Mechanisms of damage recognition and incision in human nucleotide excision repair

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NUCLEOTIDE EXCISION REPAIR (NER)



XERODERMA PIGMENTOSUM

- Inherited disorder with genetic and clinical heterogeneity
- Incidence 1:10⁵ 1:10⁶
- Extreme sensitivity to UV radiation
- 2000-fold increased incidence of skin cancer
- Severe skin and eye abnormalities
- Neurological abnormalities (20% of patients)
- 30 year reduction in survival







Photos courtesy of Prof. Reinhard Dummer, Dermatologische Klinik, Zürich

NER SUBSTRATES DISTORT THE DNA HELIX









A MODEL FOR NUCLEOTIDE EXCISION REPAIR



DAMAGE RECOGNITION IN NER



PHOTOACTIVE/FLUORESCENT NER SUBSTRATES



PHOTOREACTIVE DERIVATIVE

FLUORESCENT DERIVATIVE





SYNTHESES OF AAF ADDUCTS IN DNA



HARTWIG-BUCHWALD COUPLING OF 8-BrdG



Gillet, L., Schärer, O.D. **Org. Lett.** *4*, 4205-4208 (2002)

Ludovic Gillet

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ACYLATION AND CHANGE OF PROTECTION



Gillet, L., Schärer, O.D. Org. Lett. 4, 4205-4208 (2002) Ludovic Gillet

MODIFIED DNA SYNTHESIS OF AAF OLIGOS



Jawad Alzeer, Ludovic Gillet

ANALYSIS OF dG-AAF AND dG-AF OLIGOS



Gillet LCJ, Alzeer J, Schärer OD (2005) NAR 33, 1961

IN VITRO NER ASSAY



Jacqueline Enzlin, Ludovic Gillet

AAF/AF DERIVATES AS NER SUBSTRATES



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PHOTOACTIVE PROBES FOR NER



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XPC/HR23B BINDS TO THE DAMAGED AND NON-DAMAGED STRANDS OF DNA



Ludovic Gillet

ENDONUCLEASES IN NER



THE XPF/ERCC1 HETERODIMER





IDENTIFYING THE ACTIVE SITE OF XPF/ERCC1



CLEAVAGE OF XPF USING FENTON CHEMISTRY



N-terminal sequencing: Bands 1-4 correspond to N-terminus of XPF Band 5 could not be sequenced

CLEAVAGE OF XPF USING FENTON CHEMISTRY



Enzlin JH, Schärer OD EMBO J. 21, 2045-2053(2002)

IRON-MEDIATED CLEAVAGE SITES IN XPF



ACTIVITY OF XPF ACTIVE SITE MUTANTS

VT
WT
D676A
R678A
E679A
E679A
E679A
E679A
E701A
E701A
E714A
E715A
K716A
D720A

0.4 mM MnCl₂

*..... ππητ *<u>....</u> *-----

, *<u>.....</u>

.....

2 mM MgCl₂

Enzlin JH, Schärer OD EMBO J. 21, 2045-2053(2002)

IN VITRO NER ASSAY WITH XPF MUTANTS



Jacqueline Enzlin

A CONSERVED NUCLEASE MOTIF IN XPF

Residue D676 R678 E679 R681 E701 D704 E714 R715 K716 D720

	Nuclease activit	у -	(+)	+	++	++	-	+	(+)	-	-	
	Cleavage by Fe ²	+ -	+	-	+	+	-	-	+	+	+	
	DNA bindin	g +	+	+	+	+	+	+	+	+	+	
In	vitro NER activit	у -	+	-	+	+	-	-	+	-	-	
									1			
	HS XPF 673 IVV DMREF	SELPS	LIHRRGIDI	EPVTLEV	G YI-L-	TPEM-	CV	SIS LIGS	LNNG-RI	Y-SQCISM		
	Mm XPF 686 IVVDM <mark>R</mark> F	RSELP	SLIHRRGID	EPVTLEV	/G) YI-L	TPEL	CV	SVS LIG	SLHSG-F	LY-SQCLAI	м	
	Cg XPF 681 IVV DMR FF	SELPS	LIHRRGIDI	EPVTLEV	G YI-L-	TPEL-	CV	SVS LIGS	LNSG-R	LY-SQCLAM		
	Dm Mei9 655 VIVDMR F	RSDLP	CLIHKRGLE	VLPLTITI	IG > YI-L	TPDI-	CV	SIS LIGS	L <mark>NS</mark> G-RL	Y-NQCVQM		
	CeXPF 688 IIV MREFN	SELPT	VLYTKGYNV	/VATTIEI(G YI-L-	SPNI	—AI	ALD LTQS	L <mark>QS</mark> G-R <mark>V</mark>	F-KQIEQM		
	At XPF 726 VIV MREFM	SSLPN	VLHQKGMI	KIIPVTLEN	/G⊃YI-I <mark>r</mark>	SPSI	CV	SIQ LFQ	SFTSG-R	LF-HQVEMN	1	
	Sp RAD16 668 VIVDL <mark>R</mark> F	'RSSLP	SILHGNNFS	SVIPCQLL	'NG AI- <mark>I</mark>	SPK	ICV	SIR LIQ	SLSNG-F	LY-SQCEA	M	
	SC RAD1 822 VIVDTRI FI	NASIP	JILYRYGI R	VIPCMLTV	/G⊃YV- <mark>I</mark>	TPDI	CL	SIS LIGS	LQNN-RI	-A-NQCKKM	[
	Af Helic 523 VIV SRELR	SEVVK	HLREIGAKI	EIRNLEVA	A YV-V-	SDRV	AV	TVE FLN	S <mark>IIQ</mark> KER	LF-SAYSRP		_
	Mj Helic 576 IV VRE <mark>K</mark> NM	[AKL]	LHNY-ANIE	LKTLEVG	YV-L	SDRV	—vv	TAE FVNS	STIDK-RLI	F-SQLKNL		
	Mt Helic 520 IAY S 3VN	SRVLR	ELKKIGVDE	FELKPLA	VGDYQ- <mark>I</mark>	SED	тп	TTQ FIGS	TIDK-RI.	KQAREM		
	Ph Helic 538 VVIDSR <mark>E</mark> LR	SEVVK	KLKT-GIKII	WRTLDV	G YI-V-	SEDV-	AI	SAN FIQS	IIDG-RL <mark>F</mark>	-DQVKRL		
	Pa Helic 543 VVVDSR LF	RSEVVI	RLKTLGVE	IEVKTLD	VG YI- <mark>I</mark>	SDEV	/AI	SAN FIQ	S <mark>IID</mark> G-R <mark>L</mark>	F-DQVKRL		
	Ss Helic 6 IYA DR <mark>K</mark> AS	GIPEL	LKELGITVI	SGLTVA	YV-I	TDDV	-AV	SVN LVNS	VFDK-RF	F-DQISRL		
	Sc Mus81 352 PH HREIK	<mark>S</mark> QSDREFF-	-SRAFERK(JMKSEIR (QLALG <mark>D</mark> II	MVAKNKT-	GLQ	CVLNTTIVS	RKRLD D	LALSIRDN-I	R-FMEQKN	RL
	Sp Mus81 296 LLIDTR IR	SPLDRNLII-	-DKLTNDFG	VNCQVR	SLELG <mark>D</mark> AI	LWVARDM	ES (GQEVVLDFV	V <mark>ER</mark> RYI	D LVASIKD	G-R-FHEQ	KARL
	Ce Mus81 211 LIADN <mark>R</mark> H	RNNPRFKS	/I—EHLVKK	EDIRVDIR	RSLSVG DY	IWICRKID-	GTI	EIVMD W VV 🛛	R KT W D D	LQSSIRGG	-R-YDEQK	GRL
	Dm Mus81 155 LLVD <mark>T</mark> Q	T -SGKNKRV	LDQTRSYL	ESLGARH	EVRRL <mark>T</mark> IC] FLWVAÇ	DQE	GNELVLP	YIVI R	MDDLASSI	RDG-R-FH	EQKHRL
	Mm Mus81 76 LCV <mark>IG</mark> 1	RGAGHRPE	MLRELQRL	RVPHT	VRKLH <mark>V</mark> G	DFVWVAQ	ETRPR	DPERPGEL	LDHIV 3	RLD LCS	SIIDG-R-F	REQKER
	Hs Mus81 196 LCV IG T	RGGGHRPE	LLRELORLE	IVHTV	RKLHVGD	FVWVAOE	TNPRD	PANPGELVI	DHIV N	RLDLCSS	SIIDG-R-FF	EOKFRL

Consensus hhhD-RE--<u>h-hG<mark>D</mark>ah-h--</u> --h-----h<u>ERK</u> ----<u>D</u>----Sh----R-a---Q----h

Enzlin JH, Schärer OD EMBO J. 21, 2045-2053(2002)

OKHRL REQKFRL

DNA BINDING DOMAINS OF ERCC1/XPF



DNA BINDING DOMAINS OF ERCC1/XPF



Tsodikov OV, Enzlin JH, Schärer OD, Ellenberger T PNAS 102, 11236 (2005)

A MODEL FOR DNA BINDING BY ERCC1/XPF



THE XPG ENDONUCLEASE





ENDONUCLEASES IN NER



XPG SUBSTRATE BINDING AND CLEAVAGE



Hohl, M., Thorel, F., Clarkson, S.G., Schärer, O.D. (2003) JBC 278, 19500.

DELETIONS IN THE SPACER REGION OF XPG



ACTIVITY OF XPG MUTANTS ON Y SUBSTRATES



ACTIVITY OF XPG MUTANTS ON BUBBLES



IN VITRO NER ACTIVITY OF XPG MUTANTS





Dunand-Sauthier I, Hohl M, Thorel F, Jaquier-Gubler P, Clarkson SG, Schärer OD (2005) JBC 280, 7030

MUTANT XPG IS NOT FOUND IN NER SITES



MUTANT XPG HAS LOWER AFFINITY FOR TFIIH



A FEN1-XPG HYBRID PROTEIN



Marcel Hohl

FEN-1/XPG CLEAVES BUBBLE SUBSTRATES



Marcel Hohl

FEN1-XPG PREFERS DOUBLE FLAPS



Marcel Hohl

FEN-1/XPG IS NOT FOUND IN NER SITES



Isabelle Dunand-Sauthier

A ROLE FOR THE C-TERMINUS OF XPG IN NER?



Lidija Staresincic

FXFX, BUT NOT FX IS FOUND IN NER SITES



Isabelle Dunand-Sauthier

MODEL FOR NER COMPLEX FORMATION



FROM NER INCISION TO REPAIR SYNTEHSIS



THE PEOPLE WHO DID THE WORK

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