Exercises Cancer Biology 1, Week 3

Discussion of Zou and Elledge: Science **300**, 1542.

Questions:

All Figures: Be able to explain the Figures

Figure 1:
Figure 1A: ORC2 was detected as a loading control for chromatin-bound RPA34. Is this control valid for soluble RPA34?

Figure 1C,D: RPA70 is downregulated using siRNA. However, off-target effects of siRNAs are not uncommon. What additional experiments could you perform to exclude off-target effects?

In Figure 1B, colocalization of ATR and RPA70 is demonstrated. How could you test if there is a physical interaction between ATR and RPA?

Figure 2:
Figure 2: Can you estimate the fraction of CHK1 that becomes phosphorylated at S345 upon HU or UV treatment?

Figure 3:
Figure 3: What other experiment could you have done to quantify the effects of RPA for the binding of ATRIP to single stranded DNA (i.e. measure the dissociation constant)?

Figure 4:
Figure 4B. It is concluded on page 1545 that "the vast majority of ATR is present in complexes with ATRIP in human cells". Do you agree with this general statement?

Figure 4C. Does Rad17 phosphorylation depend on ATR-ATRIP, RPA and ssDNA ?

Figure 5:
Figure 5B, C: What is being detected on these gels ?
What are the reaction ingredients you need to carry out a PCR ?
Where would you situate the primers with respect to the double strand break?

Figure 5C-E: Why was the experiment repeated in nocodazole-treated cells?

Figure 6:
Figure 6B: Given the evolutionary distance (split 1500 Mio years ago), does it surprise you that human RPA can recruit yeast Ddc2 to ssDNA?

Figure 7:
Explain models

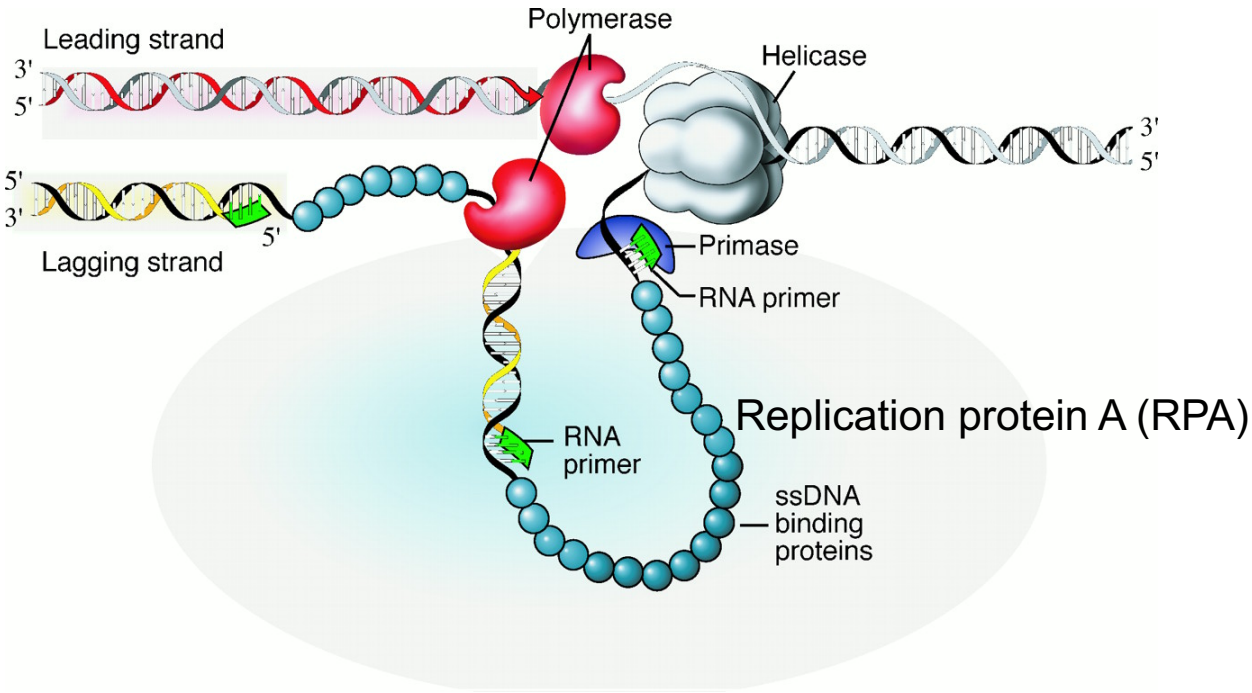
Why were many of the experiments in this paper carried out with cancer cells? Is this problematic?

Background information:

RPA: essential protein required for DNA replication, repair and ATR-dependent checkpoint activation (this paper). ssDNA is generally associated with RPA.
ATR; named **Mec1** in budding yeast (*S. cerevisiae*).
ATRIP: ATR-interacting protein; named **Ddc2** in budding yeast.
The **CHK1 protein kinase** is phosphorylated by ATR on Ser^{345} in response to DNA damage.
Degron tag: peptide that confers rapid degradation of the tagged protein.
Hydroxyurea (**HU**): antineoplastic drug used in hematological malignancies, which leads to a reduction of dNTPs (it inhibits ribonucleotide reductase which catalyzes dNTP synthesis from NTPs).
FLAG-tag: DYKDDDDK peptide tag against which high affinity monoclonal antibodies are available (Western blot analysis; immunofluorescence, immunoprecipitation). Often three FLAG-peptides are expressed in tandem fused to the protein of interest.
HO-endonuclease: recognizes specific 24 bp long DNA sequence (involved in mating type switching in *S. cerevisiae*). Used in the paper to induce DNA cleavage at one specific site in the genome.
Nocodazole: interferes with polymerization of microtubules:→arrest at prometaphase.
HCT116: colon cancer-derived cell line
HeLa: cervical cancer-derived cell line (isolated in 1951)
U2OS: osteosarcoma-derived cell line

Checkpoint proteins involved in the ATR-dependent checkpoint:
RAD17-RFC2-5: RAD17 forms a complex with RFC2-5. This protein complex binds to the junction of ssDNA/dsDNA and loads the 9-1-1 complex checkpoint protein complex onto chromatin after DNA damage.

RAD9-RAD1-HUS1 (9-1-1 complex): Chromatin-bound RAD9 is phosphorylated by ATR on S387 which further activates ATR (through a mechanism involving TopBP1; to be discussed next week).



Replication protein A (RPA):
heterotrimer, composed RPA1 (RPA70) (70kDa subunit), RPA2 (RPA32) (32kDa subunit) and RPA3 (RPA14) (14kDa subunit). The three RPA subunits contain six OB-folds (oligonucleotide/oligosaccharide binding), with DNA-binding domains (DBD) that bind RPA to single-stranded DNA

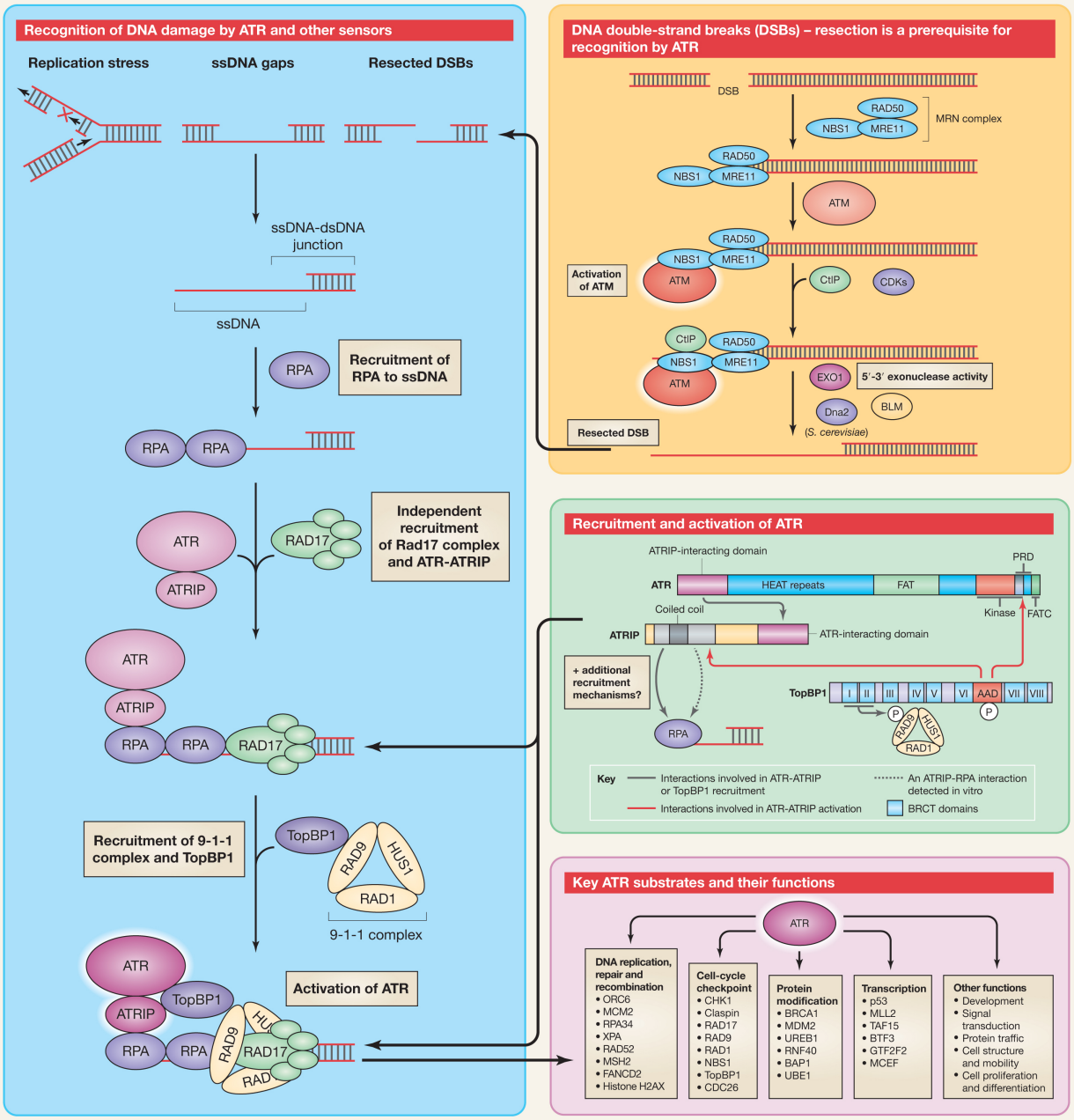
Function of DNA Replication Fork Proteins:

Proteins	Functions
RPA	Heterotrimer; single-stranded DNA binding; stimulates DNA polymerases; facilitates helicase loading
RFC	5 subunits; DNA-dependent ATPase; primer template binding; displaces pol α; loads PCNA onto 3' primer/template junction and then dissociates
PCNA	Homo-trimer forms a ring; stimulates processivity of DNA pol δ; stimulates RFC ATPase
Polα/primase	4 subunits; p48 synthesizes ~12-nucleotide RNA primer followed by ~20 nt DNA which is synthesized by p180
Polδ	4 subunits; replicative DNA polymerase; 3 '-5 ' exonuclease
Pol ε	4 subunits; replicative DNA polymerase; leading strand synthesis.
MCM	MCM2-7; 3'-5' replicative helicase; helicase activity detected for MCM4/6/7 subcomplex
FEN1	Nuclease for removal of RNA primers
RNase HI nuclease	Removal of RNA primers
DNA ligase I	Ligation of DNA
T antigen	DNA helicase for SV40; primosome assembly
Topo 1/2	Release of tortional stress during pol movement and for decatenation of replicated sister molecules.

- **Cell cycle checkpoints** exist at specific points in the cell cycle to prevent them from progressing to the next phase of the cell cycle in the event of DNA damage or another condition which would make cell division dangerous for the cell.
- Two kinases– ataxia telangiectasia mutated (ATM), and ATM- and Rad3-related (ATR) – are master regulators of two major checkpoint pathways.
- ATM is primarily activated by DNA double-strand breaks (DSBs), whereas ATR responds to a much broader spectrum of DNA damage, including DSBs and many types of DNA damage that interfere with DNA replication.
- **ATR** senses single stranded DNA via ATR-interacting protein (ATRIP), which binds directly to RPA- coated ssDNA.

ATR is an essential gene
Hypomorphic alleles

Clinical symptoms (Seckel syndrome):
Microcephaly (small circumference of the head)
Dwarfism
Large eyes, low ears, small chin
Severe mental retardation
Hematological abnormalities and chromosome breaks



Abbreviations: AAD, ATR-activating domain; ATM, ataxia telangiectasia mutated; ATR, ATM- and RAD3-related; ATRIP, ATR-interacting protein; BAP1, BRCA1-associated protein 1; BLM, Bloom syndrome protein; BRCA1, breast cancer type 1 susceptibility protein; BRCT, BRCA1 carboxy-terminal domain; BTF3, RNA polymerase B transcription factor 3; CDC26, cell-division cycle protein 26; CDK, cyclin-dependent kinase; CHK1, checkpoint kinase 1; CtIP, CtBP-interacting protein; Dna2, DNA replication mutant 2; DSB, double-strand break; dsDNA, double-stranded DNA; EXO1, exonuclease 1; FANCD2, Fanconi anemia group D2 protein; FAT, FRAP-ATM-TRRAP domain; FATC, FAT carboxy-terminal domain; GTF2F2, general transcription IIF subunit 2; HEAT repeat, Huntington-elongation-factor-3–PP2A–TOR repeat; HUS1, hydroxyurea-sensitive mutant 1; MCEF, major CDK9 elongation-factor-associated protein; MCM2, minichromosome maintenance protein 2; MLL2, mixed-lineage leukemia protein 2; MRE11, meiotic recombination protein 11; MSH2, MutS protein homolog 2; NBS1, Nijmegen breakage syndrome protein 1; ORC6, origin recognition complex subunit 6; PRD, PIKK regulatory domain; RAD, radiation-sensitive mutant; RNF40, RING finger protein 40; RPA, replication protein A; RPA34, DNA-directed RNA polymerase subunit RPA34; TAF15, TATA-box-binding-protein-associated factor; ssDNA, single-stranded DNA; TopBP1, topoisomerase II binding protein 1; UBE1, ubiquitin-activating enzyme E1; UREB1, upstream regulatory element binding protein 1; XPA, xeroderma pigmentosum A.