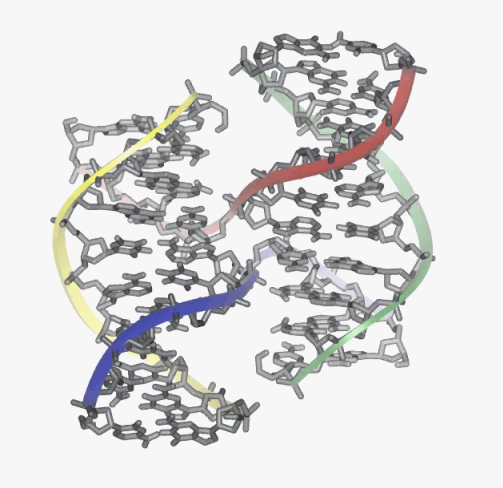
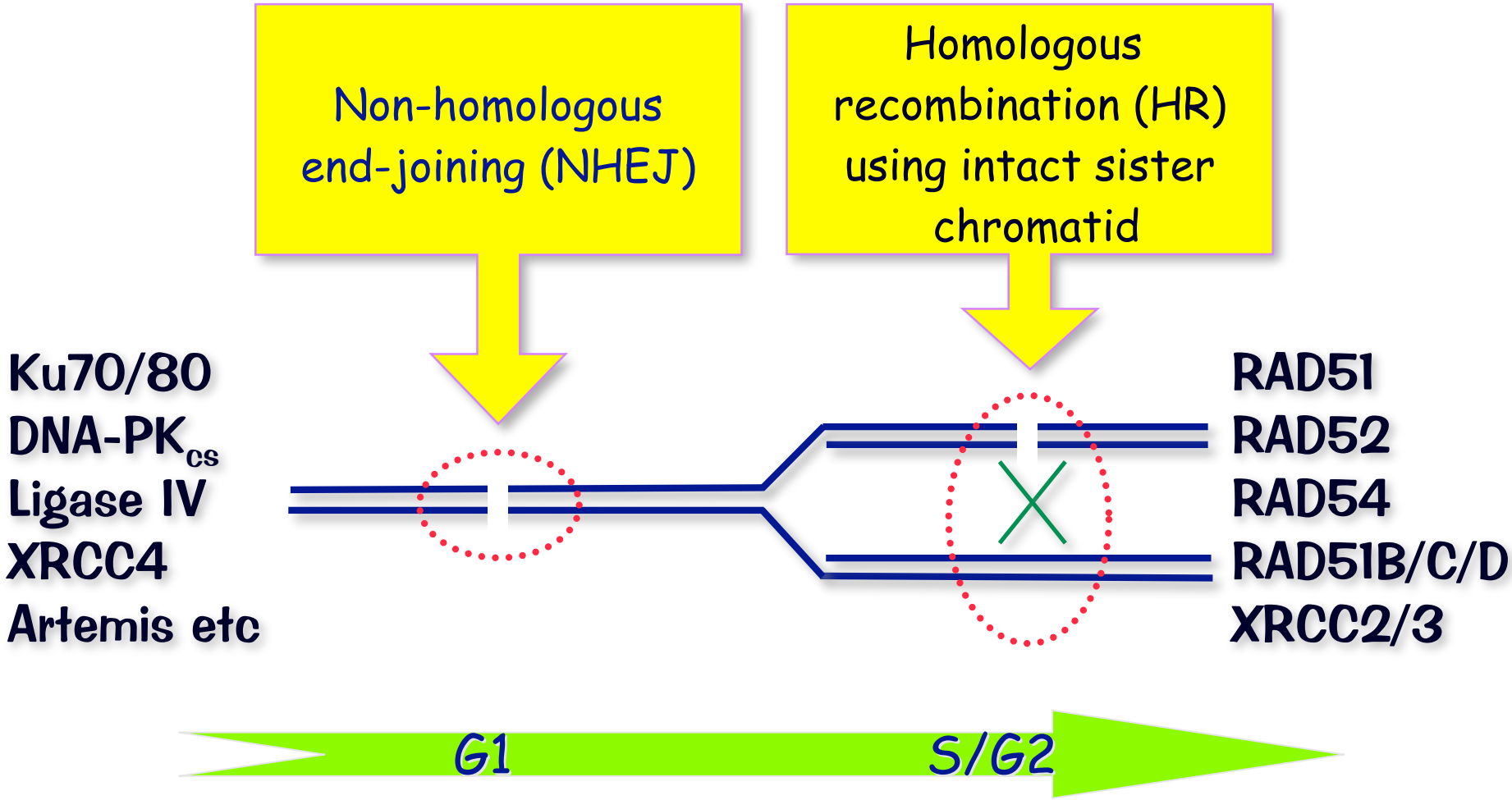


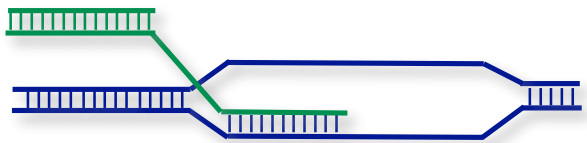
New insights into the Mechanisms of BRCA2-regulated DSBR

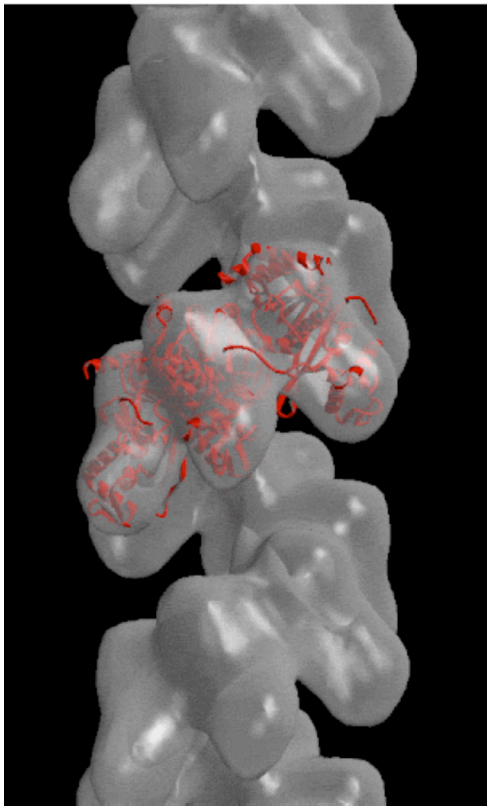
Steve West
Cancer Research UK
London Research Institute
Clare Hall Laboratories

Human Cells Have Two Primary Mechanisms for Double-Strand Break Repair



RAD51 Recombinase





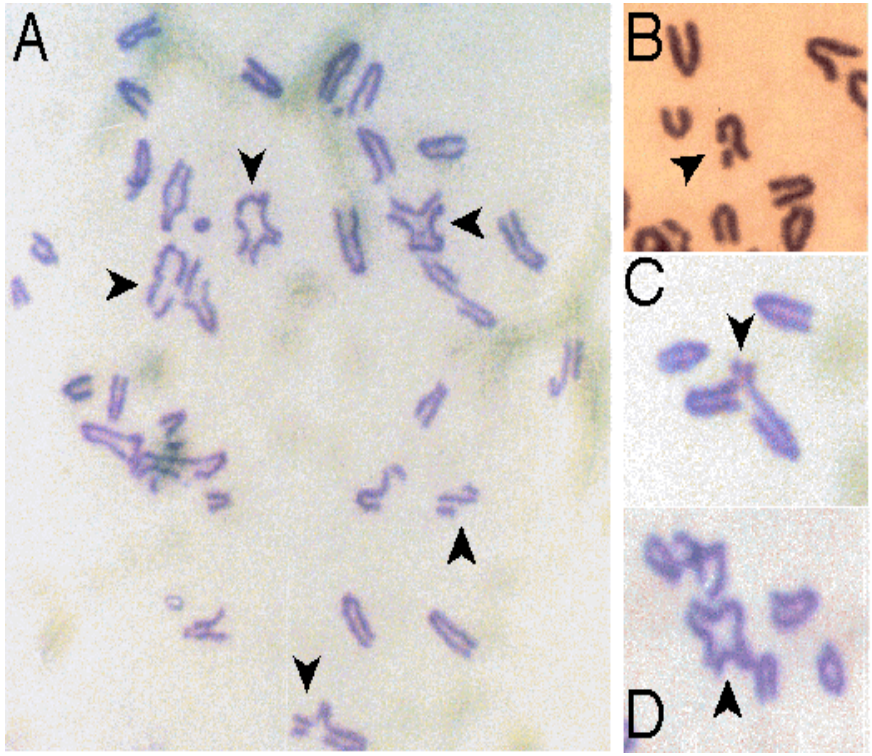
- Promotes strand invasion & exchange
- Highly conserved in bacteria, yeast, man
- Essential in mammalian cells (k/o's are embryonic lethal)
- Required for mitotic and meiotic recombination, DSBR
- In mammalian cells, RAD51's activities are controlled by BRCA2

Some basic facts about BRCA1 and BRCA2

- BRCA1/BRCA2 mutations predispose individuals to cancer at specific sites (breast, ovary, pancreas)
- Inheritance of 1 defective allele is sufficient for cancer predisposition
- Tumours from predisposed individuals show loss of heterozygosity
- Approx 70% of women carrying mutant BRCA1 or BRCA2 alleles will develop cancer

Genome Instability Associated with Mutations in BRCA1 or BRCA2

Yu et al.,
Genes Dev 2000



Chromosome breaks

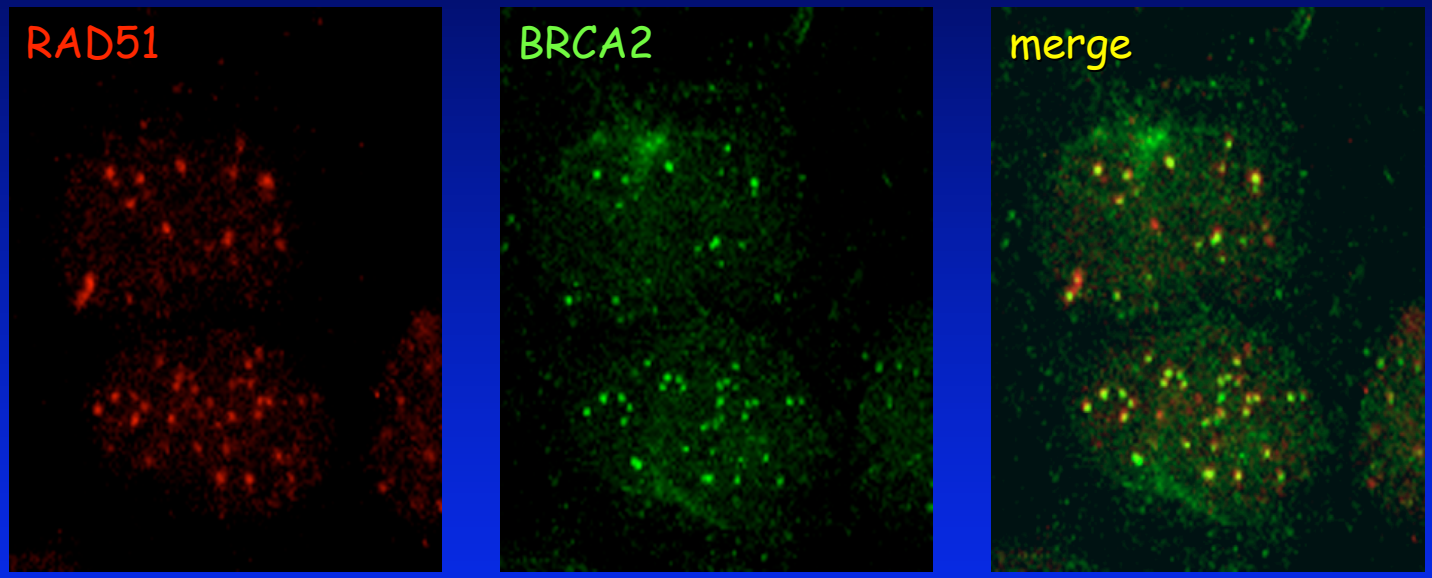
Triradials

Quadri-radials

BRCA2 deletions cause:

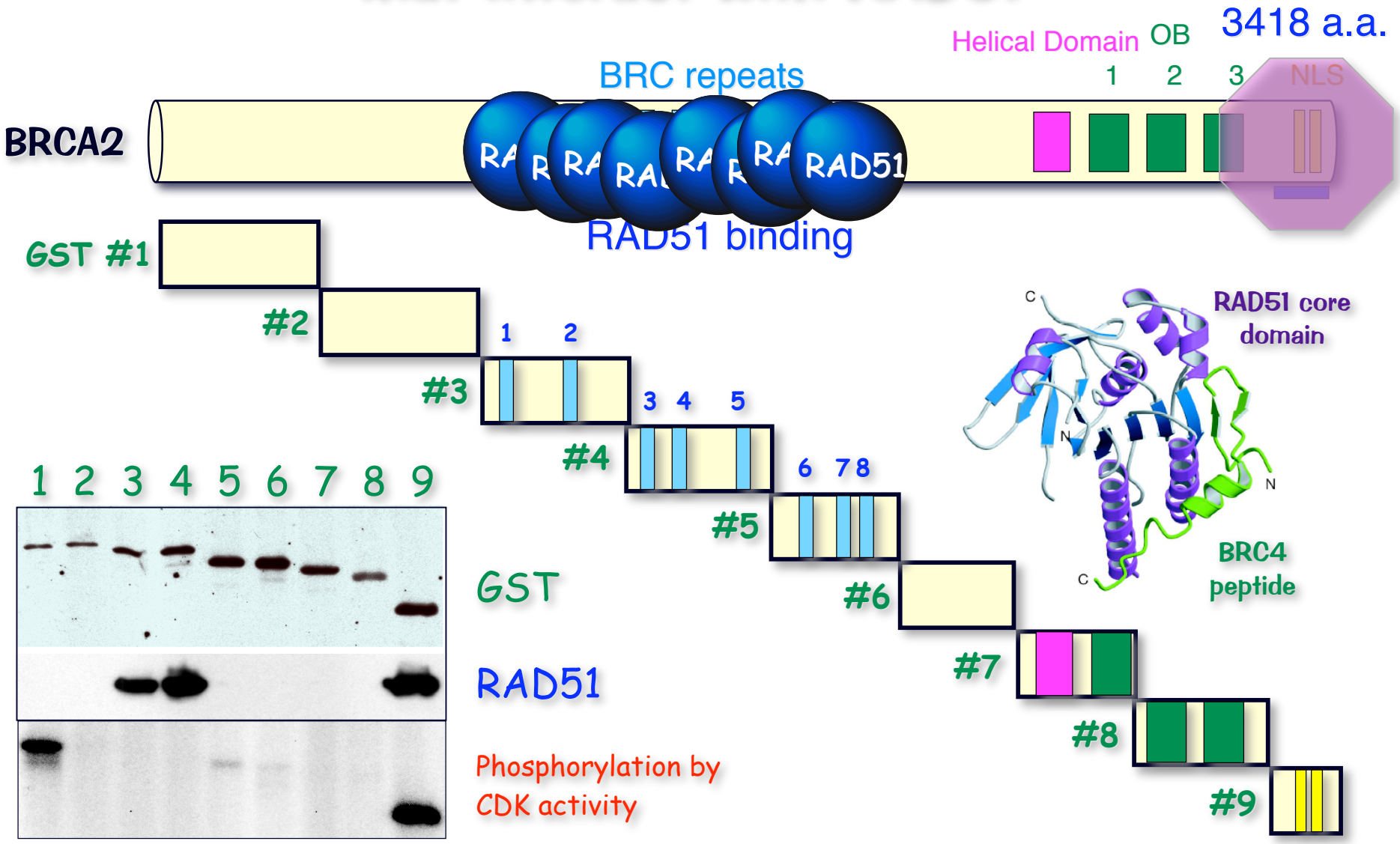
Spontaneous gross chromosomal rearrangements
X-ray sensitivity
Recombination deficiency

BRCA2 is Associated with the RAD51 Recombination Repair Protein

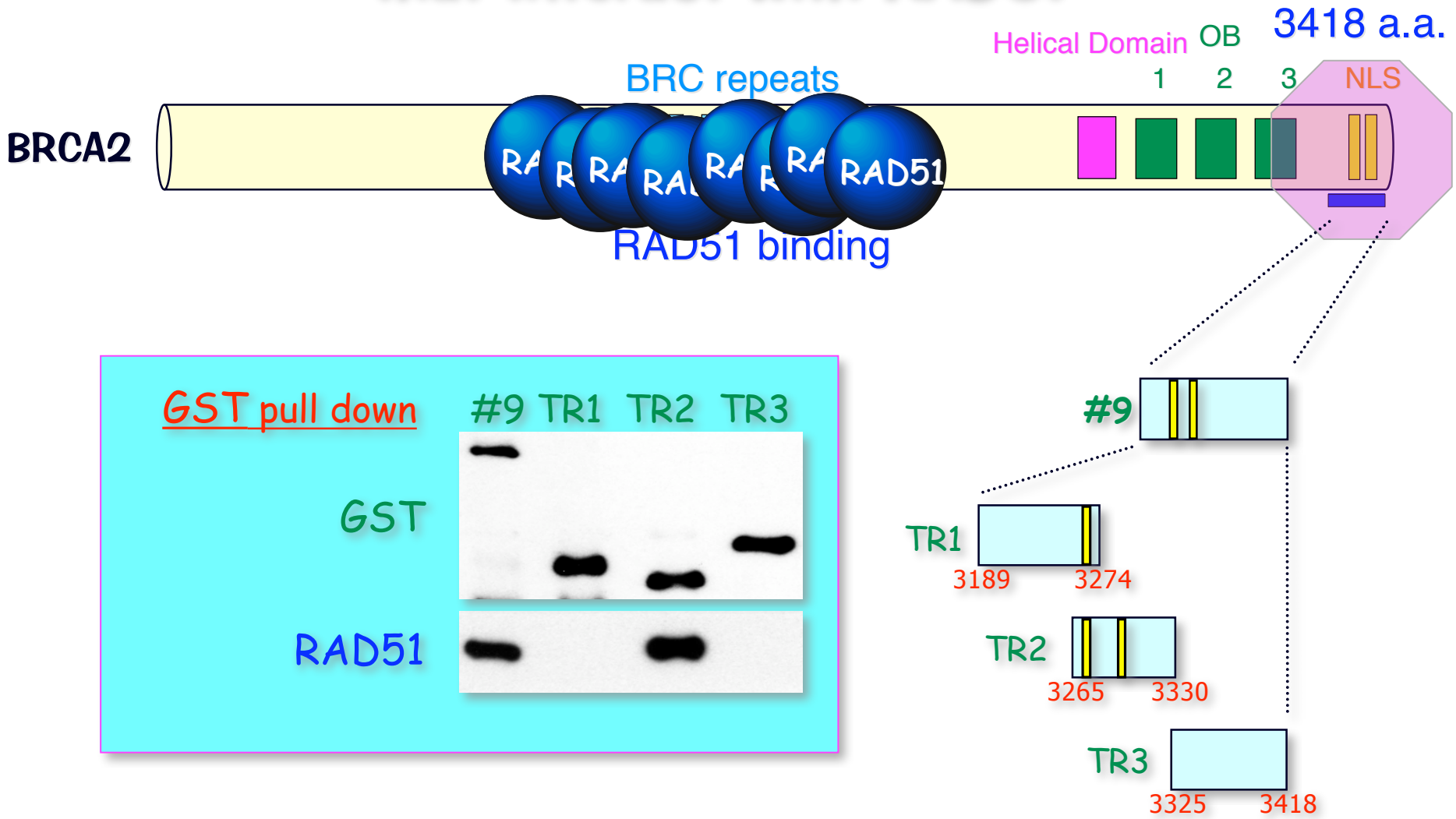


- BRCA2 interacts directly with RAD51
- BRCA2 colocalises with RAD51 to DNA-damage induced nuclear foci
- BRCA2 is required for the formation of RAD51 foci after DNA damage

Identification of Regions of BRCA2 that Interact with RAD51



Identification of Regions of BRCA2 that Interact with RAD51



S3291 of BRCA2 is phosphorylated by Cyclin-dependent kinase (CDKs)

```
                 ••                                  ••        ••                ••
Human   3189 PKWSTPTKDCTSGPYTAQIIPGT-----GNKLLMSSPNCEIYYQSPLSLCMAKRKSVSTPV 3244
Dog     3228 PKLSTPTKDYASEPHTAQIVLGI-----GNKFLMSSPNNEMNYQSPLSLCKPKEKSVPIPG 3283
Rat     3116 PKWSTPNKDPTREPYPASTCSASDLASGG-QLPRSSPTDQQSYRSPL C          AH 3175
Mouse   3107 SQVSTPNKDPTREPHAASTCCASDLLGSGGQFLRISPTGQQSYQSP              AH 3167
                                          ▼▼                ••      ••
Human   3245 SAQMTSKSCKGE-KEIDDQKNCKKRRALDFLSRLPLPPVSPICTFVSPAAQKAFQPPRSC 3304
Dog     3284 STQMTSKSYCKEEKEMDDPKTCKKRRALDFLSRVPLPPSVSPICTFVSPAAQKAFQPPRSC 3344
Rat     3176 SAWMAAKSCSGEN-EIEDPKTCRKKRALDLLSRLPLPPPLSPVCTFVSPAAQKAFQPPRSC 3235
Mouse   3168 SAQMAAKSWSGEN-EIDDPKTCRKRRALDFLSRLPLPSPVSPICTFVSPAAQKAFQPPRSC 3227
                 ••        ••  ••
Human   3305 GTKYETPIKKKELNSPQMTP-FKKFNEISLLESNSIADEELALINTQALLSGSTGEKQFIS 3364
Dog     3345 GTKYETLMKKE-LNSPQMTP--RKFNDLSLLESDSIADEELAMINTQALLLGSPGEHQLVS 3402
Rat     3236 GTKYPTPLKKEGPSSPWSRAPFQKASGVSLLDCDSVADEELALLSTQALVPHSVGGSEQVF 3296
Mouse   3228 GTKYATPIKKE-PSSPRRTP-FQKTSGVSLPDCDSVADEELALLSTQALTPDSVGGNEQAF 3286

Human   3365 VSESTRTAPTSSEDYLRLK-RRCTTSLIKEQESSQASTEECEKNKQDTITTKKYI       3418
Dog     3403 VSDSTRTAPTSSKDYLGLK-RHSTAPGVRGPESPQACTRKREPRVQNTSDLKRTI       3456
Rat     3297 PSDSTRTEGPSASTEARPANRSKRESLRDCRDDSDGKLAA-ETVPDYS-------       3343
Mouse   3287 PGDSTRNPQPAQRPDQQVGPRSRKESLRDCRGDSSEKLAV-ES------------       3328
```

S3291

•• ; (T/S)P, CDK target consensus sequence

▼▼ ; RxL, Cyclin recognition motif

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Mouse  3107 SQVSTPNKDPTREPHAASTCCASDLLGSGGQFLRISPTGQQSYQSP      AH 3167

Human  3245 SAQMTSKSCKGE-KEIDDQKNCKKRRALDFLSRLPLPPVSPICTFVSPAAQKAFQPPRSC 3304
Dog    3284 STQMTSKSYCKEEKEMDDPKTCKKRRALDFLSRVPLPPSVSPICTFVSPAAQKAFQPPRSC 3344
Rat    3176 SAWMAAKSCSGEN-EIEDPKTCRKKRALDLLSRLPLPPPLSPVCTFVSPAAQKAFQPPRSC 3235
Mouse  3168 SAQMAAKSWSGEN-EIDDPKTCRKRRALDFLSRLPLPSPVSPICTFVSPAAQKAFQPPRSC 3227

Human  3305 GTKYETPIKKKELNSPQMTP-FKKFNEISLI
Dog    3345 GTKYETLMKKE-LNSPQMTP--RKFNDLSLI
Rat    3236 GTKYPTPLKKEGPSSPWSRAPFQKASGVSLI
Mouse  3228 GTKYATPIKKE-PSSPRRTP-FQKTSGVSLI

Human  3365 VSESTRTAPTSSEDYLRLK-RRCTTSLIKEQ
Dog    3403 VSDSTRTAPTSSKDYLGLK-RHSTAPGVRGI
Rat    3297 PSDSTRTEGPSASTEARPANRSKRESLRDCI
Mouse  3287 PGDSTRNPQPAQRPDQQVGPRSRKESLRDCI
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Rat    3236 GTKYPTPLKKEGPSSPWSRAP                                       QVF 3296
Mouse  3228 GTKYATPIKKE-PSSPRRTP-FQKTSGVSLPDCDSVADEELALLSTQALTPDSVGGNEQAF 3286

Human  3365 VSESTRTAPTSSEDYLRLK-RRCTTSLIKEQESSQASTEECEKNKQDTITTKKYI       3418
Dog    3403 VSDSTRTAPTSSKDYLGLK-RHSTAPGVRGPESPQACTRKREPRVQNTSDLKRTI       3456
Rat    3297 PSDSTRTEGPSASTEARPANRSKRESLRDCRDDSDGKLAA-ETVPDYS-------       3343
Mouse  3287 PGDSTRNPQPAQRPDQQVGPRSRKESLRDCRGDSSEKLAV-ES------------       3328
```

S3291

K

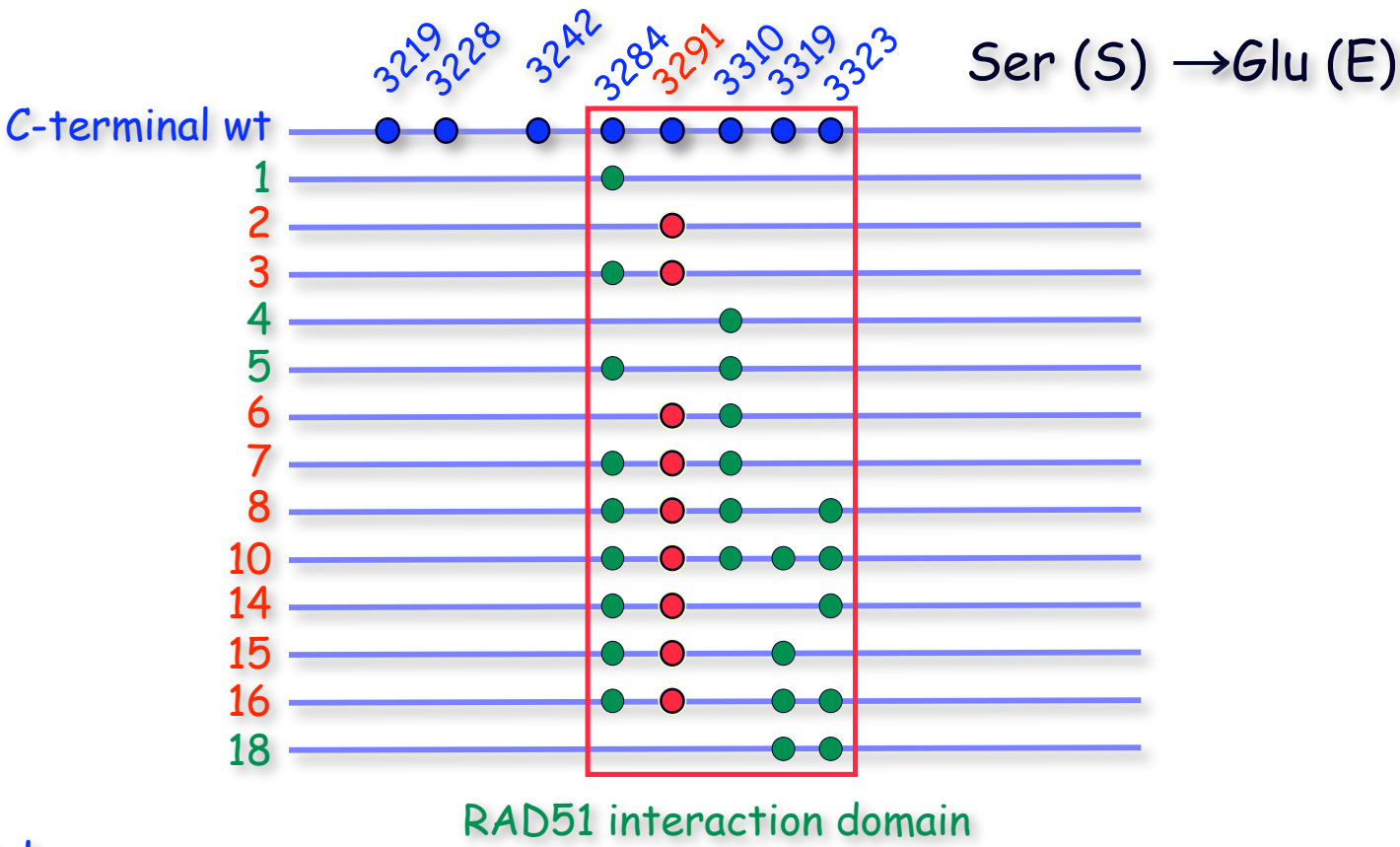
L

Tumour associated mutations

•• ; (T/S)P, CDK target consensus sequence

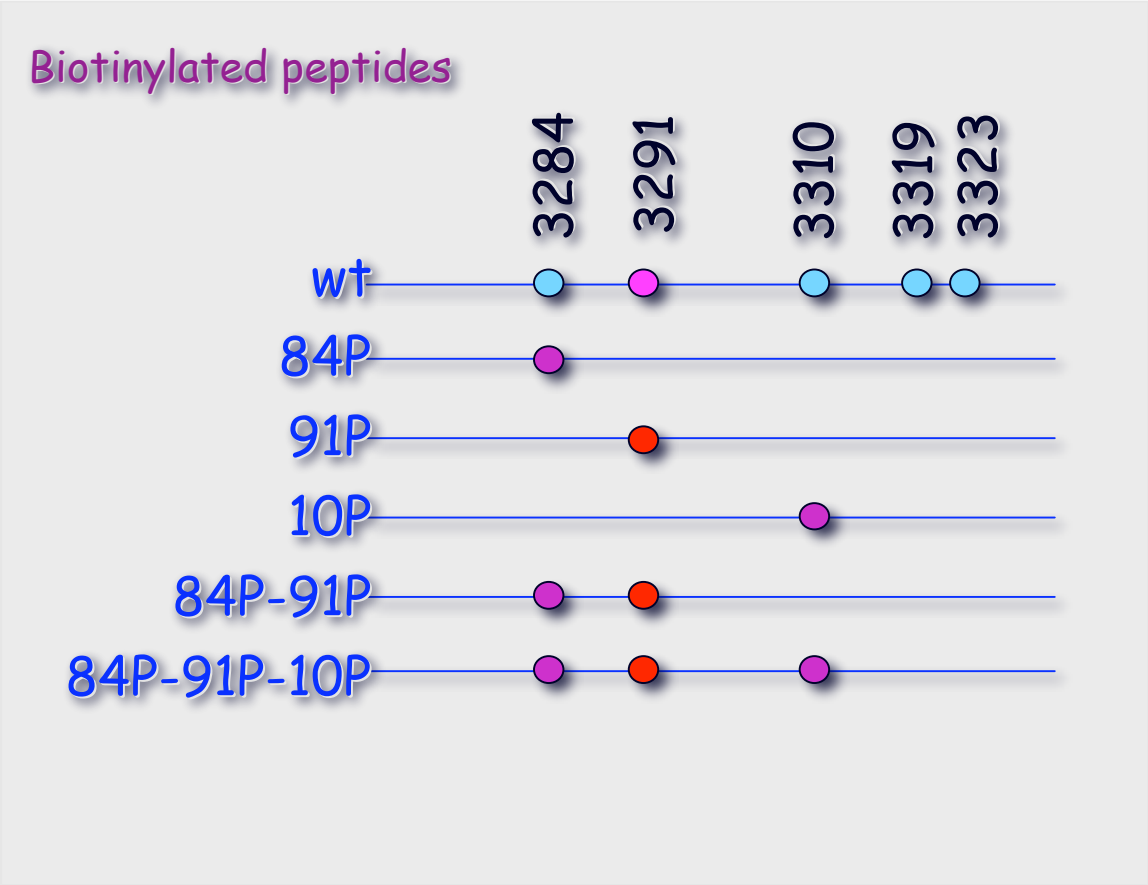
▼▼ ; RxL, Cyclin recognition motif

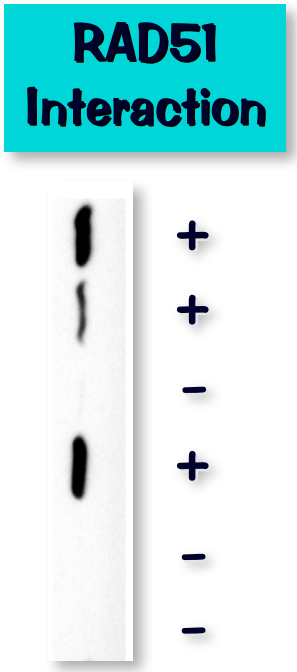
The BRCA2 S3291E Mutant Fails to Interact with RAD51



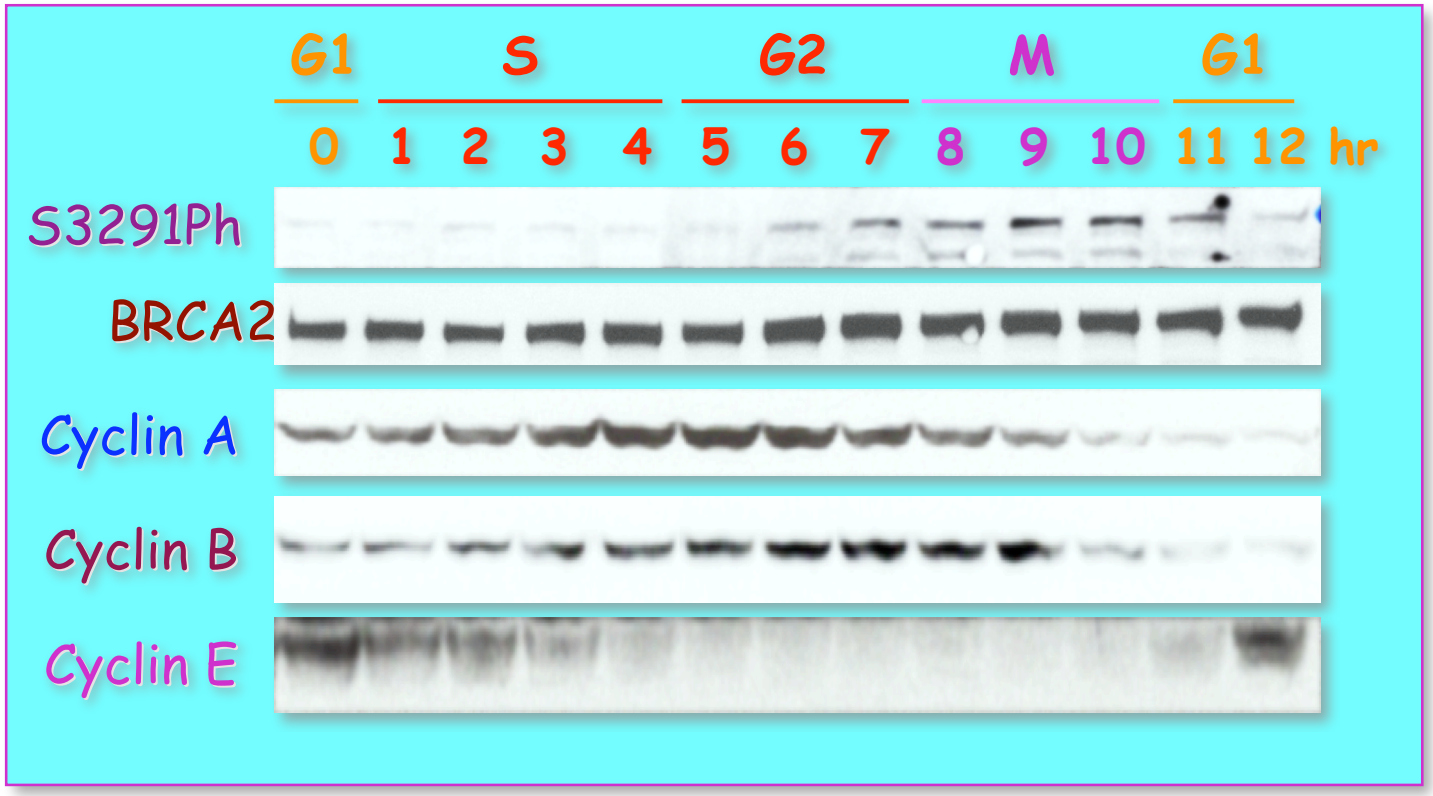
GST pull down

Phosphorylation of S3291 of BRCA2 Blocks Interaction with RAD51

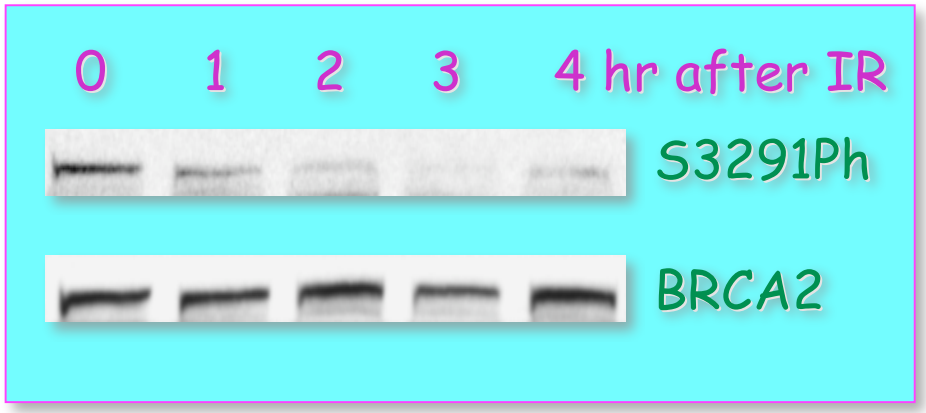


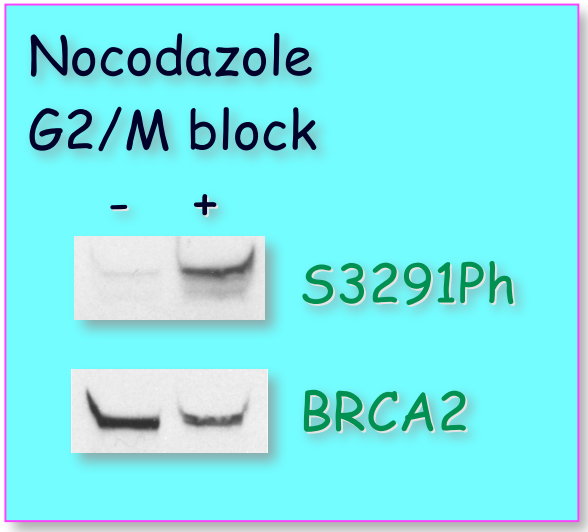


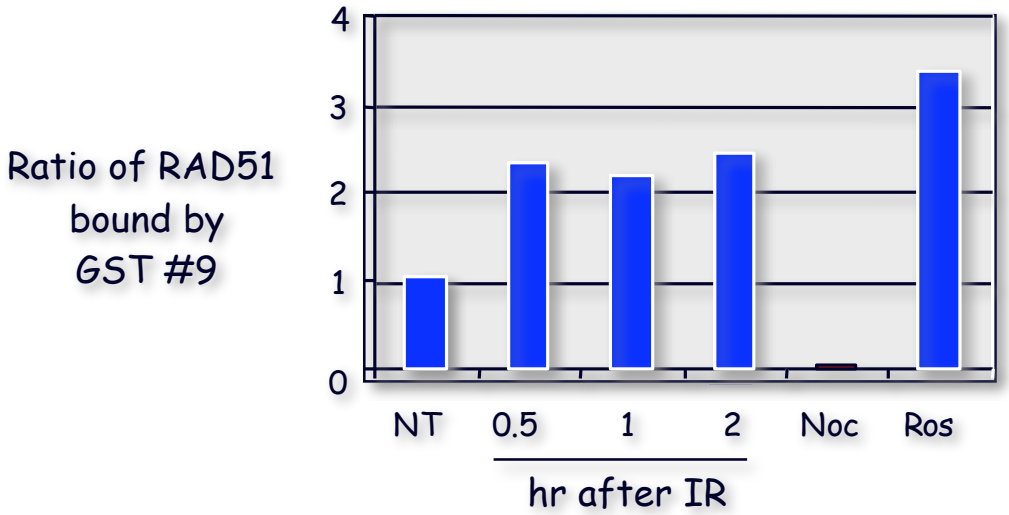
Regulation of S3291 Phosphorylation of BRCA2 Throughout the Cell Cycle



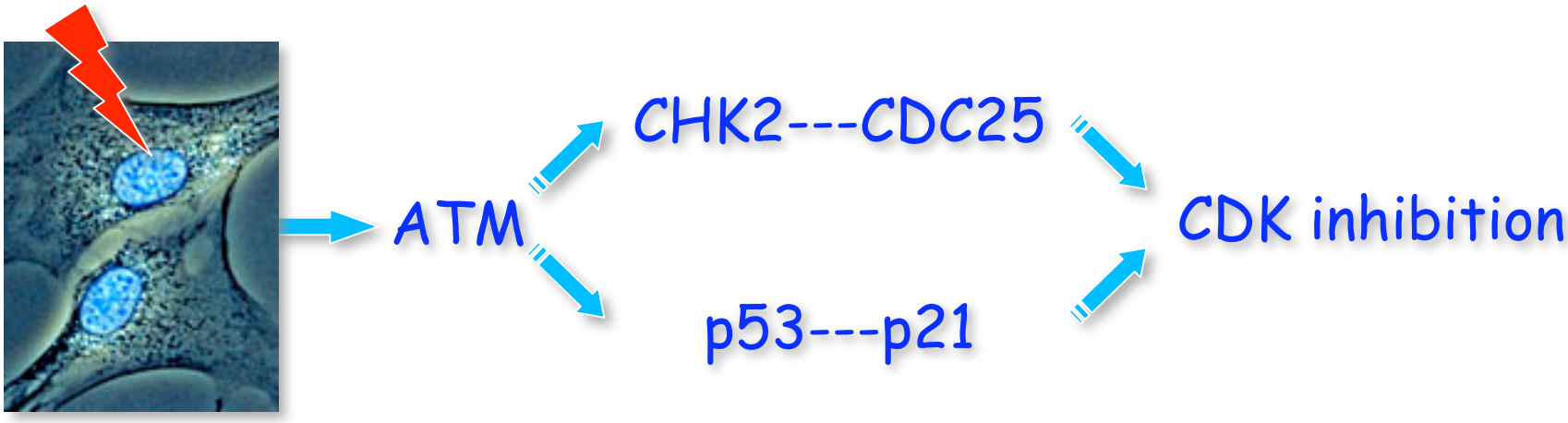
Modulation of S3291 Phosphorylation by Radiation Treatment and Cell Cycle

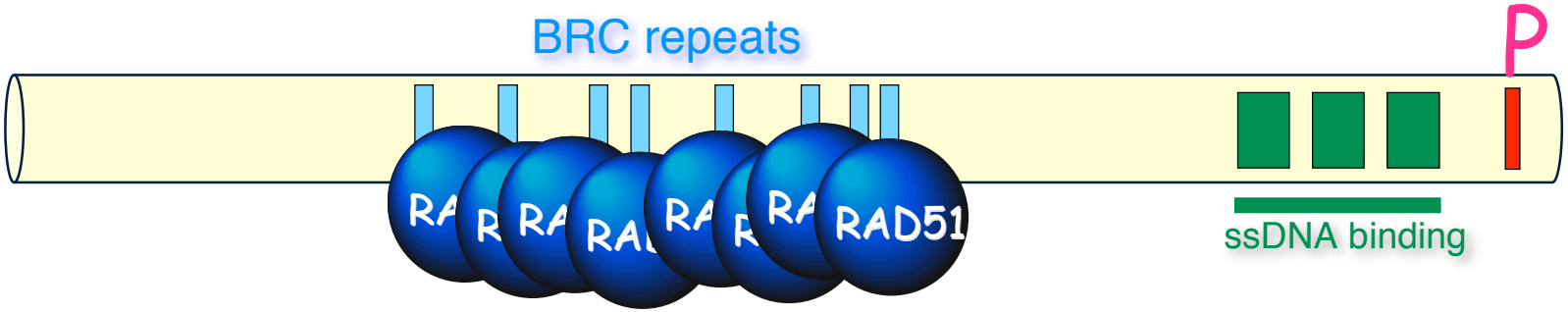






Model for the Control of RAD51 by BRCA2





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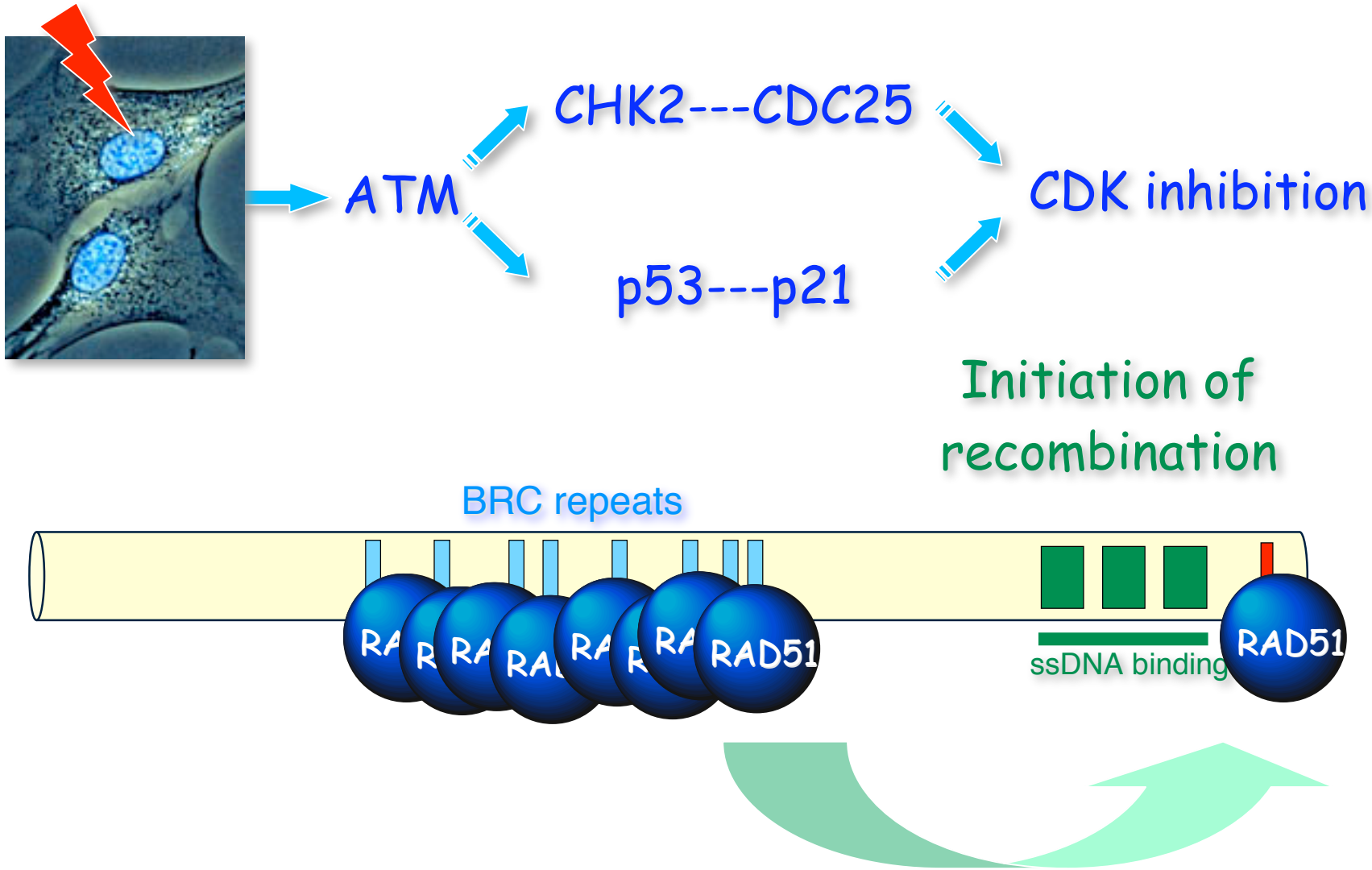
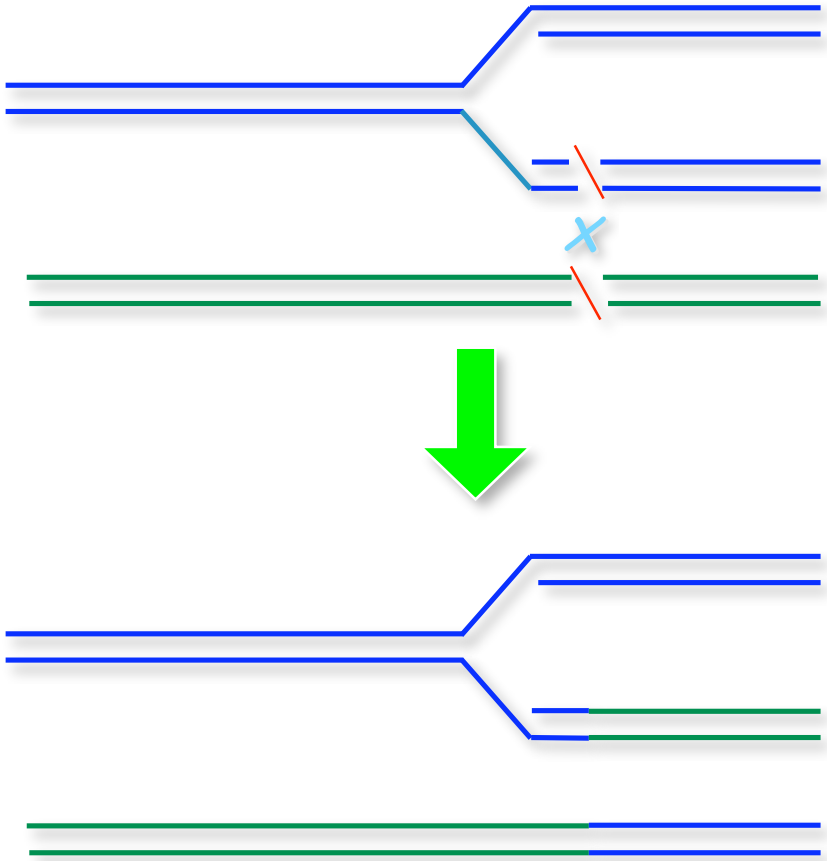
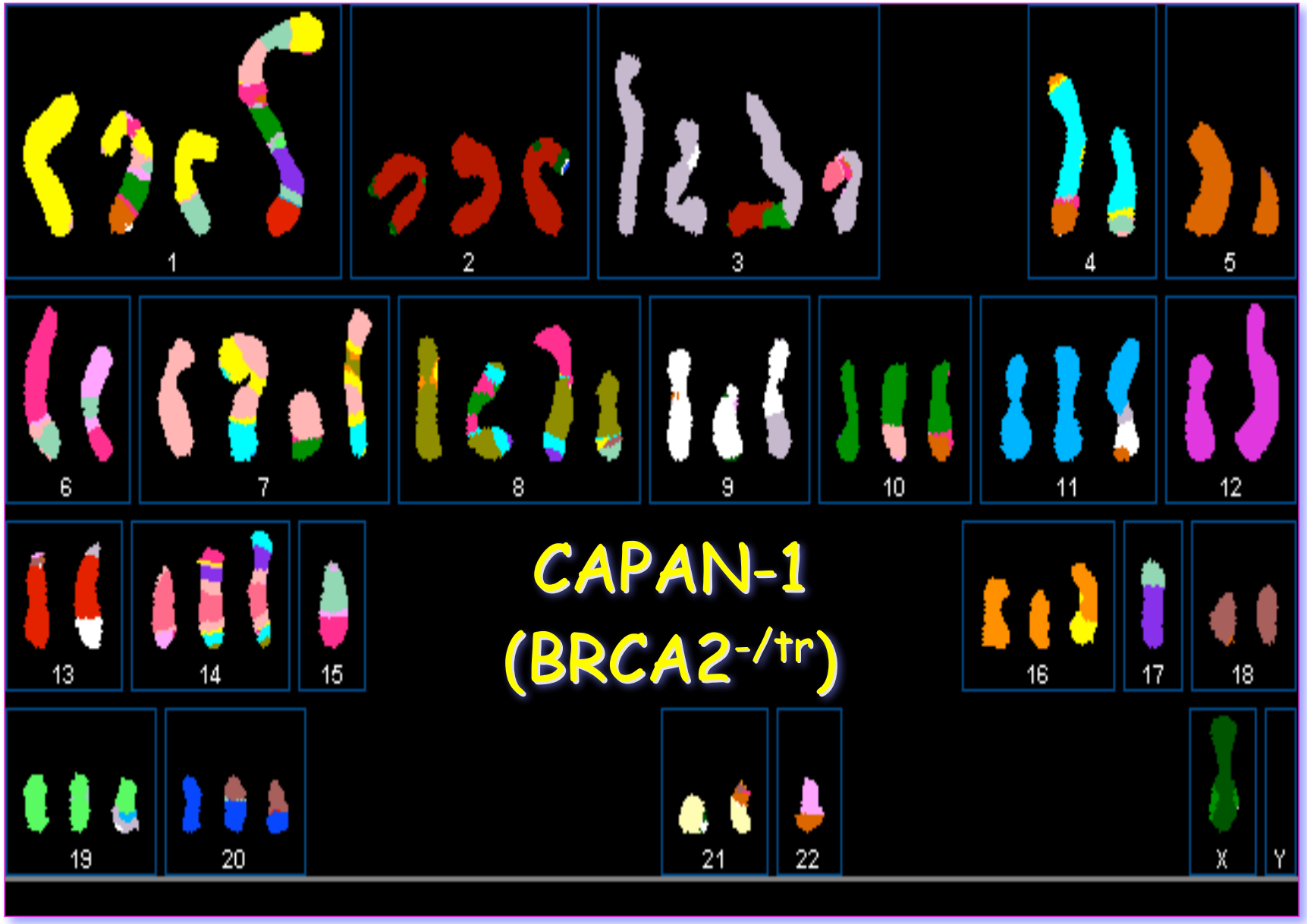


Defective Homologous Recombination Leads to Chromosomal Instability

Inaccurate repair by non-homologous end-joining will fuse ends from different chromosomes

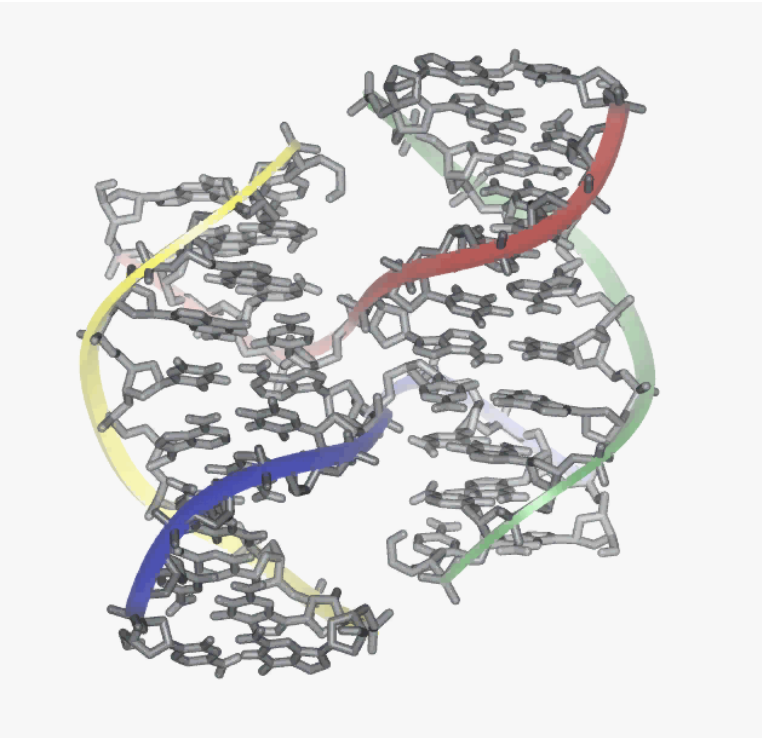


Chromosome Rearrangements



Photograph courtesy of Joanne Davidson & Paul Edwards (Cambridge)

Holliday Junction Resolution in Human Cells



3-D structure of a HJ
[Eichman et al 2000]

1990:

HJ resolvase activity detected in extracts

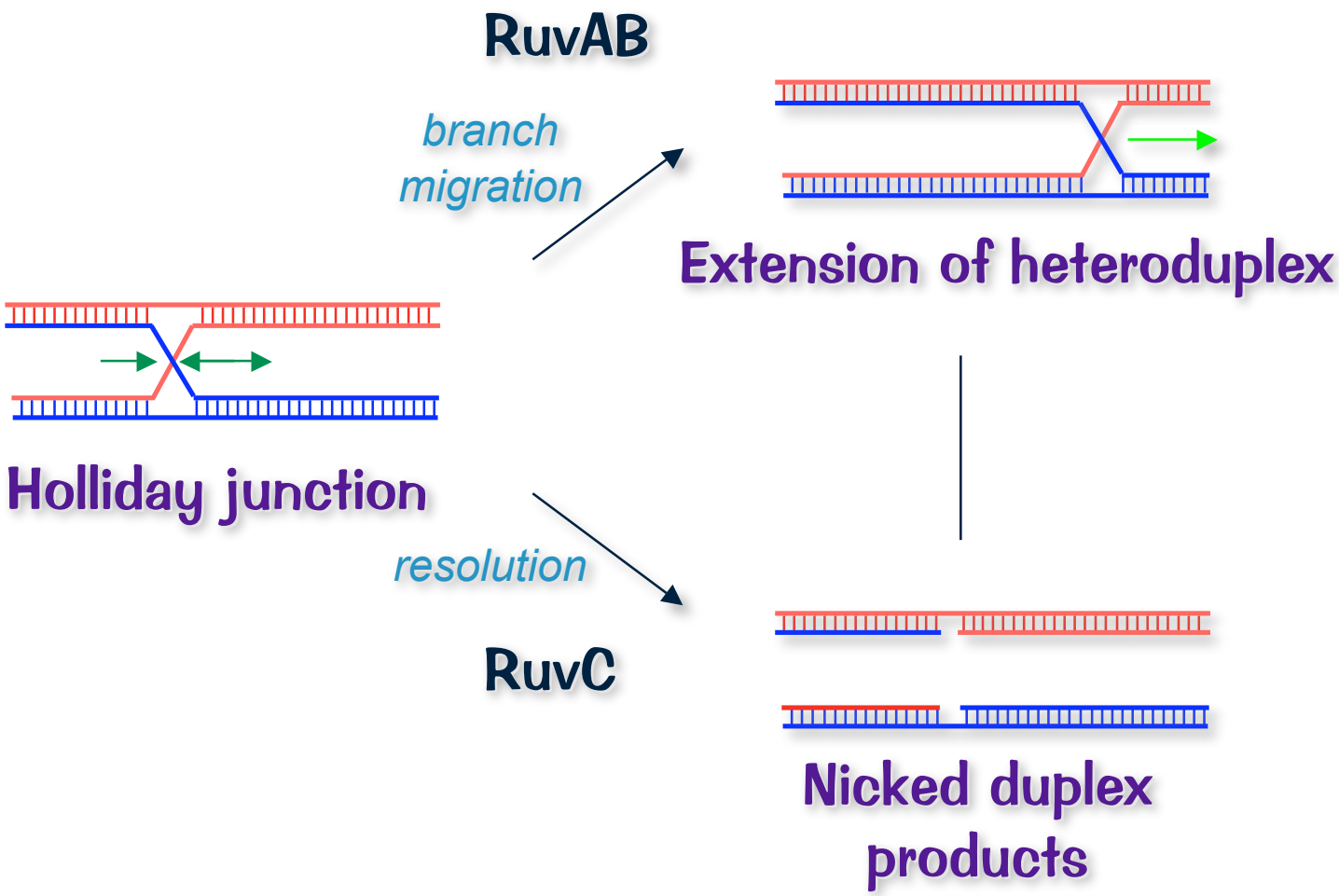
The activity fits *E. coli* RuvC paradigm

- Holliday junction specific
- Symmetric cleavage
- Ligatable nicks

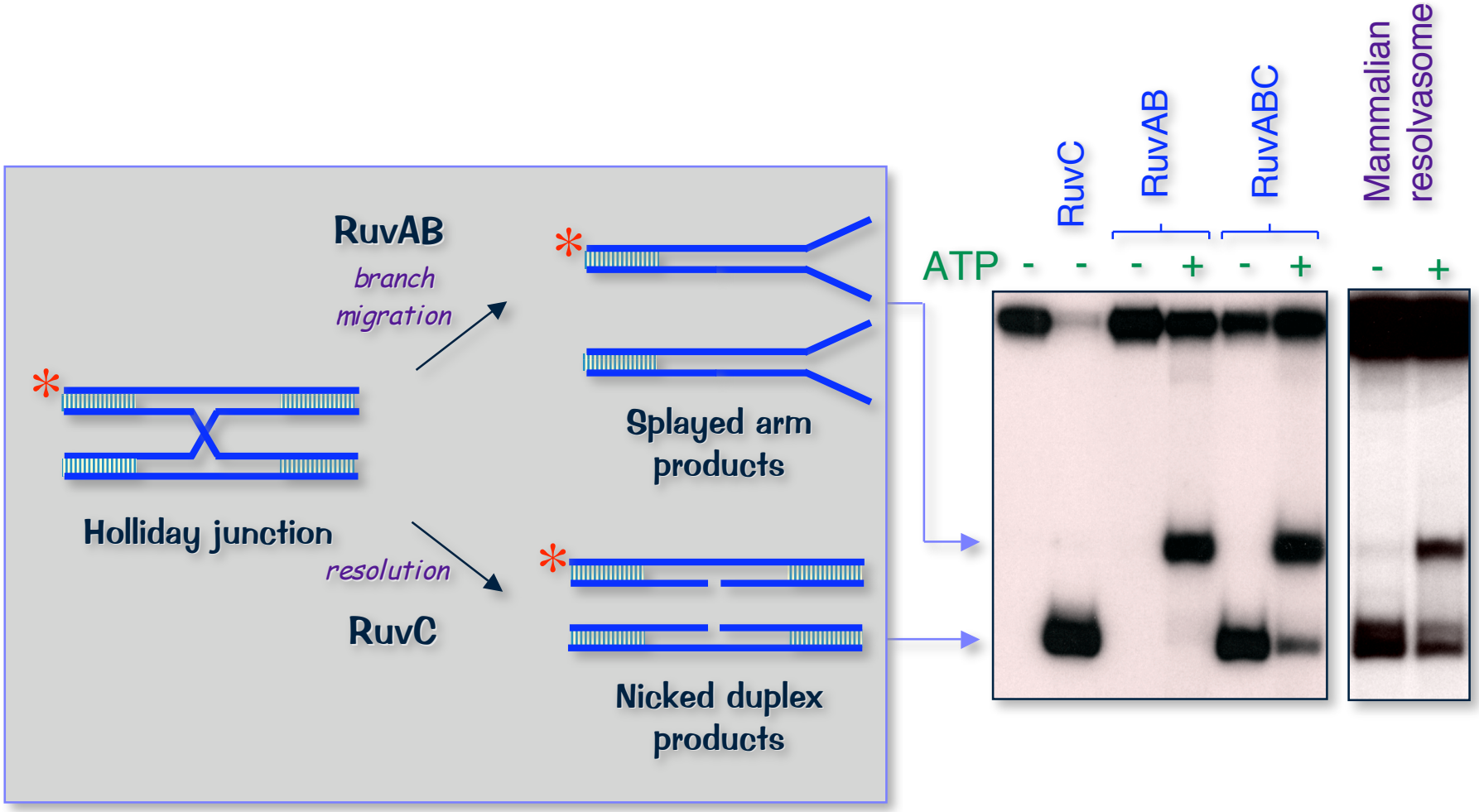
2004:

Resolvase activity shown to be dependent upon the RAD51C and XRCC3 proteins (2 of the 5 RAD51 paralogs)

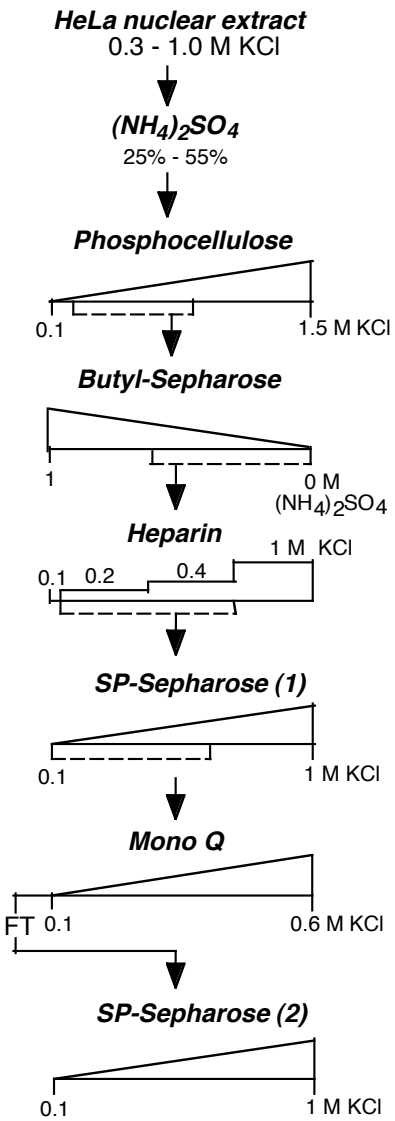
Holliday Junction Processing in *E. coli*

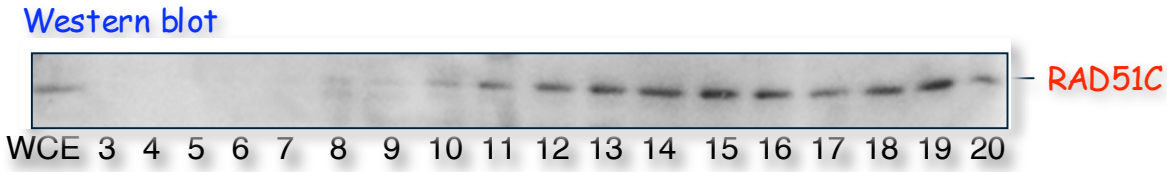


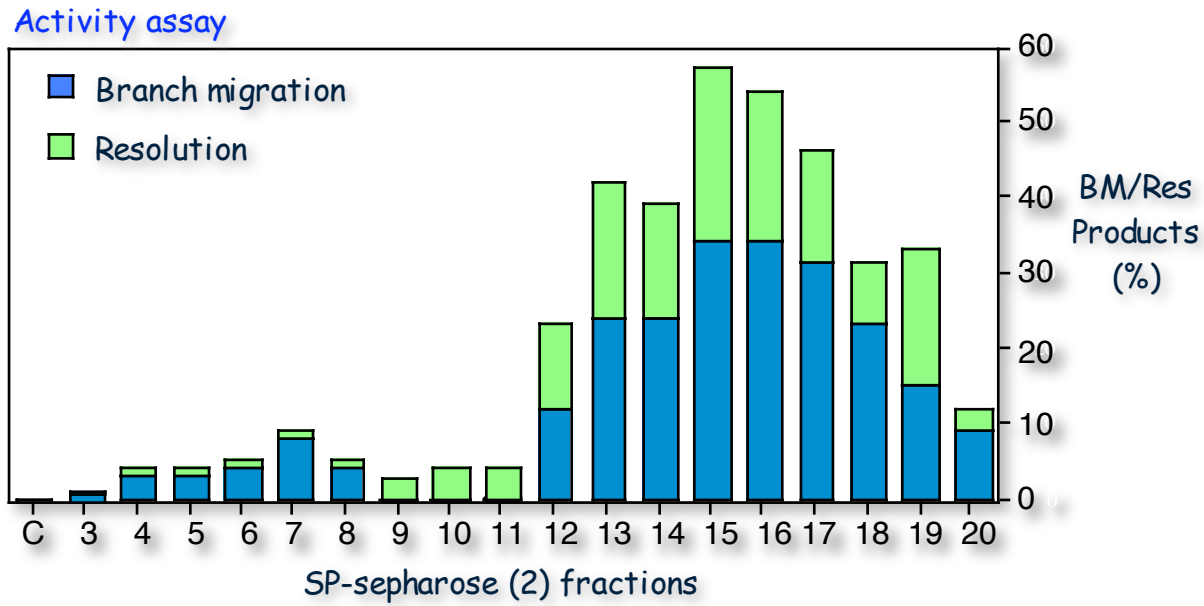
Activities from Human Cells Promote Reactions Similar to those Catalyzed by *E. coli* RuvABC



Purification of the Human HJ Resolvasome

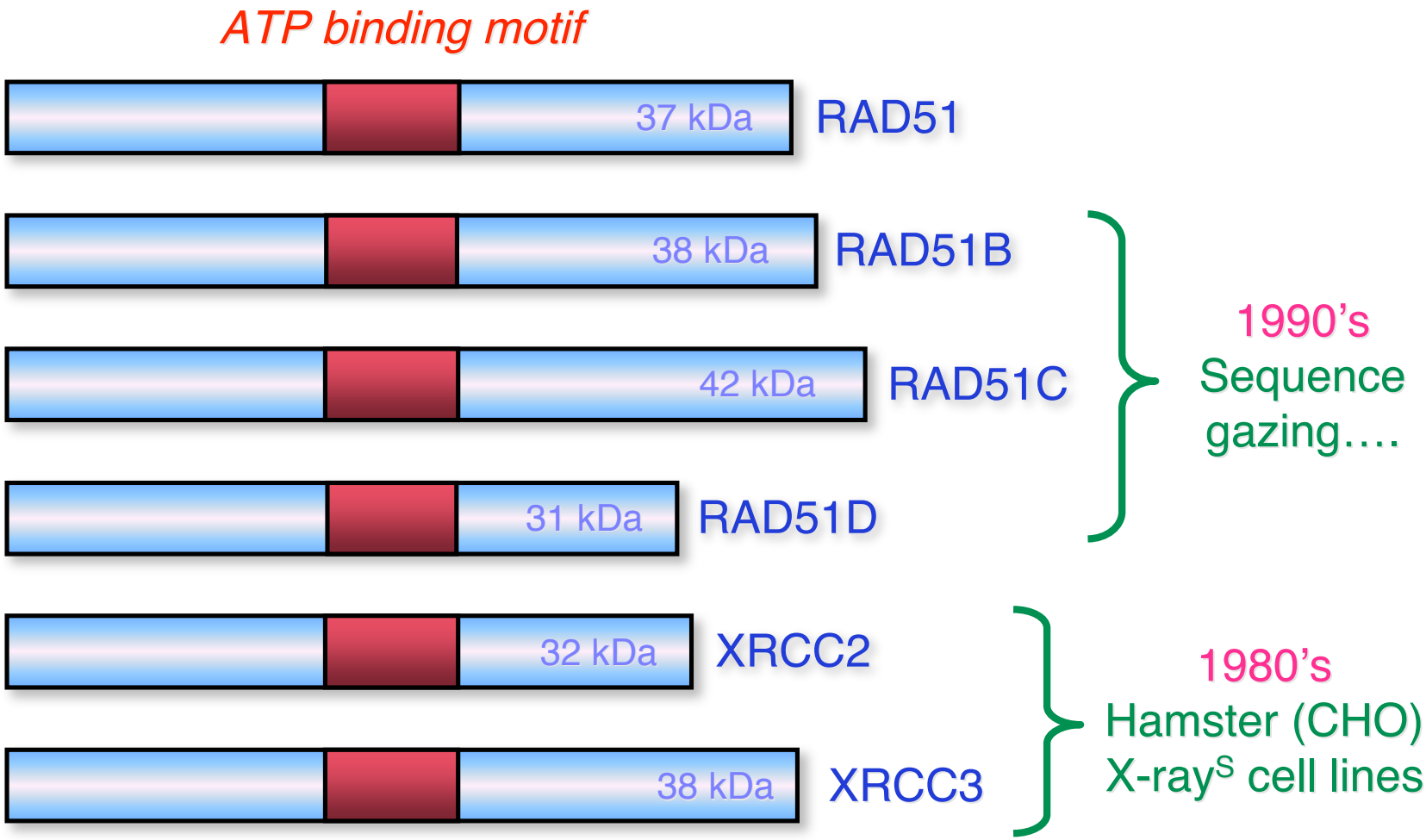






Resolvase activity can be immunodepleted using RAD51C antibodies

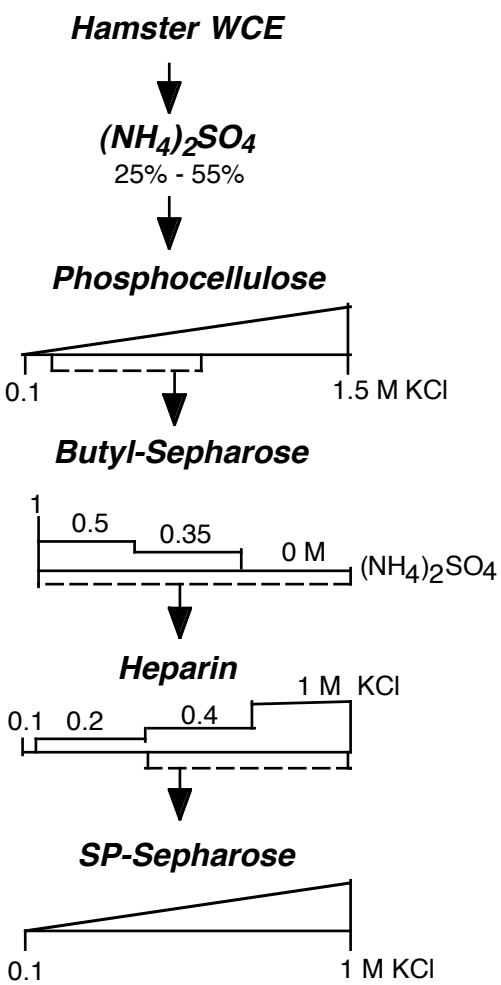
RAD51C is One Member of a Family of Proteins Known as the RAD51 'paralogs'

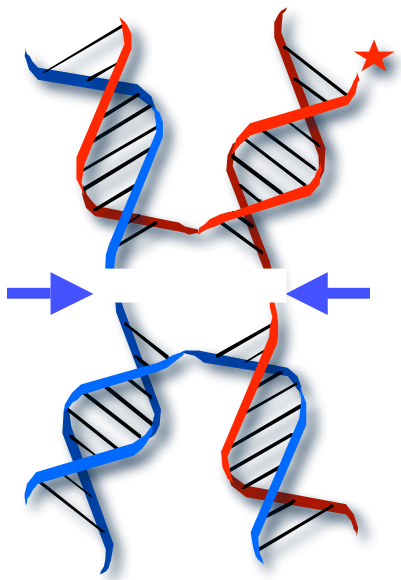


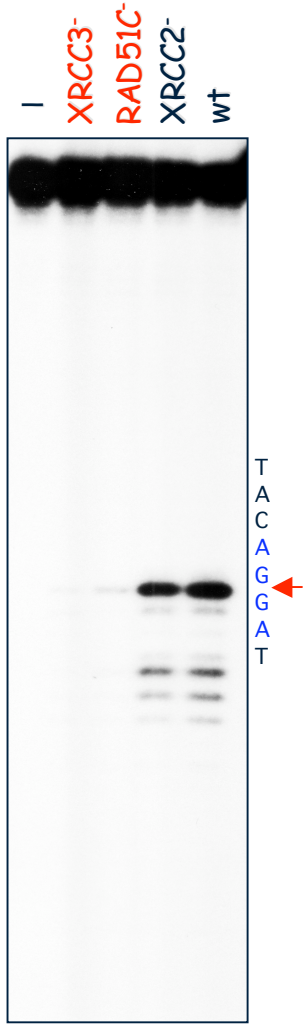
RAD51C is One Member of a Family of Proteins Known as the RAD51 'paralogs'

- ✓ Mouse RAD51 paralog k/o's are embryonic lethal
- ✓ Mutant chicken & hamster cell lines exhibit HR deficiency:
  - ➢ accumulate spontaneous chromosomal breaks
  - ➢ sensitive to mitomycin C, IR etc
  - ➢ defective in targeted integration
  - ➢ defective in IR-induced RAD51 focus formation

Holliday Junction Resovase Activity is Lacking in irs3 (*RAD51C*⁻) and irs1SF (*XRCC3*⁻) cells







Biochemical Evidence for a Late Role for RAD51C-XRCC3 in HR

- RAD51C is found in highly purified resolvase fractions
- RAD51C depletion causes a loss of both branch migration and resolution activity
- Full complementation can be achieved by addition of purified protein complexes containing RAD51C
- irs3 (RAD51C$^-$) and irs1SF (XRCC3$^-$) extracts contain severely reduced resolvase activity
- RAD51C-XRCC3 complex binds HJs in vitro