DNA mutation, damage and repair

DNA修复与人类生活密切相关

长期吸烟和酗酒会导致DNA损伤，削弱和抑制身体DNA修复能力；

紫外线照射、辐射损伤DNA

DNA mutation

What is a mutation?

- Substitution, deletion, or insertion of a base pair.
- Chromosomal deletion, insertion, or rearrangement.

Somatic mutations occur in somatic cells and only affect the individual in which the mutation arises.

Germ-line mutations alter gametes and passed to the next generation.

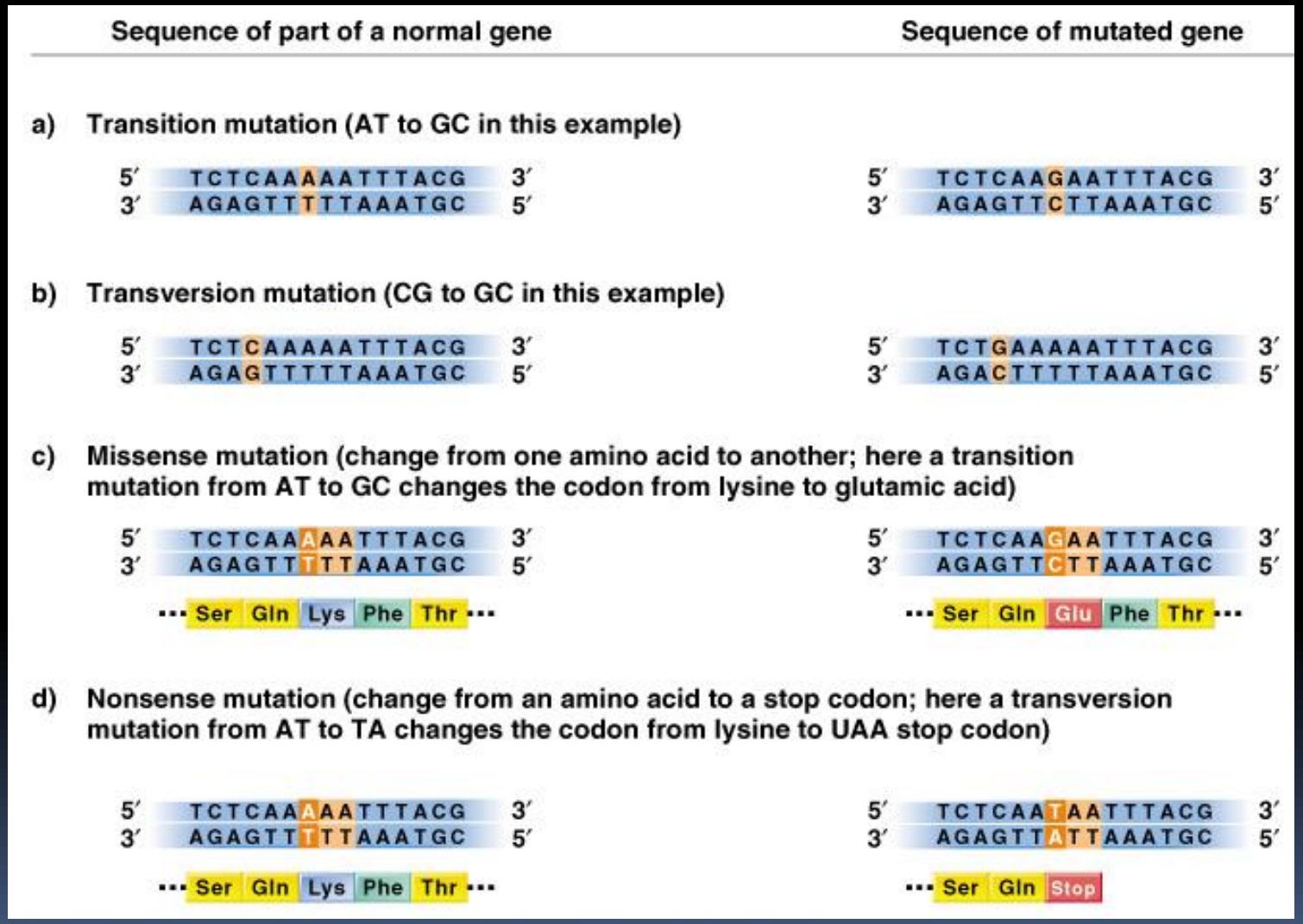
Types of base pair substitutions and mutations

转换

颠换

错义突变

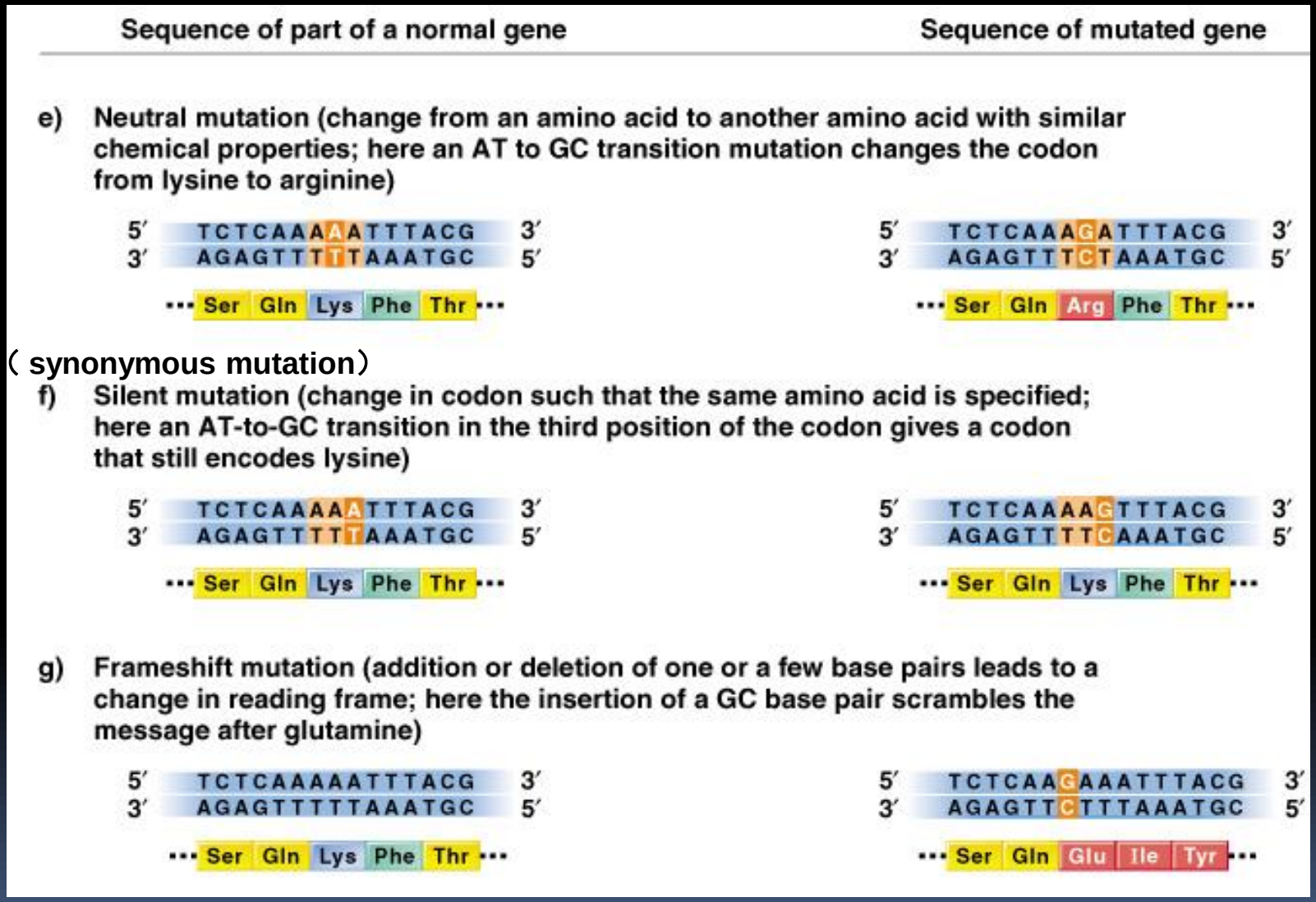
无义突变



中性突变

同义突变（synonymous mutation）

移码突变

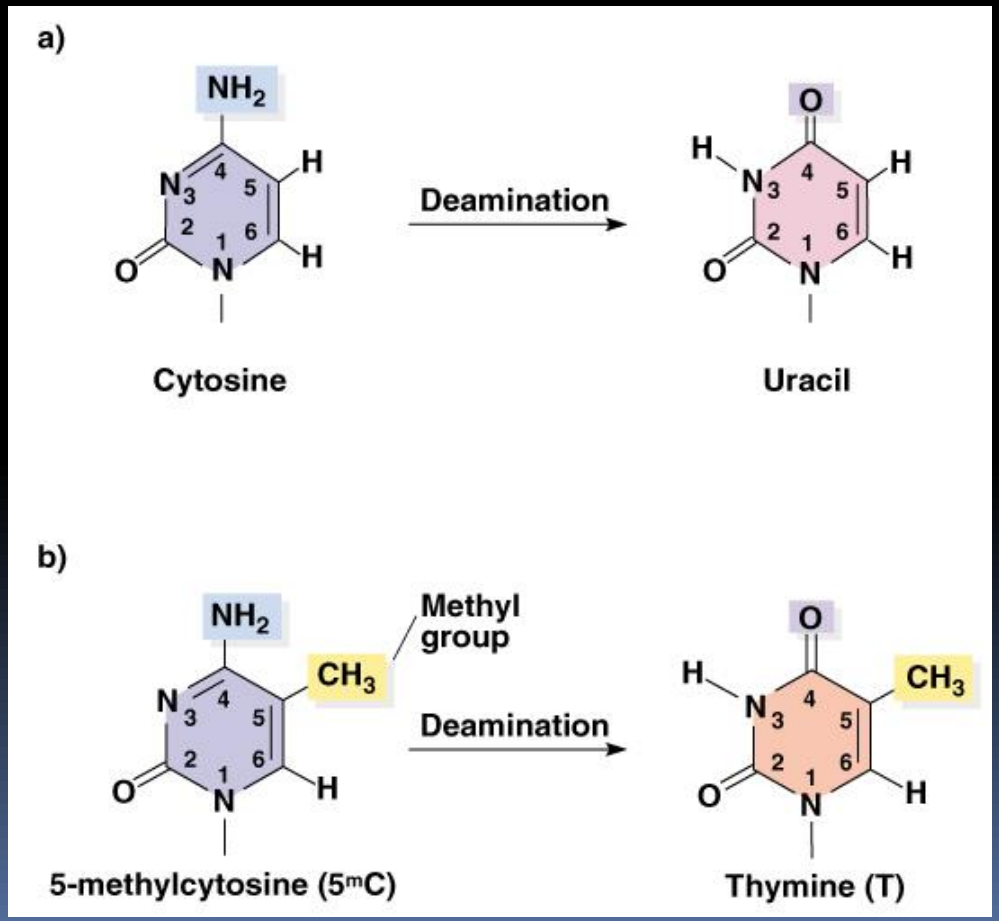


Spontaneous mutations differ from induced mutations:

- Spontaneous mutations can occur at any point of the cell cycle.
- Movement of transposons (mobile genetic elements)
- Mutation rate = ~10^{-4} to 10^{-6} mutations/gene/generation
- Rates vary by lineage, and most spontaneous errors are repaired.

Spontaneous chemical changes can cause mutation

Example : Deamination: C → U, C → T

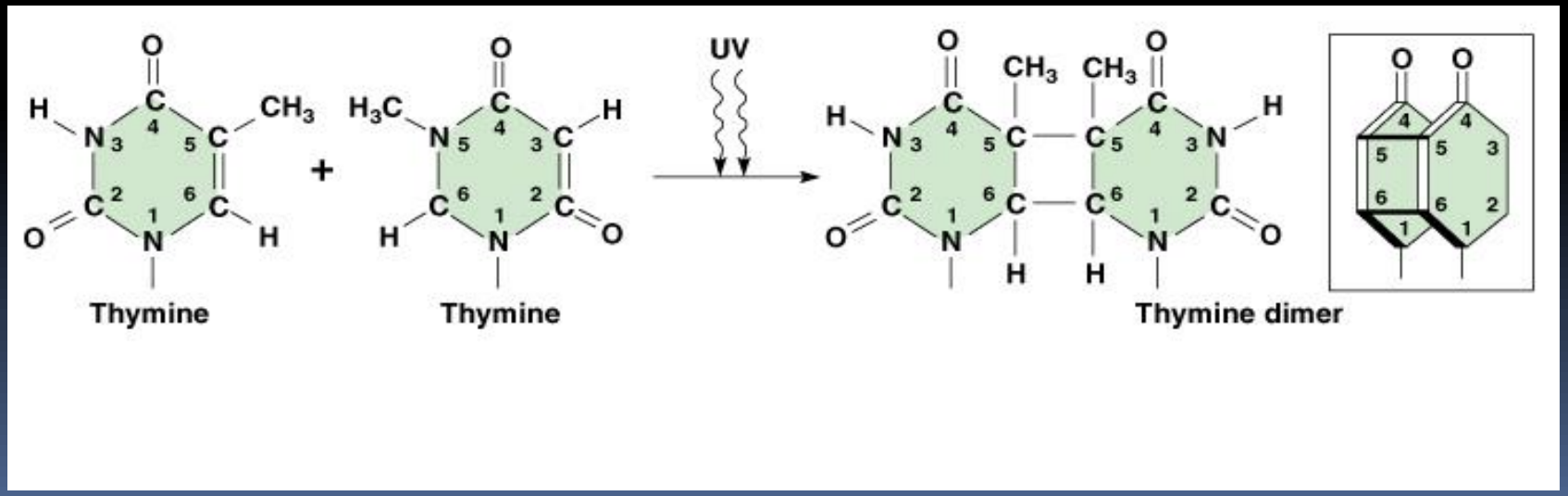


Induced mutations

Radiation (e.g., X-rays, UV)

Ionizing radiation breaks covalent bonds including those in DNA and is the leading cause of chromosome mutations.

UV (254-260 nm) causes purines and pyrimidines to form abnormal dimer bonds and bulges in the DNA strands.



Induced mutations: **chemical mutagens**

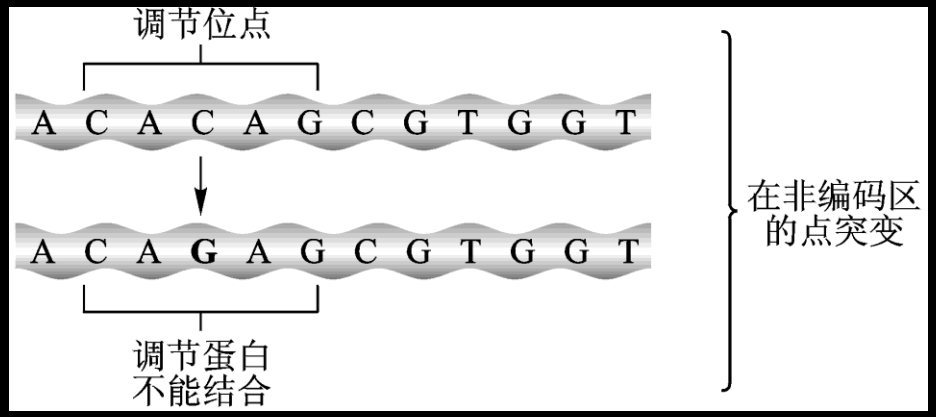
Base analogs

- Similar to normal bases, incorporated into DNA during replication.
- Some cause mis-pairing (e.g., 5-bromouracil).

Base modifying agents, act at any stage of the cell cycle:

- Deaminating agents
- Hydroxylating agents
- Alkylating agents

突变发生在基因的非编码区



调节区和非编码区的DNA序列:

DNA水平：包括RNA聚合酶、特定转录因子的结合位点；

RNA水平：包括核糖体结合位点，真核生物mRNA 外显子交接区5′和3′端拼接位点，以及调节mRNA进入细胞特定区域和组分的翻译调节和定位信号位点。

转录因子结合位点被破坏可能改变基因在特定时间、组织或特定环境中的表达量，某些结合位点的突变可能完全阻遏基因正常表达。

如果RNA聚合酶或剪接因子（ splicing factor）的接合位点发生突变则可使基因产物完全失活或阻断其产生。

调节位点突变通常只改变产生蛋白质丰度而不是其结构。

功能缺失型突变与功能获得型突变

loss-of-function mutation：

由于突变导致基因功能的丧失或减弱，这种突变称为功能缺失型突变。

Null mutations/knockout mutations：

如果由于DNA序列插入、丢失或重要碱基替换造成的蛋白质功能完全丧失，或导致转录产物的提前终止，使得基因功能完全丧失，则称为零突变或敲除突变。

Hypomorphic mutation：

如果基因突变仅造成基因表达水平或基因产物活性的降低，这种突变称为亚效突变。

功能获得型突变（gain-of-function mutation）

这种突变赋予了蛋白质异常的活性，并可能产生新的表型。很多这类突变发生在基因的调节序列而不是编码区，因而其后果有多种情况。

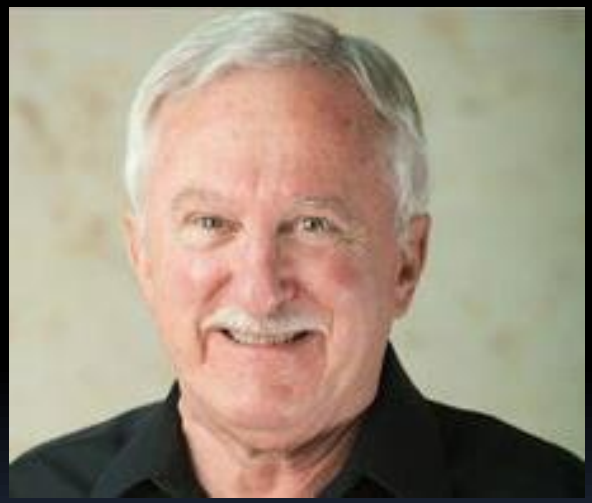
如果基因表达的空间方式被改变，即基因表达产物在基因原来不表达的部位积累，这种表达方式叫异位表达（ectopic expression）。基因的异位表达经常导致超出预期的表型变化，例如在果蝇任何非眼组织（腿、嘴、腹和翅等）异位表达*eyeless*可导致复眼的部分组织以及完整的眼色素的产生。

DNA damage and repair

Tomas Lindahl
Francis Crick Institute
Clare Hare Laboratory

Aziz Sancar
UNC,Chapel Hill

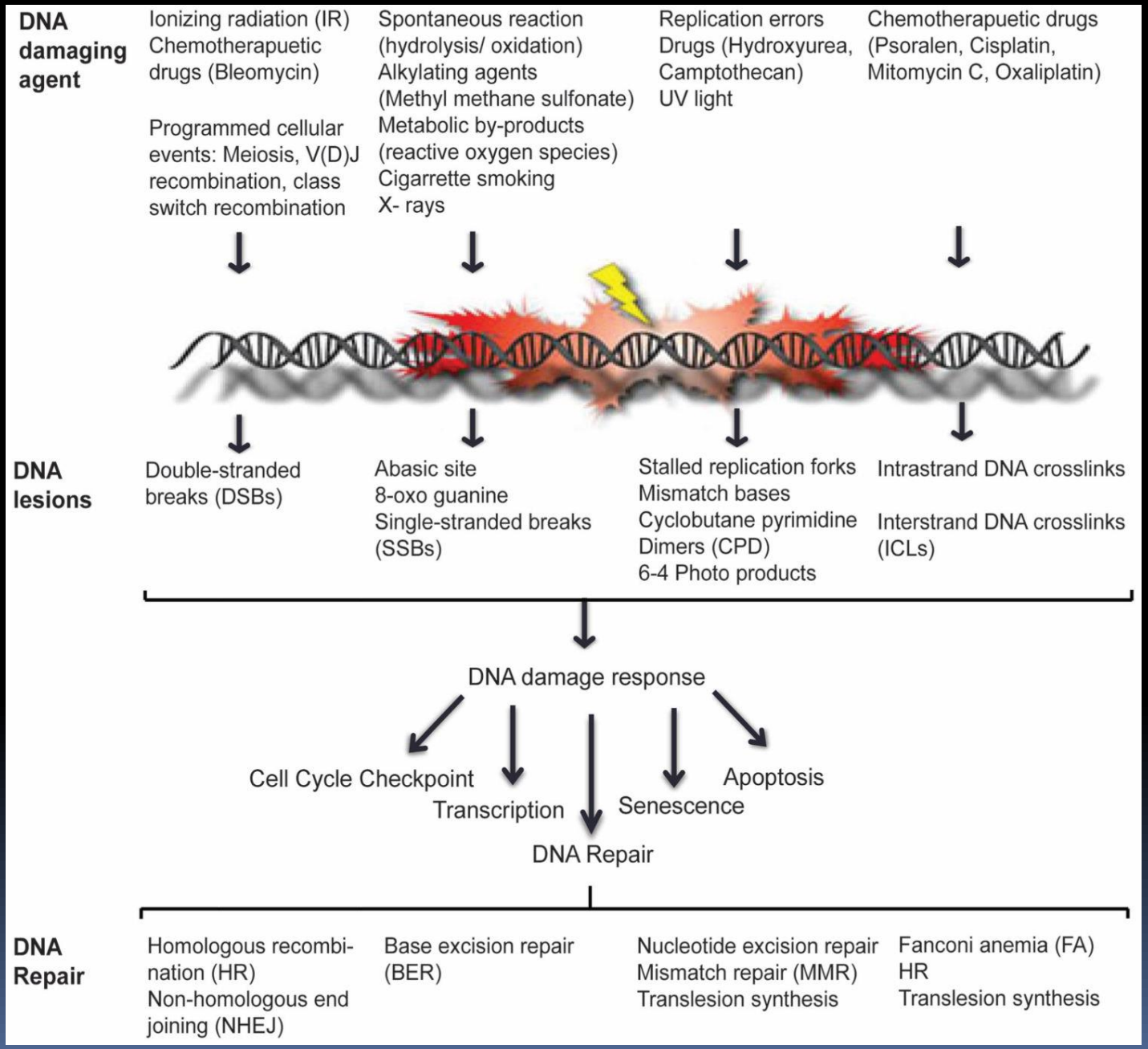
Paul Modrich
HHMI
Duke U



Base excision repair
碱基切除修复

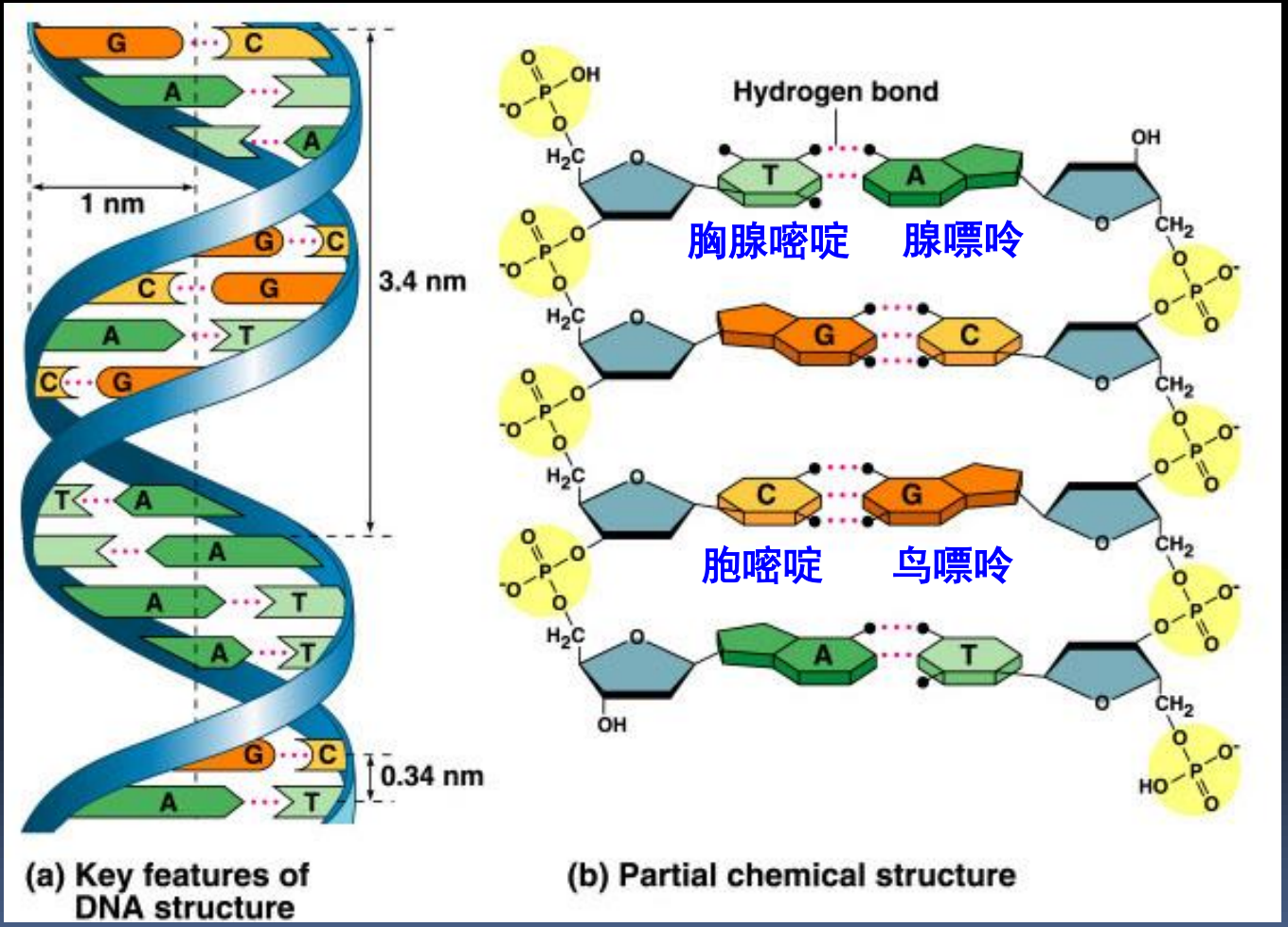
Nucleotide excision repair
核苷酸切除修复

Mismatch repair
错配修复


DNA damaging agent
Ionizing radiation (IR)
Chemotherapuetic drugs (Bleomycin)
Programmed cellular events: Meiosis, V(D)J recombination, class switch recombination
Spontaneous reaction (hydrolysis/ oxidation)
Alkylating agents (Methyl methane sulfonate)
Metabolic by-products (reactive oxygen species)
Cigarrette smoking
X- rays
Replication errors
Drugs (Hydroxyurea, Camptothecan)
UV light
Chemotherapuetic drugs (Psoralen, Cisplatin, Mitomycin C, Oxaliplatin)
DNA lesions
Double-stranded breaks (DSBs)
Abasic site
8-oxo guanine
Single-stranded breaks (SSBs)
Stalled replication forks
Mismatch bases
Cyclobutane pyrimidine Dimers (CPD)
6-4 Photo products
Intrastrand DNA crosslinks
Interstrand DNA crosslinks (ICLs)
DNA damage response
Cell Cycle Checkpoint
Transcription
Senescence
Apoptosis
DNA Repair
DNA Repair
Homologous recombi-nation (HR)
Non-homologous end joining (NHEJ)
Base excision repair (BER)
Nucleotide excision repair
Mismatch repair (MMR)
Translesion synthesis
Fanconi anemia (FA)
HR
Translesion synthesis

DNA分子组成及双螺旋结构

磷酸基团、脱氧核糖、碱基



常见致DNA损伤因素

外源因素：离子辐射，X-ray，UV，化疗药物（Hydroxyurea,camptothecin, bleomycin),化学试剂（MMS，EMS）,吸烟

内源因素：减数分裂，VDJ 重组，复制错误，ROS，氧化等;

DNA损伤类型及修复途径

双链断裂	碱基缺失 单链断裂	复制叉倒塌 碱基错配	嘧啶二聚体 6-4 光产物	DNA交联 DNA-蛋白质交联
⬇	⬇	⬇	⬇	⬇
双链断裂修复	碱基切除修复	错配修复	核苷酸切除修复	FA途径 跨损伤修复 同源重组

主要DNA修复途径

碱基切除修复：Base excision repair (BER）

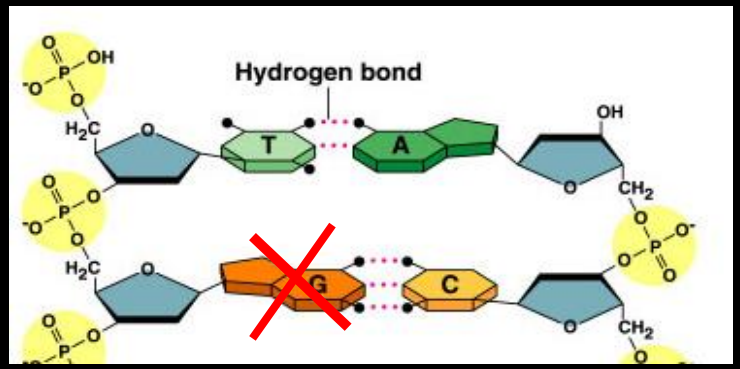
错配修复：Mismatch repair (MMR)

跨损伤合成：Translesion synthesis (TLS)

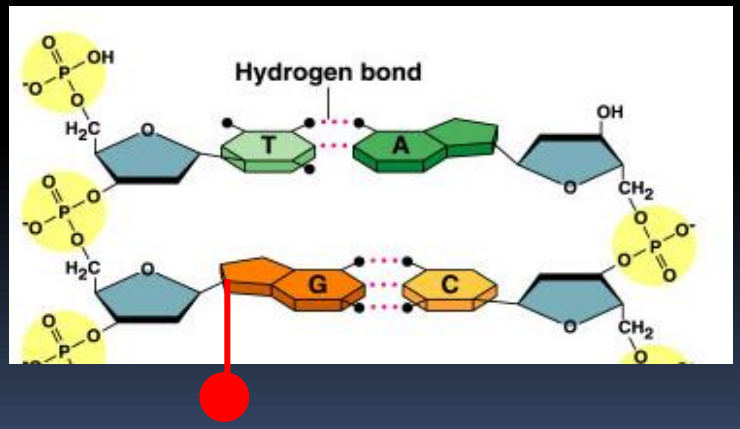
核苷酸切除修复：Nucleotide excision repair (NER)

双链断裂修复：DNA double-strand break repair (DSB)

1. 碱基切除修复 （Base excision repair，BER)



碱基缺失、损坏



化学修饰

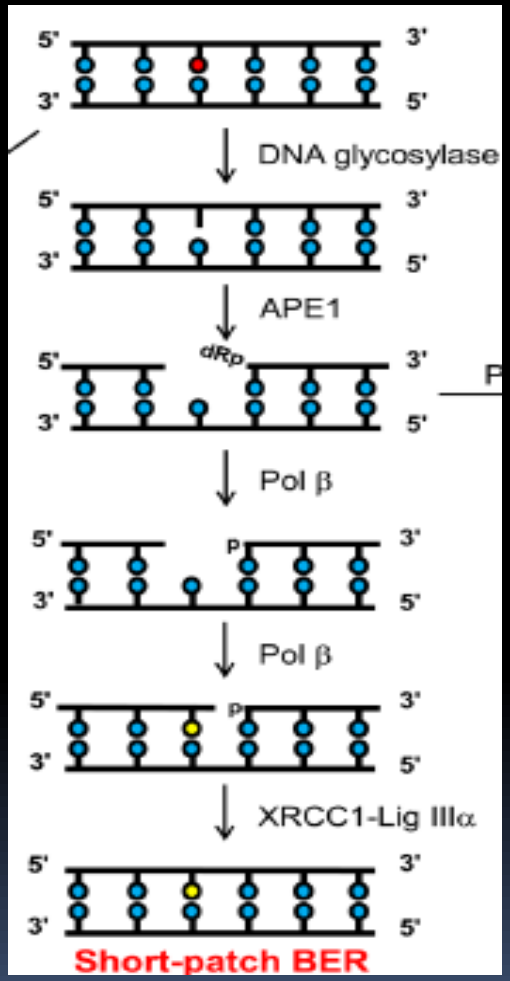
（甲基化，脱氨基）

切除损坏碱基

切除磷酸二酯键

切除dRP， 加入核苷酸

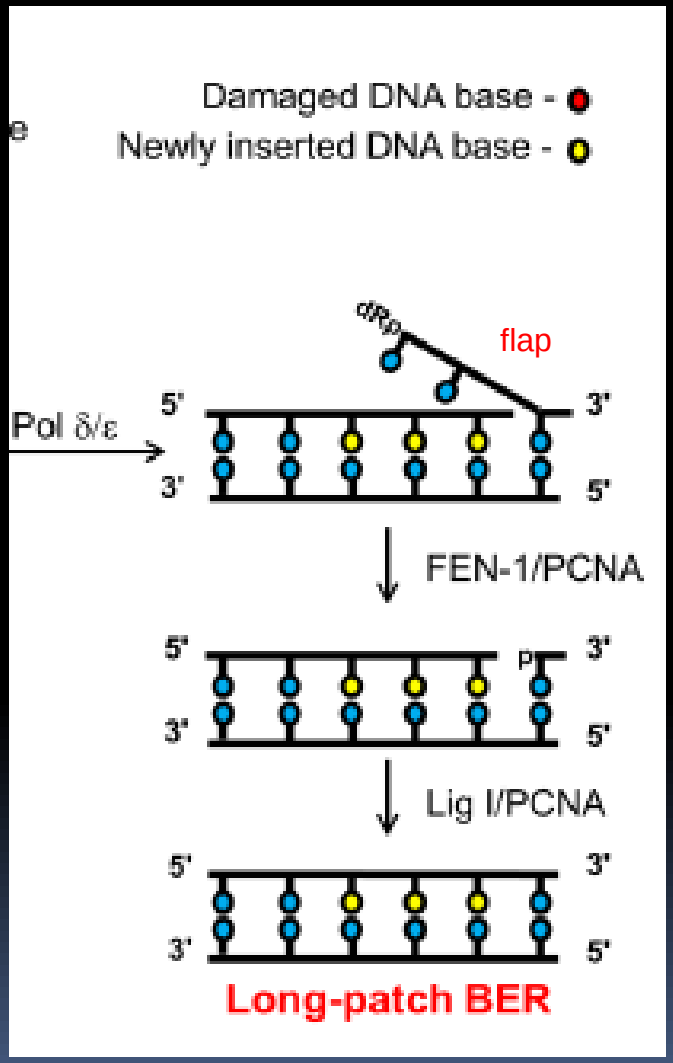
连接完成修复



dRP: deoxyribosephosphate

切除flap

连接完成修复



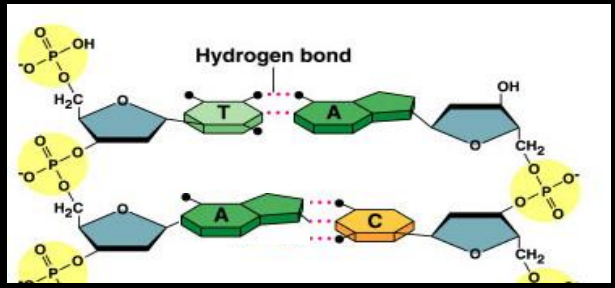
当dRP没有被切除时

dRP: deoxyribosephosphate

2. 错配修复（Mismatch repair）

纠正复制过程错配的碱基 （保真性提高100倍）

MMR缺陷导致复制错误或重复序列不稳定

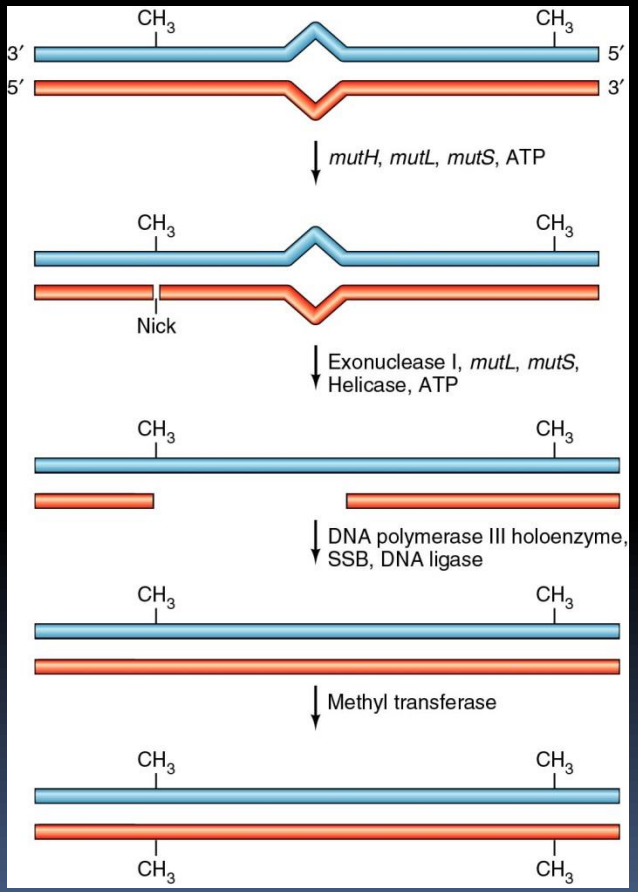


mutS 扫描 DNA 并招募 *MutL*, 后者激活 *mutH*酶.

mutH 识别附近的GATC并在没有发生甲基化的新链是产生切口.

解旋酶和核酶切除DNA

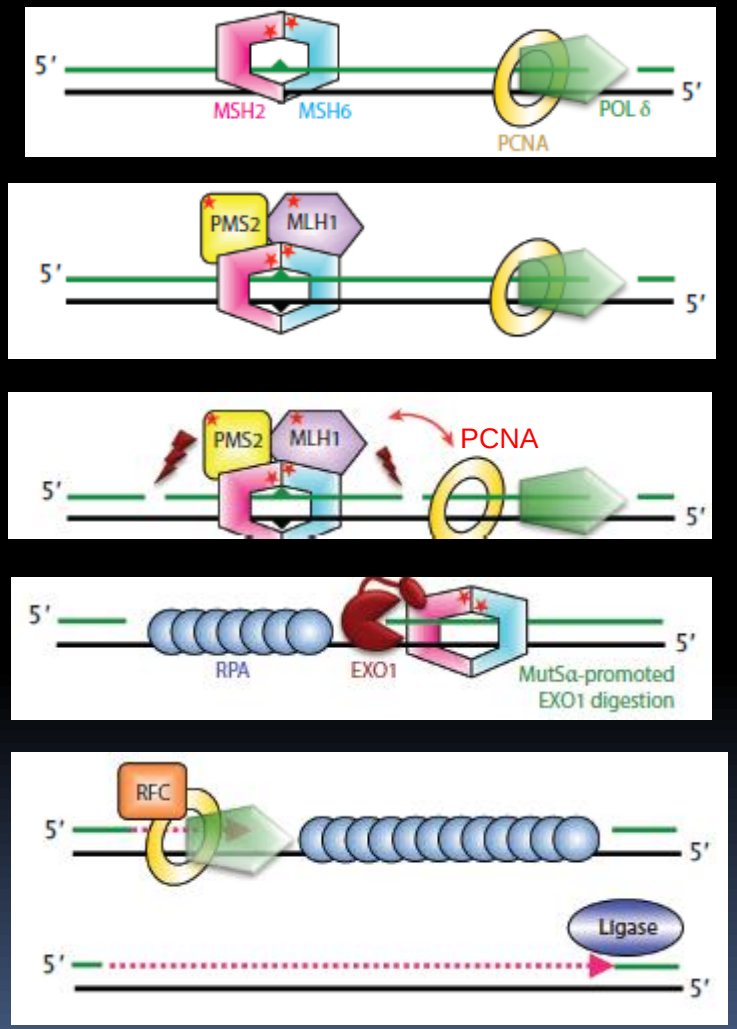
DNA 聚合酶 III和连接酶完成修复



人类细胞错配修复

从遗传性非息肉性大肠癌中分离出来的一组遗传易感基因

真核生物如何识别新生链？



MSH2-MSH6识别错配

↓

MutLα招募

↓

PCNA激活MutLα
在新生链上产生切口

↓

Exo1切除片段

↓

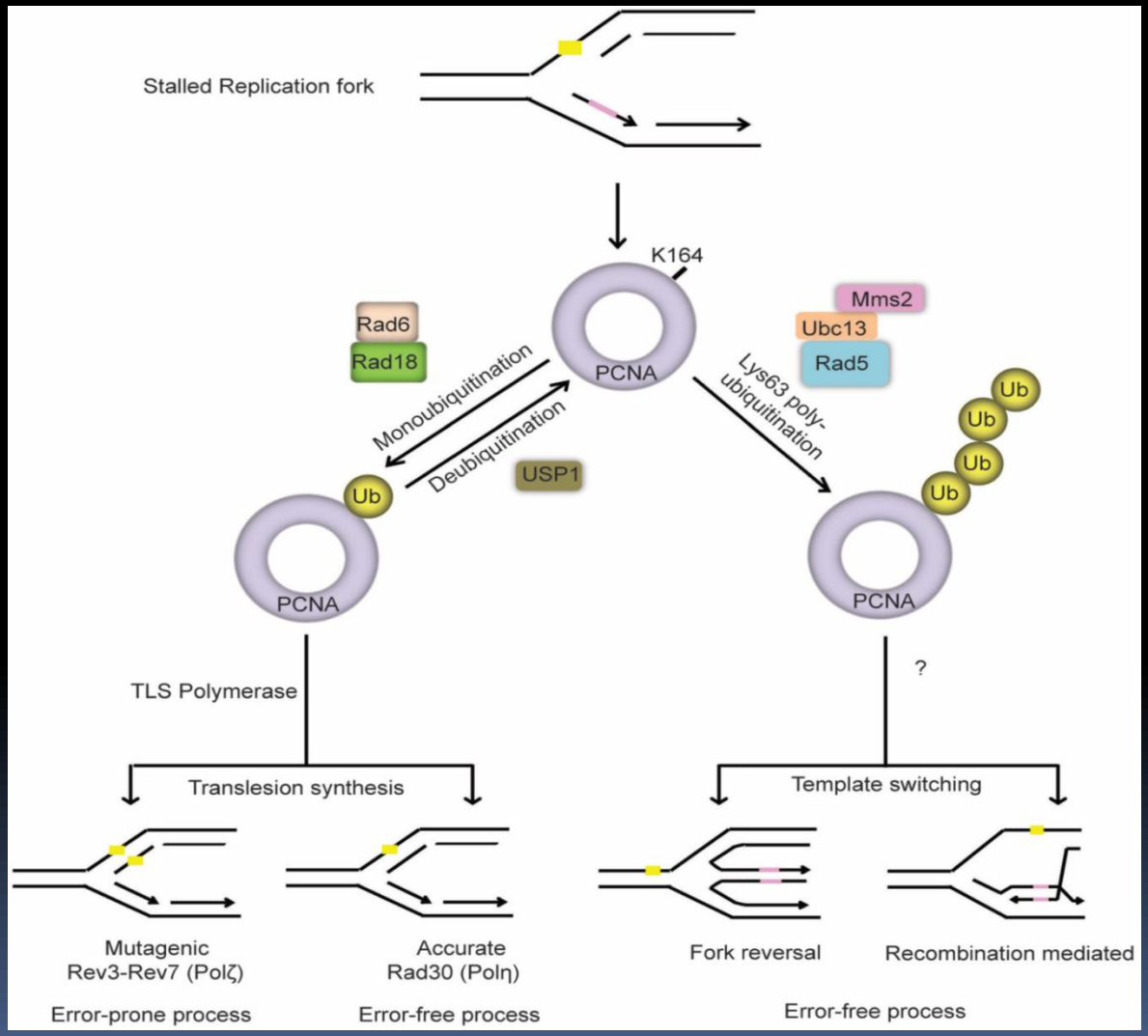
合成和连接

3. 跨损伤合成（Translesion synthesis）

enables replication to proceed across DNA damage （tolerance）

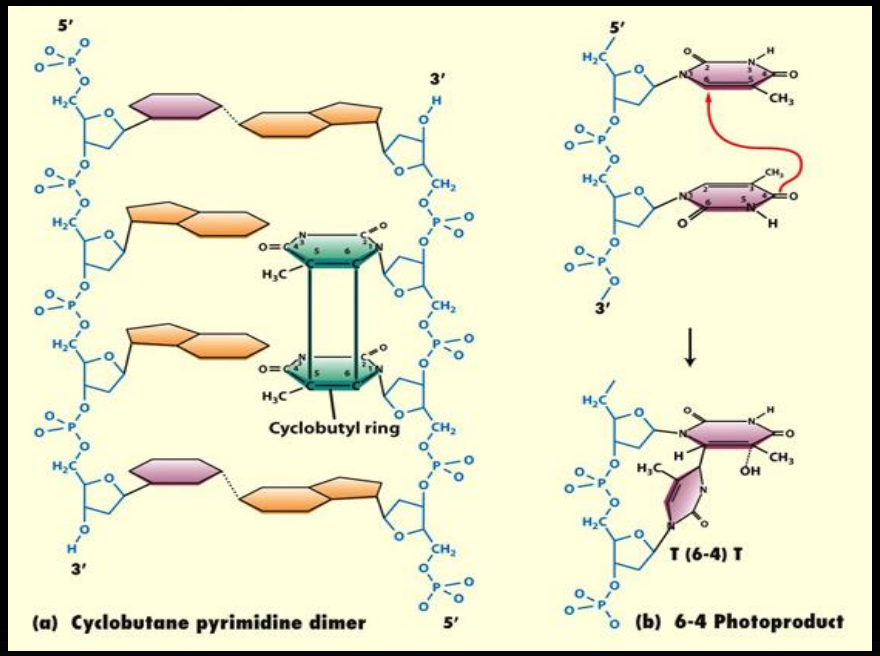
- **Occurs when the above repairs are not efficient enough**
- **the last resort mechanism, prevent the worse fate of an incompletely replicated chromosome**

Translesion synthesis



4. 核苷酸切除修复： Nucleotide excision repair (NER)

嘧啶二聚体　　6-4光产物



NER修复方式：

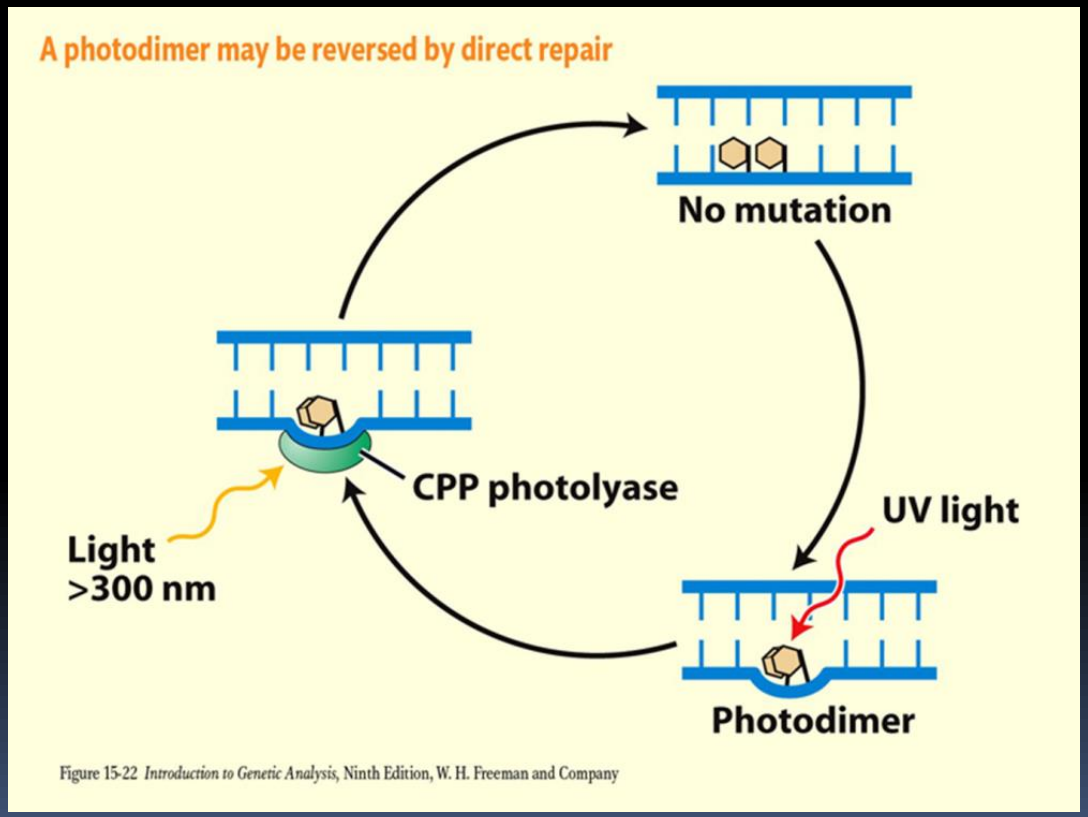
光修复

全基因组修复（global genomic repair）

转录偶联修复（transcription-coupled repair）

1） 在原核和部分真核细胞中存在光修复

DNA光裂合酶　与环丁基嘧啶二聚体结合　（被蓝光和可见光激活）



人类细胞不存在光修复机制

Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 4389–4393, May 1993
Biochemistry

Evidence for lack of DNA photoreactivating enzyme in humans

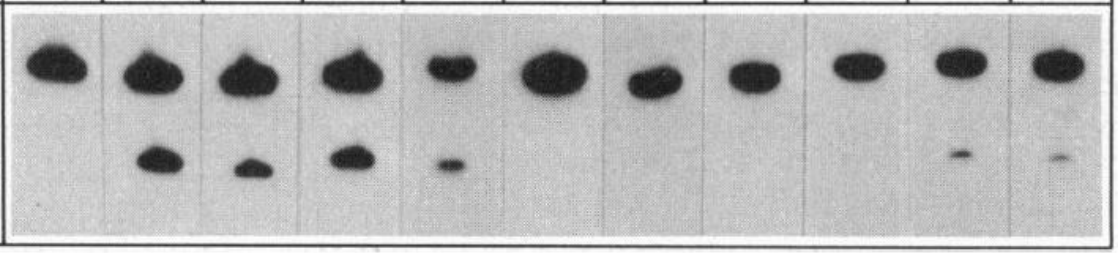
(thymine dimer/skin cancer/mammals/reptiles)

YWAN FENG LI, SANG-TAE KIM, AND AZIZ SANCAR

Department of Biochemistry and Biophysics, University of North Carolina, School of Medicine, Chapel Hill, NC 27599

Communicated by Mary Ellen Jones, January 27, 1993 (received for review November 13, 1992)

Source	–	PL	EC	SC	CH	DH	YL	TM	JR	EC	EC+JR
Protein (μg)	0	2.7×10^{-4}	42	44	6	19	4.8	4.5	13	4.2	4.2+13
% T<>T Repaired	0	27	13	23	16	0	0	0	0	3.6	2.3
Lane	1	2	3	4	5	6	7	8	9	10	11



纯化的酶 大肠杆菌 酵母 响尾蛇 人 大肠杆菌

2）发现UV照射导致胸腺嘧啶二聚体形成；

早期研究发现很多大肠杆菌对紫外照射敏感

Beukers, R., and W. Berends, *Biochim. Biophys. Acta*, 41, 550，1960.

Isolation and identification of the irradiation product of thymine.

紫外线诱导产生的胸腺嘧啶二聚体对细胞有害　　PNAS，1962

EVIDENCE THAT ULTRAVIOLET-INDUCED THYMINE DIMERS IN DNA CAUSE BIOLOGICAL DAMAGE

By Richard B. Setlow and Jane K. Setlow

BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY*

Communicated by Alexander Hollaender, May 2, 1962

发现胸腺嘧啶二聚体的切除与修复有关

RELEASE OF ULTRAVIOLET LIGHT-INDUCED THYMINE DIMERS FROM DNA IN E. COLI K-12

By Richard P. Boyce and Paul Howard-Flanders

DEPARTMENT OF RADIOLOGY, YALE UNIVERSITY SCHOOL OF MEDICINE

UV敏感菌株缺乏TT切除能力，存活率低。

Setlow et al, PNAS 1964, Boyce et al, PNAS 1964, Pettijohn et al, JMB 1964； Rosmussen et al， 1964

3） 发现NER与人类疾病关联 （GGR）

Defective Repair Replication of DNA in Xeroderma Pigmentosum

by

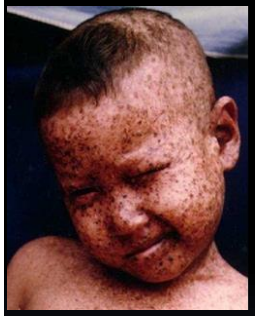
J. E. CLEAVER

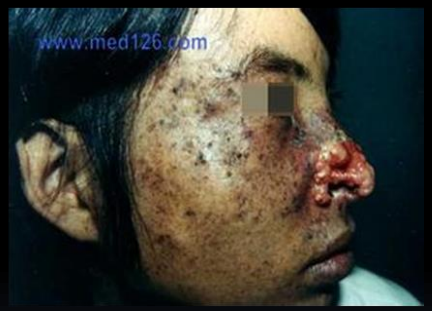
Laboratory of Radiobiology,
University of California Medical Center,
San Francisco, California

Normal skin fibroblasts can repair ultraviolet radiation damage to DNA by inserting new bases into DNA in the form of small patches. Cells from patients with the hereditary disease xeroderma pigmentosum carry a mutation such that repair replication of DNA is either absent or much reduced in comparison to normal fibroblasts. Patients with xeroderma pigmentosum develop fatal skin cancers when exposed to sunlight, and so the failure of DNA repair in the skin must be related to carcinogenesis.

Xeroderma Pigmentosum

（着色性干皮病， **Kaposi，1882**）





J.E. Cleaver，Nature，1968 （UCSF）

着色性干皮病（XP）：第一个与DNA损伤修复缺陷有关的人类疾病；

患者存在DNA损伤修复功能缺陷，皮肤缺乏核酸内切酶，不能修复被紫外线损伤的DNA；

皮肤炎症，患者发生皮肤癌的可能性几乎是100% （比正常人高2000倍）。

Cockayne氏综合征 （CSB， CSA） 与TCR关联

Edward Alfred Cockayne

Trichothiodystrophy 毛发低硫营养不良

小头、纹状体小脑钙化；身材矮小，面容苍老；

视网膜萎缩和耳聋综合征；精神发育迟滞；

皮肤对光敏感；

4） 发掘与克隆NER修复相关基因

Genetic Heterogeneity of Xeroderma Pigmentosum demonstrated by Somatic Cell Hybridization

E. A. DE WEERD-KASTELEIN
W. KEIJZER
D. BOOTSMA

Department of Cell Biology and Genetics, Rotterdam Medical Faculty, Netherlands

Received November 16, 1971.

不同家系的病人表现出的症状有所不同（异质性）；

不同家系病人突变基因不同；

De Weerd-Kastelein， Nature New Biology，1972

酵母中NER相关基因的克隆 （通过筛选突变体）

Yeast nucleotide excision repair factors (NEFs)

NEFs	Components	Function or Activity
NEF1	Rad1, Rad10, Rad14	DNA endonuclease, DNA damage recognition
NEF2	Rad4, Rad23	DNA damage binding, Tethering of NEF1 with NEF3
NEF3	Rad2, Rad3, Rad25, SSL1, TFB1, TFB2, TFB3	DNA endonuclease, DNA helicase
NEF4	Rad7, Rad16	DNA dependent ATPase, DNA damage recognition
RPA	p69, p36, p13	DNA damage recognition

Prakash et al， 2000

酵母和人类NER同源蛋白

	S. cerevisiae gene	Human gene	Biochemical activities
	RAD7	Not known	Rad7–Rad16 complex, a DNA dependent
	RAD16	Not known	ATPase, binds UV-damaged DNA in an ATP dependent manner
	RAD14	*XPA*	Damage binding protein
	RAD4	*XPC*	Rad4–Rad23 complex binds UV damaged DNA
	RAD23	*HR23B*	
TFIIH	*RAD3*	*XPD*	5′ → 3′ DNA helicase
TFIIH	*RAD25*	*XPB*	3′ → 5′ DNA helicase
TFIIH	*SSL1*	*P44*	–
TFIIH	*TFB1*	*P62*	–
TFIIH	*TFB2*	*P52*	–
TFIIH	*TFB3*	*MAT1*	–
	RAD1	*XPF*	Rad1–Rad10 nuclease cuts damaged DNA on the 5′-side of the lesion
	RAD10	*ERCC1*	
	RAD2	*XPG*	Rad2 nuclease cuts damaged DNA on the 3′-side of the lesion
	MMS19	Not known	None detected
	RAD26	*CSB*	DNA dependent ATPase
	RAD28	*CSA*	–

识别

解旋

剪切

发现至少10个不同基因：

XPA、XPB、XPC、XPD、XPE、XPF、XPG、XPH、XPI、XPV

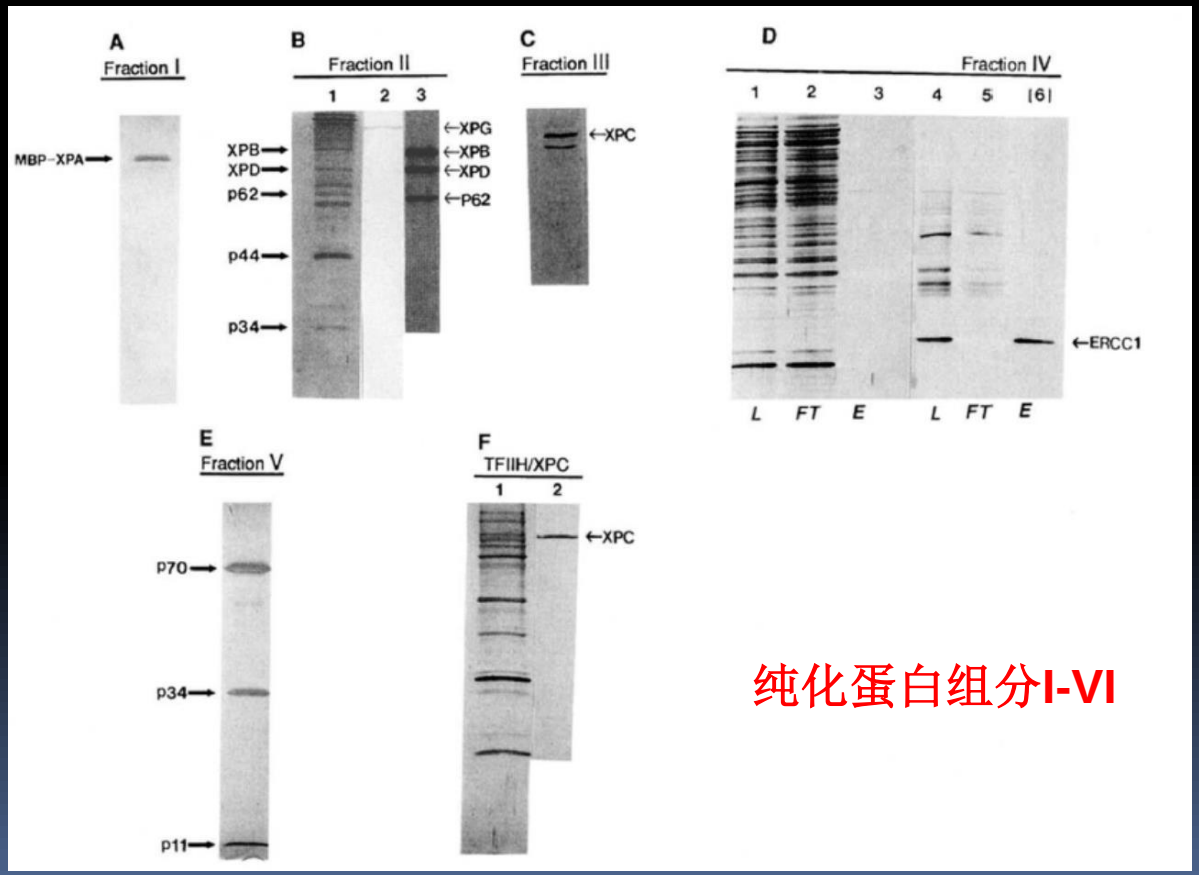
5） 体外重建NER

Reconstitution of Human DNA Repair Excision Nuclease in a Highly Defined System*

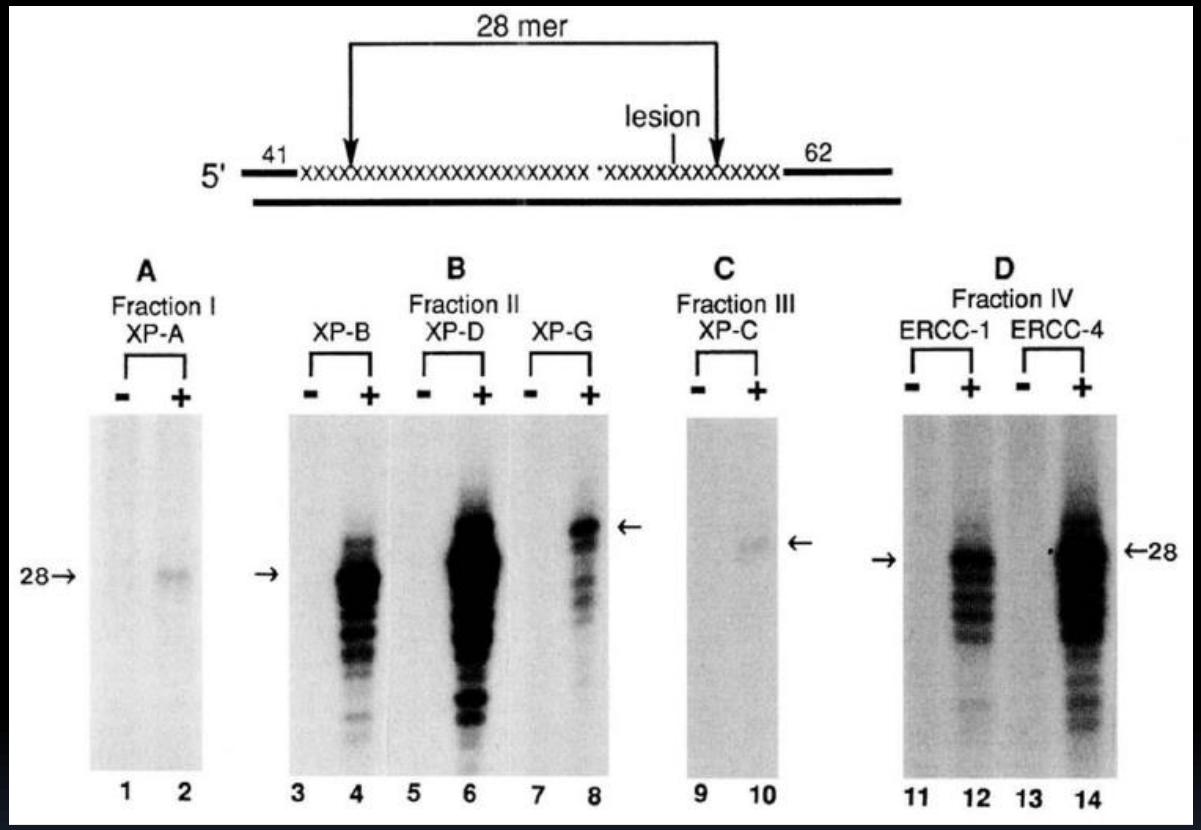
(Received for publication, November 23, 1994, and in revised form, December 18, 1994)

David Mu‡, Chi-Hyun Park, Tsukasa Matsunaga, David S. Hsu, Joyce T. Reardon, and Aziz Sancar§

From the Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599-7260

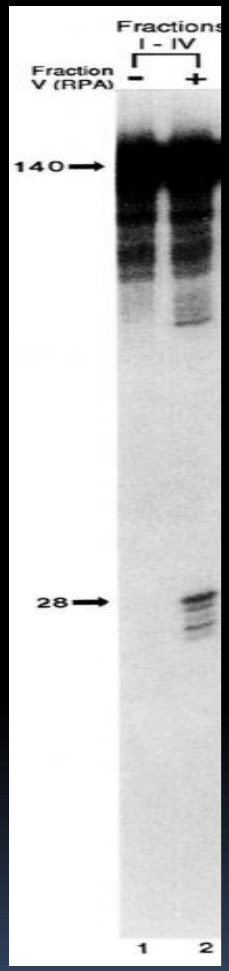


纯化蛋白组分**I-VI**



Cell-free extract

证明纯化的蛋白或复合物具有生化活性



（XPA，TFIIH(XPB and XPD)，XPC, XPF，ERCC1, XPG， RPA）组建成了最简单的NER机器；

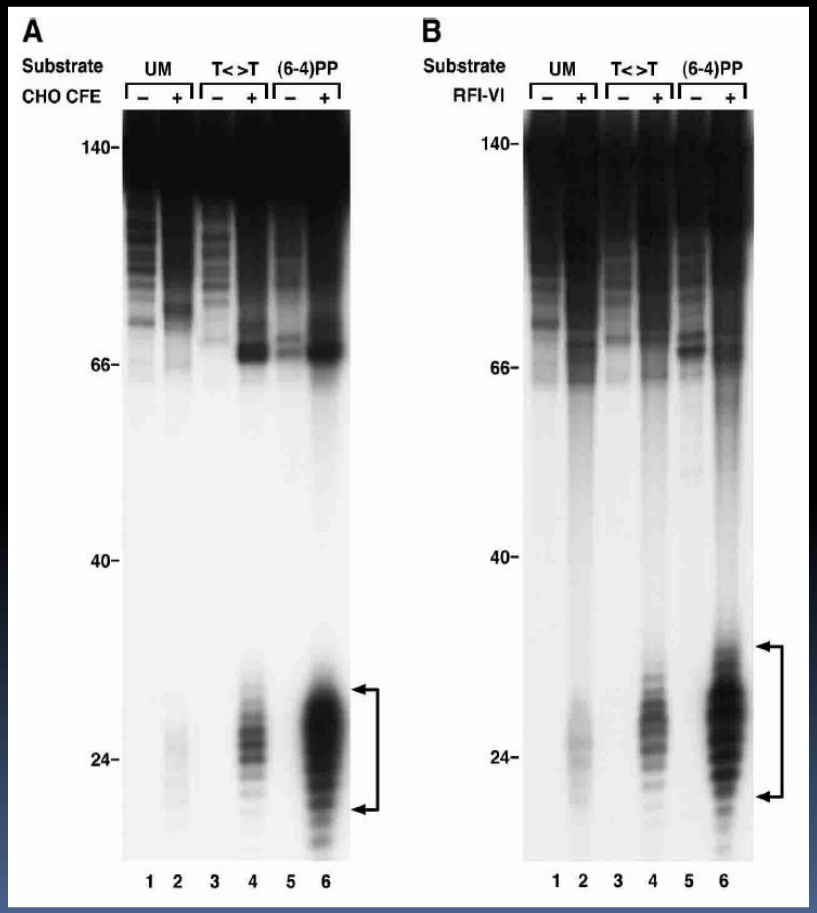
能否特异识别并切割UV导致的异常DNA结构？

Recognition and repair of the cyclobutane thymine dimer, a major cause of skin cancers, by the human excision nuclease

Joyce T. Reardon and Aziz Sancar[1]

Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599, USA

细胞提取物　　组建蛋白复合物



RF I-VI 体外特异切除TT二聚体和6-4PP

与体内速率相似

6）细胞对于转录区和非转录区域NER修复有区别吗？

MOLECULAR AND CELLULAR BIOLOGY, Jan. 2006, p. 39–49 Vol. 26, No. 1
0270-7306/06/$08.00+0 doi:10.1128/MCB.26.1.39–49.2006

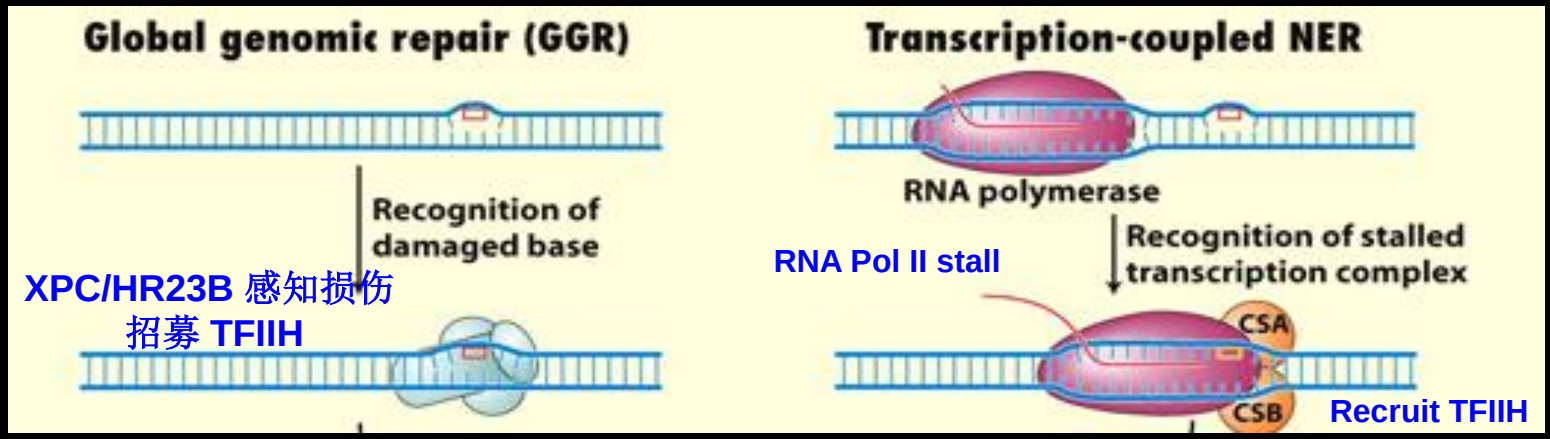
Recruitment of DNA Damage Checkpoint Proteins to Damage in Transcribed and Nontranscribed Sequences

Guochun Jiang and Aziz Sancar*

Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599

Received 6 September 2005/Returned for modification 5 October 2005/Accepted 6 October 2005

活跃转录区和非转录区NER识别机制不同



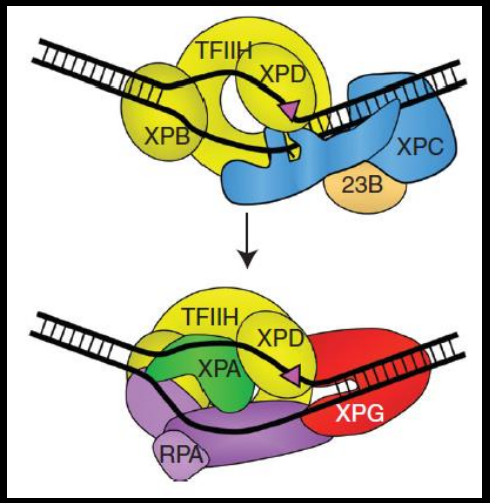
全基因组

活跃转录区

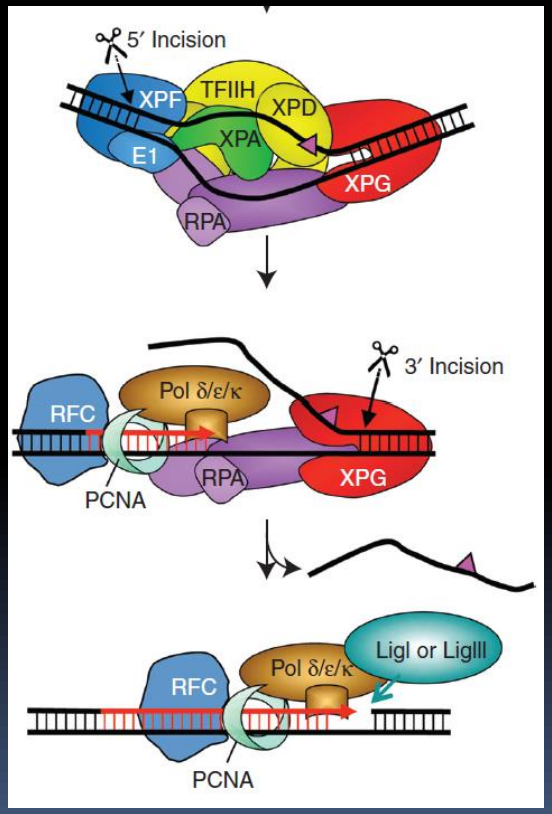
招募TFIIH

XPB 和XPD打开DNA双链

XPA, RPA, XPG结合



Preincision complex



招募XPF/ERCC1

5’端剪切

DNA合成

3’端剪切

连接缺口

核苷酸切除修复研究发展历程

发现UV敏感突变体 （1960s）

↓

与人类疾病关联 （1960s）

↓

克隆相关基因 （1970s-1990）s

↓

体外验证蛋白功能 （1990s）

↓

体外重构NER （1990s）

↓

细胞生物学研究（2000-）

↓

NER调控机制 （2000-）

5. DNA双链断裂修复

1. Non-homologous end joining (NHEJ)

2. Homologous recombination (HR)

a.Single strand annealing (SSA)

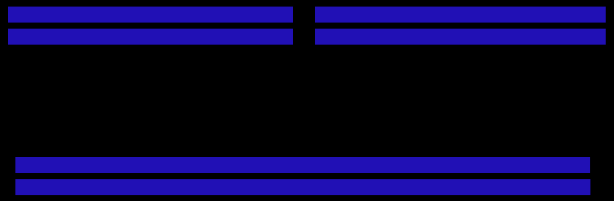
b.Synthesis-dependent strand annealing (SDSA)

c.Break induced repair (BIR)

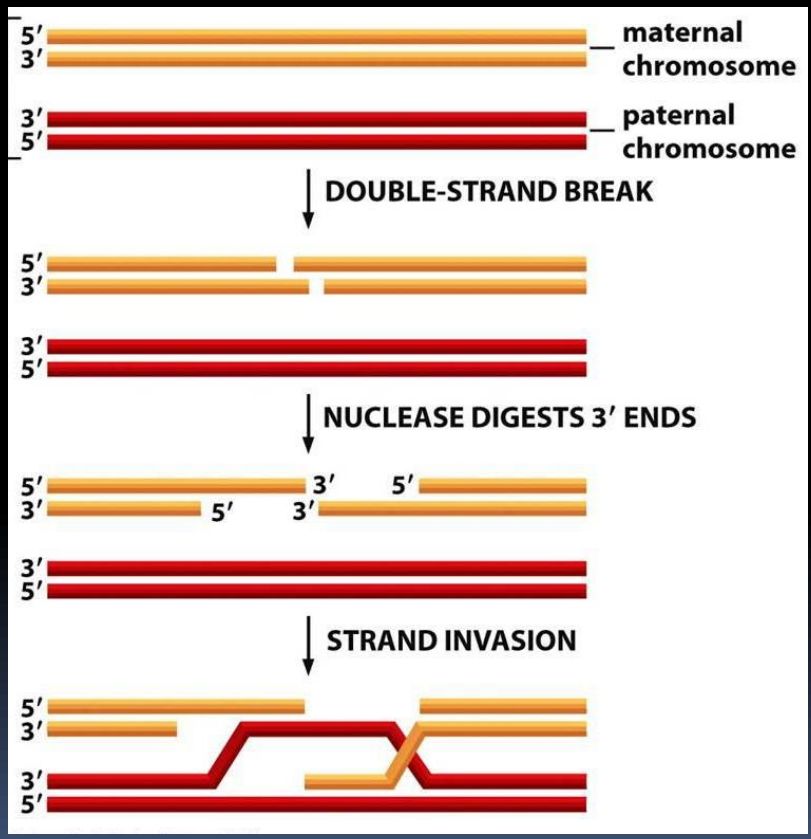
d.Homologous recombination

经典DNA双链断裂修复途径

Non-Homologous End Joining (NHEJ)
Error-prone



Homologous recombination (HR)
Error-free

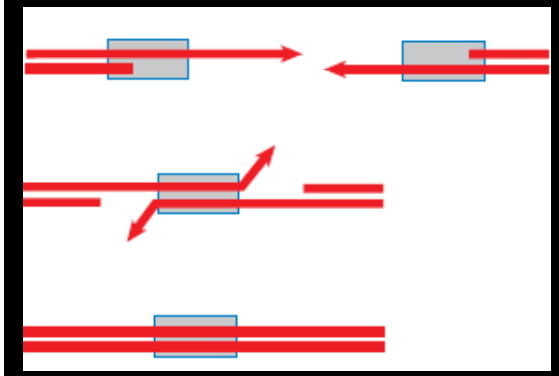


resection

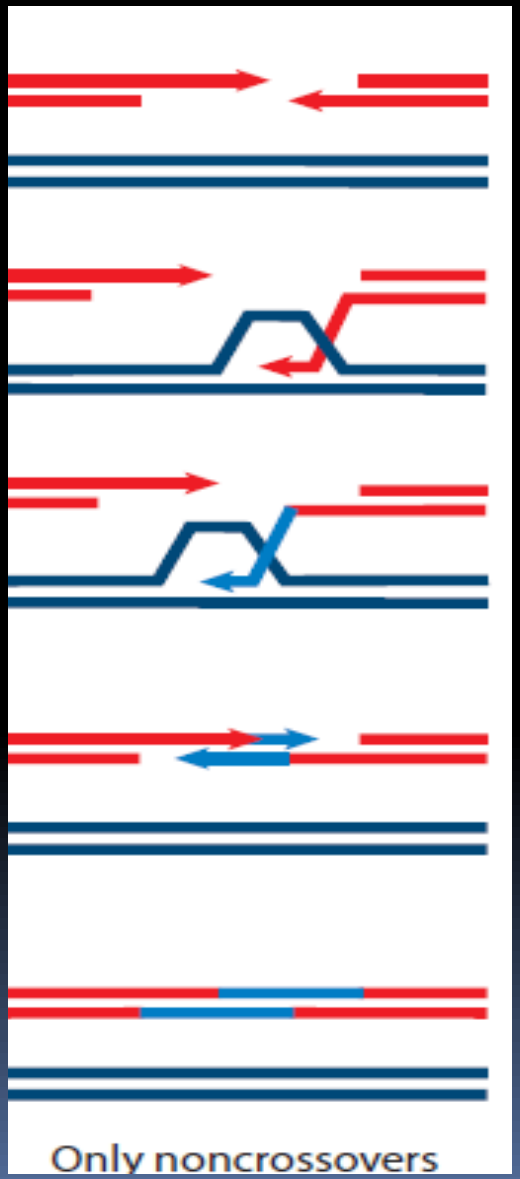
Strand invasion

Modified from Kwan et al, 2011

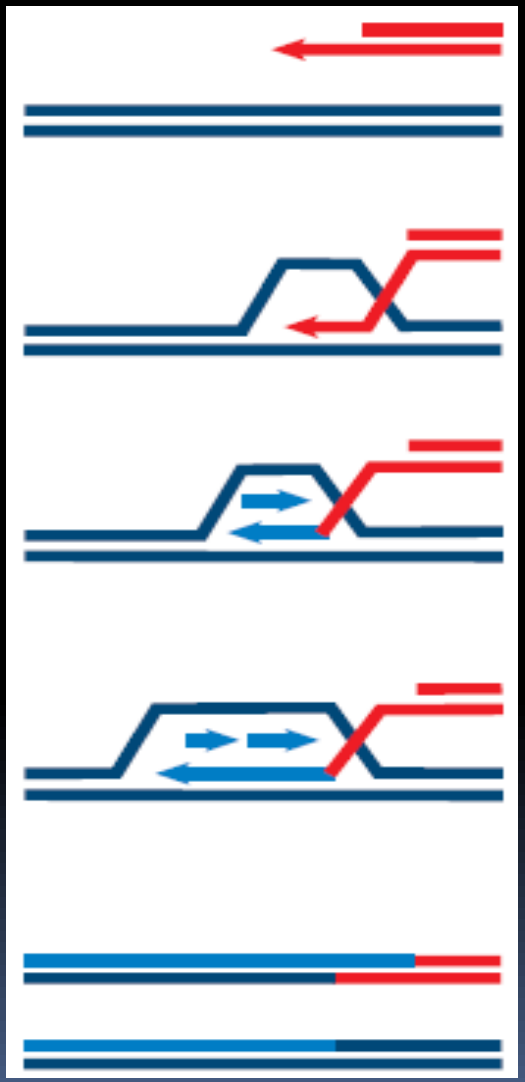
SSA



SDSA

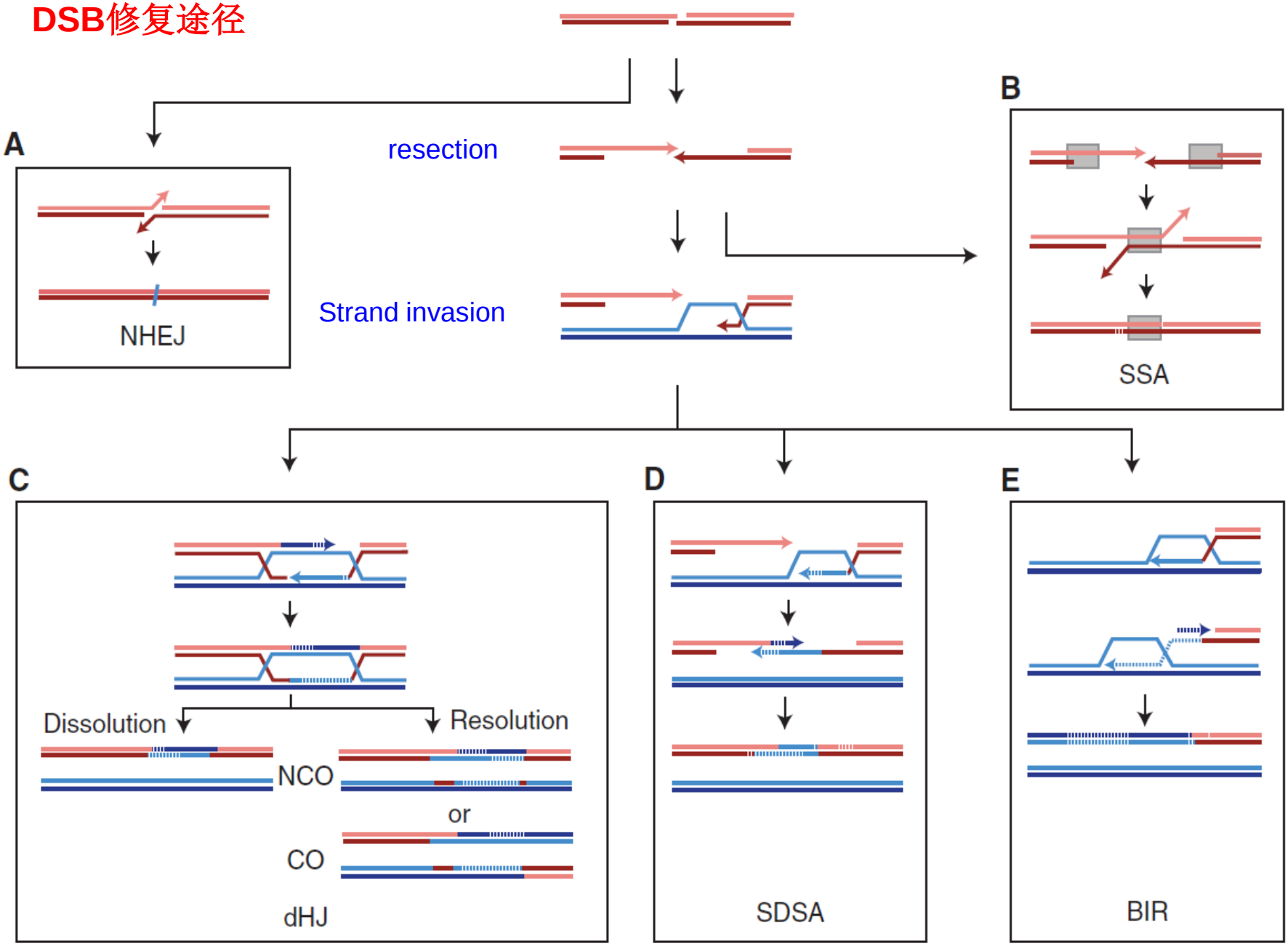


BIR



Direct repeat

DSB修复途径


A
NHEJ
resection
Strand invasion
B
SSA
C
Dissolution
Resolution
NCO
or
CO
dHJ
D
SDSA
E
BIR

Double-strand break (DSB)

DSB is the most deleterious DNA lesion. It can cause cell death and tumorigenesis.

DSB occurs:

1) Exogenous: Ionizing radiation, some classes of chemotherapeutic drugs.

2) Programmed recombination events: meiosis, yeast mating type switch, V(D)J recombination (generate diverse immunoglobulin and T cell receptors).

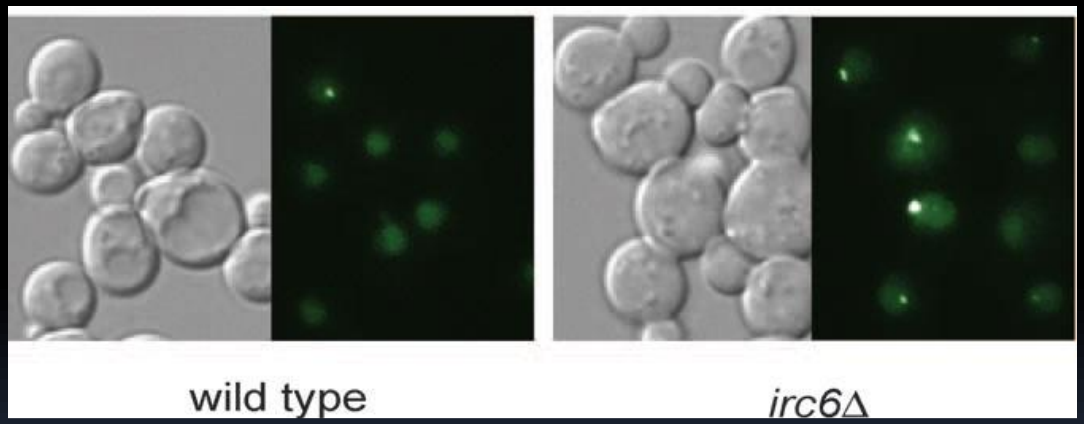
3) Normal cellular metabolism: replication fork collapse; replication through a single strand nick or fragile site; ROS

Kasparek et al, DNA repair, 2011; Polo, et al. Gene & Dev 2011

Spontaneous DSB formation during DNA replication

Breaks occur in approximately 20 – 50 % of S phase cells

Rad52-GFP



Alvaro et al, PLoS Genetics, 2007

DSB repair and human diseases

Developmental defects

Neurodegeneration and aging

Immunodeficiency

Radiosensitivity

Cancer predisposition

(Mckinnon et al, 2007, Annual Rev Genom. Human Genetics; Aguilera et al, 2008, Nat Rev Genet; Jackson et al, 2009, Nature)

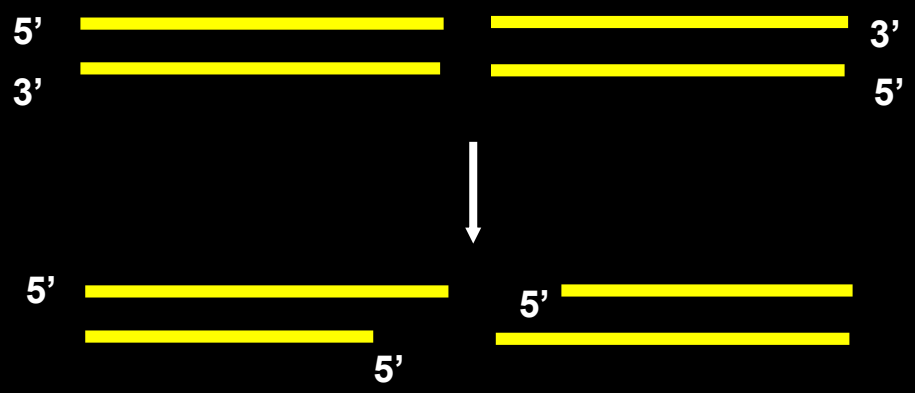
Defective HR is tightly linked to human cancers

Table 1
Summary of homologous recombination genes which are reported to harbour mutations or epigenetic alterations leading to reduced function in human cancer.

Cancer Site	HR genes	References
Head and Neck	*ATM FANCF*	[51,143
Meduloblastoma	*FANCD1/BRCA2*	222,223
Nasopharyngeal	*ATM*	136,140,141
Oral	*ATM*	137
Thyroid	*XRCC3*	204
Lung	*NBS1 BRCA1 FANCF XRCC2*	51,176,177,206
Breast	*ATM RAD50 NBS1 BRCA1 BRCA2 RAD51 FANCJ*	23,44,45,48,52–61,63–73,118,120, 122–134,142,144–147,167,170,171, 190–192,194,195,199,200,226
Stomach	*ATM ATR BRCA1 BRCA2*	46,48,49,96,122,153–156,217
Pancreas	*BRCA2 RAD51 FANCC*	47,91–95,122
Colorectal	*ATM MRE11 BRCA1 BRCA2*	[46,49,124,165]
Ovarian	*MRE11 RAD50 BRCA1 BRCA2 FANCF*	44–46,48,55,63,66,74–83,85–90,99, 122,129,150,151,166,202,230–232
Endometrial	*ATR*	154–156
Cervical	*BRCA1 FANCF*	50,233
Prostate	*ATM NBS1 BRCA2*	48,80,98–100,135,170,171
Multiple Myeloma	*RAD51*	193
Leukaemia	*ATM NBS1 RAD51 FANCA FANCC FANCF FANCG*	122,138,175,196,197,220,224,225,229
Melanoma	*NSB1 XRCC3, BRCA2*	48,49,172,174,203
Lymphoma	*NSB1*	130,138,173

Cerbinskaite et al, 2011, Cancer treatment reviews

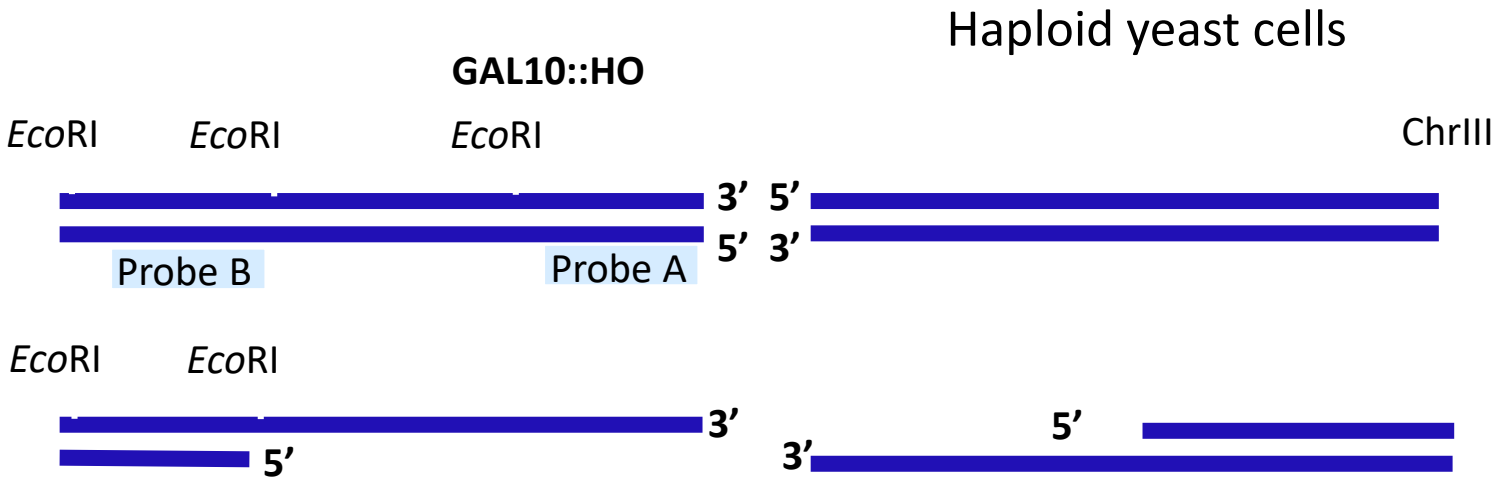
DNA双链断裂修复途径选择----末端加工

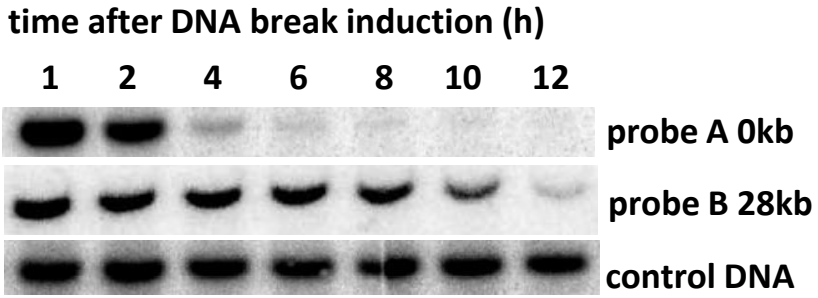


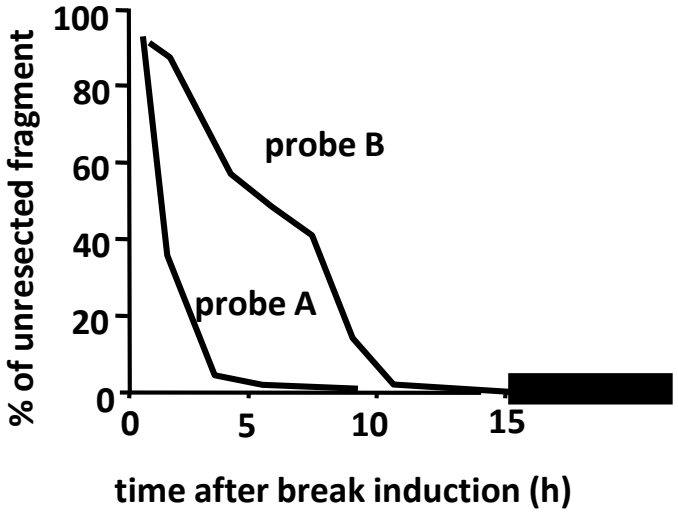
?

哪些蛋白参与末端加工?

An inducible system to measure resection of DSB ends



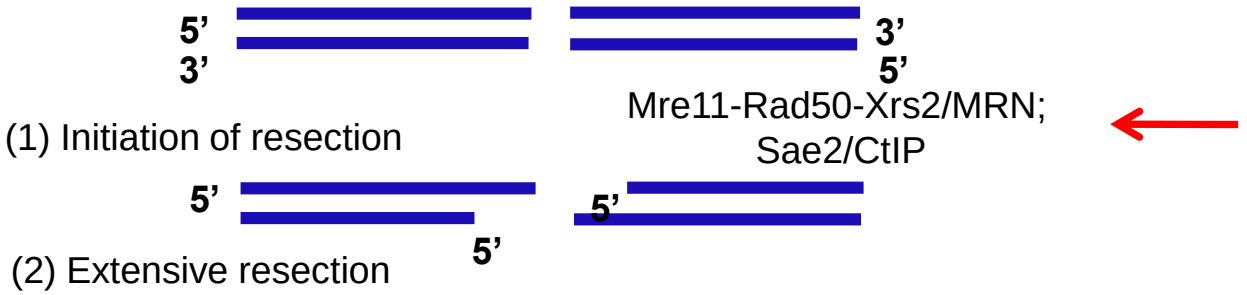


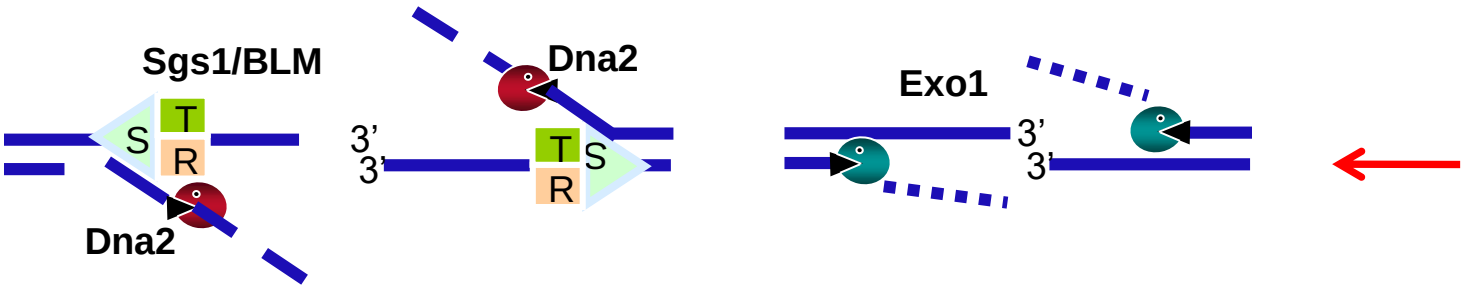


Enzymes processing DSB ends

Activation of DNA damage checkpoint

Essential for strand invasion in homologous recombination





STR: Sgs1-Top3-Rmi1

Sgs1 Helicase and Two Nucleases Dna2 and Exo1 Resect DNA Double-Strand Break Ends

Zhu Zhu,[1,3] Woo-Hyun Chung,[1,3] Eun Yong Shim,[2] Sang Eun Lee,[2] and Grzegorz Ira[1,*]
[1]Department of Molecular & Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030
[2]Department of Molecular Medicine and Institute of Biotechnology, University of Texas Health Science Center at
15355 Lambda Drive, San Antonio, TX 78245, USA
[3]These authors contributed equally to this work
*Correspondence: gira@bcm.edu
DOI 10.1016/j.cell.2008.08.037

nature Vol 455|9 October 2008|doi:10.1038/nature07312

ARTICLES

Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing

Eleni P. Mimitou[1] & Lorraine S. Symington[1]

DNA ends exposed after introduction of double-strand breaks (DSBs) undergo 5′–3′ nucleolytic degradation to generate single-stranded DNA, the substrate for binding by the Rad51 protein to initiate homologous recombination. This process is poorly understood in eukaryotes, but several factors have been implicated, including the Mre11 complex (Mre11–Rad50–Xrs2/NBS1), Sae2/CtIP/Ctp1 and Exo1. Here we demonstrate that yeast Exo1 nuclease and Sgs1 helicase function in alternative pathways for DSB processing. Novel, partially resected intermediates accumulate in a double mutant lacking Exo1 and Sgs1, which are poor substrates for homologous recombination. The early processing step that generates partly resected intermediates is dependent on Sae2. When Sae2 is absent, in addition to Exo1 and Sgs1, unprocessed DSBs accumulate and homology-dependent repair fails. These results suggest a two-step mechanism for DSB processing during homologous recombination. First, the Mre11 complex and Sae2 remove a small oligonucleotide(s) from the DNA ends to form an early intermediate. Second, Exo1 and/or Sgs1 rapidly process this intermediate to generate extensive tracts of single-stranded DNA that serve as substrate for Rad51.

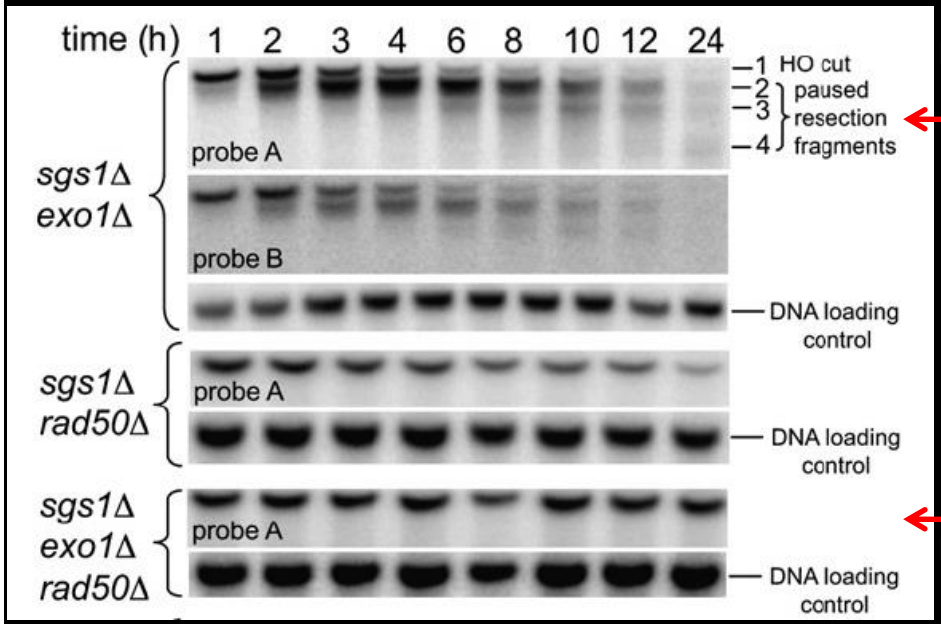
RESEARCH COMMUNICATION

DNA helicases Sgs1 and BLM promote DNA double-strand break resection

Serge Gravel, J. Ross Chapman, Christine Magill, and Stephen P. Jackson[1]

The Wellcome Trust and Cancer Research UK Gurdon Institute, and the Department of Zoology, University of Cambridge, Cambridge CB2 1QN, United Kingdom

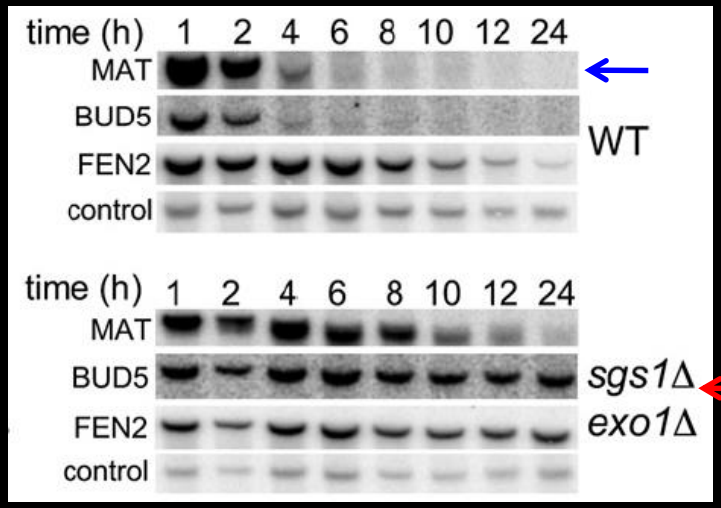
MATa (0.2 kb)



Poor cut

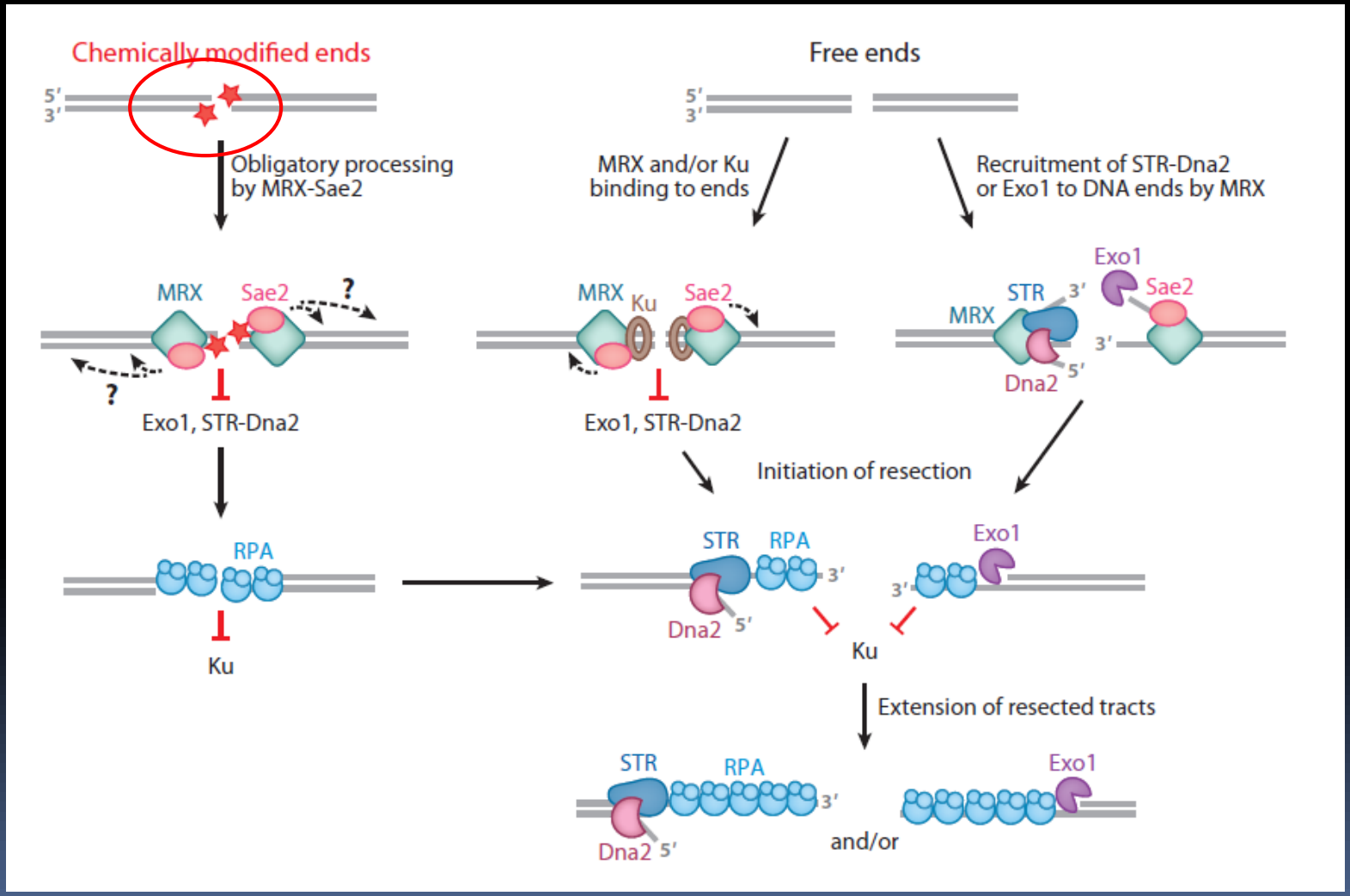
No cut

control

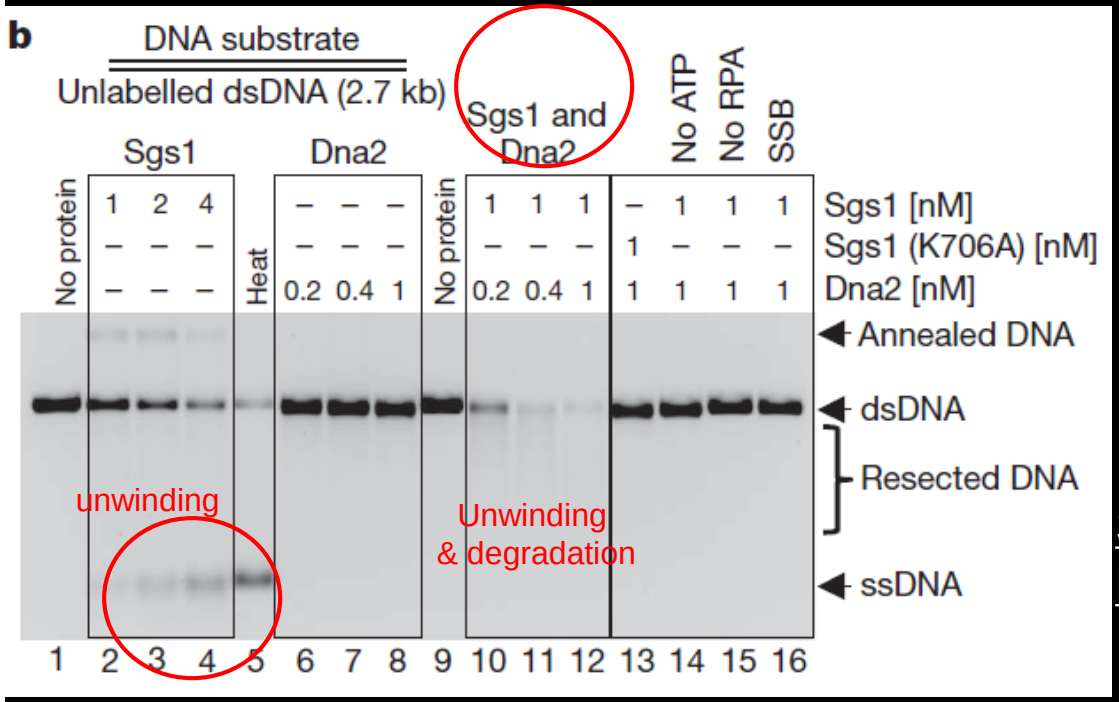


No cut

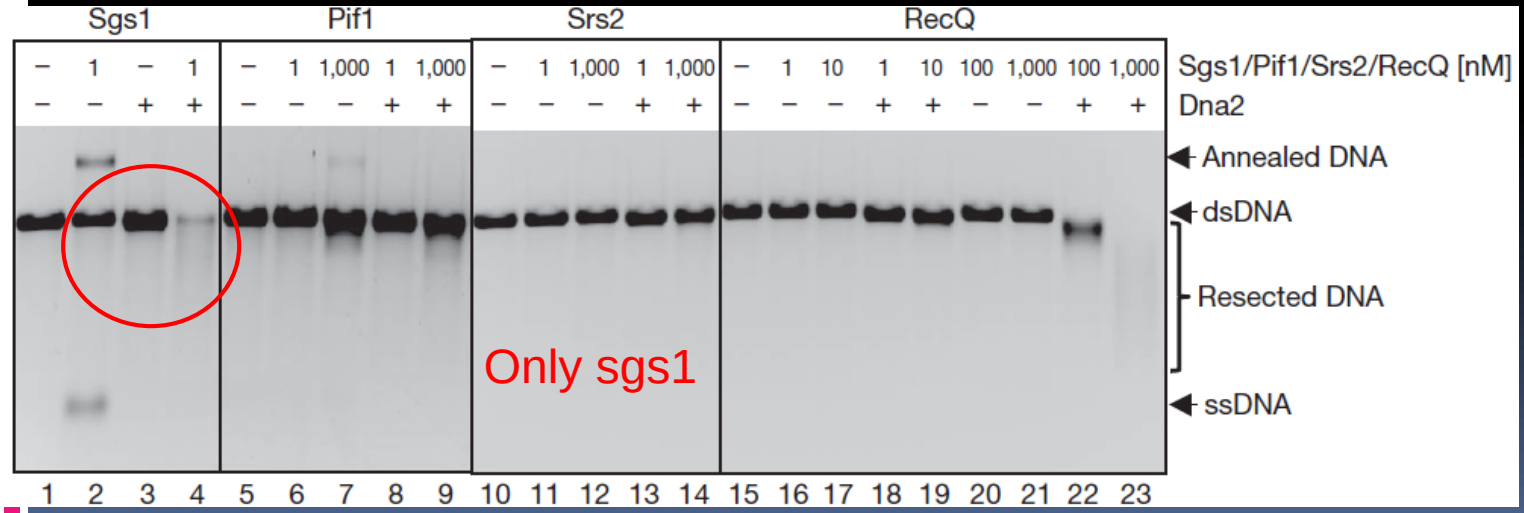
Processing of “dirty” ends requires Sae2



Biochemical evidence

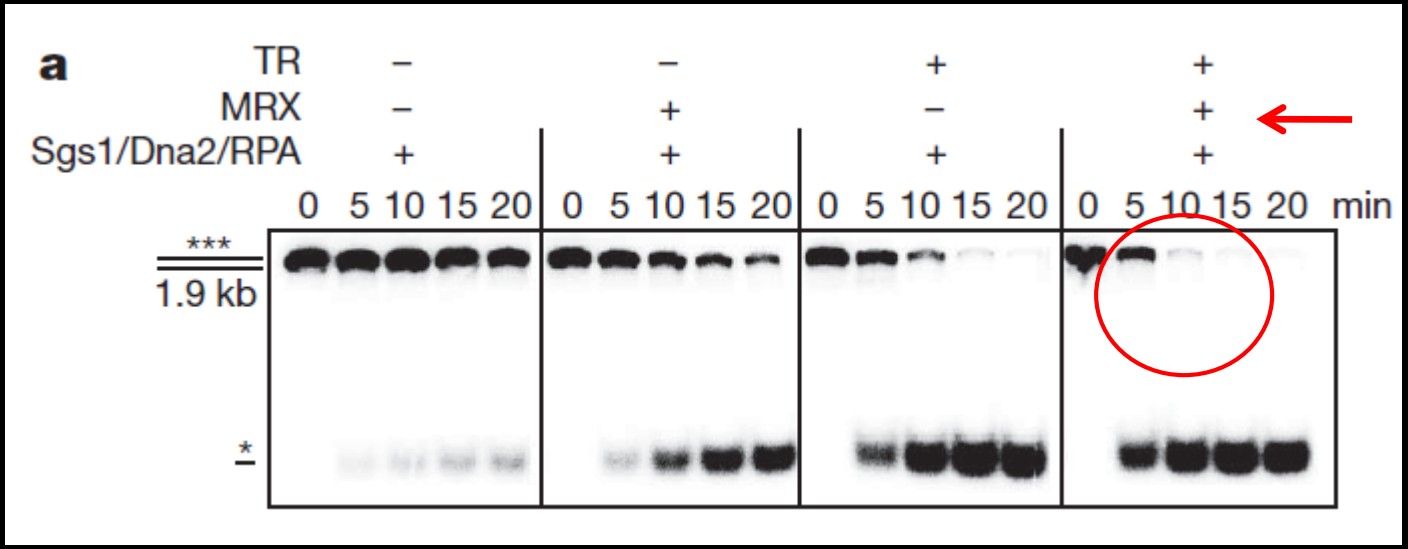


Sgs1 helicase cooperates with Dna2 nuclease



Cejka et al ,2010, Nature

Niu et al, 2010, Nature



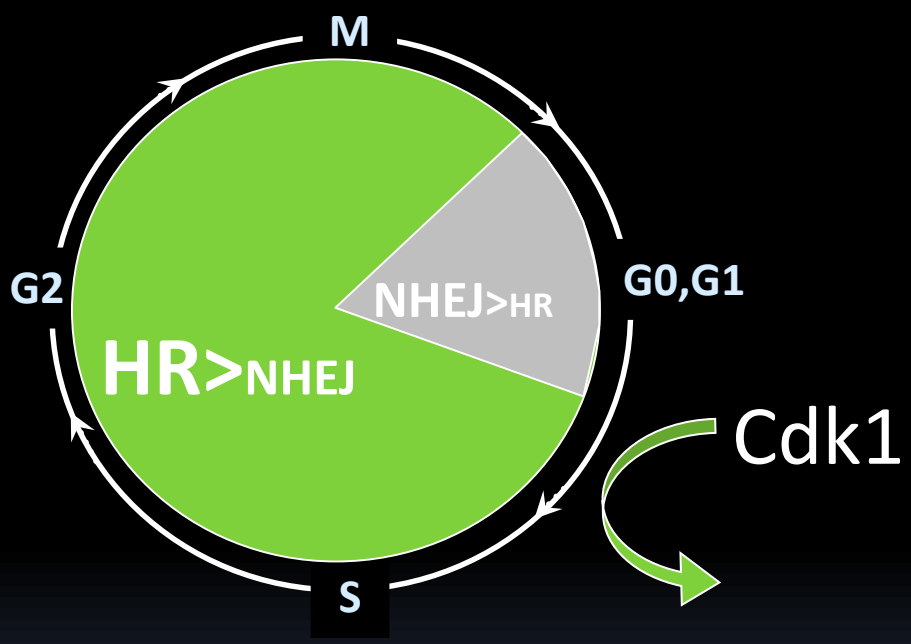
Adding of MRX complex stimulates resection

In mammlian cells

Nimonkar, A. V. *et al.* BLM-DNA2-RPA-MRN and EXO1-BLM-RPA-MRN constitute two DNA end resection machineries for human DNA break repair. *Genes Dev.* 25, 350–362 (2011).

DNA双链断裂修复的关键调控因素

细胞周期

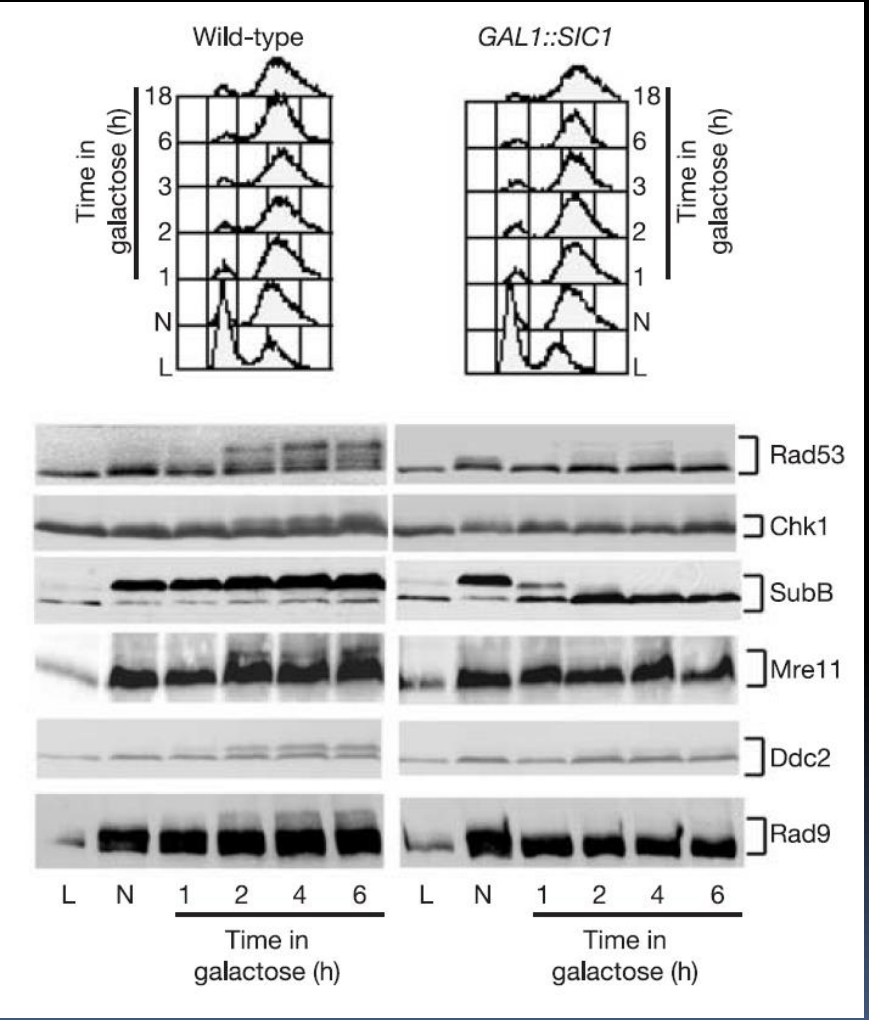


染色质结构状况

Nature (2004), 431:1011-7
EMBO J. (1998)17:5497-508
MCB (2003) 23:5706-5715

Cdk1 is required for checkpoint activation and HR

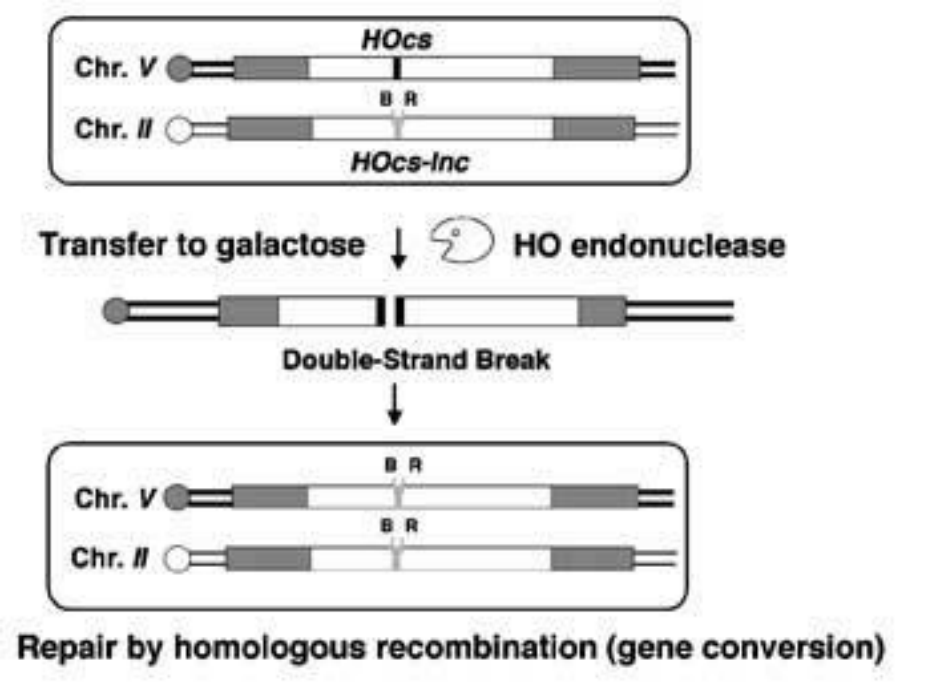
Checkpoint activation



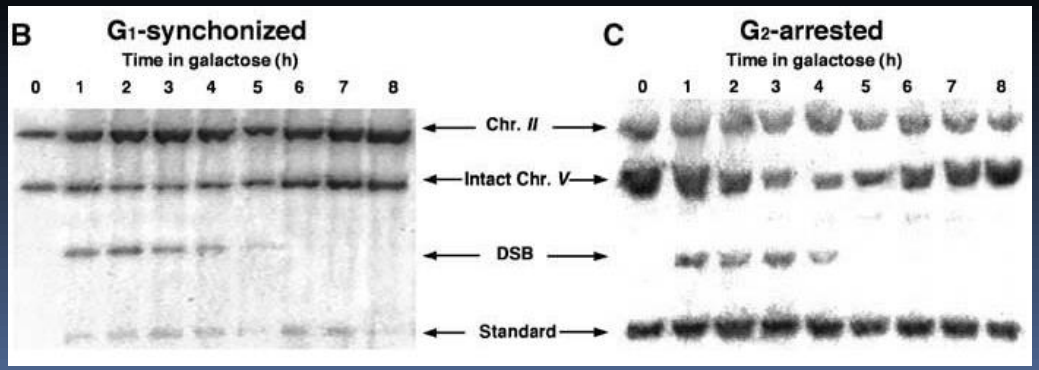
Cdk1 inhibitor

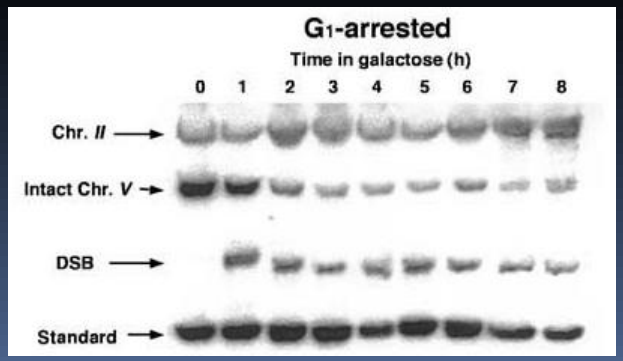
Ira et al, 2004, Nature; Aylon et al, 2004, EMBO J

Cdk1 is required for checkpoint activation and HR



Assay to measure HR





Ira et al, 2004, Nature; Aylon et al, 2004, EMBO J

Cell cycle-dependent (Cdk1) regulation of DSB end processing

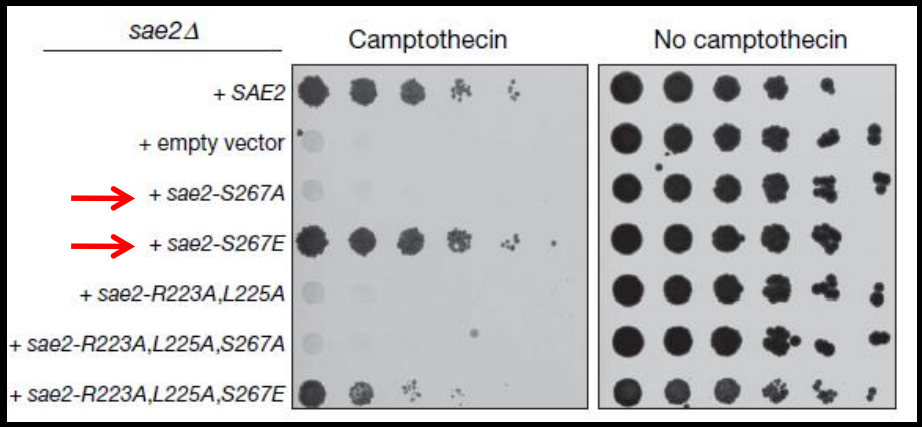
Vol 455|2 October 2008|doi:10.1038/nature07215 nature

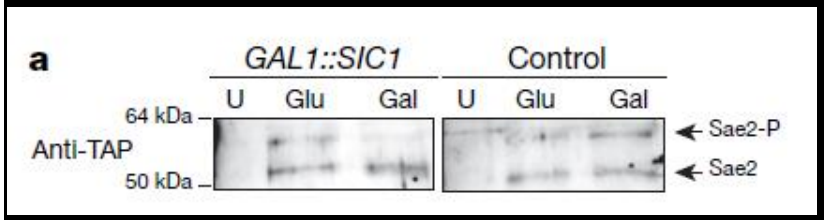
LETTERS

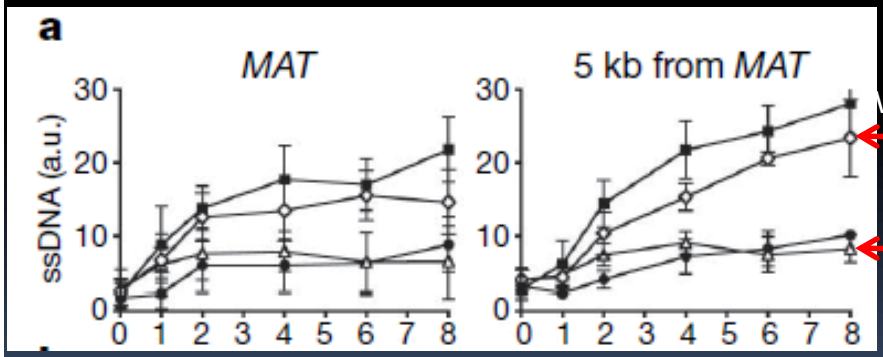
CDK targets Sae2 to control DNA-end resection and homologous recombination

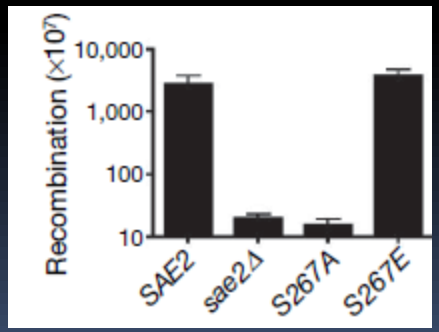
Pablo Huertas[1], Felipe Cortés-Ledesma[2], Alessandro A. Sartori[1]†, Andrés Aguilera[2] & Stephen P. Jackson[1]

Sae2 (yeast)



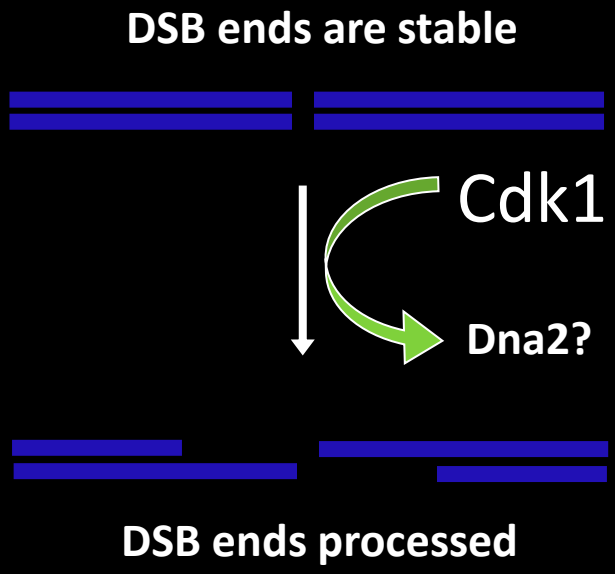




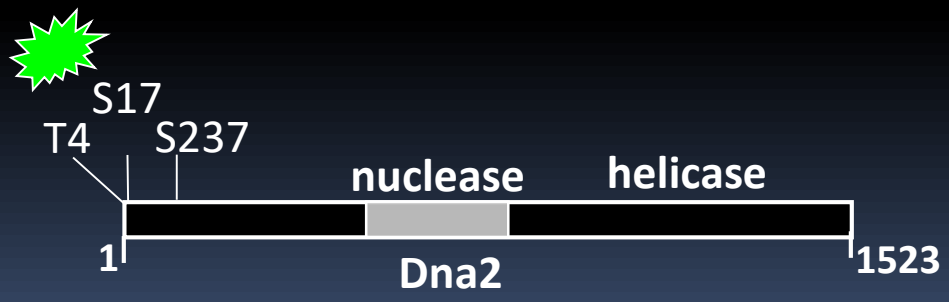


Phosphorylation of CtIP, the human otholog of Sae2, on T847 promotes resection and HR

Dna2 is an essential nuclease/helicase involved in Okazaki fragments processing and DSB resection



Dna2 (yeast)

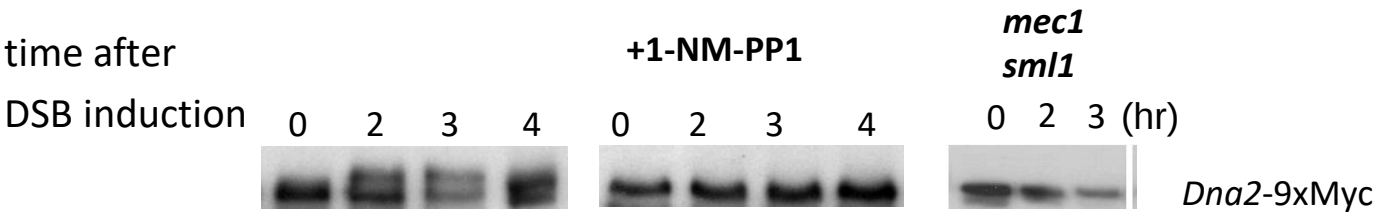


Dna2 contains 3 full Cdk1 phosphorylation consensus sites (S/T-P-X-K/R)

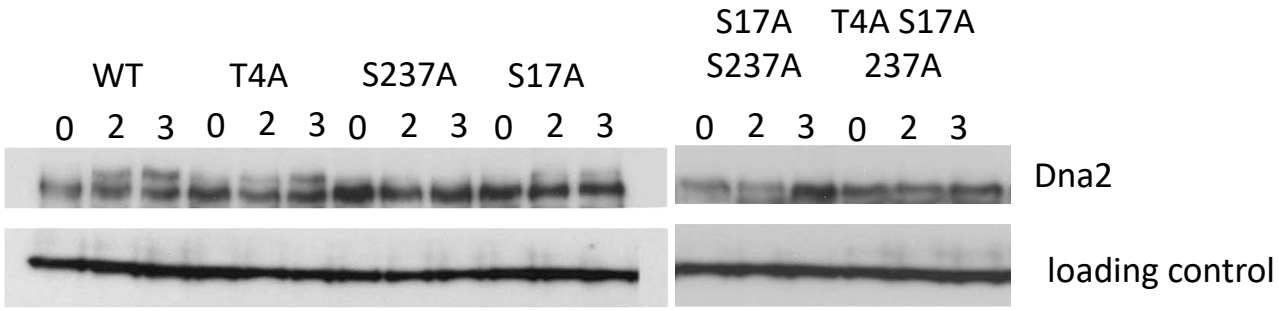
Nature 2003, 425: 859-64

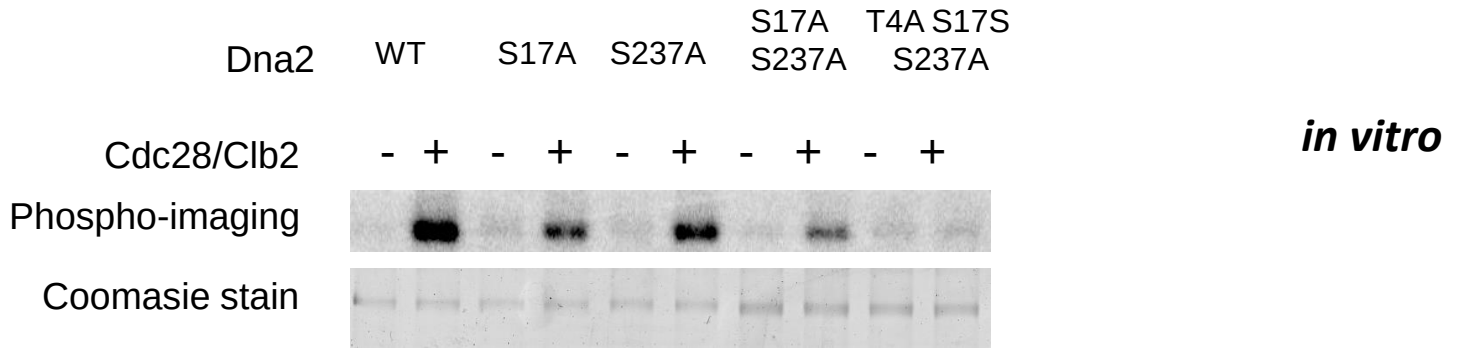
Dna2 is phosphorylated on Thr4, Ser17 and Ser237 by Cdk1 in response to DSB

1) Dna2 is phosphorylated by Cdk1 and Mec1 in response to DNA damage

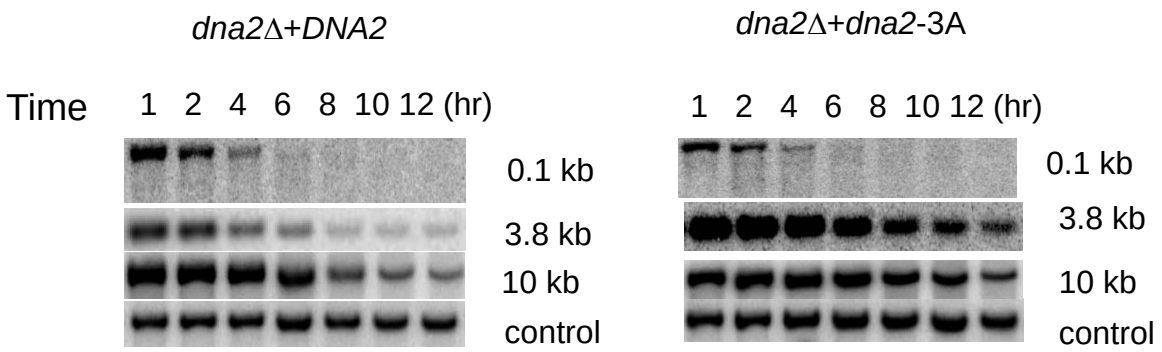


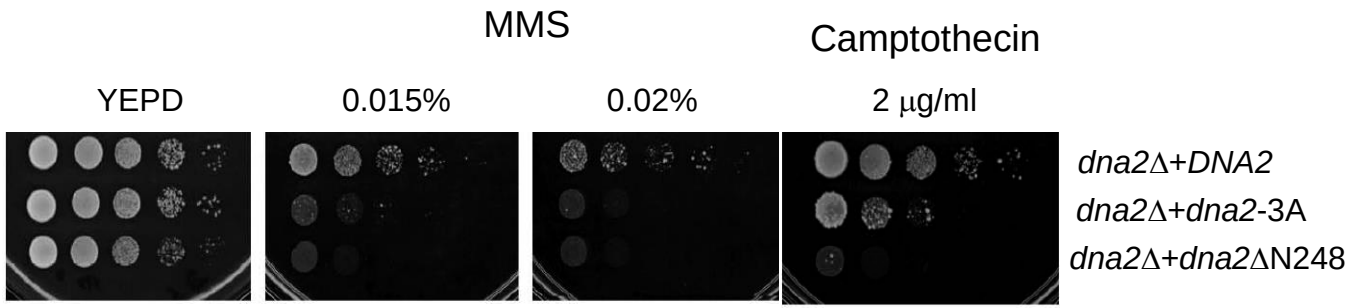
2) Phosphorylation of Dna2 on Thr4, Ser17 and Ser237 by Cdk1



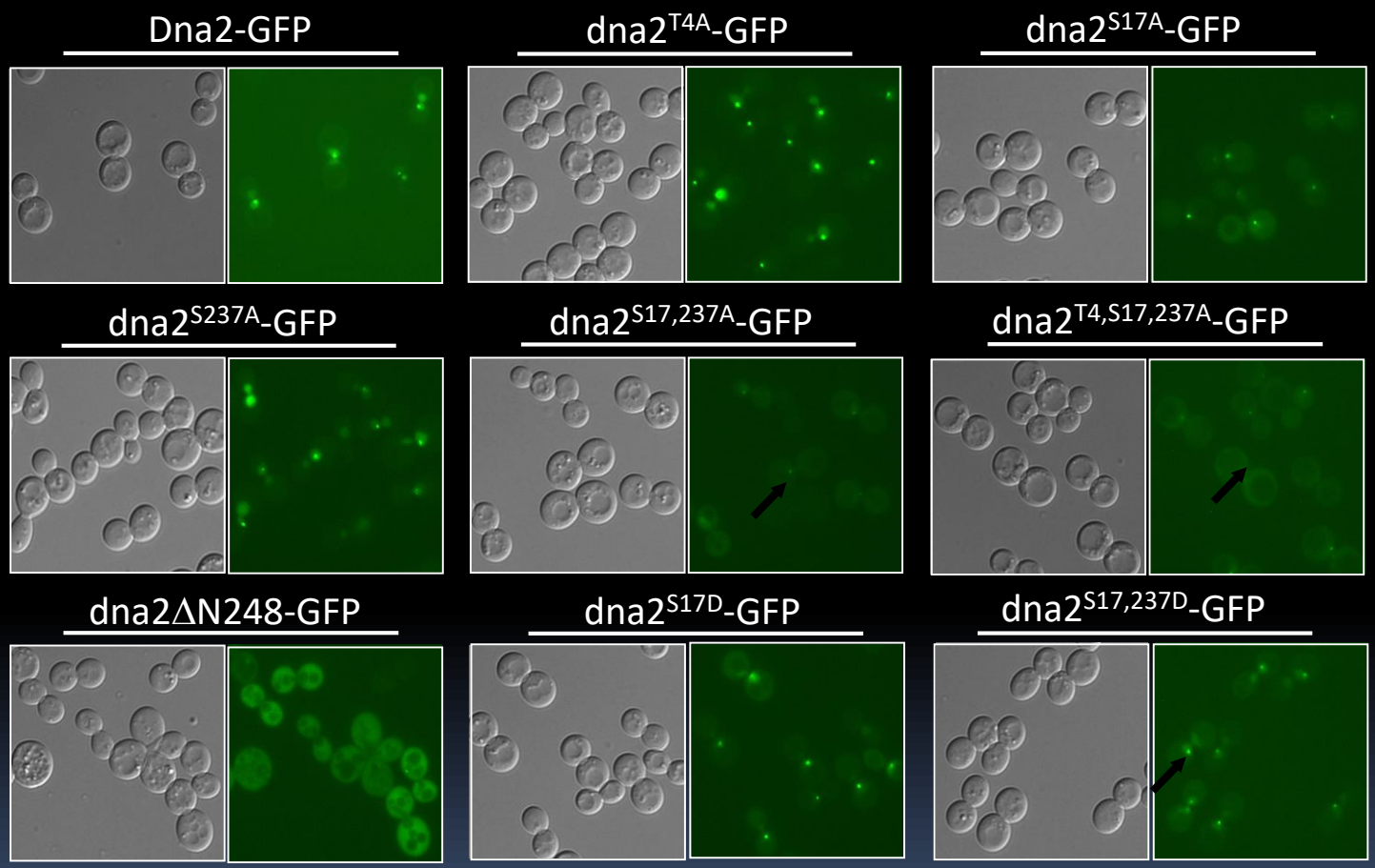


Dna2 phosphorylation by Cdk1 promotes resection and DNA damage response





Cdk1-dependent phosphorylation of Dna2 is required for its recruitment to DSB ends



Chen et al, Nature Structural & Molecular Biology, 2011

nature COMMUNICATIONS

ARTICLE

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Phosphorylation of EXO1 by CDKs 1 and 2 regulates DNA end resection and repair pathway choice

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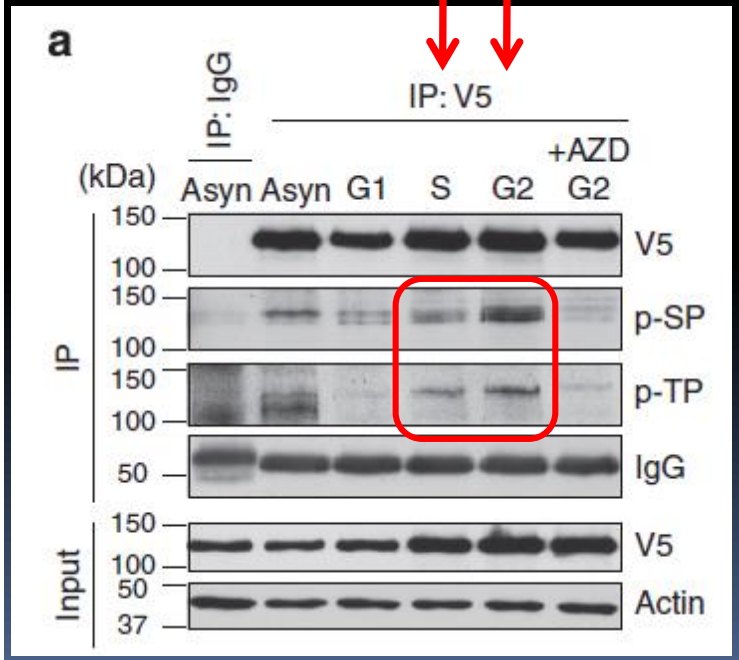
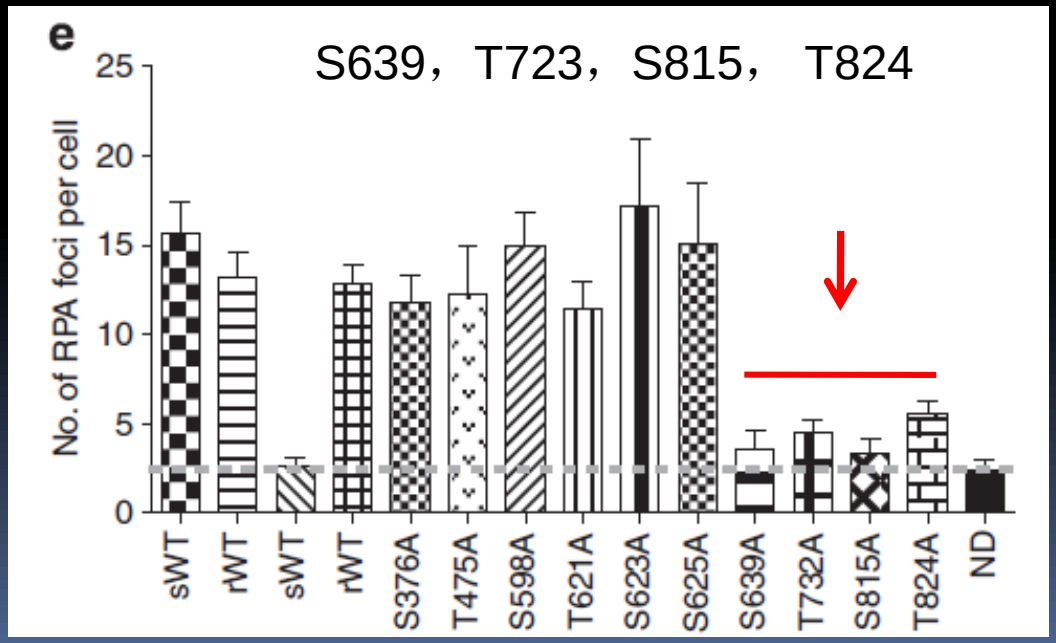
Exo1 (mammalian cells)

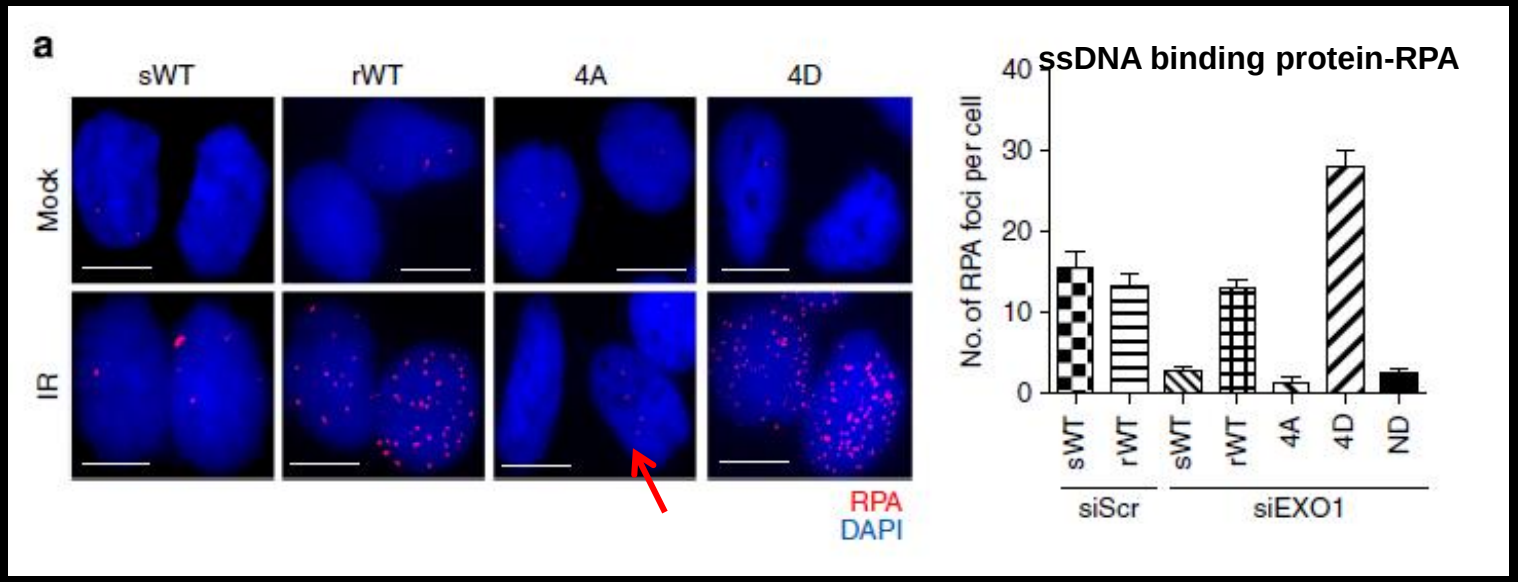
Exo1 phosphorylation in S and G2 phases

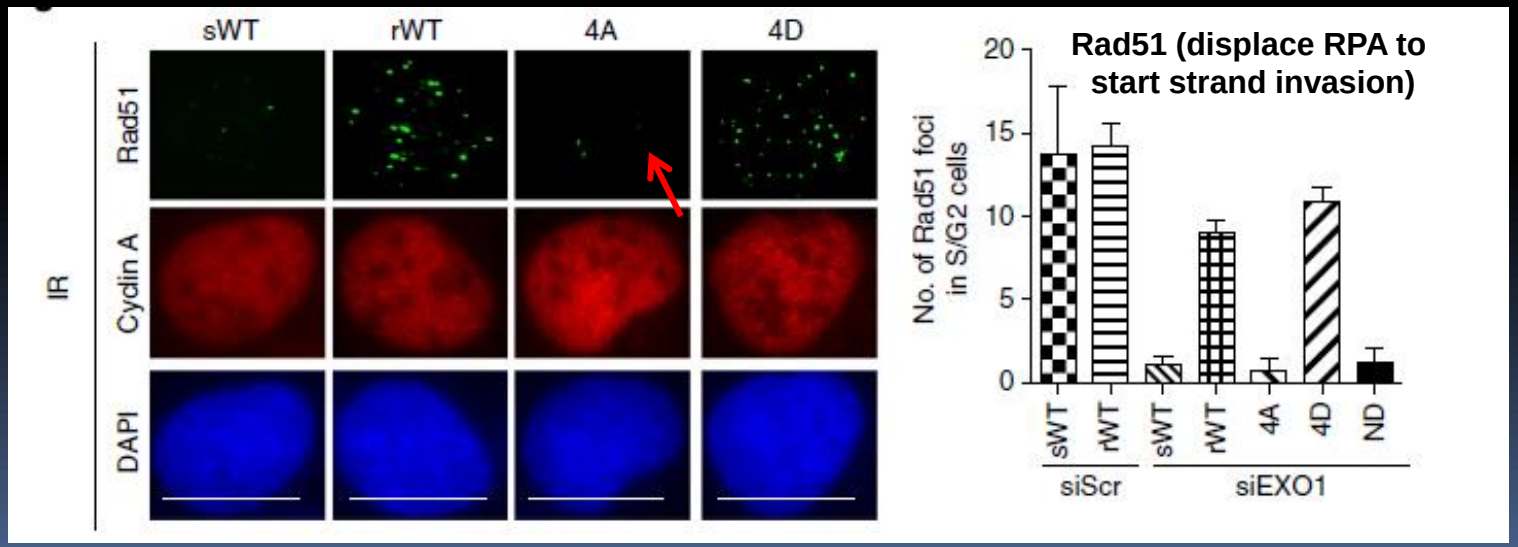


单链结合蛋白RPA结合减少

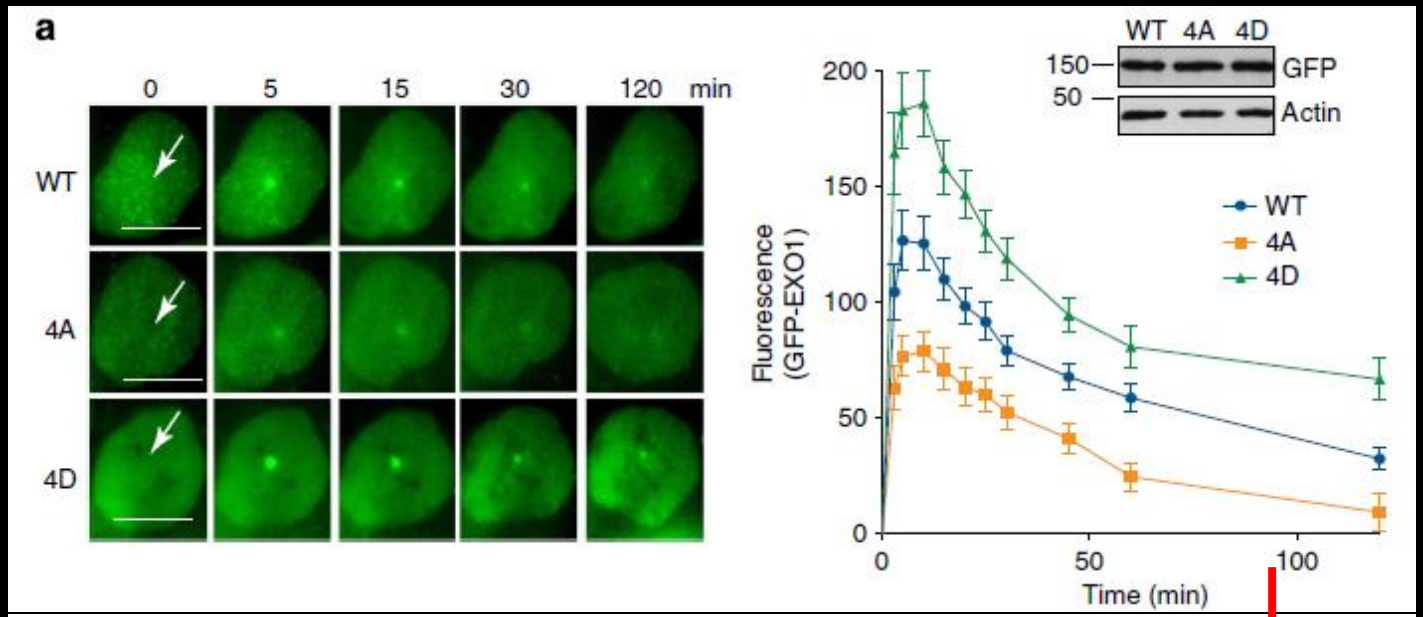


Phosphorylation of Exo1 by CDKs promotes end resection

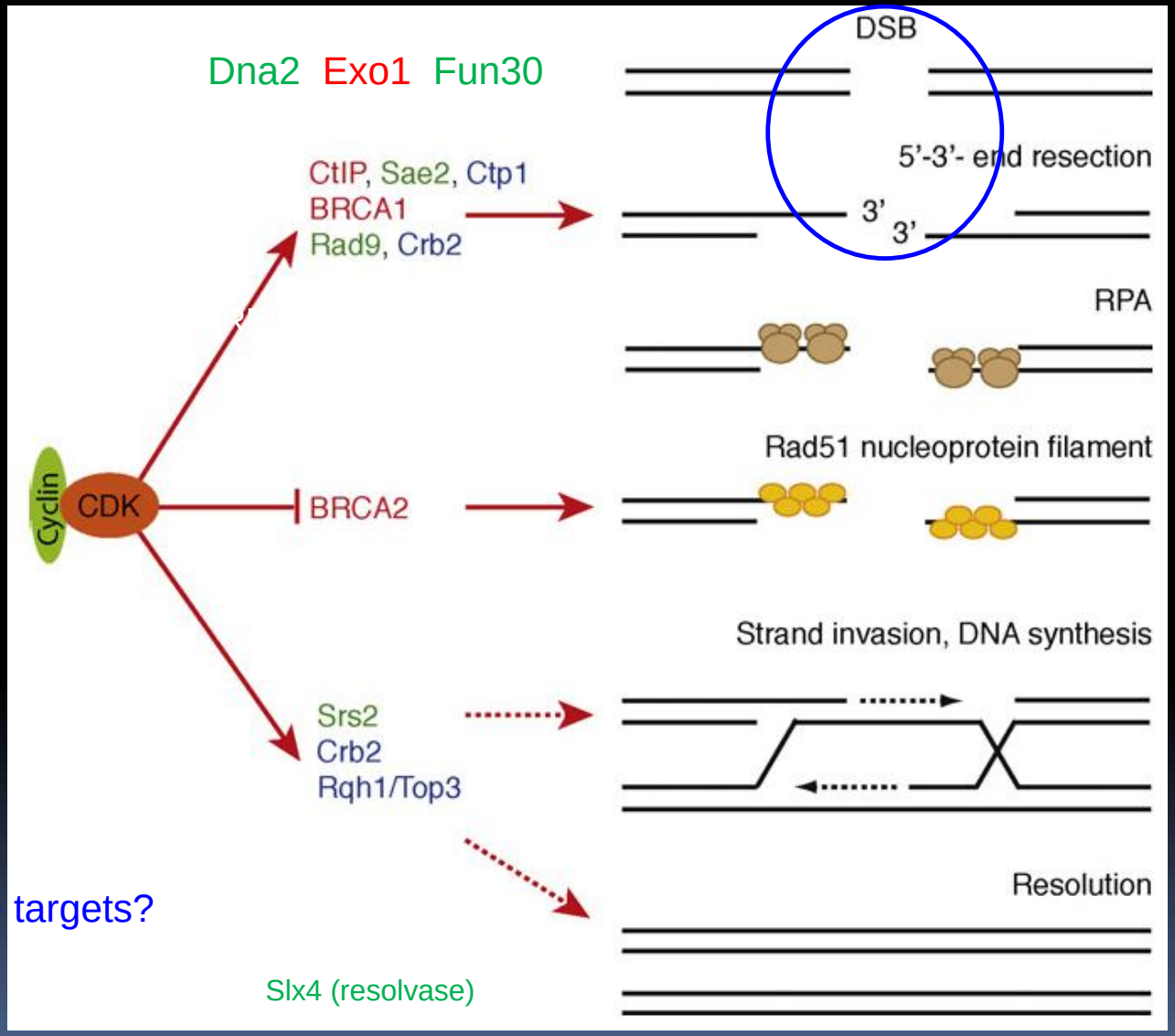




Exo1 phosphorylation promotes its recruitment to break sites and regulates repair pathway choice

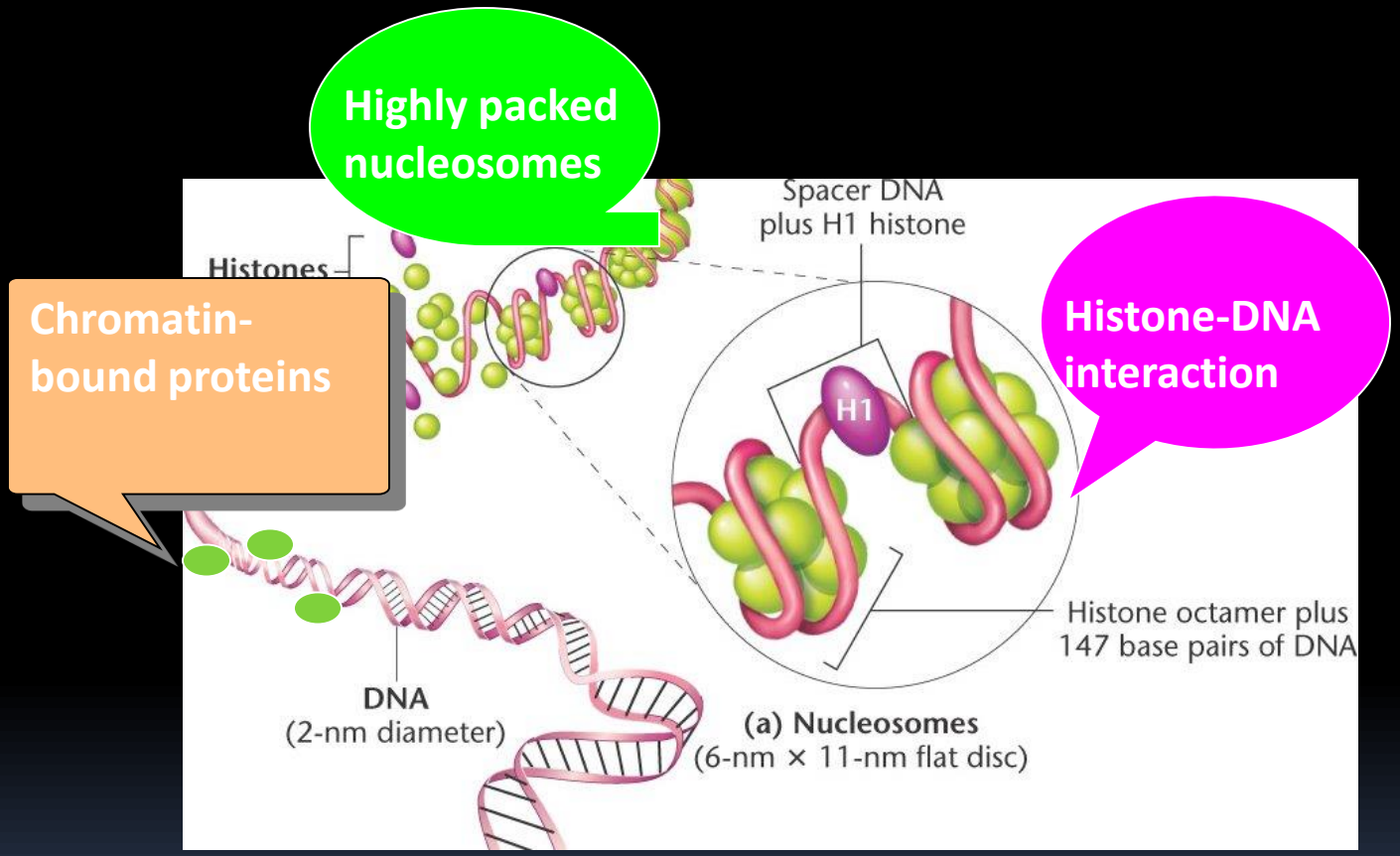


Cdk1 targets implicated in DSB repair



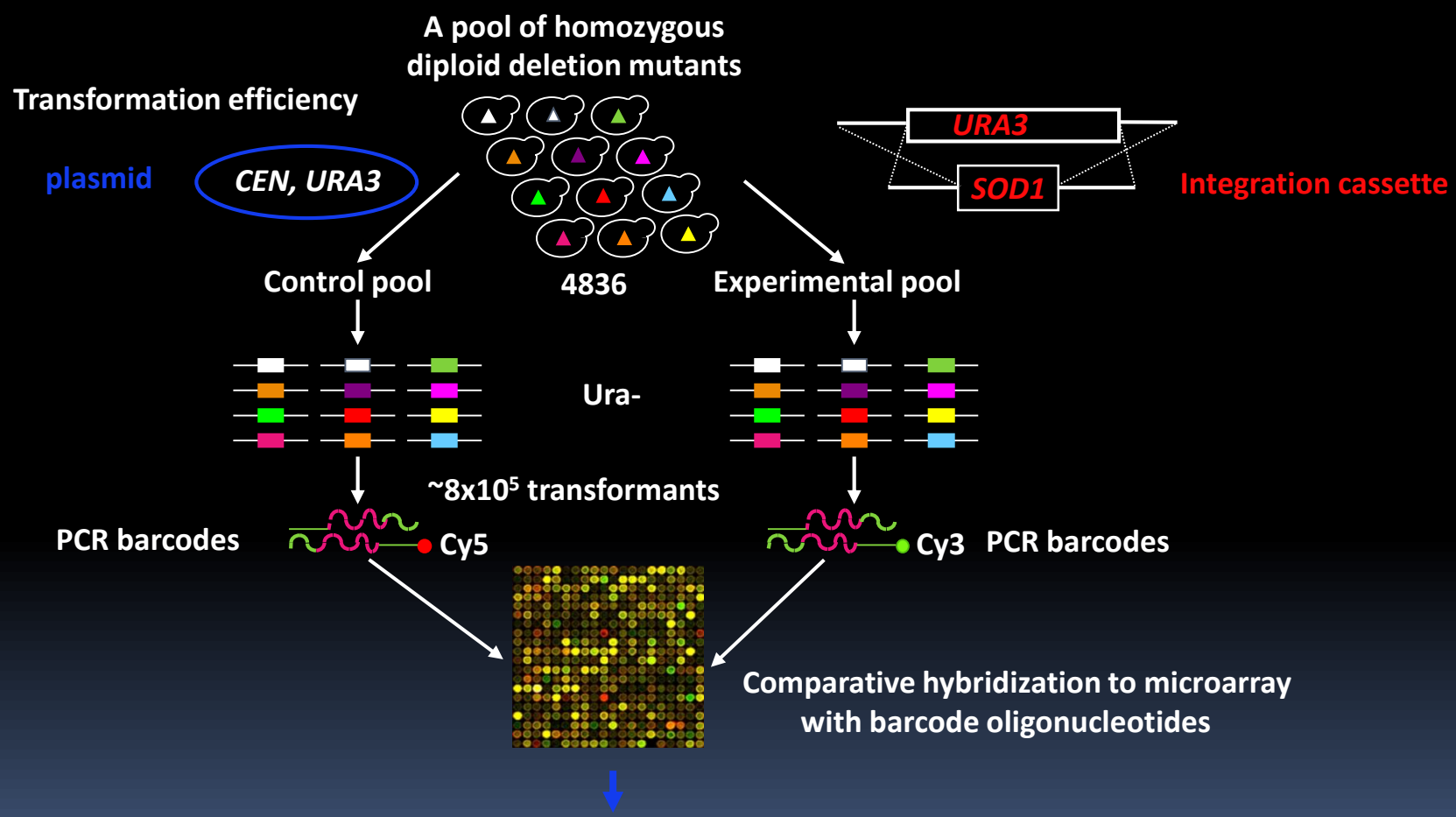
Modified from wohlbold et al, 2009 DNA repair

The roles of chromatin remodeling factor in DSB repair



We identified that the ATP-dependent remodeling factor Fun30 plays a unique role in promoting resection and HR on chromatin context.

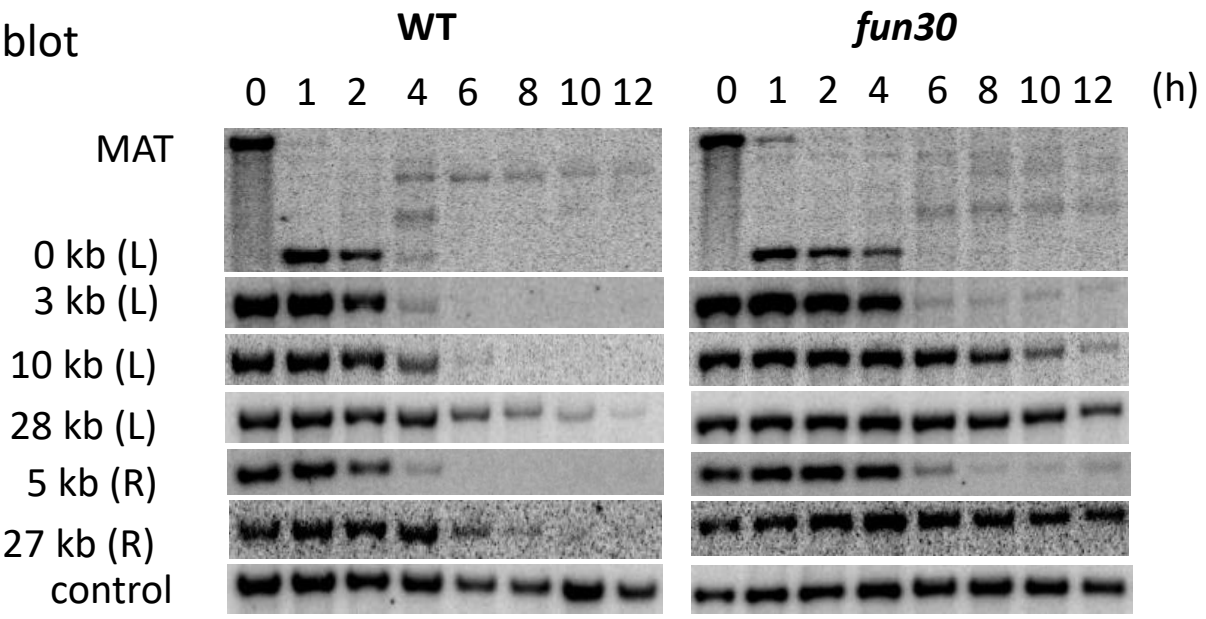
A genome-wide screen for mutants with altered HR frequency



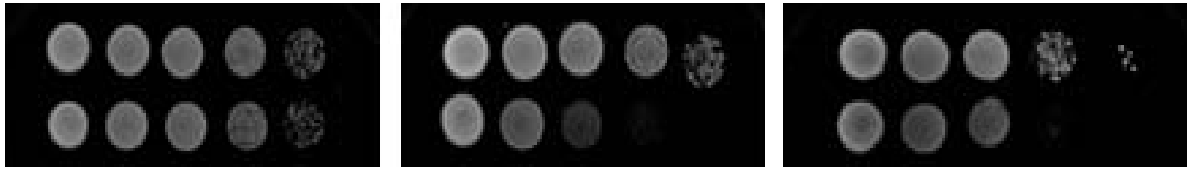
Fun30 was identified as a regulator of homologous recombination

The chromatin remodeling factor Fun30 promotes resection and DSB repair

Southern blot

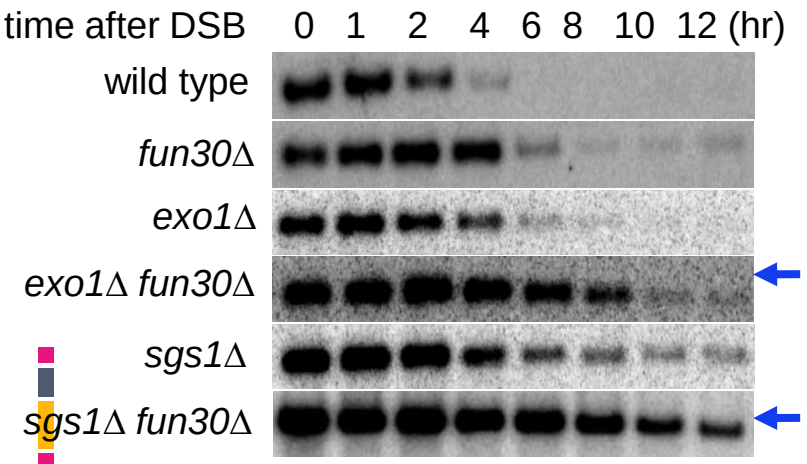


DNA damage sensitivity



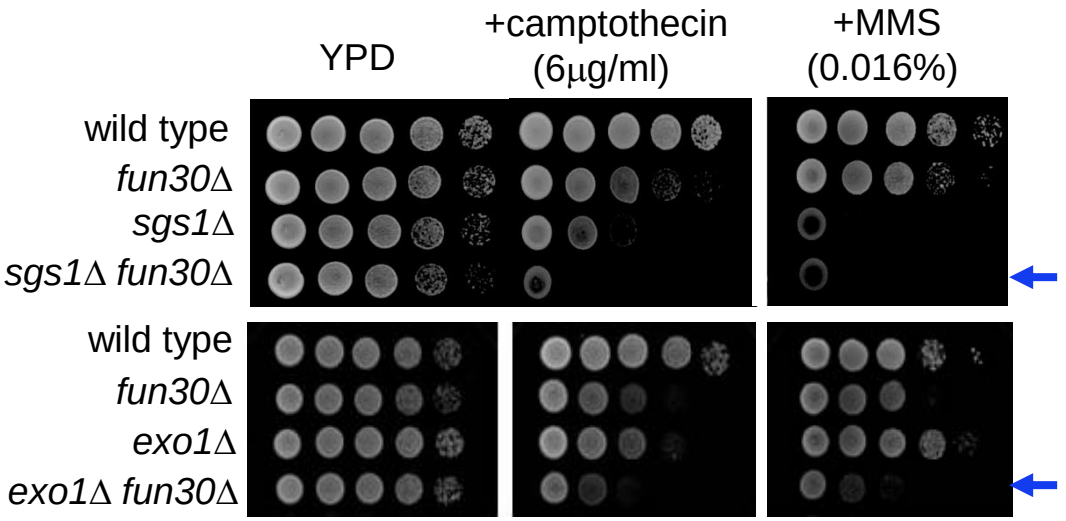
Fun30 promotes both Sgs1- and Exo1-mediated resections

resection at 5 kb

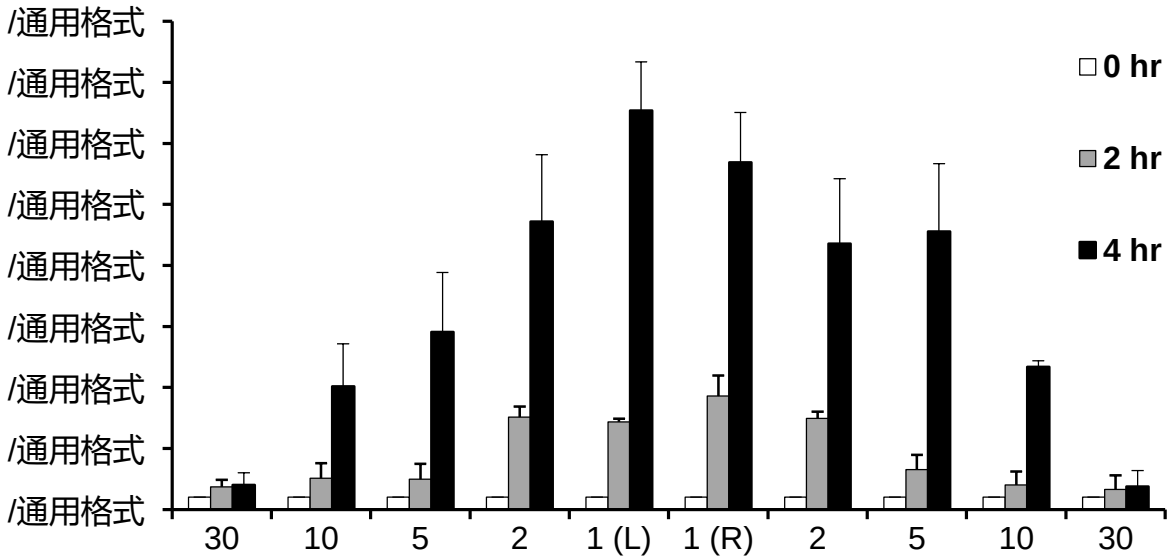


Southern blot

response to DNA damage

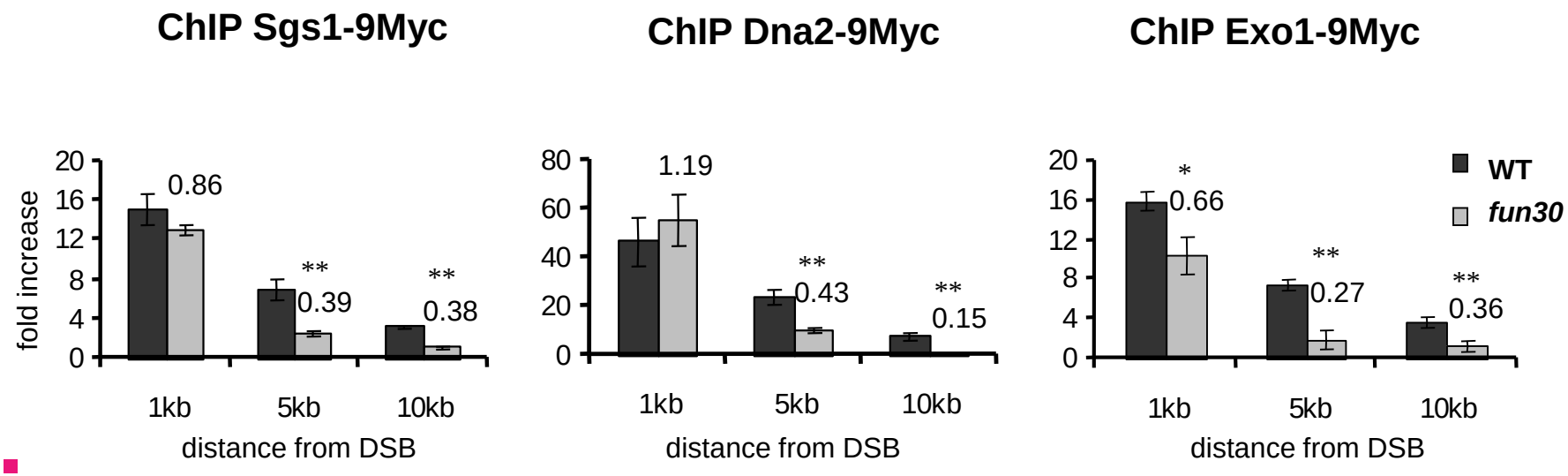


How does Fun30 promote resection?



Fun30 is robustly recruited to DSBs and spread along chromatin.

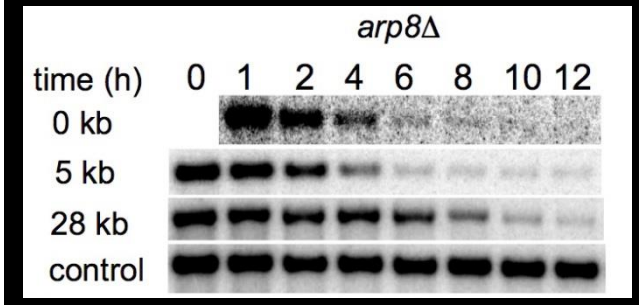
Fun30 facilitates the progression of resection enzymes on chromatin

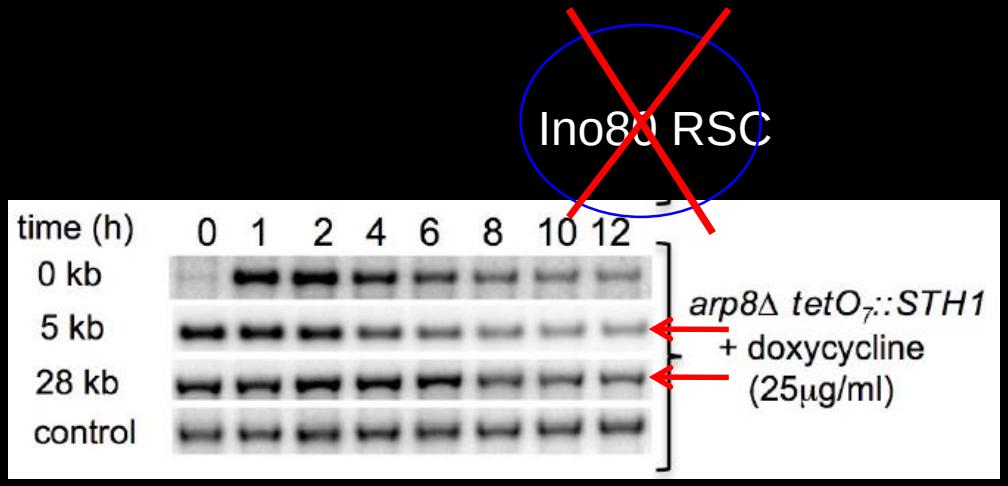


* P < 0.05 ** P < 0.01, t-test

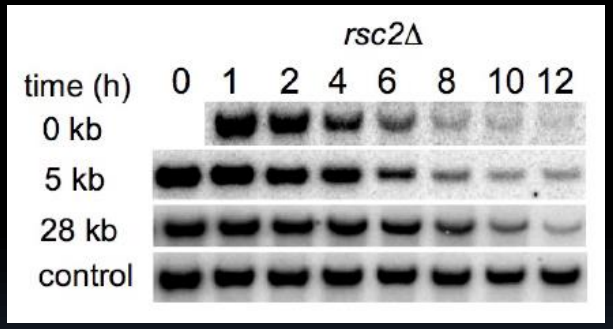
Ino80, RSC and Fun30 cooperate to stimulate resection and checkpoint

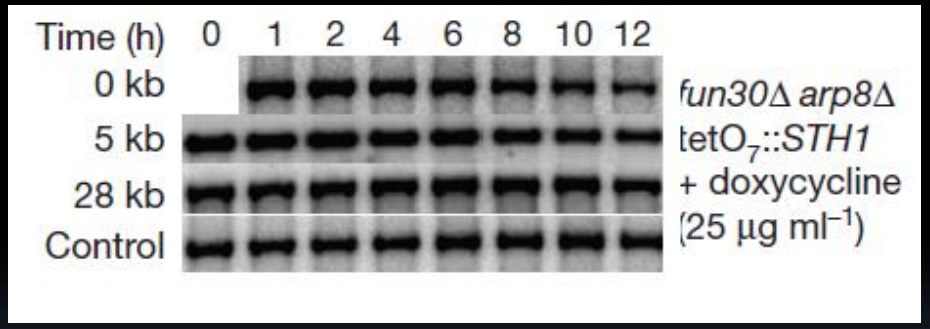
Ino80 complex



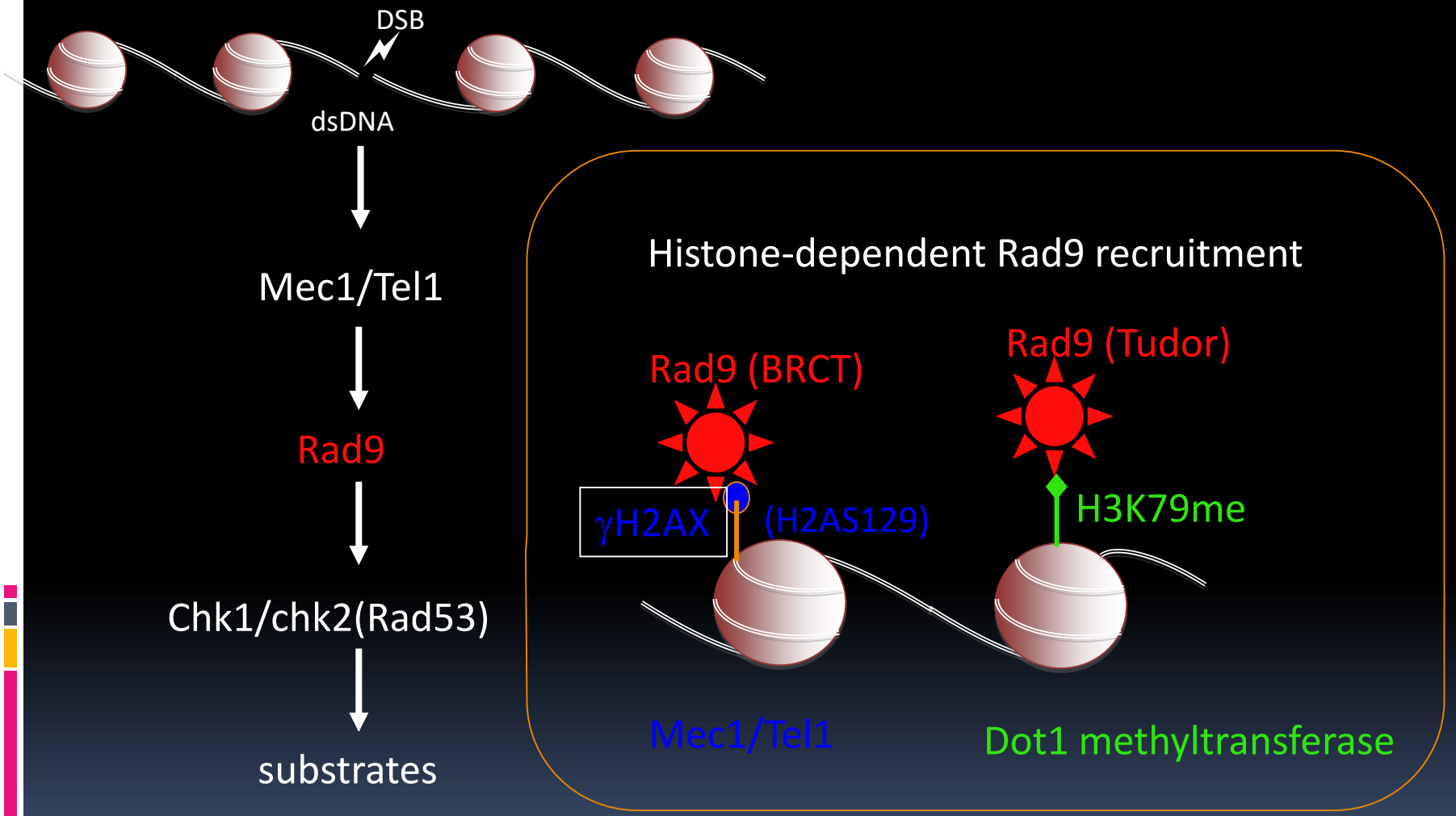


RSC complex

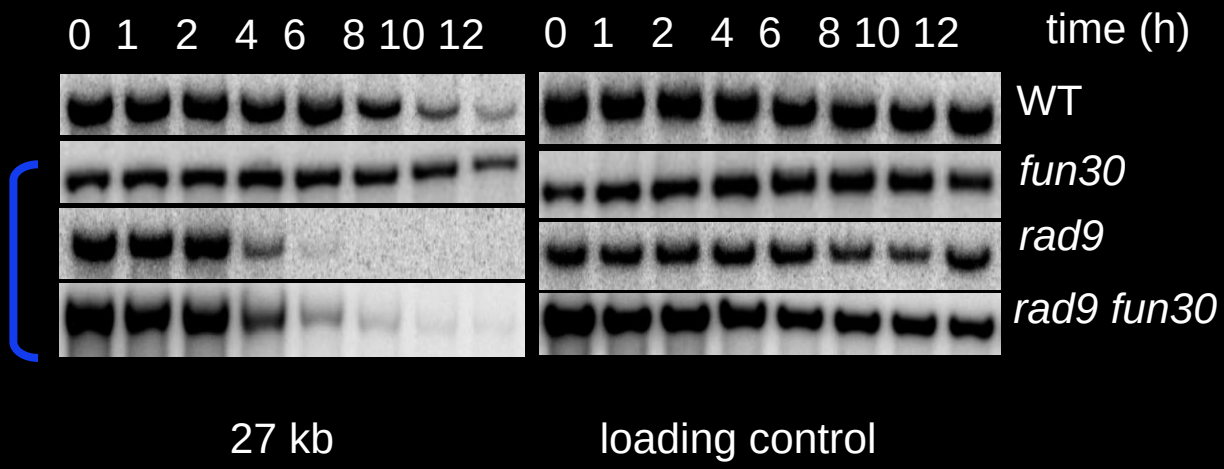




DNA damage-induced Rad9 recruitment to chromatin

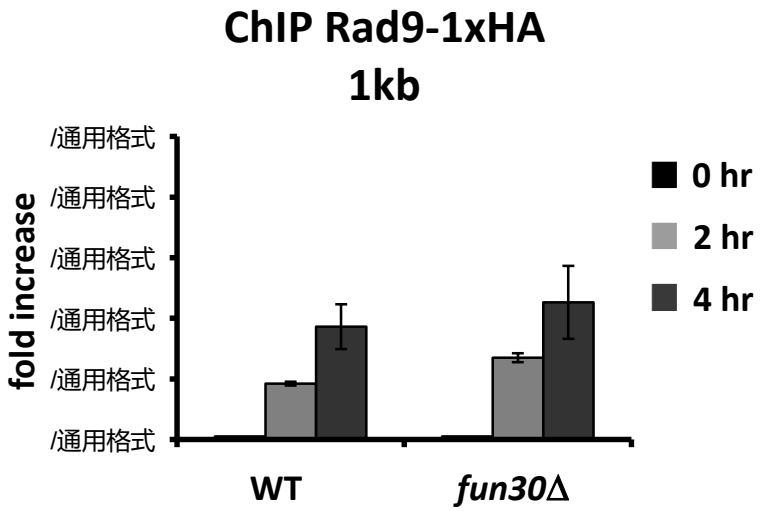


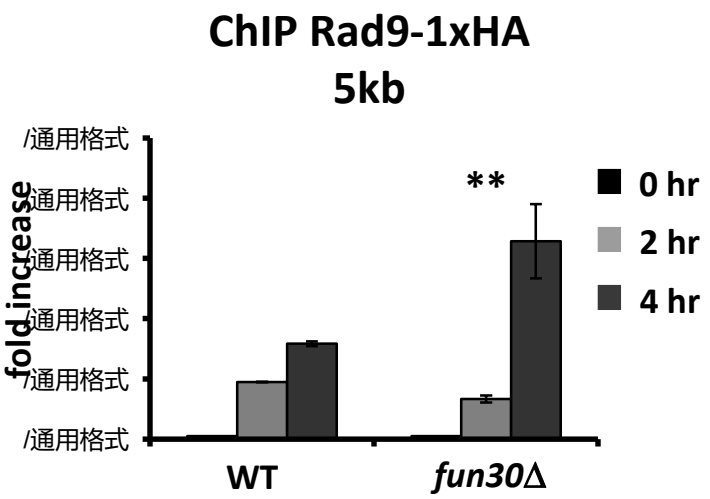
Fun30 becomes less important for resection in the absence of histone-bound Rad9



Similarly, Fun30 becomes less important in the absence of the histone modifications γH2A or H3K79me which mediate the recruitment of Rad9 to chromatin.

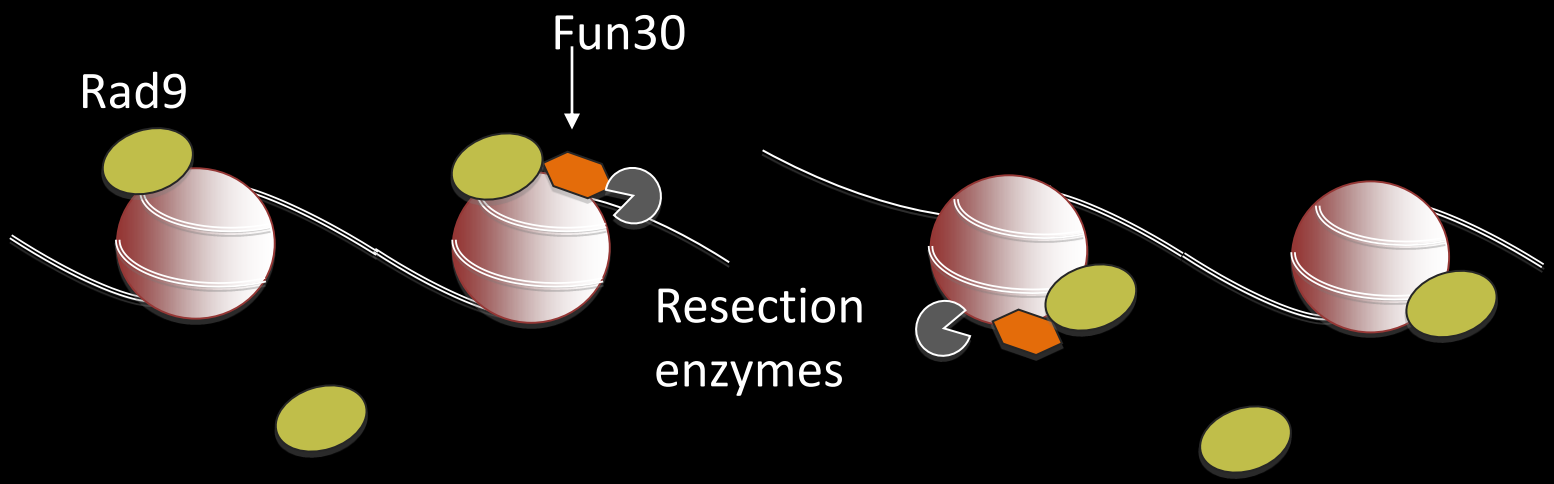
Fun30 likely promotes resection by removing the histone-bound Rad9 barriers





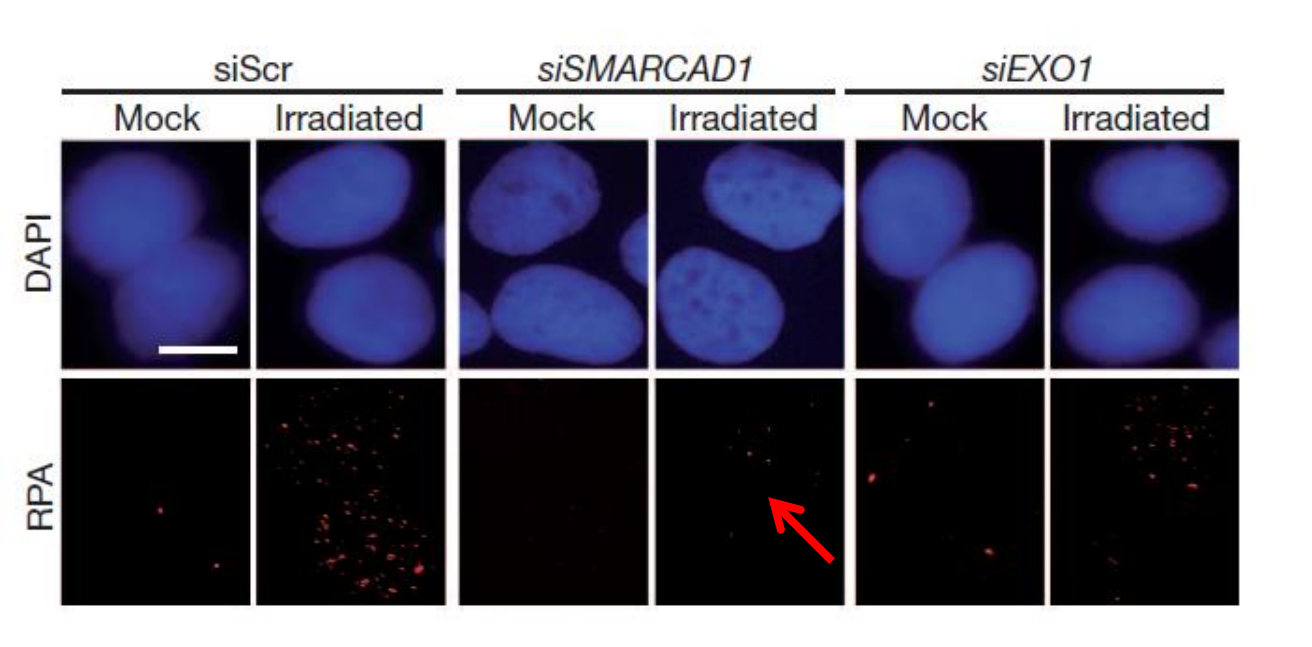
In the absence of Fun30, there is more Rad9 associated with chromatin.

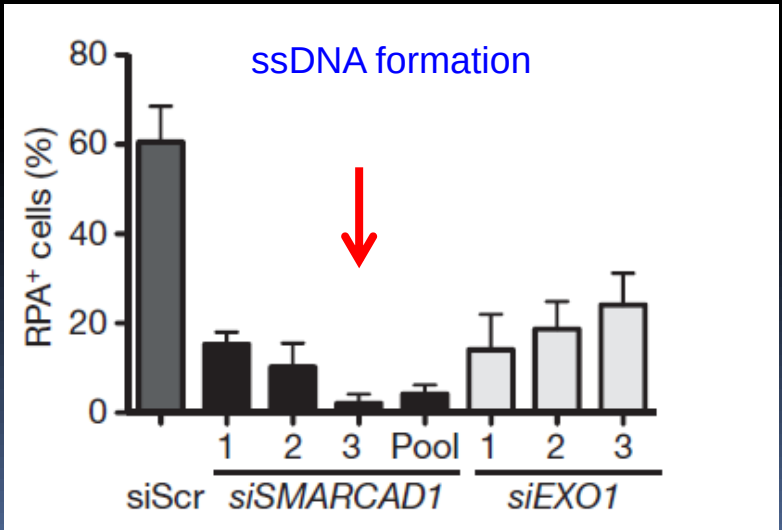
A Model for Fun30's role in resection

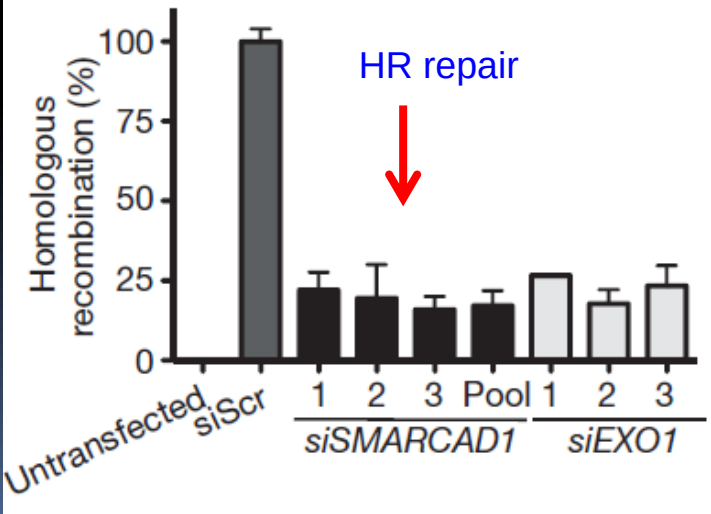


Chen et al, Nature, September 2012

Human SMARCAD1 promotes resection and repair by HR



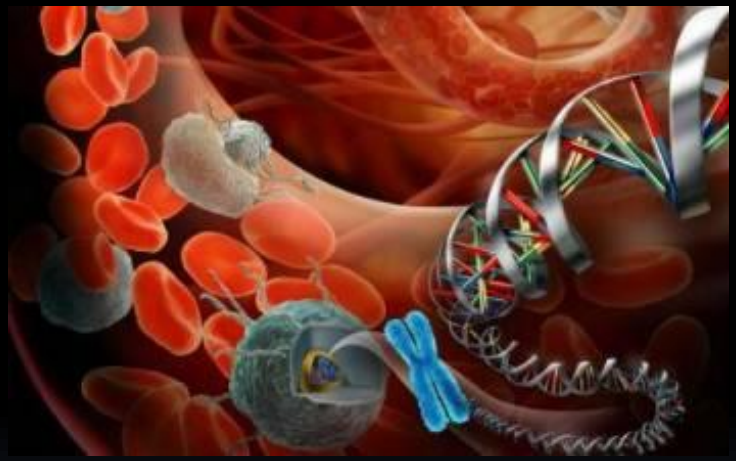




Costello et al, 2012, Nature

DNA修复的应用—分子诊断及疾病预测

分子诊断(Molecular diagnostics）



Circulating Tumor Cell

发掘易感基因—癌症诊断

疾病预测

发掘新的分子标记

确定病症所处时期

帮助选择何种化疗药物

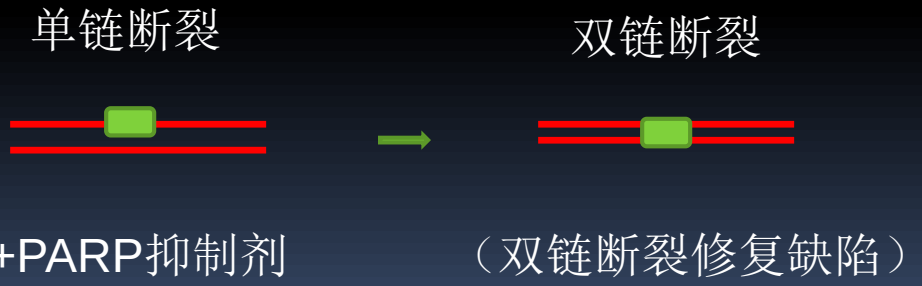
检测化疗效果如何

DNA修复的应用--癌症治疗药物开发

利用癌细胞已经受损的DNA修复系统，加速癌细胞死亡（凋亡）。

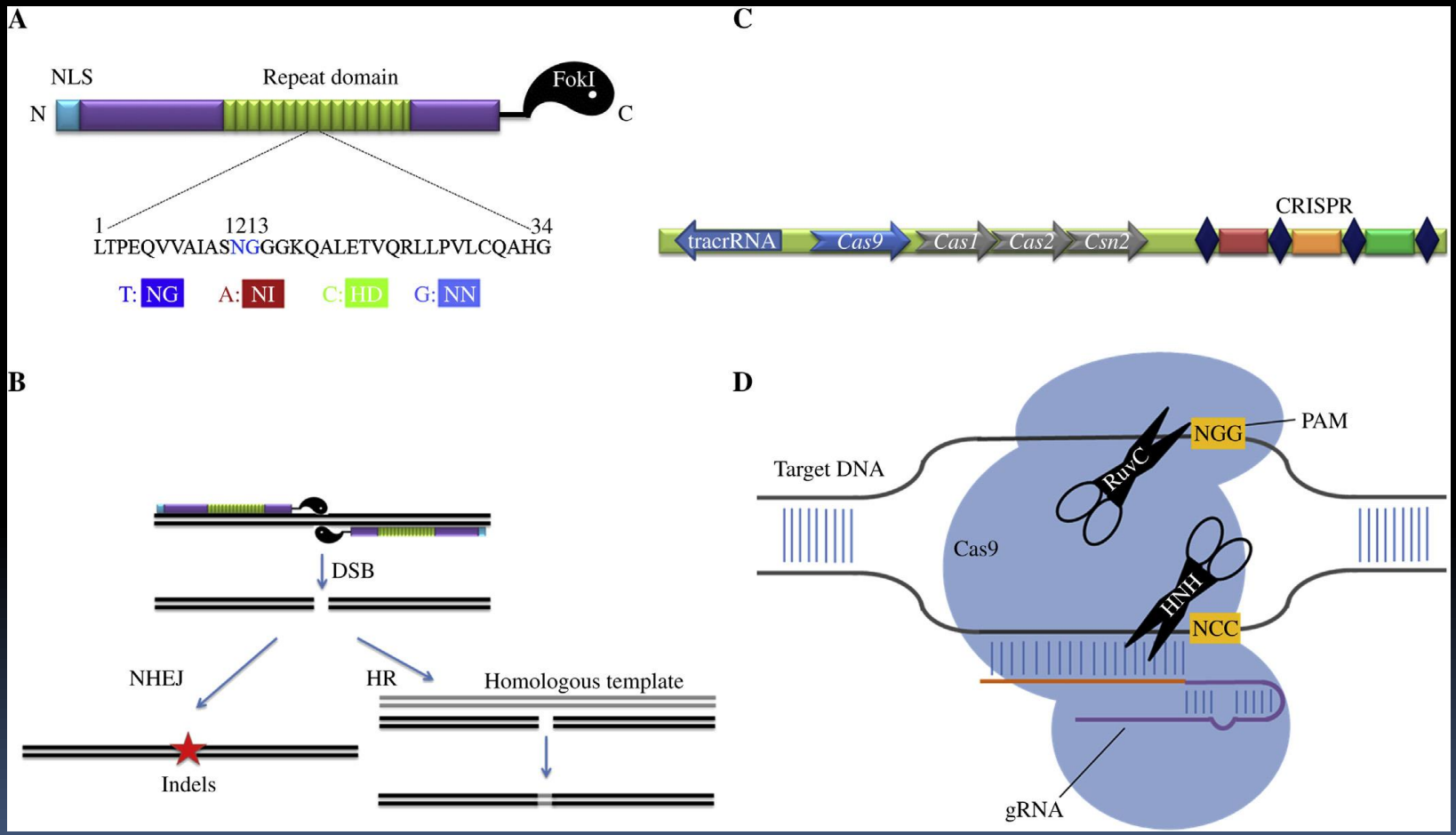
聚腺苷酸二磷酸核糖转移酶（PARP）抑制剂，这是利用DNA修复原理研制的一种新癌症治疗药物。

PARP参与碱基切除修复对DNA单链缺口的修复，抑制其活性能够增强放疗和DNA损伤类化疗药物的效果。



DNA修复的应用—基因组编辑

基因组编辑技术(基于DNA双链断裂)　　TALEN, CRISPR cas9



结语

DNA修复研究为人类开发新的基因组编辑技术、开发新药物及潜在的遗传疾病基因治疗提供了广阔前景

解决科学理论与实践问题：

揭示细胞不同DNA修复途径的分子机理与调控机制；

揭示高等生物维持基因组稳定的奥秘；

阐明DNA修复相关疾病的发病机制，为药物开发和临床诊疗提供指导；