

DNA Repair in Eucaryotes G1/C4

Photoreactivation

Nucleotide Excision Repair

Yeast

C80

Humans

Preferential Repair

ER in vitro

DNA per person = 2×10^{11} meters = 0.02 light yrs

Photoreactivation

Cyano	A. nid	✓	Yes
Bact.	E. coli S. gris		Yes
	H. inf B. subt M. luteus		No
Fungi	-		+/-
Plants			Yes
Protozoa			Yes
Molluscs			Yes
Echinods			Yes
Arthropods			Yes
Fish			Yes
Amphibians			Yes
Mammals	Placental		No
	Marsupials		Yes

Yeast ER

- Rad 3 ATPase, 5' → 3' helicase
- Rad 14 homology to XPAC protein
- RAD 8 thymid. Kinase
- CD19 DNA ligase
- Phr photoreactivation

Human genes... use CHO cell complementation

1) mutagenize repair-competent CHO cells

2) screen cells for decr. UV resistance

3) transfect these w/ human DNA

4) select for UV resistance

5) isolate ~~single~~ human clone

many compl. groups

1) fuse cells

2) screen for

~~UV sensitivity~~

UDS in

non DNA replication

conditions

Group	UV sensitiv.	Complem. human gene	Yeast homolo
1	++	ERCC1	RAD10
2	++	" 2	RAD3
3	++	" 3	SSL2
4	++	" 4	
5	+	" 5	RAD2
6	+	" 6	

Human Diseases w/ Repair Defects

	<u>cellular sensitivity,</u>	<u>cancer?</u>	<u>compl grps</u>	<u>clinical</u>
xp	uv, chemical mut	skin, internal	7	skin, eye sensit
ataxia	x-rays	lymphomas	4	neuro. abnorm. <u>immunodef, ataxia</u>
fancoria	cross-linking	leukemia	3	
Bloms	?	lymp, leuk		
Cockayne	uv	none	3	prem. aging

xp

v. rare

defect in ER of damaged DNA

	uv sensit	cloned?	clinical	protein
A	++		neurolog.	DNA binding
B	++		neurolog; Cockayne	helicase
C	+			
D	++		neurolog.; some CS	
E	+			recogn. of damage
F	+			
G	++		neur, some CS	

XPA

gene ... single zinc finger (DNA binding)
protein ... binds DNA (R. Wood)

XPB

gene ... seven motifs like helicase
patient ... pt mutation in one allele
other not expressed

XPE

- protein studied by identifying protein that binds
damaged DNA - deficient in XPE

- * - over expression in tumor cells
- flx ... recognize damaged DNA

Preferential Repair - in Yst, E. coli, Humans

- ⊙ transcribed region
- ⊙ transcribed strand

<u>Disease</u>	<u>Bulk Repair</u>	<u>Prof. Repair</u>
XPA	-	-
XPC	-	+
Cock.	+	-

Repair in Vitro

UV irradiated plasmid $\xrightarrow[\text{Pyr. glycols}]{\text{remove}}$ plasmids w/ other lesions \rightarrow gap filling
(w/ E. coli Nth prot. & gradient centrifugation)

- "background" may be repair of undamaged DNA

- ⊙ repair is low in XPA, XPC
- ⊙ can get complementation (mix XPA & XPC)
- ⊙ then can check for XPA-compl. protein in various extracts