Dear Students of Cancer Biology 1

- The first exam will take place on Wednesday October 30, from 1:30pm-3:30pm. The exam will take place in room CM 1 121. Please arrive a bit earlier (by 1:25pm) to find your place and get installed.
- Auxiliary material (books, computer, handouts, printouts etc.) is not allowed.
- Please bring your camipro card.

- What is to take from week 1, are we supposed to know how to distinguish oncogene and tumor suppressor based on a rule, like the 20/20 or the fact that mutations have different distributions ? I am a bit confused because p53 doesn't follow such rules.

Regarding p53, it is indeed special as mutant p53, when mutated in the DNA binding domain has a dominant negative effect on wild type p53. P53 binds as a tetramer to DNA and all 4 subunits must be wild type for function. If a DNA binding mutant p53 co-assembles with wild type p53 into a tetramer, the complex is not able to efficiently bind to DNA.
Please see also week 4, exercises, question 7. The data shown in this Figure beautifully demonstrate the dominant negative effects of DNA binding-mutant p53.

# Most of the mutations in p53 are missense

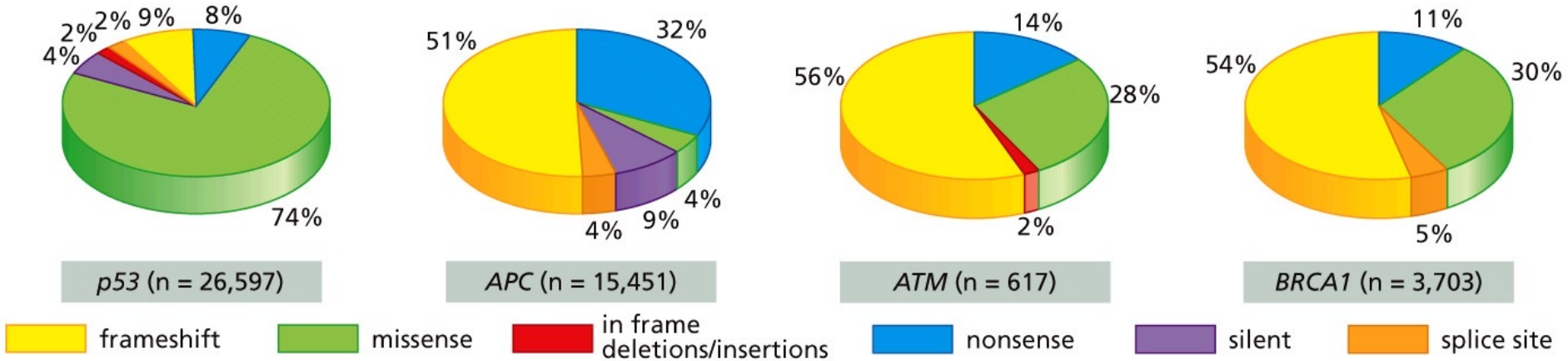



Figure 9.6a The Biology of Cancer (© Garland Science 2014)

# 20/20 rule

- Oncogene classification: >20% of recorded mutations are at recurrent positions and are missense
- Tumor suppressor gene classification: >20% of the recorded mutations in the gene are inactivating
- *TP53*: Oncogene score: 73%; TSG score: 20%

but classified as TSG because well-studied oncogenes rarely harbor premature stop codons

**B Vogelstein et al. Science (2013);339:1546-1558**

# Most of the missense mutations in p53 affect DNA-binding Domain

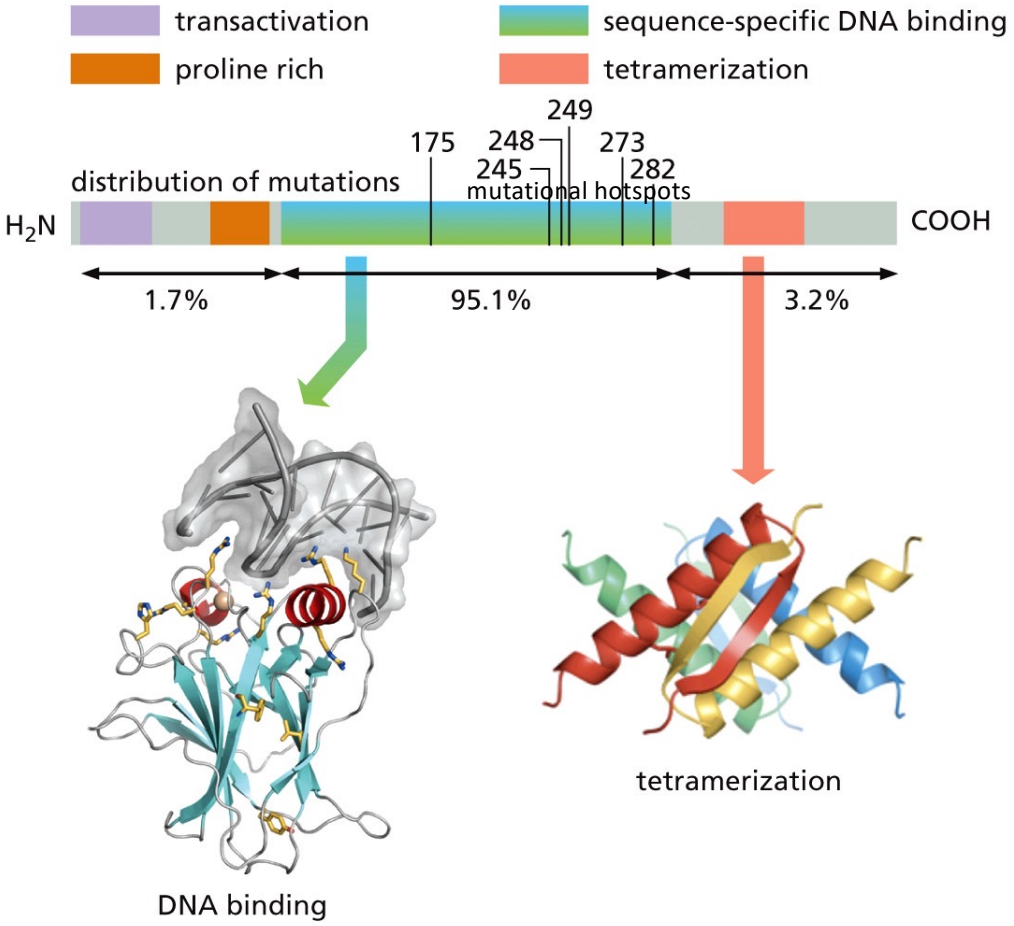



Figure 9.6b The Biology of Cancer (© Garland Science 2014)

# Dominant-Negative Mutations in p53

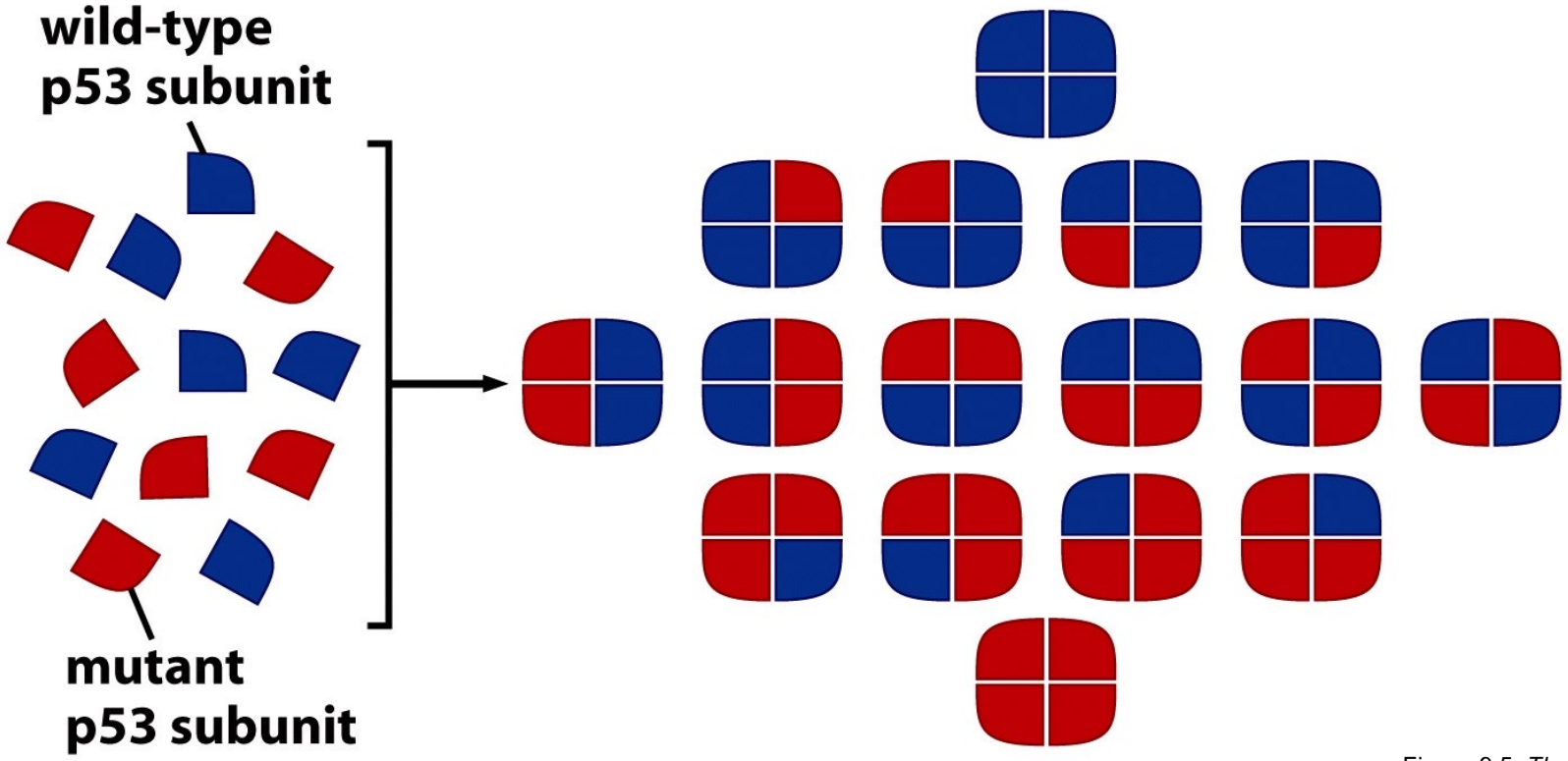



Figure 9.5 *The Biology of Cancer*

**Illustration of dominant negative effect of p53 missense mutations in myeloid malignancies:** Boettcher et al., Science **2019** 365: 599-604

**Daunorubicin** stabilizes the topoisomerase II complex after it has **broken** the **DNA** chain for replication, preventing the DNA double helix from being resealed. Nutlin-3, inhibits the p53/MDM2 interaction with IC50 of 90 nM in a cell-free assay. **Nutlin-3a induces autophagy and apoptosis in a p53-dependent manner.**

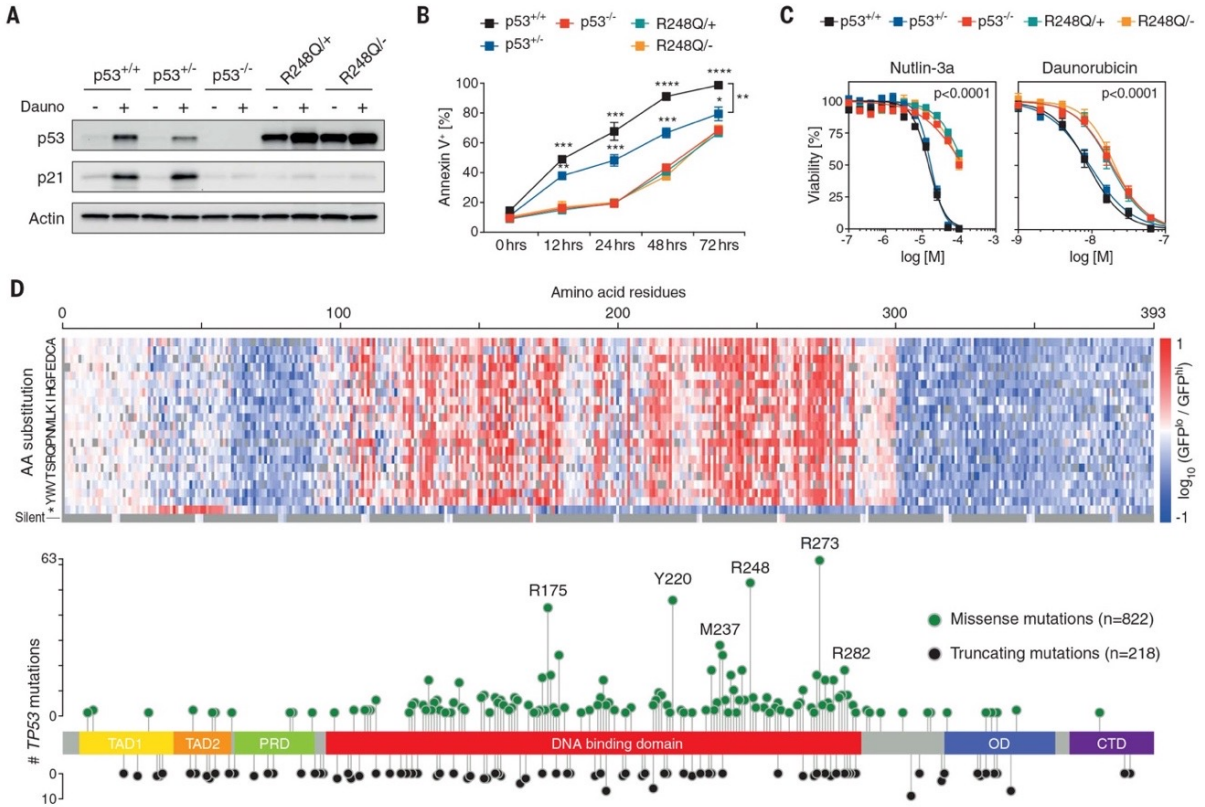
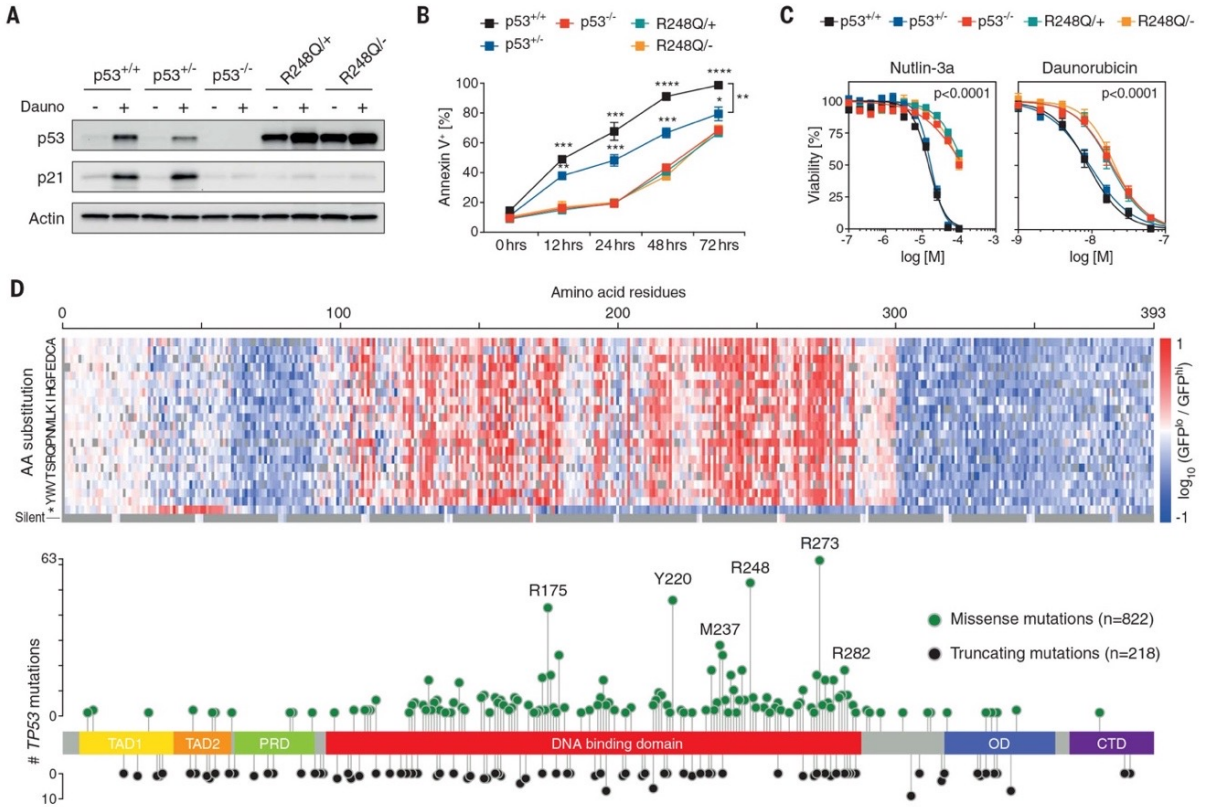



**Fig. 3. *TP53* missense mutations in the DNA-binding domain confer a DNE.** (**A**) MOLM13-*TP53* isogenic AML cell lines with $p53^{+/+}$, $p53^{+/-}$, and $p53^{-/-}$ as well as $p53^{R248Q/+}$ and $p53^{R248Q/-}$ were treated with DMSO (−) or 100 nM daunorubicin (+) for 6 hours, after which whole-cell protein lysates were collected, run on a polyacrylamide gel, and immunoblotted for p53, p21, and actin (replicates, $n = 3$; representative images are shown). (**B**) MOLM13-*TP53* isogenic AML cell lines were treated with 100 nM daunorubicin for up to 72 hours. At the indicated time points, cells were stained with Annexin V and analyzed by flow cytometry to assess total apoptotic cells (replicates, $n = 3$; symbols represent averages of experimental replicates; error bars indicate SEM; $*P = 0.05$, $**P = 0.01$, $***P < 0.001$, $****P < 0.0001$, two-tailed Student's $t$ test). (**C**) MOLM13-*TP53* isogenic AML cell lines were treated with DMSO, nutlin-3a, or daunorubicin at increasing concentrations for 72 hours, after which cell viability was assessed by using CellTiter-Glo luminescent assay (replicates, $n = 3$; symbols represent averages of experimental replicates; error bars indicate SEM). (**D**) Heatmap depicting the *TP53* saturation mutagenesis screen results after nutlin-3a treatment, shown as $\log_{10}$ of the ratio of normalized read counts in $GFP^{lo}$ over $GFP^{hi}$ cells per *TP53* variant (top panel) overlaid on a lollipop plot demonstrating *TP53* mutational data from 1040 patients with MDS, myeloproliferative neoplasms, and AML (bottom panel). Missense mutations (green circles) and truncating mutations (black circles) comprising frame-shift, nonsense, and splice mutations are shown. AA, amino acid; CTD, C-terminal domain; OD, oligomerization domain; PRD, proline-rich domain; TAD, transactivation domain.

•For Series 4, question 7b: Why is the conclusion for Daunorubicin that p53 is non-functional rather than a dominant-negative effect, as suggested for Nutlin-3a?

The R248Q mutation of p53 is dominant-negative!
$p53^{R248Q/+}$ behaves like $p53^{-/-}$ and not as $p53^{+/-}$

Daunorubicin stabilizes the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed. Nutlin-3, inhibits the p53/MDM2 interaction with IC50 of 90 nM in a cell-free assay. Nutlin-3a induces autophagy and apoptosis in a p53-dependent manner.

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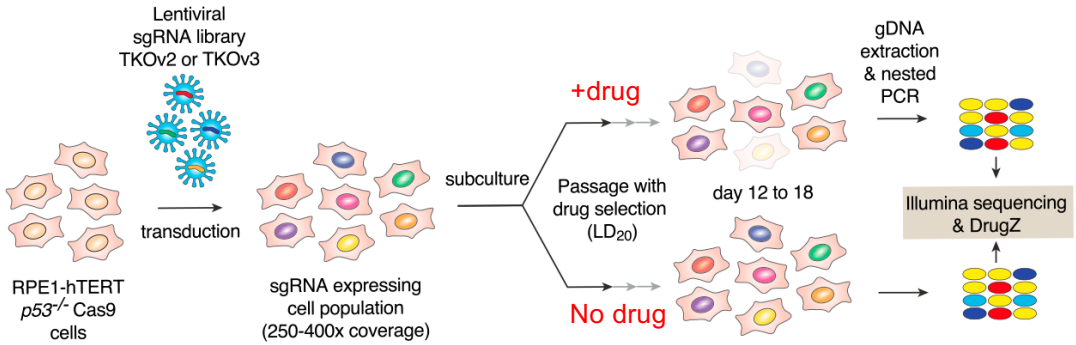
- CRISPR-Cas9 Screens: Identification of genes whose loss increases (or decreases) sensitivity to a genotoxic drug used in cancer therapy. (week 3 slide 5)
What I understood was that cells were transduced with a library of guiding RNA, target all genes to cleave these genes when these guides are associated with Cas9. After transfected with the vector, cells are cultured either in presence or absence of this drug...amplified specifically the regions that contained the guided RNA. But then what do we look at ? the presence of cells in no drug but absence in the +drug ?

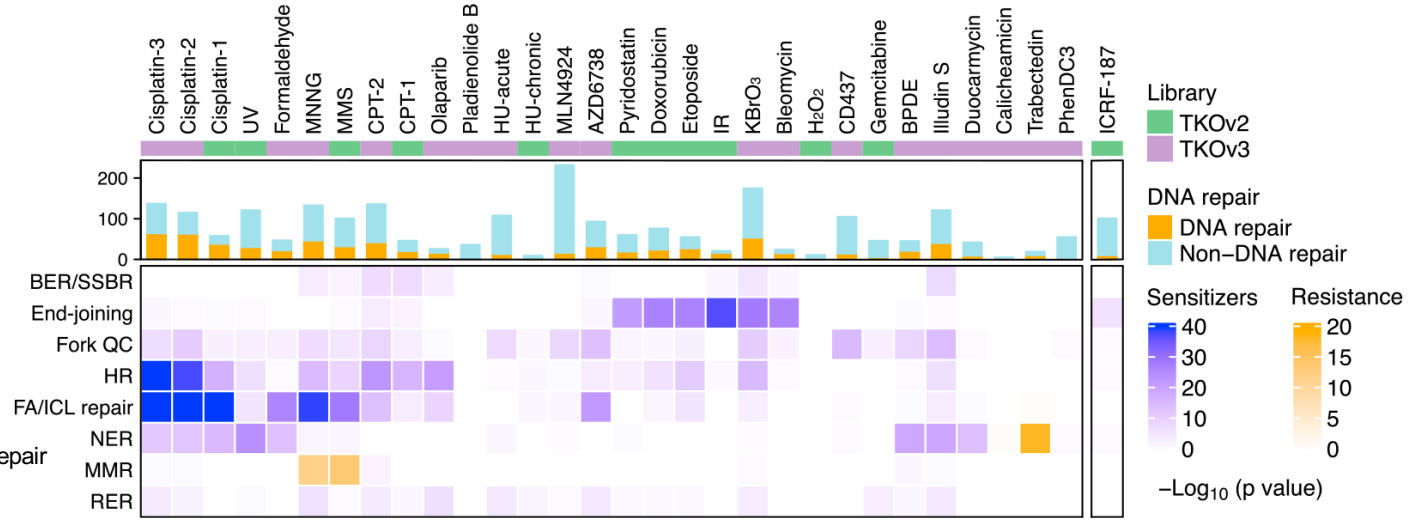
We are interested in the **guiding RNA sequence**s that are **present** in the **no drug cells but absent in the +drug cells**. The guiding RNAs that are absent in the drug-treated cells may target genes whose absence enhances the the efficiency of the drug.

Experimental Procedure: After growth in presence or absence (+/-) of the drug, cell pellets are frozen for genomic DNA (gDNA) isolation. gDNA from cell pellets are isolated and **genome-integrated sgRNA sequences are** amplified by PCR and **sequenced** to determine sgRNA representation in each sample.

# CRISPR-Cas9 Screens: Identification of Genes whose Loss Increases (or Decreases) Sensitivity to a Genotoxic Drug (used in cancer therapy)

**RPE**: retinal epithelial cells.
**hTERT**: cells are immortalized.
**Cas9**: guide RNA-directed DNA endonuclease.
**p53 -/-** : to avoid cell cycle arrest that may be induced by these drugs.
Essential gene products will not be identified.



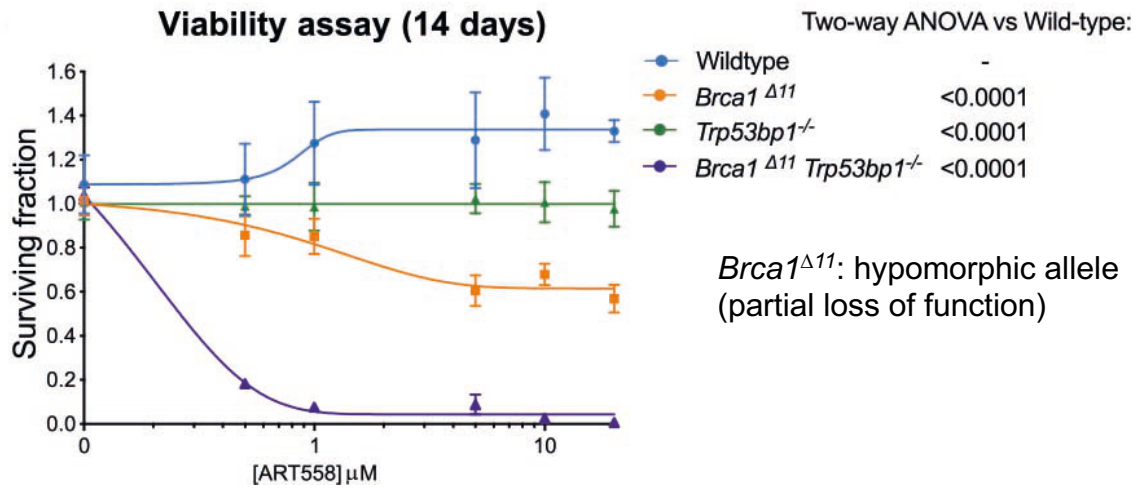




BER: Base Excision Repair
HR: Homologous Recombination
NER: Nucleotide Excision Repair
MMR: Mismatch Repair

From Cell 182, 481-496 (2020)

## Pol θ (teta) Inhibitor Targets even a PARPi Resistant *BRCA1-mt* Cells

(NatComm 12: 3636 (2021))



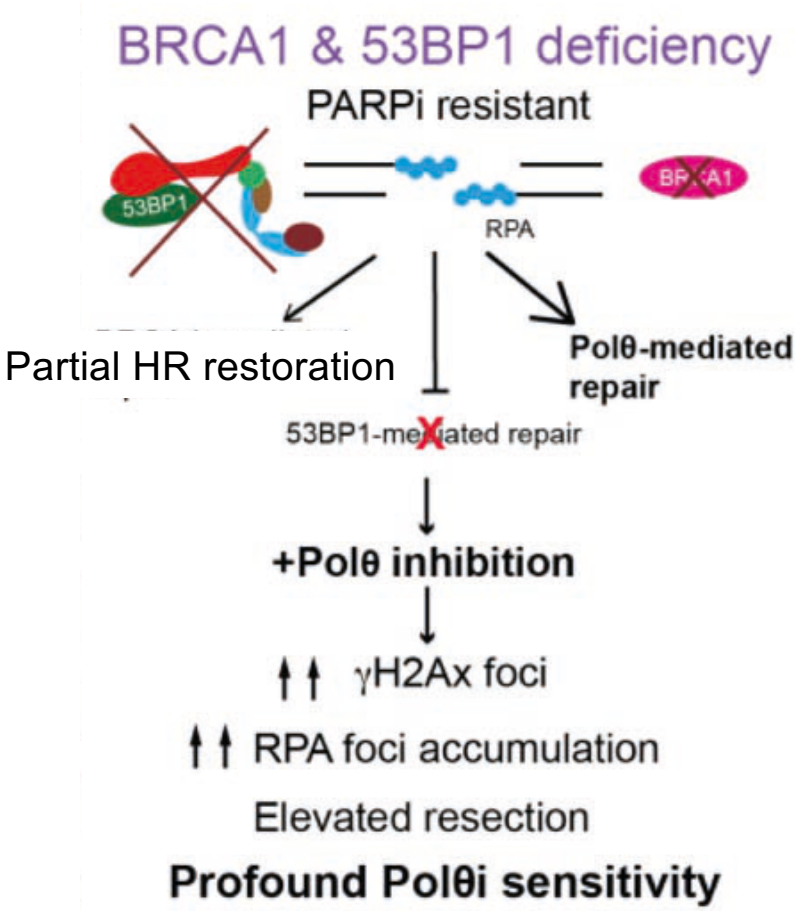

*Brca1*$^{\Delta 11}$: hypomorphic allele (partial loss of function)

I do not quite understand why the lethality of the MMEJ Pol theta inhibitor is increased in the 53BP1 double mutant case compared to just the BRCA1 mutant alone. I would have expected that NHEJ and other pathways that do not depend on Pol theta would still be present in both the single and double-mutant cases, resulting in the survival of some cells and a similar lethality rate in both cases.

## Pol θ (teta) Inhibitor Targets PARPi Resistant *BRCA1-mt* Cells

(NatComm 12: 3636 (2021))

Model: the authors observed elevated DNA end resection upon Pol θ inhibition.
Therefore, they speculate that when both BRCA1 and 53BP1 function become impaired, Pol θ becomes essential for repairing resected ssDNA caused by the exposure of DSB ends due to 53BP1-loss.
I.e.This would correspond to a MMEJ-independent function of pol θ.
…to be investigated further.



-Week 4 slide that I attached pdf of, why is that an unusual gene structure? Is it because the ARF is skipped by exon splicing, I am a bit confused there.

Two things are unusual. First that two genes are overlapping. Second that ARF uses an alternative reading frame when compared to p16. Thus, the protein sequences derived from exon 2 are different between p16 and ARF!

# Control of Apoptosis by ARF

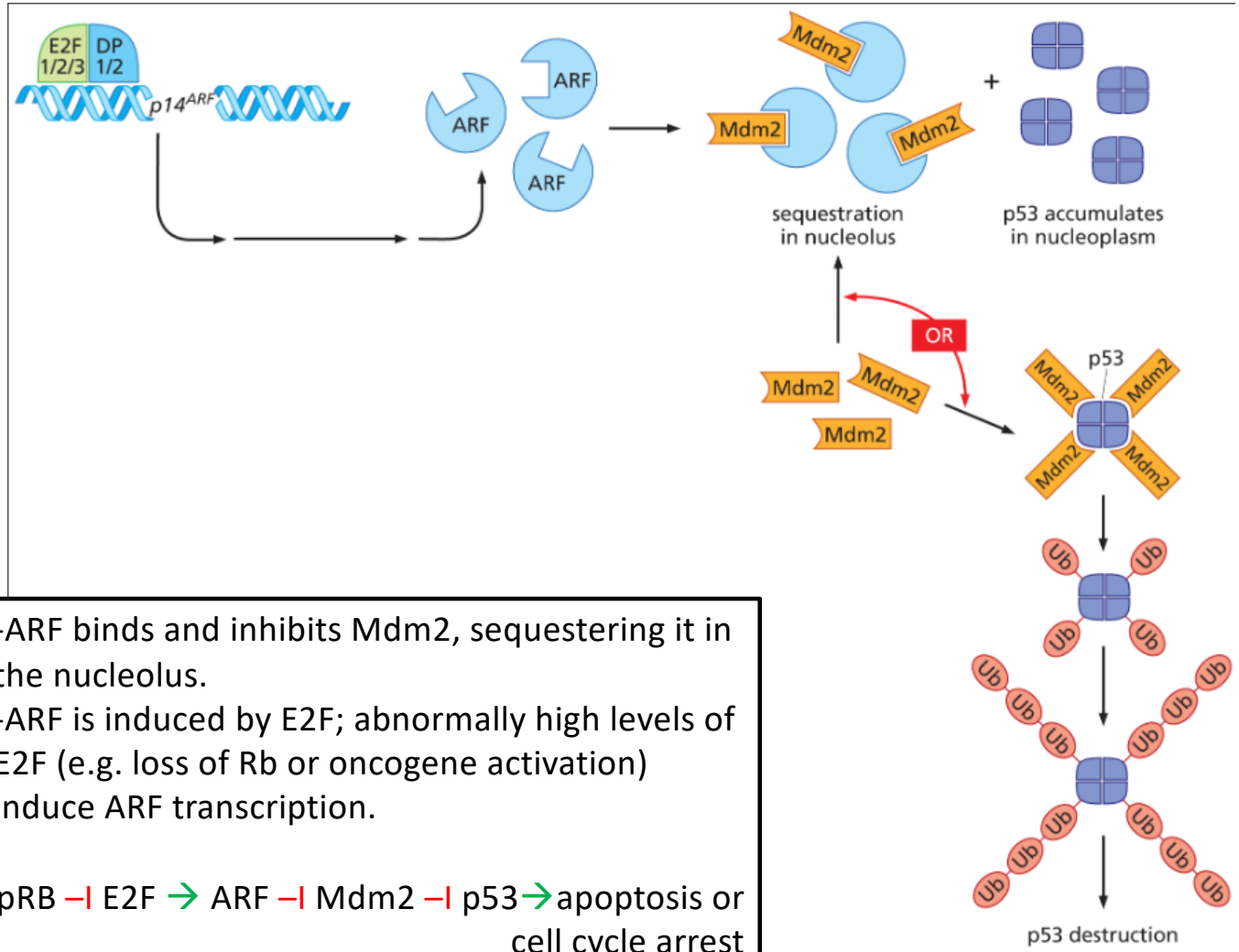



Figure 9.14. Weinberg, The Biology of Cancer

-ARF binds and inhibits Mdm2, sequestering it in the nucleolus.
-ARF is induced by E2F; abnormally high levels of E2F (e.g. loss of Rb or oncogene activation) induce ARF transcription.

pRB –| E2F → ARF –| Mdm2 –| p53 → apoptosis or cell cycle arrest

# Unusual Gene Structure of the Mdm2 antagonist $p14^{ARF}$

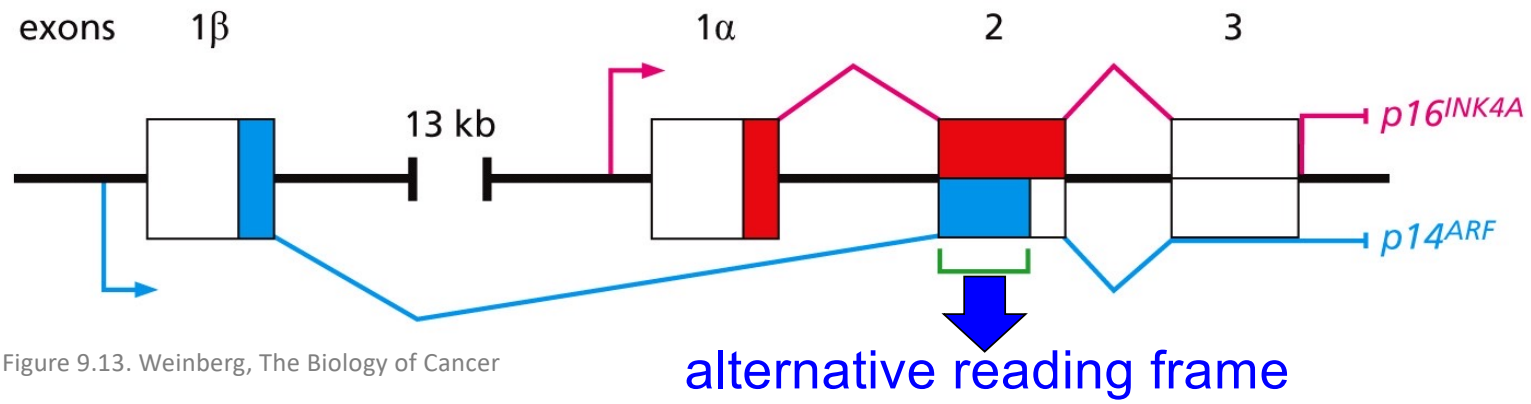



Figure 9.13. Weinberg, The Biology of Cancer

p16INK4A is an important inhibitor of CDK4 and CDK6 which phosphorylate Rb (week 6). $p14^{ARF}$ (p19 in mice) shares its 2nd exon with p16 but contains an alternative reading frame! The boxes indicate exons, while the filled areas indicate reading frames.

week 4 slide 72-73
about Transgenic Mice that O/E myc and bcl-2 from the IgG-Promoter, so they made mice that carried either one or both transgene, on the graph I see that only the bcl-2 alone have a 100% survival rate, and the mice develop lymphomas only when myc is overexpressed...But I thought Bcl-2 was an oncogene ? What is there to take here, is it that myc and bcl2 collaboration creates an aggressive malignancy on the B-lymphocyte lineage ? Is bcl2 more an anti-apoptotic than an oncogene ?

Bcl2 is antiapoptotic. It is overexpressed in ~ 50% of human tumors.
It acts as an oncogene when overexpressed (i.e. it induces cancer).

## Death Due to Lymphomas

**Synergy of Bcl2 with Myc. Both genes were overexpressed from the IgG promoter in B lymphocytes.**

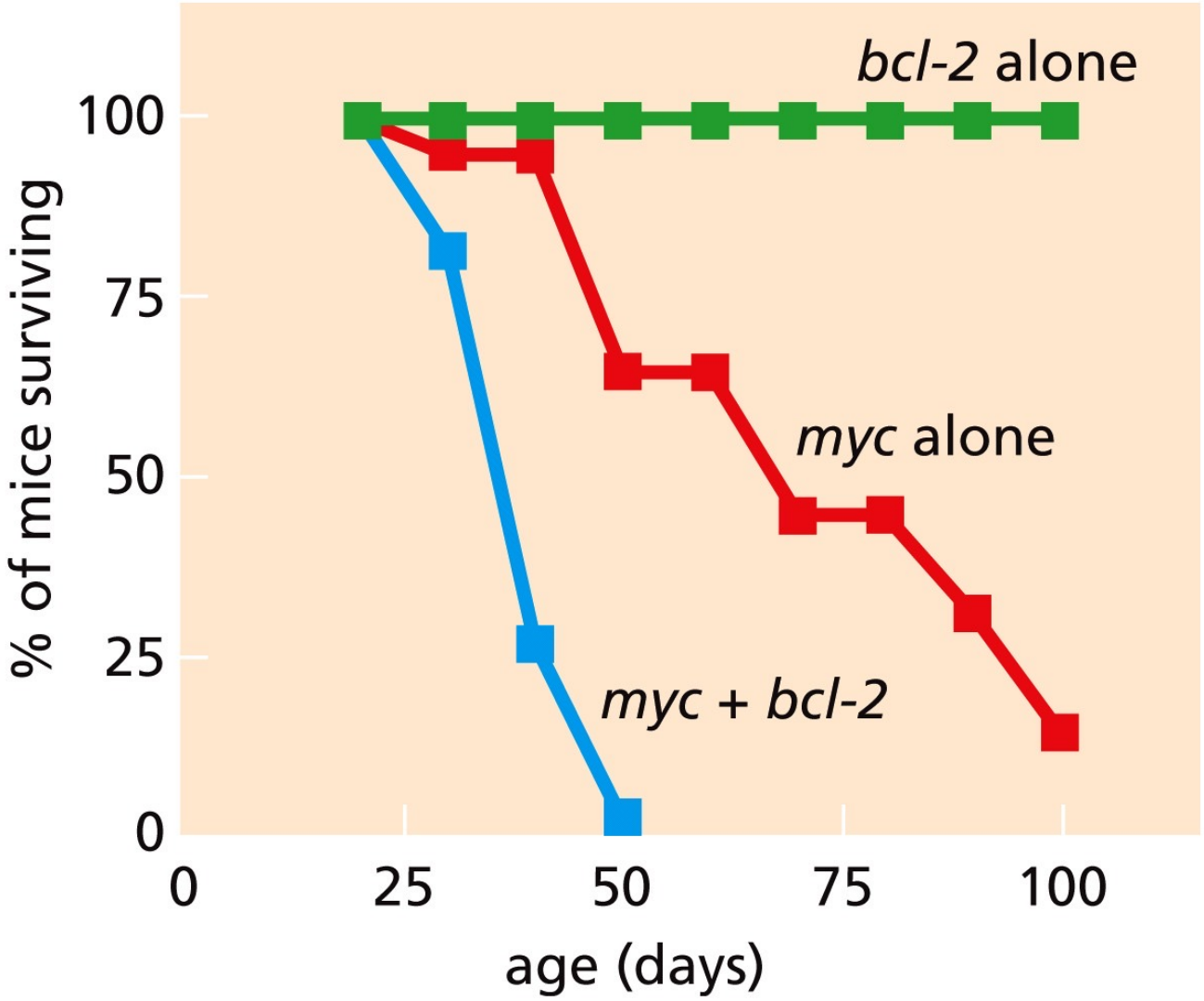



Figure 9.20. Weinberg, The Biology of Cancer

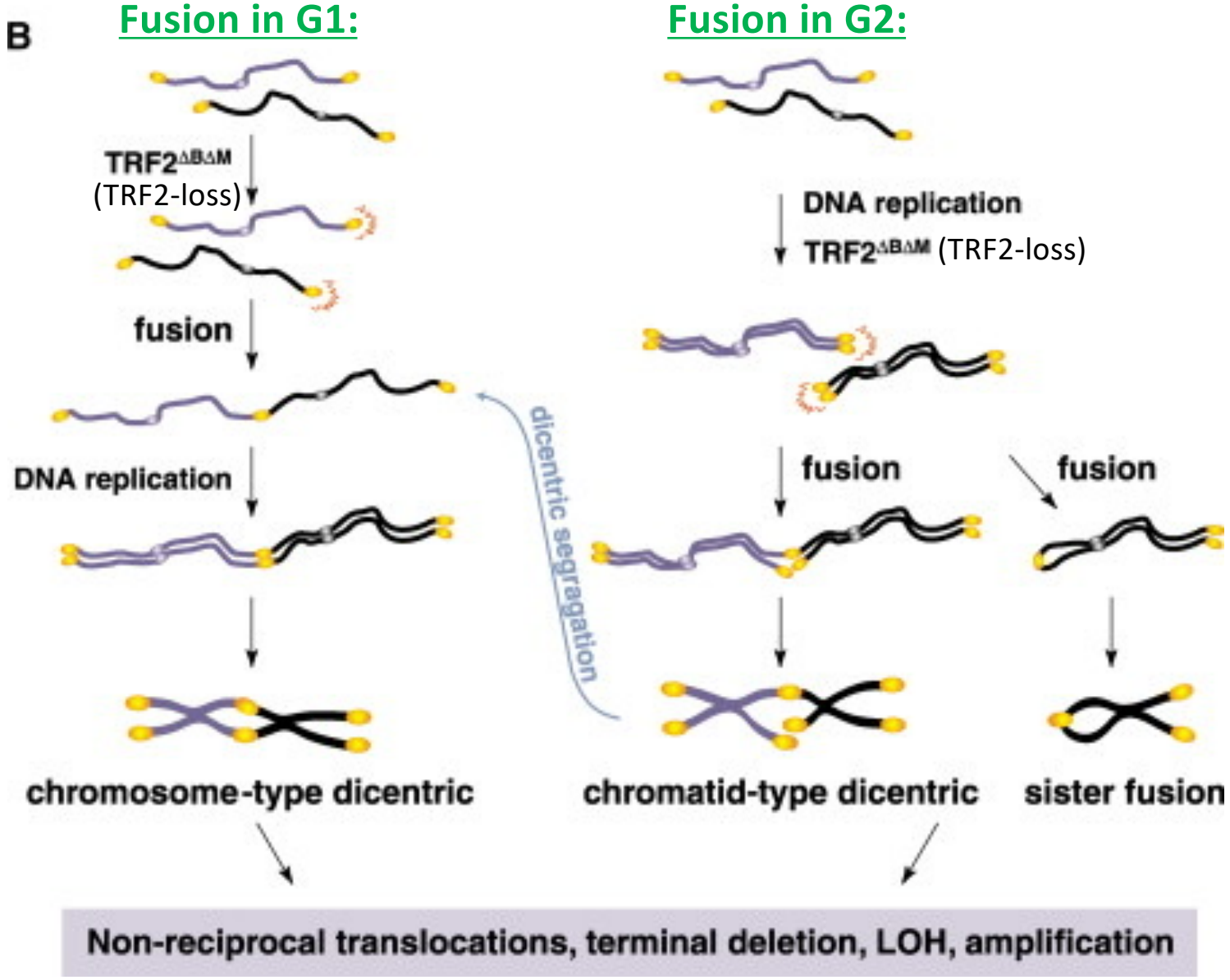
## -week 5 ; What factor is decisive to know if a cancer rely on telomerase for maintaining telomeres or rely on ALT ? Can a cancer rely on both or is it one or the other exclusively ?

- Most cancers rely on telomerase for maintaining telomeres but roughly 10-15% rely on ALT (mostly of mesenchymal origin; some brain tumors).

- ALT occurs in a smaller fraction of sarcomas (cancer of connective or supportive tissue; bone, cartilage, muscle etc.), many glioblastomas. It is not known, why ALT is mostly observed in sarcomas and glioblastomas.

- Generally, human cancers activate either telomerase or ALT, but not both.

exercises week 6 Q4: « Upon loss of telomere capping, telomere fusions can occur before (G1) and after DNA synthesis (G2). What will be the consequences for the involved chromosomal DNA molecules during M phase? Draw the involved chromosomes during the different cell cycle stages for a fusion that took place (A) in G1 and (B) in G2. »


B
Fusion in G1:
Fusion in G2:
TRF2ΔBΔM
(TRF2-loss)
fusion
DNA replication
DNA replication
TRF2ΔBΔM (TRF2-loss)
fusion
fusion
dicentric segregation
chromosome-type dicentric
chromatid-type dicentric
sister fusion
Non-reciprocal translocations, terminal deletion, LOH, amplification

- In Figure 1B of Series 5, I'm having trouble understanding the SELEX question.

Figure 1 :
For SELEX method (Figure 1B, see page 3 of this handout)
How could you confirm that the Bound (B) complex indeed contains the indicated polypeptides and is not an agglomerate of DNA only?

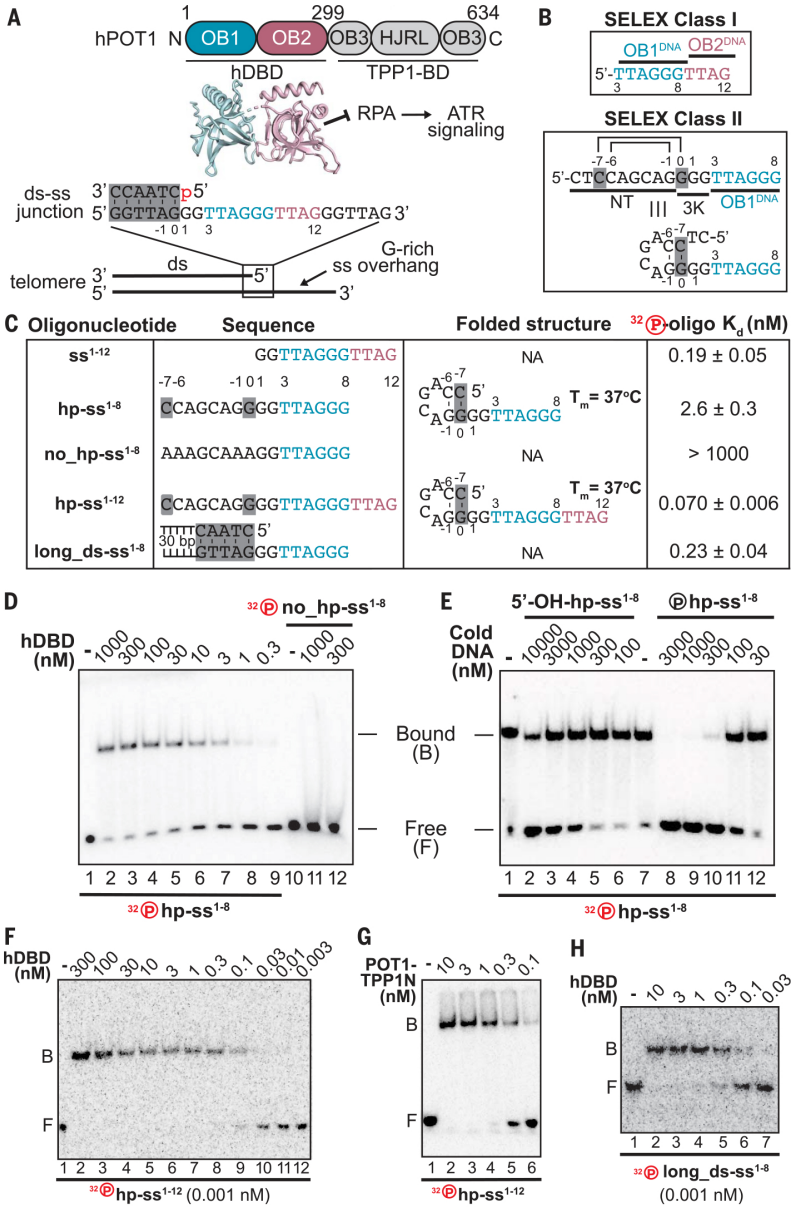
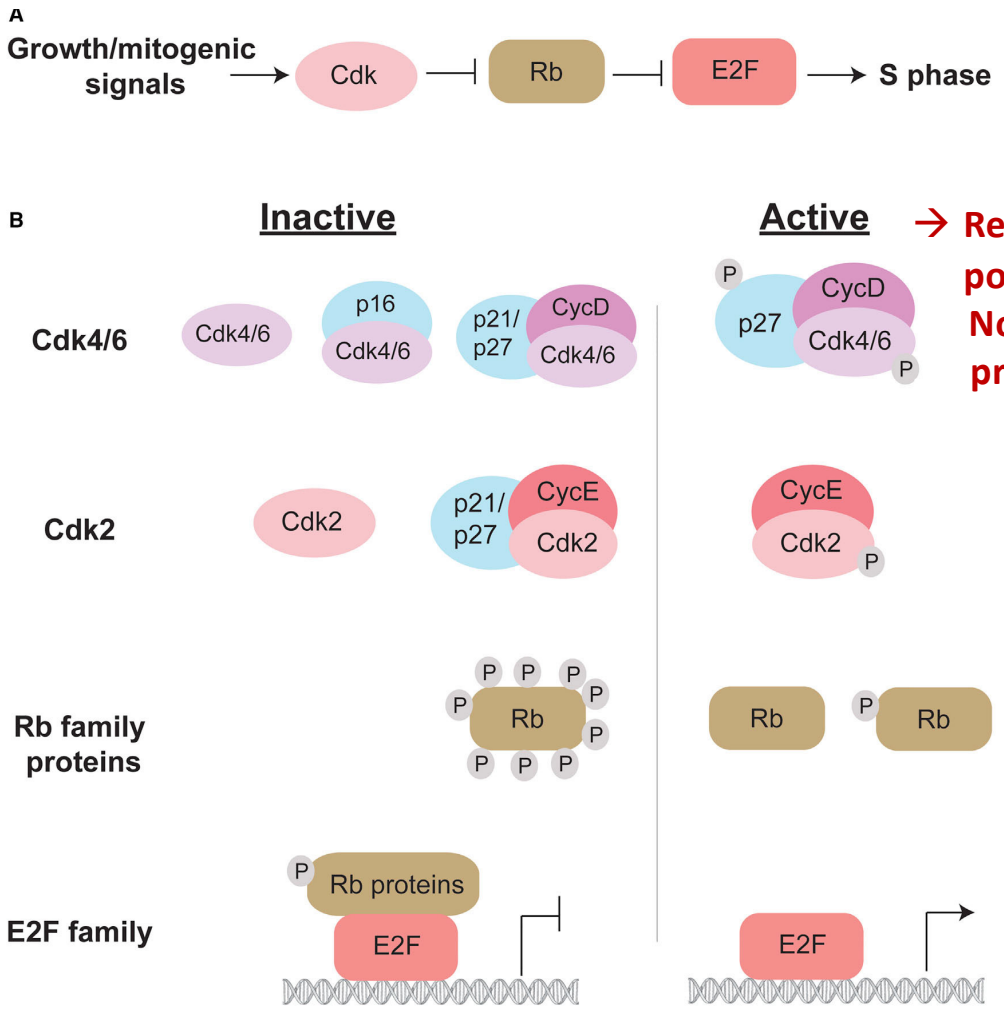



**Fig. 1. Human POT1 recognizes the 5′-phosphorylated ds-ss junction of telomeres.** (**A**) (Top) Schematic of hPOT1 includes binding domains for ssDNA (hDBD) and TPP1 (TPP1-BD). hDBD (PDB: 1XJV) is composed of OB1 and OB2. The current model suggests that POT1 outcompetes the ssDNA-binding RPA complex to prevent ATR signaling at telomeres. HJRL, Holliday junction resolvase-like. (Bottom) Mammalian chromosomes end in a ds-ss junction containing ATC-5′ (predominantly) and a ss G-rich overhang. Numbering starts with the first overhang nucleotide. (**B**) A previous SELEX study revealed two hPOT1-binding DNA classes (*24*). Class I harbors the known sites for OB1 and OB2, denoted as $OB1^{DNA}$ (cyan) and $OB2^{DNA}$ (pink), respectively. Class II revealed a consensus containing a seemingly nontelomeric (NT) sequence upstream of $OB1^{DNA}$ that can potentially fold into a hp; "K" indicates a G or T nucleotide, and the shaded area indicates the sequence of the first bp at the telomeric ds-ss junction. (**C**) Annotated name, sequence, predicted hp structure [with $T_m$ (where $T_m$ is the temperature at which 50% of dsDNA is denatured) calculated by the UNAFold web server], and mean $K_d$ and SD (of binding to hDBD) of the oligonucleotides used in EMSA analysis. NA indicates not applicable. (**D** to **H**) EMSA of indicated proteins (hDBD or POT1-TPP1N heterodimer) and 5′-$^{32}$P–labeled DNA oligonucleotides. (D), (F), (G), and (H) indicate direct binding experiments, and (E) indicates a competition experiment. In (D), 0.1 nM 5′-$^{32}$P-hp-ss$^{1-8}$ was used; the number of experimental replicates $n$ = 5 for hp-ss$^{1-8}$ (full and partial titrations); $n$ = 3 for no_hp-ss$^{1-8}$. In (E), 100 nM hDBD and 0.1 nM 5′-$^{32}$P–labeled hp-ss$^{1-8}$ were incubated with indicated amounts of unlabeled hp-ss$^{1-8}$ (cold DNA) containing either a 5′-OH or a 5′-P; $n$ = 3. In (F), 0.001 nM 5′-$^{32}$P-hp-ss$^{1-12}$ was used; $n$ = 3. In (G), 0.01 nM 5′-$^{32}$P-hp-ss$^{1-12}$ was used; $n$ = 3. (H) 0.001 nM 5′-$^{32}$P-long_ds-ss$^{1-8}$ was used; $n$ = 3. Circled red "P" indicates radiolabeled; circled black "P" indicates nonradiolabeled. Bound (B) indicates DNA bound to protein; Free (F) indicates free, unbound DNA.

•In Week 6, 2nd handout, slide 5: In my understanding, when Rb is hypophosphorylated, it blocks E2F, is there an error in the image (I attached a screenshot)?




→ Refers to the individual polypeptide;
Not the final outcome for progression into the S phase

**Figure 1. Components of the Cdk-Rb-E2F Pathway Controlling the G1/S Transition**

(A) Simplified linear model for the pathway.

(B) Inactive and active states of the key players in the Rb pathway. Cdk4/6 have relatively high sequence homology among Cdks. They are inactive as monomers, bound to p16 family proteins, or bound by unphosphorylated p21/p27 proteins. Cdk4/6 are activated by association with CycD family proteins, but full activity also requires a phosphorylated form of p27 in the complex and phosphorylation on the kinase activation loop. Cdk2 is inactive as a monomer or in complex with p21/p27 family proteins, and it is activated by CycE binding in G1 (or by CycA later in the cell cycle) and activation loop phosphorylation. Rb is considered active when hypophosphorylated or monophosphorylated; in this state, it binds and inhibits E2F. Hyperphosphorylation of Rb leads to its inactivation, dissociation from E2F, and subsequent E2F activation.