MRC Genome Damage and Stability Centre

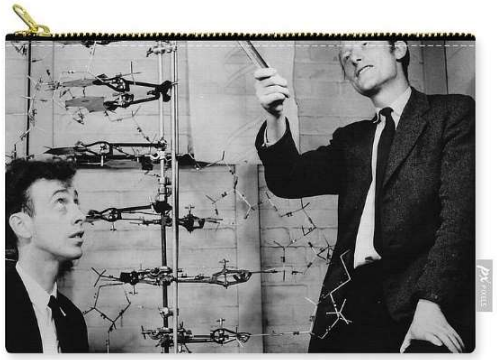
University of Sussex

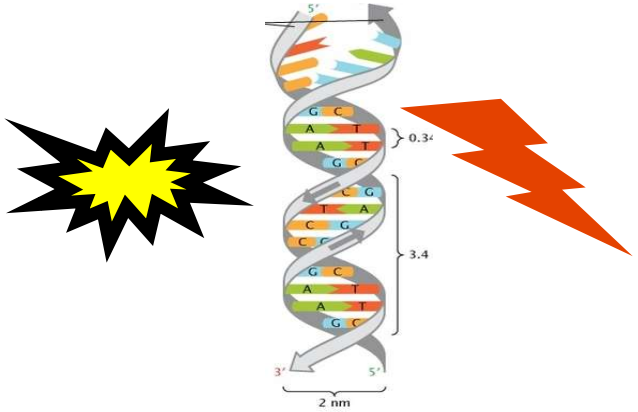
Radiation induced DNA damage and repair plus radiation sensitive disorders

Penny Jeggo, Genome Damage and Stability Centre, University of Sussex

November 11th 2024

Watson and Crick (aided by data from Rosalind Franklin) gained a Nobel prize for identifying the structure of DNA

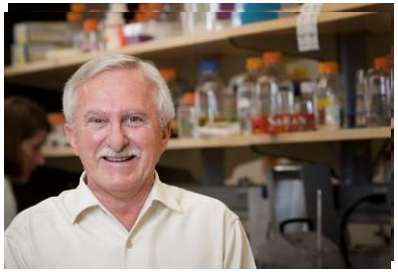




In 2015 – these three scientists gained the Nobel prize for research showing that our DNA frequently incurs damage – and revealing the mechanisms that repair DNA damage

Tomas Lindahl

Aziz Sancar



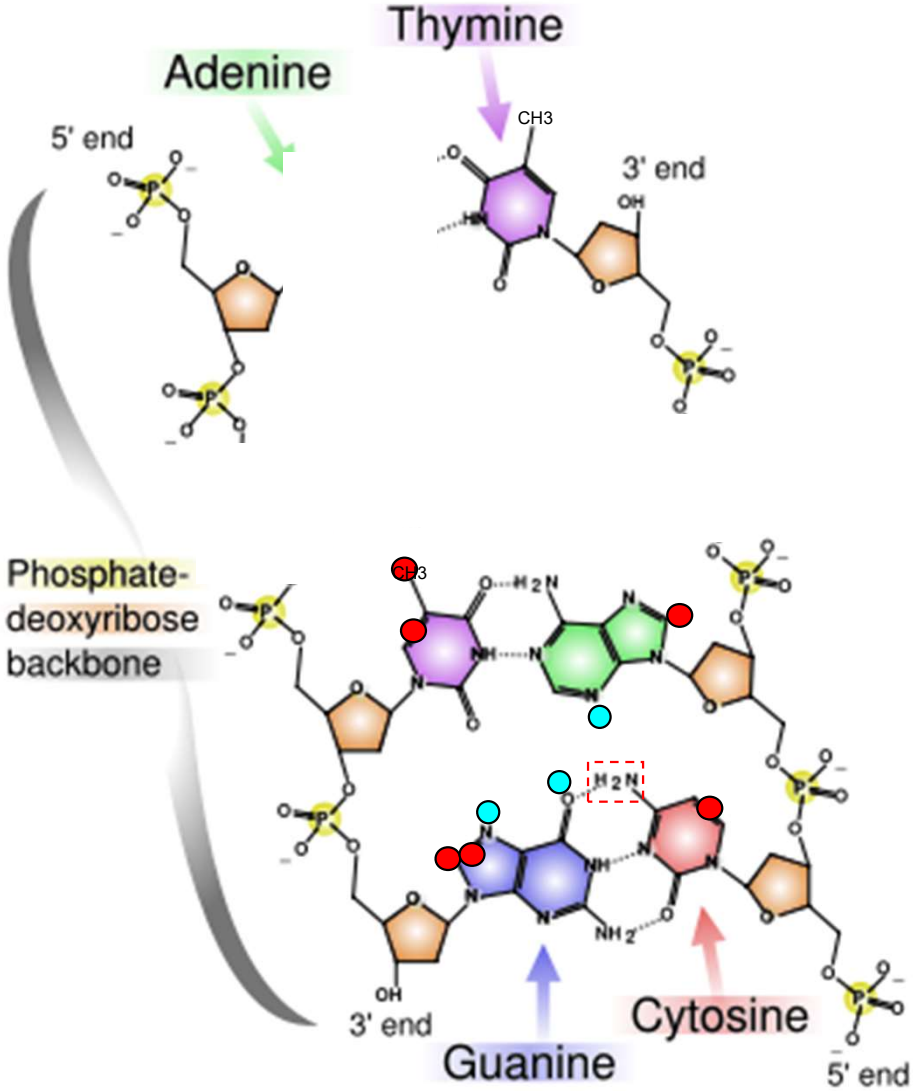
Paul Modrich

Lecture overview.

- Distinction between endogenous damage and radiation induced damage.
- The two major DSB repair pathways - NHEJ and HR (plus minor pathways)
- DNA damage response signalling by DSBs.
- Steps in the formation of radiation induced foci
- The impact of ATM on DSB repair – thinking about misrepair
- Cell cycle checkpoint arrest
- Apoptosis.
- (Radiation sensitive syndromes)

Our DNA encounters substantial damage daily;

Our cells are equipped with damage response mechanisms to repair or respond to this damage.



Endogenous DNA damage

>50,000 lesions per cell per day

20,000 single-strand breaks

10,000 depurination/depyrimidation

5,000 alkylating lesions

2,000 oxidative lesions

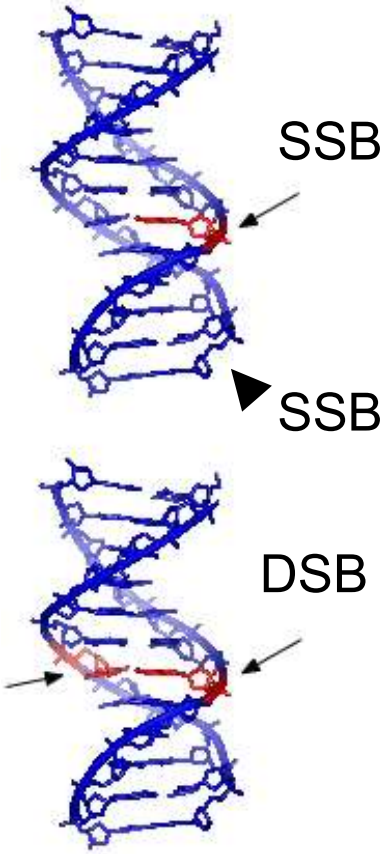
600 deamination events

10-20 double strand breaks

10-20 double strand breaks

DNA double strand breaks

DSBs represent breaks in both strands of the DNA – The breaks may be at the same bp of the DNA strand or several bp apart.



- unrepaired DSBs usually lead to loss of genetic material causing cell death

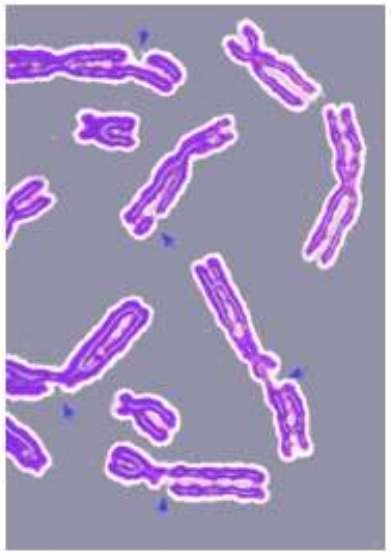
- mis repair can cause translocations (loss of tumour suppressor genes; activation of oncogenes)

- Therefore need to ensure **both** the efficiency and fidelity of repair.

- DSBs frequently have associated damaged bases + SSBs

therefore have to co-ordinate with additional repair processes

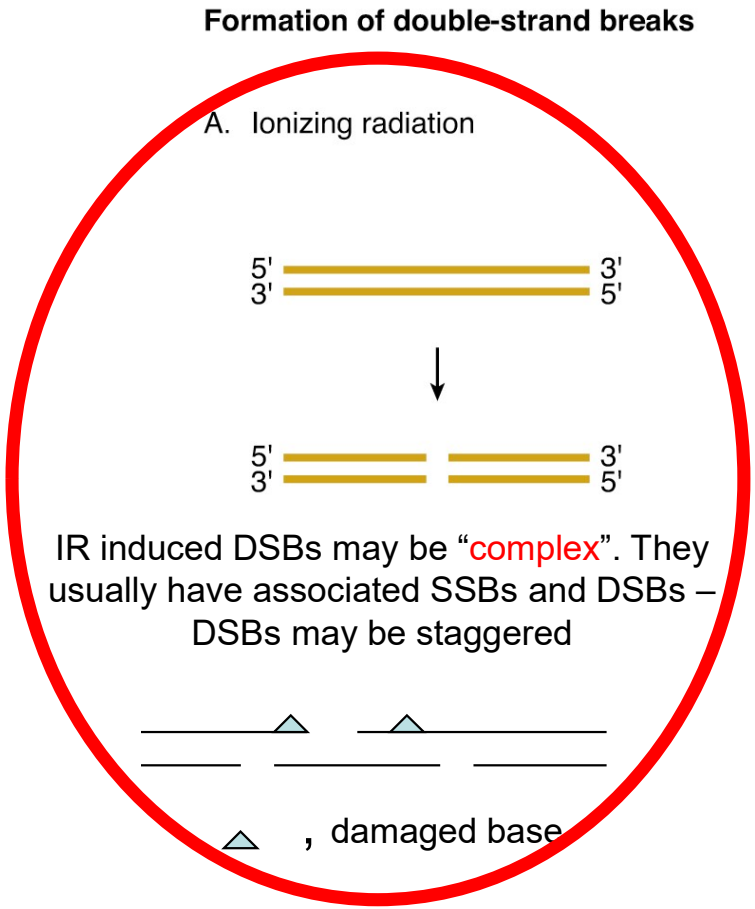
RADIATION AVIDLY INDUCES DSBs



Shows two chromatids of a DNA molecule but each chromatid is double stranded. The arrows therefore represent a double strand break

How do DSBs arise? Endogenously and exogenously

- Processes involving DNA such as transcription and replication
- certain developmental processes - eg V(D)J recombination (process that creates diversity during immune development) and meiosis.
- oxidative damage (Reactive oxygen species (ROS) - DSBs arise from two overlapping ssbs – but this is quite rare (endogenous damage).
- DNA damaging agents such as ionising radiation (ie exogenous DNA damage).



The unique feature of radiation induced DSBs is that they frequently have associated base damage or nearby SSBs.

These are called complex DSBs

And these DSBs are hard to repair

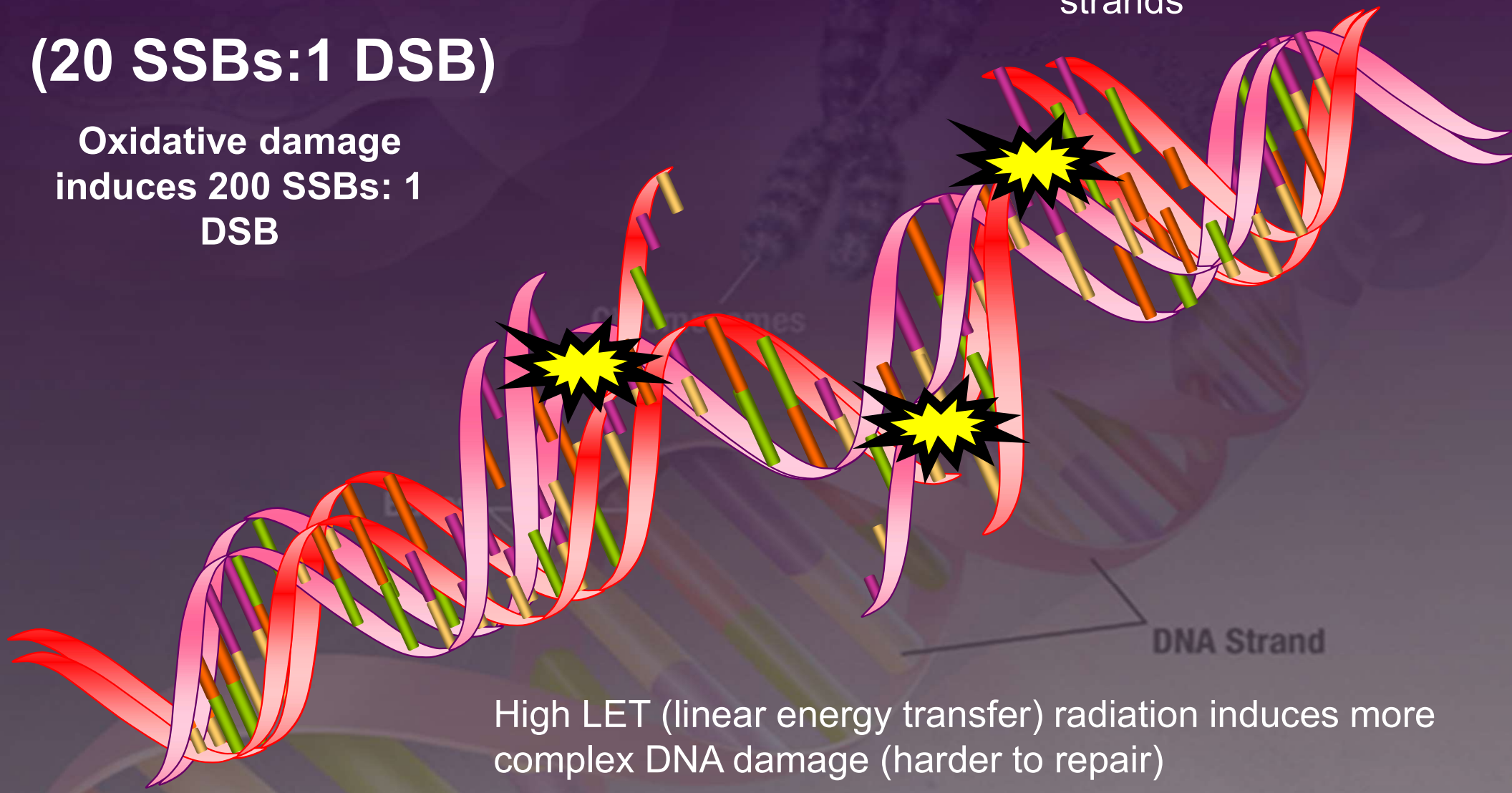
Radiation induced DSBs are distinct to endogenous DSBs.

Radiation induces a high frequency of DSBs

(20 SSBs:1 DSB)

Oxidative damage induces 200 SSBs: 1 DSB

Unique feature of radiation is the complexity of the damage giving multiple lesions in close proximity. This can cause loss of sequencing information in both strands



High LET (linear energy transfer) radiation induces more complex DNA damage (harder to repair)

DNA double strand breaks

2 Strategies for defence:

Optimising recovery or eliminating

DSB

REPAIR

SIGNAL TRANSDUCTION

cell cycle checkpoint arrest

Optimises time for repair and prevents proliferation of damaged cells

apoptosis

Eliminates damaged cells

Two major DSB repair pathways are:

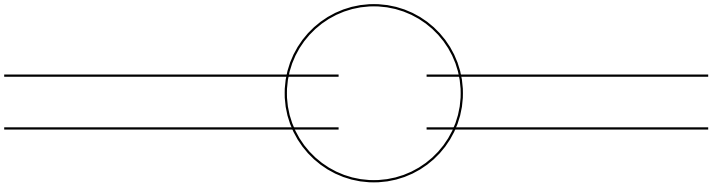
1) Non-homologous end-joining (NHEJ)

2) homologous recombination (HR)

There are some additional pathways, which I will also mention

Non-homologous end-joining.

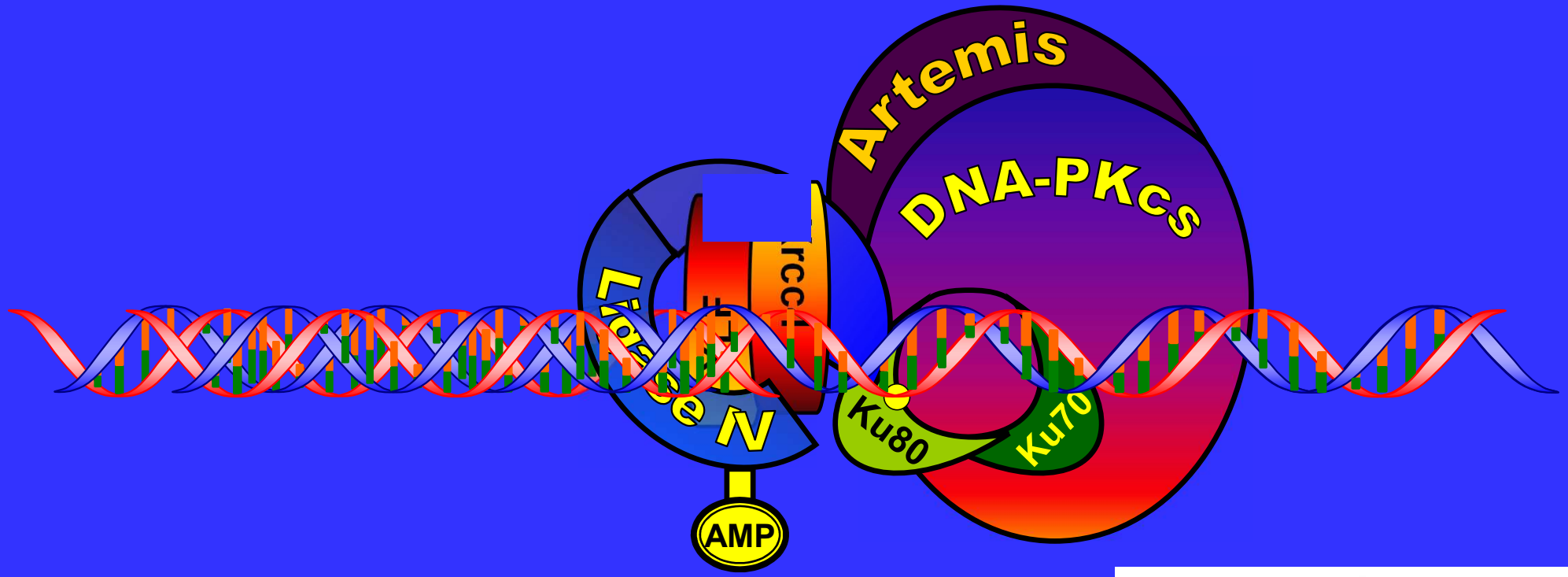
- NHEJ requires little or no homology at the DNA ends.
- functions in all cell cycle stages and is the major mechanism that functions in G0/G1 phase.
- Argued to be an error prone but accuracy is unknown – likely to be inaccurate for complex DSBs with loss of sequences at both ends of the DSB



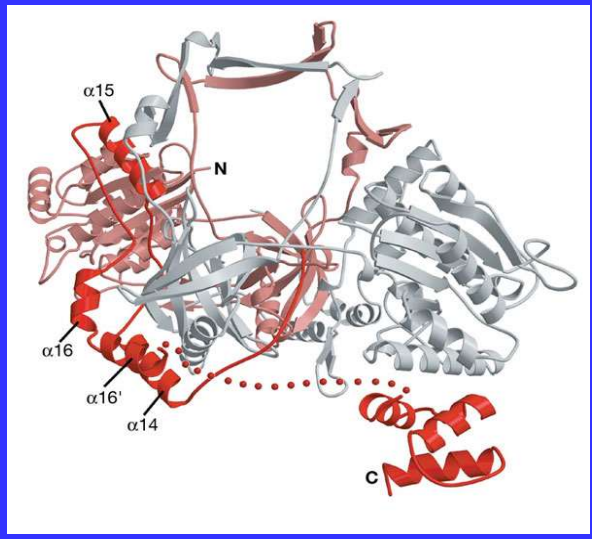
ONE ACHILLES HEEL OF NHEJ

if base pairs lost from both strands at the site of the break, these cannot be reconstituted by NHEJ

Non-Homologous End-Joining



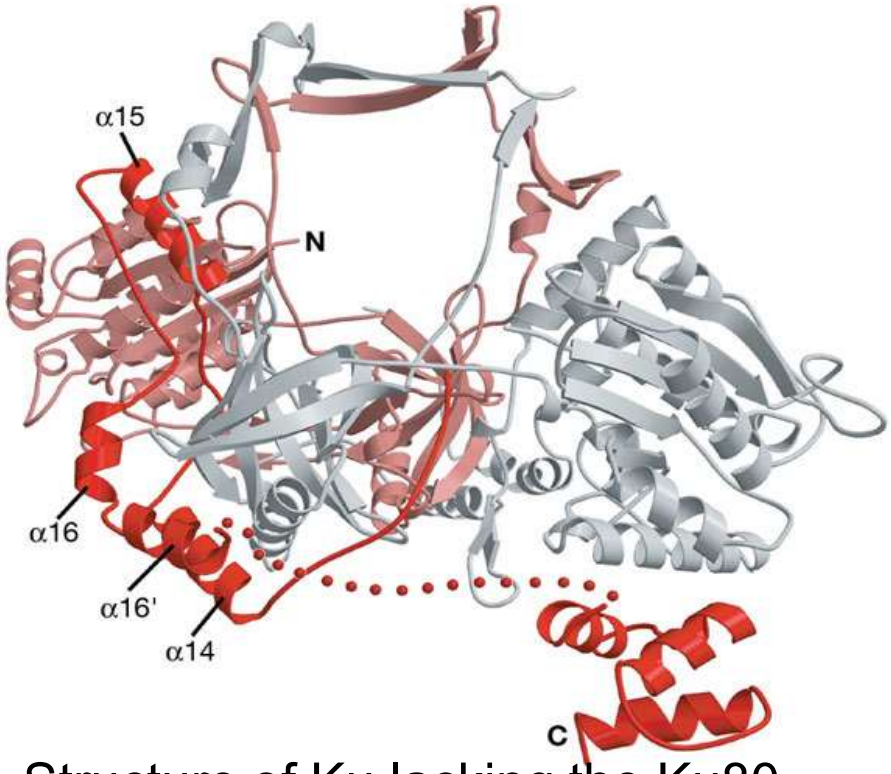
1. DSB protection
2. DSB end-bridging
3. DSB end-processing
4. DSB ligation
5. DSB resealed



Ku heterodimer.

• The Ku heterodimer has 70 and 80 kDa subunits (Ku70 and Ku80) (the 80KDa subunit is slightly larger)

• Stability of Ku70 and 80 are mutually dependent -2 proteins are tightly folded.



Structure of Ku lacking the Ku80 C-terminus

•Ku (and DNA-PKcs) is highly abundant – less abundant in rodents

•Ku has STRONG ds DNA end binding activity (which is sequence independent) – the dsDNA passes through the hole in the structure (see next slide)

•The high abundance + strong end-binding activity results in Ku being the first responder to all DSB ends – and protects the ends against nucleolytic degradation.

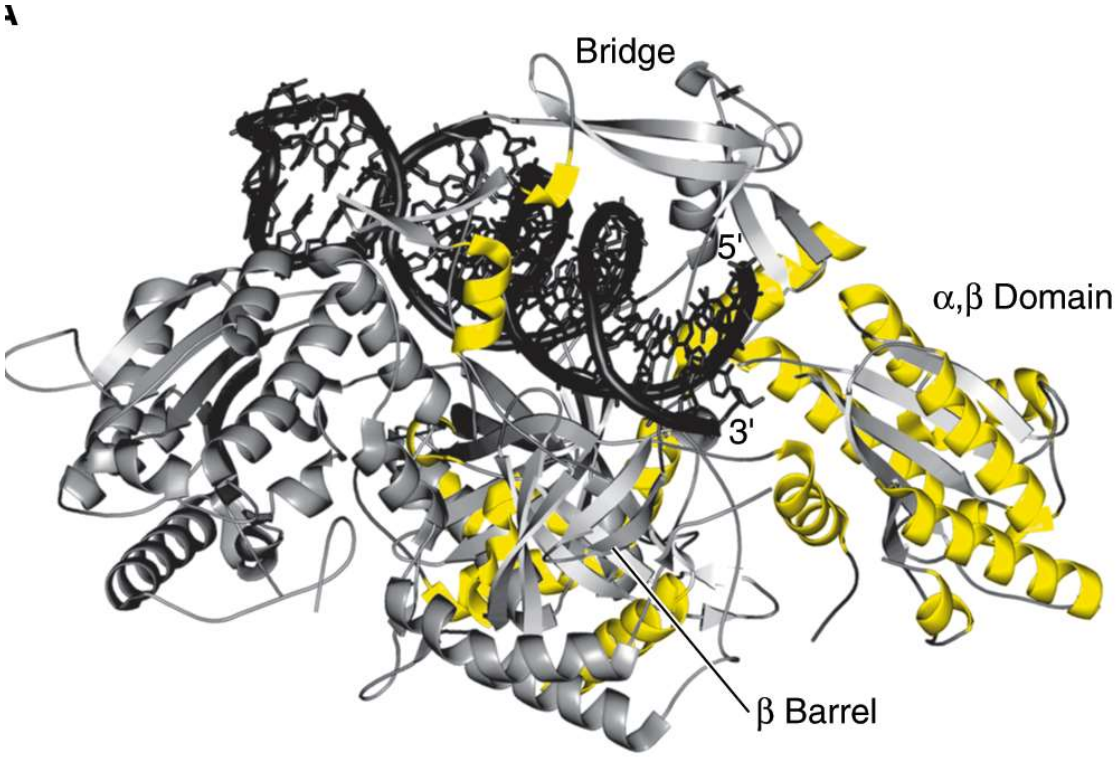
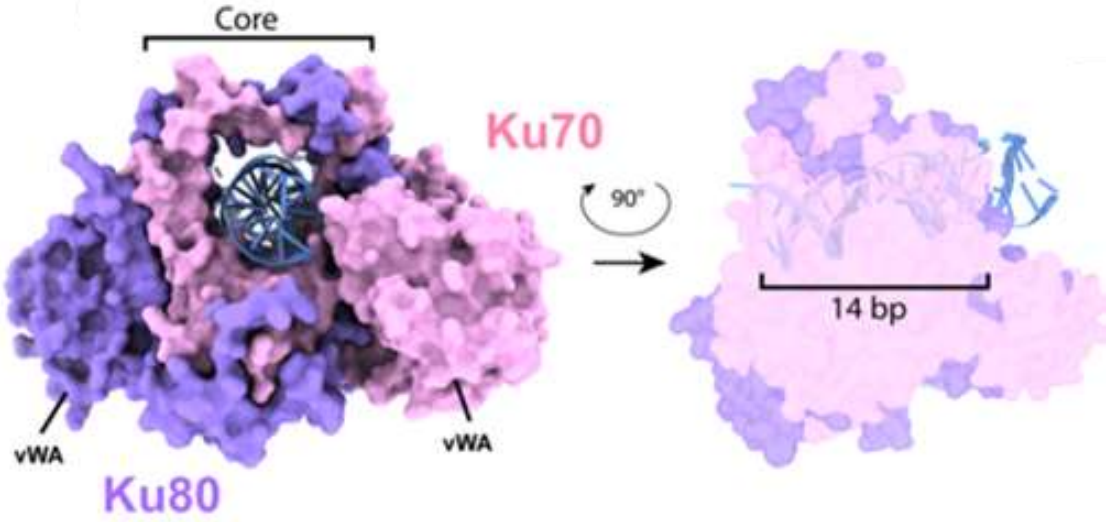


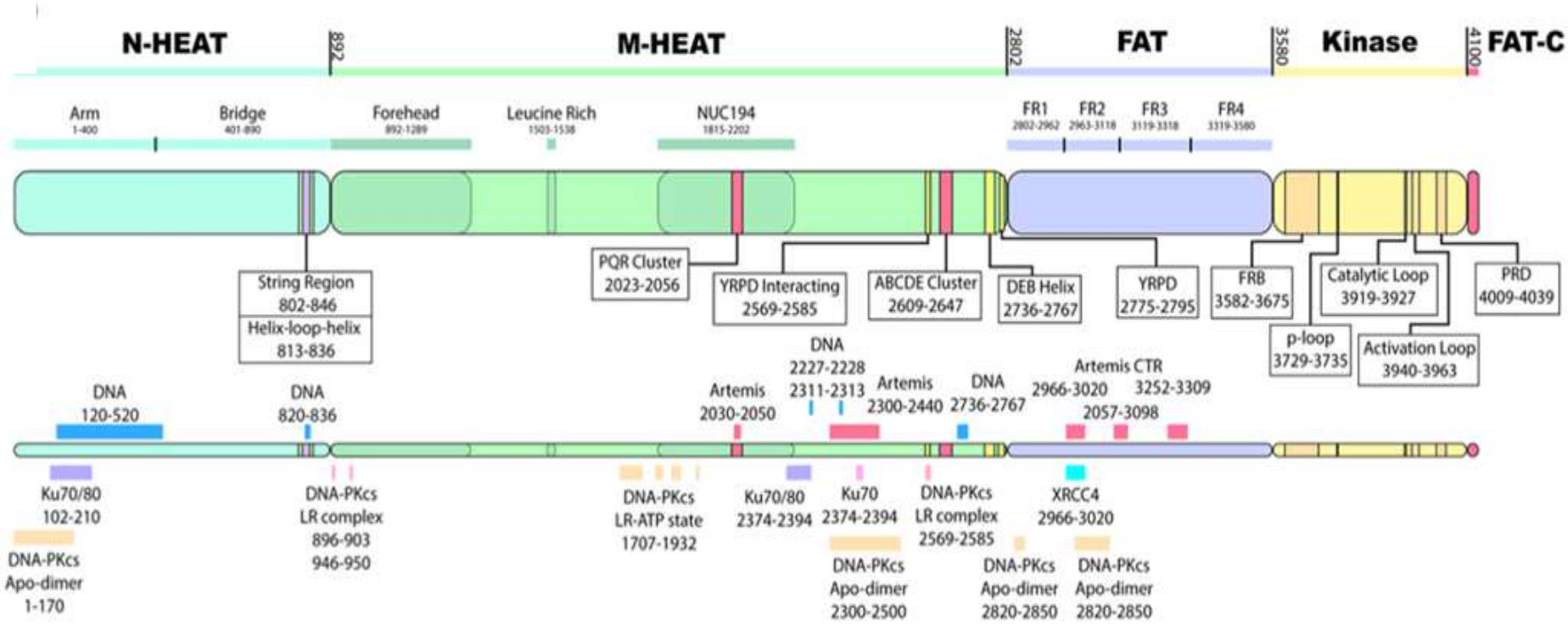
Figure shows the structure of Ku with DNA bound – there is little contact with DNA bases.

DNA goes through the centre space in the Ku structure

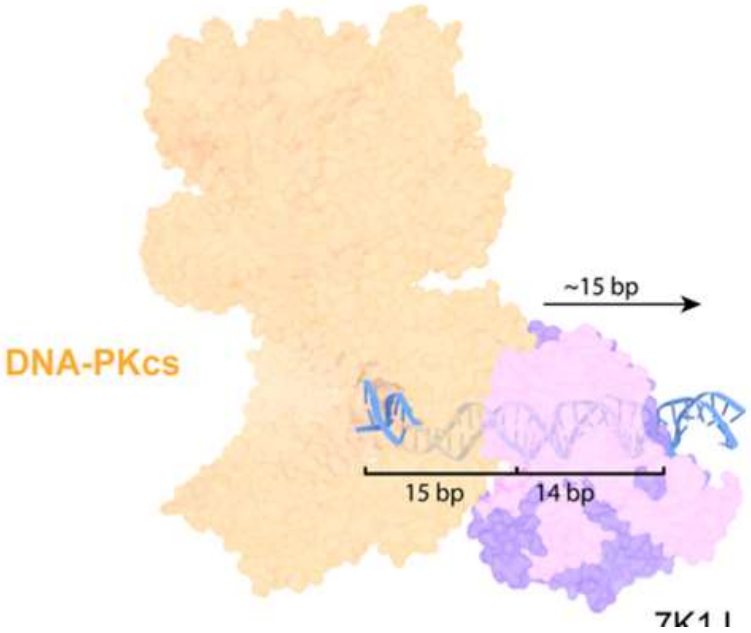


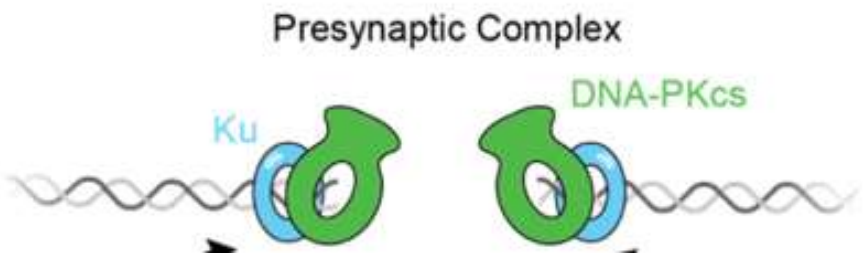
Ku bound to DNA occupies around 14 bp DNA (called a footprint)

- **DNA-PKcs – a large protein- is then recruited to the DNA end:**
- DNA-PK is a phosphatidylinositol 3-kinase like kinase (PIKK).
- Kinases are proteins that phosphorylate other molecules – DNA-PK is a protein kinase
- DNA-PKcs is one of the largest proteins - 14 Kb cDNA (one of the largest cDNAs known).

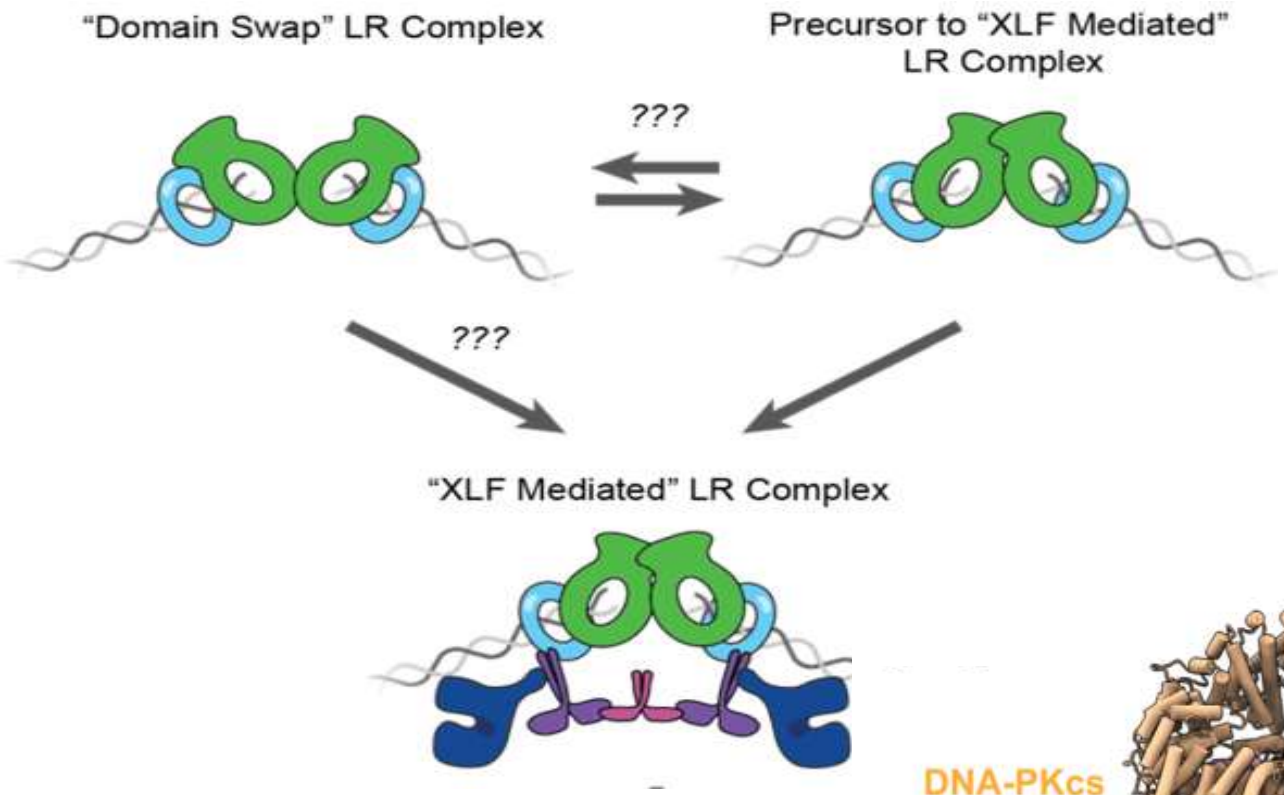


The DNA dependent protein kinase holozenyme

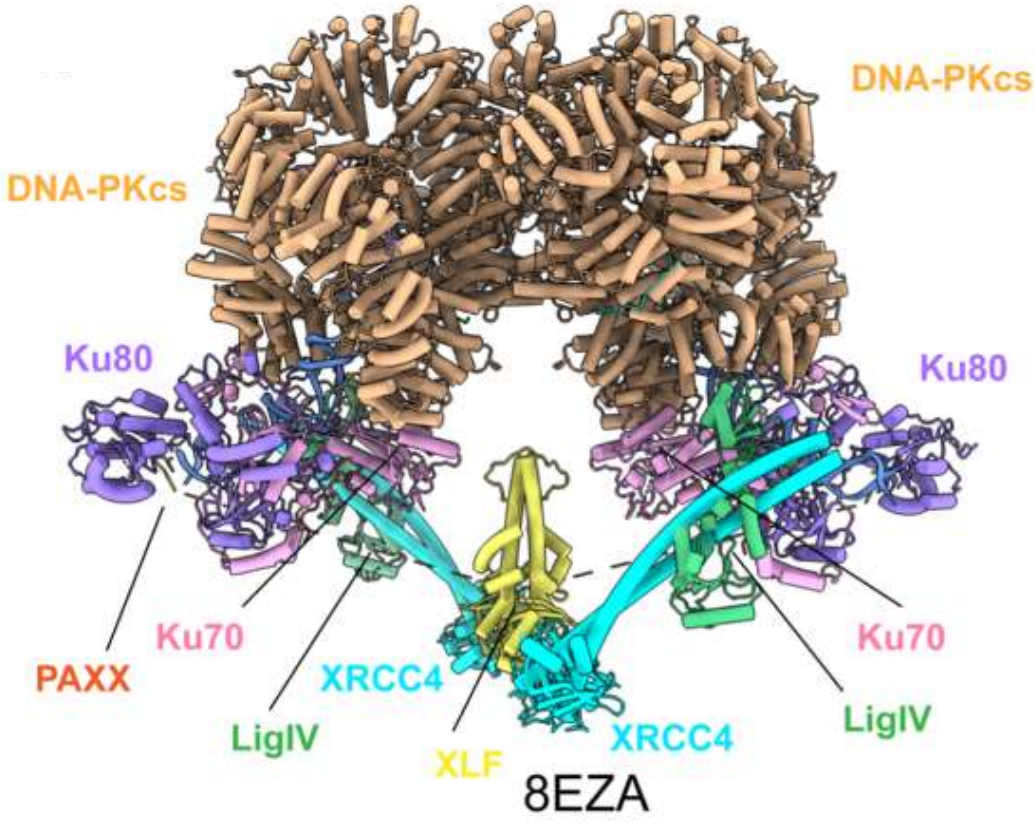




- Ku and DNA-PKcs exist separately
- But when bound to a DNA end, Ku interacts with DNA-PKcs creating the DNA-PK holoenzyme
- Interaction with Ku causes a conformational change which activates DNA-PKcs as a protein kinase
- DNA-PKcs pushes Ku inwards on the DNA and the DNA-PK footprint is 28-29 bp
- The first stage is DNA-PKcs bound to Ku on each end – presynaptic complex
- DNA-PK then can create a synaptic complex by forming a dimer



An important step in NHEJ is the recruitment of the other NHEJ proteins (XLF, XRCC4, DNA ligase – plus end processing factors.
Many are bound to Ku but DNA-PKcs is central to the assembly process



- DNA-PK phosphorylates all components of the NHEJ machinery including Ku, Xrcc4 and DNA ligase IV.

- Also undergoes autophosphorylation – which regulates end-processing and the release of DNA-PKcs from Ku

- Co-ordinates formation of a synaptic complex, end-processing and ligation

- DNA-PK interacts with Artemis, a nuclease involved in NHEJ (see later).

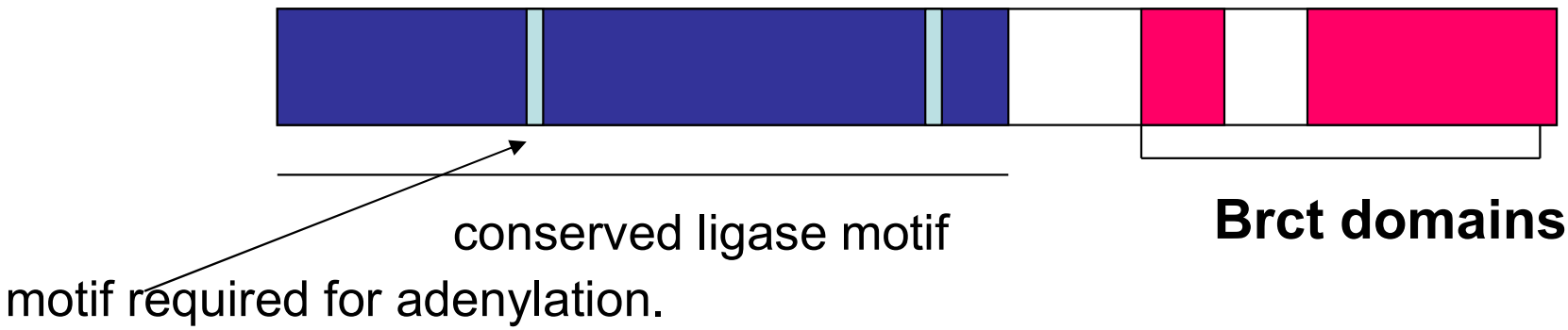
Figures taken from:
Alex Vogt and Yuan He: Structure and mechanism in NHEJ. DNA Repair; 130, 2023
Joseph Lopara: Holding it together: DNA end-synapsis during NHEJ DNA Repair: 130, 2023

Final step: end rejoining

- carried out by a complex involving DNA ligase IV , Xrcc4 and XLF (Xrcc4/LigIV interacting factor). (XLF also called Cernunnos) and PAXX (a recently identified factor).
- DNA ligase IV and Xrcc4 form a tight complex and are essential for ligation.
- XLF has structural similarity to Xrcc4.
- XLF and XRCC4 seem to have a role in synapsis of the ends as well
- PAXX –has sequence similarity to XLF and XRCC4 – but function is unknown

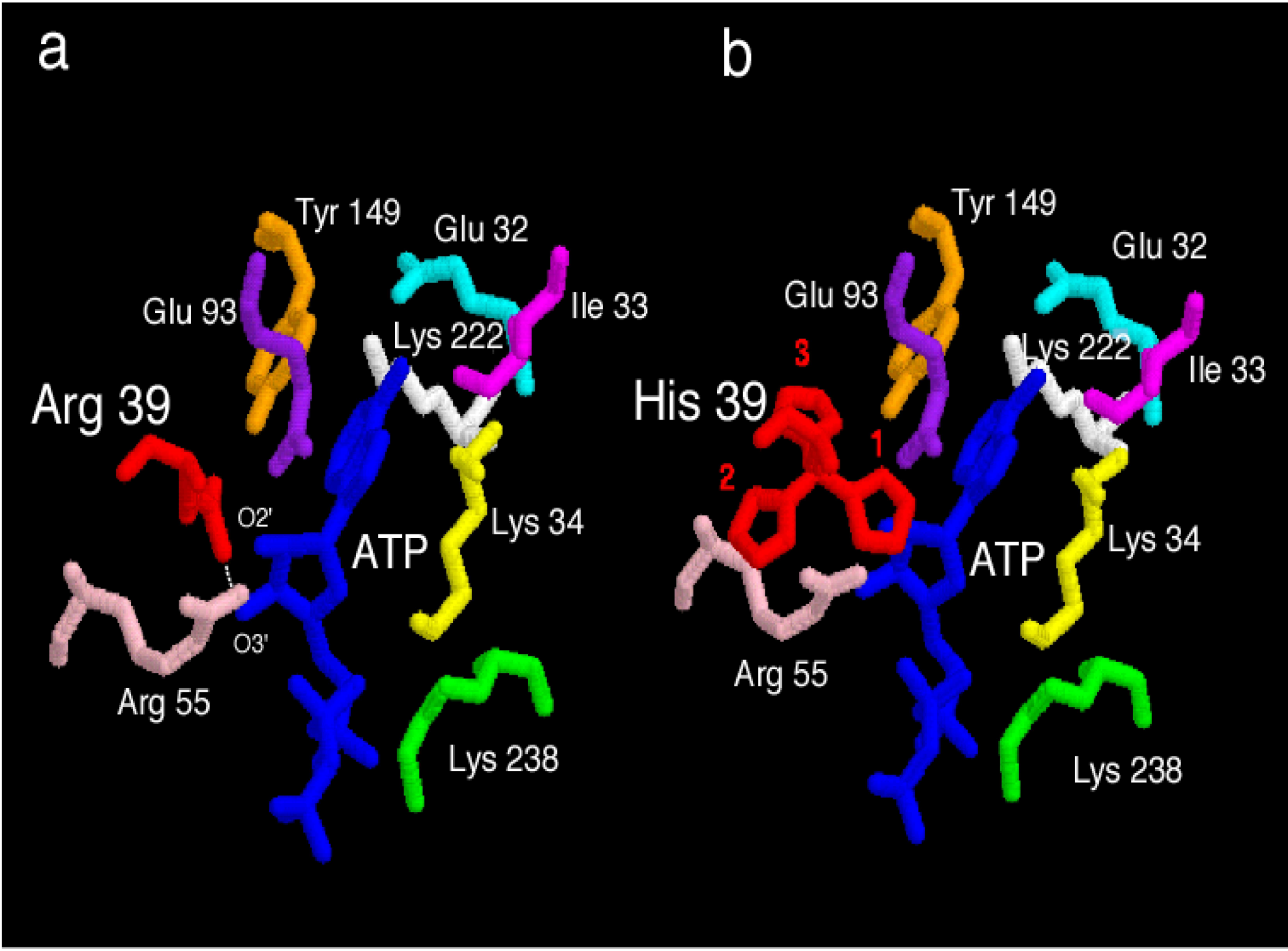
The rejoining step of NHEJ

DNA ligase IV (LIG4) has a conserved DNA ligase domain at its N-terminus and two Brct domains (tandem BRCT motif) at is C-terminus



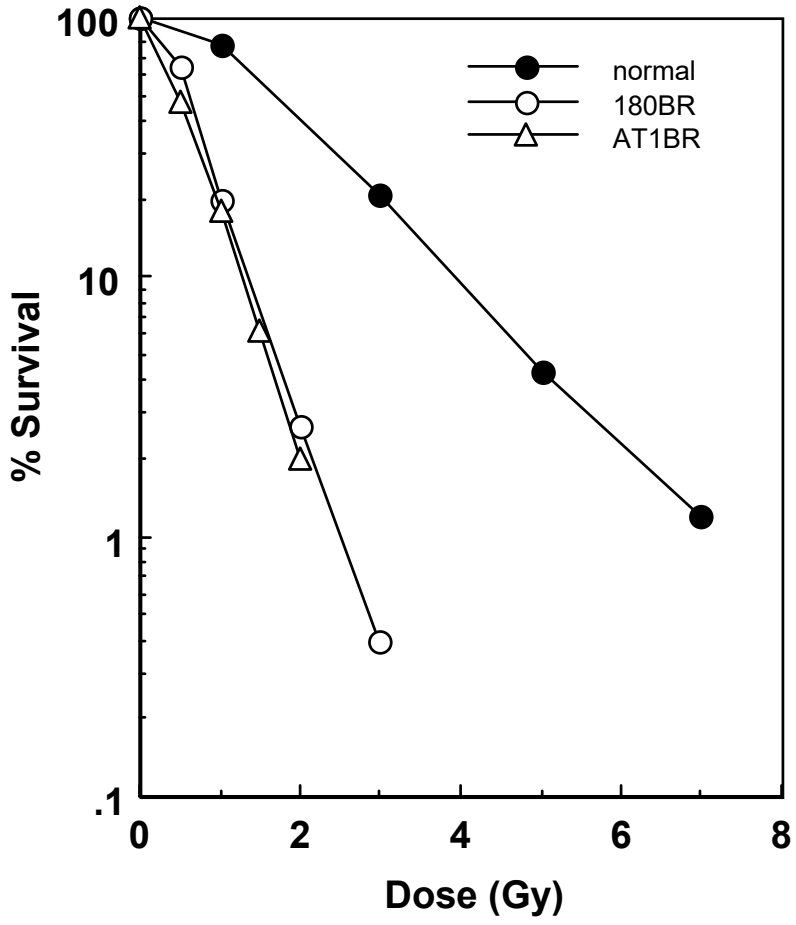
•BRCT motifs are found in many DNA damage response proteins – they are phospho binding motifs

• First step of the ligation reaction is the formation of a complex with ATP, which provides the energy – adenylate complex


a
Tyr 149
Glu 32
Glu 93
Ile 33
Lys 222
Arg 39
O2'
ATP
Lys 34
O3'
Arg 55
Lys 238
b
Tyr 149
Glu 32
Glu 93
3
Lys 222
Ile 33
His 39
1
2
Lys 34
ATP
Arg 55
Lys 238

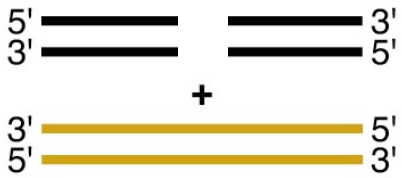
Mutants lacking NHEJ proteins are VERY radiation sensitive

- Demonstrating the major role that NHEJ plays in repairing IR-induced DSBs.



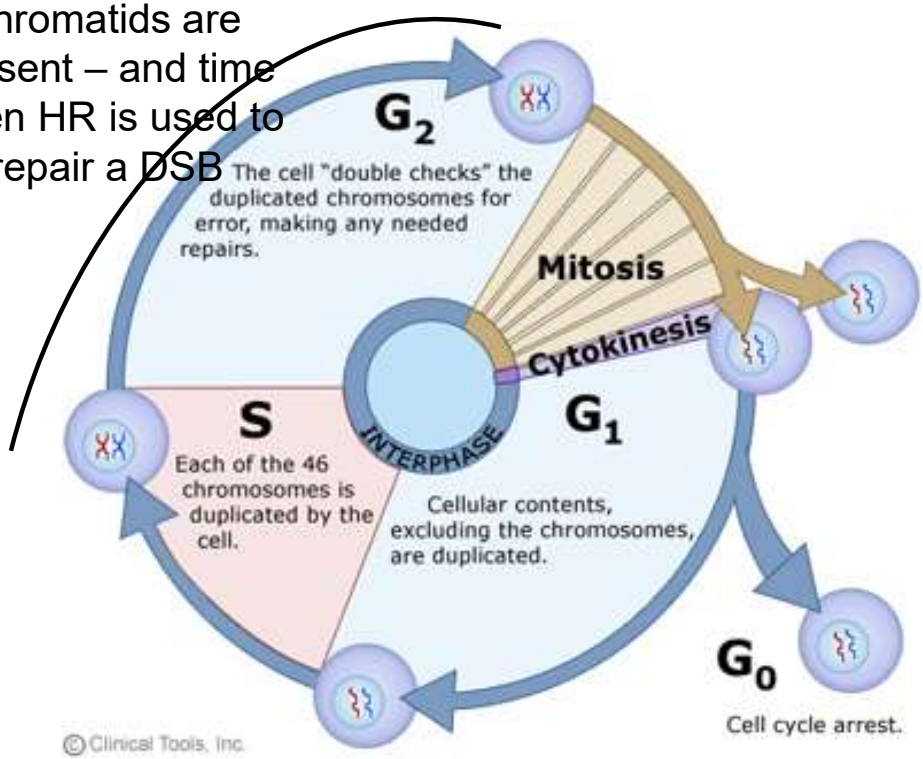
180BR is a human cell line mutated in DNA ligase IV (will discuss later)

Homologous Recombination is 2nd DSB repair process. It uses an undamaged template to restore information lost at the DSB site



• In mammalian cells, although there are two copies of every chromosome (homologues), HR does not use the homologous chromosome.

Time when sister chromatids are present – and time when HR is used to repair a DSB



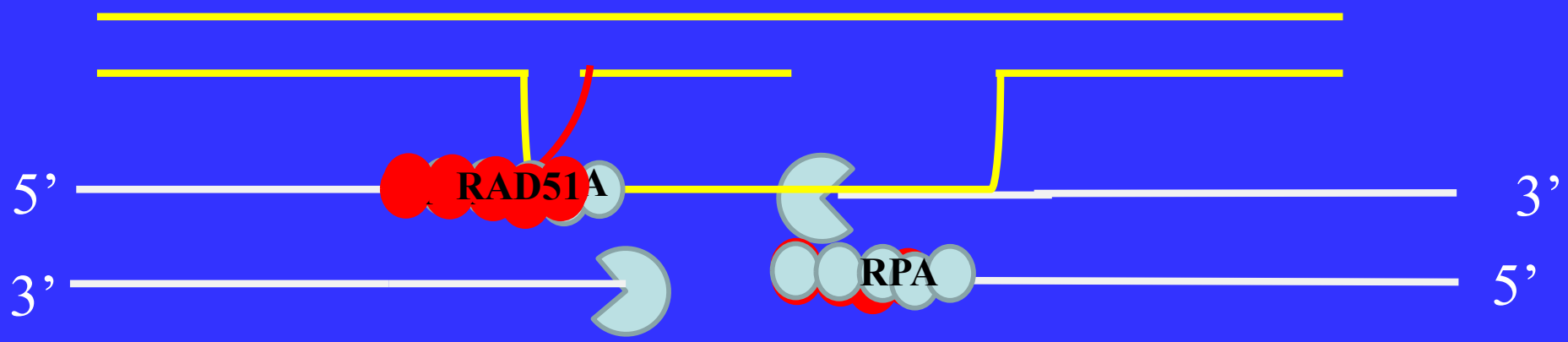
•Instead HR functions following replication (in late S/G2 phase) using the replicated sister (sister chromatid) for information

Homologous recombination

Steps of HR in brief:

- 5’ to 3’ resection of the DSB to generate a 3’ overhang
- Invasion onto the undamaged strand giving a D-loop
- Repair using the undamaged strand as a template.

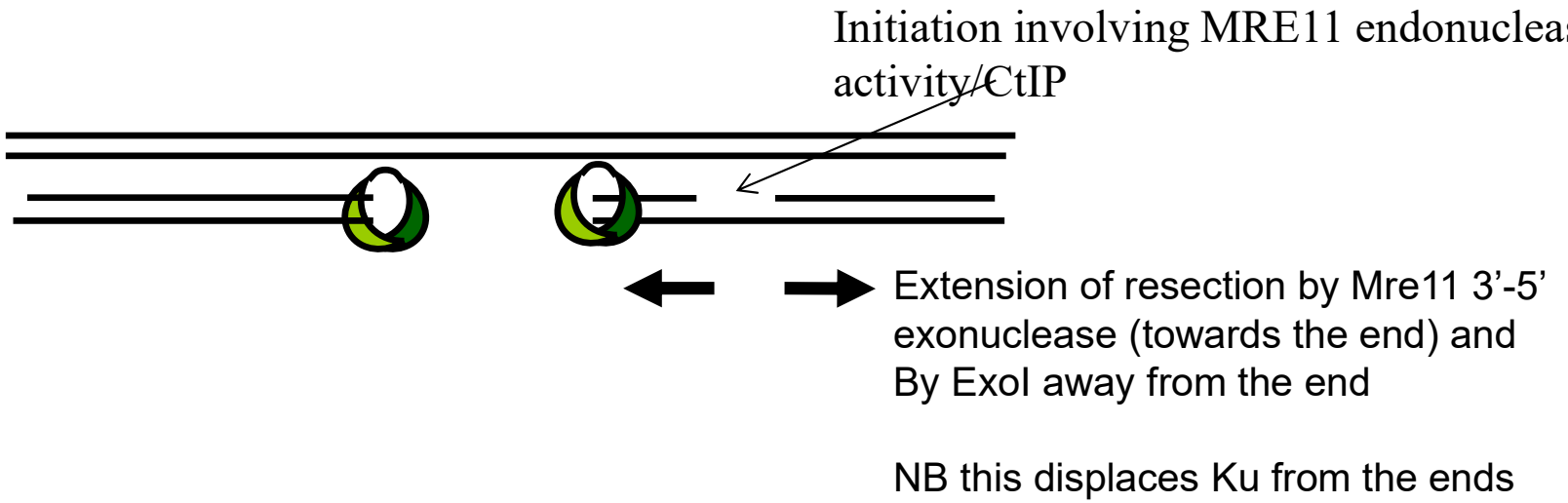
Only uses a sister chromatid – so only functions in late S/G2



The **initiating** step in HR is **resection** of the DSB

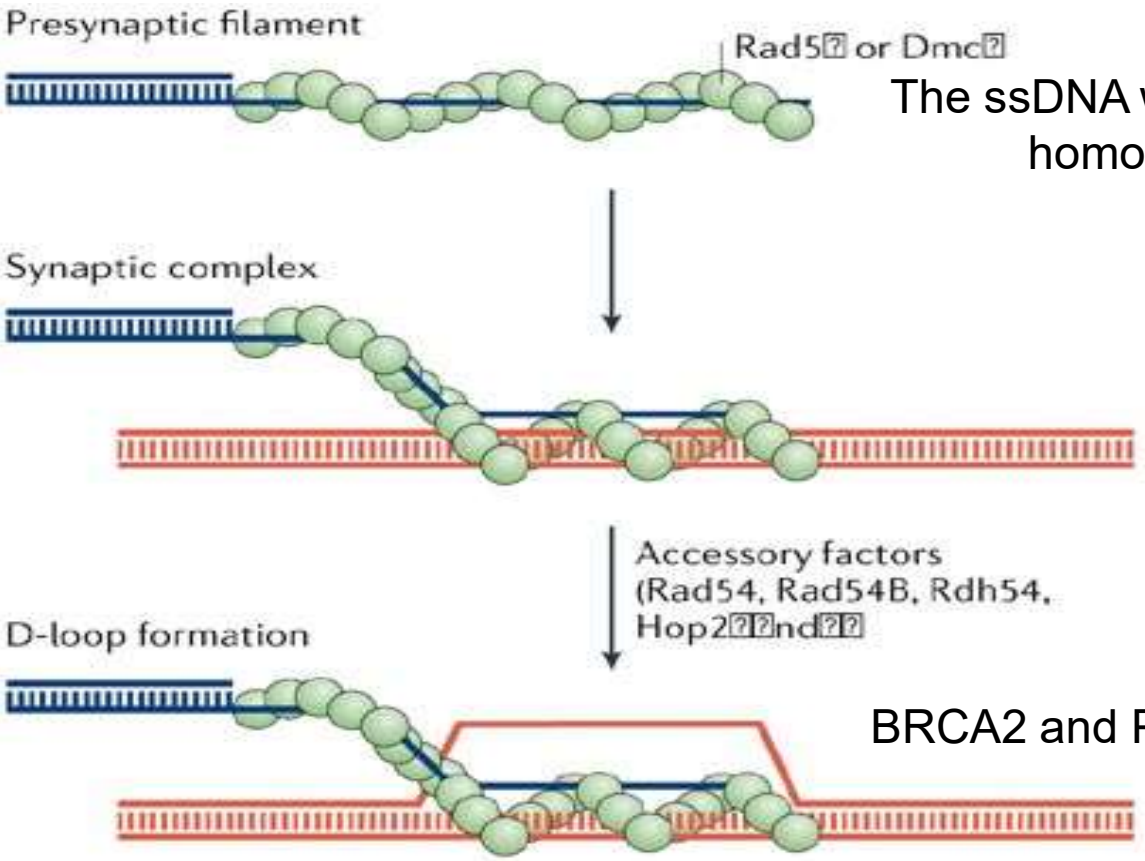
NB – Ku binds to all DSBs ends – and stops resection – so Ku must be removed to allow HR to proceed.

1st Step – involves an endonucleolytic incision by the MRN (MRE11, RAD50, NBS1)



1) MRE11 endonuclease activity creates a nick 5' to the DSB end (not shown in previous slide) – this also required CtIP
2) MRE11 exonuclease activity (3' to 5') digests towards the end
3) Ku is displaced (precise mechanism unclear)
4) ExoI (5' to 3') digests away from the end to enlarge the region of ss DNA

Rad51 replaces RPA bound ssDNA – then RAD51 coated DNA engages with the undamaged strand and displaces one strand to form a D loop



The ssDNA with Rad51 bound invades a homologous DNA molecule

BRCA2 and PALB2

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Nature Reviews | Molecular Cell Biology

Sung and Klein *Nature Reviews Molecular Cell Biology* **7**, 739–750 (October 2006) | doi:10.1038/ nrm2008

nature REVIEWS GENETICS

HR versus NHEJ.

• **HR** functions only in late S and G2

• **Major function** is to promote recovery from stalled replication - **replication fork restart –** Replication fork DSBs have a particular nature (called one ended DSBs) and are not suitable for rejoining by NHEJ.

• Forks stall during most rounds of replication. Therefore HR is essential.

• HR does repair some radiation-induced DSBs in G2 phase – including those in transcriptionally active regions.

• HR is likely accurate because it uses an undamaged template to recover lost sequences

• HR requires extensive homology – so less likely to rejoin the wrong DNA ends (rejoining the wrong ends forms a translocation)

• **NHEJ** functions in G1 (S and G2) – ie all cell cycle phases;

• NHEJ is unable to efficiently repair complex DSBs where information is lost on both strands

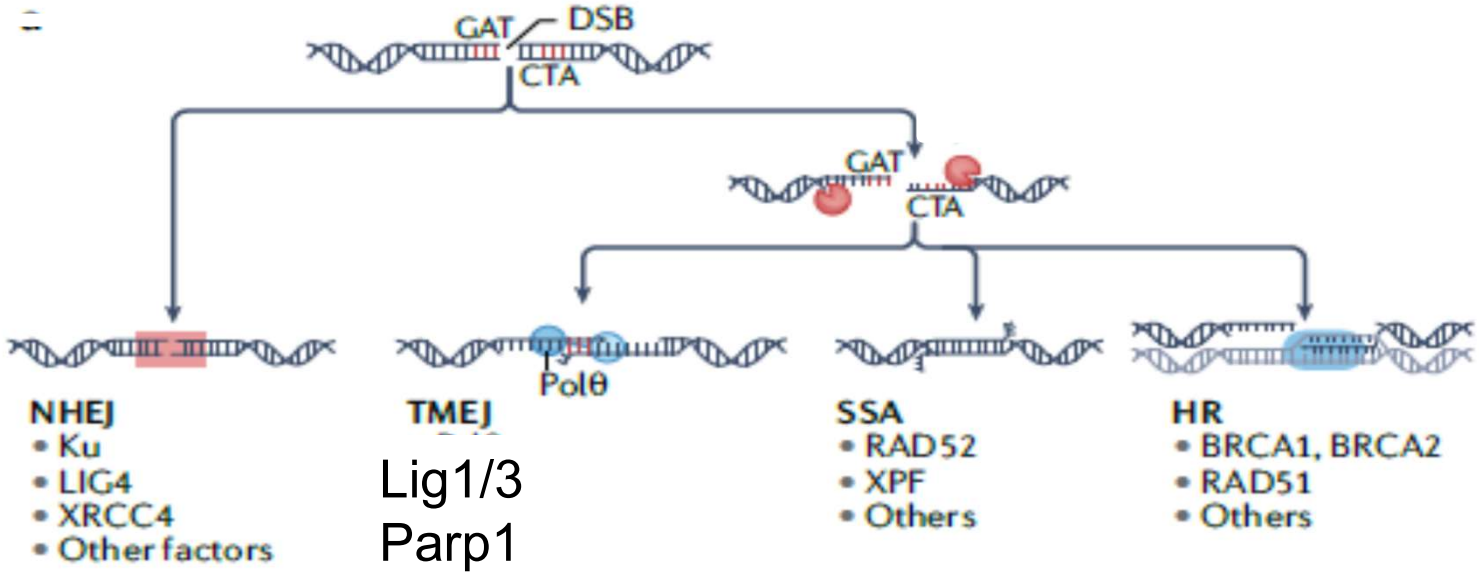
• Ku inhibits HR and has to be removed for HR to ensue.

• Ku can rejoin the “wrong” DNA ends – because does not use homology

NHEJ plays the major role in repair of IR-induced DSBs – HR more involved in repairing replication induced DSBs.

NHEJ mutants are VERY radiation sensitive suggesting that NHEJ is the major mechanism to repair IR induced DSBs.

Other processes of DSB rejoining.



Alternative end-joining (Alt-NHEJ): uses short regions of microhomology (MH) to rejoin the ends and often Lig1/3 to rejoin single ends

Recently pol theta: polθ has been shown to play a role in alternative end-joining pathways – and functions in most alt-NHEJ – hence called TMEJ – theta mediated end-joining.

SSA (single strand annealing) uses longer regions of MH and often arises when HR fails

Both TMEJ and SSA cause deletions – therefore inherently error prone.
Both cause genomic instability and are used by cancer cells to generate mutations

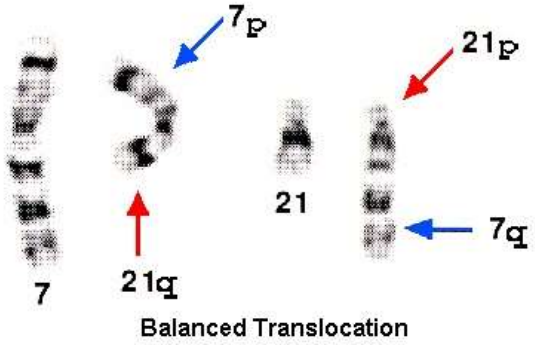
DSB misrepair?

Two classes of DSB misrepair induced by radiation

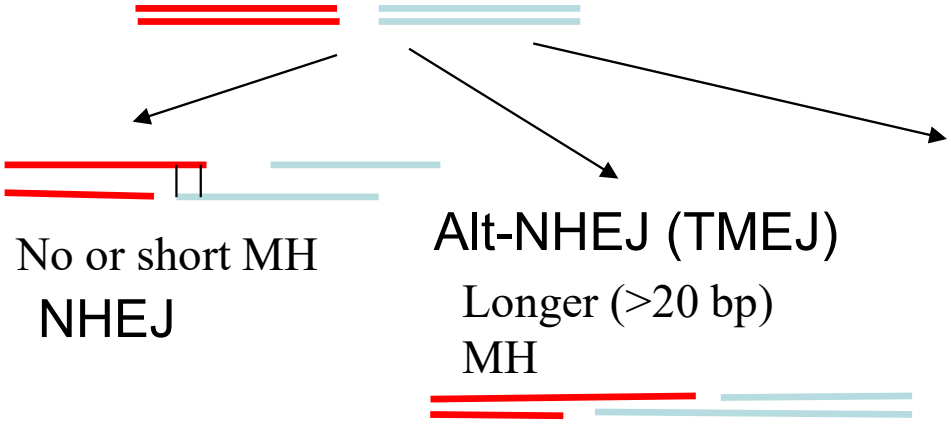
Correct end rejoining with deletions

2bp deletion
No microhomology

Rejoining of the incorrect ends (translocations/big deletions/inversions



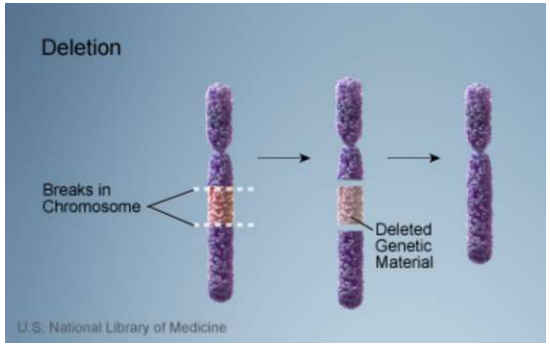
Balanced Translocation



No or short MH
NHEJ

Alt-NHEJ (TMEJ)
Longer (>20 bp) MH

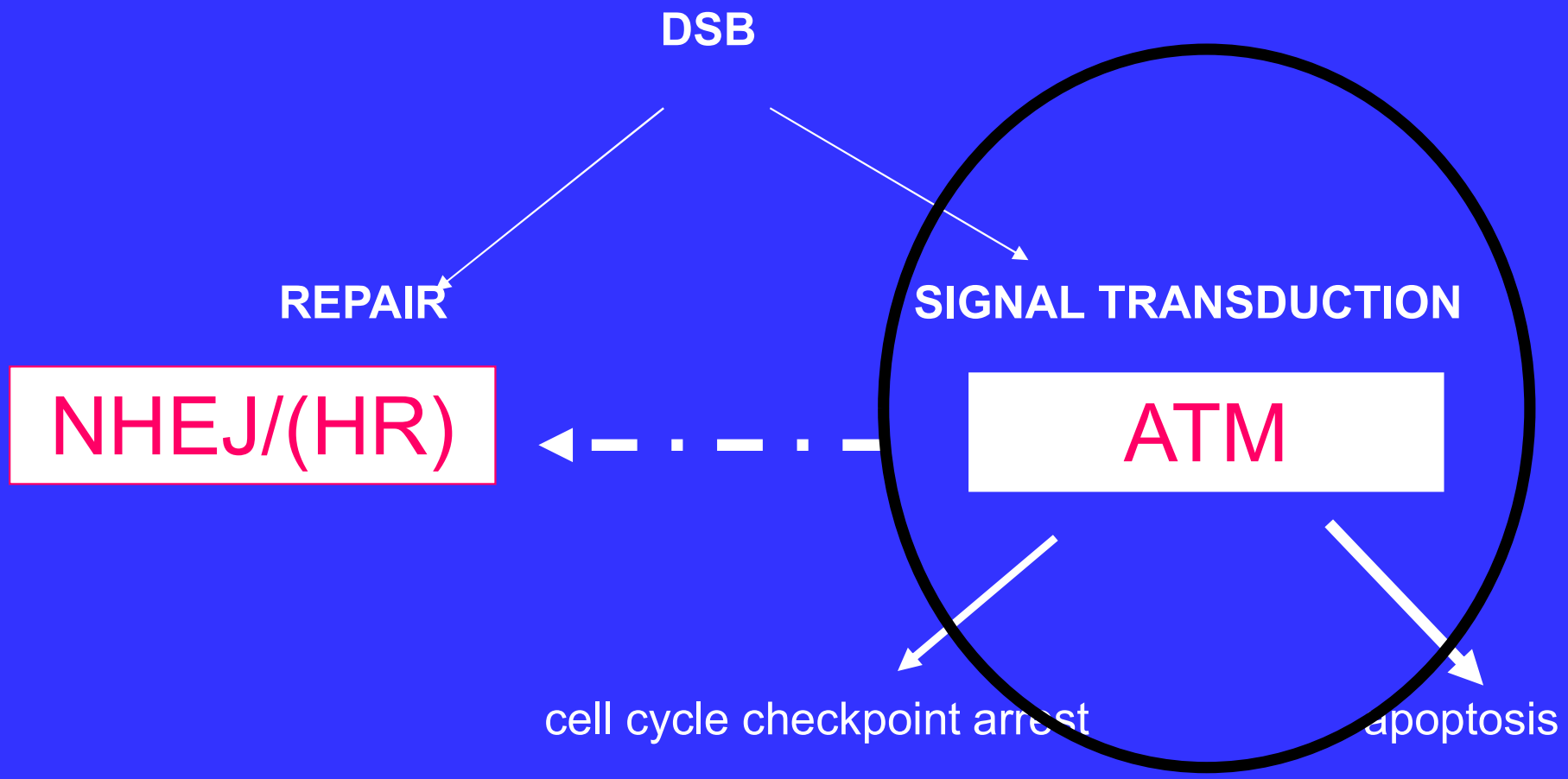
Single strand annealing (SSA) – occurs in G2 – in place of HR



Microhomology mediated end joining

Important type of misrepair after IR

Damage response to DNA double strand breaks (DDR) – encompasses repair + a signalling response

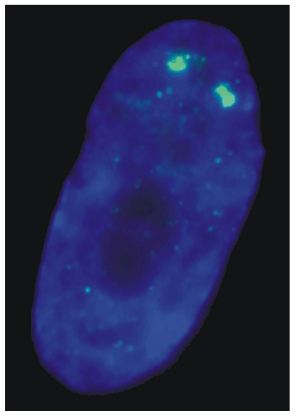


ATM signalling

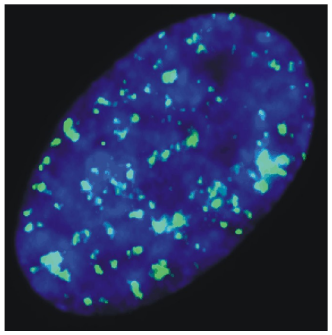
- ATM (ataxia telangiectasia mutated) is a kinase that activates a signalling response to a DSB
- A related kinase, ATR, regulates an overlapping response to ssDNA regions (> 25 bp) (which arise following replication).
- SSBs (just a nick/gap) do not initiate signalling.
- IRIF (irradiation induced foci) arise at DSBs due to the ATM-dependent signalling response.
- IRIF involve the recruitment of multiple proteins and histone modifications at DSBs. The changes can extend Mega base pairs around the DSB.

What are IRIF??

wild-type nucleus



Wild type cell with no IR



15 min after exposure to low dose radiation. Stained for phosphorylated H2AX (γ-H2AX) (primary antibody recognises gH2AX: secondary antibody is fluorescent and recognises first antibody.

- With time these foci are lost and the kinetics of loss correlates with the kinetics of DSB repair.
- They remain in DSB repair defective mutants.

ATM - ataxia telangiectasia mutated

- large protein kinase (11 Kb) belongs to the same family of kinases as DNA-PKcs and ATR – PIKK family)
- PIKK=phosphatidyl inositol 3-kinase like kinase (like a PI3 lipid kinase)
- ATM is non-essential - many A-T patients (individuals who have mutations in ATM) have no residual protein - knock out mice viable
- Is activated by DNA DSBs
- Loss of ATM causes MAJOR radiation sensitivity (and cancer predisposition)
- BUT most DSBs are repaired in absence of ATM – which demonstrates the importance of the signalling process

Steps in Signalling from a DSB-

It involves an ordered assembly of proteins and histone modifications at the DSB – a choreography

Activators/sensors

Recognise DNA damage and initiate the signal

Mediator proteins

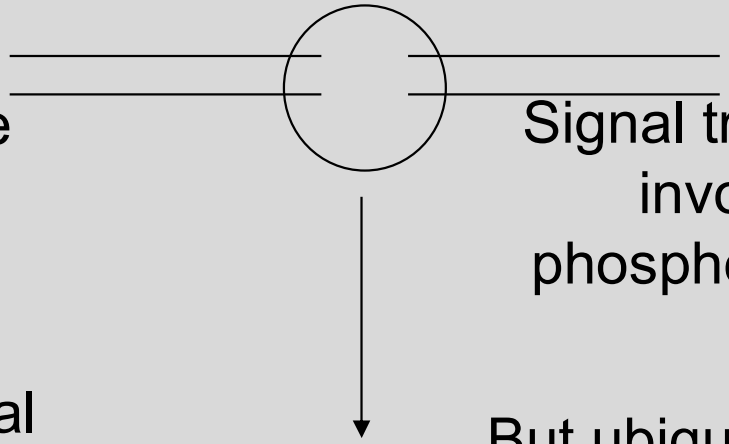
help to transmit the signal

Transducers

relay the signal via further phosphorylation or transcription – Chk2

Effectors

cause the final effect



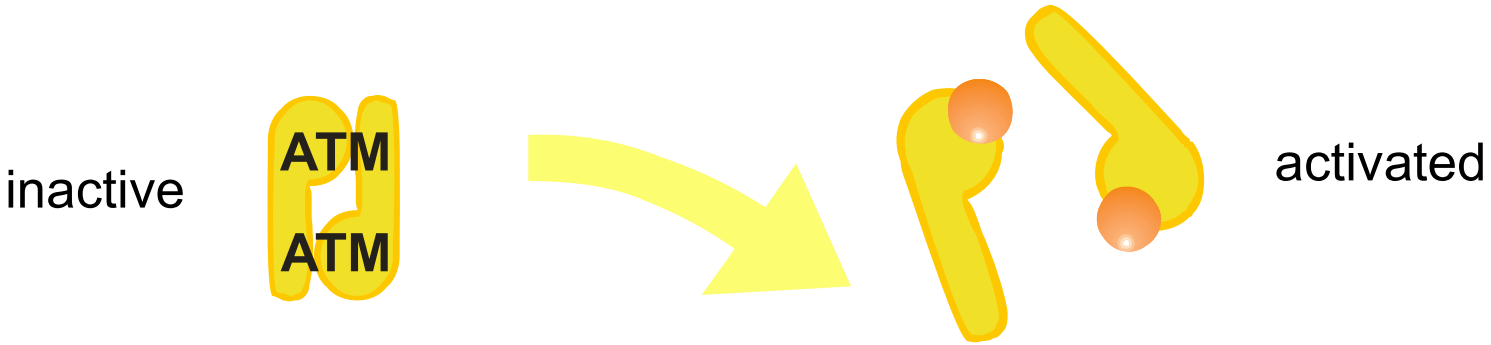
Signal transduction process involves **PROTEIN** phosphorylation to transmit signals

But ubiquitylation, sumoylation and methylation epigenetic modifications arise

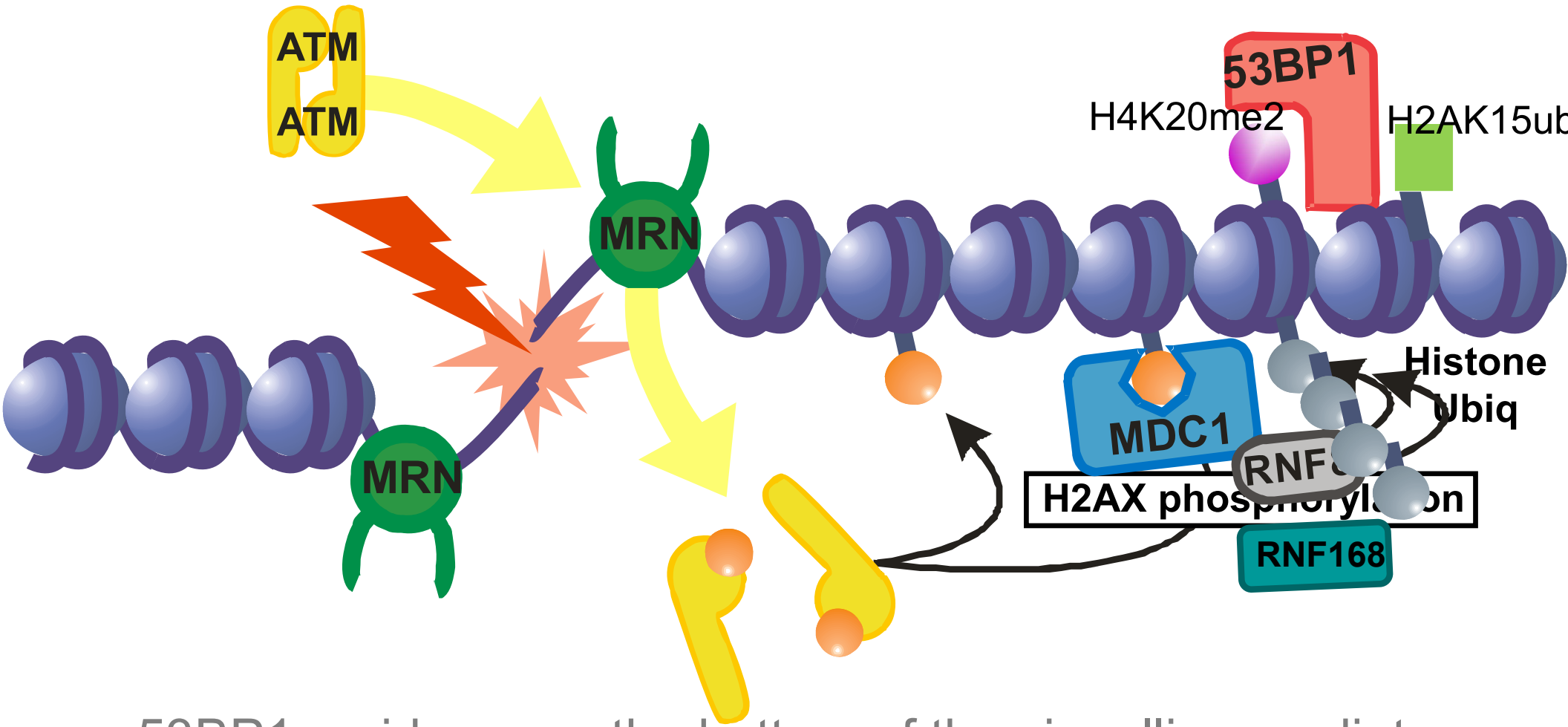
Mediator proteins:
H2AX is a variant form of the histone H2A
MDC1= Mediator of DNA damage checkpoint 1
53BP1 = p53 binding protein 1
BRCA1= breast cancer susceptibility gene 1
RNF – ring finger protein – they are ubiquitin ligases

Sensing and ATM activation.

- MRE11/NBS1/RAD50 (MRN) is the DSB sensor (not ATM)
- The 3 PIKKs (ATM, ATR and DNA-PKcs) have partner proteins that localise them to DNA damage.
- DNA-PKcs partner protein is Ku.
- ATM partner protein is the Mre11/Rad50/NBS1 complex (ATM binds to NBS1)
- MRE11-RAD50 recognises the DSB ends.
- NBS1 binds ATM via its (NBS) C-terminus).
- ATM exists in undamaged cells as a dimer.
- When in contact with MRN, undergoes autophosphorylation causing its dissociation into monomers and activation



The ATM signalling mediator hierarchy

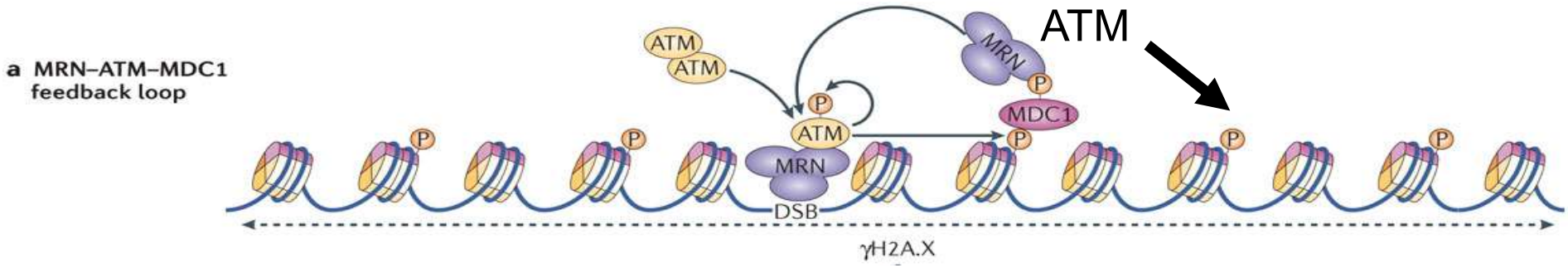


53BP1 resides near the bottom of the signalling mediator hierarchy (it recruits PTIP and RIF1).

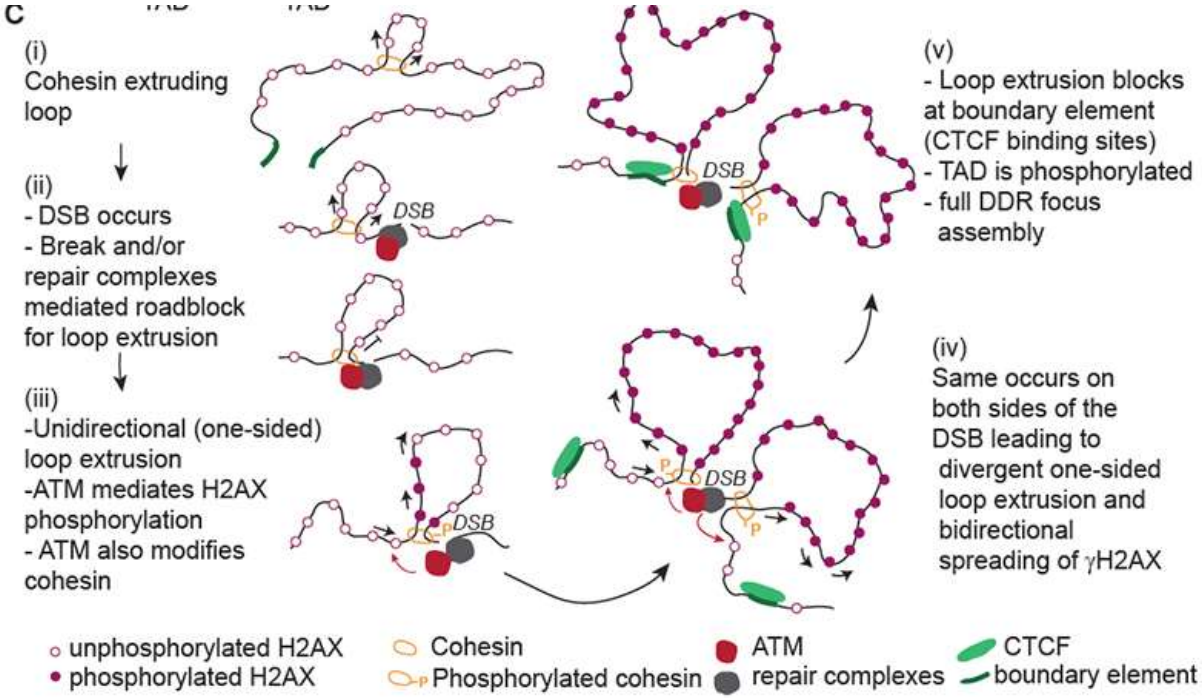
This protein assembly generates irradiation induced foci (IRIF).

TWO MODELS FOR FOCI EXPANSION

- γH2AX binds MDC1, a mediator protein. MDC1 is phosphorylated by ATM. **pMDC1 interacts with MRN which further tethers MRN at the DSB. This allows the binding of more**



More recent concept: loop extrusion model (Arnould .. Legube – Nature 2021 590, 660-665



TADs (topologically associated domains) represent domains of DNA organisation – important for transcription. They are important for repair and hold the DSBs at a fixed position to result in one-sided cohesion mediate loop extrusion – occurs on either side of the DSB

Recruitment and Role of 53BP1

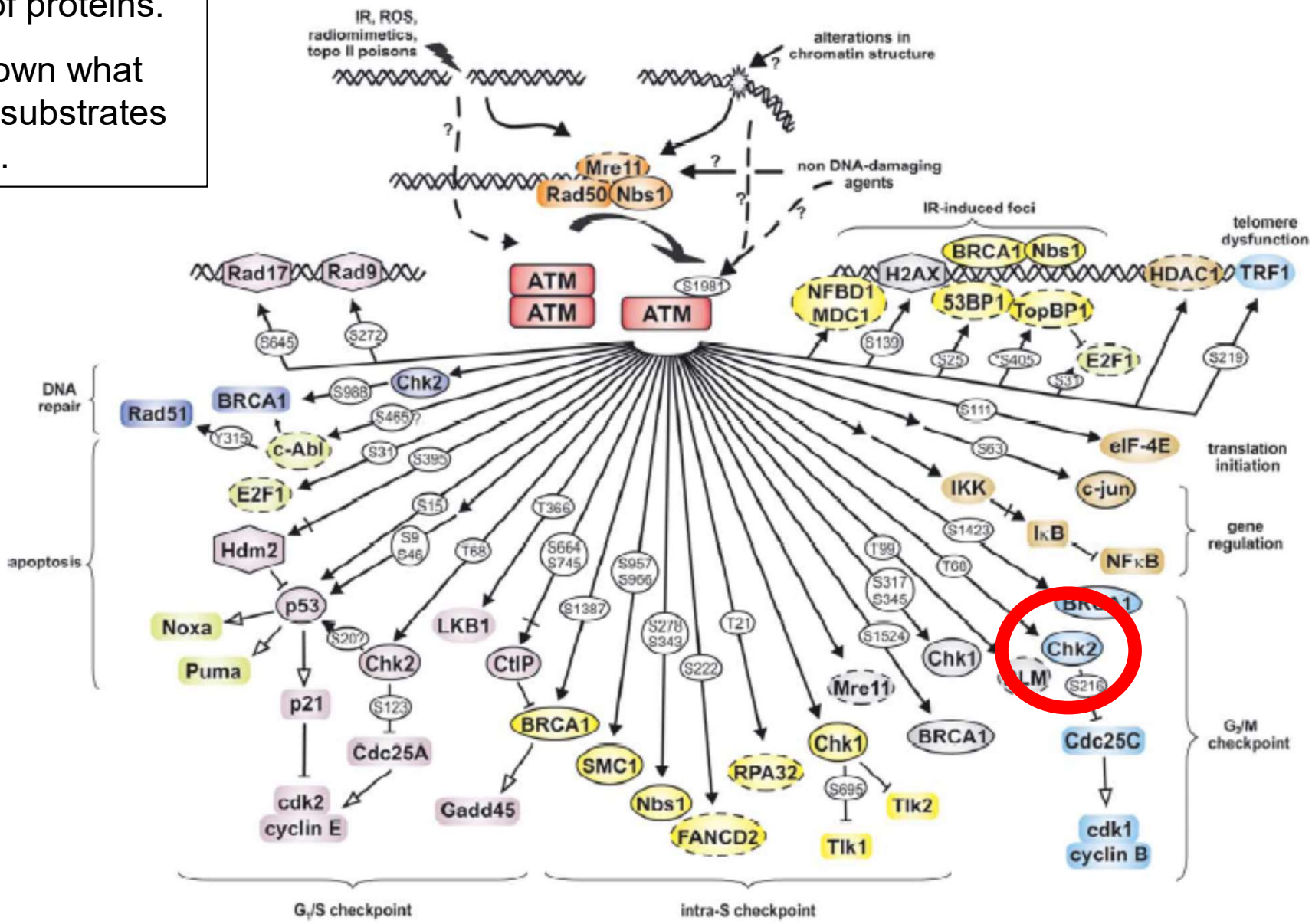
- 53BP1 is recruited by two histone modifications
  H4K20me2 and H2AK15Ub
- 53BP1 recruits RIF and SHIELDIN. These factors promote chromosome condensation or repression around the DSBs
- Thus, 53BP1 prevents resection (ie 53BP1 is pro-NHEJ)
- The RNF8/RNF168 ubiquitin chains also result in the recruitment of a complex involving BRCA1, Abraxas, Merit40, BRCC36, BRCC45 and RAP80.
- BRCA1 can counteract 53BP1 by causing it to move away from the break end
- Thus BRCA1 promotes resection and hence HR
- Very recently – it has been appreciated that 53BP1 forms as nano-foci – and that these confer a phenomenon called “phase separation”

Targets of ATM phosphorylation

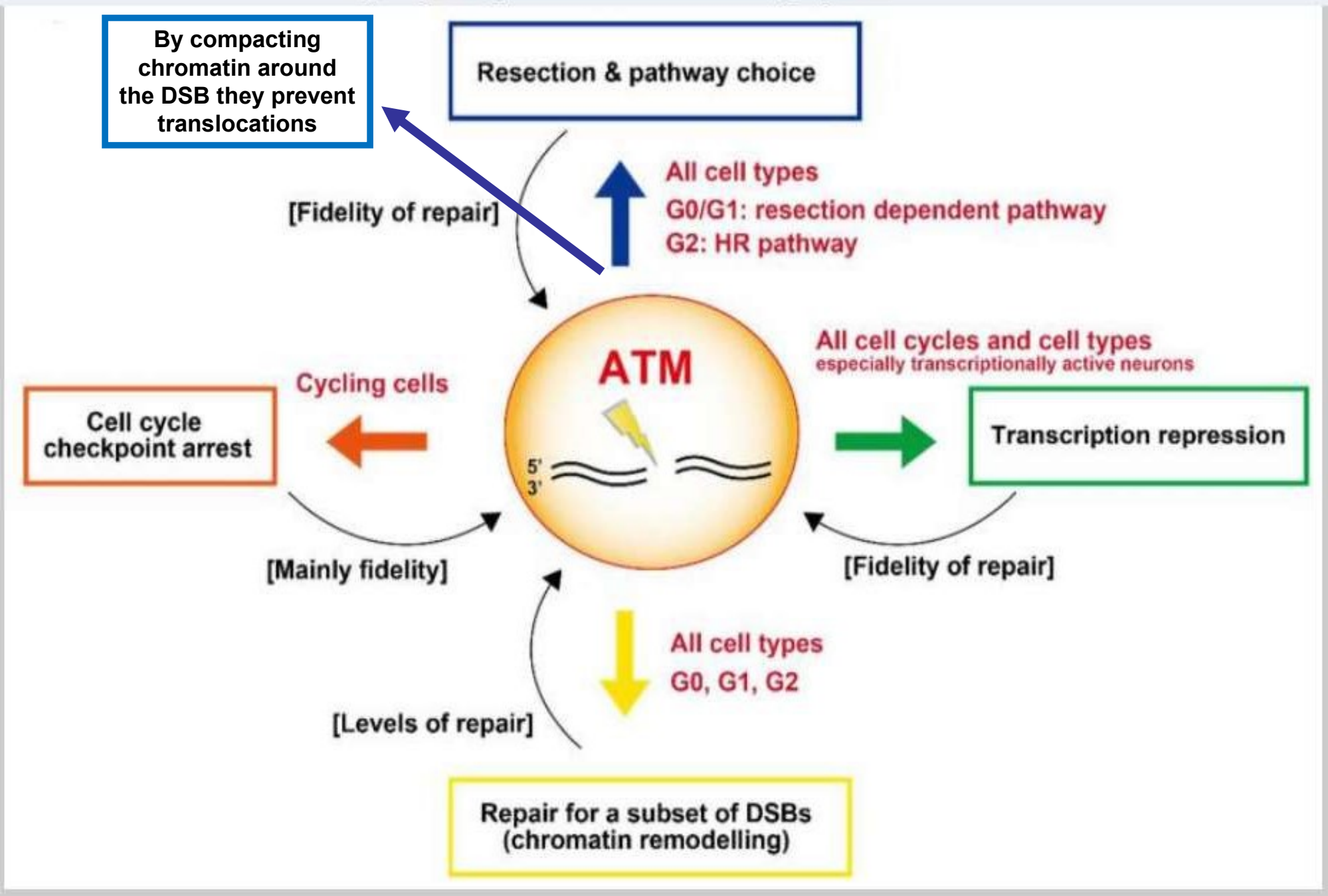
ATM phosphorylates a huge array of proteins.

It is not known what many of the substrates do.

Chk2 is the main transducer for ATM signalling

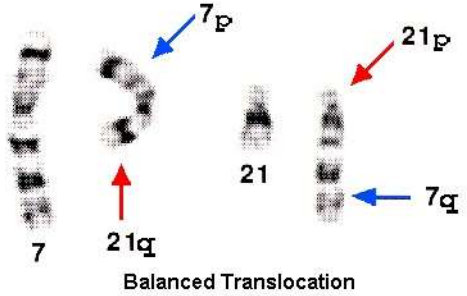


What does ATM and these foci do:



1. ATM signalling enhances "correct" end rejoining – most likely via chromatin changes in the DSB vicinity

Translocations (rejoining of the wrong ends) are a potential weakness of c-NHEJ because it does not require homology.
Therefore need to maintain synapsis



ATM creates a repressive environment to maintain synapsis.

Loss of ATM enhances IR induced translocations

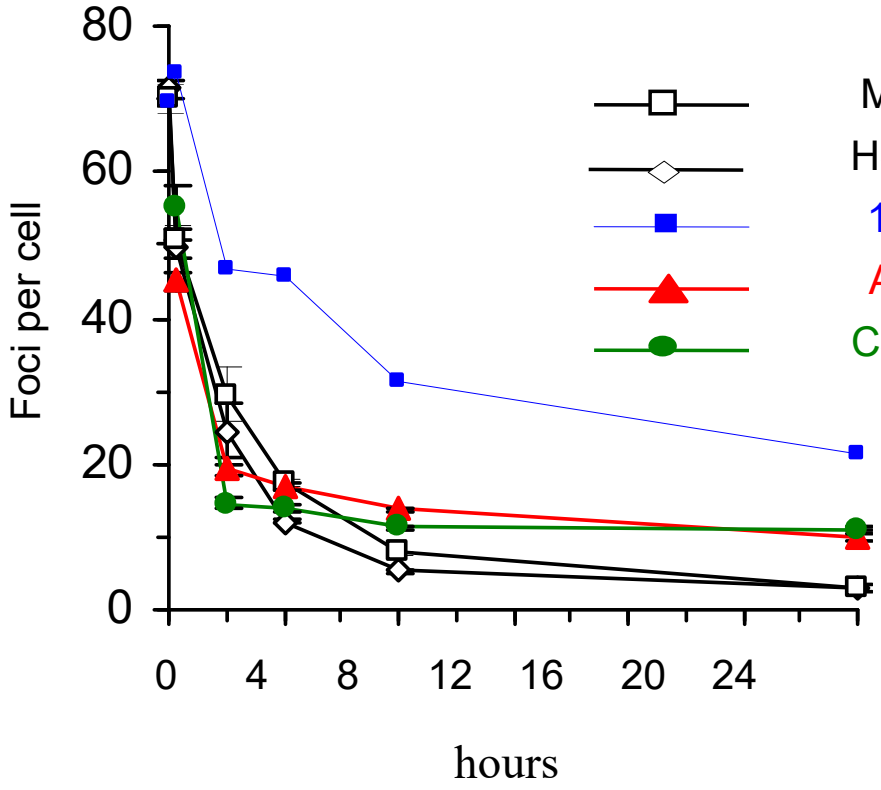
Classical cytogenetics has suggested that translocations are a major lethal lesion following irradiation in G1 phase

3. Role of ATM in DSB repair

DSBs are repaired with fast and slow kinetics.

NHEJ (Ku, DNA-PKcs, XRCC4, ligase IV, XLF) proteins are required for both the fast and slow repair process.

BUT ATM, Artemis and all the ATM signalling proteins (MRN, H2AX, MDC1, RNF8, RNF168 and 53BP1) are required ONLY for the slow DSB repair process

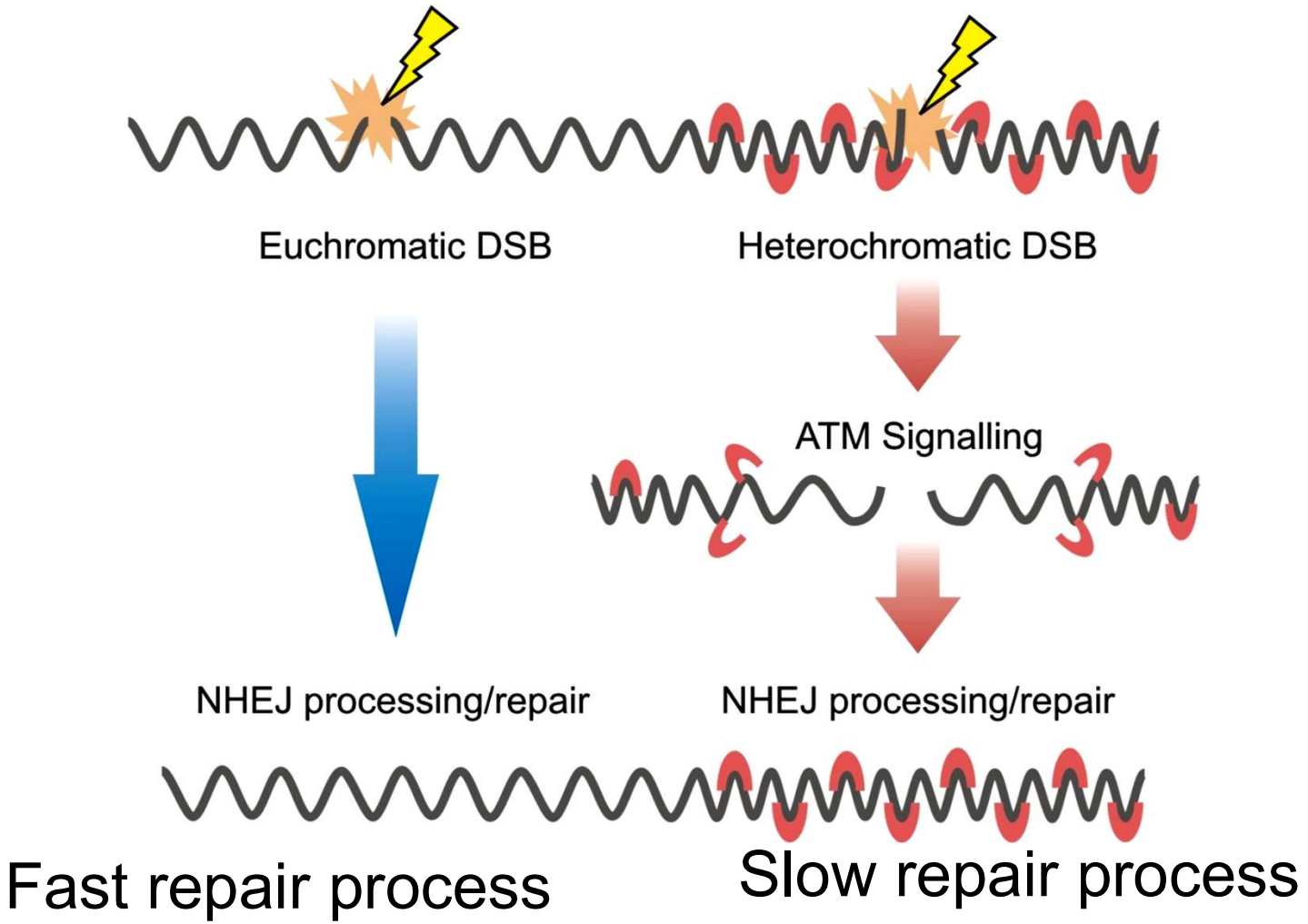


AT1BR (AT) lack ATM

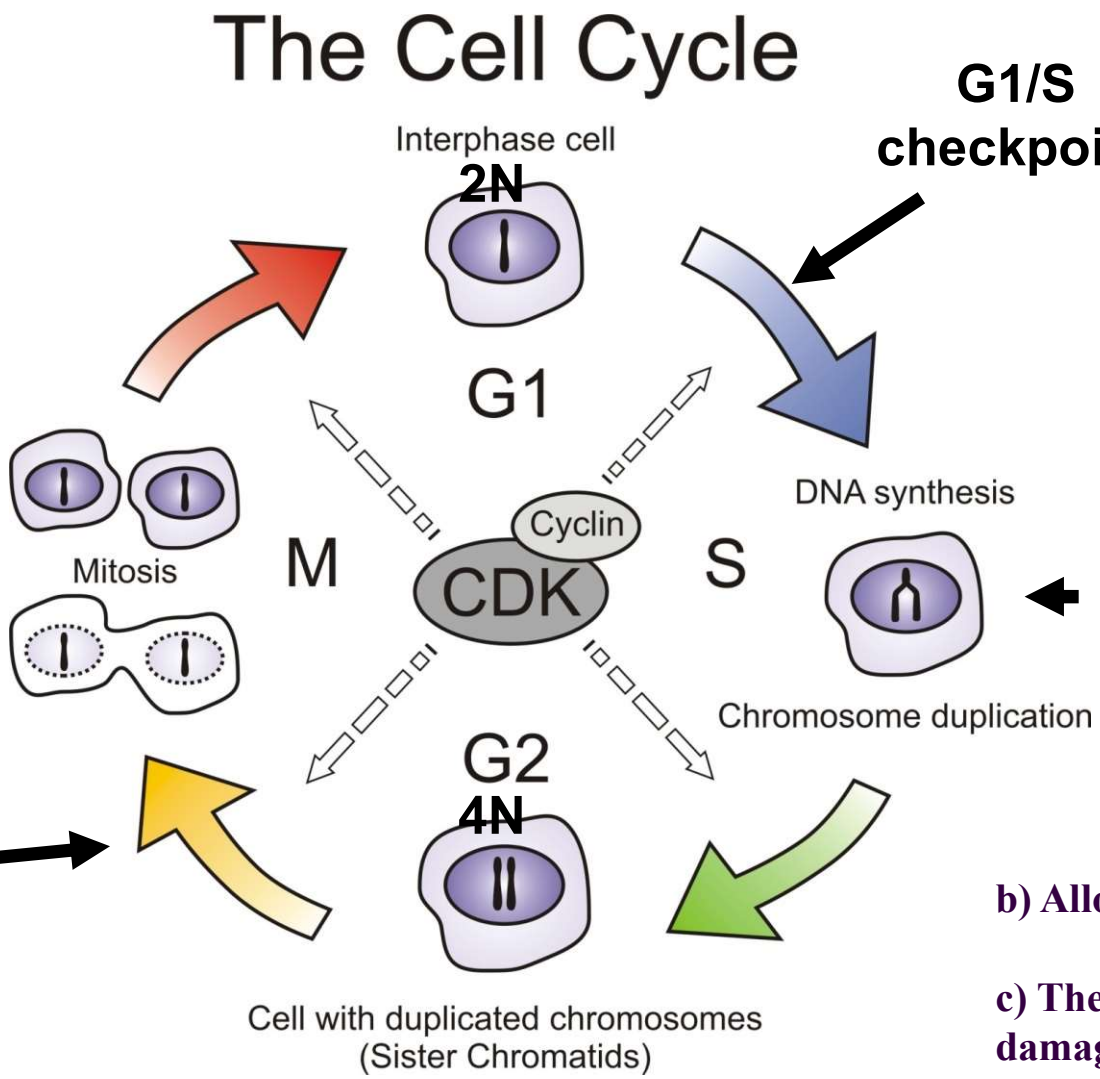
CJ179 (Art) lack the nuclease **Artemis**

What is special about these DSBs that require ATM (and other factors) for repair.

We don't precisely know but there is evidence that they are DSBs located in regions of compacted chromatin



5. Cell Cycle Progression-*Checkpoints*



G1/S checkpoint

Intra-S checkpoint

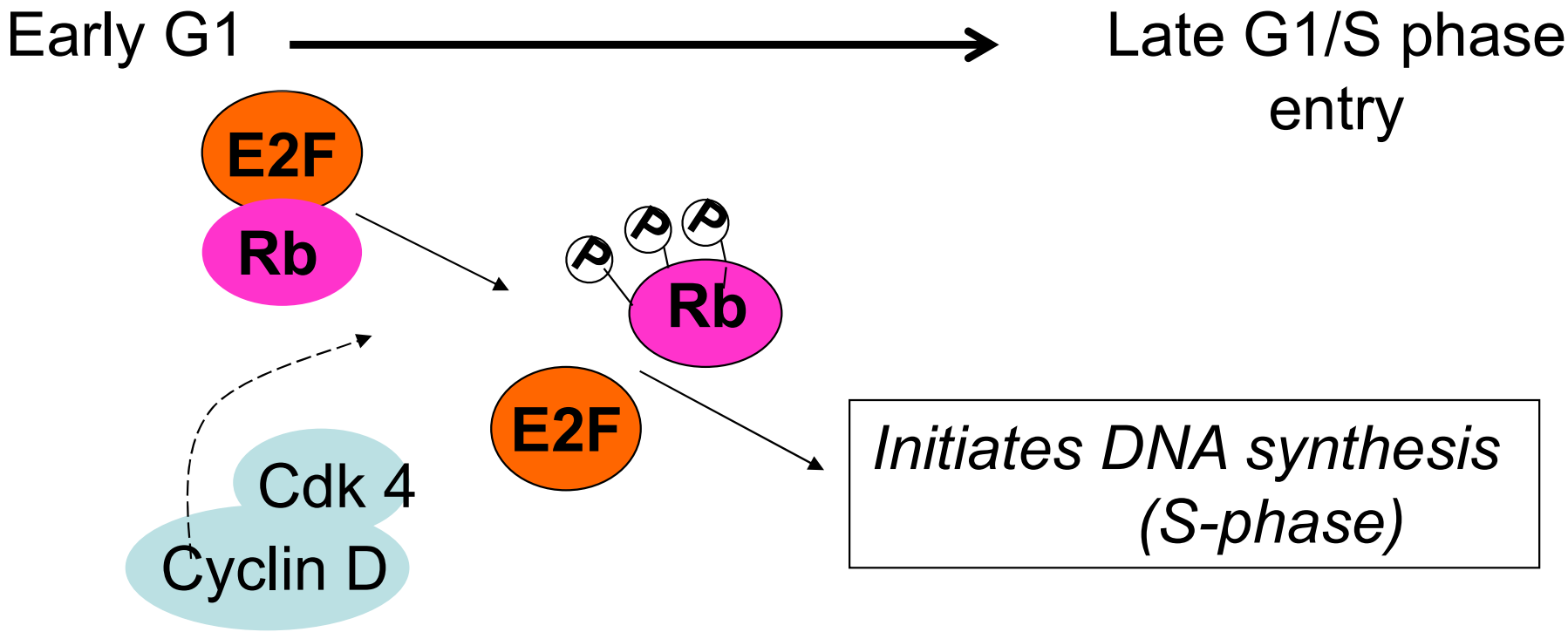
G2/M check point

DNA damage Checkpoints prevent cell cycle progression in the presence of DNA damage: G1/S and G2/M are the most significant and will be discussed

b) Allow more time for repair.

c) They can be permanent and prevent damaged cells cycling.

G1/S progression requires pRb.



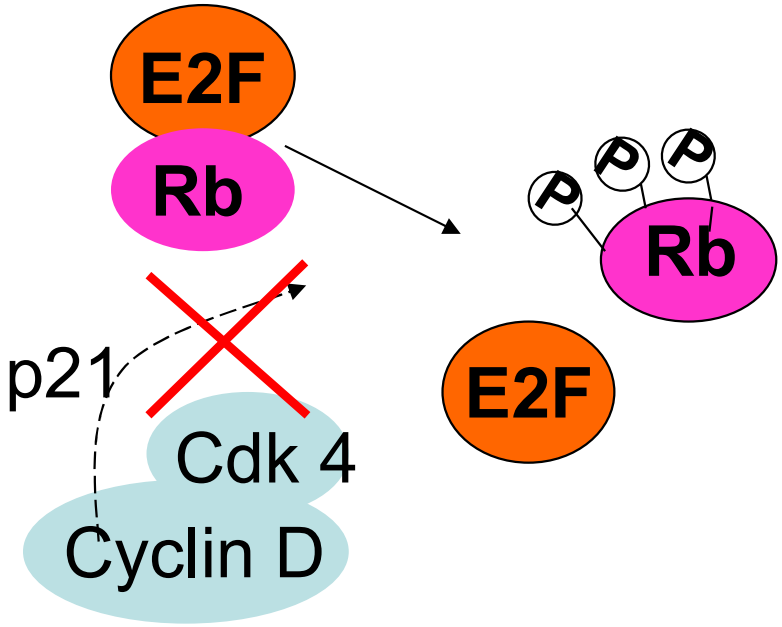
- G1/S progression is regulated by Cyclin D/CDK4 phosphorylation of Rb
- When Rb is fully phosphorylated it is released from EF2. The restriction point represents the point when EF2 is released from Rb.
- E2F is a transcription factor and transcribes factors required for replication.

G1-S checkpoint arrest

DNA damage activates p53, which transcriptionally activates, p21, a Cdk inhibitor

ATM → P53 activation —*Transcriptional regulation*→ p21$^{Cip/Waf1}$ *Accumulation*

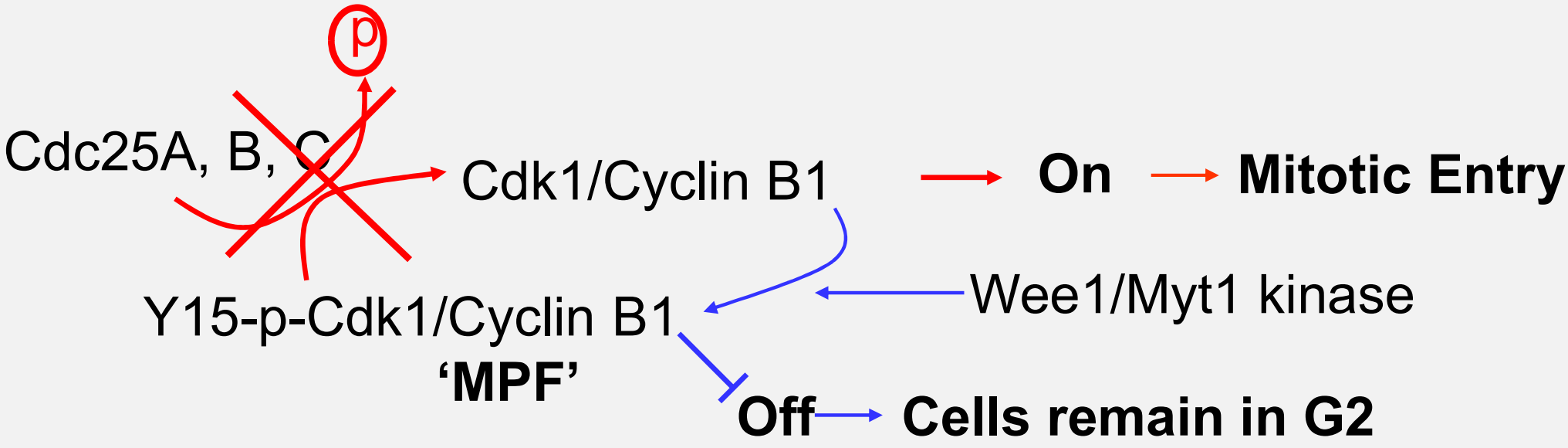
Cdk Inhibitor



NB: ATM activation of p21 requires transcription.

Therefore G1/S arrest is a slow process.

G2-M phase progression



Mitotic entry is regulated by Cdk1/Cyclin B1 (called MPF – mitosis promoting factor)

Cdk1 is phosphorylated by the Myt1 and Wee1 kinases on Thr14 and Tyr15.
This phosphorylation **inhibits** Cdk1
Cdk1 is progressively dephosphorylated by Cdc25 phosphatases (a, b or c) – **need dephosphorylation for Cdk1 to be active**.

ATM and ATR phosphorylate Chk2 and Chk1, respectively.
Chk1 and Chk2 phosphorylate Cdc25. This inhibits Cdc25 activity and causes Cdc25 degradation.
Hence entry into mitosis is prevented.
Chk2 is a transducer kinase

6. Apoptosis is a highly **orchestrated** form of cell suicide

Orchestrated = a defined set of events= programmed.

•Cell death by apoptosis is regulated – ie the death is not due to an inability to survive but is a positively, regulated death process

•genes are required for apoptosis and their loss can hence survival

- Apoptosis can remove cells harmful to the organism.
- The contents of the cell become available to and benefit the organism

- ATM phosphorylates p53, which activates genes enhancing apoptosis.
- p53 is the most commonly mutated gene in cancer cells.

- Not all cells die by apoptosis after IR treatment (most differentiated cells do not die by apoptosis)
- some cells sensitively activate apoptosis and these cells are radiosensitive (eg lymphocytes)

Two pathways for Apoptosis.

- Mitochondrial mediated pathway (resembles pathway in C. elegans) – signals arise from the mitochondria – intrinsic apoptosis (typically initiated by DNA damaging agents)

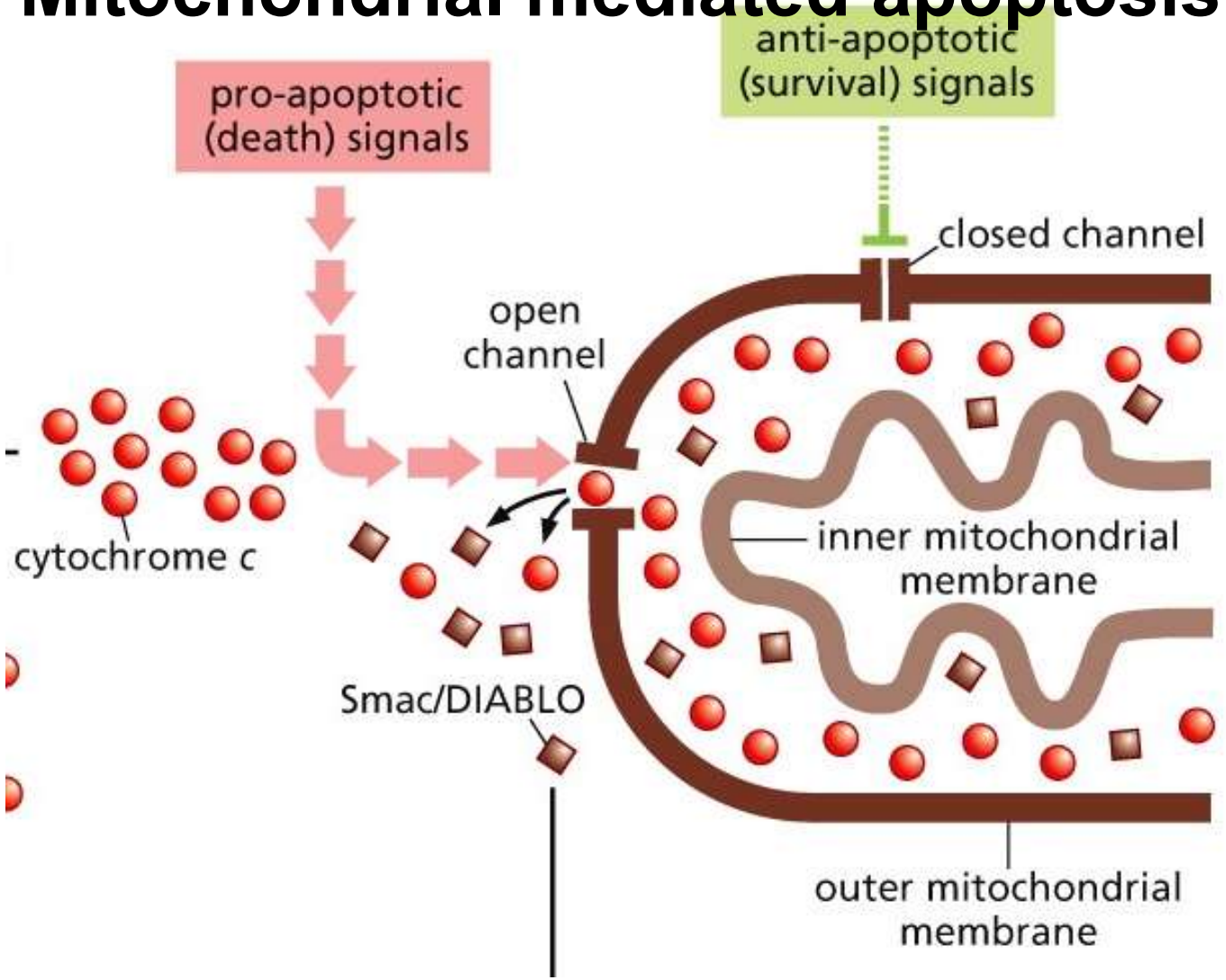
- Receptor mediated signalling at the plasma membrane – signals come from outside the cell – extrinsic apoptosis (initiated by death receptors at the cell membrane).

Both signal to CASPASES

Caspases

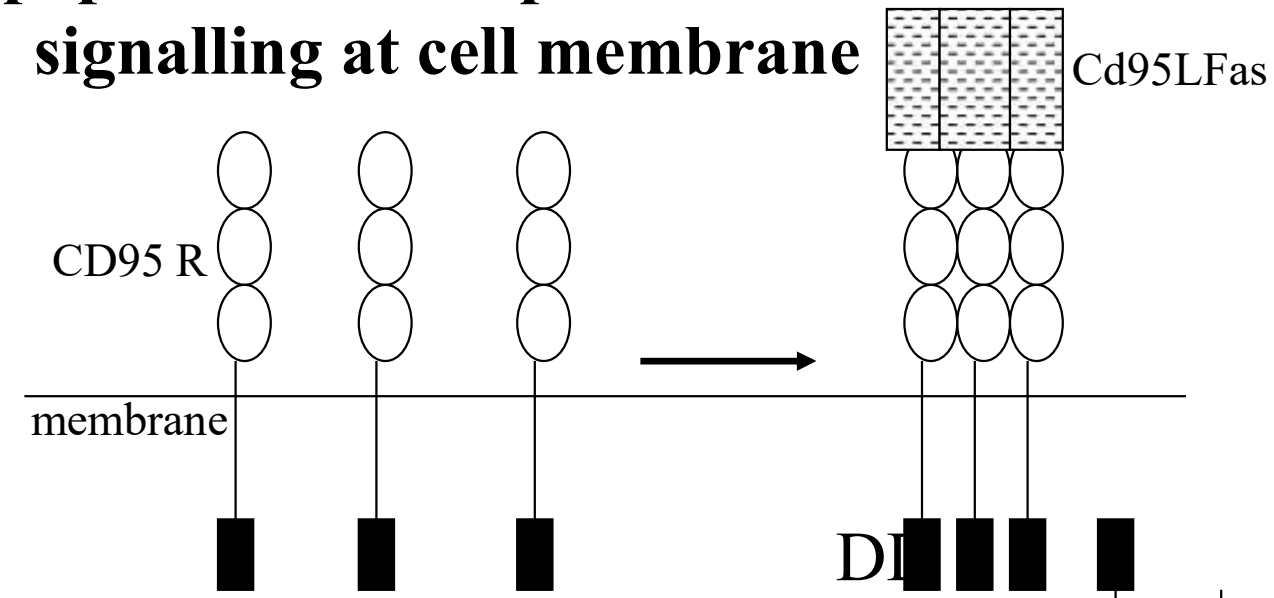
- Either process of apoptosis activates caspases
- Caspases are proteases, which digest proteins during apoptosis (canabolistic enzymes)
- They promote digestion of DNA (laddering effect)
- They degrade lamin and other essential nuclear proteins.
- They degrade enzymes (eg PARP and DNA-PK) which promote cell survival after DNA damage.
- This process causes cell death and release of cell material to the organism

Mitochondrial mediated apoptosis



- cellular stress signals converge at the mitochrondria to induce mitochondrial outer-membrane permeabilisation (MOMP).
- MOMP leads to release of Cytochrome C, the major factor from the mitochondria leading to apoptosis (via caspase activation).
- MOMP is regulated by a balance of pro-apoptotic and anti-apoptotic members of the Bcl2 (B-cell lymphoma 2) family

Apoptosis via Receptor mediated signalling at cell membrane



CD95L/Fas

DD death domain

DED death effector domain

Caspase recruiting domains

Steps;

the ligand binds to the receptor

this recruits an adaptor protein (FADD)

this recruits and activates the effector caspase

FADD

Caspase prodomain

Caspase 8

Card

Activation of caspase 8

Large and small subunit of caspase 8

Activation of downstream caspases eg. Caspase 3, 6 or 9.

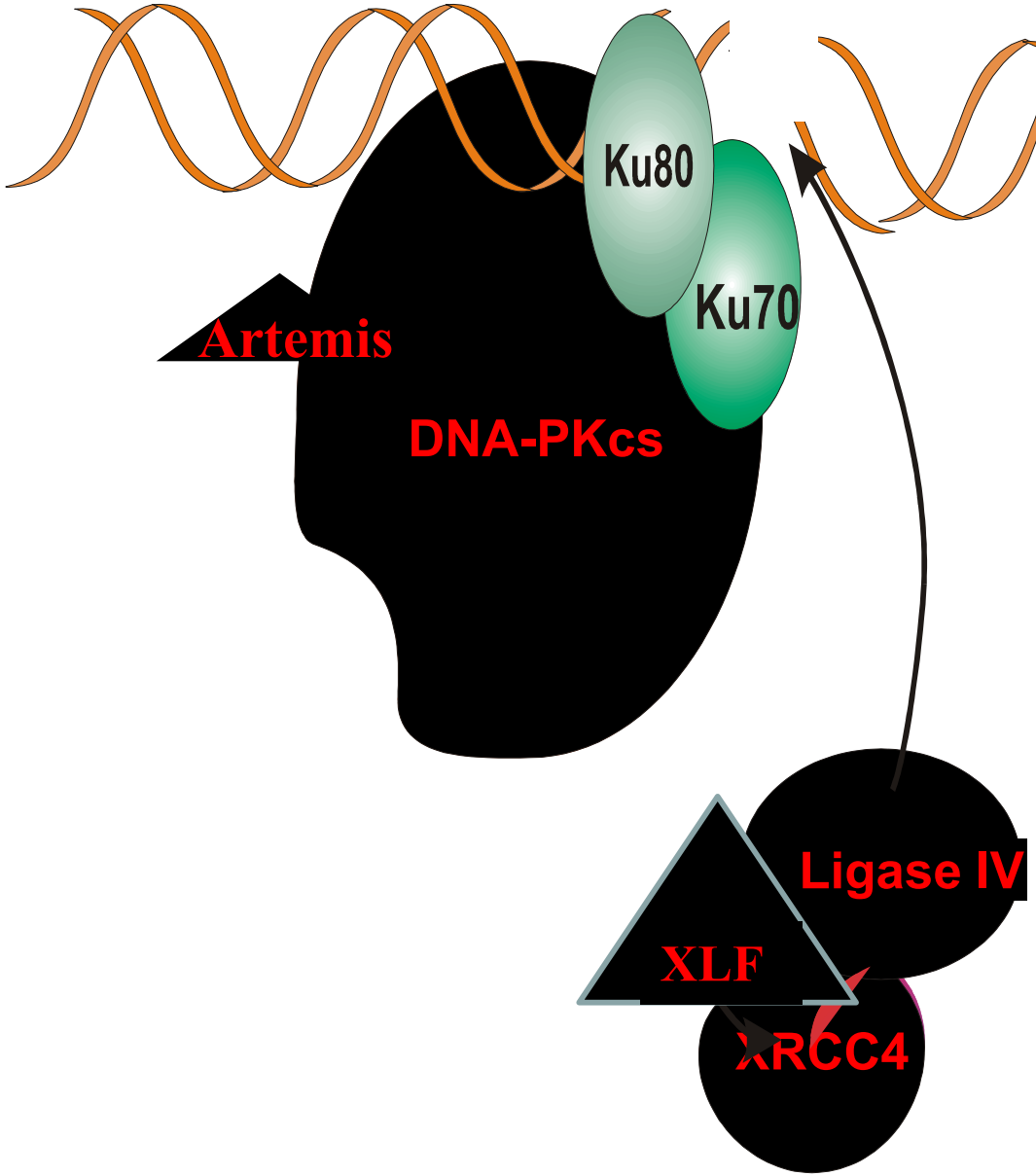
Lecture overview.

- Distinction between endogenous damage and radiation induced damage.
- The two DSB repair pathways - NHEJ and HR
- DNA damage response signalling by DSBs.
- Steps in the formation of radiation induced foci
- The impact of ATM on DSB repair
- Cell cycle checkpoint arrest
- Apoptosis.
- (Radiation sensitive syndromes)

DNA damage response disorders:

- Damage response disorders are rare inherited genetic disorders – here we focus on those conferring radiosensitivity due to failure to respond to DSBs
- Immunodeficiency often observed in these disorder -
- Developmental abnormalities are common in DDR disorders (microcephaly and ataxia – neuronal development often in radiosensitive disorders)
- Cancer predisposition is sometimes seen- especially lymphoid cancer
- How can we exploit our knowledge to treat these disorders

Patients with defects in NHEJ proteins (all have been identified in patients except)



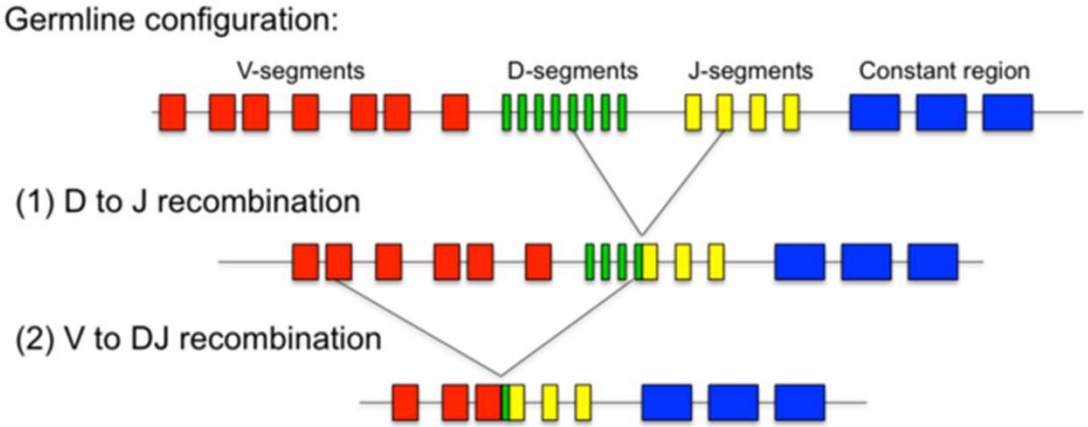
They (usually) display:

- clinical radiosensitivity (Radiotherapy) + sensitivity to drugs used for immunosuppression for bone marrow transplants
- marked or moderate immunodeficiency (SCID or CID)
- microcephaly (small heads), dysmorphic facial features and growth delay.
- Sometimes lymphoid cancers

NHEJ and the immune system. Programmed DSB formation.

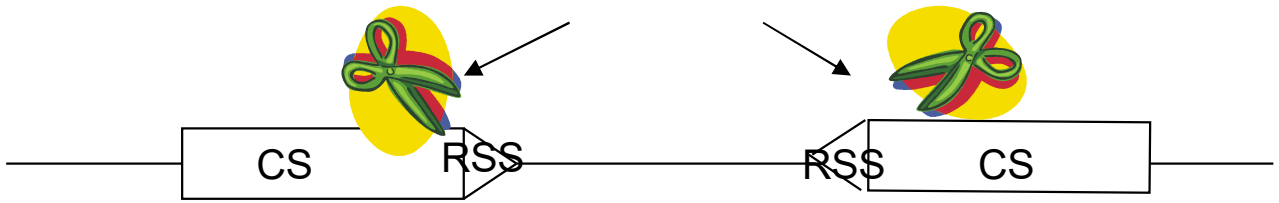
The immune response aims to create genetic diversity to enable recognition of different antigens to which we are exposed

V(D)J recombination (variable (diversity)joining) is one processing achieving that



V(D)J recombination creates T and B cells

Site specific DSBs are introduced by an endonuclease to allow rearrangement of V, D or J segments – and then rejoined with diversity by NHEJ



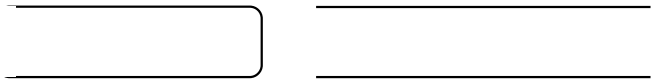
Severe combined immunodeficiency (SCID) **(NO or very few T or B cells)**

combined immunodeficiency (CID) **(reduced but residual T or B cells)**

Most NHEJ genes (eg LIGIV) are essential for viability (ie for an embryo to develop) – therefore patients have hypomorphic (leaky) mutations (some residual function) – hence they are usually CID

Artemis is only required for the repair of a subset of IR induced DSBs – therefore it is non-essential. But is essential for VDJ recombination so patients often SCID

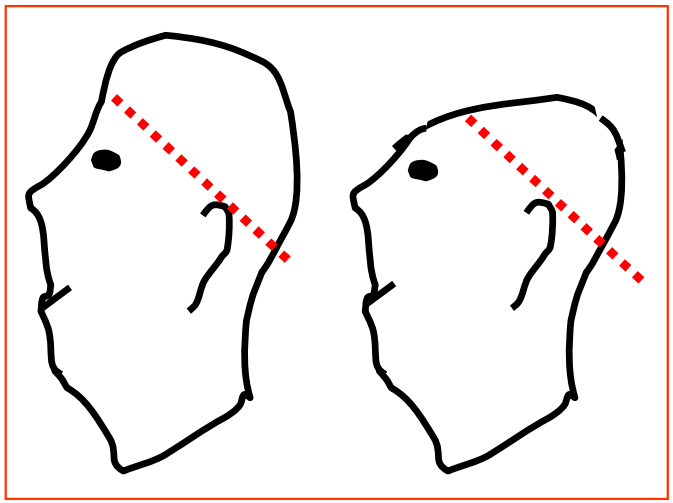
In brief –DSBs induced during VDJ recombination have hairpin ends – and Artemis cleaves the hairpin



SCID or CID with high radiosensitivity is usually caused by NHEJ deficiency BUT XRCC4 patients do not display immunodeficiency – redundancy with XLF?

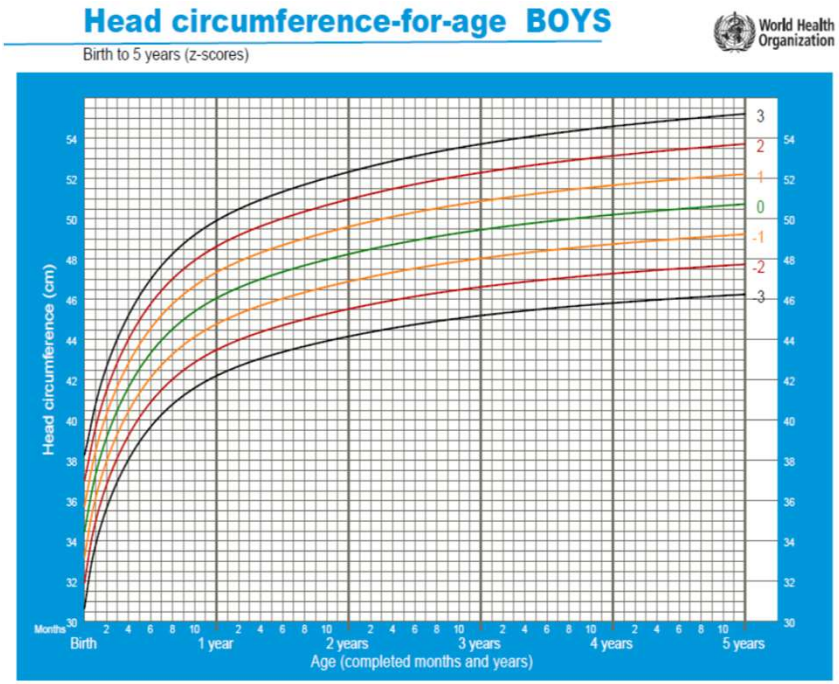
Microcephaly

Reduced occipitalfrontal (head) circumference

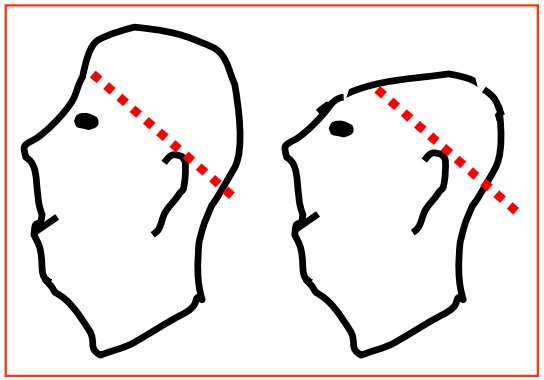


NB the pronounced nose – bird like features - now called dysmorphic facial features arise due to the reduced head circumference

NB it is the forebrain that is reduced in size



NHEJ and microcephaly/growth delay



- Microcephaly is present at birth but is not progressive (ie arises during embryogenesis)
- Embryonic neuronal development (neurogenesis) involves a stage of rapid proliferation when high endogenous DSBs arise in the neuronal stem cells
- Failure to rapidly repair these DSBs in progenitor stem cells leads to rapid cell death via apoptosis (NB non dividing cells can tolerate unrepaired DSBs)
- Post birth lost stem cells cannot be replaced because the stem cell regions diminish

- Loss of DNA ligase IV, DNA-PKcs, XLF, XRCC4 can lead to **microcephaly** –
- but **Artemis deficiency does not** (DSB repair defect is less severe).

NHEJ patients can develop lymphoid tumours

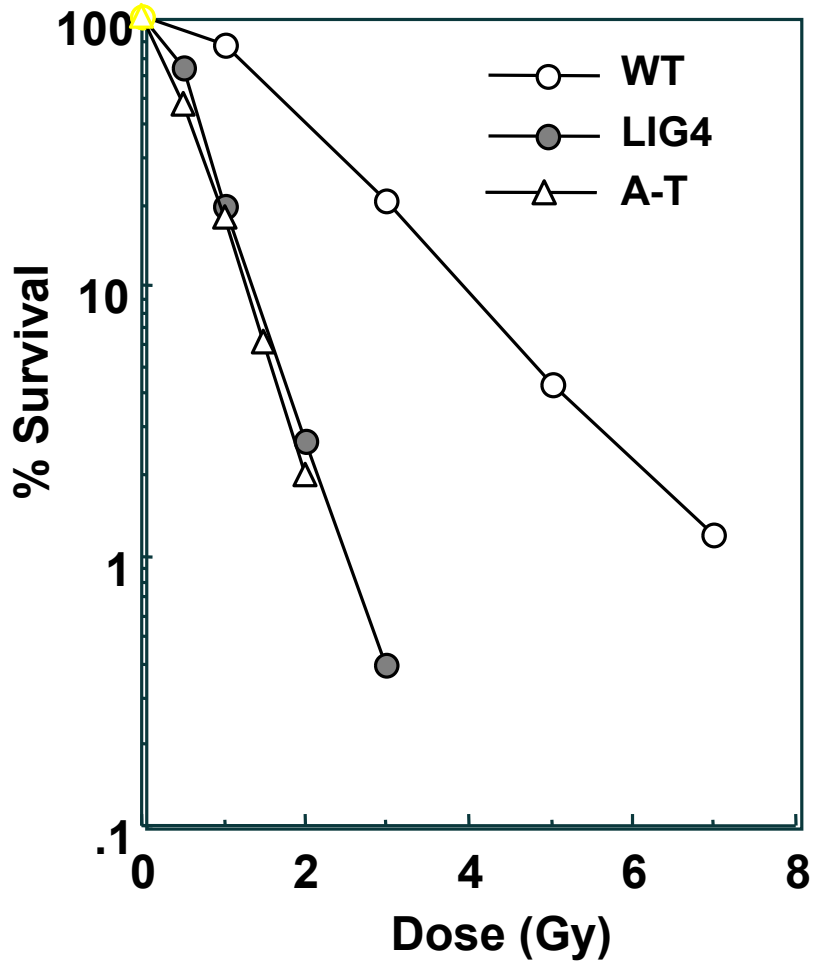
- Perversely –patients with less severe immunodeficiency are more at risk of tumours because they have T and B cells

- Partly due to incorrect VDJ rearrangements

- But also due to viruses (eg EBV) that can cause lymphoid tumours which develop due to immunodeficiency

Diagnosis of DNA DSB Repair Defective patients

Cellular Radiosensitivity

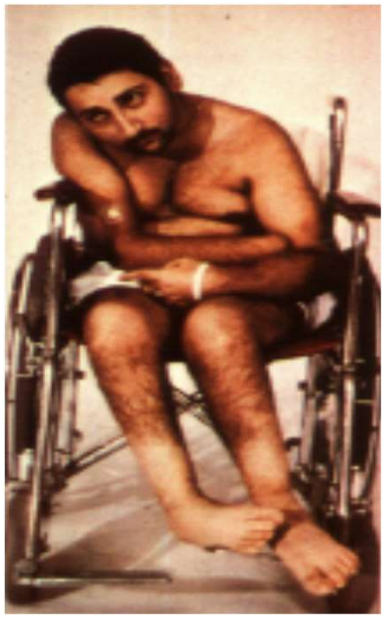


- Clonogenic survival using Primary skin fibroblasts
- Marked radiosensitivity (NB A-T patients are also very RS)
- NB often few T/B lymphoctyes (due to the immunodeficiency)– hence a skin biopsy to obtain fibroblasts (rather than a blood sample) is usually used for diagnosis.
- Fibroblasts are irradiated, then plated to give ~ 200 cells per dish, then incubated to allow growth of the cells and survival estimated.
- Diagnosis can be backed up by gH2AX analysis

BENEFIT OF DIAGNOSIS

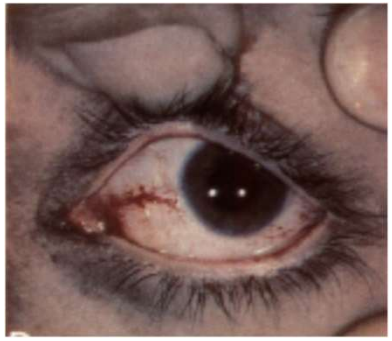
- Cannot yet cure this inherited disorder
- But diagnosis prevents radiation treatment for therapy – previously some Ligase IV patients died from radiation morbidity after radiation therapy
- Some have bone marrow transplants – this involves suppression of the immune response to allow grafting, The normal regime for BMT involves agents that induce DSBs – now that is modified for NHEJ deficient patients.

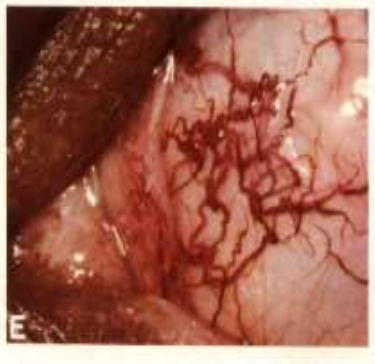
Ataxia telangiectasia
Web page for parent support group
http://www.atsociety.org.uk/



Ataxia means lack of order; muscle control, balance and speech; (uncoordinated involuntary movement of limbs);

Telangiectasia-dilated blood vessels

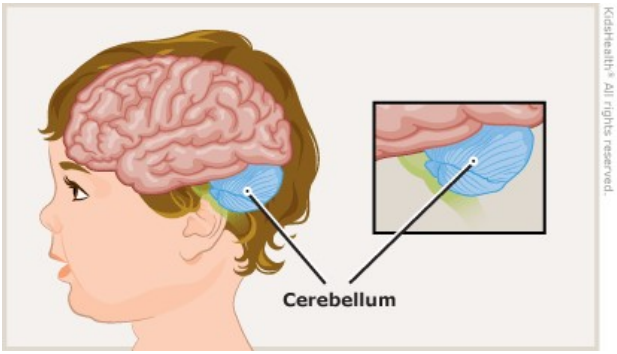




Ataxia telangiectasia – clinical presentation

Clinically:

- progressive ataxia
- Patients normal at birth but unsteady gait (ataxia) by 2-3 years. Wheel chair bound by teens.
- Due to degeneration and loss of Purkinje cells in the cerebellum region of the brain - note that this is not the forebrain, where Microcephaly arises in the NHEJ patients

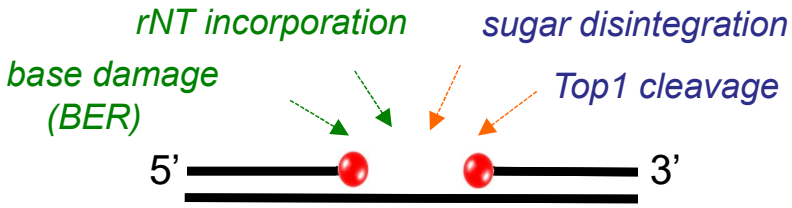


- Also dysarthia (problems with speech) and unco-ordinated movements
- Telangiectasias (normally seen as blood vessels in the eyes)
- Mildish immunodeficiency and lung abnormalities
- Cancer prone BUT normal intelligence

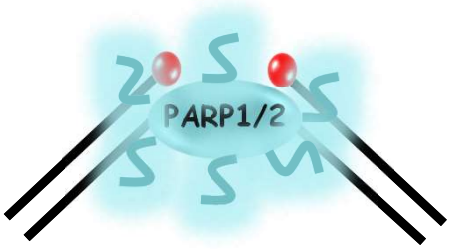
Other disorders displaying Ataxia

- Progressive ataxia is displayed by some other disorders with defects in SSB repair (will not cover but see next slide)
- Thus, progressive ataxia does not necessarily mean the patient has A-T.
- These patients have milder RS

Single-Strand Break Repair



Damage Detection



Ataxia Oculomotor Apraxia-4 & Microcephaly with Early Onset Seizures

End Processing

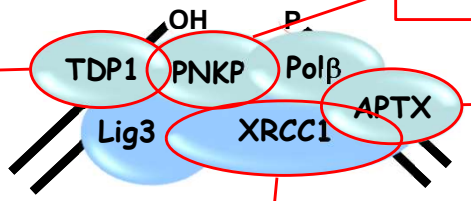
Spinocerebellar Ataxia with Axonal Neuropathy-1

SCAN1

AOA4/MCSZ

AOA1

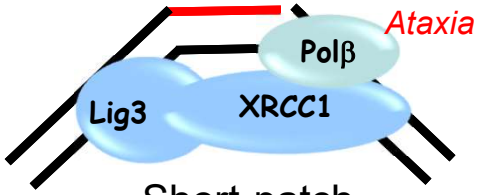
Ataxia Oculomotor Apraxia-1



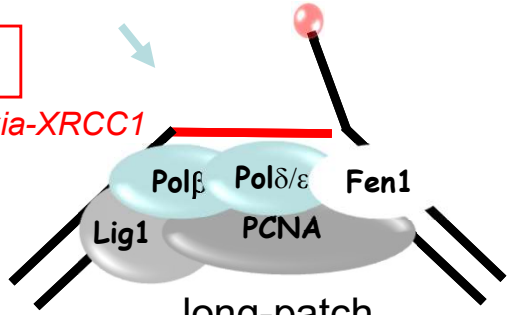
AOA-XRCC1

Ataxia Oculomotor Apraxia-XRCC1

Gap Filling

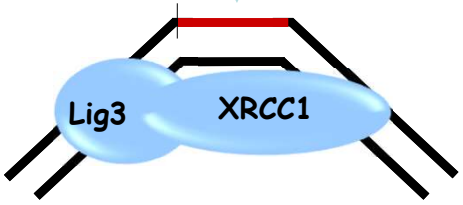


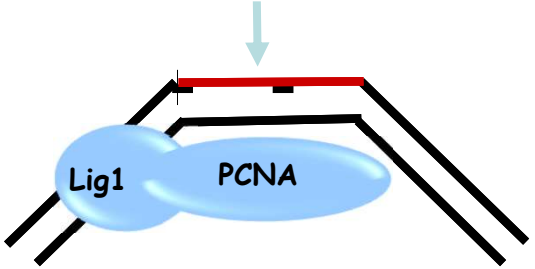
Short-patch



long-patch

Ligation





Disorders caused by mutations in the MRN complex

- MRN can promote ATM and ATR signalling.
- MRN is essential so mutations are Hypomorphic (ie not null) – this can cause unexpected features if mutations affect specific functions.
- Phenotypes not entirely as might be expected.
- NBS1 defects – called Nijmegen Breakage Syndrome – have very defined abnormal faces – seems to be a distinct role for NBS1
- MRE11 mutations – called A-T like disorder (can be progressive ataxia OR microcephaly).
- RAD50 – only a couple of patients – are like NBS.

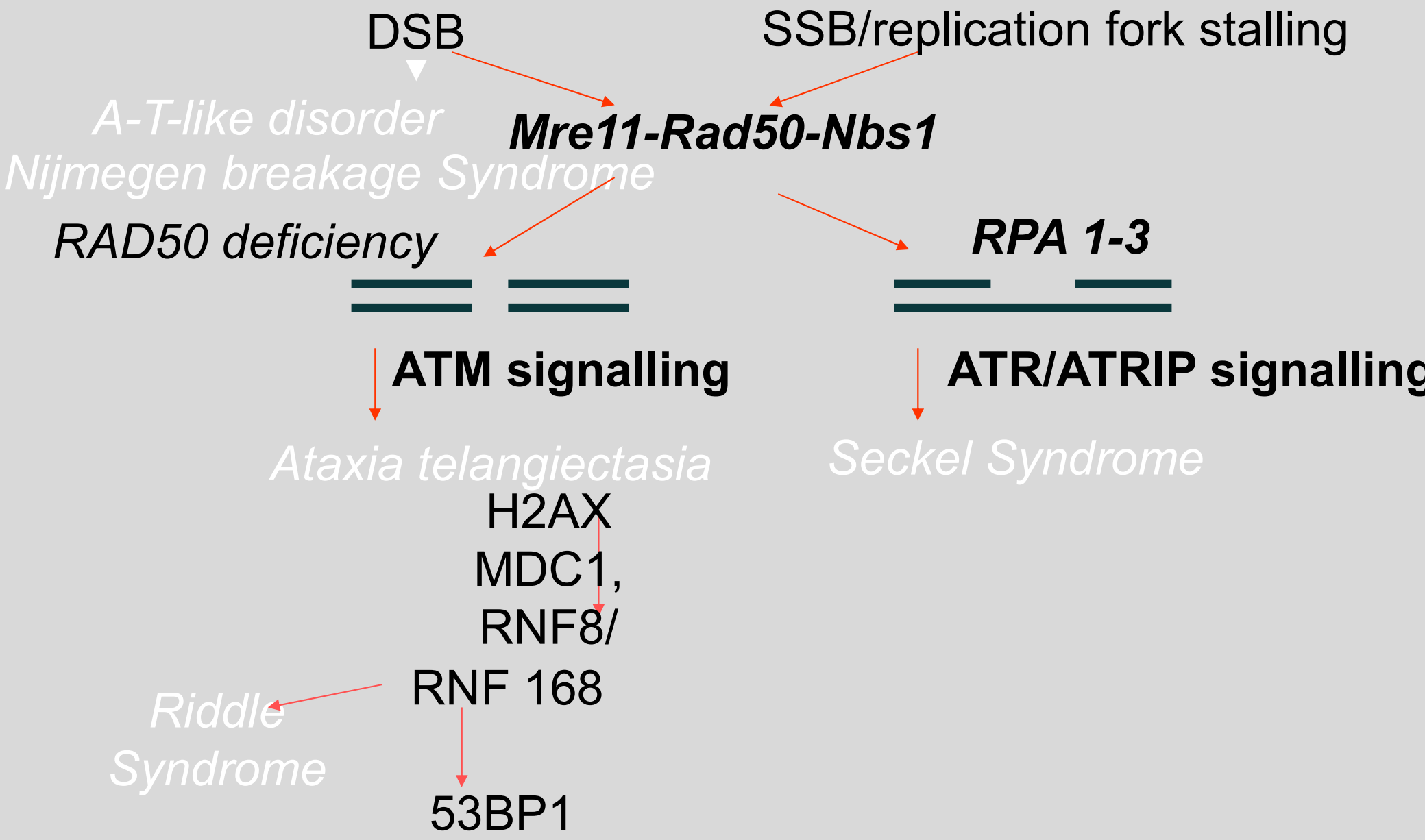
RIDDLE Syndrome

Radiosensitivity
Immunodeficiency
Dysmorphic Features
Learning difficulties

Due to mutations in RNF168, the ubiquitin ligase required for ATM and ATR signalling.

have reduced 53BP1 and BRCA1 foci formation

Syndromes associated with signalling defects.



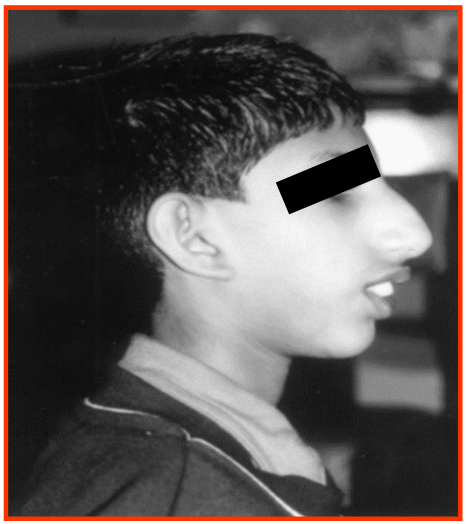
ATR is defective in some patients

with Seckel Syndrome.

•ATR is essential –mutations in patients are “leaky” - **hypomorphic**.

•Seckel Syndrome is characterised by dramatic microcephaly (small heads), developmental delay and dysmorphic facial features.

•(NB –mutations in other genes can also cause Seckel Syndrome)



A 10-year old boy

Britain’s smallest child is also an ATR-deficient patient

Photos taken from the Mail Online (March 14 2013)

Seckel Syndrome patients do not display any significant radiosensitivity

Discussion Questions (to help understanding):

1.Radiation induced DSBs are easier to repair than ROS induced DSBs True or False?

2. 53BP1 is a core c-NHEJ protein - True or False
Is it essential for c-NHEJ ; does it promote c-NHEJ or HR

3. ATM is essential for c-NHEJ - True or False
How does it affect DSB repair?

4. ATM is an essential protein – True or False – discuss why

5. ATM is an oncogene – true or false?

6. cNHEJ is more accurate than HR – true or false?

7. Ku functions during HR.