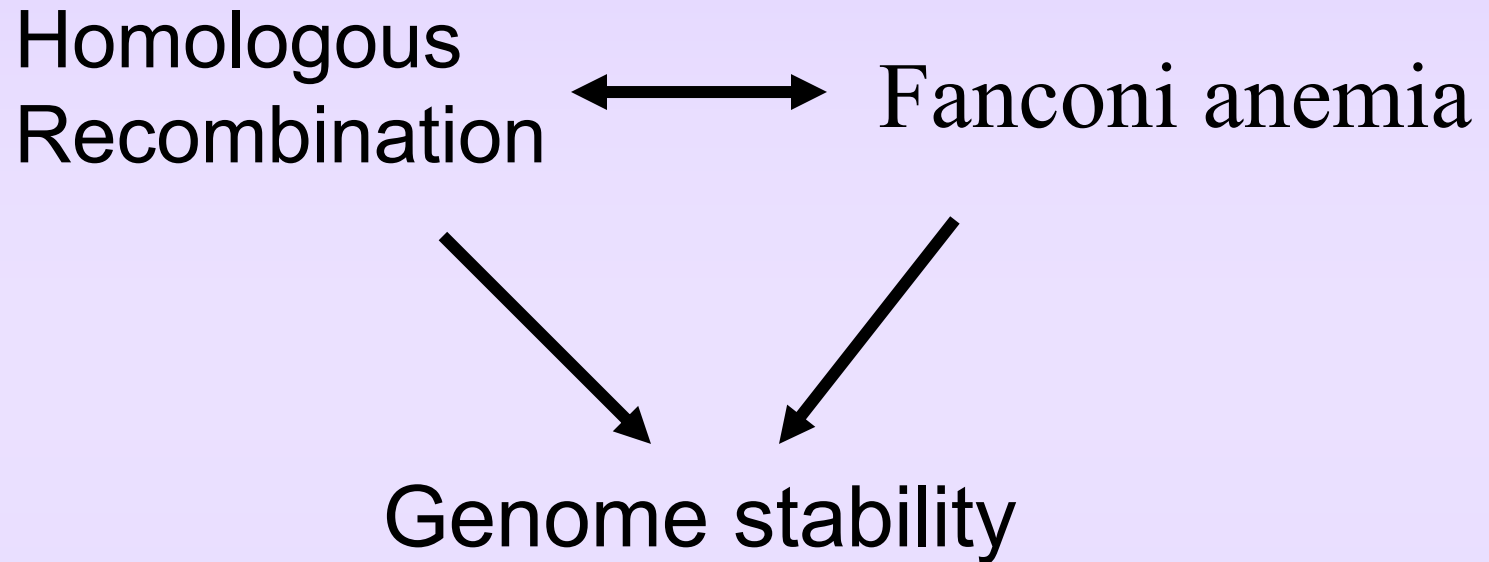# Distinct genomic instabilities associated with deficiencies in homologous recombination or the Fanconi anemia FANCD2 monoubiquitination pathway

April 19th, 2005

John Hinz
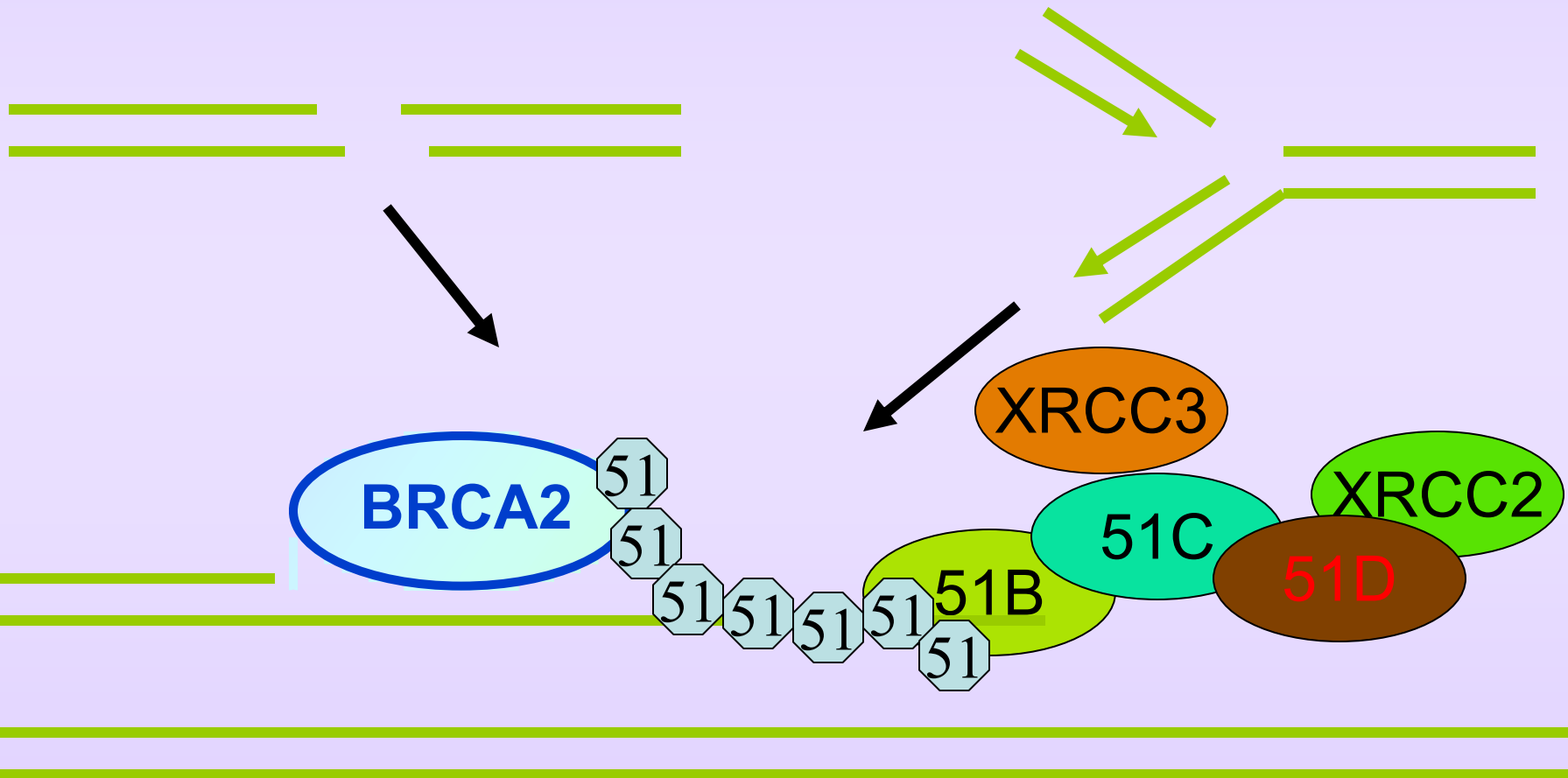Lawrence Livermore National Laboratory
Livermore, CA

Summary:

Homologous
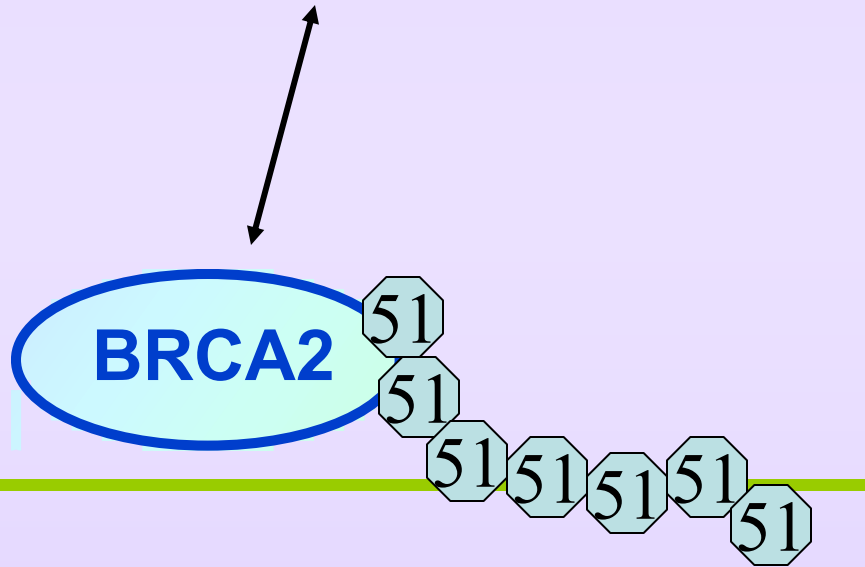Recombination ⟷ Fanconi anemia

Genome stability

Model discussion

Homologous Recombination repair:
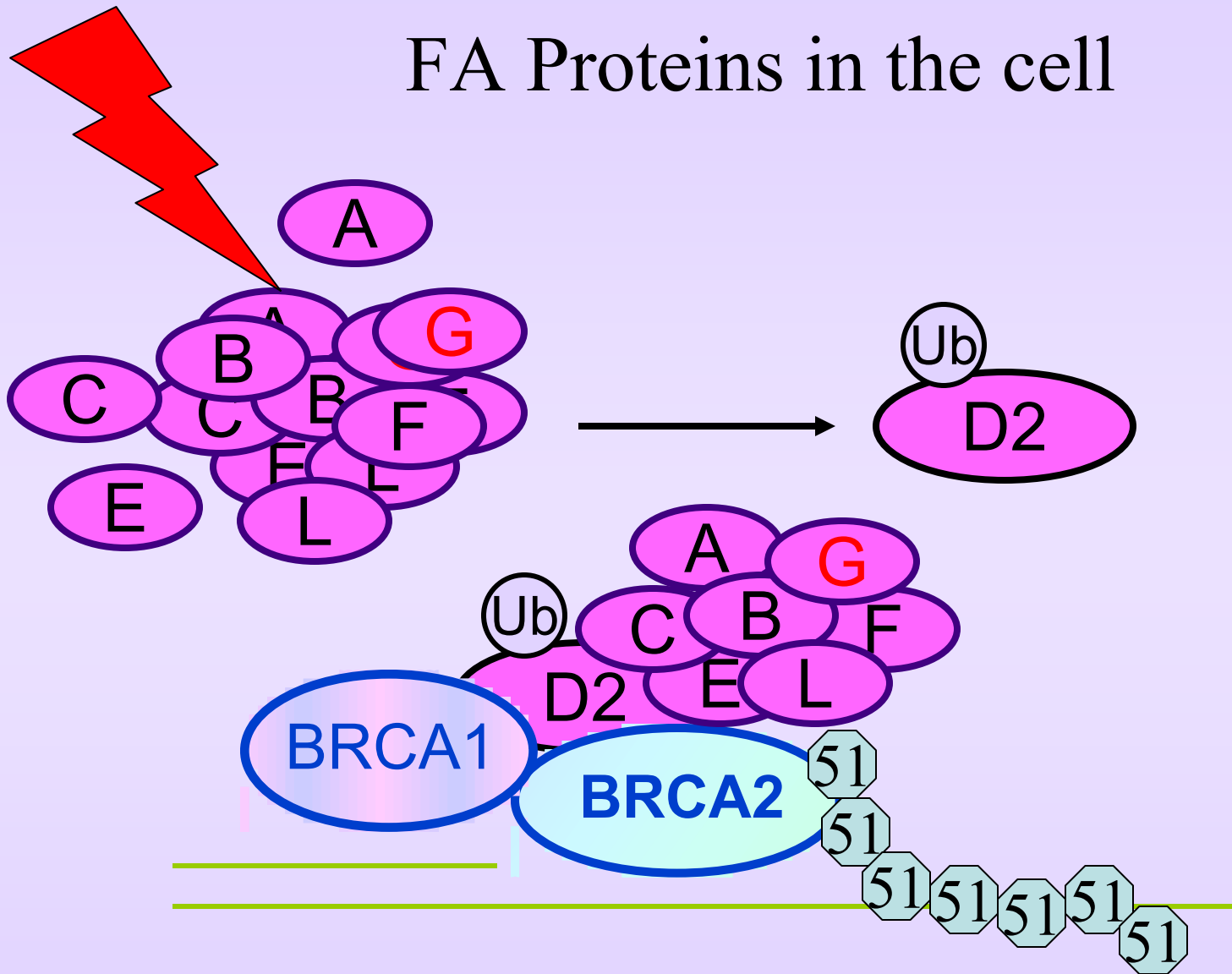
Repairing DSBs & restarting replication forks

Fanconi anemia complementation groups:

FancA, B, C, D1, D2, E, F, G, L

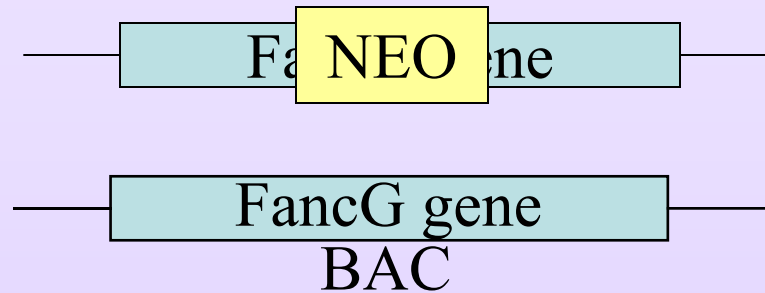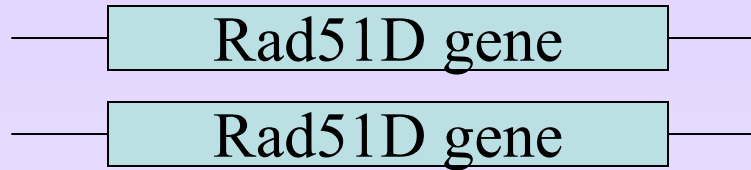# FA Proteins in the cell

Construction of a FancG knockout

AA8 — FancG gene

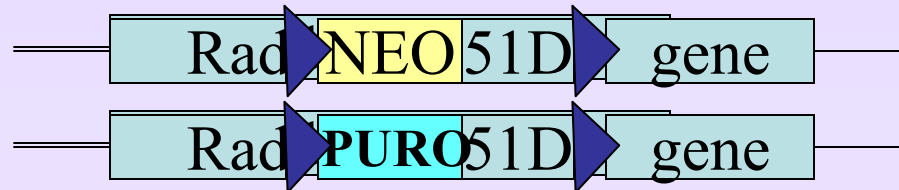**KO40** — FancG gene / NEO

BP6 — FancG gene / NEO

FancG gene

BAC

Construction of a Rad51D knockout

AA8

| Rad51D gene |

| Rad51D gene |

51D1Lox

Rad NEO 51D gene

Rad PURO 51D gene

51D1

Rad gene

Rad gene

51D1.3

| Rad51D gene |

BAC
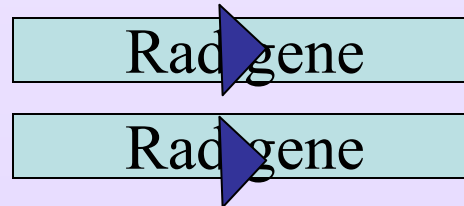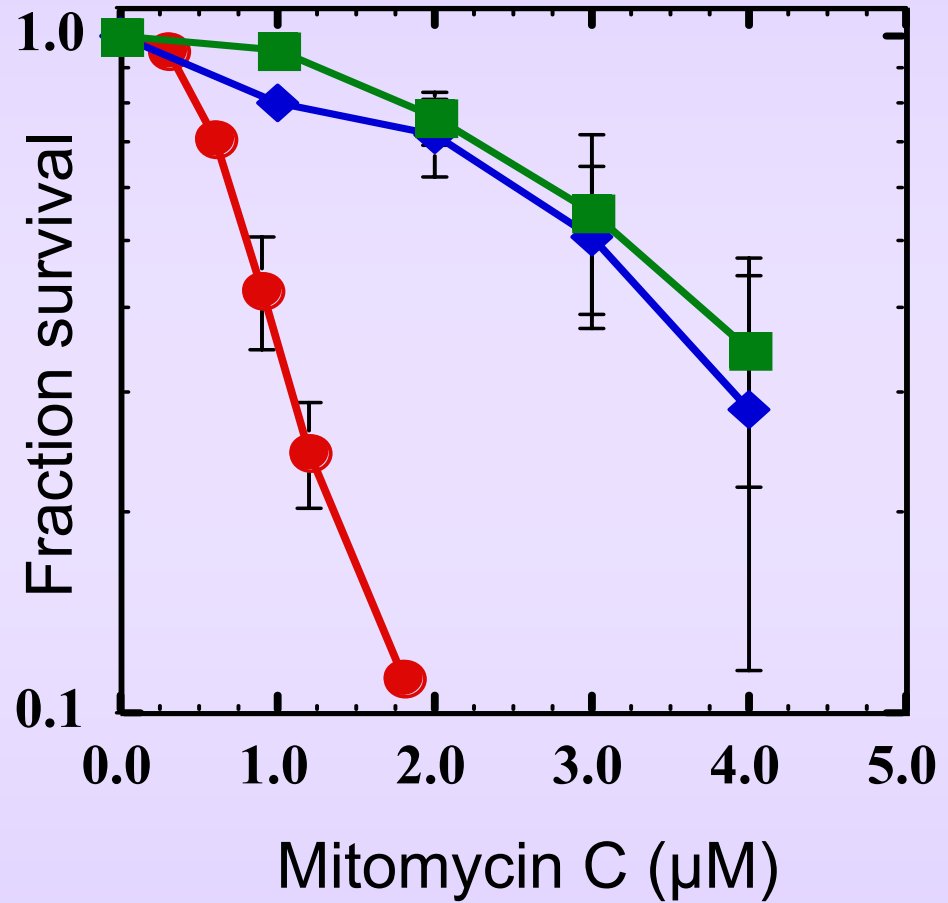
Colony Formation Assays: Cross-link sensitivity

AA8
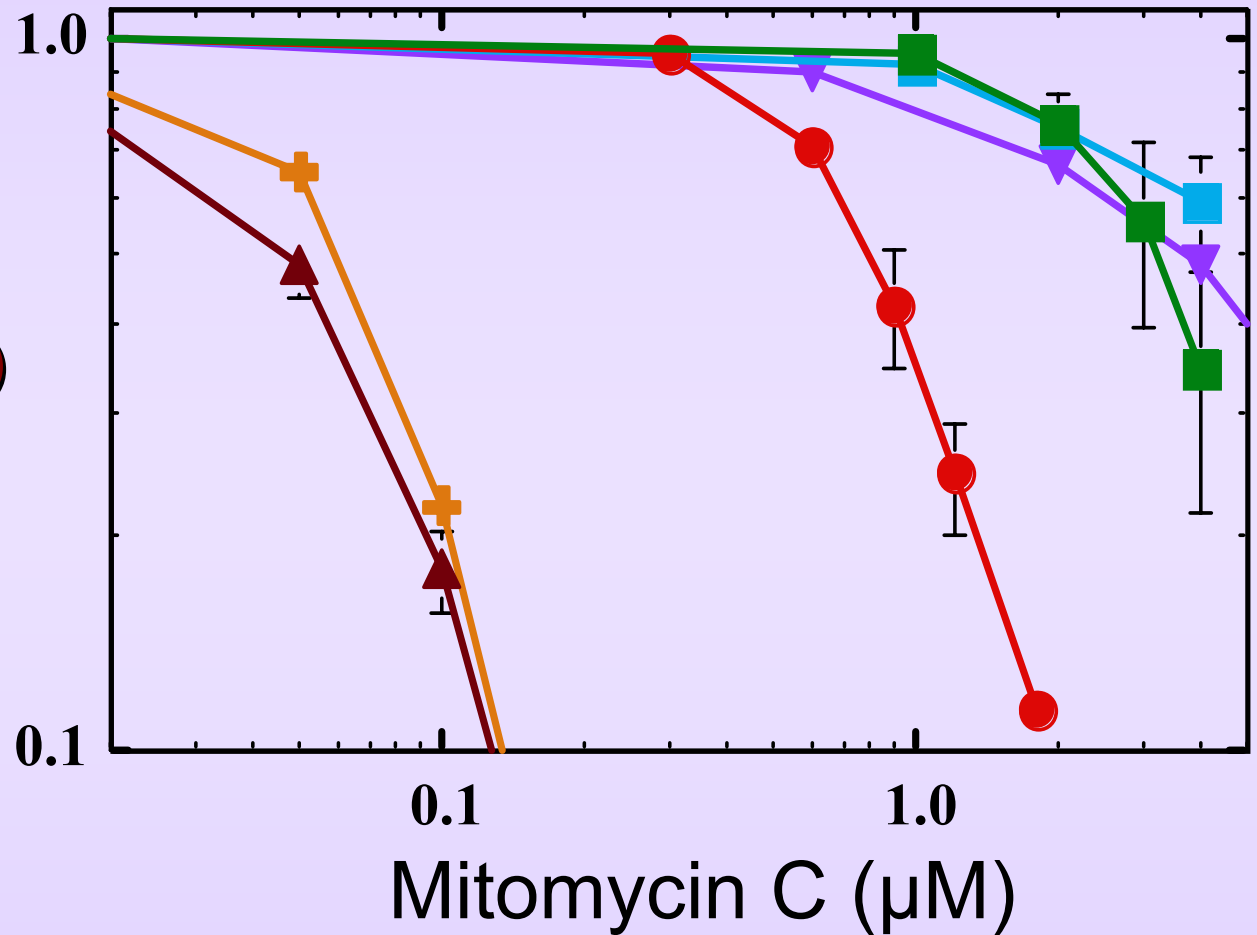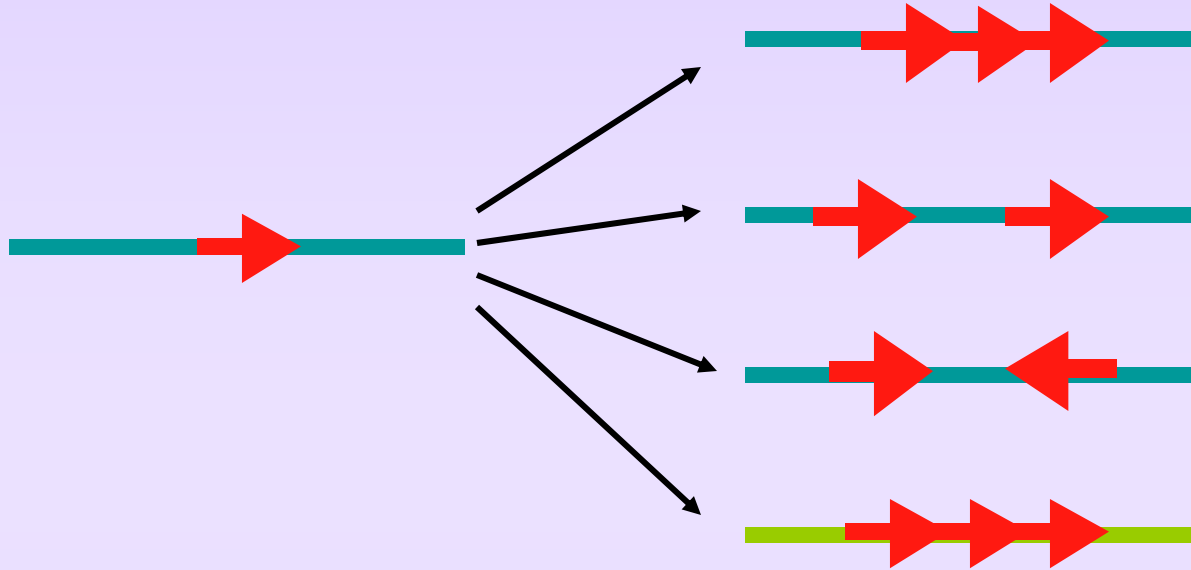KO40 (*fancg*)
BP6

AA8
51D1Lox

51D1 (*rad51d*)
irs1SF (*xrcc3*)

51D1.3

KO40 (*fancg*)

Mitomycin C (µM)

Gene Amplification

Importance of DSBs
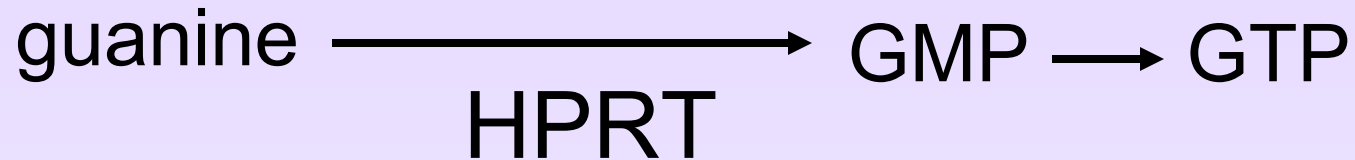
Amplifications drive tumorgenesis

Amplification Rate at *dhfr*
(units = mutations/*dhfr* locus/generation)

Cell line	Rate (x 10^{-6})	
AA8	8.8 ± 0.2	
KO40 (*fancg*)	27 ± 2	(↑~3x)
BP6	9 ± 2	
51D1Lox	7 ± 4	
51D1 (*rad51d*)	95 ± 4	(↑~10x)
irs1SF (*xrcc3*)	60 ± 14	(↑~7x)
51D1.3	3 ± 2	

Measuring mutation rate at *hprt*

guanine ⟶ GMP ⟶ GTP

HPRT

6-thioguanine ⟶ TOXIC

Large deletions/rearrangements not detectable

FancG mutant has decreased mutation rate

Mutation Rate at *hprt*

(units = mutations/hprt locus/generation)

Cell line	Rate (x 10^{-7})	
AA8	7 ± 1	
KO40 (*fancg*)	<2.0 ± .3	(⬇>3x)
BP6	4 ± 1	

HR mutants have increased mutation rate

Mutation Rate at *hprt*

Cell line	Rate (x 10^{-7})	
AA8	7 ± 1	
51D1Lox	5.0 ± .6	
51D1 (*rad51d*)	90 ± 20	(⬆~12x)
irs1SF (*xrcc3*)	220 ± 50	(⬆~28x)
51D1.3	6.8 ± .8	

Hprt mutation spectra
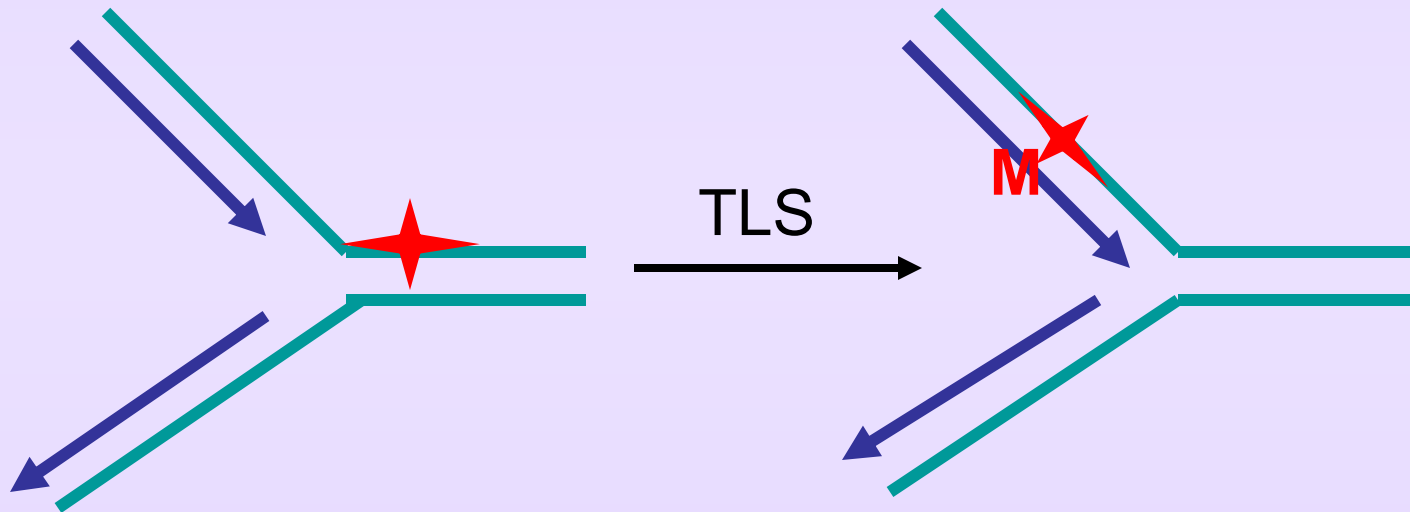
AA8 = ~70% point mutations

KO40 (*fancg*) = large deletion (1 clone)

51D1 (*rad51d*) = all deletions (12 clones)
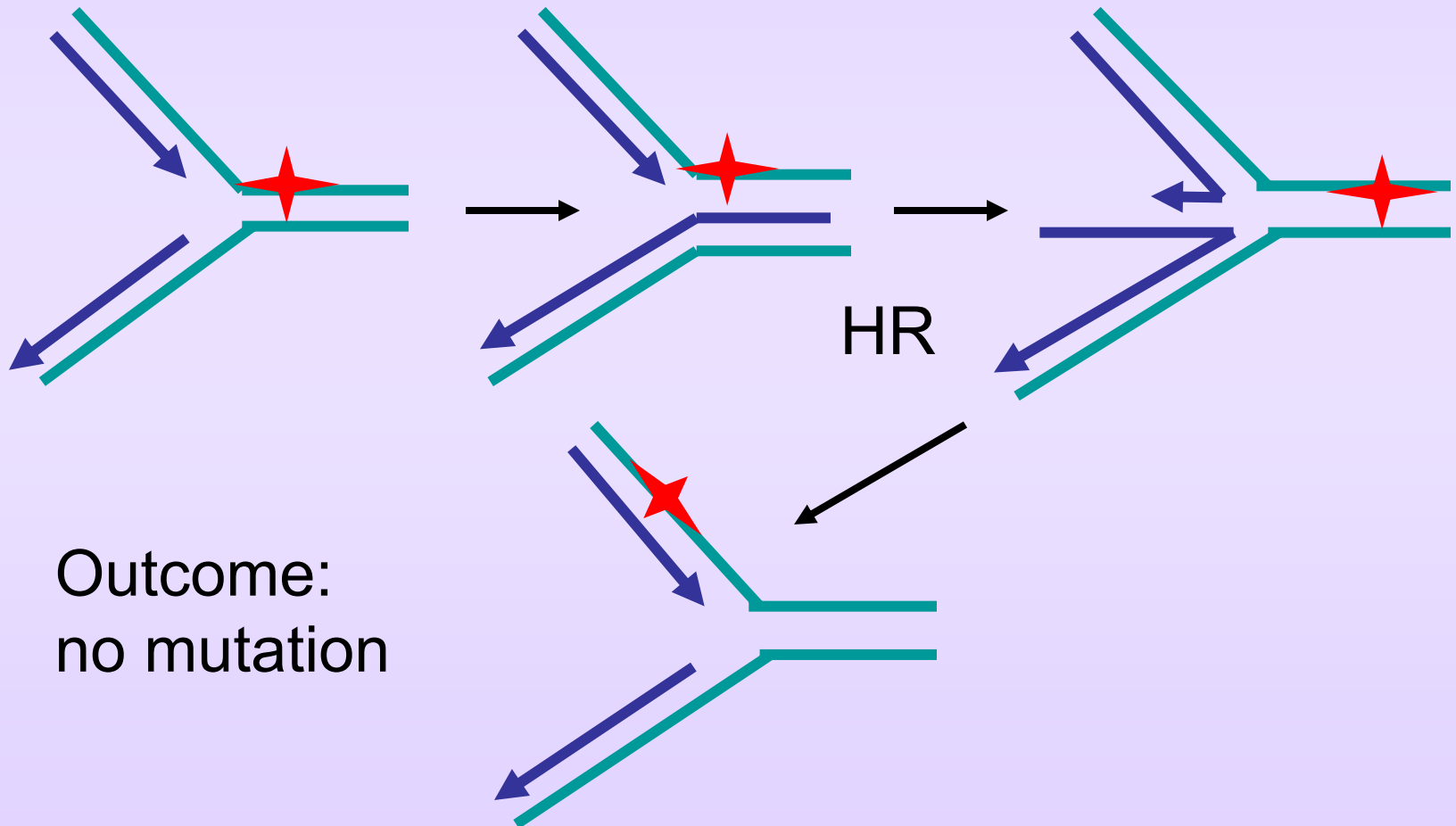
Role of FA and HR in mutagenesis?

Model involves replication forks
and lesion bypass

Replication forks bypassing a lesion:
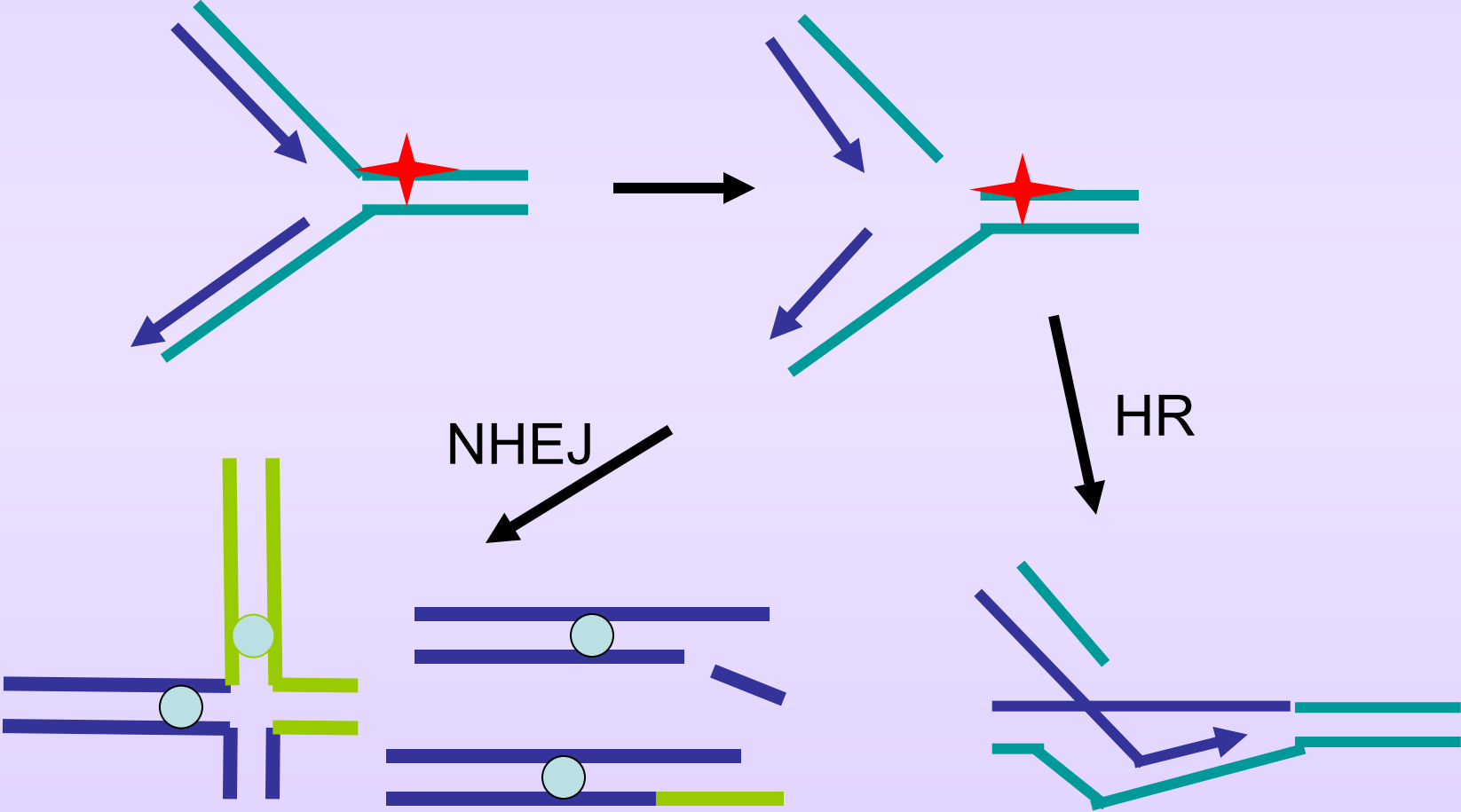
Trans-Lesion Synthesis (TLS)

TLS

M

Outcome: possible point mutation

Replication forks bypassing a lesion:
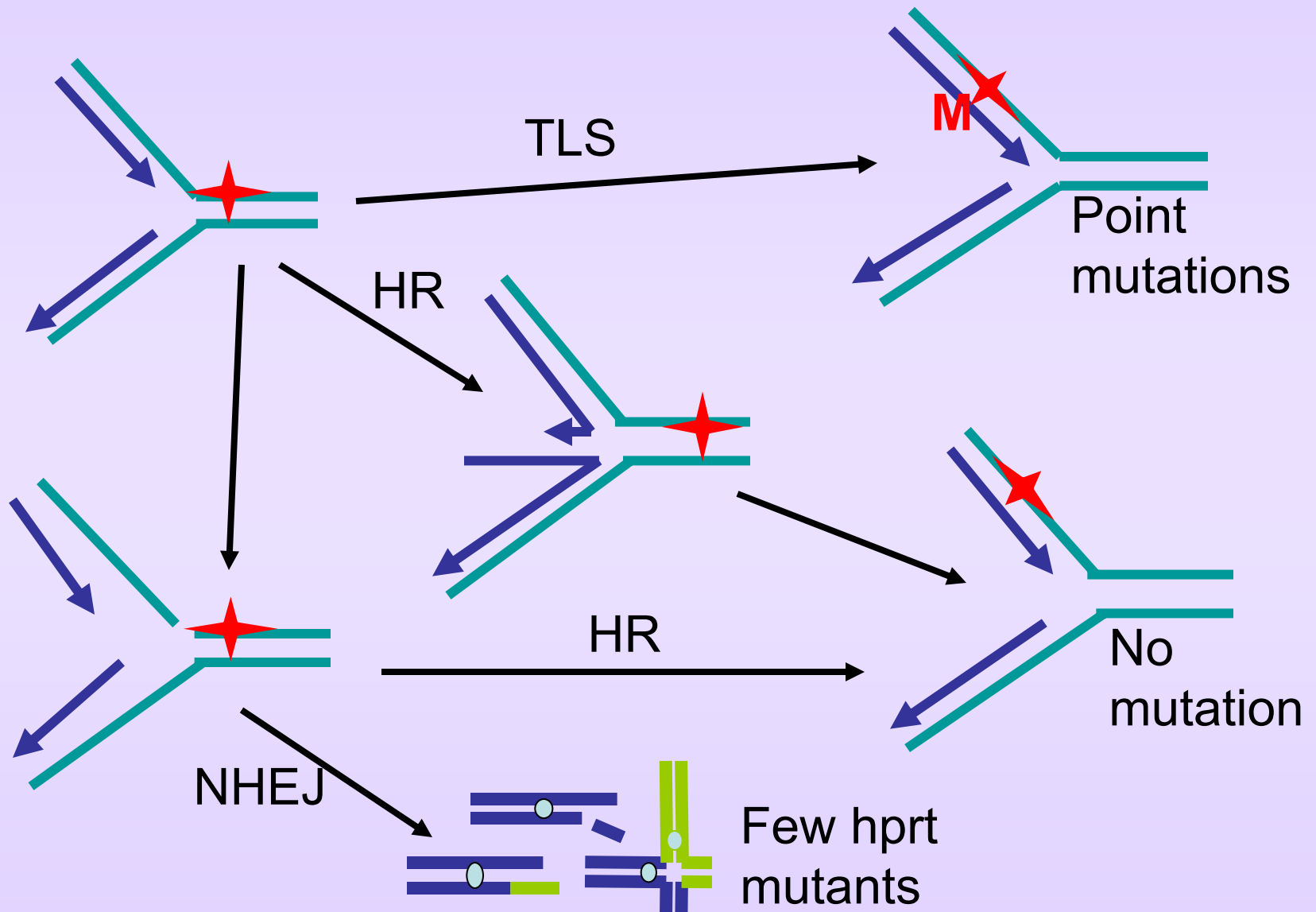
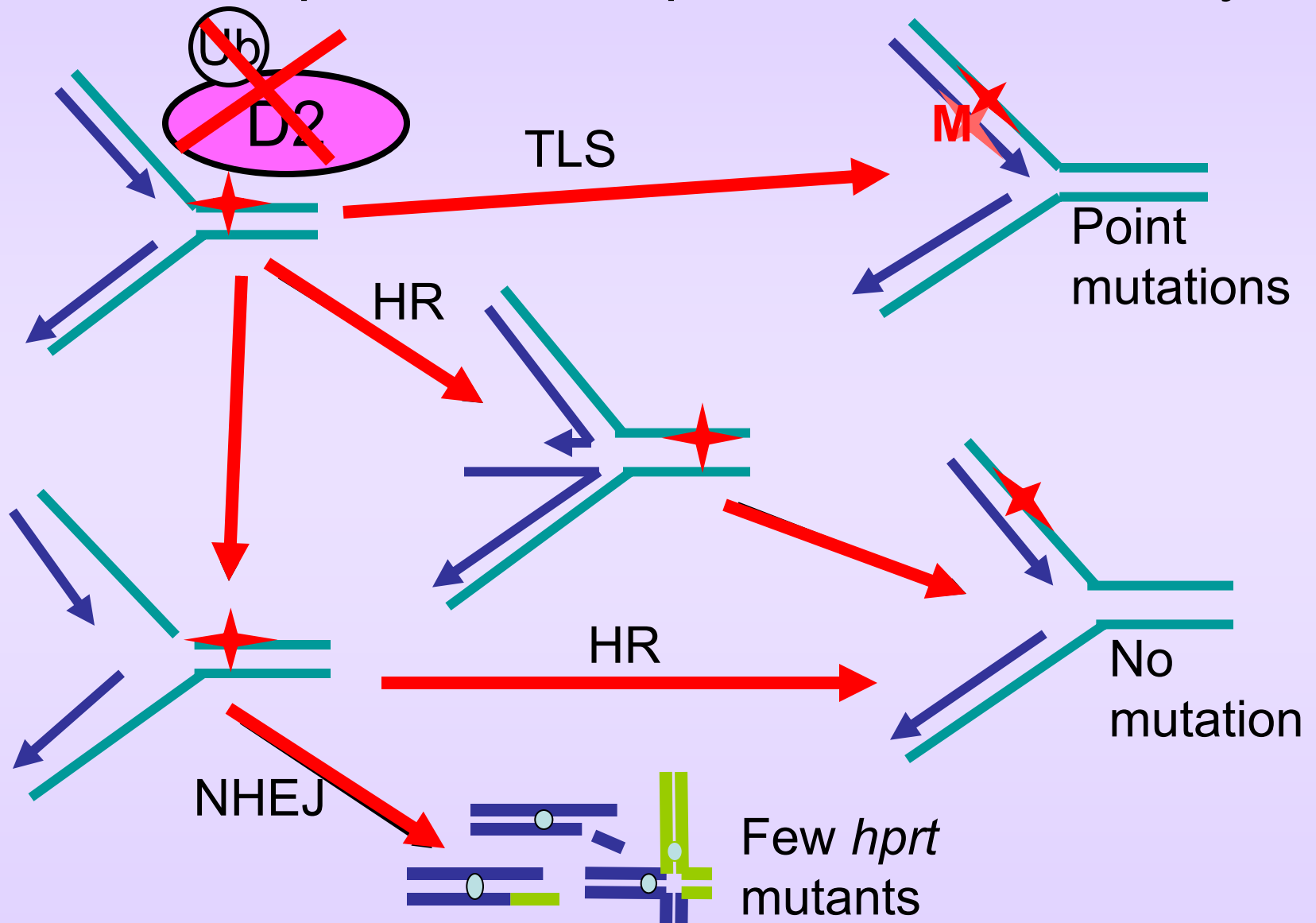Fork regression (Chicken foot structure)

HR

Outcome:
no mutation

Replication forks arresting at a lesion:

Fork breakage and collapse

Model: FA proteins in replication fork stability

TLS

M

Point
mutations

HR

HR

No
mutation

NHEJ

Few hprt
mutants

Model: FA proteins in replication fork stability

Ub

D2

TLS

M

Point
mutations

HR

HR

No
mutation

NHEJ

Few *hprt*
mutants

Model: HR proteins in replication fork stability
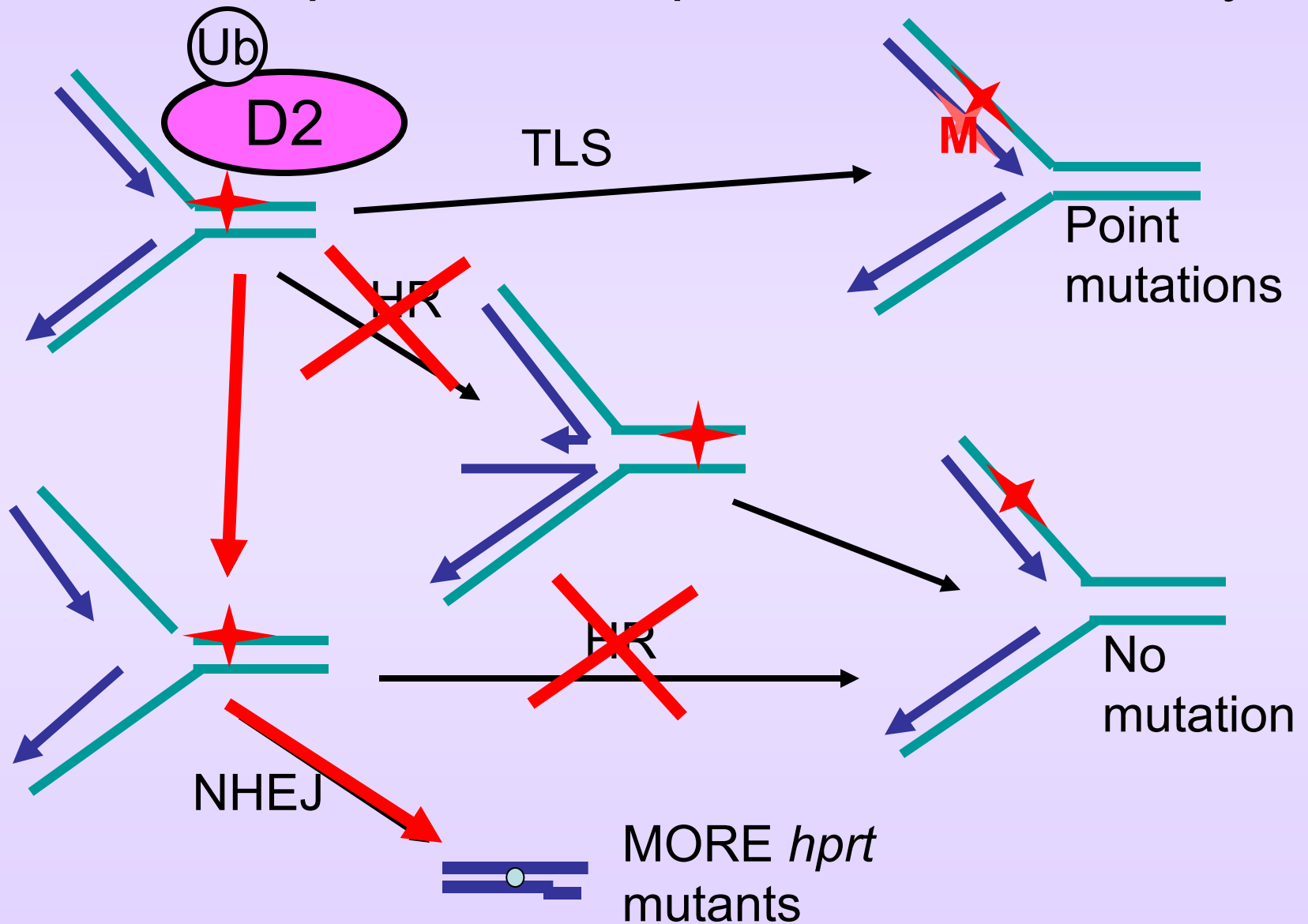
Acknowledgements

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