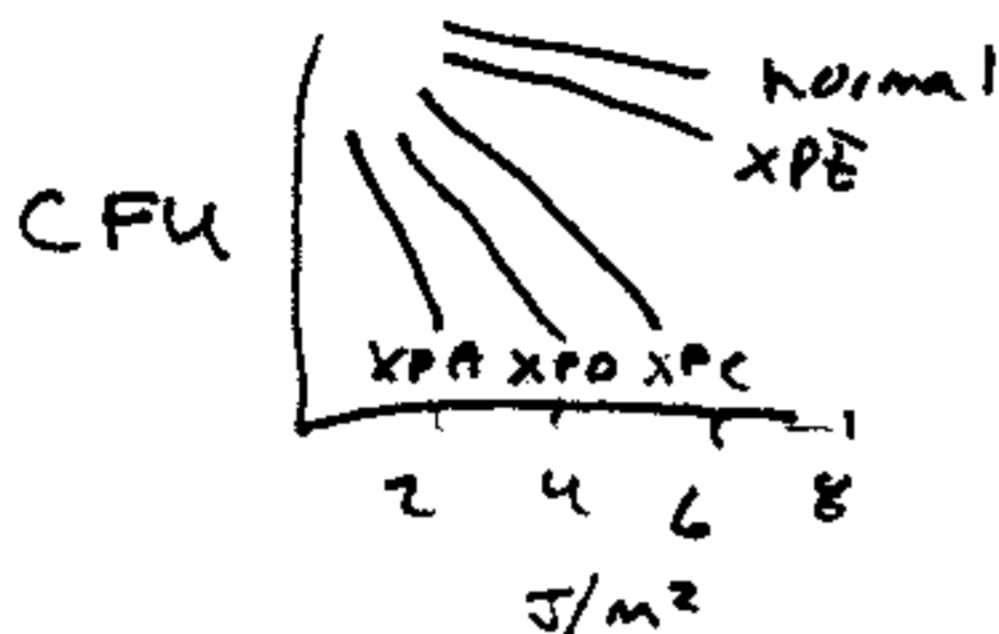


Rick Wood: NER in Mammalian Cells


2.25.99

XP - recognized as Genetic in 1960s.

1968 - Cleaver-deficient in NER



Repair Synthesis

cells → extracts →  (damaged + undamaged)
quantify p32 incorporation

XPA

- purified factor that complemented XPA mutations

XPA	binds damaged DNA	RAD14	
XPB	helicase (repair + tx)	SSB	<u>TFIIH</u>
XPC	SSDNA binding	Rad4	XPB
XPD	helicase (repair + tx)	Rad3	XPD
XPG	endonuclease	Rad2	p62 ✓
ERCC1	} Endonuclease	Rad10	p44 ✓
XPF		Rad1	p52 ✓
			p40
			p34
			p32

Cisplatin - Monoadduct

But need two additional proteins *in-vitro* for excision

IF7 :

RPA = SSB (3 subunits)

For synthesis

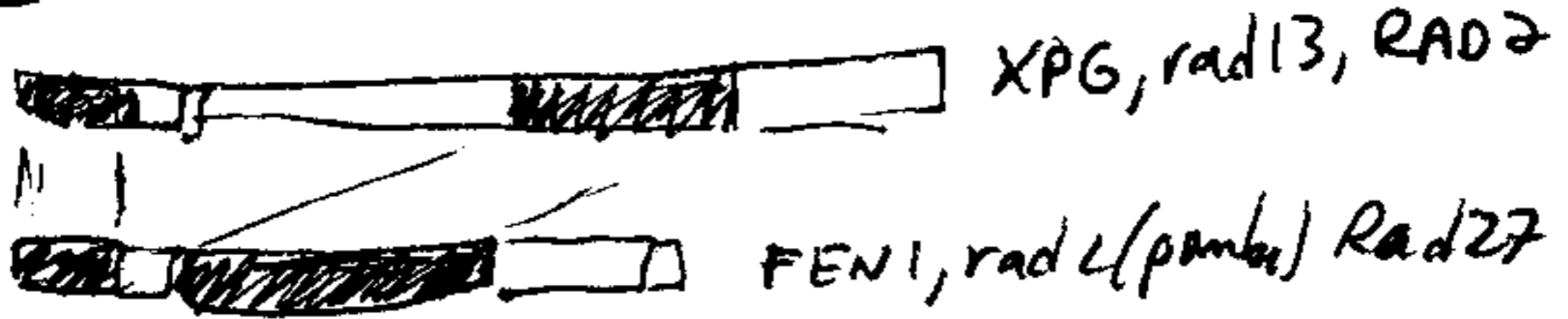
Pol E -

PCNA - clamp

RFC - ATP dependent loader

DNA-ligase -

XPG



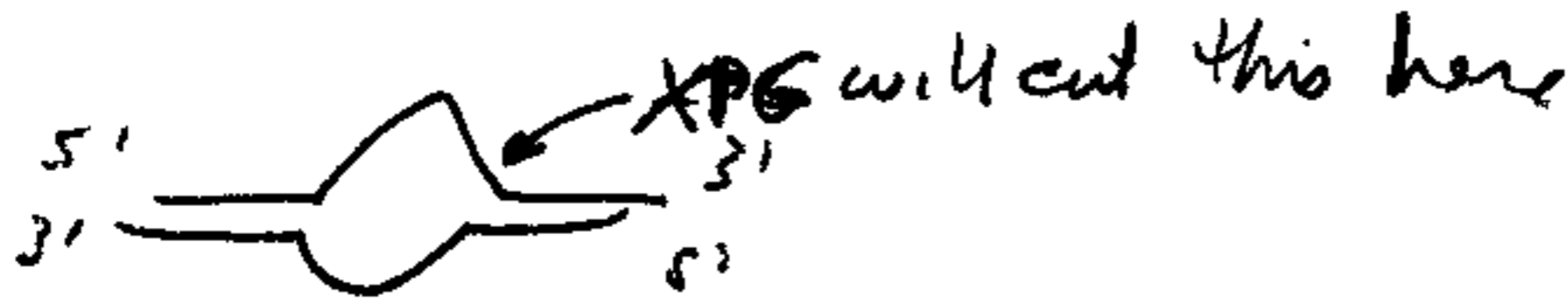
→ remove RNA primers

related to pol I 5' → 3' exo

- TH RNase H

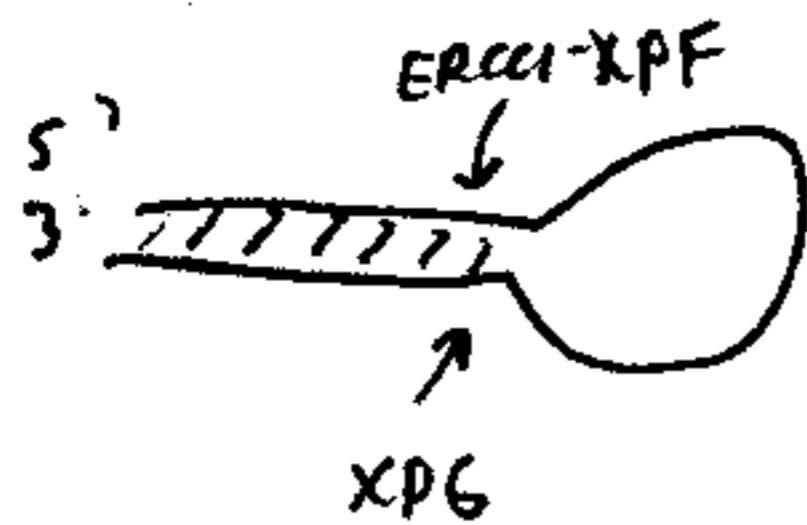
- TS exonuclease

} XPC hHR23E



Rad10, Rad1

<u>E. coli</u>	Rad10	Rad1
<u>S. pombe</u>	Swi10	rad16
<u>Drosophila</u>	.	mei-9
<u>Human</u>	ERCC1	XPF/ERCC4



Stem Loop in DNA

- XPA, XPG extracts defective in both cutting
 - XPF only defective in 5' incision
- So what opens up bubble?

TFIIH?

