Chromatin Responses to DNA Damage

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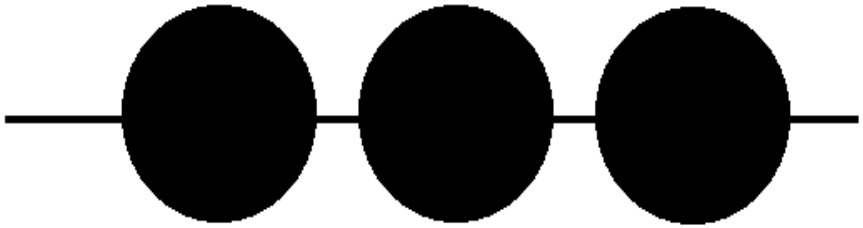
Eukaryotic genome is contained in chromatin

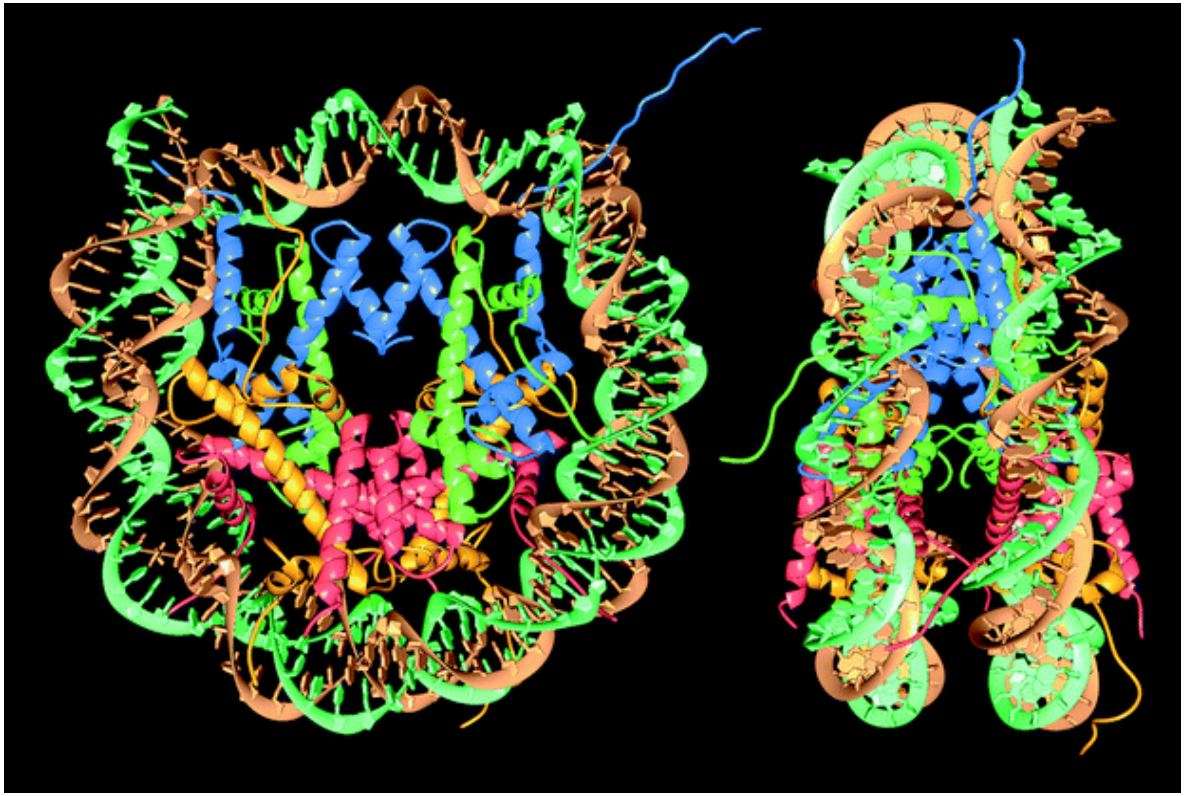
DNA

Transcription
Replication
Recombination
Repair



"The Chromatin Problem"

Nucleosome is the basic unit of chromatin



Richmond

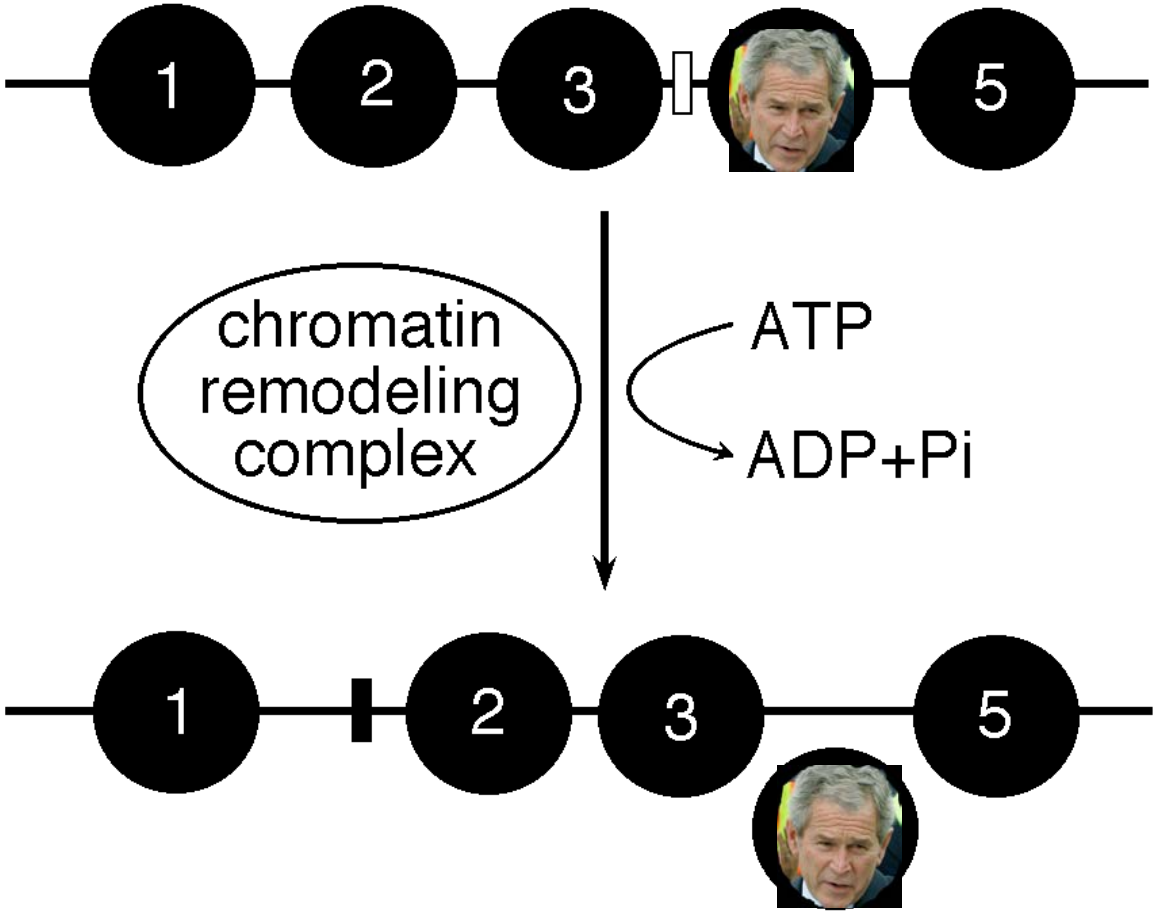
Nucleosome = histone octamer + DNA

Histone octamer = 2 H2A/H2B dimers + H3/H4 tetramer

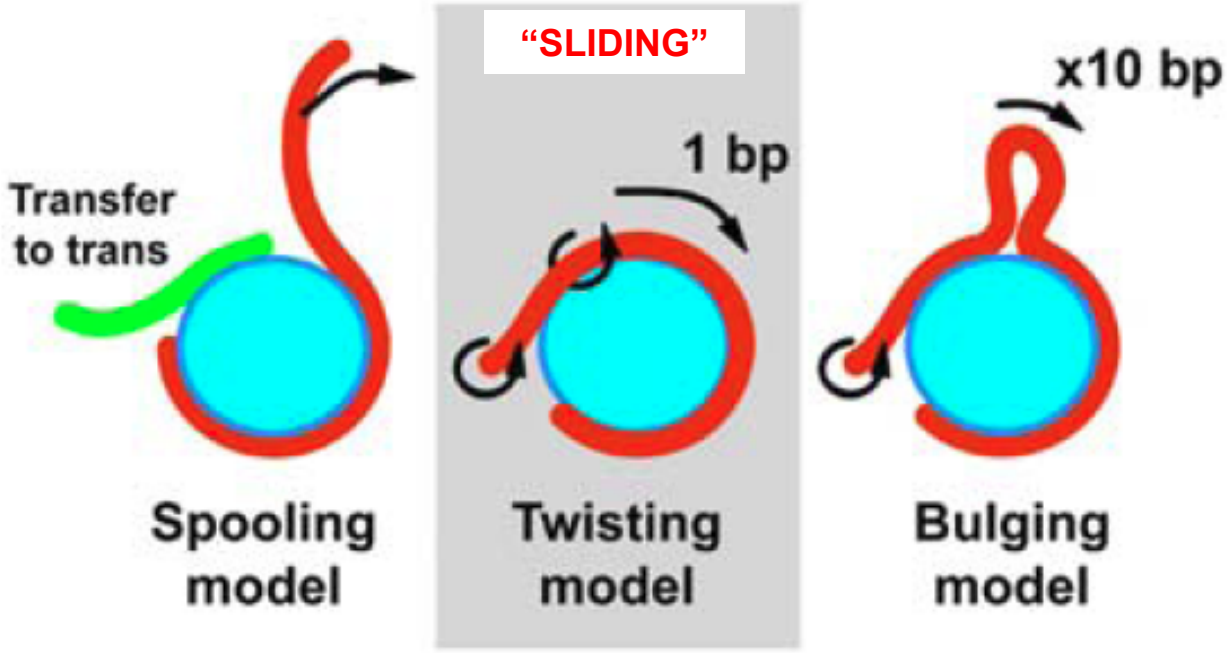
Three major ways to modify chromatin

- **Histone variants (H2AZ, H2AX marks chromatin)**
- **Secondary modifications of histones (P, Ac, Me…)**
- **ATP-dependent chromatin remodeling**

ATP-dependent chromatin remodeling

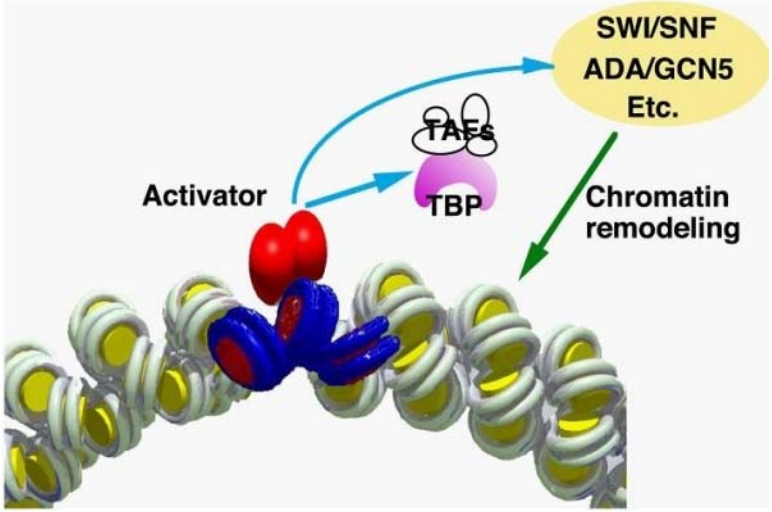


Models for ATP-dependent chromatin remodeling



Langst & Becker 2001 JCS 114 2561-8

Chromatin and Transcription
1990s - present



YFG

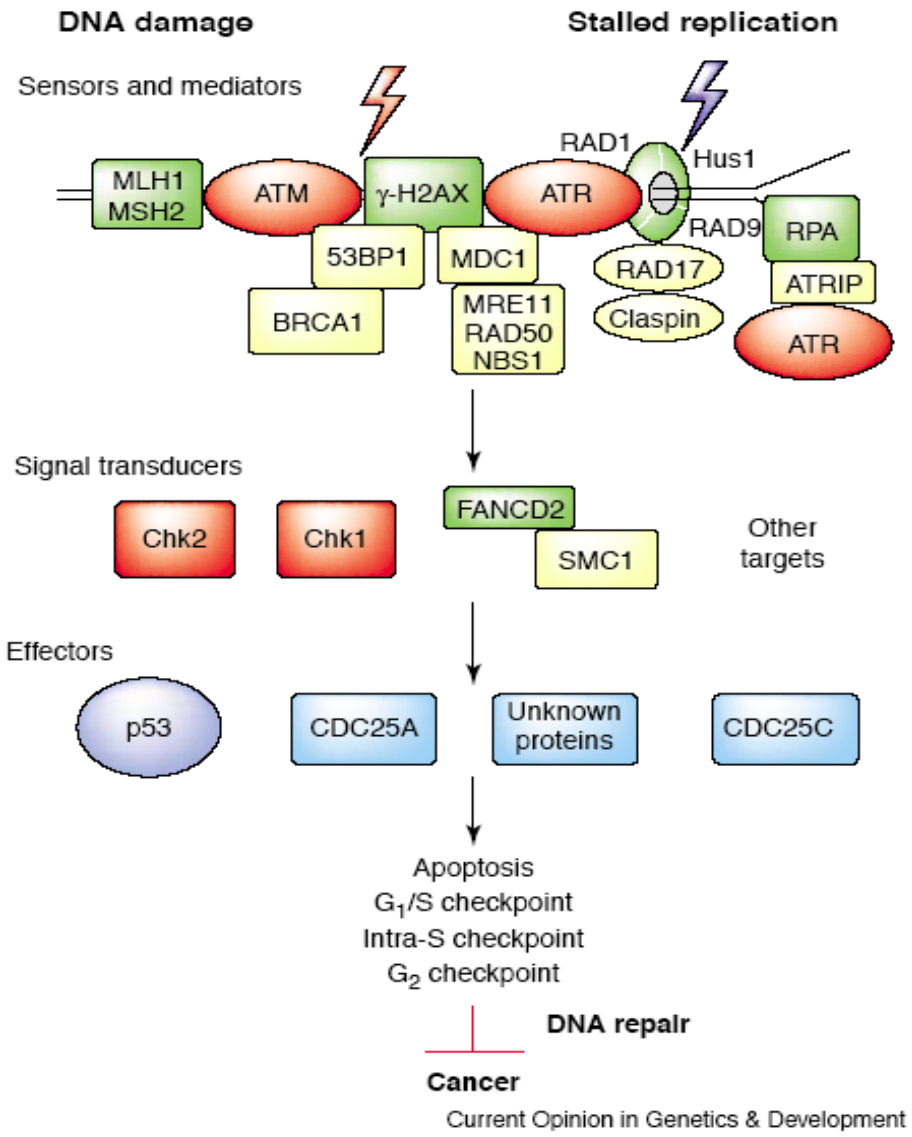
Multiple chromatin modifying complexes (both histone modifying and ATP-dependent) are often needed to regulate one gene.

Chromatin in other nuclear function?

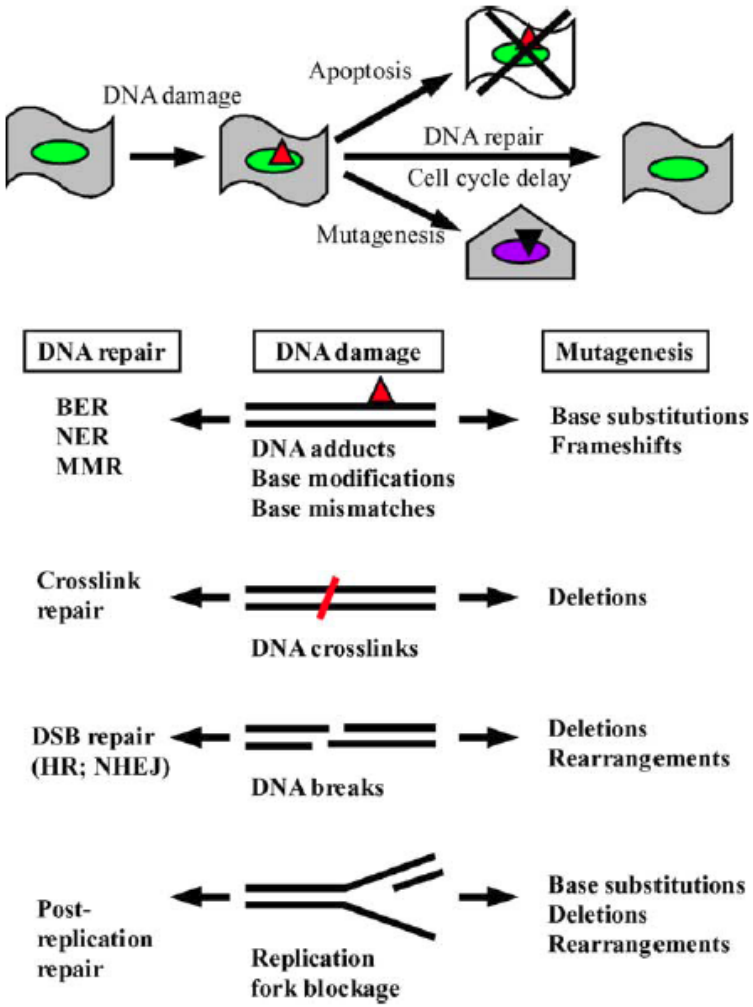
* DNA repair
* Checkpoint regulation
* DNA replication

Chromatin responses to DNA damage

Sensing DNA damage



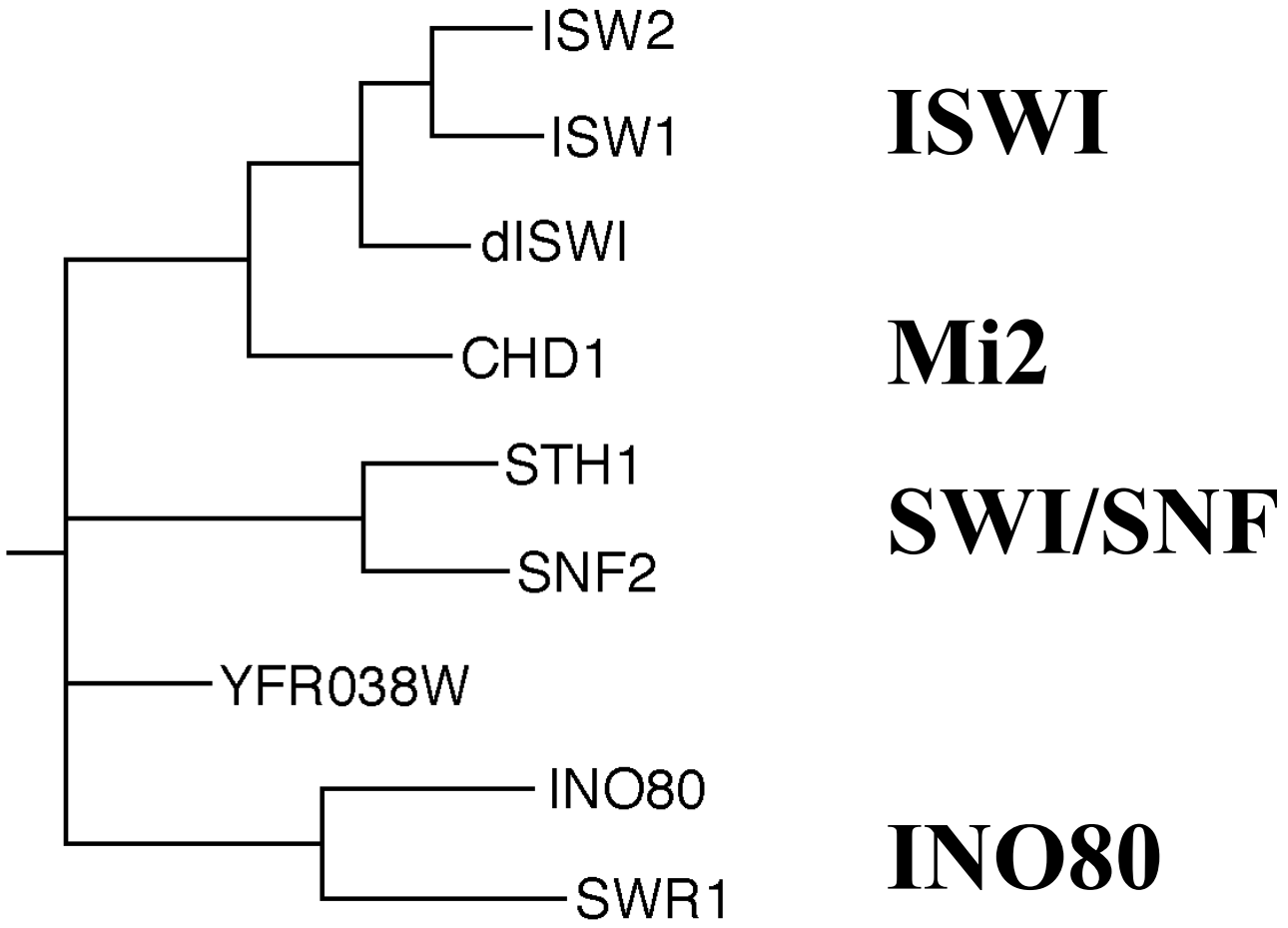
Repairing DNA damage



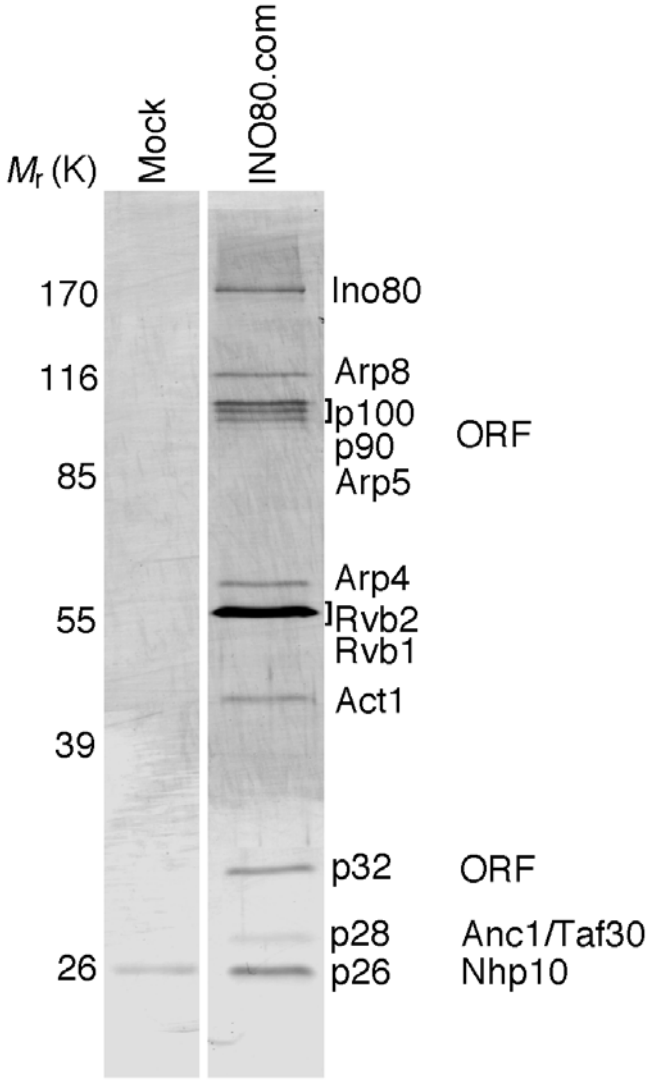
Defects in the DNA Damage Response Result in Disease

Pathway	Gene	Syndrome	Cancer Type
Mismatch repair	*MSH1, MSH2, MLH1, MSH6, PMS1, PMS2,*	HNPCC	Colon cancer
Nucleotide excision repair	*XPA–XPG*	Xeroderma pigmentosum (XP)	Skin cancer
Replication bypass	*XPV*	XP variant	Skin cancer
Replication fork integrity; double-strand break repair	*NBS1*	Nijmegen breakage syndrome	Lymphoma
	MRE11	A-T-like disorder	Lymphoma/leukemia
	WRN	Werners syndrome	Various
	BLM/RECQL3	Blooms syndrome	Leukemia, carcinomas
	BRCA1, BRCA2	Familial breast cancer	Breast/ovarian cancer
DNA crosslink repair	*FANCA-FANCG*	Fanconi anemia	Leukemia
DNA damage signaling; cell cycle checkpoints	*ATM*	Ataxia telangiectasia (A–T)	Lymphoma/leukemia
	TP53	Li-Fraumeni syndrome	Various
	RB1	Retinoblastoma	Retinoblastoma

INO80, a conserved class of SWI/SNF ATPases



The INO80 complex



Shen et al. *Nature* 2000

INO80 biology

- **Important transcription regulator**

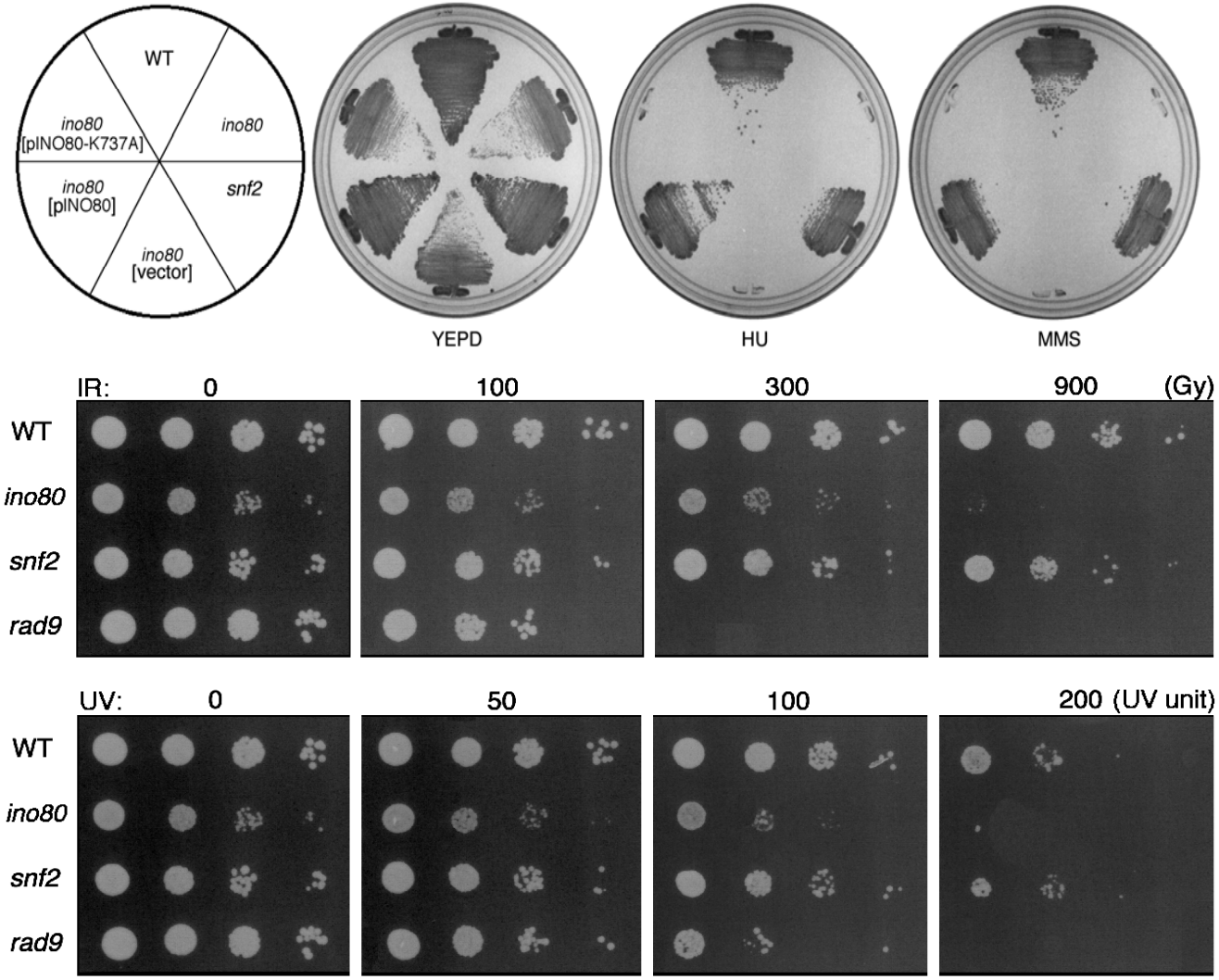
  Shen et al. *Nature* 2000

  Mizuguchi et al. *Science* 2004

- **Connection to inositol signaling**

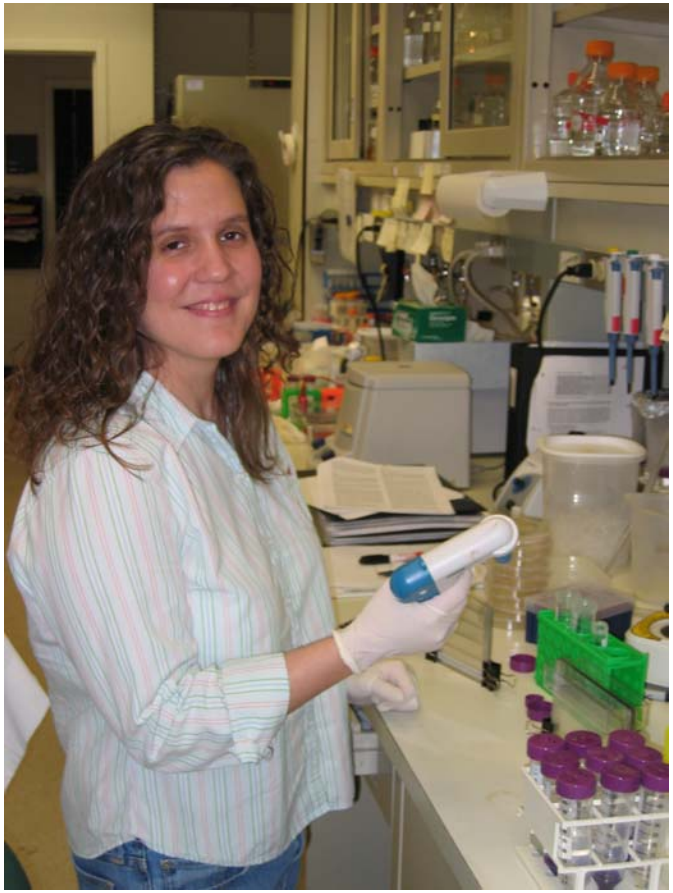
  Shen et al. *Science* 2003

ino80 mutant is sensitive to DNA damaging agents



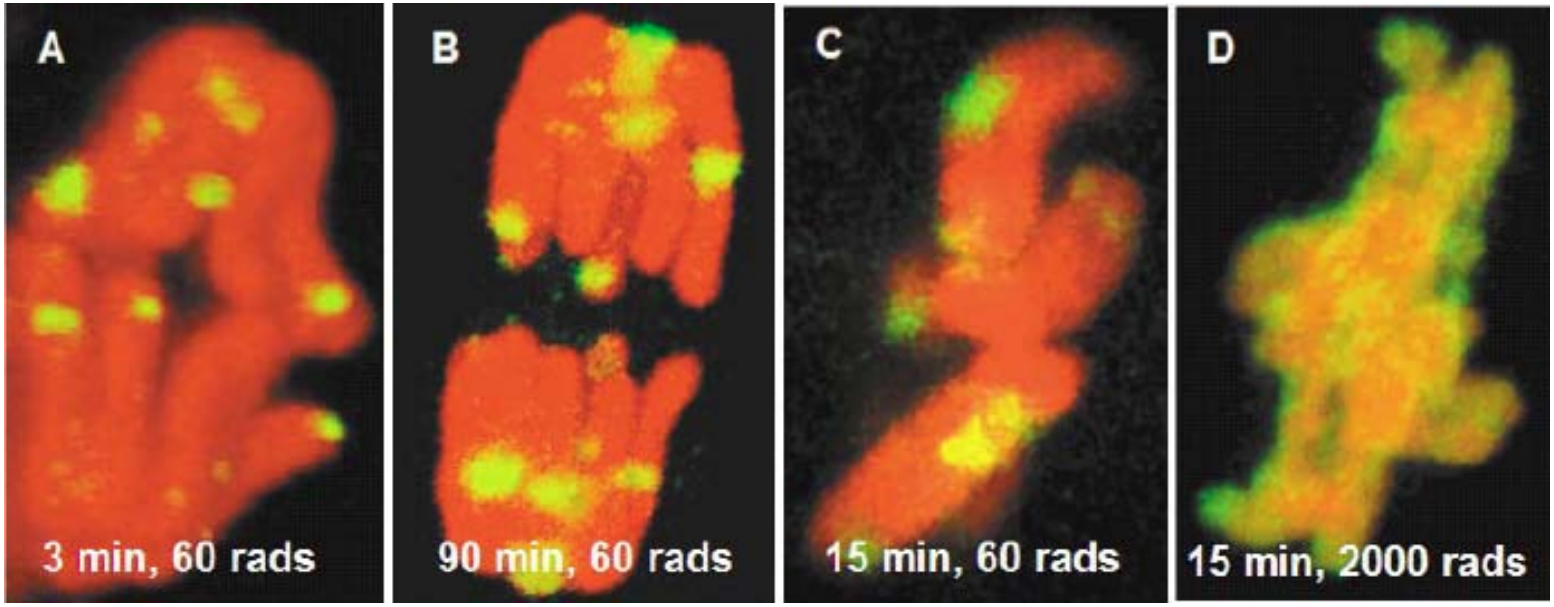
Is INO80 directly involved in DNA repair?

(such as DSB repair)



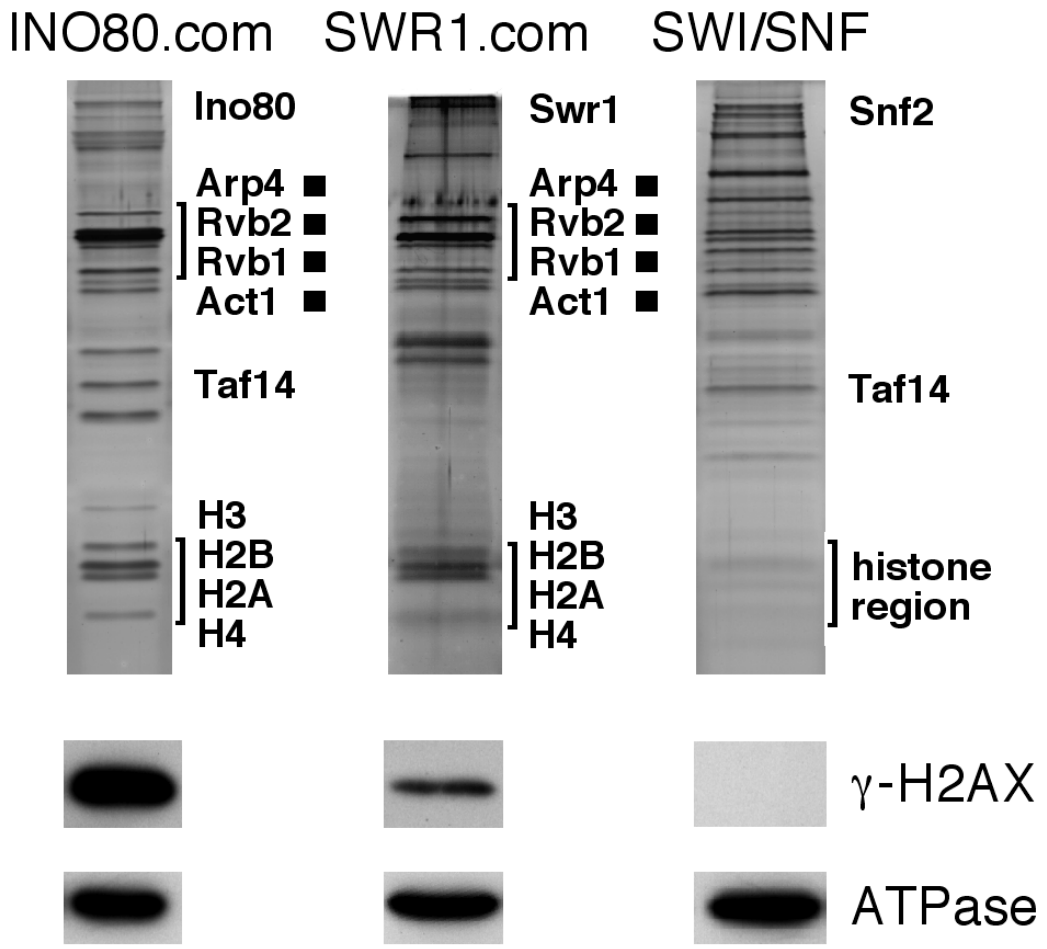
Ashby Morrison

The Chromatin connection: Histone is involved (histone variant H2AX)

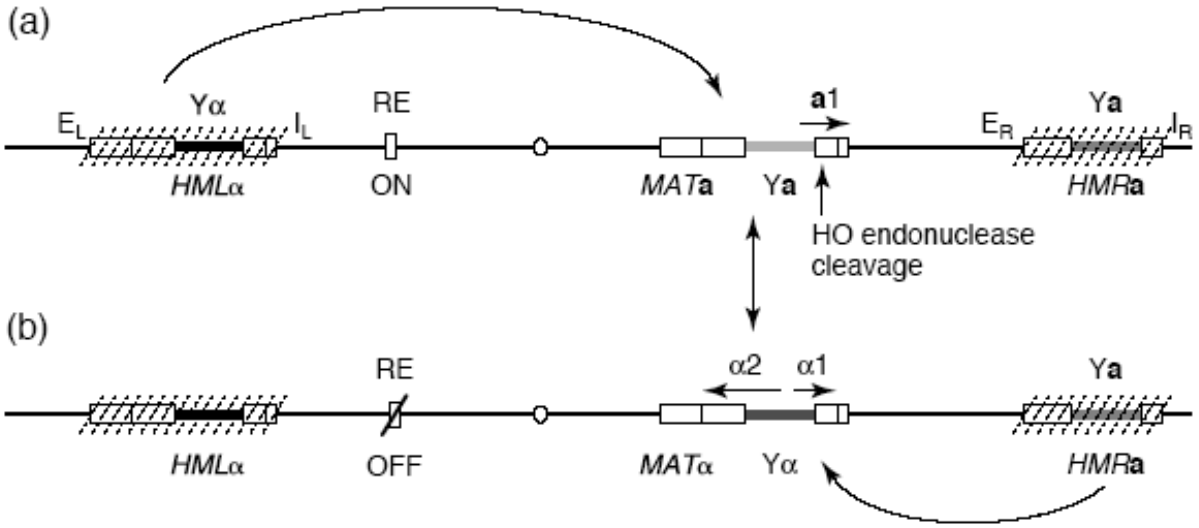


γ-H2AX rapidly accumulates at DSB (Bonner)

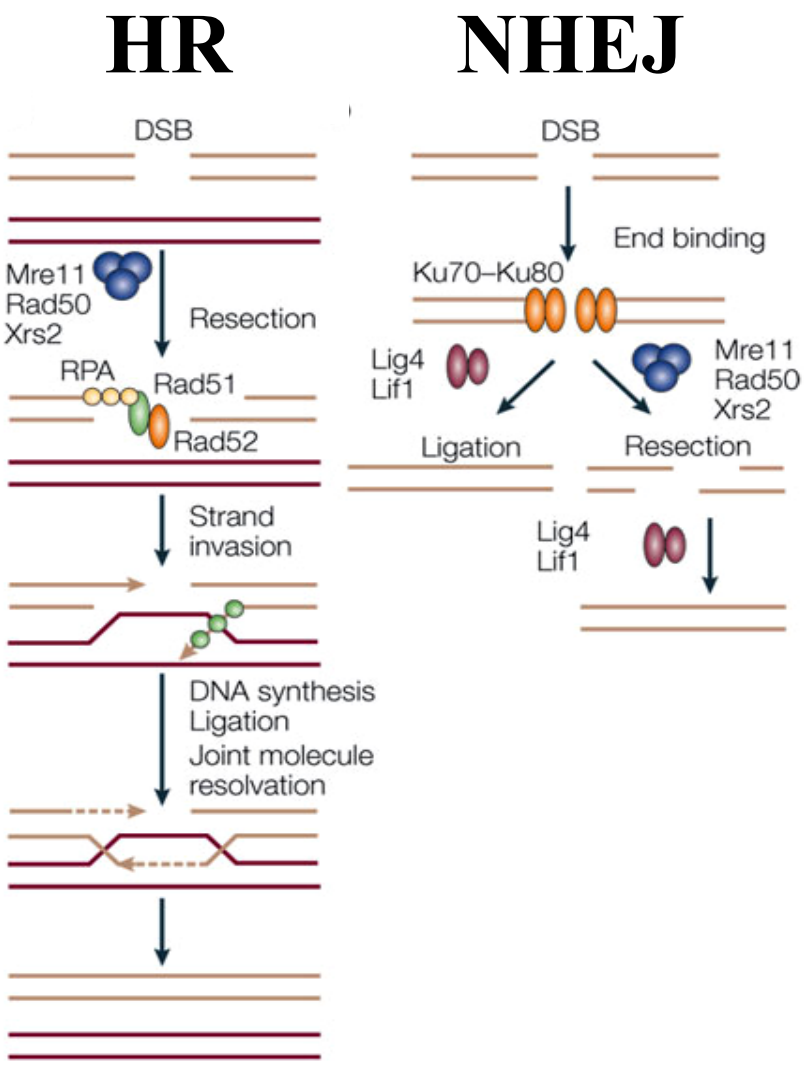
Interaction between INO80 and γ-H2AX (DSB)



Introducing a single DSB in yeast (the Haber system)

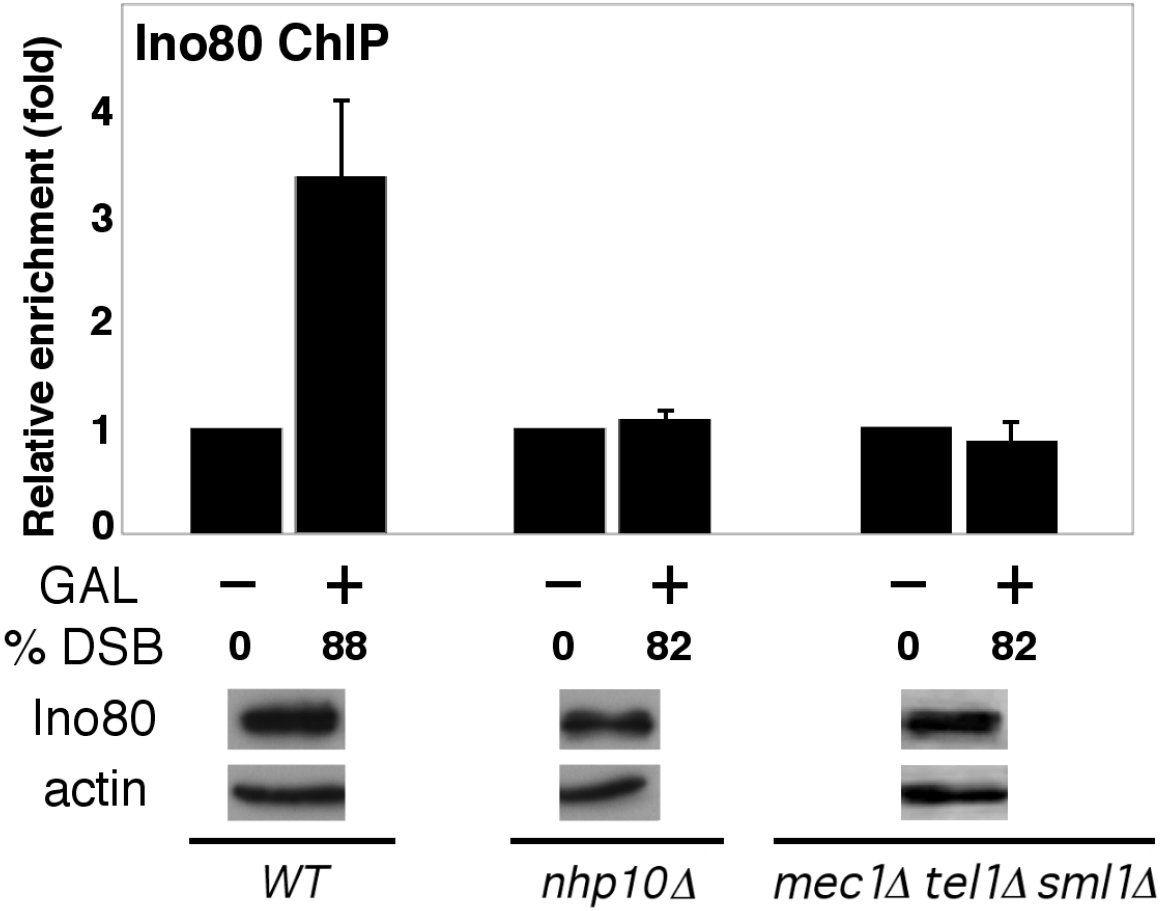


DSB Repair can be dissected with the HO-DSB system



van Attikum et.al., 2005

Interaction between INO80 and γ-H2AX is required to target INO80 to DSB



INO80 and γ-H2AX Interaction Links ATP-Dependent Chromatin Remodeling to DNA Damage Repair

Morrison et al. *Cell* 2004

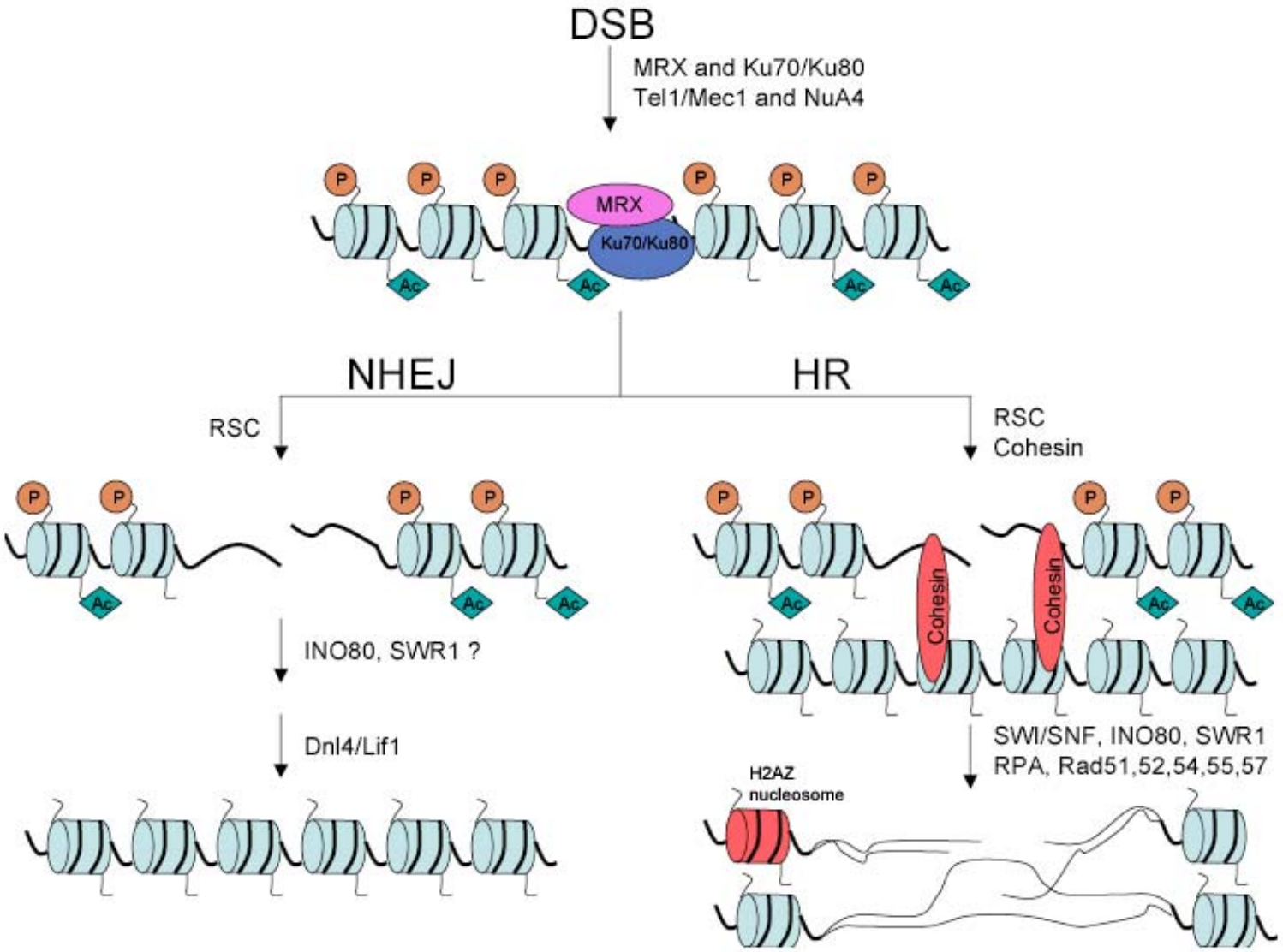
(Gasser, Cote labs)

Emerging roles of INO80 at DSB

(Shen, Gasser, Osley, Cote, Peterson, Hohn and Shi labs)

1. **Involved in both NHEJ and HR**
2. **Involved in resection in HR**
3. **Involved in nucleosome depletion at DSB**

Like regulating a gene, DSB repair requires multiple chromatin modifying activities

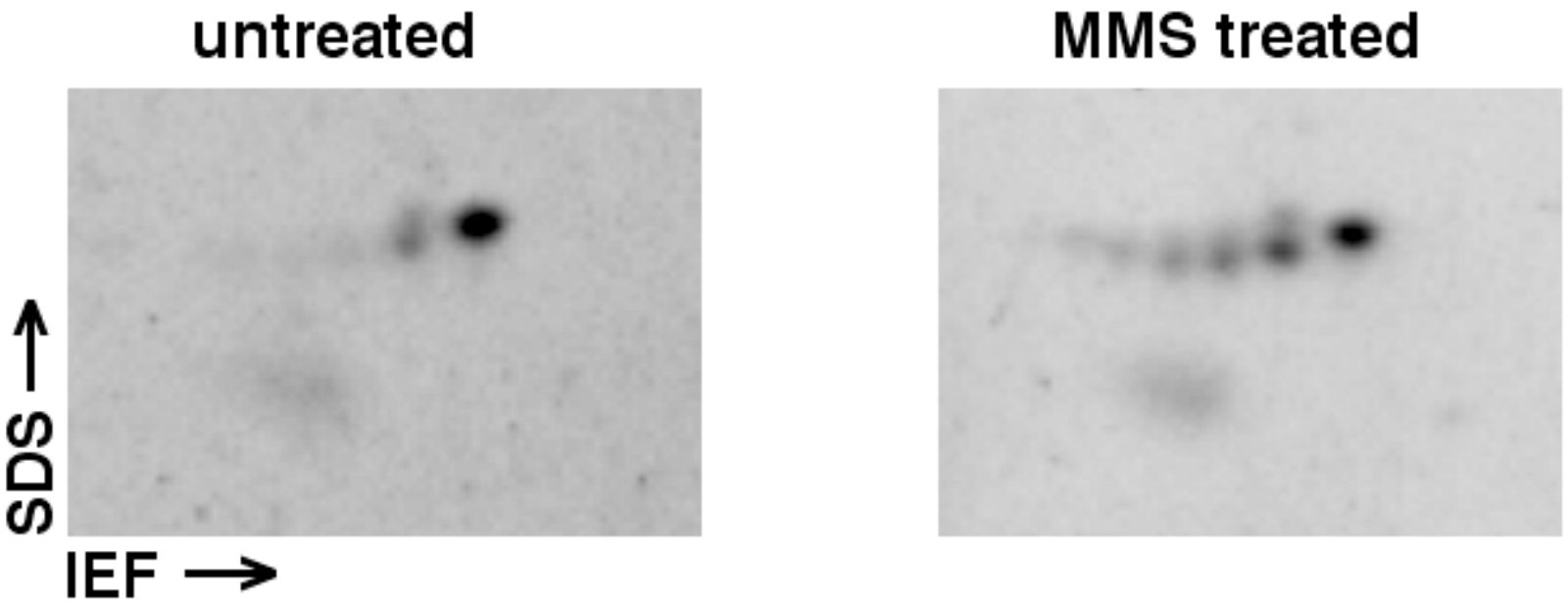


A mystery
(a general question)

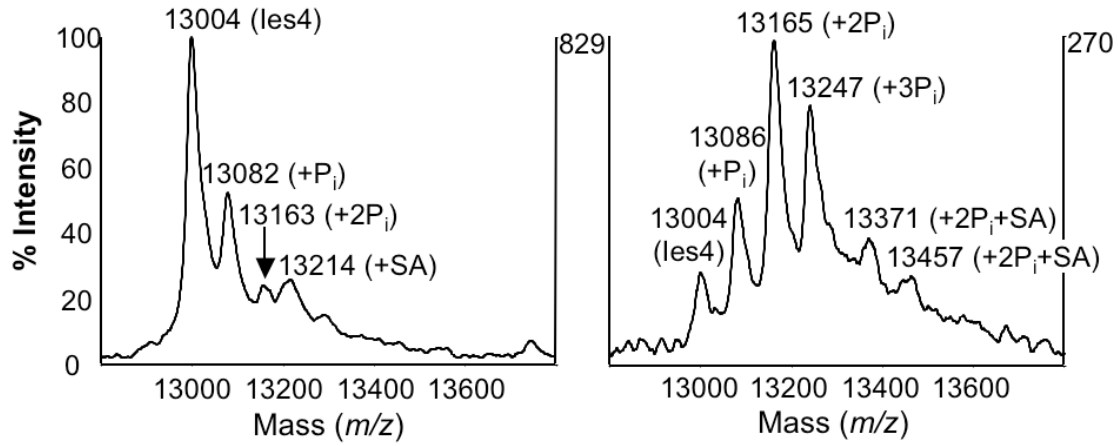
INO80 is involved in both transcription and DNA repair, how does it "switch" between the two modes?

(Ashby Morrison)

Damage-induced modifications of a INO80 subunit

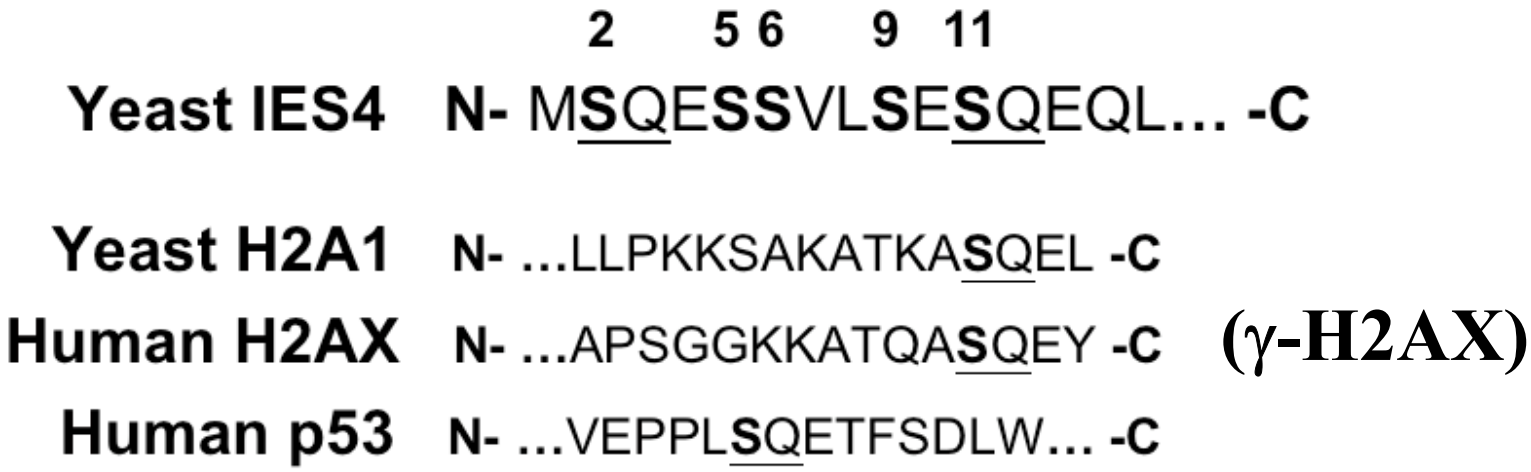


Ies4 phosphorylation identified by TOF



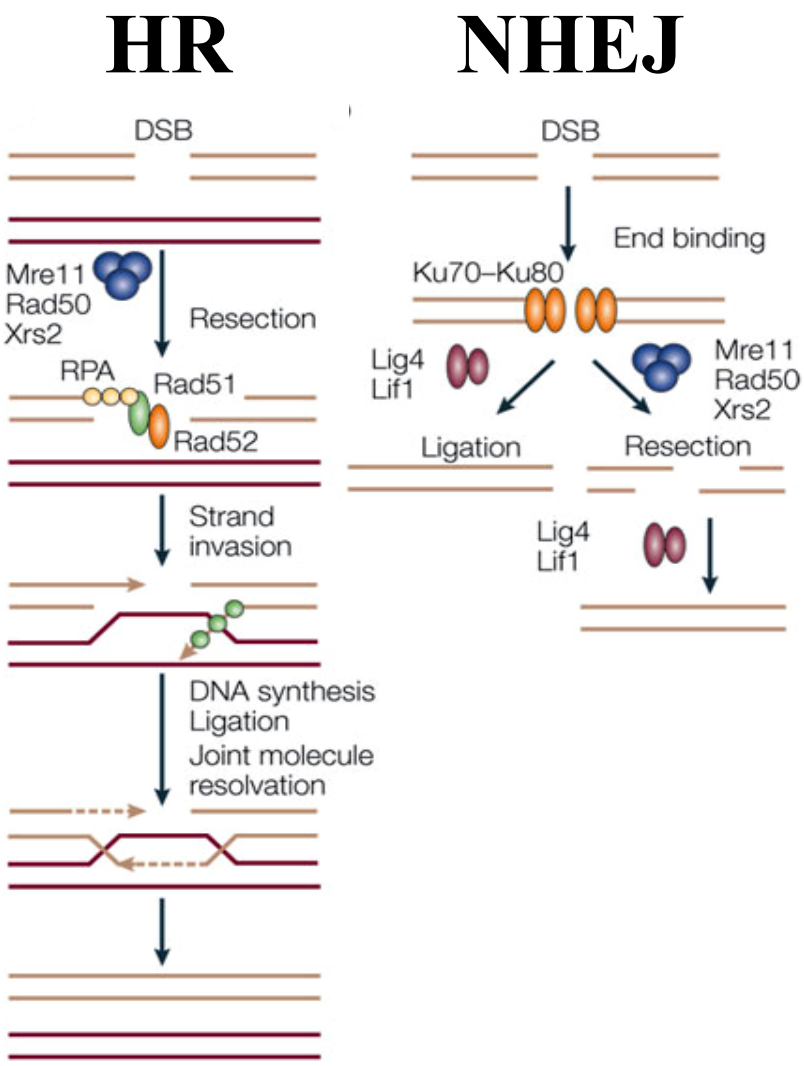
(Person)

A phospho-switch



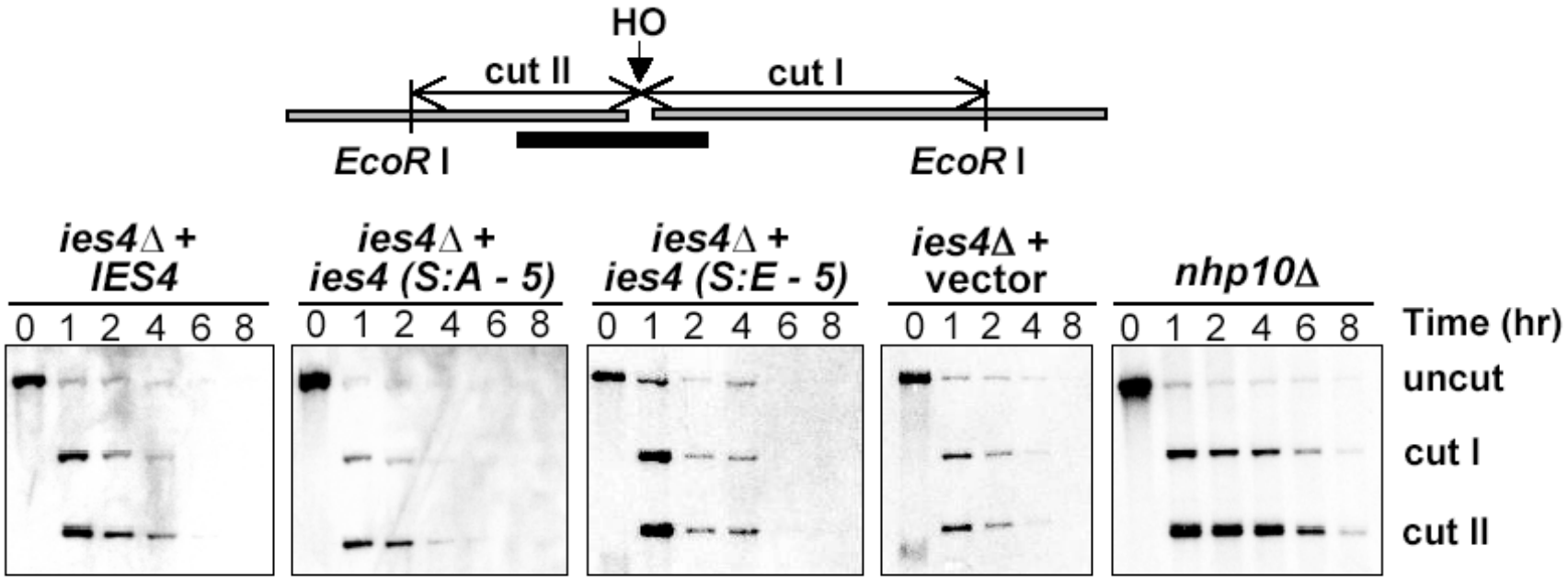
Ies4 is phosphorylated at the conserved ATM/ATR target sites - “S/TQ” motifs

Phospho-switch does not regulate DSB repair



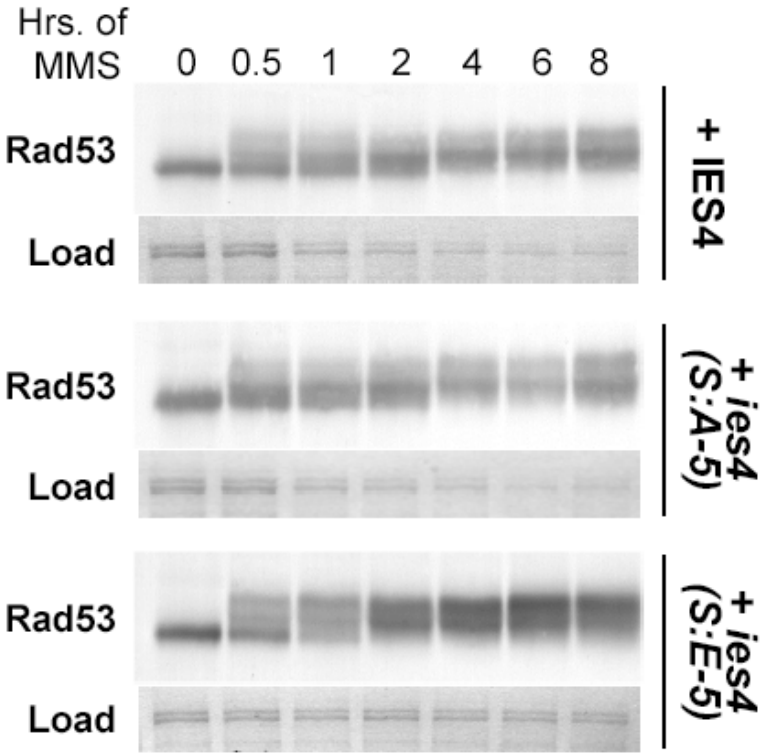
van Attikum et.al., 2005

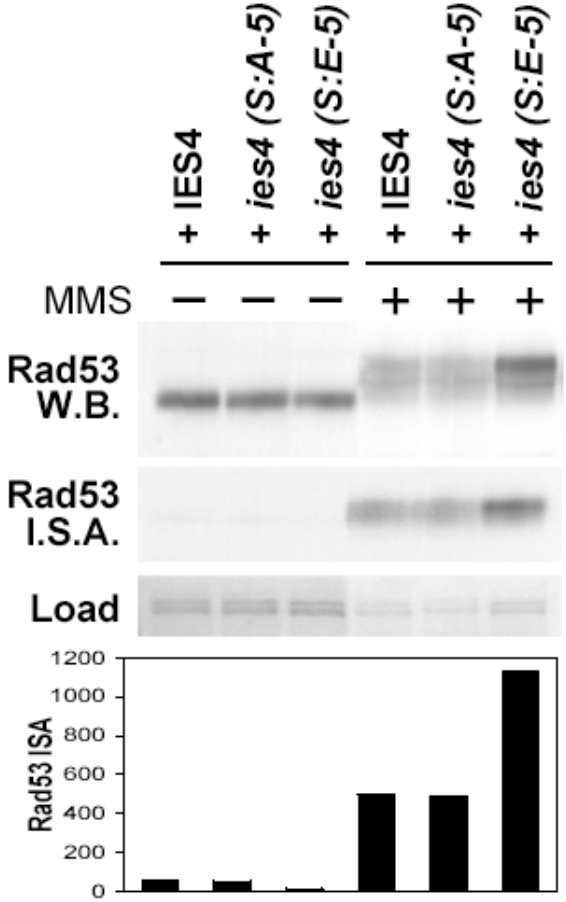
Minor role in DNA resection



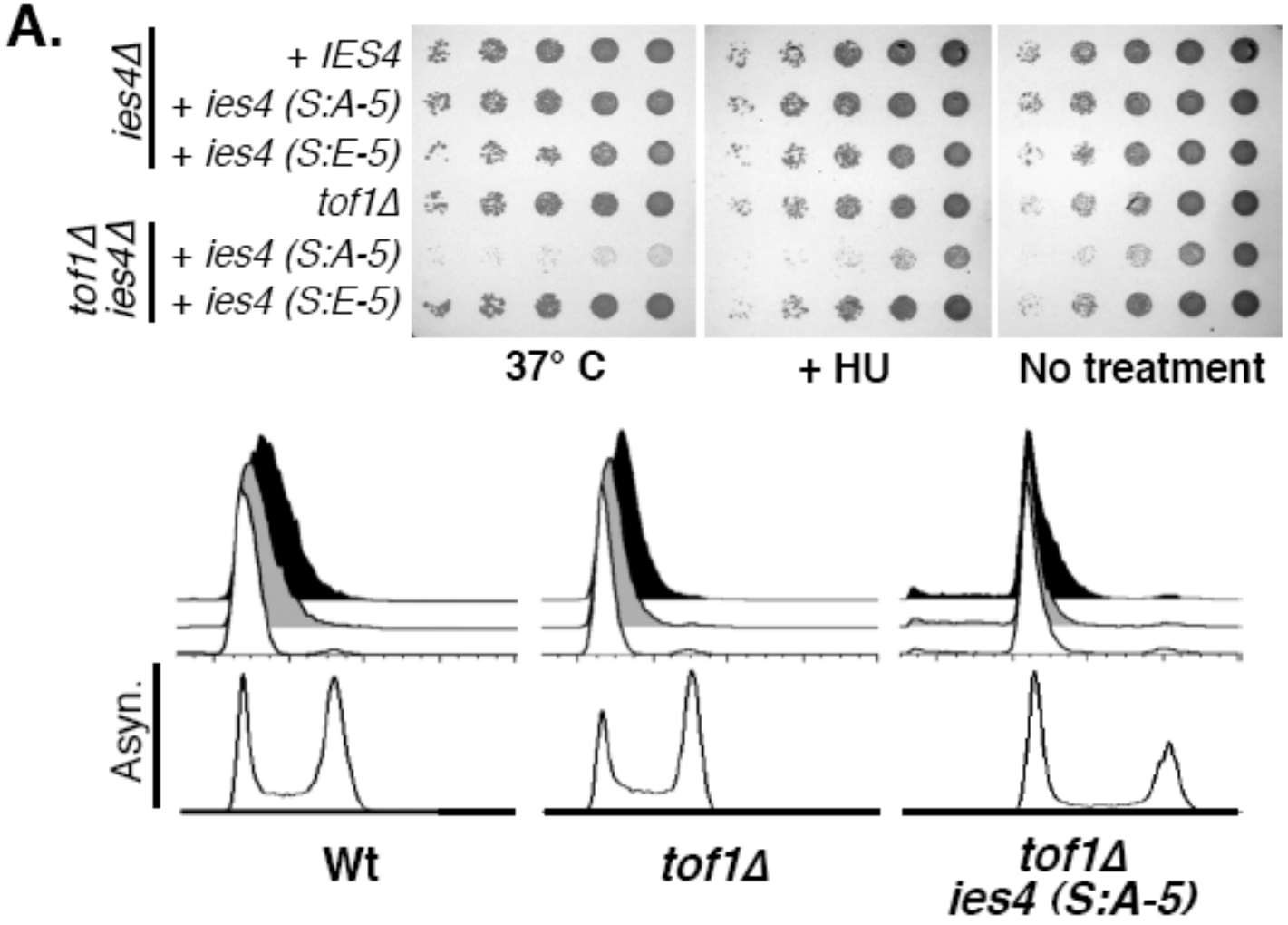
(Haber)

Ies4 phospho-mimic hyper-activates Rad53 (Chk2) checkpoint





Ies4 phosphorylation backs up Tof1, a replication checkpoint factor involved in fork stall and recovery



(HU)

ATM/ATR-mediated Ies4 phosphorylation does not affect DSB repair per se, it is involved in regulating DNA damage checkpoint response

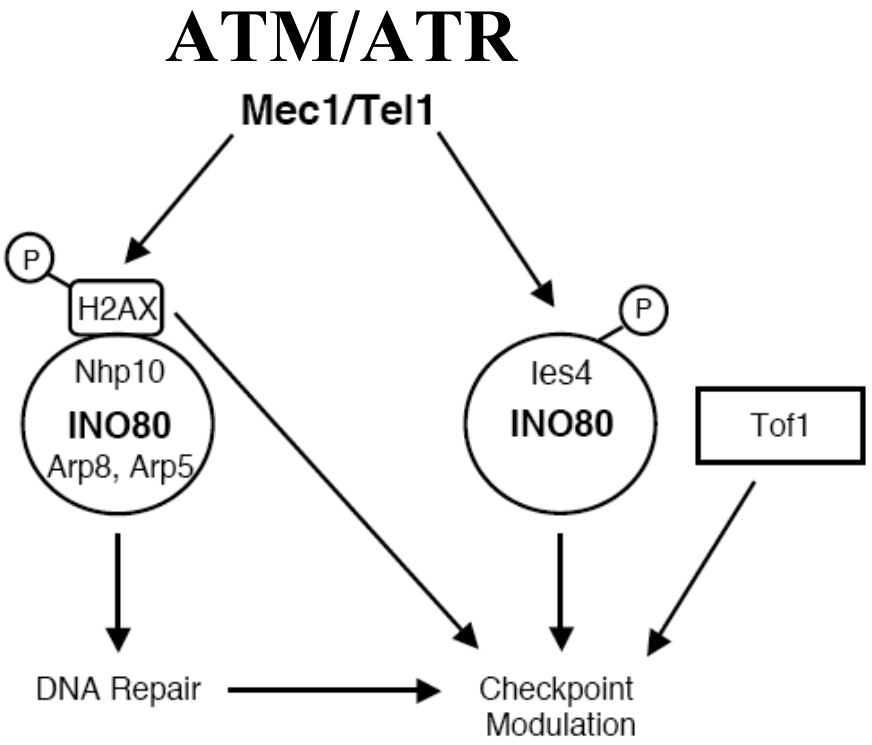
Major points

1. **Chromatin remodeling, a new target of ATM/ATR**

2. **Connecting chromatin remodeling to DNA damage checkpoint pathway.**

(Morrison et al. *Cell* 2007*)*

A paradigm of how a chromatin remodeling complex responds to DNA damage



Two distinct mechanisms

New direction

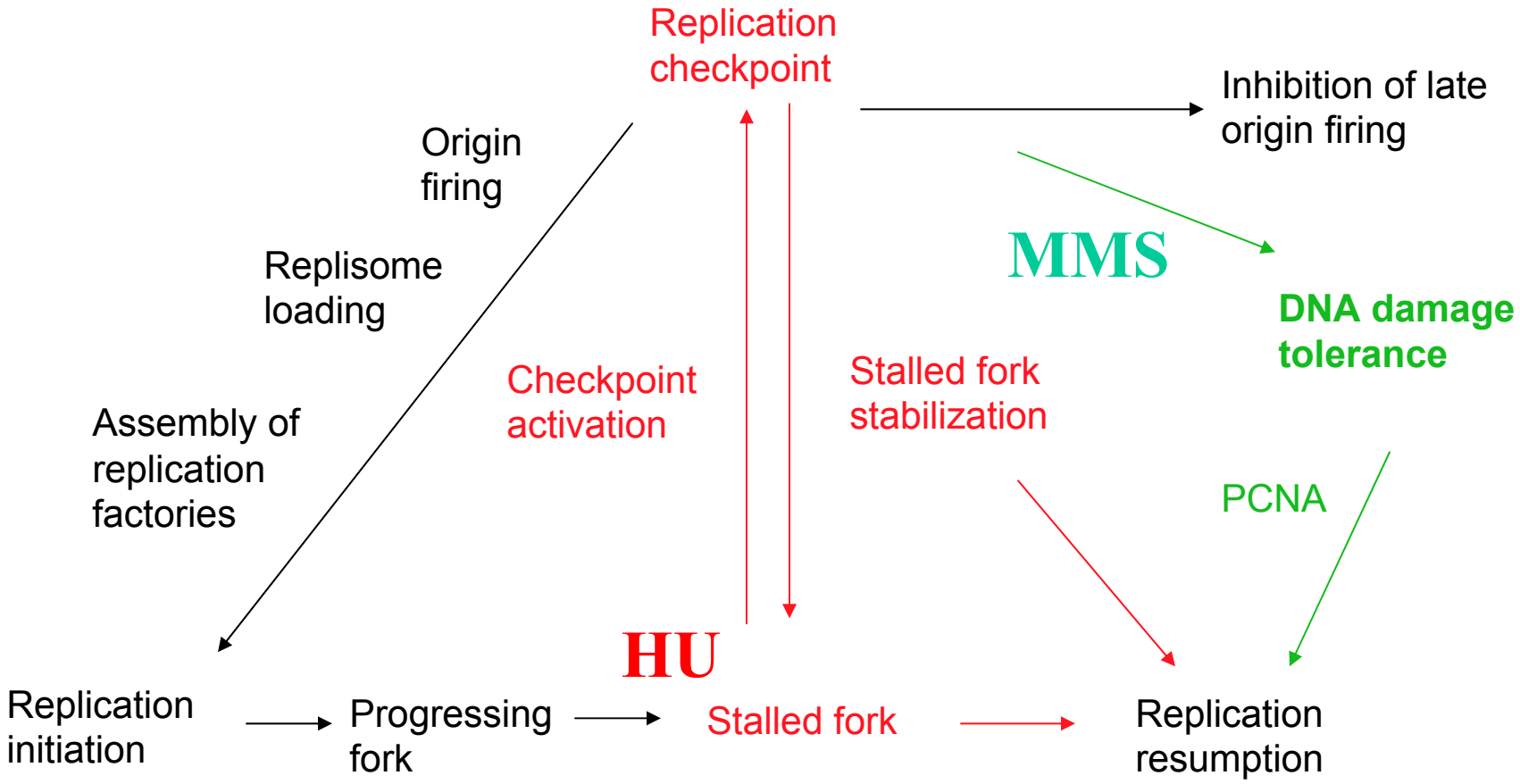
Chromatin remodeling and DNA replication?

-Precise nucleosome positioning is needed for Pre-RC formation. (Bell 2001)

- Specific roles of chromatin remodeling in DNA replication poorly understood.

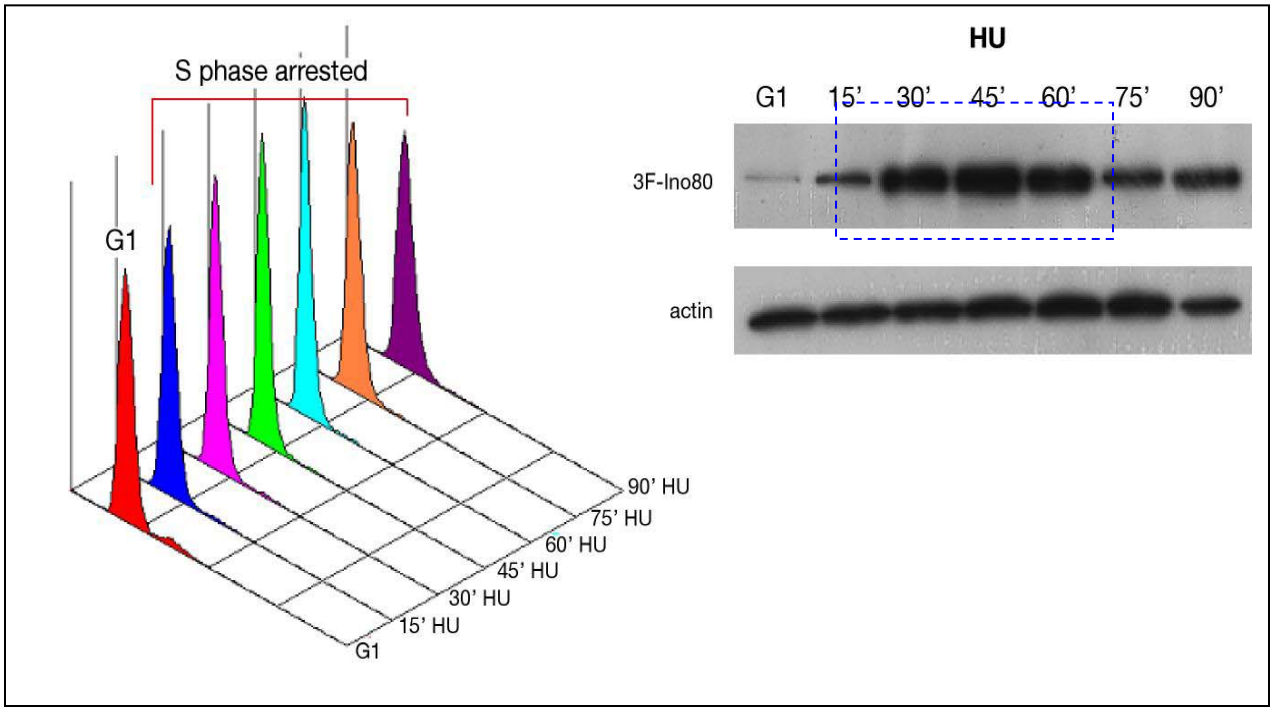
Karina Falbo

Where might INO80 function?



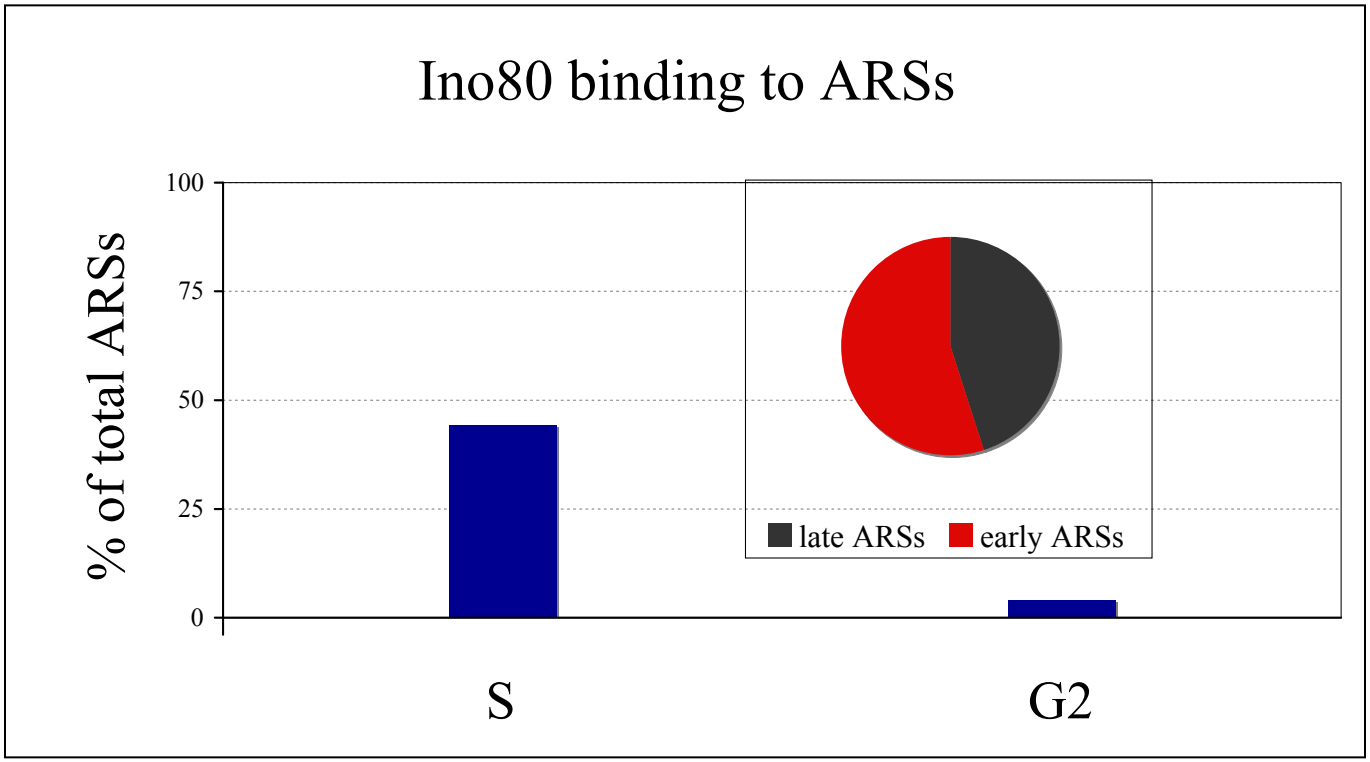
Adapted from Branzei and Foiani

Hint: *ino80* mutant is hypersensitive to HU

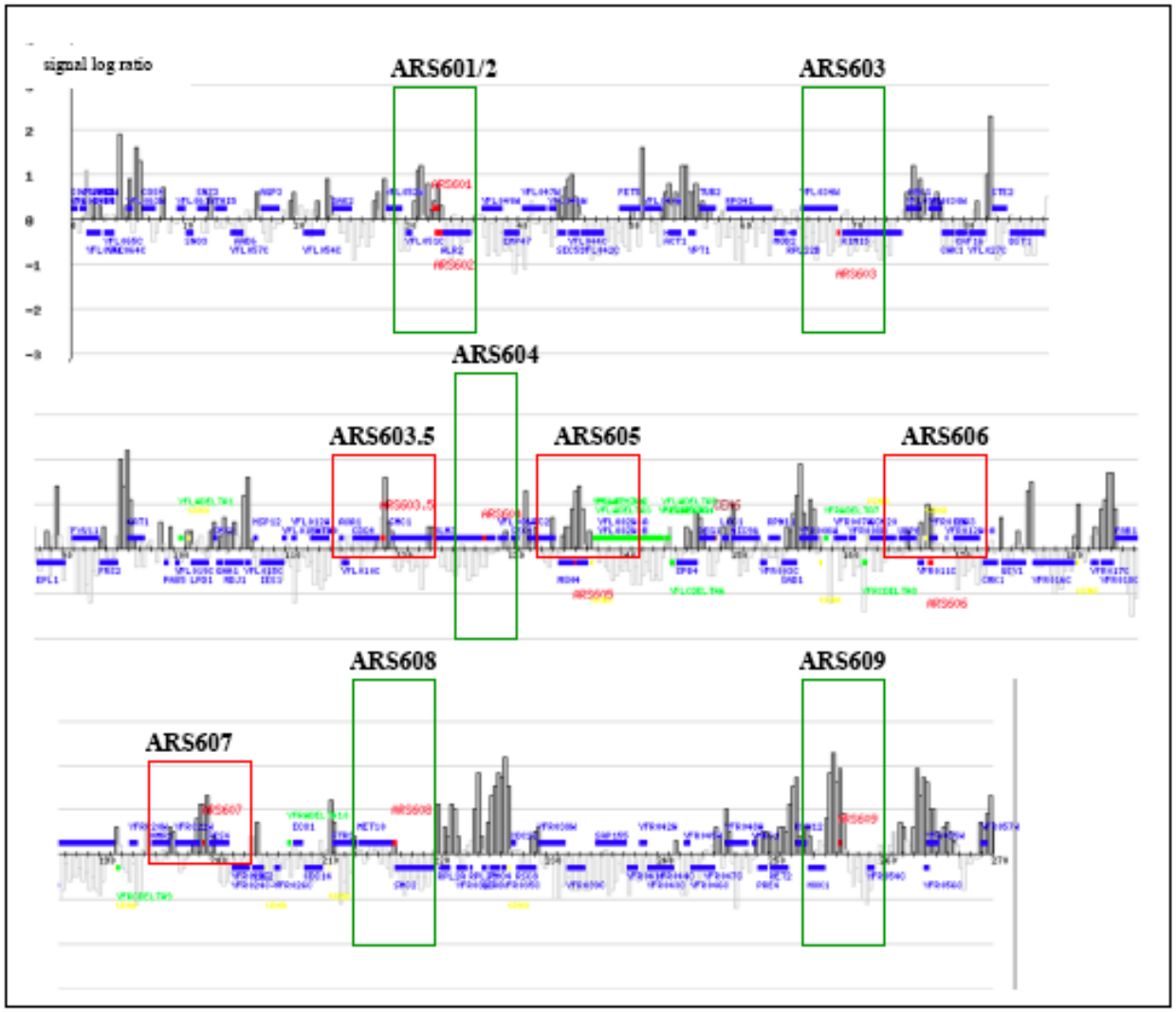


Ino80 is up-regulated upon replication stress

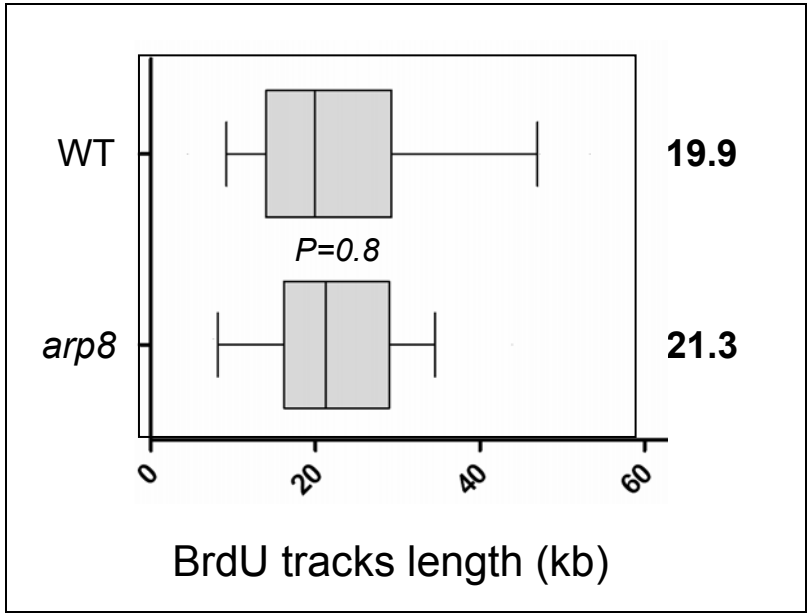
Ino80 binds to origins of replication (ARS) during S phase

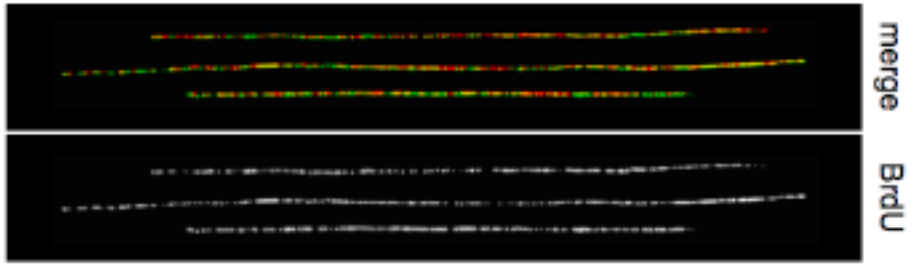


INO80 is present in both early and late ARSs



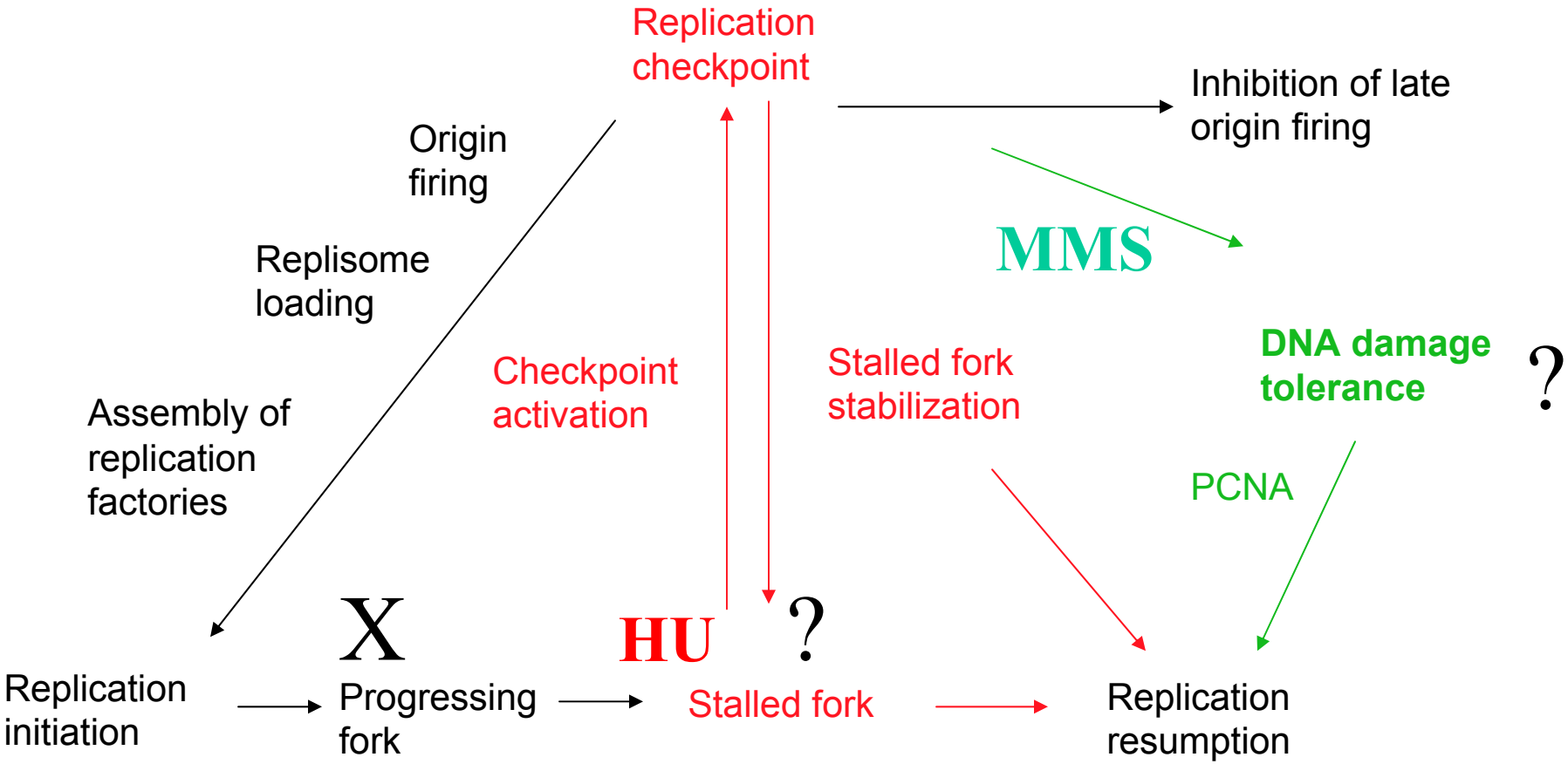
Ino80 is dispensable during “normal” replication





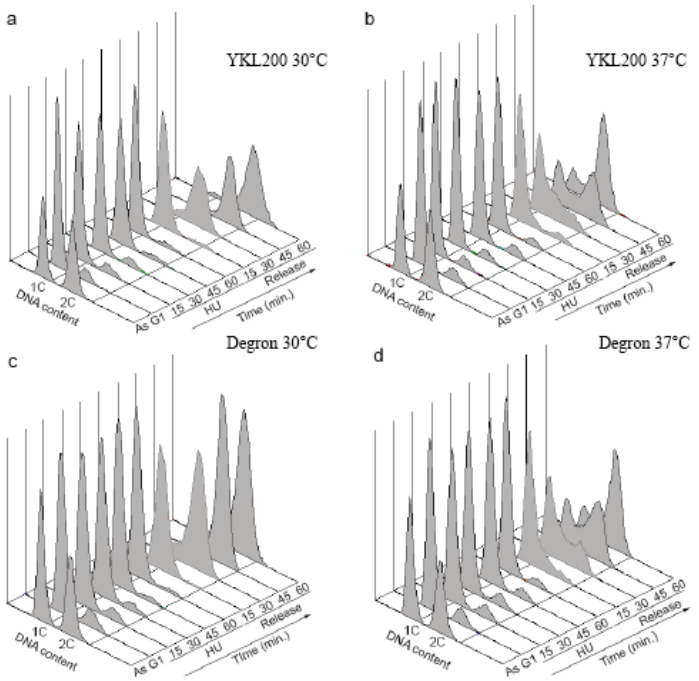
DNA combing directly measure DNA synthesis

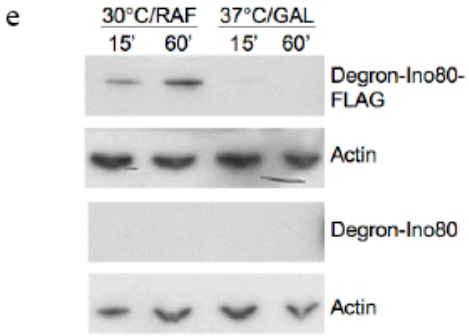
HU and MMS activate distinct stress pathways

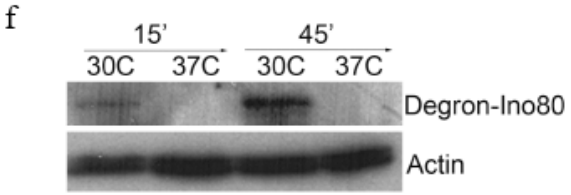


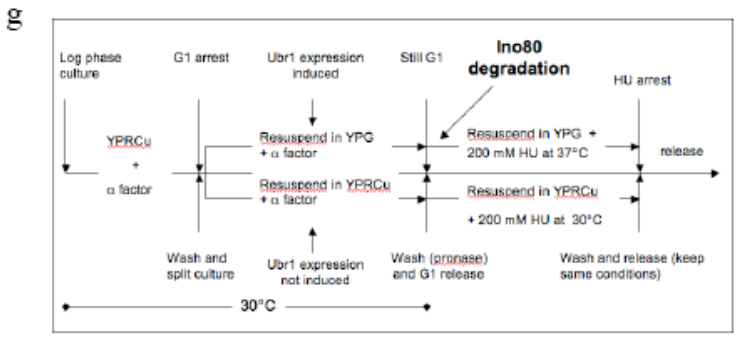
Adapted from Branzei and Foiani

INO80 does not affect fork stalling with HU or recovery after HU

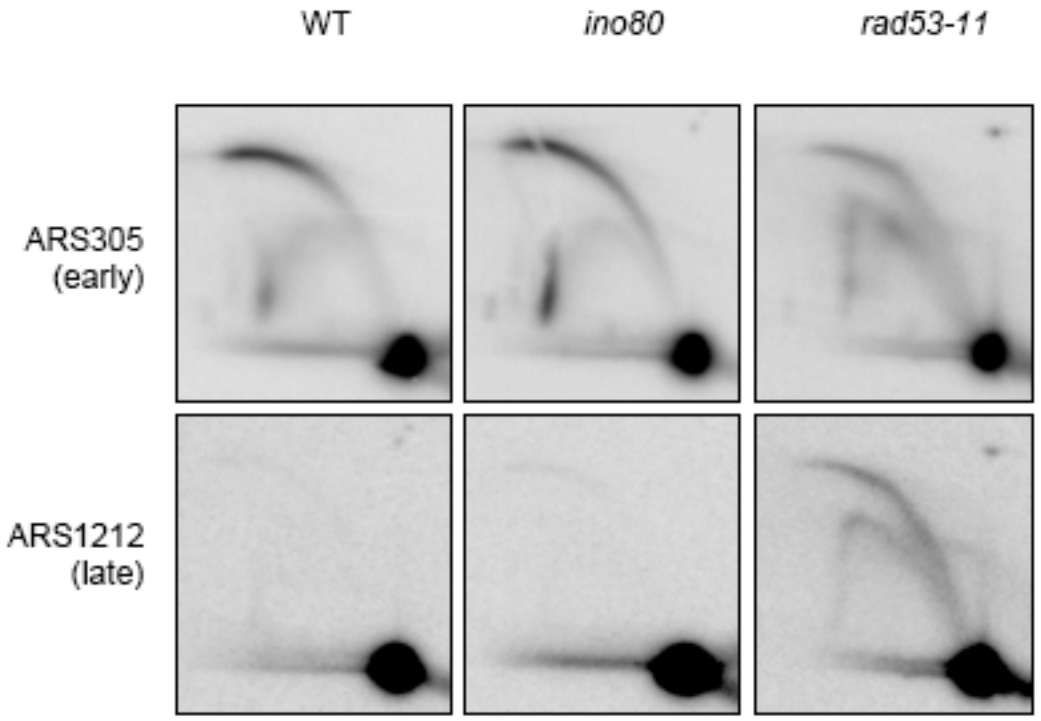




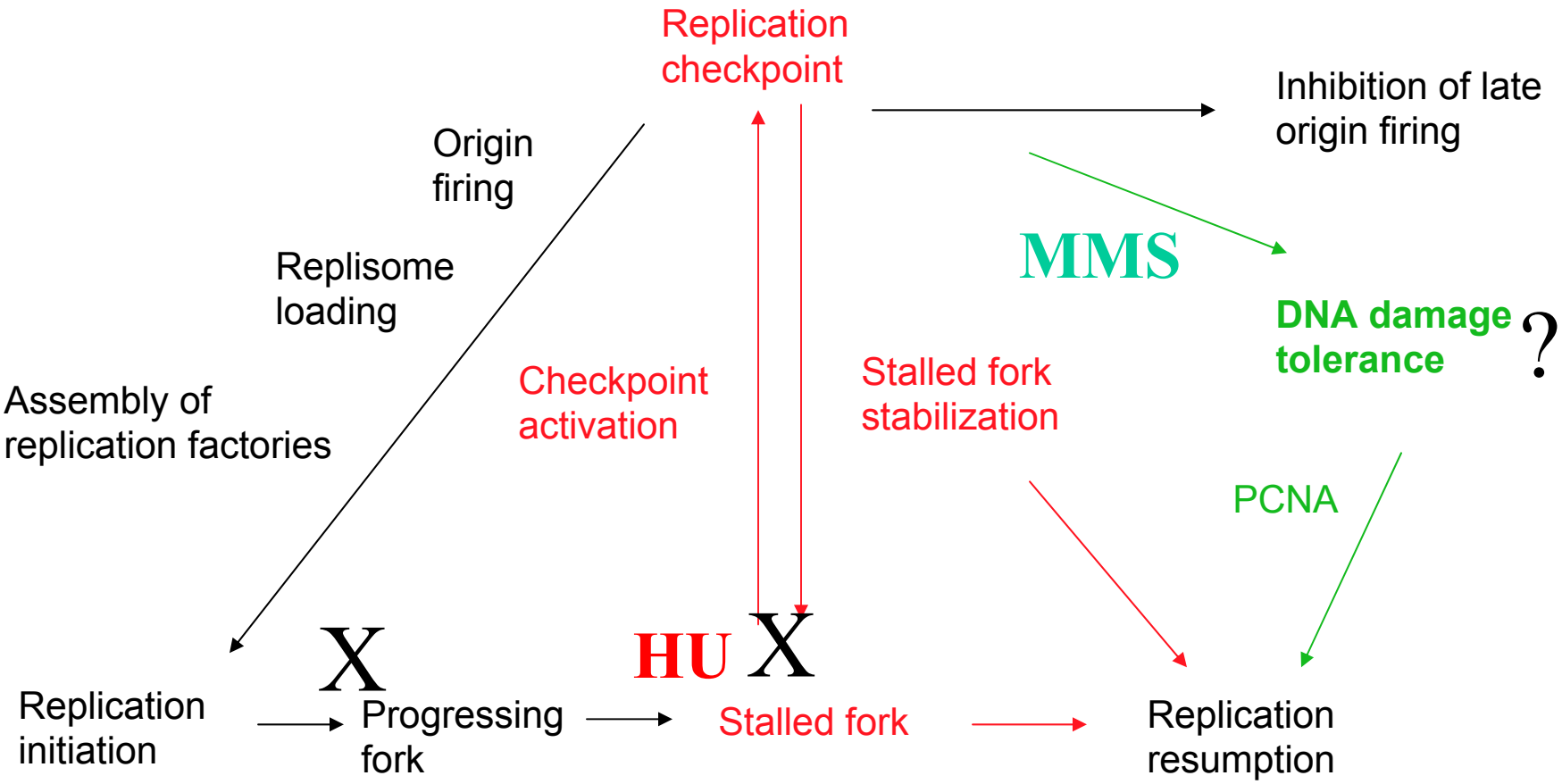




INO80 does not affect fork stability or repression of late firing origin under HU stress

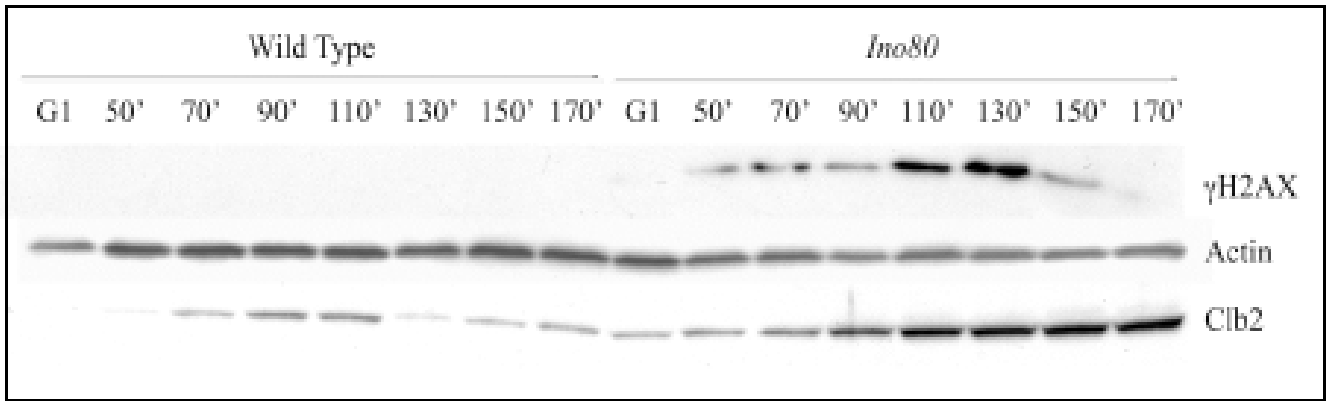


MMS-induced DNA damage tolerance?



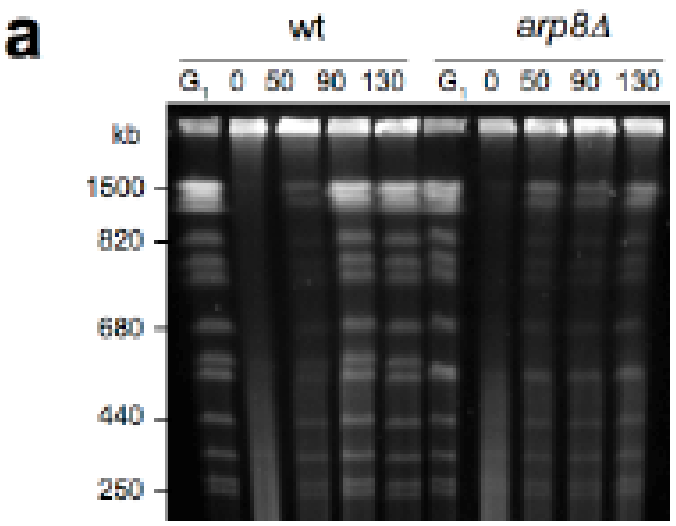
Adapted from Branzei and Foiani

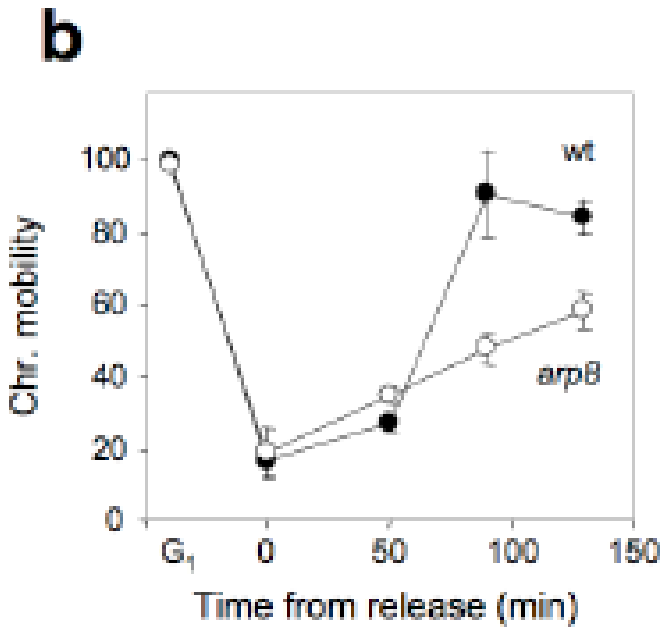
INO80 is required to tolerate DNA damage induced by MMS in S phase



MMS activates DNA damage tolerance pathways

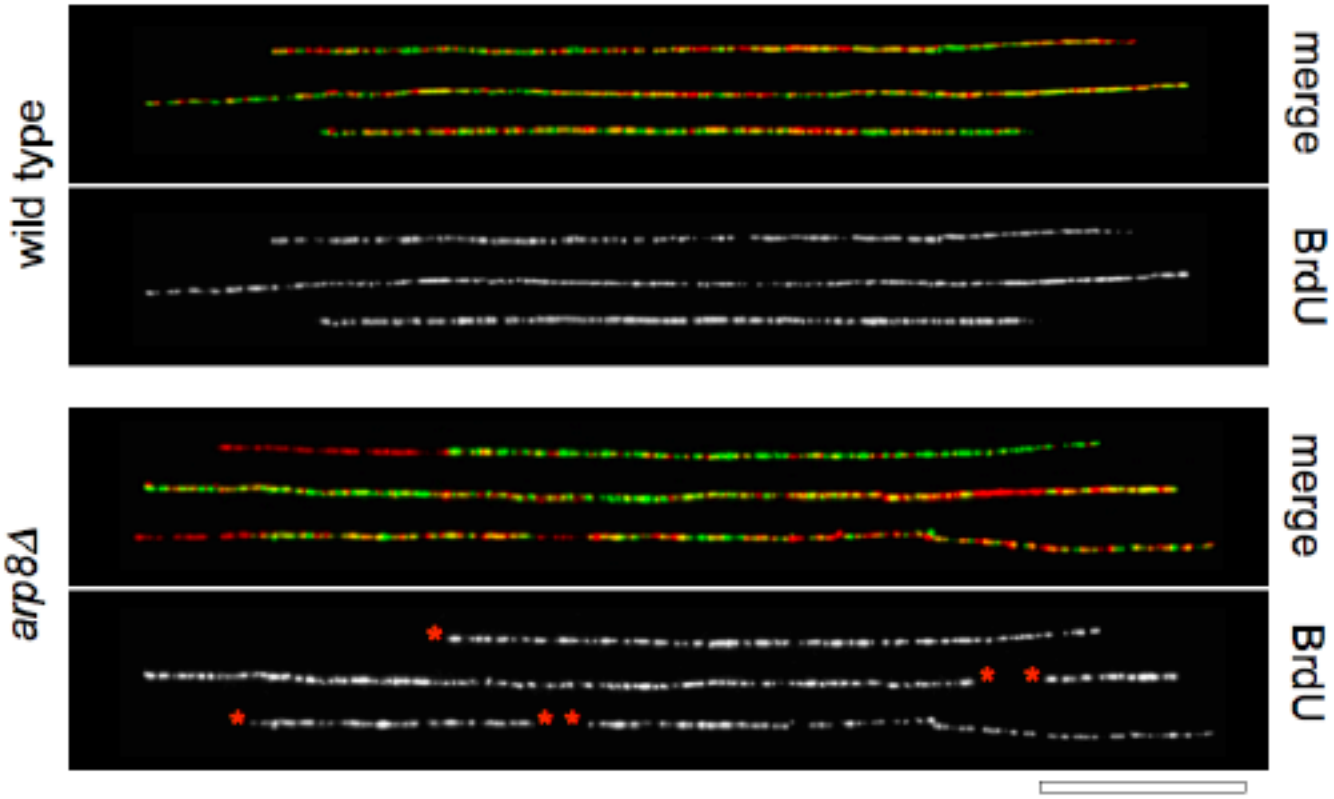
INO80 is required for replication restart after MMS



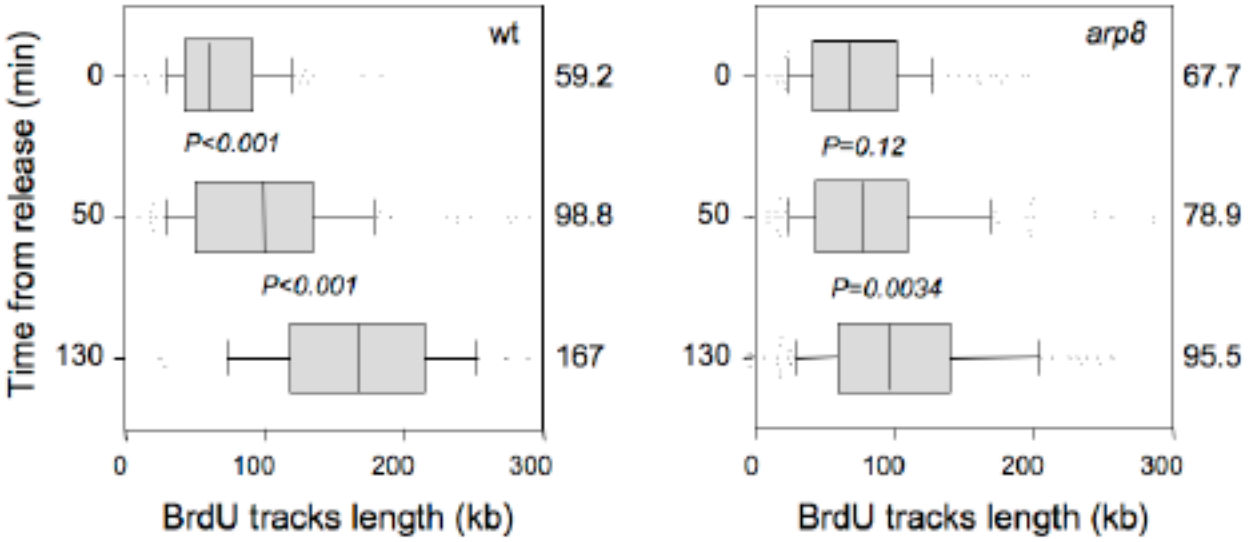


PFGE

Un-replicated gaps accumulate in *ino80* mutant after MMS

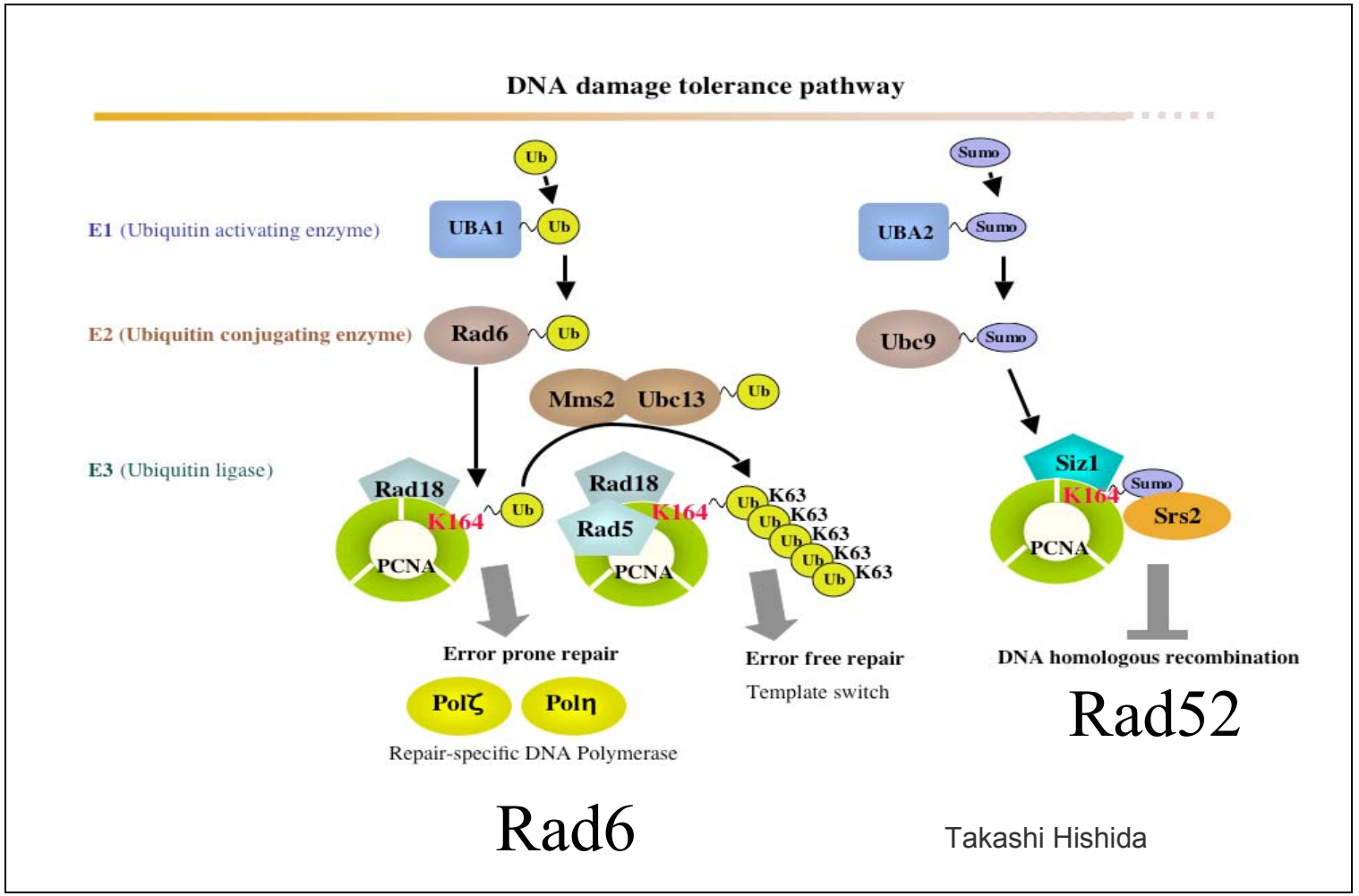


Fork can not efficiently restart after MMS in the absence of INO80



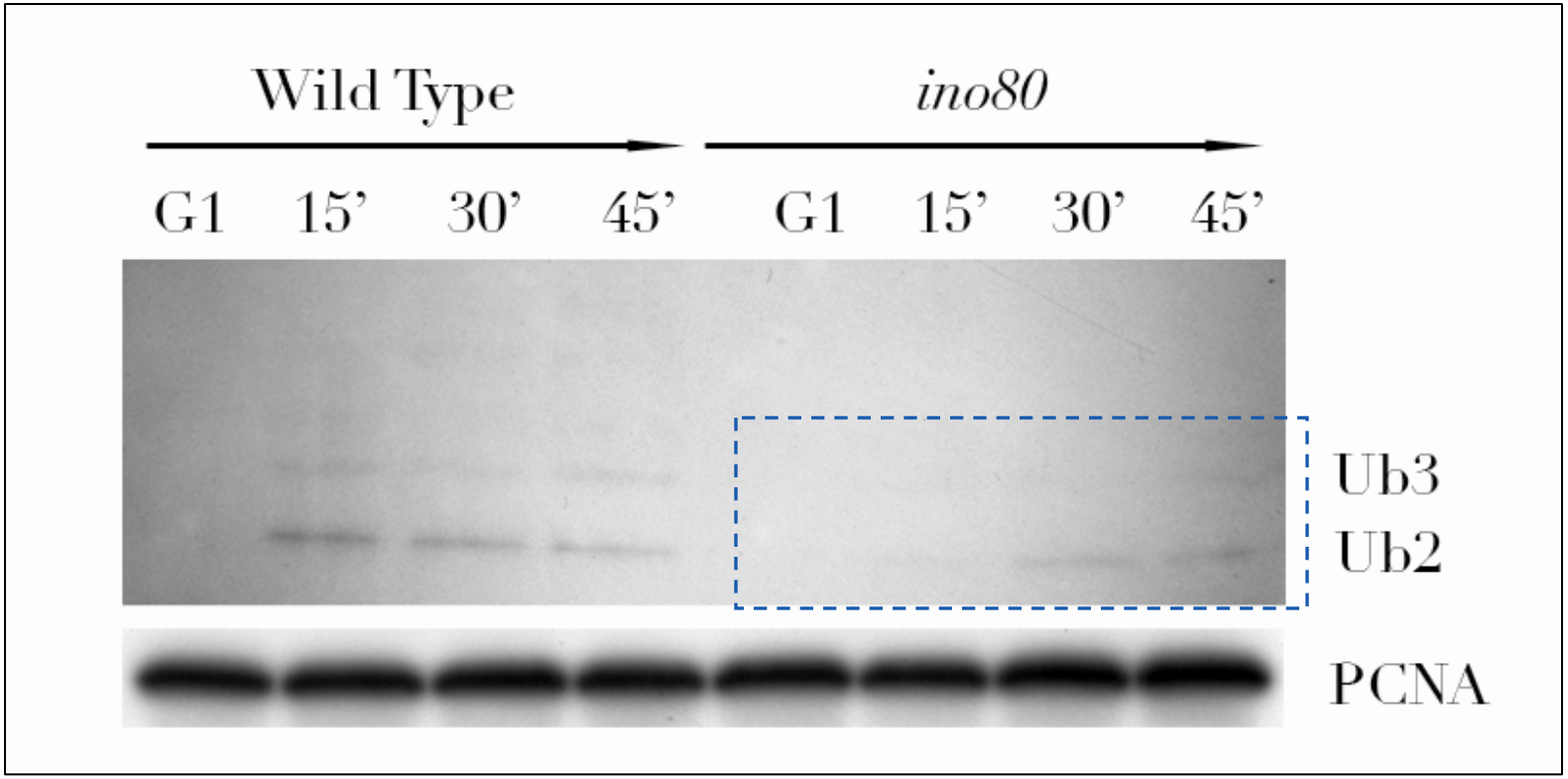
mechanism?

PCNA modification controls DNA damage tolerance pathways



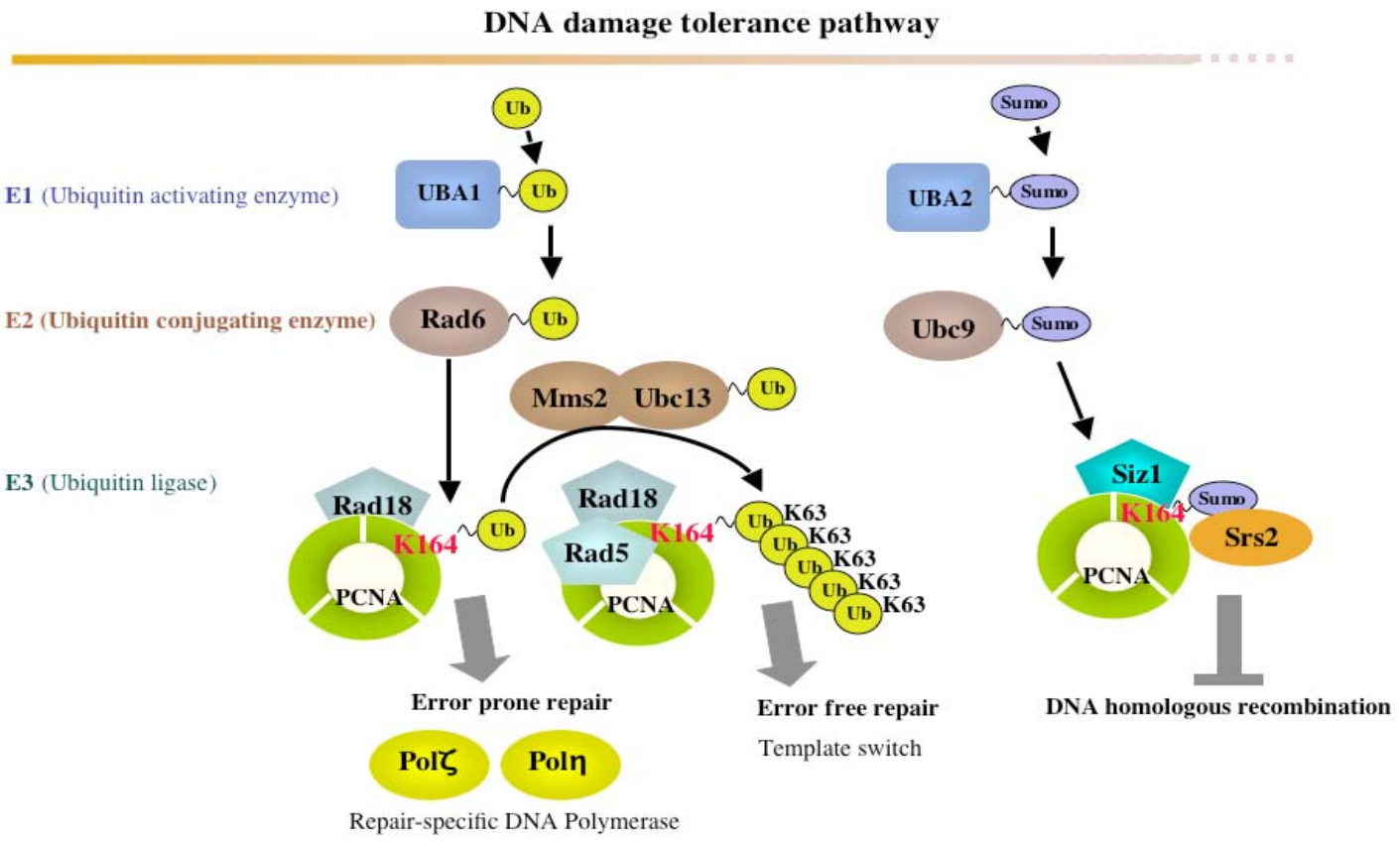
Does Ino80 affects PCNA ubiquitination?

Ino80 is required for efficient PCNA ubiquitination



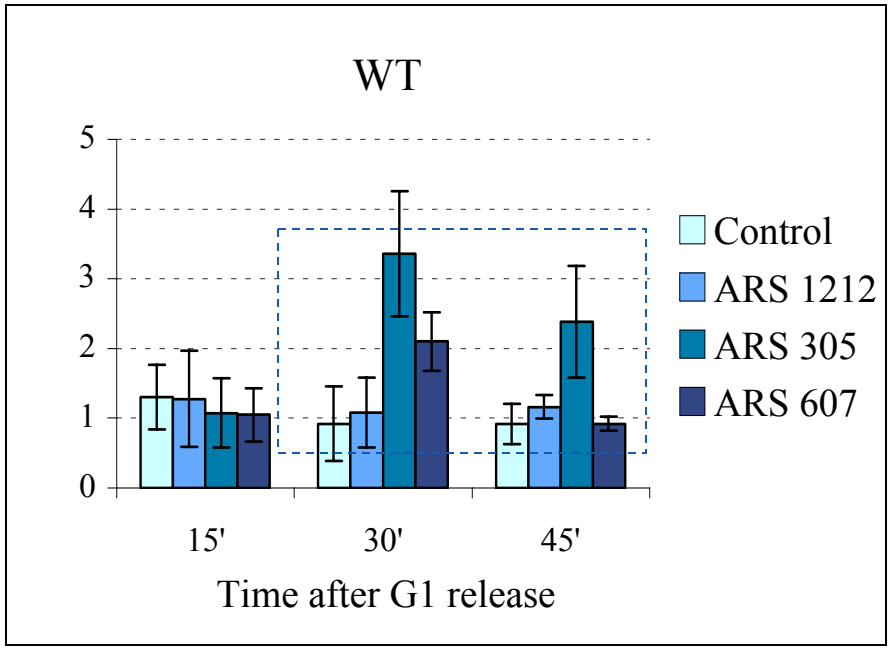
INO80 chromatin remodeling activity needed

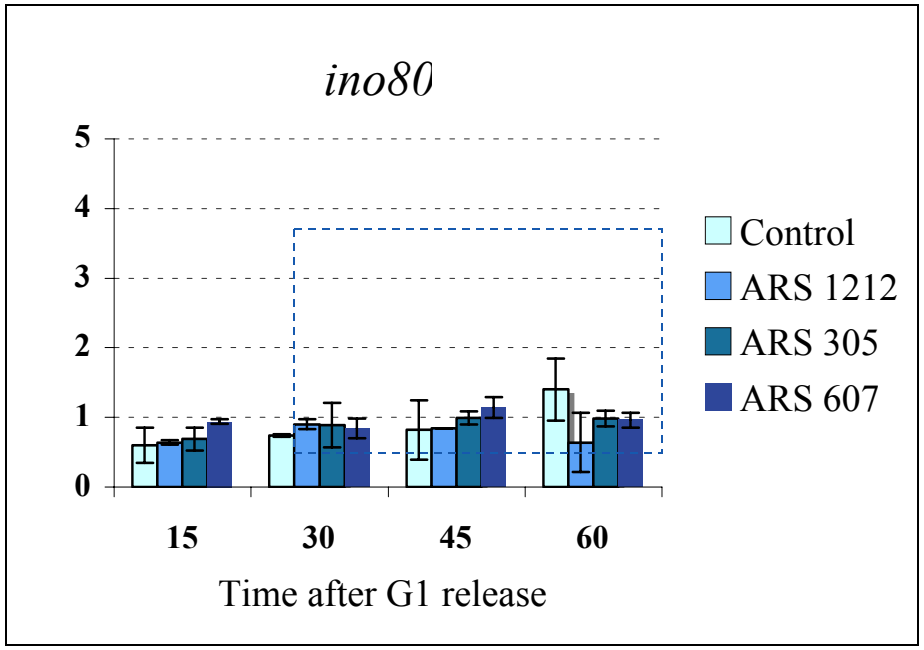
INO80 chromatin remodeling affects factor recruitment?



Takashi Hishida

INO80 is required for Rad18 recruitment to RFs





INO80 affects recombination at RF mediated by Rad51

Rad18

↘

Rad51

↓

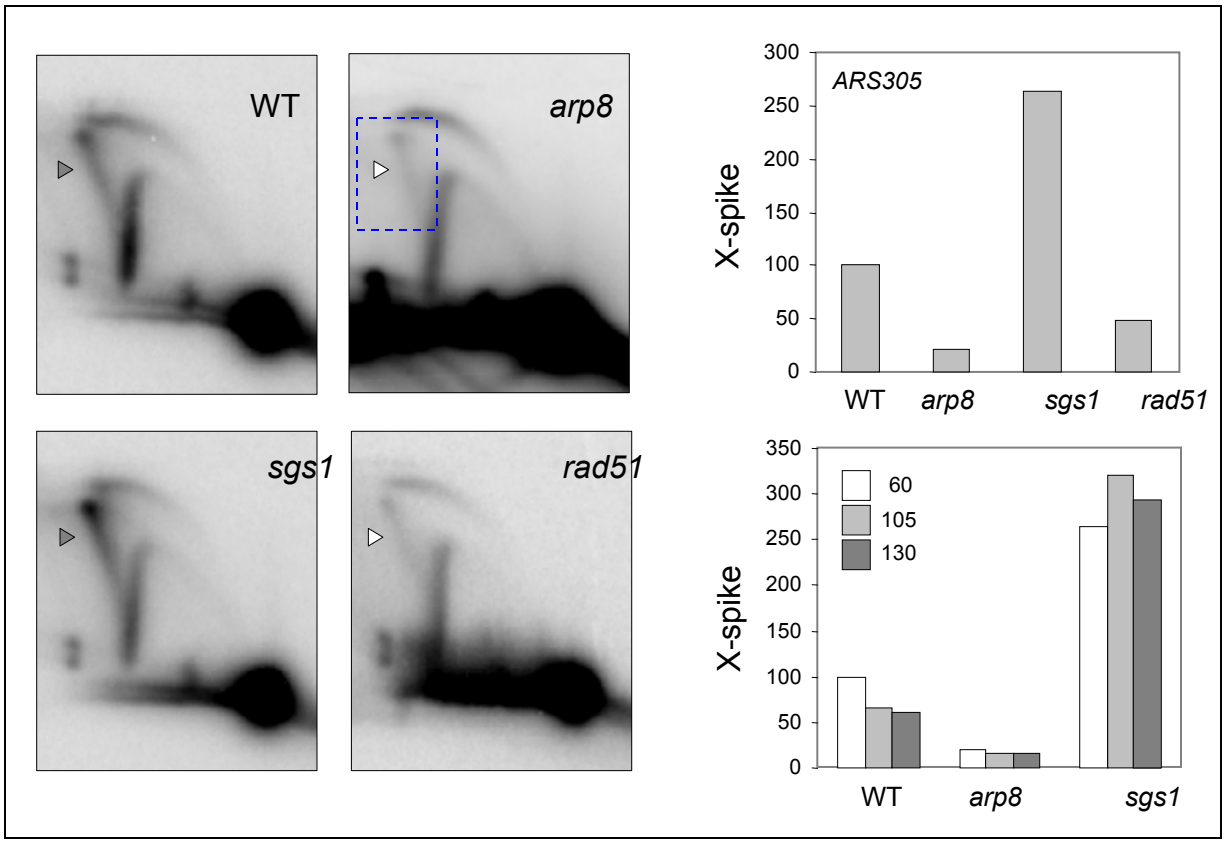
X spike structures

↓

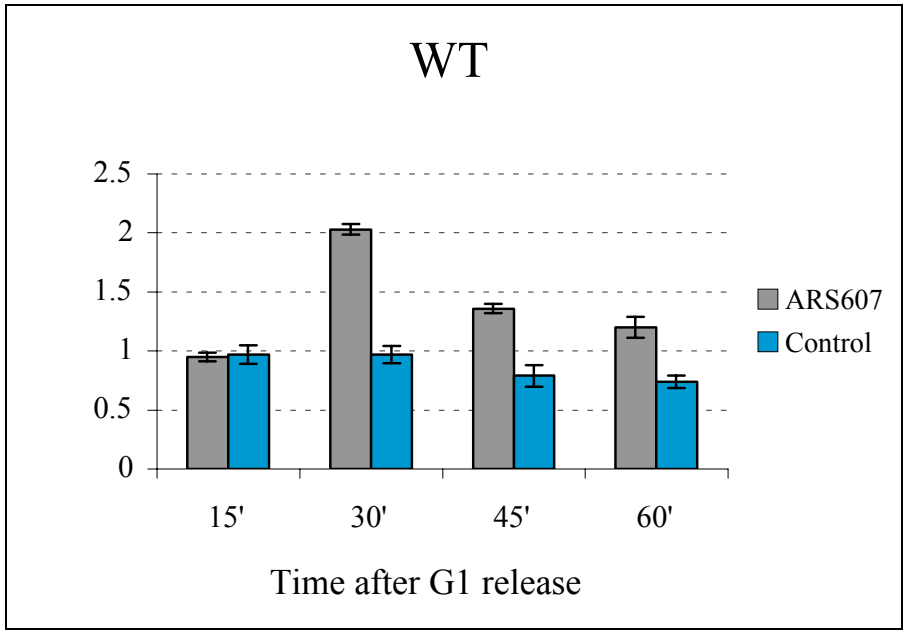
Sgs1

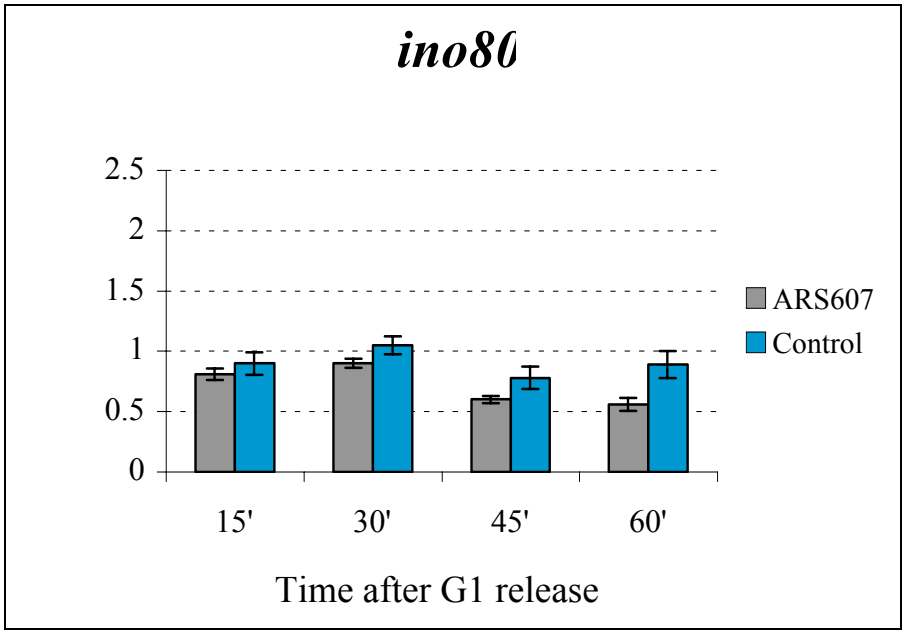
↓

resolution

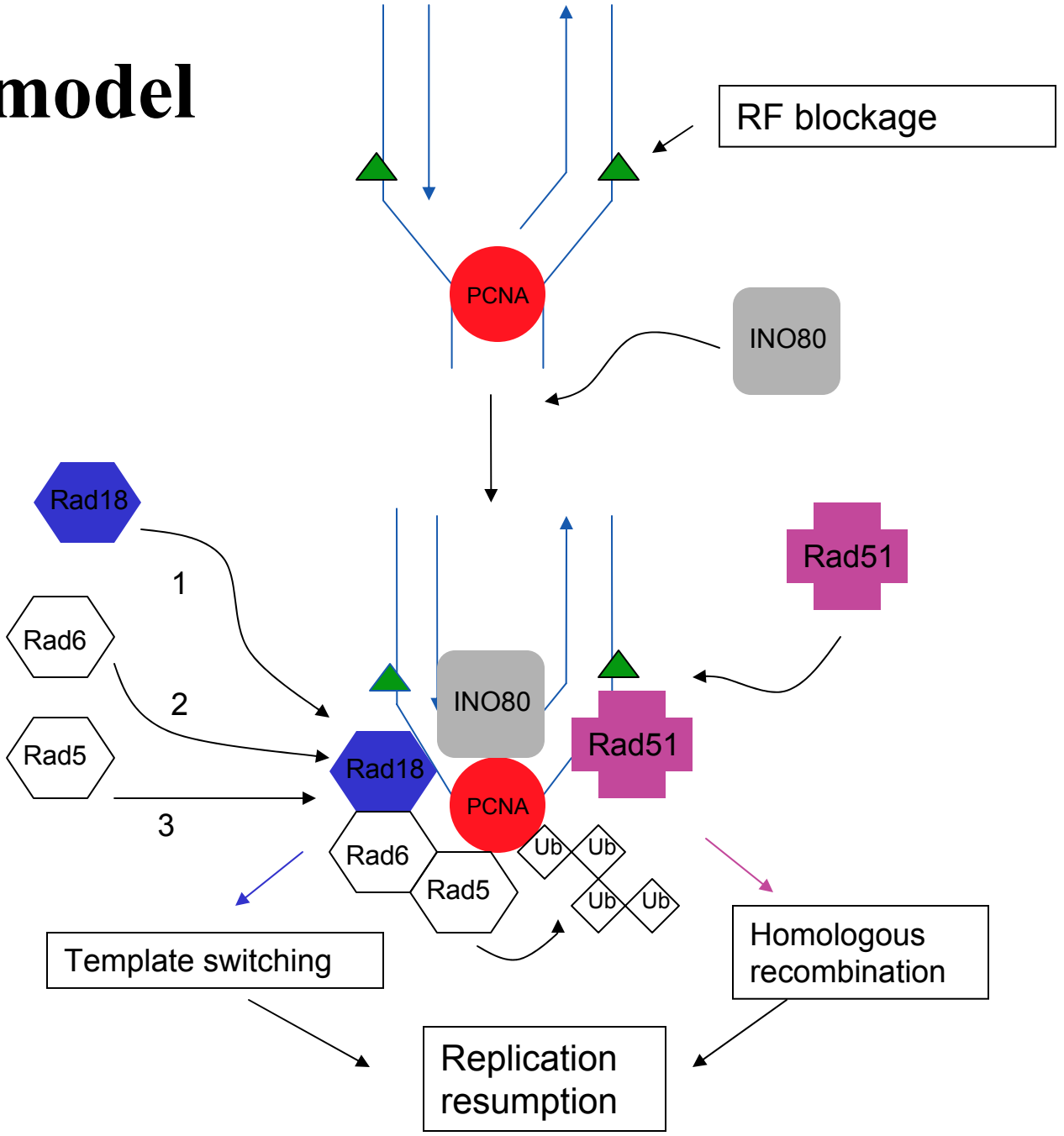


Ino80 is necessary to recruit Rad51 to RFs

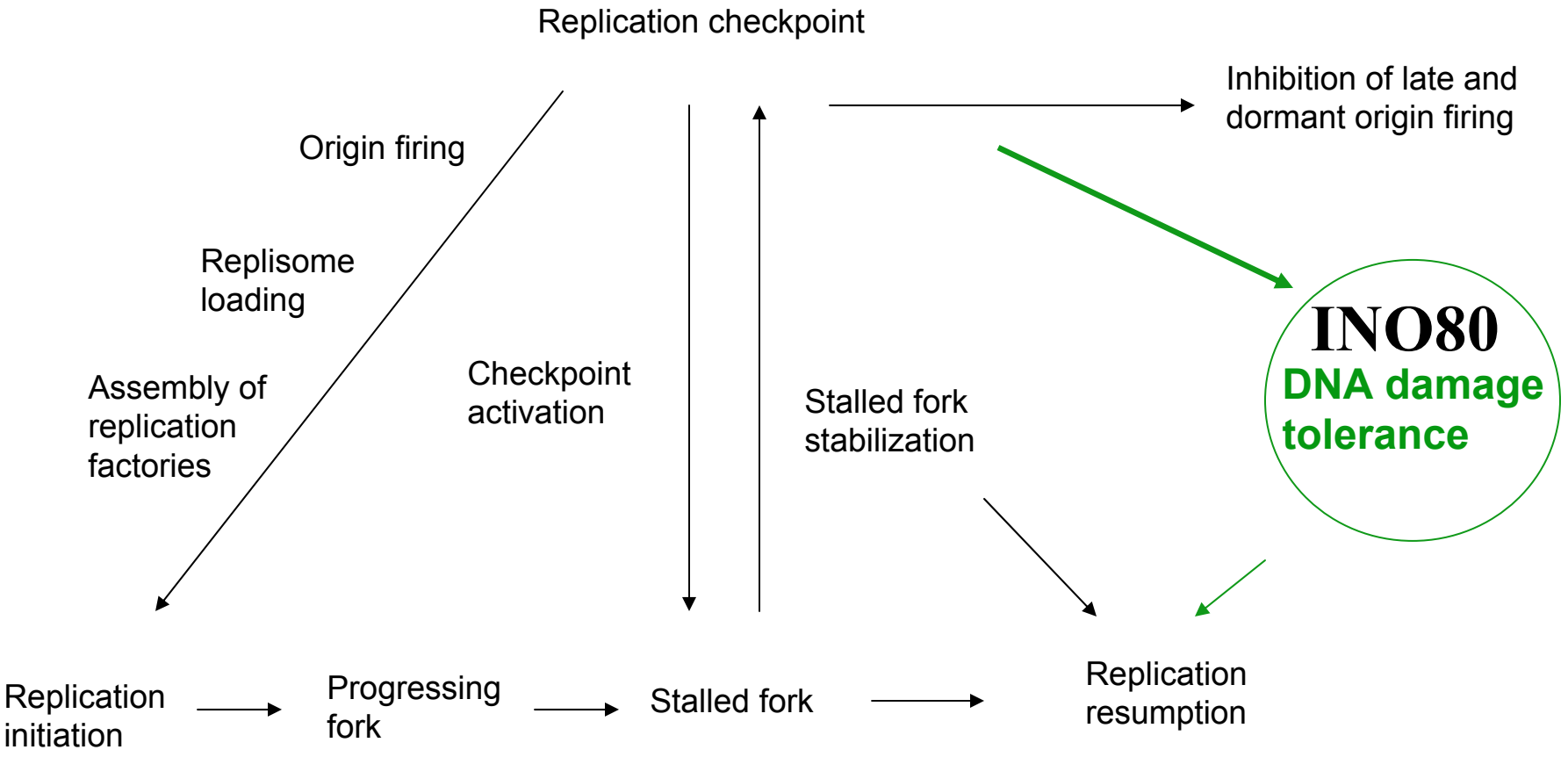




Karina's model



A new role of INO80 in DNA replication



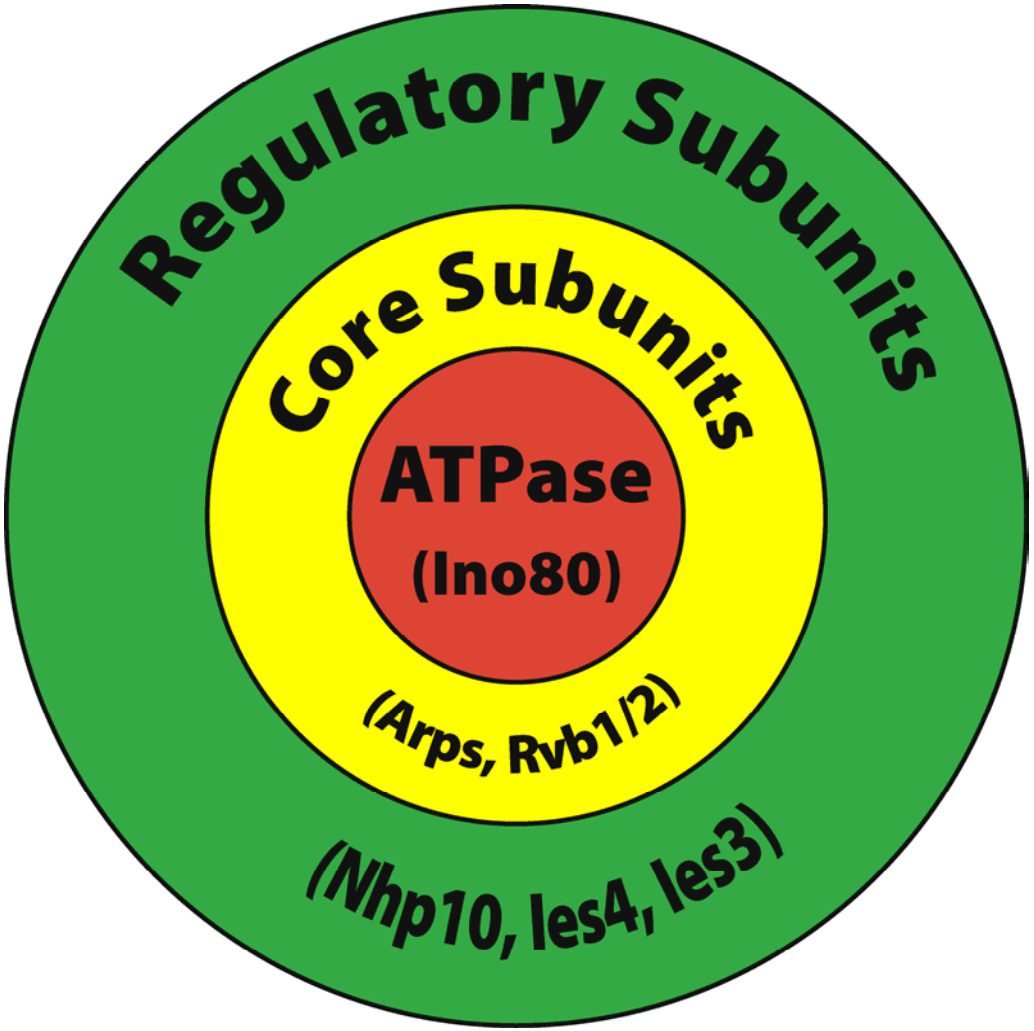
Falbo et al. ***NSMB*** 2009

Chromatin remodeling complexes are new players in genome maintenance

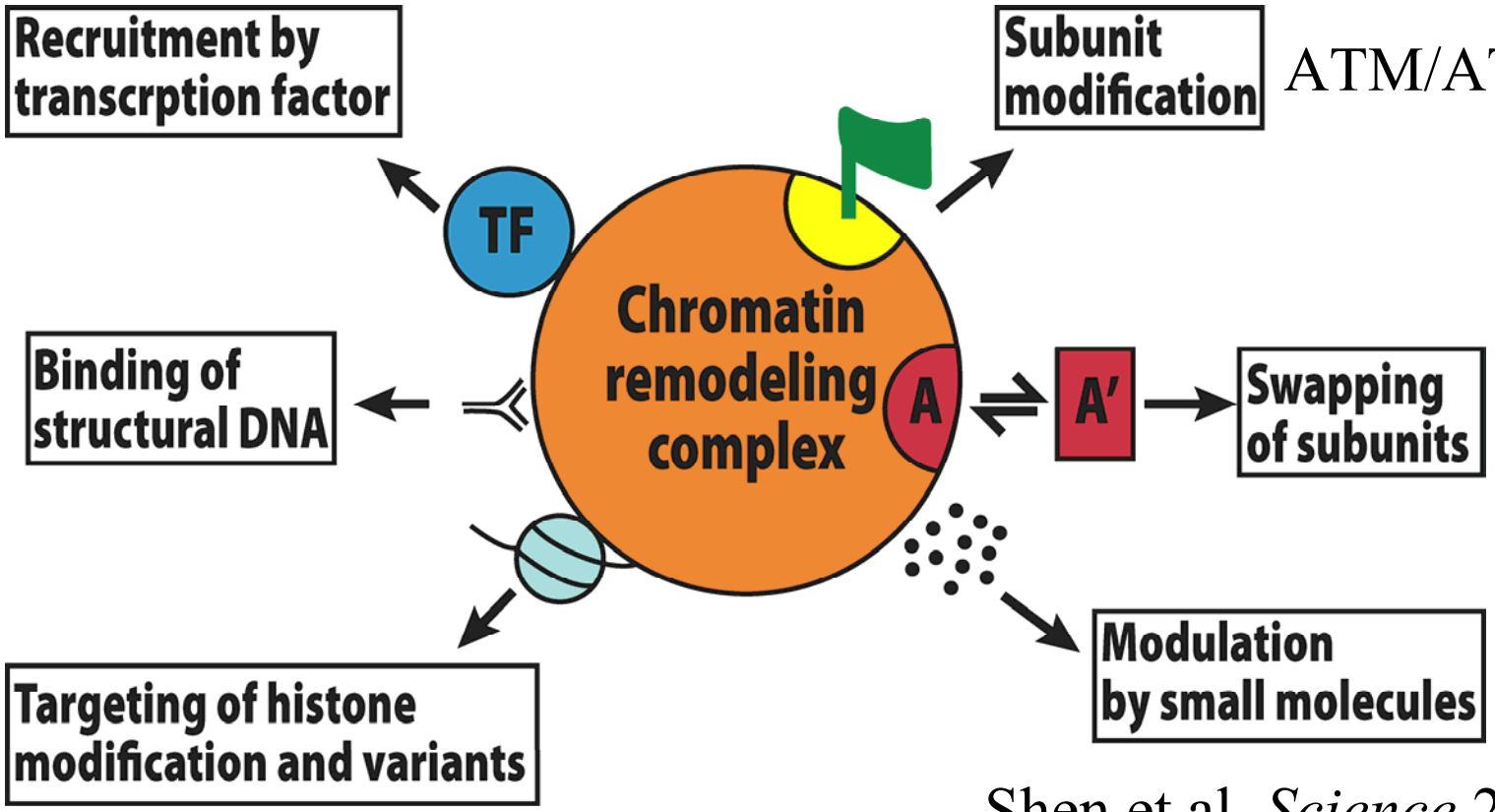
- DNA repair
- Checkpoint regulation
- DNA replication

Through distinct targeting mechanisms

A functional hierarchy



Regulating a single chromatin remodeling complex



Shen et al. *Science* 2003
(inositol signaling)

Three major ways to modify chromatin

- **Histone variants (H2AZ, H2AX marks chromatin)**
- **Secondary modifications of histones (P, Ac, Me…)**
- **ATP-dependent chromatin remodeling**

Three mechanisms all connected!

Collaborations

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