

**GENOMIC INSTABILITY AND CANCER:
INSIGHTS FROM ANALYSIS OF THE
BLOOM'S SYNDROME HELICASE**

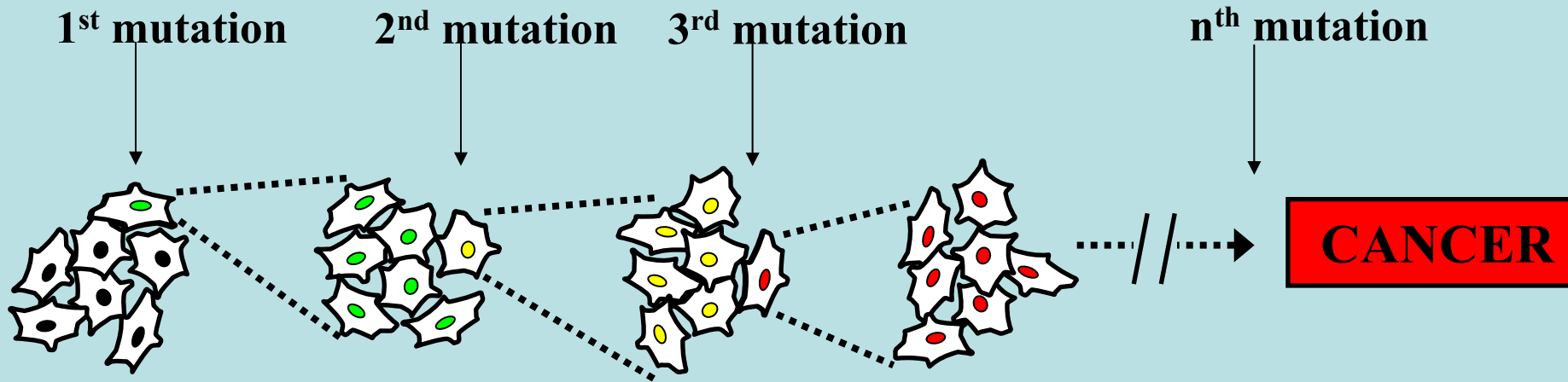
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**CR-UK Oxford Cancer Centre,
Weatherall Institute of Molecular Medicine,
University of Oxford.**

PRESENTATION OUTLINE

- **How do cancers arise - what genetic changes are necessary?**
- **Genomic instability and its role in tumorigenesis**
- **Bloom's syndrome as a model for understanding how aberrant mitotic recombination can drive tumorigenesis**
- **A conserved pathway involving the BLM protein and its partners topoisomerase III α and BLAP75/RMI1 for resolution of homologous recombination intermediates**

HOW DO CANCERS ARISE?



- **Cancer is a genetic disease - but it is polygenic**
- **Results from defects in genes that control cell birth and/or cell death**
- **Cancers acquire several capabilities in order to be life-threatening**
 - **evade apoptosis**
 - **insensitive to anti-proliferative signals**
 - **ability to invade tissue and metastasize**
 - **immortal (no replicative limit)**

THE CLASSIFICATION OF CANCER GENES

- **ONCOGENES**

- Mutation renders gene constitutively active (e.g. Ras)
- Drive tumorigenesis e.g. by making cells independent of mitogenic growth signals
- One activated allele sufficient

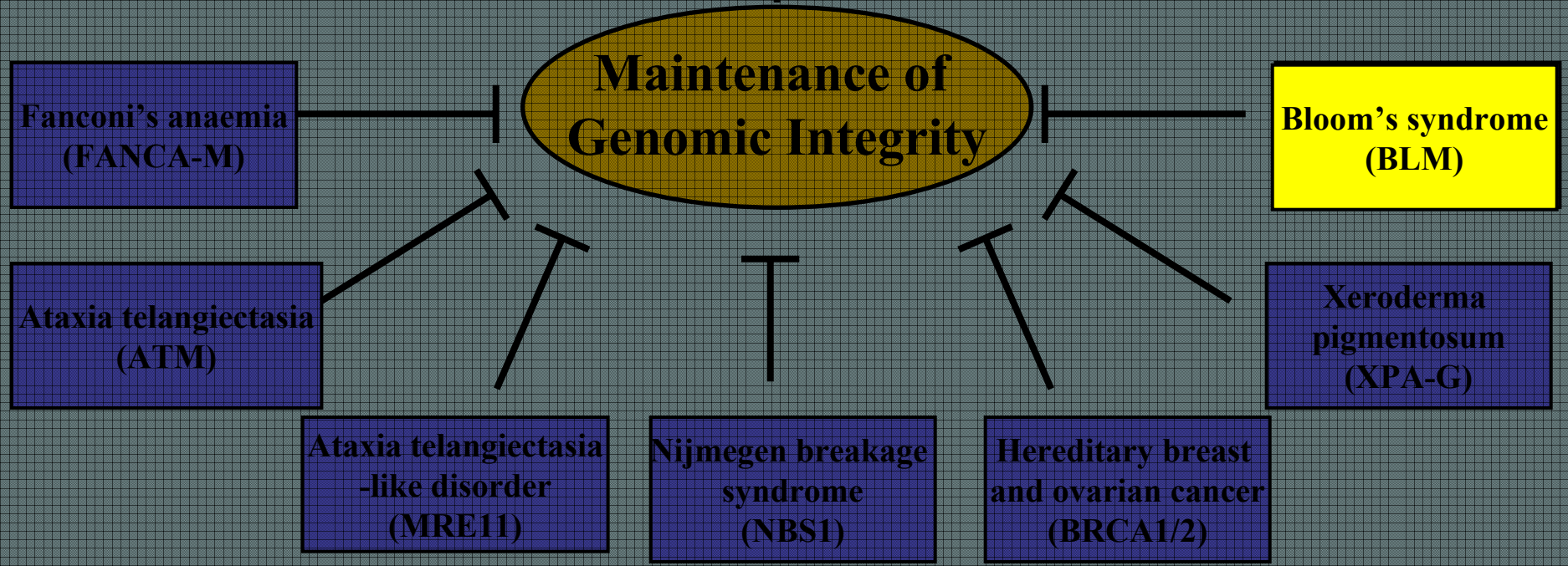
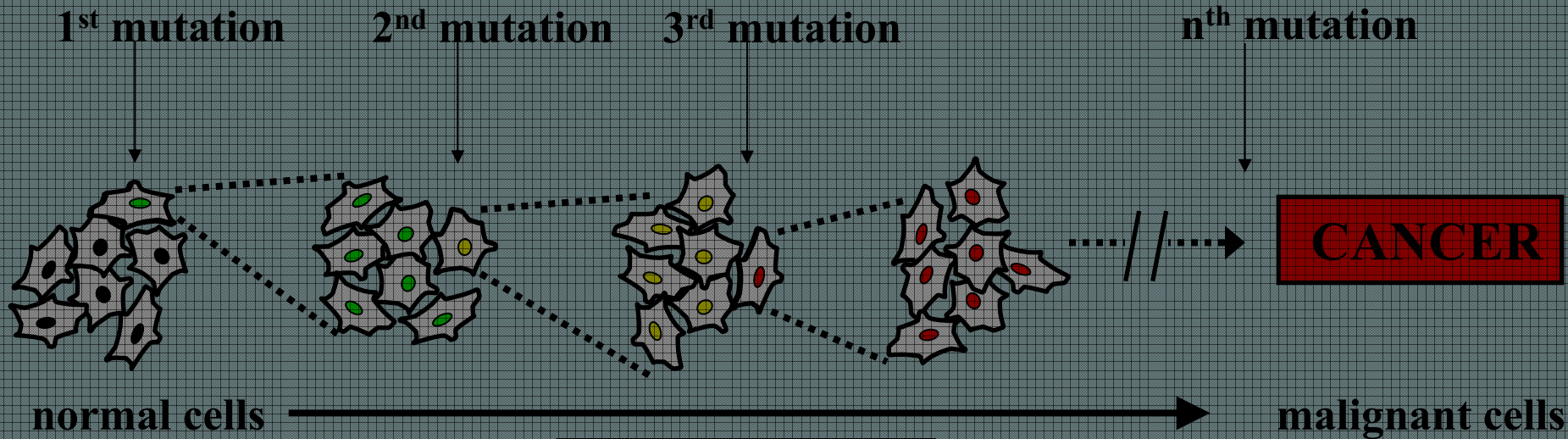
- **TUMOUR SUPPRESSOR GENES**

- Mutation renders gene inactive (e.g. Rb)
- Loss of function permits unregulated cell cycle progression etc.
- Usually both alleles inactivated

Oncogene and TS gene changes directly drive neoplastic transformation by permitting cell proliferation and/or abrogating cell death (GATEKEEPERS)

- **GENOME STABILITY GENES (CARETAKERS)**

- Mutation renders gene inactive
- Not necessarily directly involved in or rate limiting for neoplastic transformation
- Not selective; simply increase probability that oncogenes/TS genes will be hit
- Usually both alleles inactivated
- Particularly potent when defect inherited



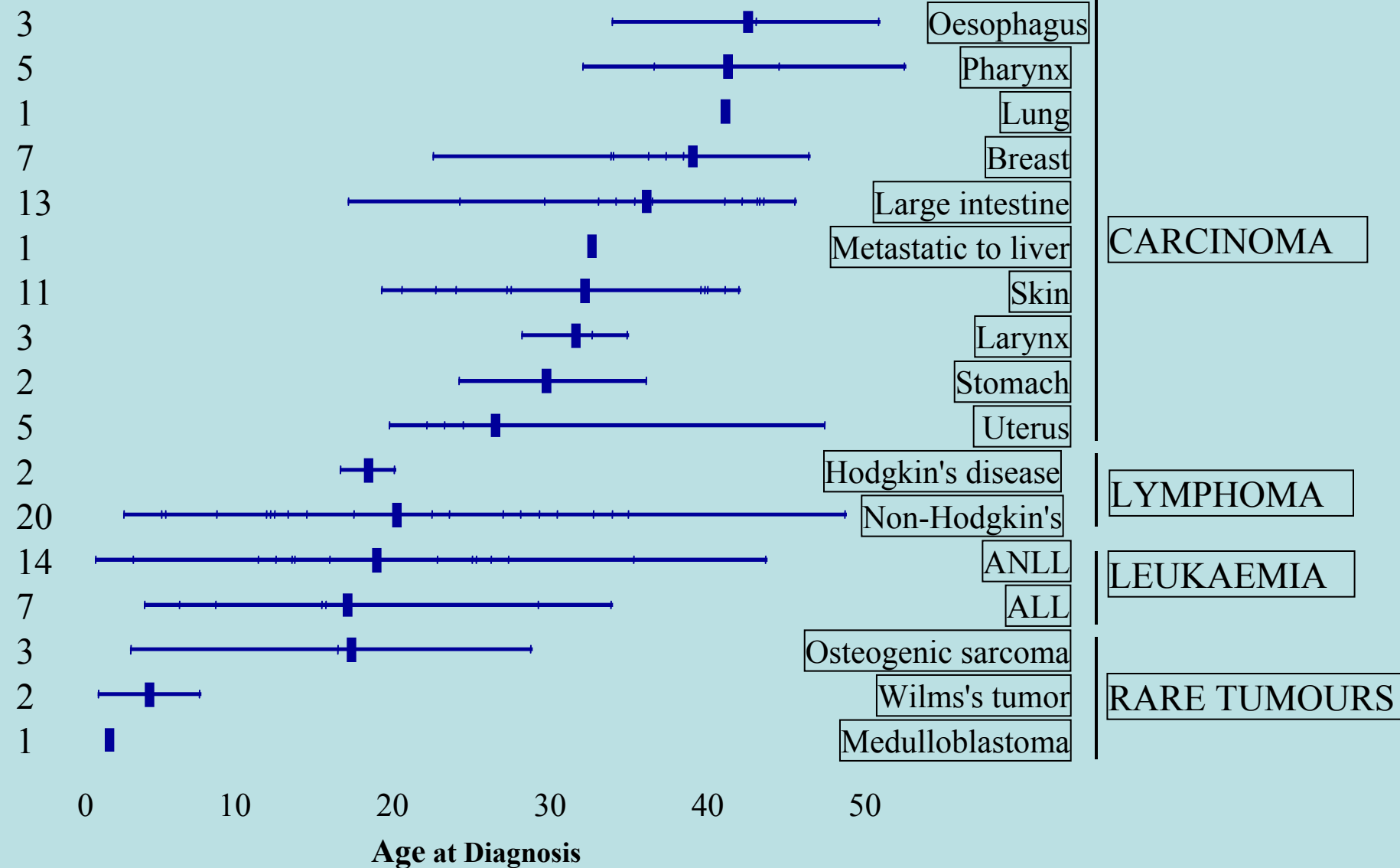


Bloom's syndrome

- Autosomal recessive disorder
- Short stature
- Skin abnormalities
- Male infertility/female subfertility
- **Predisposition to cancer**

The first 100 Cancers in the Bloom's Syndrome Registry

Cases



CANONICAL DOMAIN ORGANIZATION OF A RecQ HELICASE



7 motifs of SF2 helicases

DOMAIN STRUCTURE OF SELECTED MEMBERS OF THE RecQ HELICASE FAMILY



RecQ HELICASE DEFICIENCY DISORDERS



**Werner's syndrome
(WRN)**

**Normal until puberty
Premature ageing
Cataracts
Cancer prone**



**Rothmund-Thomson syndrome
(RECQ4)**

**Congenital skeletal abnormalities
Sparse hair and poikiloderma
Cataracts
Cancer prone**

CANONICAL DOMAIN ORGANIZATION OF A RecQ HELICASE



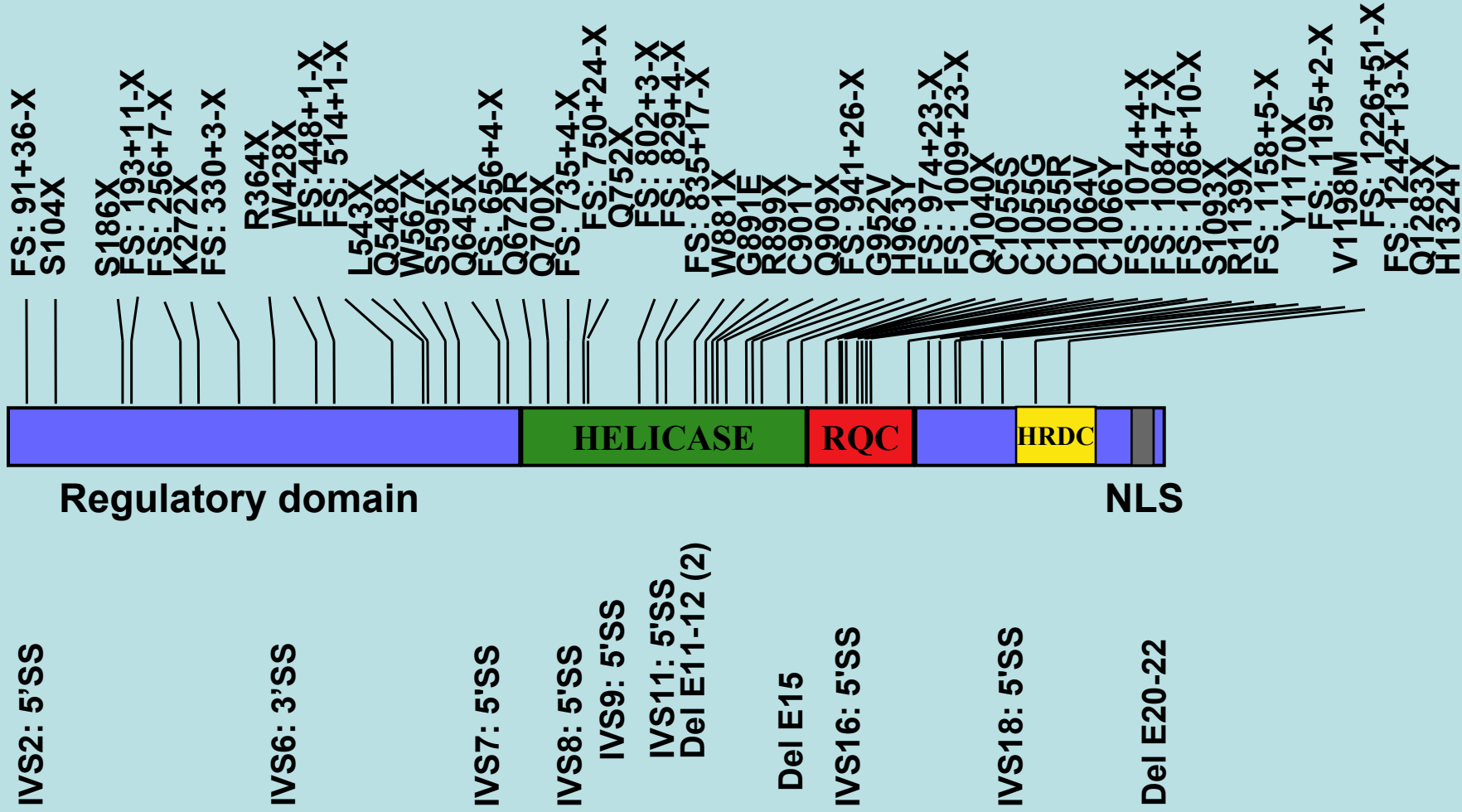
7 motifs of SF2 helicases

OVERALL STRUCTURE OF THE CATALYTIC CORE OF *E.coli* RecQ



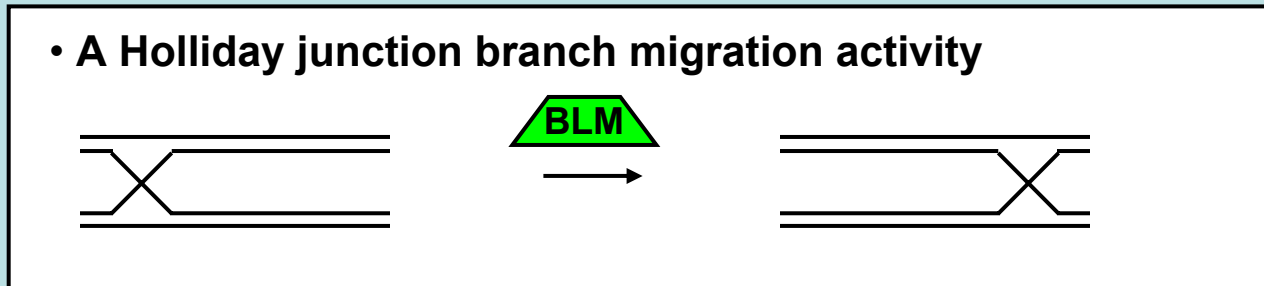
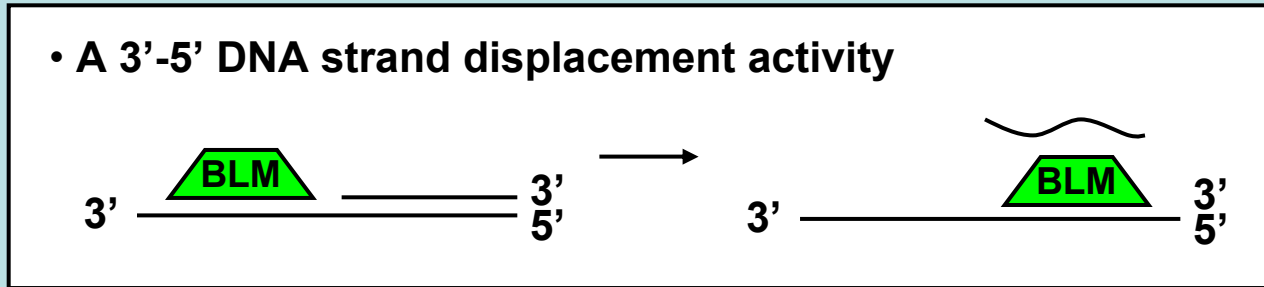
Courtesy of Dr James Keck

MUTATIONS IN *BLM*

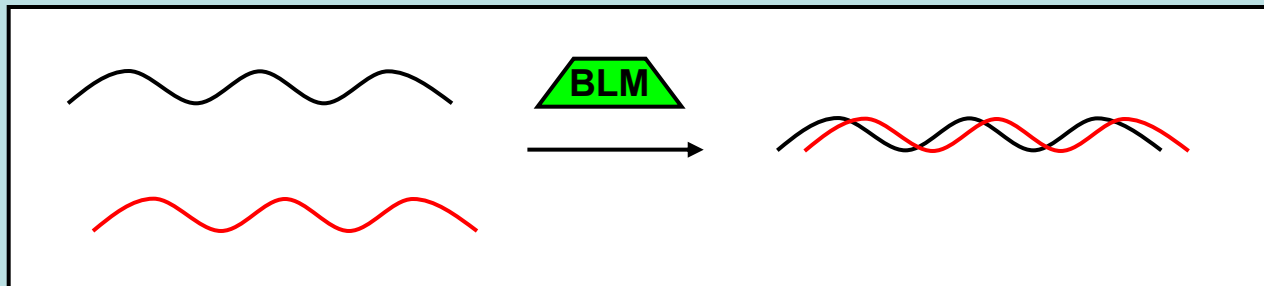


BLM contains four distinct biochemical activities

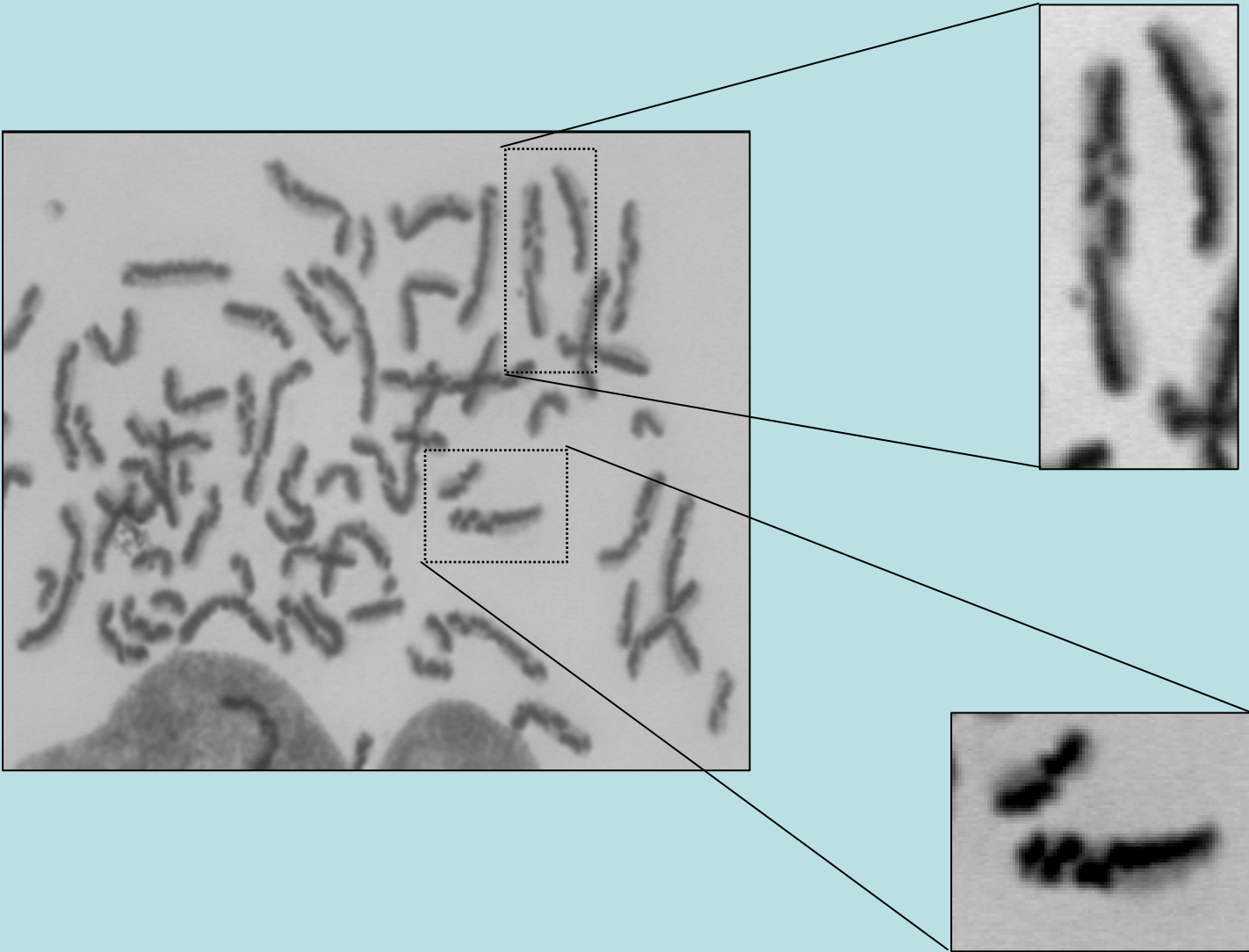
- DNA dependent ATPase :



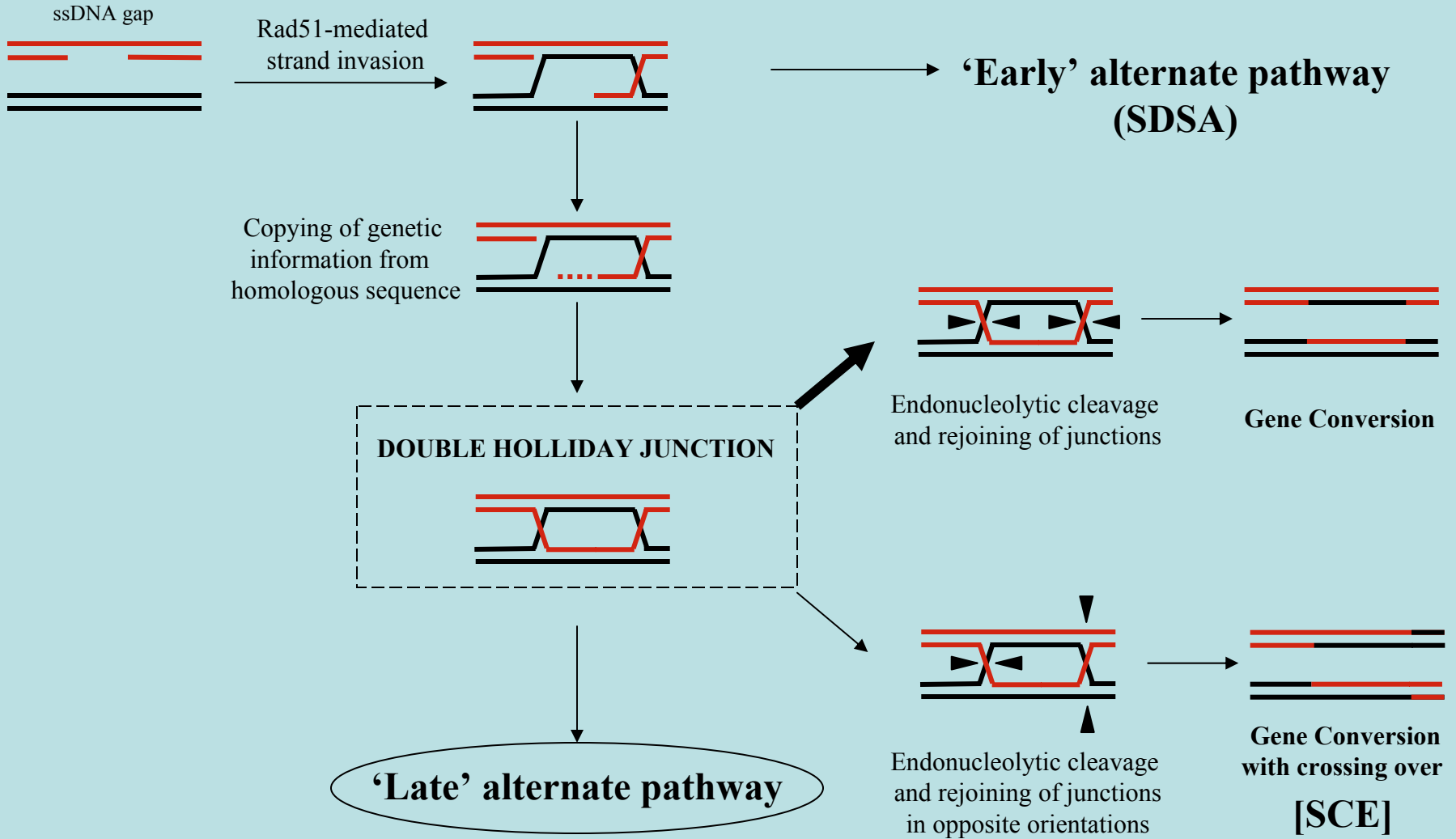
- A single stranded DNA annealing activity (ATP independent):



Elevated SCEs are diagnostic of Bloom's syndrome cells

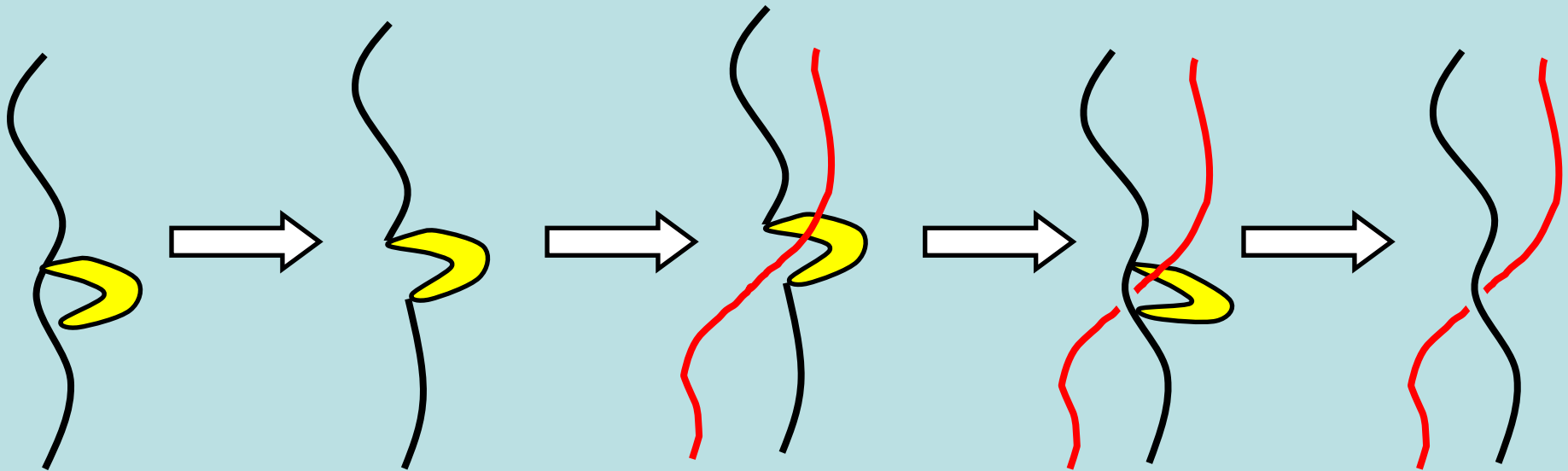


HOW ARE SCEs GENERATED ?



TOPOISOMERASES

- Ubiquitous, highly conserved enzymes
- Act to disentangle topological problems that arise during DNA metabolism

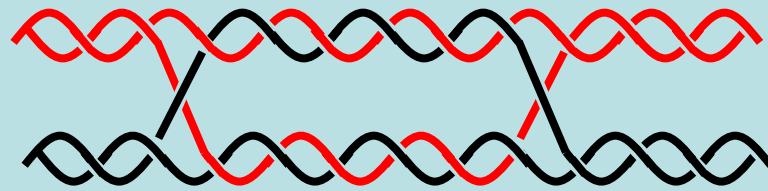


Class

Gene (*S. cerevisiae*)

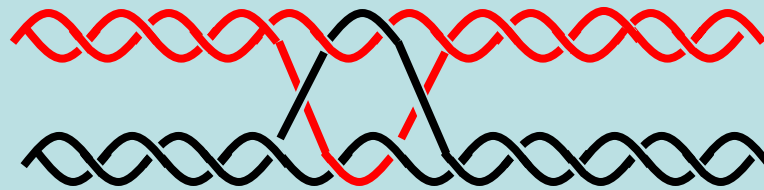
Type 1	Type 1A	<i>TOP3</i>
	Type 1B	<i>TOP1</i>
Type 2		<i>TOP2</i>

How might BLM and topo III α catalyze a non- endonucleolytic resolution of double Holliday junctions?



Branch migration

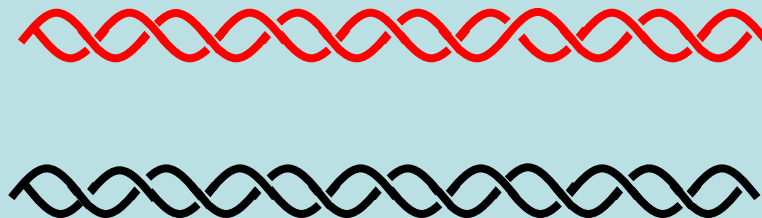
BLM?



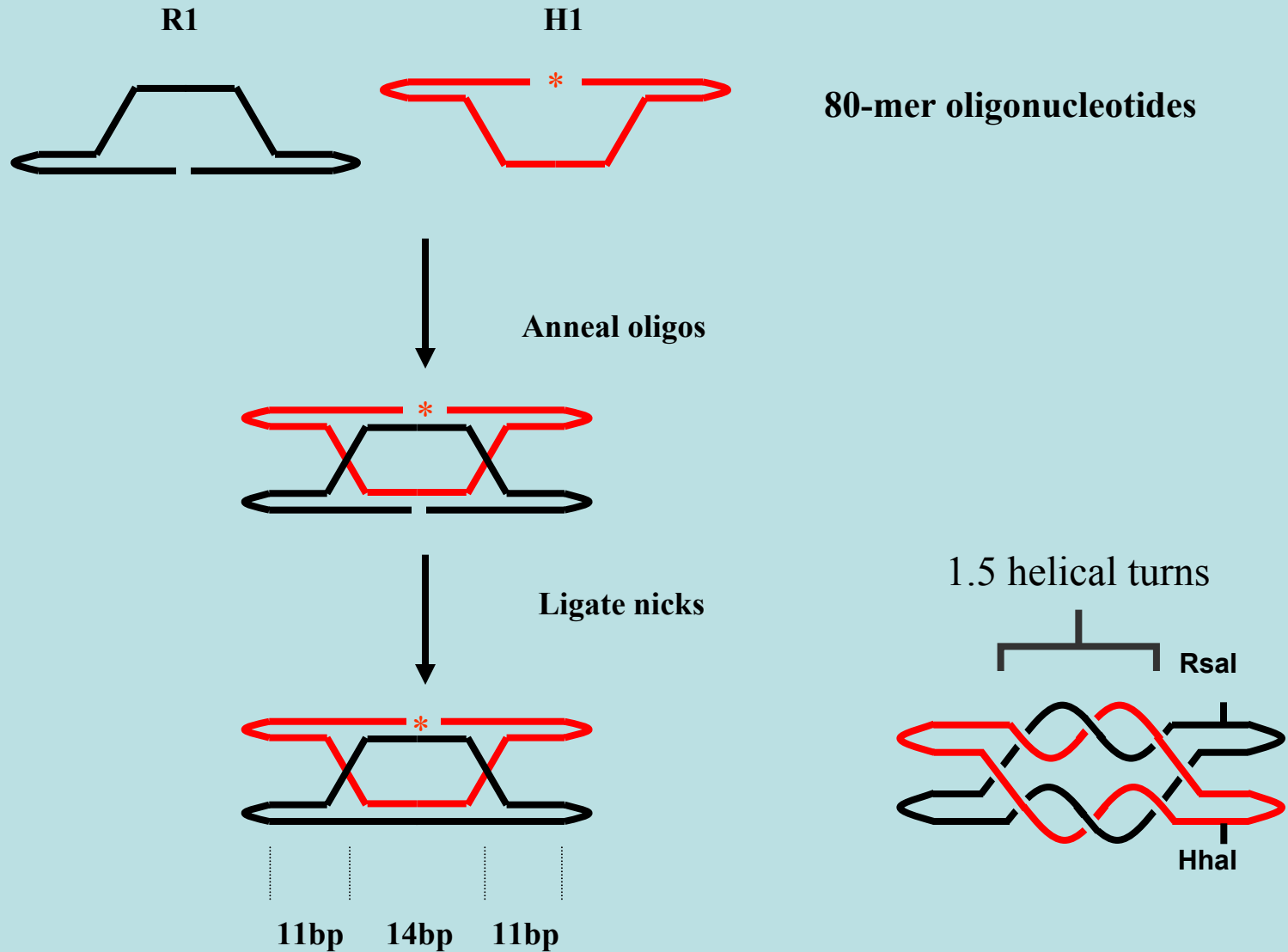
Double Holliday Junction
(Hemicatenane)



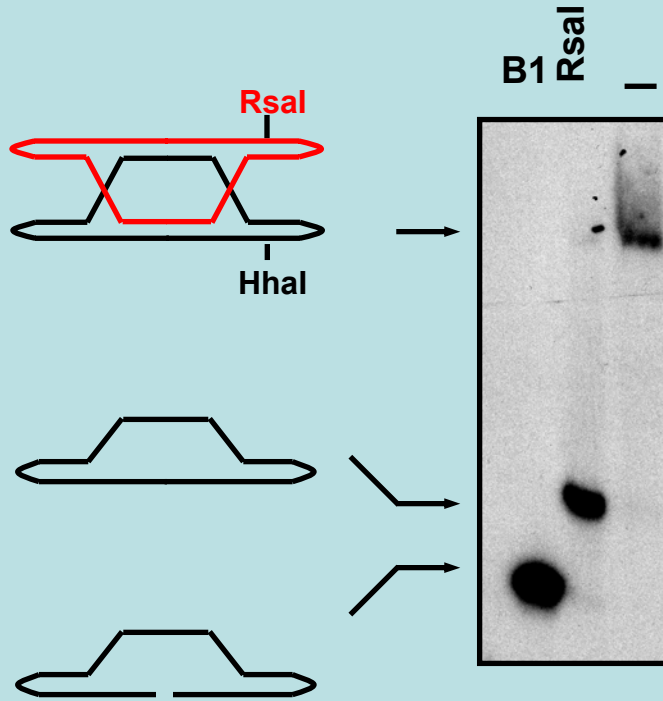
Topo III?



Generation of a DNA substrate containing two Holliday junctions

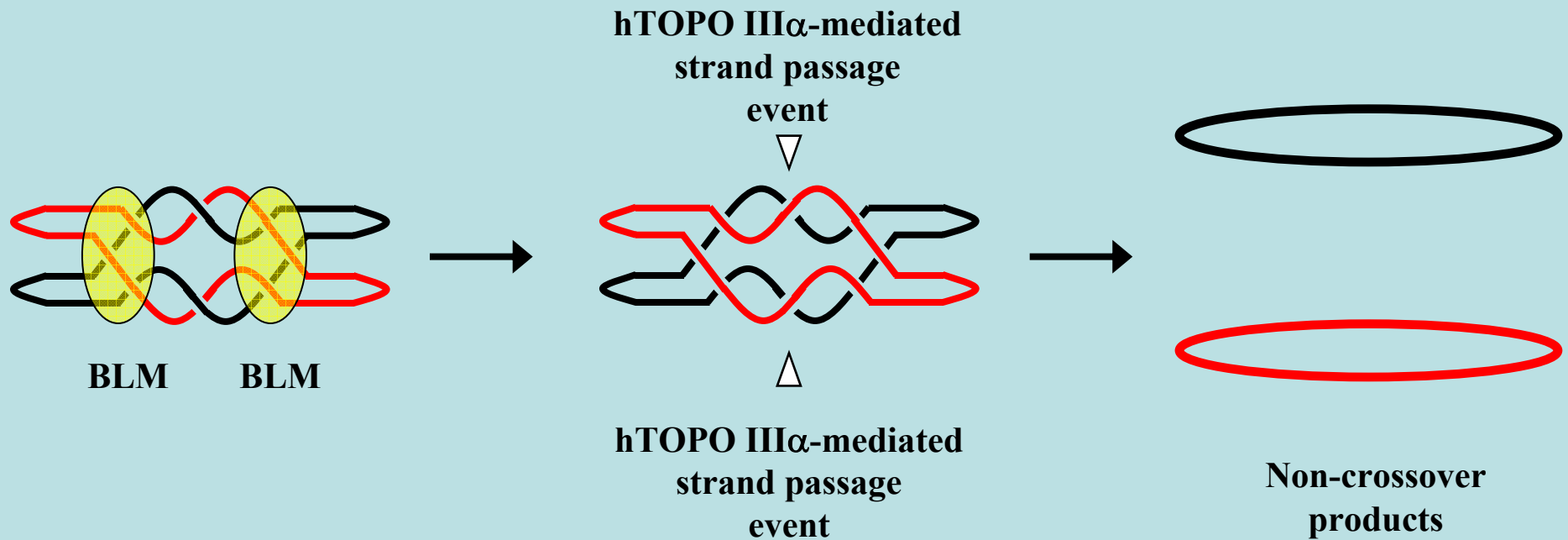


BLM and hTOPO III α cooperate to resolve DHJ



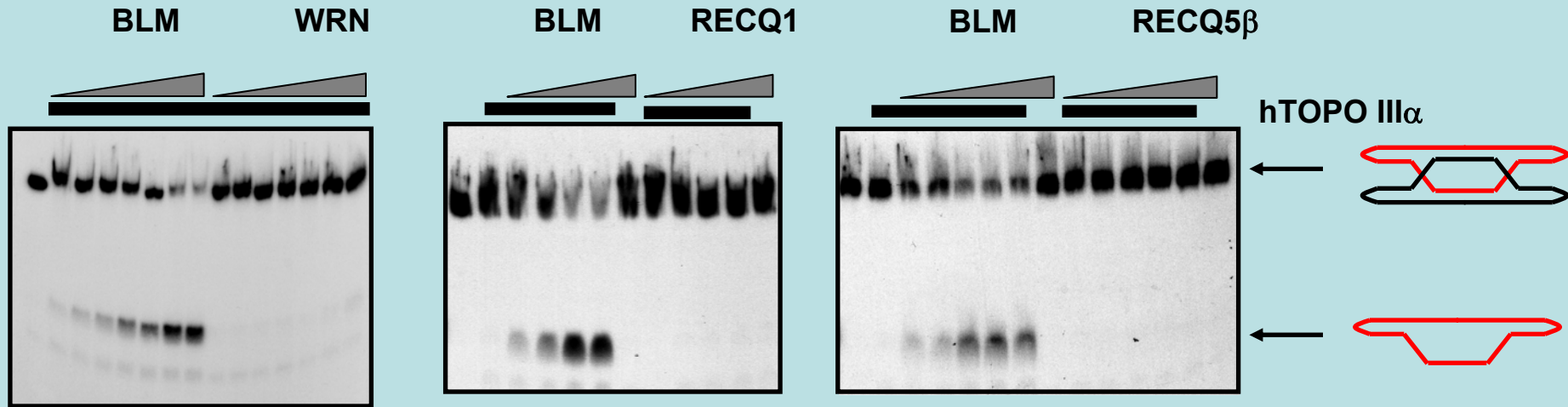
Requires the ATPase/helicase activity of BLM and the active site tyrosine of topo III α

BLM and hTOPO III α catalyze a novel mechanism to resolve double Holliday Junctions into non-crossover products



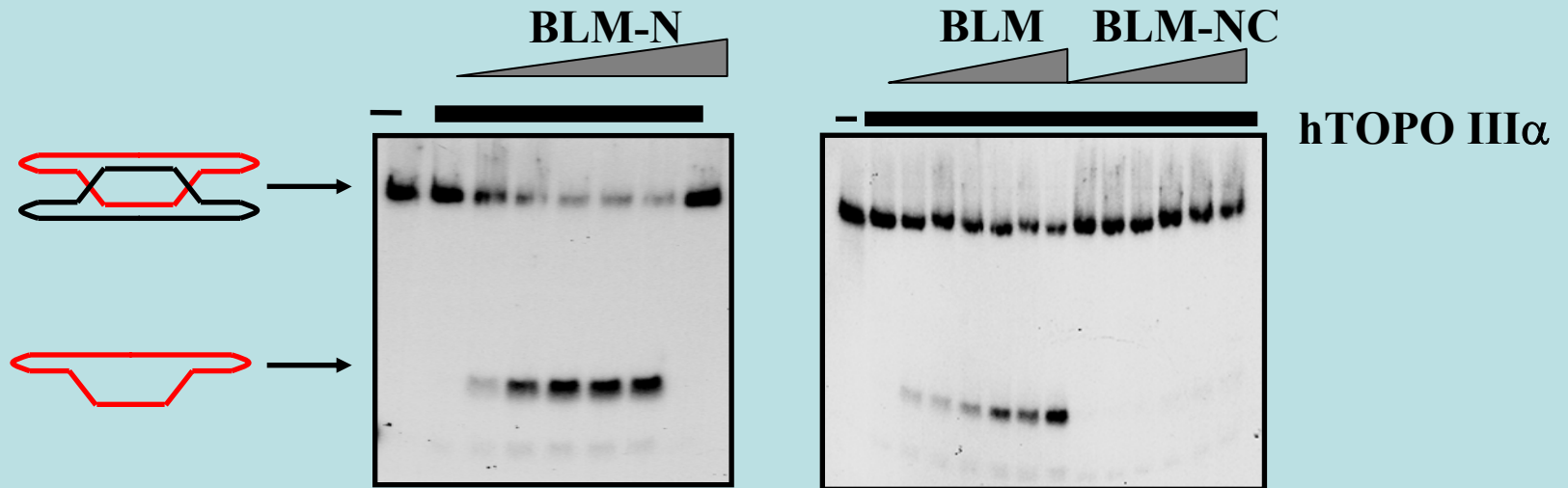
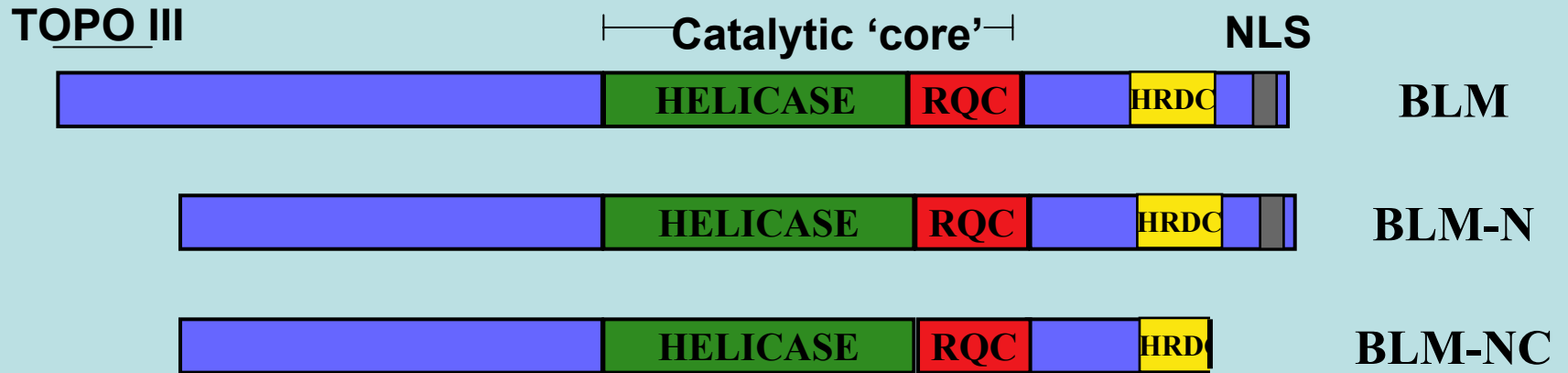
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Double junction dissolution

Double junction dissolution is catalyzed specifically by BLM



In contrast, any type IA topoisomerase is functional

The C-terminal domain of BLM is required for double junction dissolution

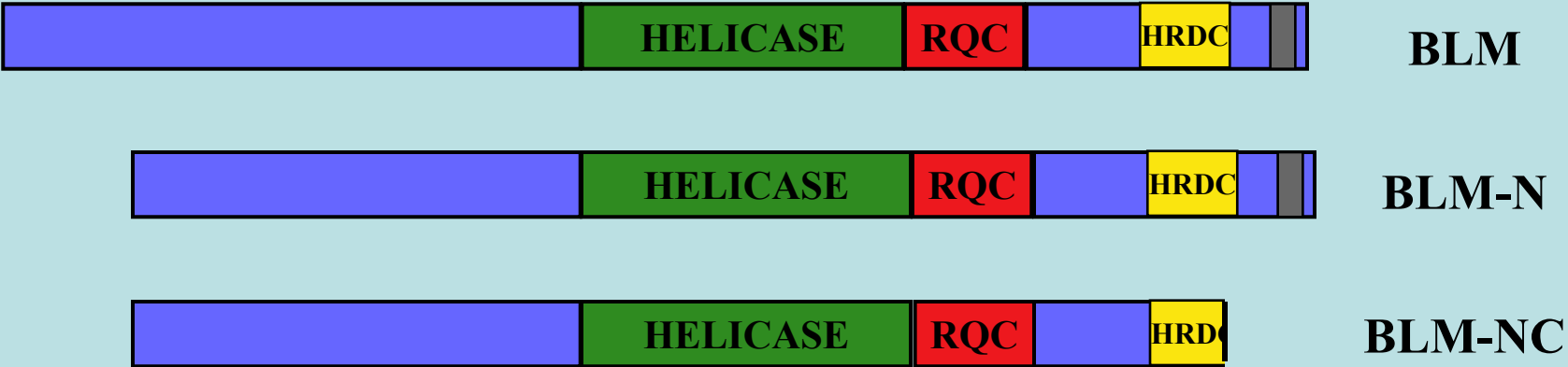


C-terminal truncation of BLM disables the HRDC domain

TOPO III

— Catalytic 'core' —

NLS

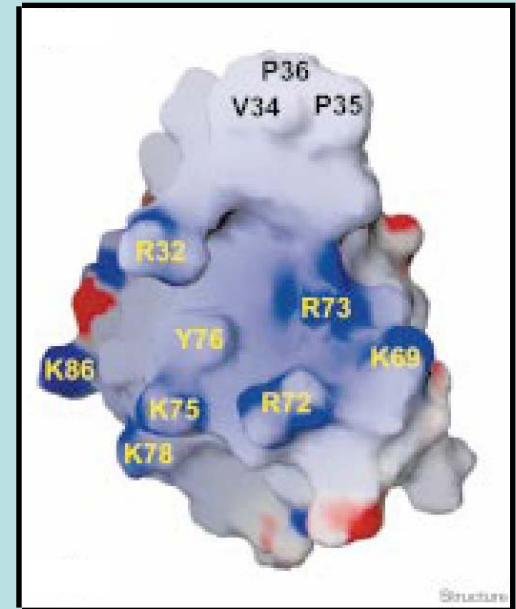


Helicase function is not impaired in BLM-NC

The HRDC domain of the RecQ helicase family forms an evolutionarily conserved protein-fold

	$\alpha 1$															$\alpha 2$																											
	20										30										40										50												
sgs1_sc	L	N	N	L	R	M	T	Y	E	R	L	R	E	L	S	L	N	L	G	N	R	.	M	V	P	P	V	G	N	F	M	P	D	S	I	L	K	K	M	A	A	I	L
blm_hu	E	E	M	V	K	K	C	L	G	E	L	T	E	V	C	K	S	L	G	K	V	F	G	V	H	Y	F	.	N	I	F	N	T	V	T	L	K	K	L	A	E	S	L
blm_dm	R	E	I	H	E	R	C	Y	T	D	L	L	D	L	C	R	T	I	A	S	Q	R	N	V	T	M	A	.	S	I	M	N	I	Q	A	L	K	S	M	A	E	T	L
wrn_hu	Q	E	T	Q	I	V	L	Y	G	K	L	V	E	A	R	Q	K	H	A	N	K	M	D	V	P	P	A	.	I	L	A	T	N	K	I	L	V	D	M	A	K	M	R
wrn_mm	L	D	A	R	T	G	L	Y	A	R	L	V	E	A	R	Q	K	H	A	N	K	M	D	V	P	P	A	.	I	L	A	A	N	K	V	L	L	D	M	A	K	M	R
wrn_xe	R	E	L	Q	T	T	L	Y	G	R	L	V	V	A	R	Q	K	I	A	S	E	R	D	I	L	P	A	.	V	L	A	T	N	K	V	L	V	D	M	A	K	L	R
recq_ce	P	E	K	I	D	Q	L	R	S	R	L	D	D	I	R	V	G	I	A	N	M	H	E	V	A	P	F	.	Q	I	V	S	N	T	V	L	D	C	F	A	N	L	R
recq_sp	I	D	V	M	T	R	C	L	K	D	L	K	L	L	R	S	N	L	M	A	I	D	D	S	R	V	S	.	S	Y	F	T	D	S	V	L	L	S	M	A	K	K	L
recq_ml	A	D	V	D	E	E	L	L	Q	L	K	A	W	R	L	S	T	A	K	E	Q	N	V	P	A	Y	.	V	V	L	T	D	N	T	L	I	A	I	A	E	L	L	
recq_hi	A	N	Y	D	K	D	L	F	A	R	L	R	F	L	R	K	Q	I	A	D	K	E	N	I	P	P	Y	.	I	V	F	N	D	A	T	L	Q	E	M	A	Q	Y	M
recq_ec	G	N	Y	D	R	K	L	F	A	K	L	R	K	L	R	K	S	I	A	D	E	S	N	V	P	P	Y	.	V	V	F	N	D	A	T	L	I	E	M	A	E	Q	M

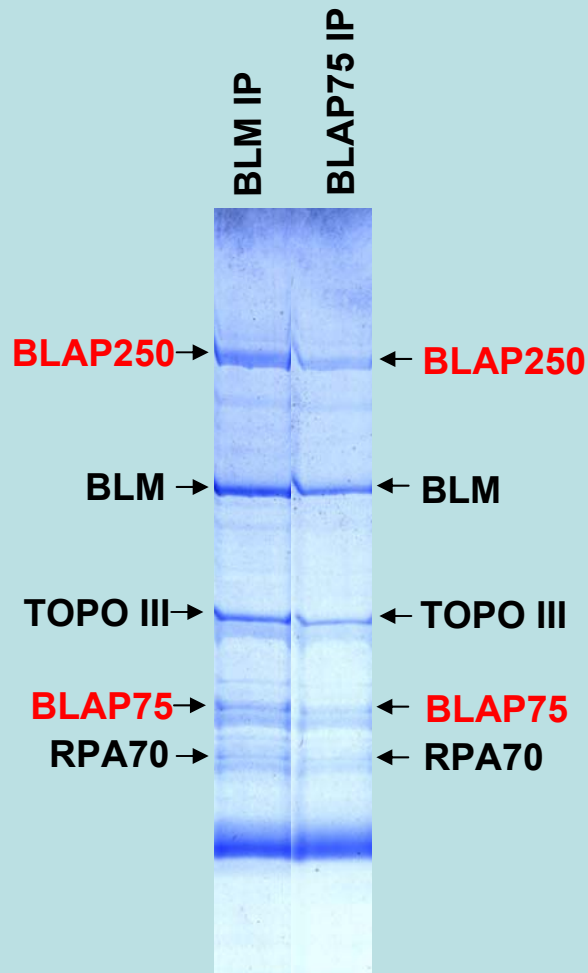
	$\alpha 3$										$\alpha 4$										$\alpha 5$																			
	60										70										80										90									
sgs1_sc	P	M	N	D	S	A	F	A	T	L	.	G	T	V	E	D	K	Y	R	R	R	F	K	Y	F	K	A	T	I	A	D	L	S	K	K	R	S	S	E	
blm_hu	S	S	D	P	E	V	L	L	Q	I	D	G	V	T	E	D	K	L	E	K	Y	G	A	E	V	I	S	V	L	Q	K	Y	S	E	W	T	S	P	A	
blm_dm	P	I	T	E	K	D	M	C	S	I	P	H	V	T	K	A	N	F	D	K	Y	G	A	K	L	L	E	I	T	S	N	Y	A	S	E	K	L	L	M	
wrn_hu	P	T	T	V	E	N	M	K	R	I	D	G	V	S	E	G	K	A	A	M	L	.	A	P	L	L	E	V	I	K	H	F	C	Q	T	N	S	V	Q	
wrn_mm	P	T	T	V	E	N	M	K	Q	I	D	G	V	S	E	G	K	A	A	L	L	.	A	P	L	V	G	V	I	K	H	F	C	Q	V	T	S	V	Q	
wrn_xe	P	T	T	S	E	N	M	K	K	L	D	G	V	S	E	A	K	S	A	M	L	.	A	P	L	L	E	V	V	K	E	F	C	I	A	N	S	L	K	
recq_ce	P	T	S	A	S	N	L	E	M	I	D	G	M	S	A	Q	Q	K	S	R	Y	G	K	R	F	V	D	C	V	V	Q	F	S	K	E	T	G	I	A	
recq_sp	P	R	N	V	K	E	L	K	E	I	H	G	V	S	N	E	K	A	V	N	L	G	P	K	F	L	Q	V	I	Q	K	F	I	D	E	K	E	Q	N	
recq_ml	P	A	D	E	A	A	L	I	A	V	P	G	M	S	V	R	K	I	E	Q	Y	G	S	D	V	L	Q	L	V	R	C	R	A	V	A	V	R	T	Q	
recq_hi	P	T	S	N	I	E	M	L	Q	I	N	G	V	G	S	I	K	L	E	R	F	G	Q	P	F	M	A	L	I	Q	E	H	K	A	I	L	A	N	A	
recq_ec	P	I	T	A	S	E	M	L	S	V	N	G	V	G	M	R	K	L	E	R	F	G	K	P	F	M	A	L	I	R	A	H	V	D	G	D	D	E	E	



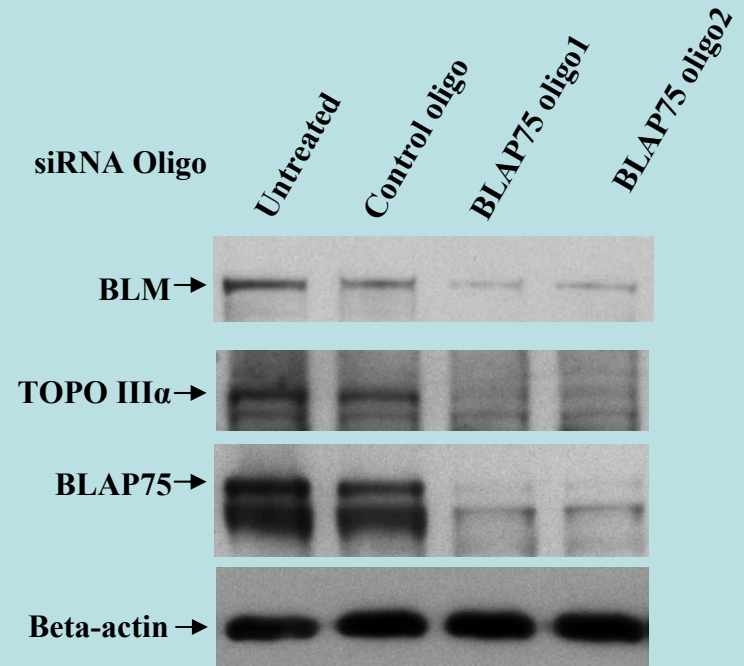
Structure

A third subunit in the BLM/topo III α complex

BLAP75, an essential component of BLM-Topo III α complex



Yin et al. EMBO J. 2005

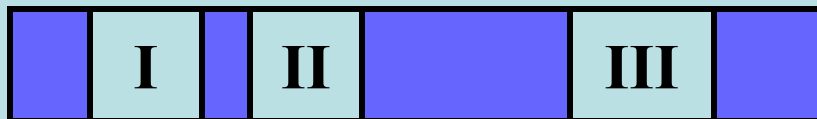


BLAP75 is required for the stability of the BLM/topo III α complex

Courtesy of Dr Weidong Wang

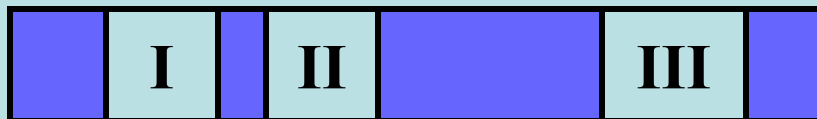
BLAP75 is conserved in yeast (Rmi1/Nce4)

Sc Rmi1



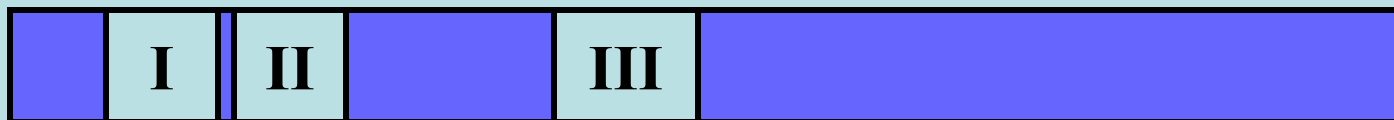
241aa

Sp Rmi1



235aa

Hs BLAP75

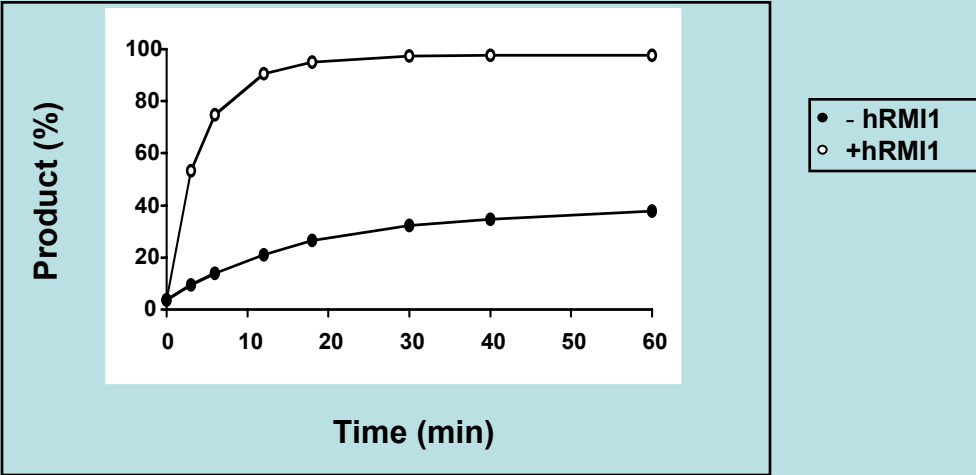
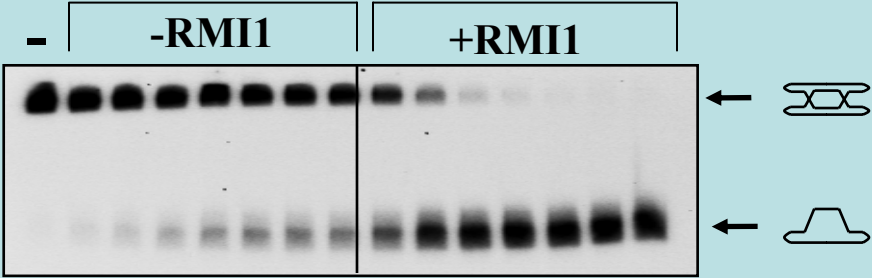


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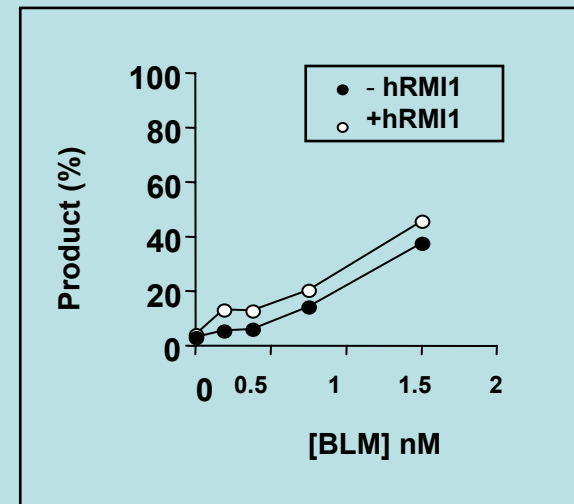
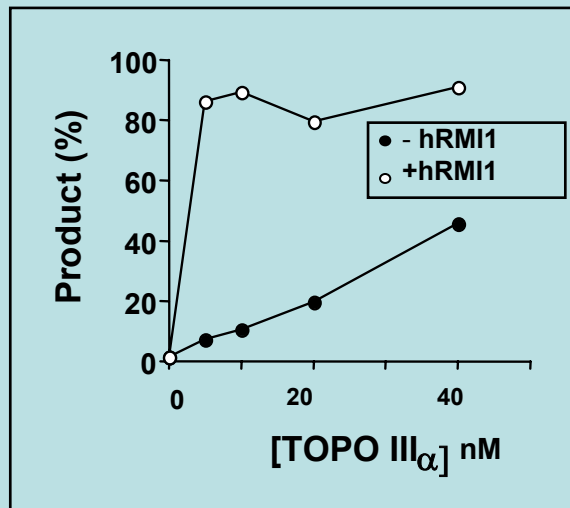
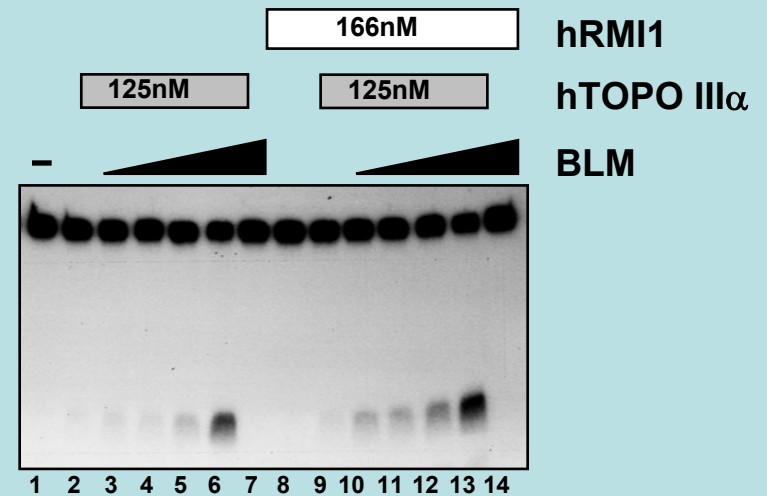
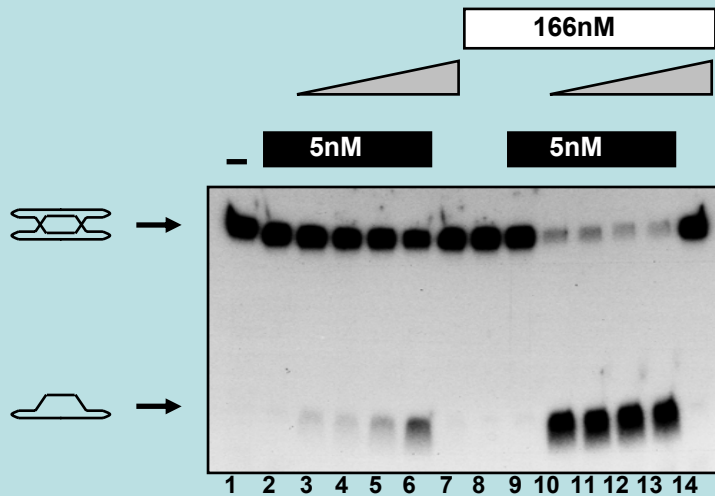
OB-fold domain

hRMI1 strongly stimulates BLM/hTOPoIII α -dependent dissolution

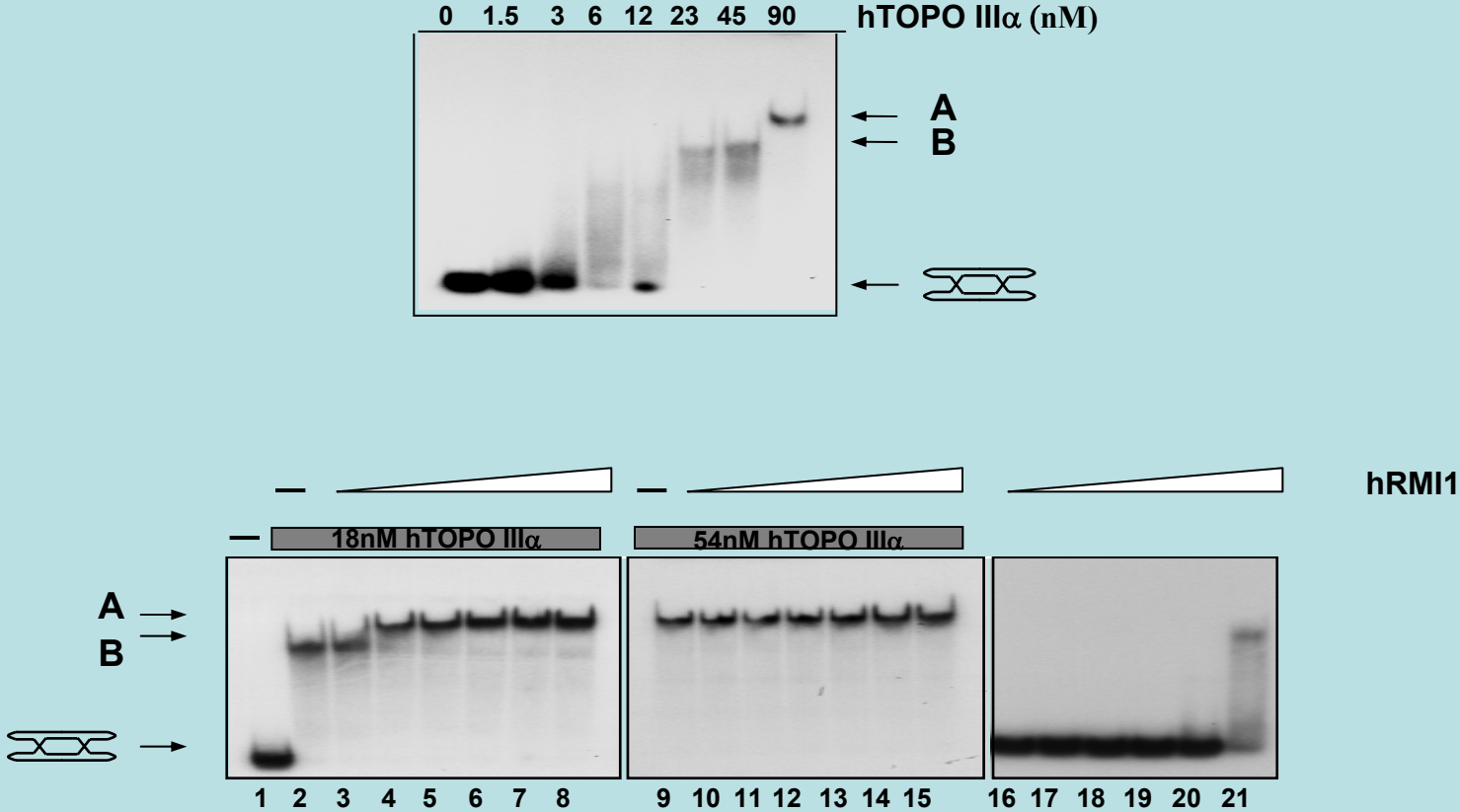
Time-course



hRMI1 STIMULATES DISSOLUTION VIA TOPO III α



RMI1 recruits hTOPOIII α to the double Holliday junction



Summary and Additional Data

BLM and hTOPO III α catalyze a novel mechanism to resolve recombination intermediates that does not involve RuvC-like endonuclease cleavage (Double junction dissolution)

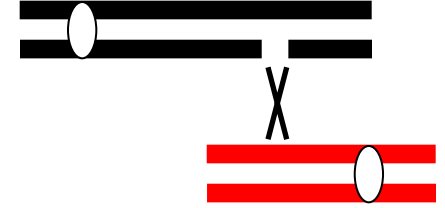
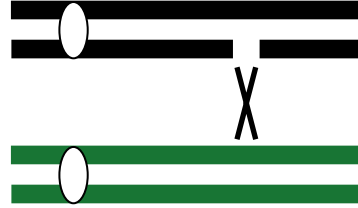
Double junction dissolution is highly specific for BLM, but requires the action of any type IA topoisomerase

The HRDC domain of BLM (and RecQ) constitutes a DNA structure-specific recognition motif

The HRDC domain of BLM (and Lys-1270) is required for efficient catalysis of dissolution

Double junction dissolution is markedly enhanced by hRMI1 via a stimulation of topo III α . hRMI1 binds directly to topo III α , and appears to promote its loading to the substrate

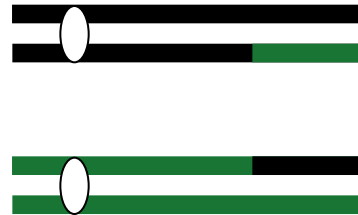
Alternative template usage during homologous recombination



Resolution into
crossover products



**sister chromatid
exchange**



loss of heterozygosity



**chromosome
instability**



CANCER