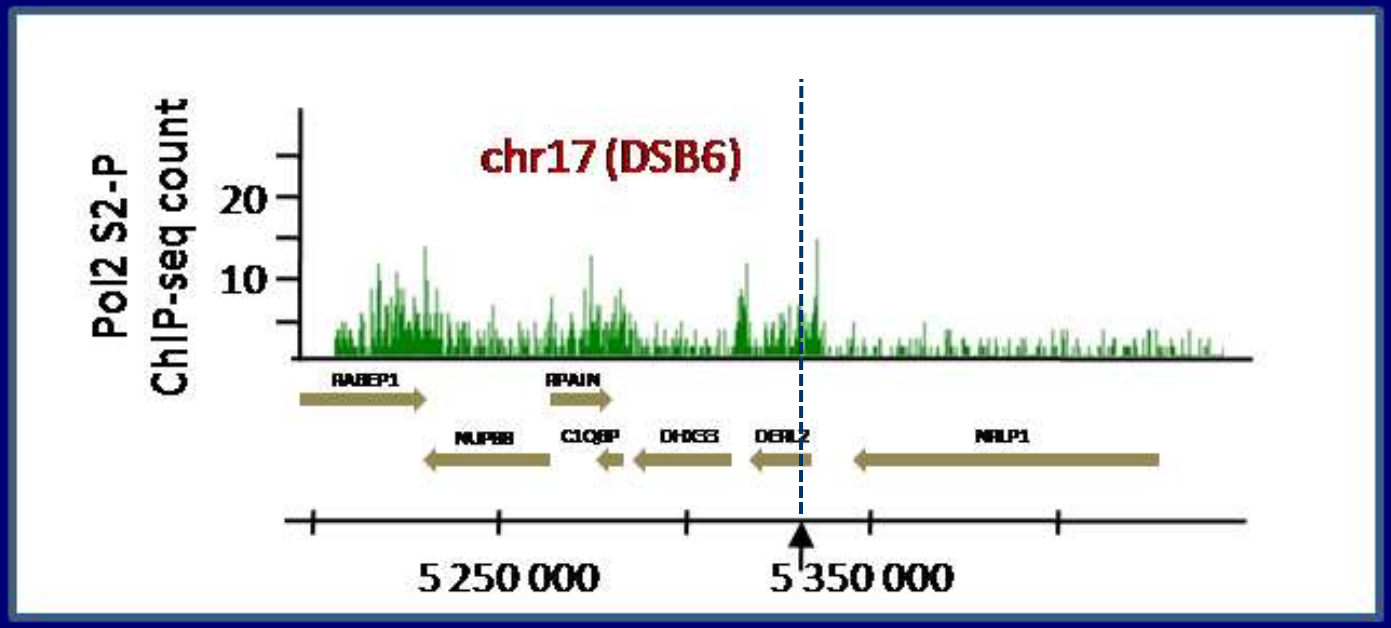
ENCODE Epigenome project

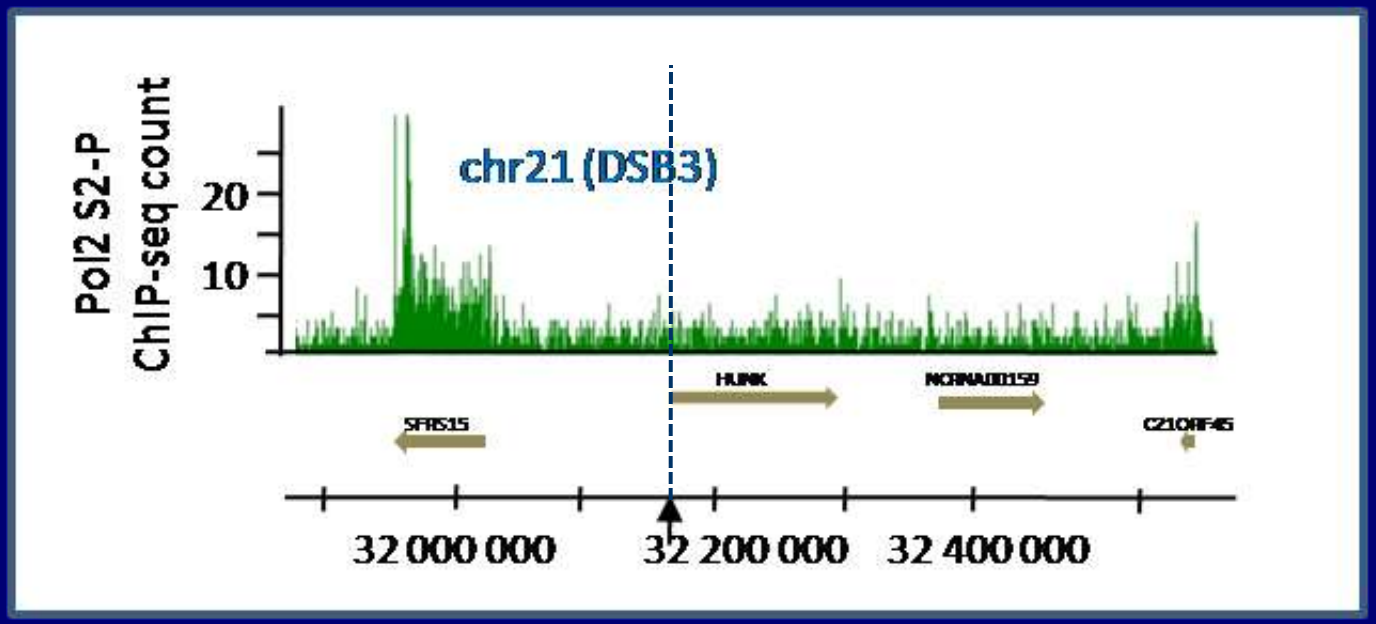


HR-prone sites lie in transcribed units

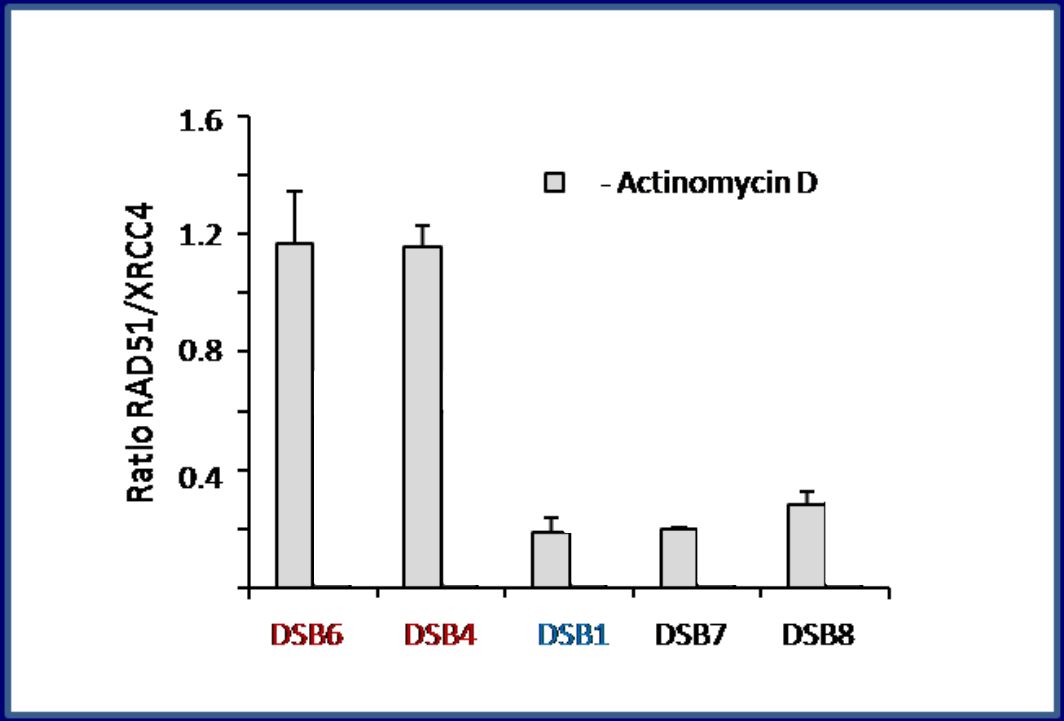


HR-prone sites lies in transcribed units



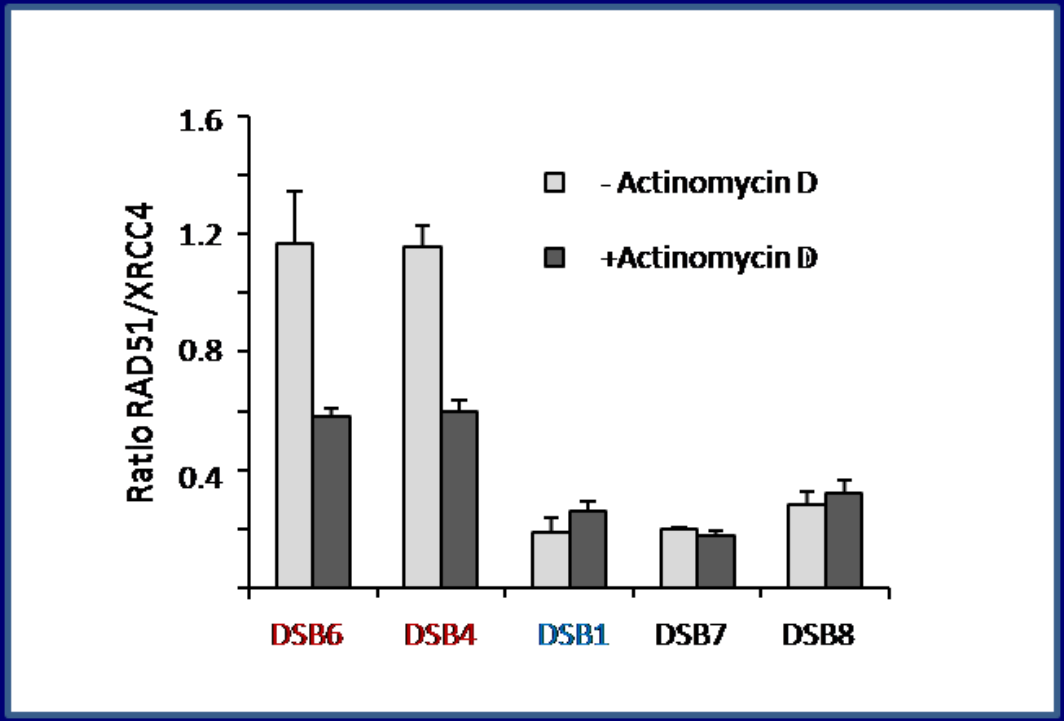


Transcription inhibition orients repair toward a RAD51 independant pathway



Francois Aymard

Transcription inhibition orients repair toward a RAD51 independant pathway

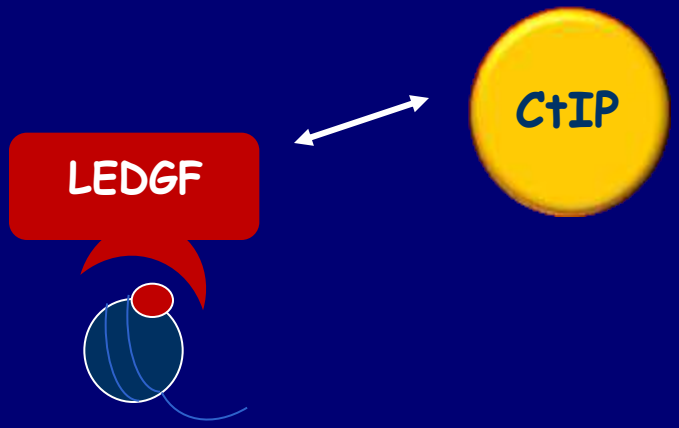


Aymard et al, 2014

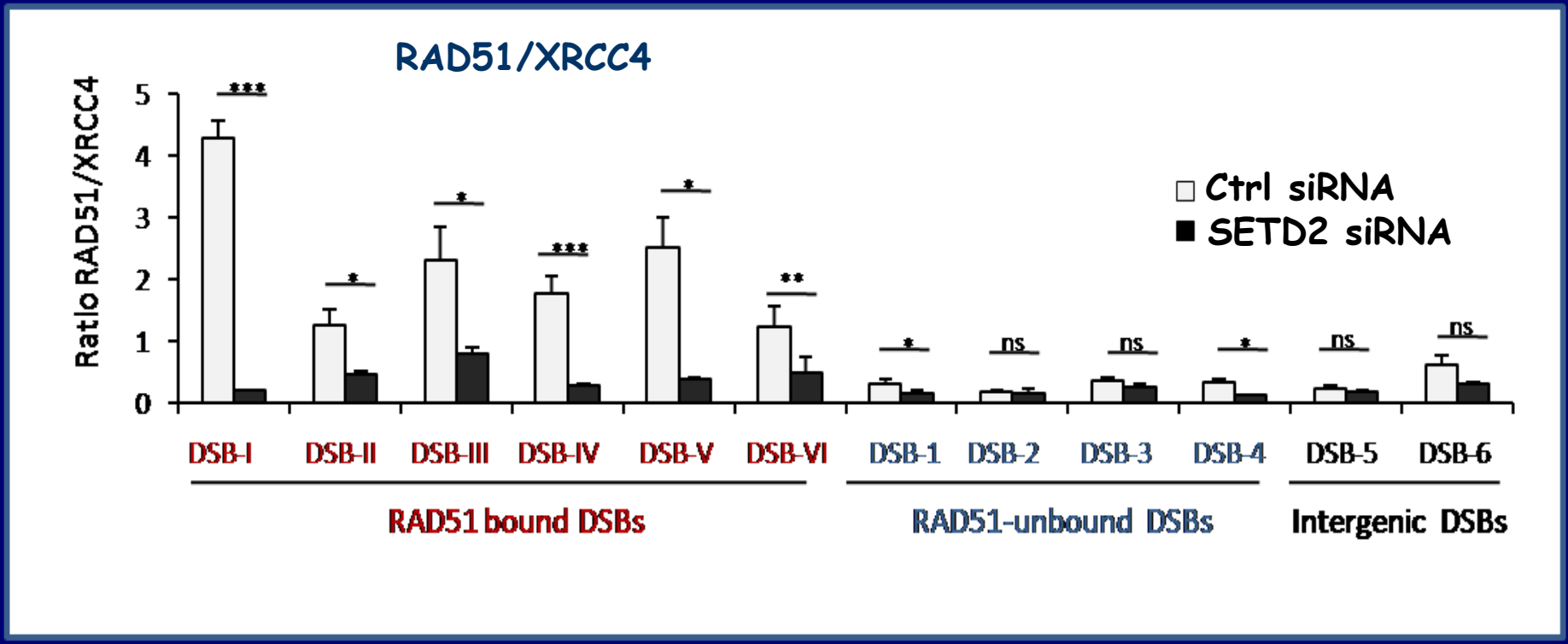
nature structural & molecular biology

LEDGF (p75) promotes DNA-end resection and homologous recombination

Mads Daugaard[1–3], Annika Baude[1,2], Kasper Fugger[4], Lou Klitgaard Povlsen[1,2,8], Halfdan Beck[4], Claus Storgaard Sørensen[4], Nikolaj H T Petersen[1,2], Poul H B Sorensen[3], Claudia Lukas[2,5], Jiri Bartek[2,6,7], Jiri Lukas[2,5], Mikkel Rohde[1,2] & Marja Jäättelä[1,2]

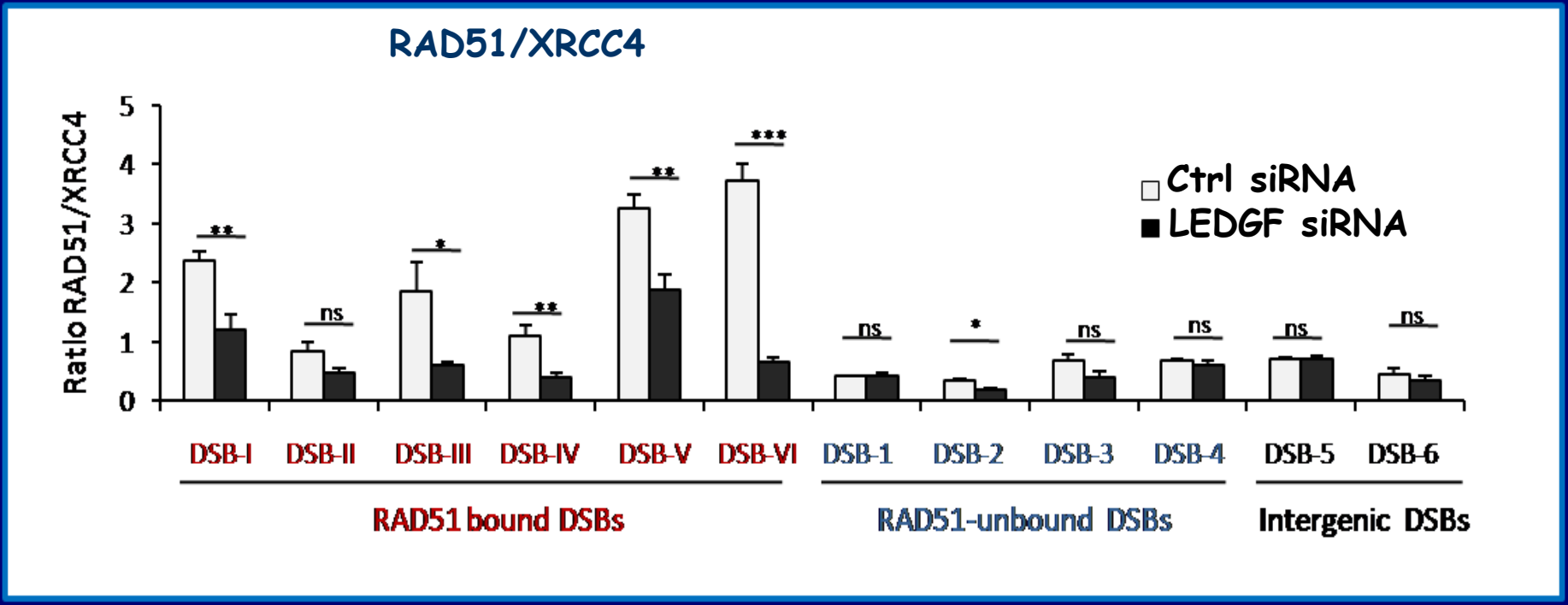


H3K36me3 is required for channeling HR at active genes

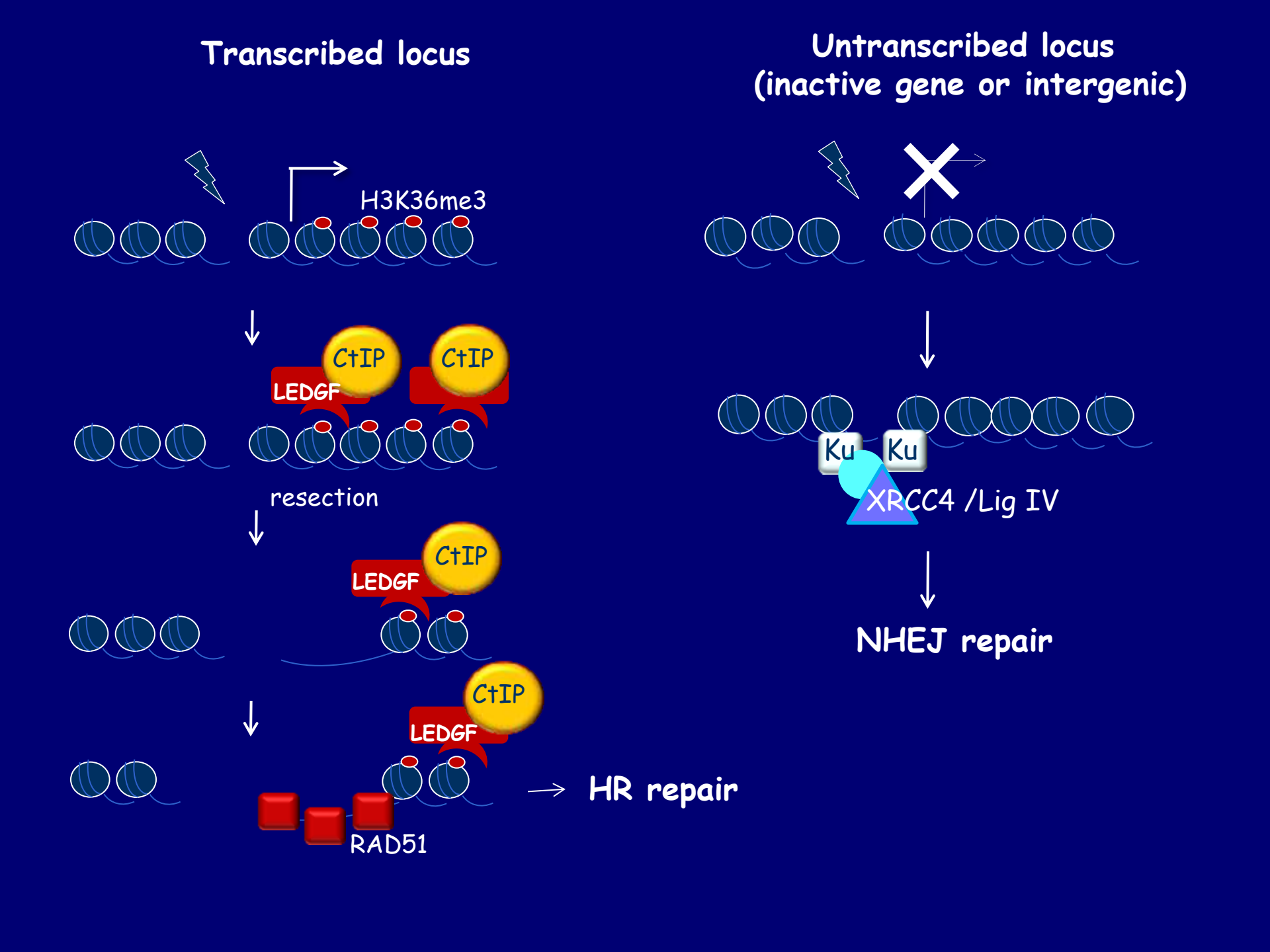


Aymard et al, 2014

The H3K36me3- interacting factor LEDGF is required for channeling HR at active genes



Aymard et al, 2014


Transcribed locus
Untranscribed locus
(inactive gene or intergenic)
H3K36me3
CtIP
CtIP
LEDGF
resection
CtIP
LEDGF
CtIP
LEDGF
RAD51
HR repair
Ku
Ku
XRCC4 /Lig IV
NHEJ repair

Aknowledgments

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Thomas Clouaire (Post Doc)

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Jonathan Choudjaye (PhD)

Virginie Daburon (AI)
Marion Aguirrebengoa
(Bioinformatic Engeneer)

EMBL Genomic Core
Vladimir Benes,
Tomi Ivacevic

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Jim Haber, Boston US
Kyle Miller, Auxin US
Tanya Paull, Auxin US
Sagar Sengupta, New Dehli India
Jason Iacovoni, Toulouse
Erwan Watrin, Rennes
Emmanuelle Fabre, Pasteur/ Kerstin Bystricky (LBME)