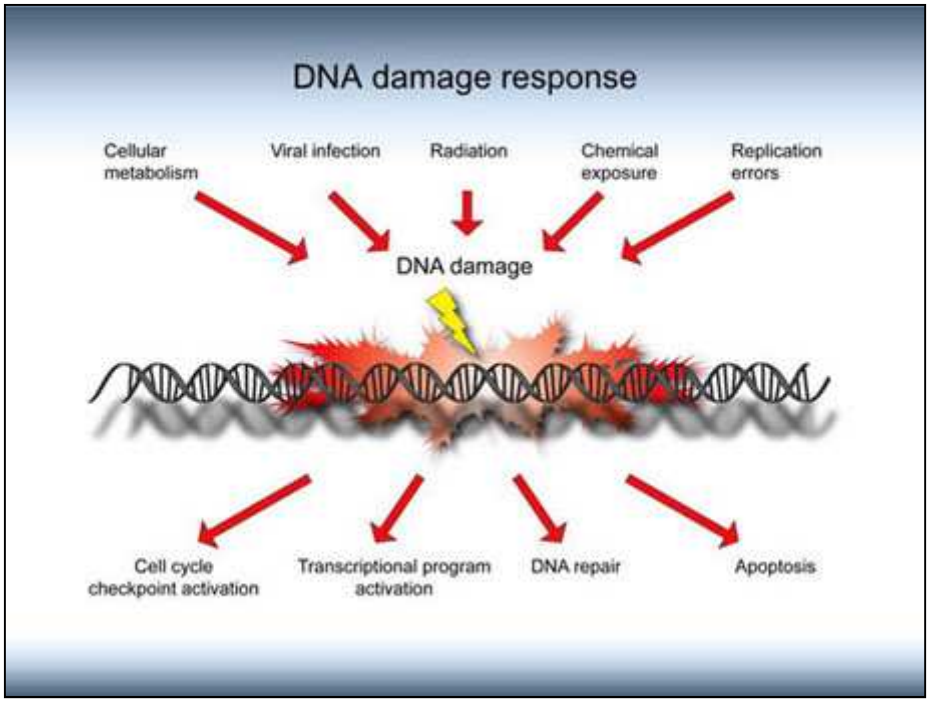
ATR = ATAXIA-TELANGIECTASIA MUTATED AND RAD3-RELATED

IN DNA DAMAGE RESPONSE (DDR)

Preservation of genome integrity via the DDR is critical to prevent disease

ATR is essential for life:

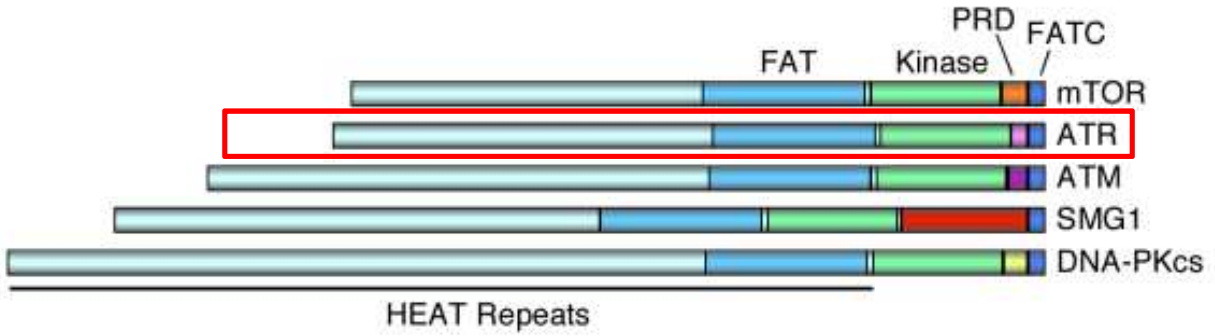
- ✓ is a master regulator of the DDR (during DNA replication)
- ✓ ATR controls and coordinates DNA replication origin firing, replication fork stability, **cell cycle checkpoints** and DNA repair



In response, the cell develops an evolutionarily conserved DDR that coordinates:
-cycle progression
-DNA repair
-DNA replication, DNA transcription

Mutation or deletion of many DDR genes → Lethality, cancer susceptibility, neurodegenerative disorders and premature aging syndrome

The DDR is regulated by PIKKs (phosphoinositide-3- kinase-related protein)



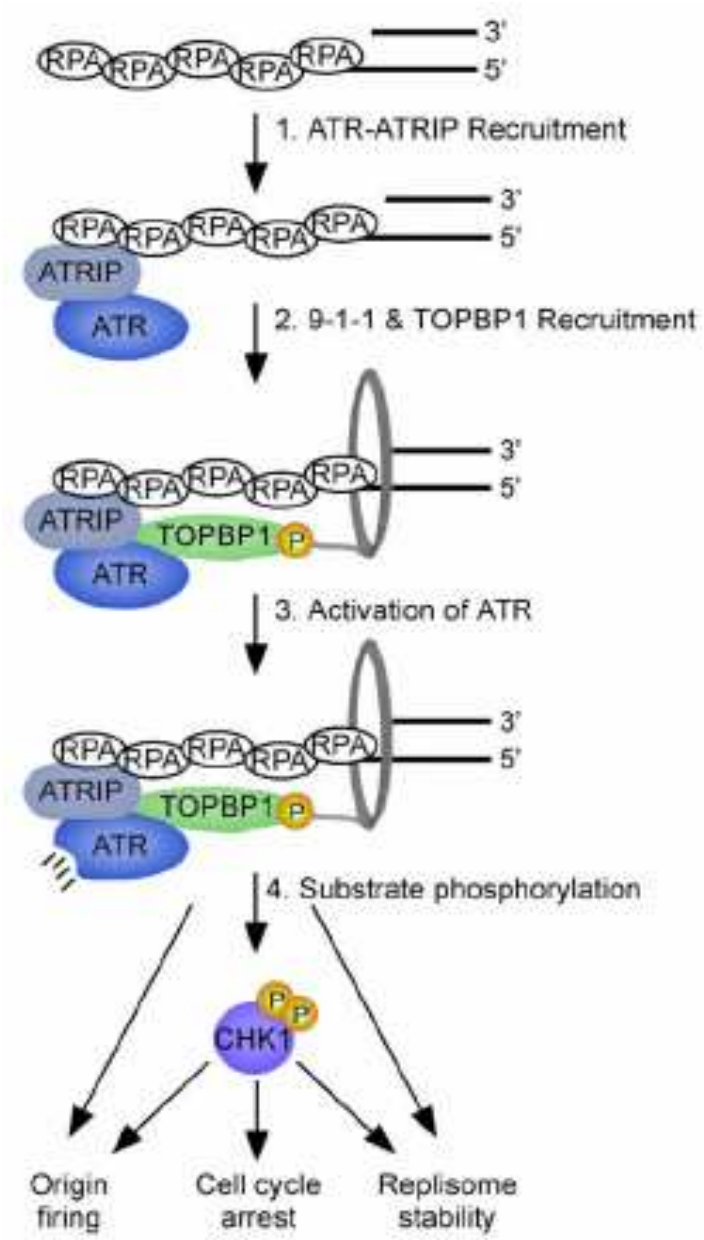
PRD (PIK regulatory domain)
FAT (named after kinases FRAP, ATM and TRRAP)

ATR: large enzyme of 2644 aa
300kDa MW

S/TQ kinase

The ATR-interacting protein (ATRIP) binds to the N-terminus of ATR

ATR signaling pathway



Apex of DDR are three related protein kinases:
ATM, ATR and DNA-dependent protein kinase (PIKK family)

Many types of DNA damage activate these protein:
DSBs, base adducts and crosslinks

Phosphorylate serines and threonines followed by a glutamine (S/TQ)

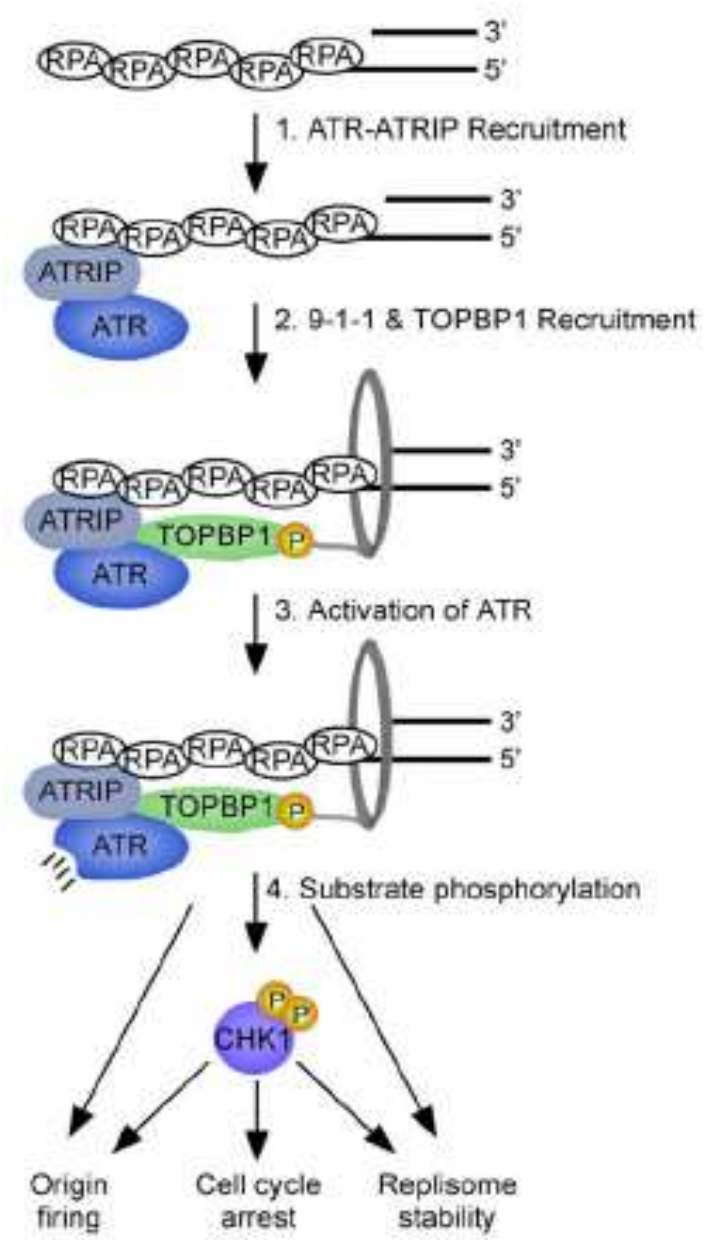
The most common signal for **ATR** activation likely involves replication stress

ATR is an essential gene in replicating cells

Homozygous loss of function mutations in ATR are not compatible with mammalian cell viability

Mutations that cause reduced ATR function are found in few patient with the rare **Seckle Syndrome**

ATR signaling pathway



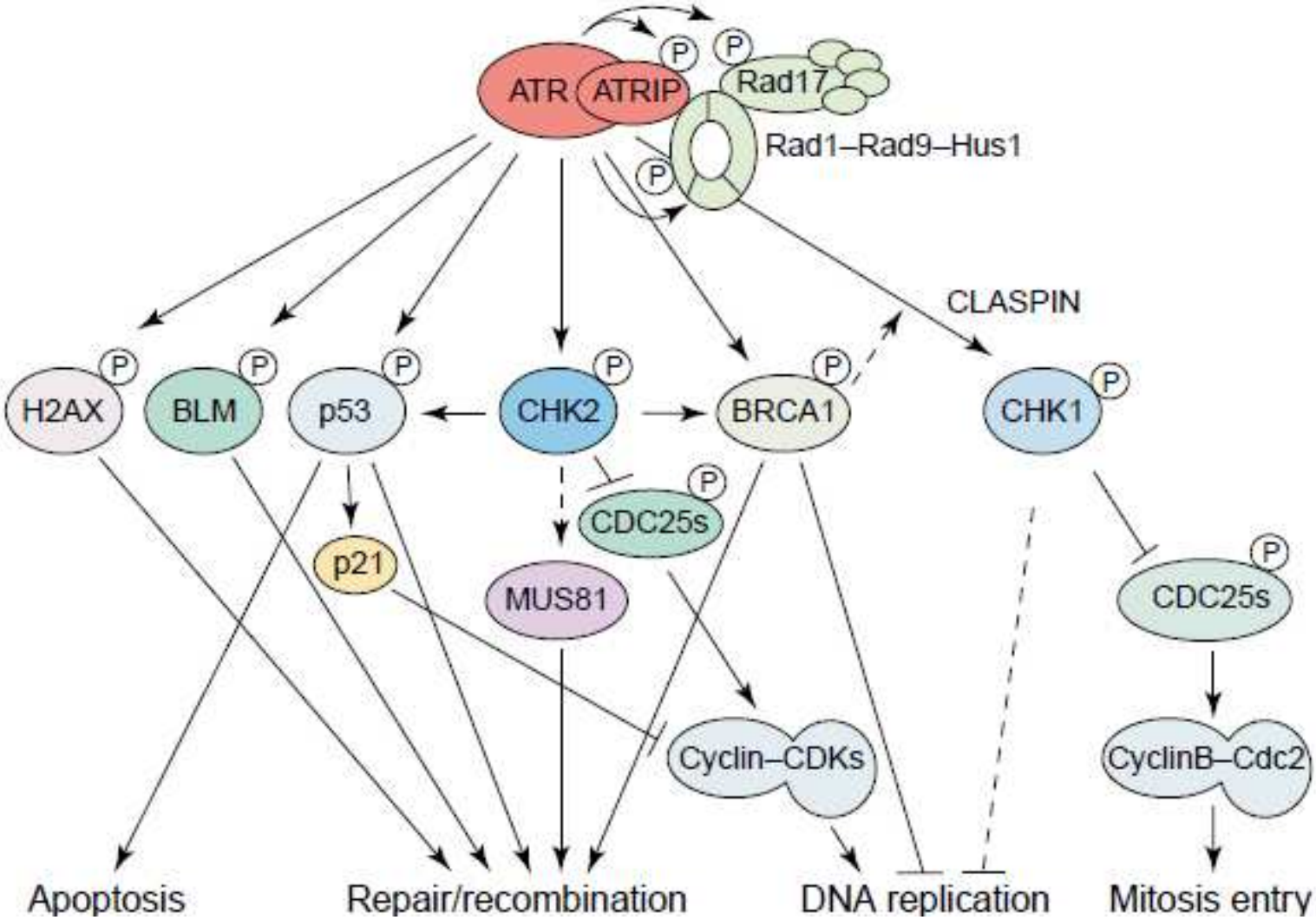
ssDNA is bound by the ssDNA binding protein Replication protein A

RPA-ssDNA is the ligand that recruits ATR and other ATR signaling components to site to replication stress

1. Recruitment of ATR via its obligate partner ATR Interacting Protein (ATRIP) to RPA-ssDNA
2. Independent recruitment of a checkpoint clamp containing ATR activator Topoisomerase Binding Protein1 (TOPBP1) to RPA-ssDNA
3. Activation of ATR by TOPBP1
4. Substrate phosphorilation: Chk1 activated by phosphorilation on Ser317 and Ser345

→ Inibition of cell cycle progression

ATR signaling pathway



DDR genes are essential for maintenance of genomic integrity

Mutations in DDR genes → failures in development

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JCB: Report

DNA structure-specific priming of ATR activation by DNA-PKcs

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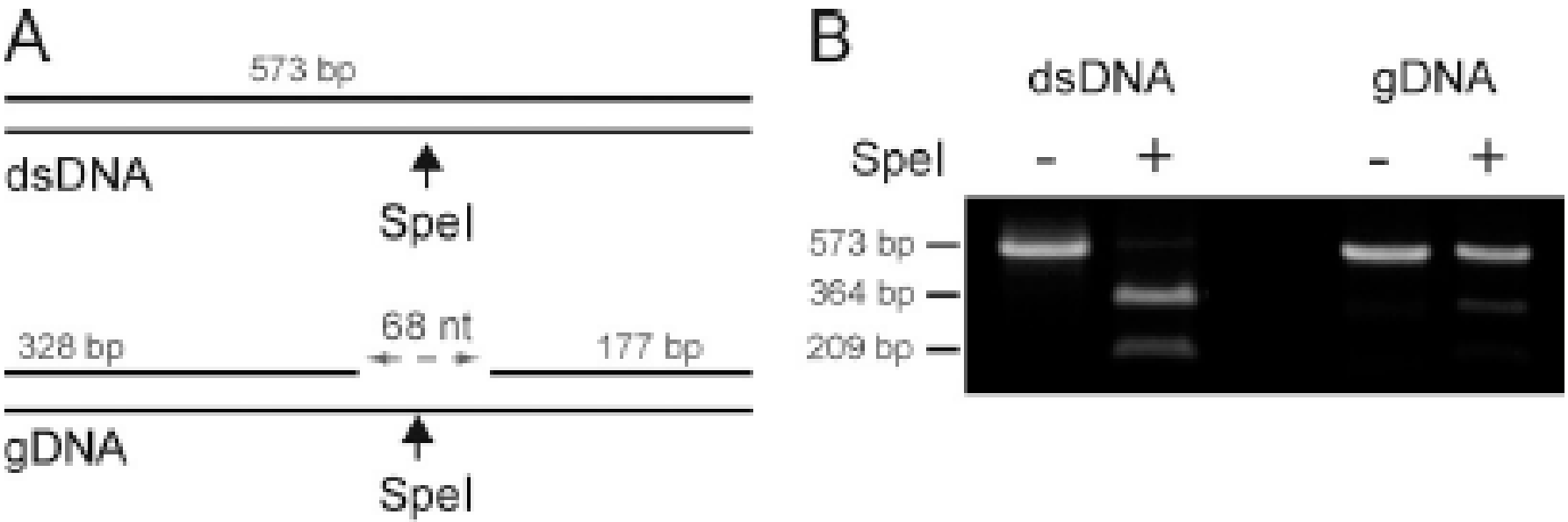
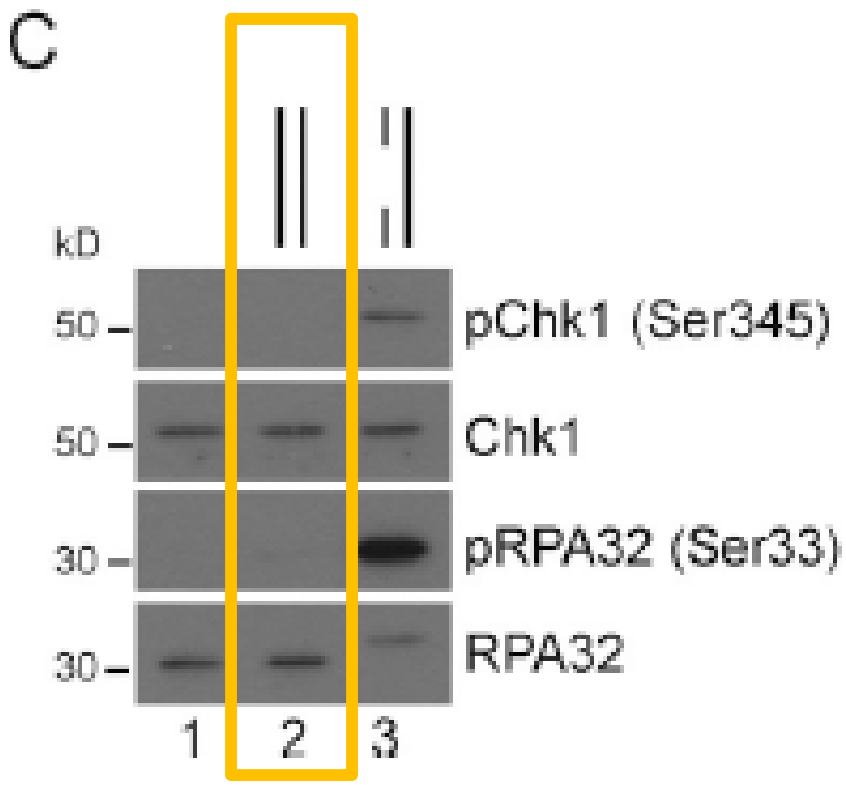
Three phosphatidylinositol-3-kinase–related protein kinases implement cellular responses to DNA damage. DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and ataxia-telangiectasia mutated respond primarily to DNA double-strand breaks (DSBs). Ataxia-telangiectasia and RAD3-related (ATR) signals the accumulation of replication protein A (RPA)–covered single-stranded DNA (ssDNA), which is caused by replication obstacles. Stalled replication intermediates can further degenerate and yield replication-associated DSBs. In this paper, we show that the juxtaposition of a double-stranded DNA end and a short ssDNA gap triggered robust activation of endogenous ATR and Chk1 in human cell-free extracts. This DNA damage signal depended on DNA-PKcs and ATR, which congregated onto gapped linear duplex DNA. DNA-PKcs primed ATR/Chk1 activation through DNA structure-specific phosphorylation of RPA32 and TopBP1. The synergistic activation of DNA-PKcs and ATR suggests that the two kinases combine to mount a prompt and specific response to replication-born DSBs.

ATR activation




C
kD
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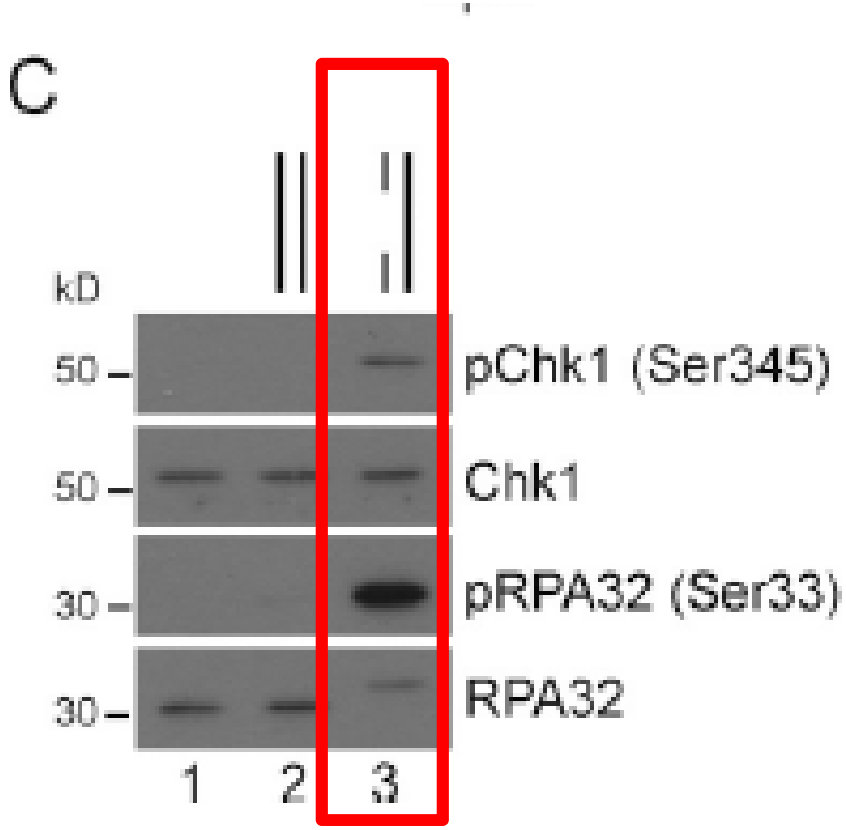
50
30
30
pChk1 (Ser345)
Chk1
pRPA32 (Ser33)
RPA32
1
2
3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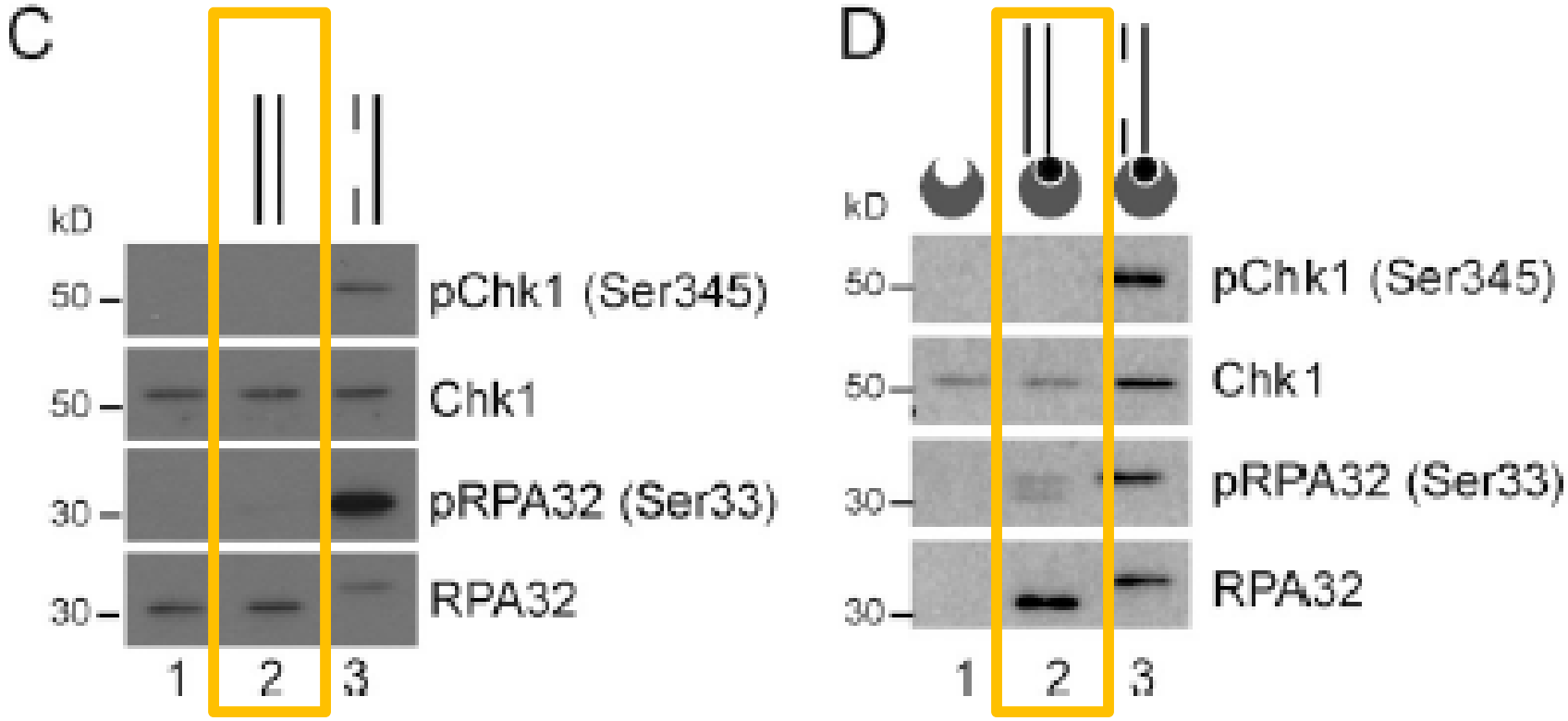
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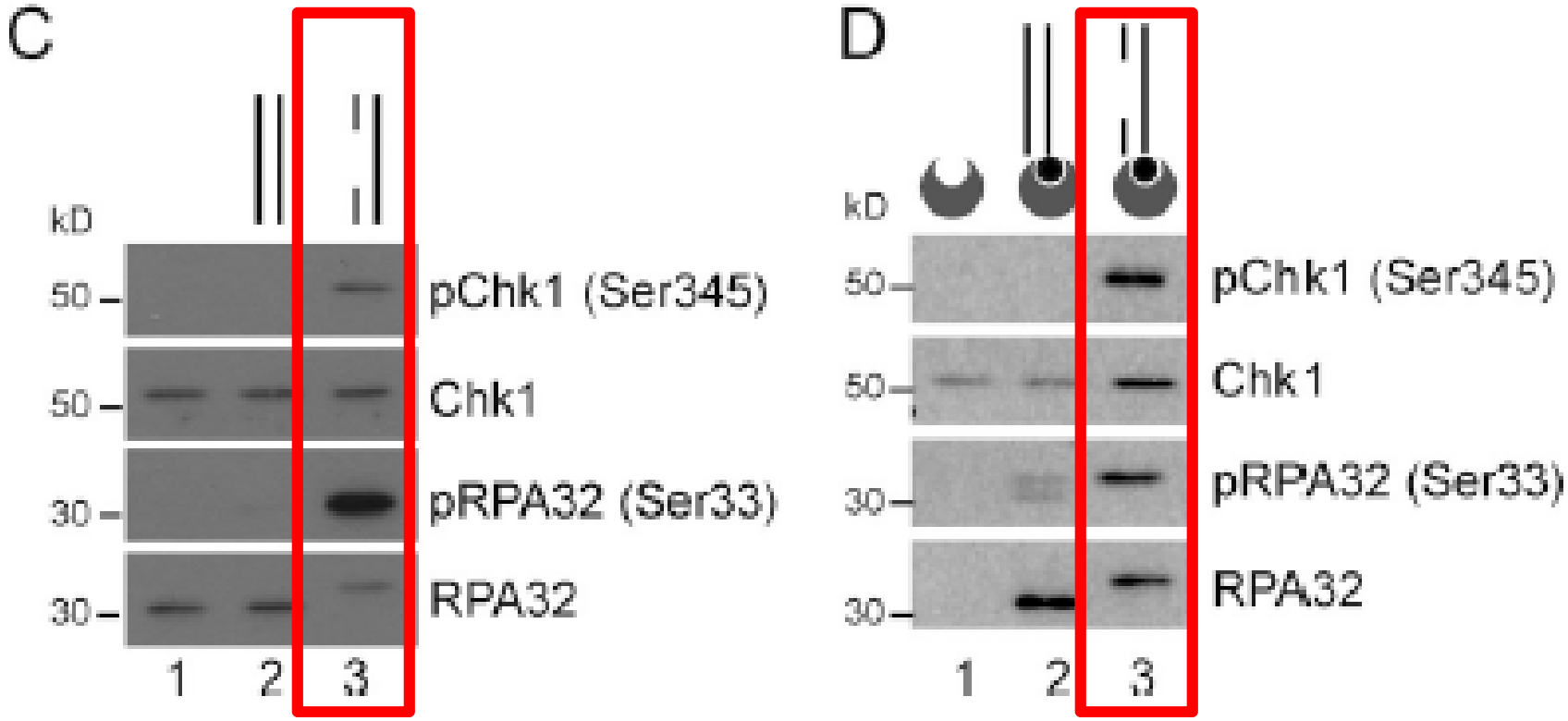
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30
pChk1 (Ser345)
Chk1
pRPA32 (Ser33)
RPA32
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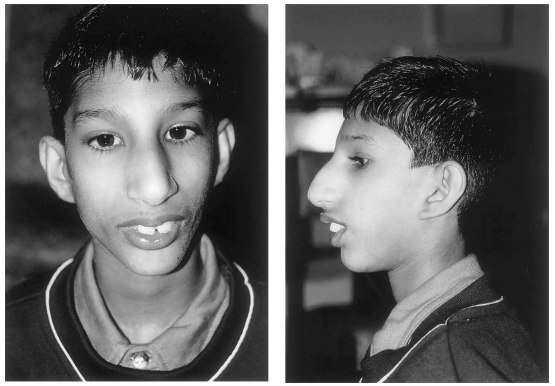
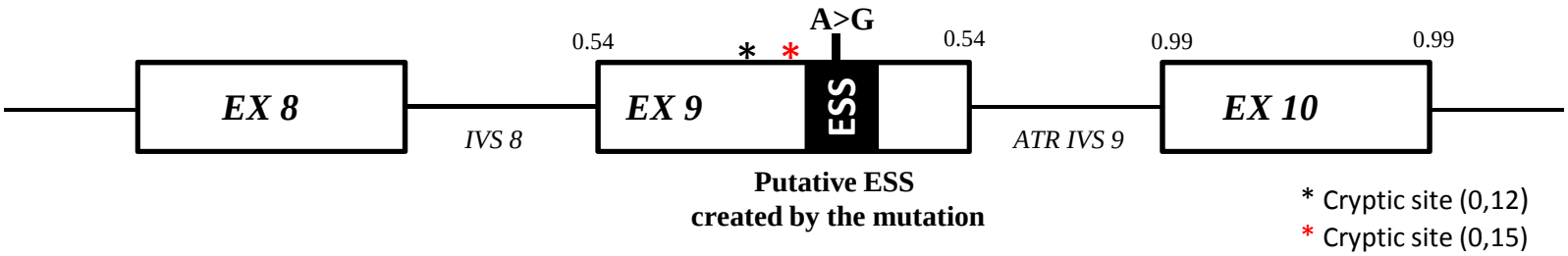
SECKEL SYNDROME

- Intrauterine and postnatal growth retardation
- Microcephaly
- Mental retardation
- Dysmorphic facial and skeletal features



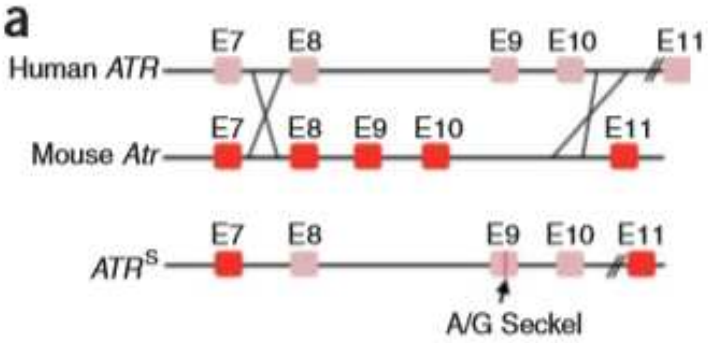
Rare disease caused by splicing mutation in ATR gene
Synonimous splicing mutation in ATR exon 9 (2101a/g)

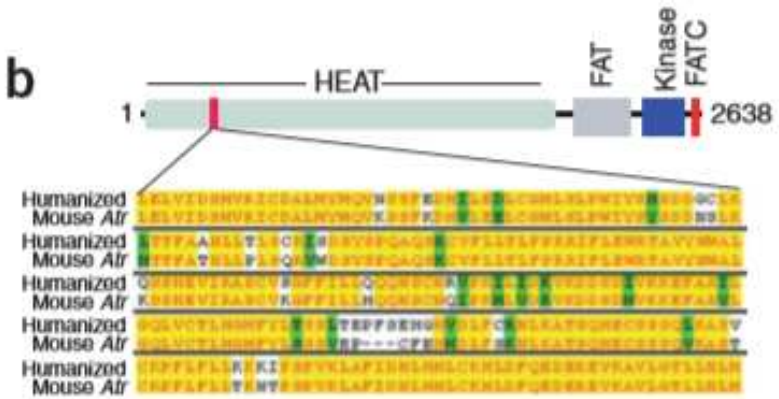


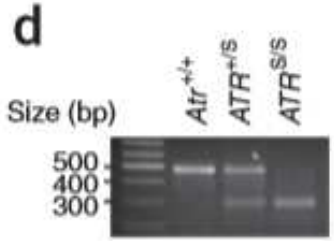
A mouse model of ATR-Seckel shows embryonic replicative stress and accelerated aging

Matilde Murga[1], Samuel Bunting[2], Maria F Montaña[1], Rebeca Soria[1], Francisca Mulero[3], Marta Cañamero[4], Youngsoo Lee[5], Peter J McKinnon[5], Andre Nussenzweig[2] & Oscar Fernandez-Capetillo[1]

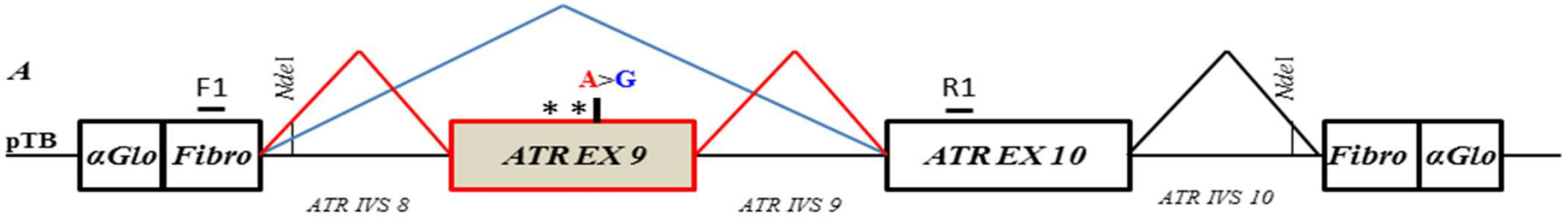
Although DNA damage is considered a driving force for aging, the nature of the damage that arises endogenously remains unclear. Replicative stress, a source of endogenous DNA damage, is prevented primarily by the ATR kinase. We have developed a mouse model of Seckel syndrome characterized by a severe deficiency in ATR. Seckel mice show high levels of replicative stress during embryogenesis, when proliferation is widespread, but this is reduced to marginal amounts in postnatal life. In spite of this decrease, adult Seckel mice show accelerated aging, which is further aggravated in the absence of p53. Together, these results support a model whereby replicative stress, particularly *in utero*, contributes to the onset of aging in postnatal life, and this is balanced by the replicative stress–limiting role of the checkpoint proteins ATR and p53.

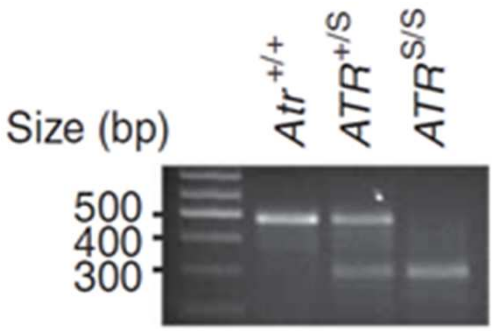


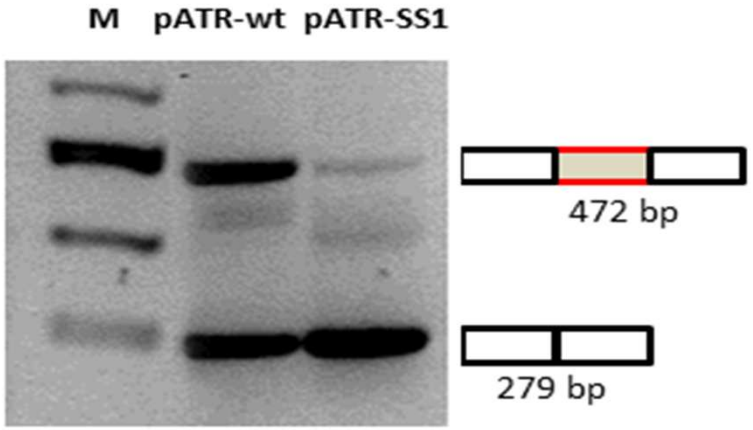




ATR splicing patterns with minigenes and in ATRs/s MEFs cells







Rescue of the correct ATR splicing

- Use AONs (antisense oligonucleotides)
- Use U1snRNA
- Both