cancers

MDPI

Review

Pharmacologic Induction of BRCAness in *BRCA*-Proficient Cancers: Expanding PARP Inhibitor Use

Rachel Abbotts [1,2,*], Anna J. Dellomo [1,2] and Feyruz V. Rassool [1,2]

1 Department of Radiation Oncology, University of Maryland School of Medicine, Baltimore, MD 21201, USA; anna.dellomo@som.umaryland.edu (A.J.D.); frassool@som.umaryland.edu (F.V.R.)
2 University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center, Baltimore, MD 21201, USA
* Correspondence: rabbotts@som.umaryland.edu

Omidreza Firuzi

Journal club presentation

25-10-1401

Update Your Profile

Feyruz V. Rassool, PhD

Academic Title: Professor

Primary Appointment: Radiation Oncology

Additional Title: Co-director, Experimental Therapeutics Program, UMGCCC

Email: frassool@som.umaryland.edu

Location: BRB, 8-037

Phone (Primary): (410) 706-5337

Phone (Secondary): (410) 971-5056

Fax: (410) 706-6666

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Education and Training

Education

- 1979 - A Levels, Camden School for Girls, London, UK
- 1983 - B.Sc., University College London, London, UK, Honors in Human Genetics
- 1990 - Ph.D., Royal Post-Graduate Medical School, University of London, London, UK, Biological Sciences

Biosketch

Feyruz V. Rassool, PhD, received her doctorate at the Royal Postgraduate Medical School, University of London, UK. She did her postdoctoral training at the University of Chicago and assumed her first independent faculty position studying DNA damage and repair in myeloid malignancies at King's College London. She has been at the University of Maryland for the last 16 years.

She is an expert in repair of potentially lethal forms of DNA damage, DNA double-strand breaks (DSBs), that play a critical role in generating genomic instability in cancer. Her work has specifically focused on the aberrant expression and activity of these repair pathways in cancer and leukemia cells that not only play a role in genomic instability, but also appear critical for cancer cell survival. These DNA repair components are attractive therapeutic targets. Thus, Dr. Rassool's work provides a framework for the development and translation of novel therapeutic strategies for patients with leukemia's and other cancers. Her recent work is focused on targeting DNA repair abnormalities in Cancer and leukemia for therapy.

Dr Rassool recently received the Ziskin Award to study the intersection between DNA damage and repair and epigenetic pathways in cancer and she is part of the SU2C stand-up to cancer Epigenetics Dream Team.

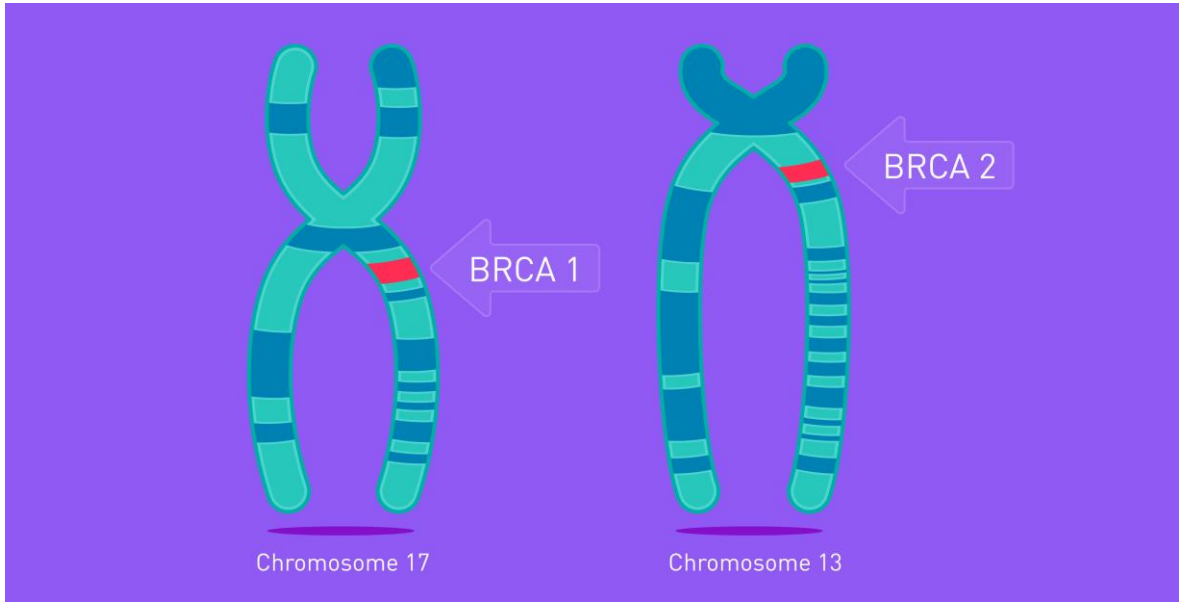
Studies to target acute myeloid malignancies and triple negative breast cancers with a combination of DNA repair and epigenetic inhibitors was recently published in Cancer Cell (2016). These studies led to a clinical trial in AML led by Dr Maria Baer, Director of hematologic malignancies at UMGCCC. A clinical trial in TNBC based o this treatment paradigm was also opened at Indiana University, in collaboration with Dr. Kenneth Nephew and Kathy Miller. The Rassool group has also extended this therapy paradigm to lung cancer. The study showed that the efficacy of this drug combination in non-small cell lung cancer (NSCLC) is further enhanced with ionizing radiation (IR) (PNAS, 2019). Notably this data suggests that DNMTis iduce a DNA double strand break repair defect that sensitizes cells to PARPis and IR.

The recent studies in the Rassooll group have linked decreased DSBR with the drug combination to activation of immune signaling. This work is based on a report that low does of DNMTis induce an increased tumor immune response through the activation of endogenous retrovirus transcription, and a cytopllasmic double stranded RNA (dsRRNA) response. This has been termed the DNMTi-induced iral mimiry (Chiappinelli et al., 2015; Chiappinelli et al., 2016). Importantly, the Rassool lab has extended this concept by showing that DNMTi's in combination with PARPis induce interferon and inflammasome signaling

BRCA1 and BRCA2 proteins play central roles in homologous recombination

- BRCA1 and BRCA2 proteins play central roles in homologous recombination, the high-fidelity pathway responsible for the repair of DSBs during DNA replication.

- They are **cancer-susceptibility genes**, but at the same time an **Achilles' heel** for cancer cells.

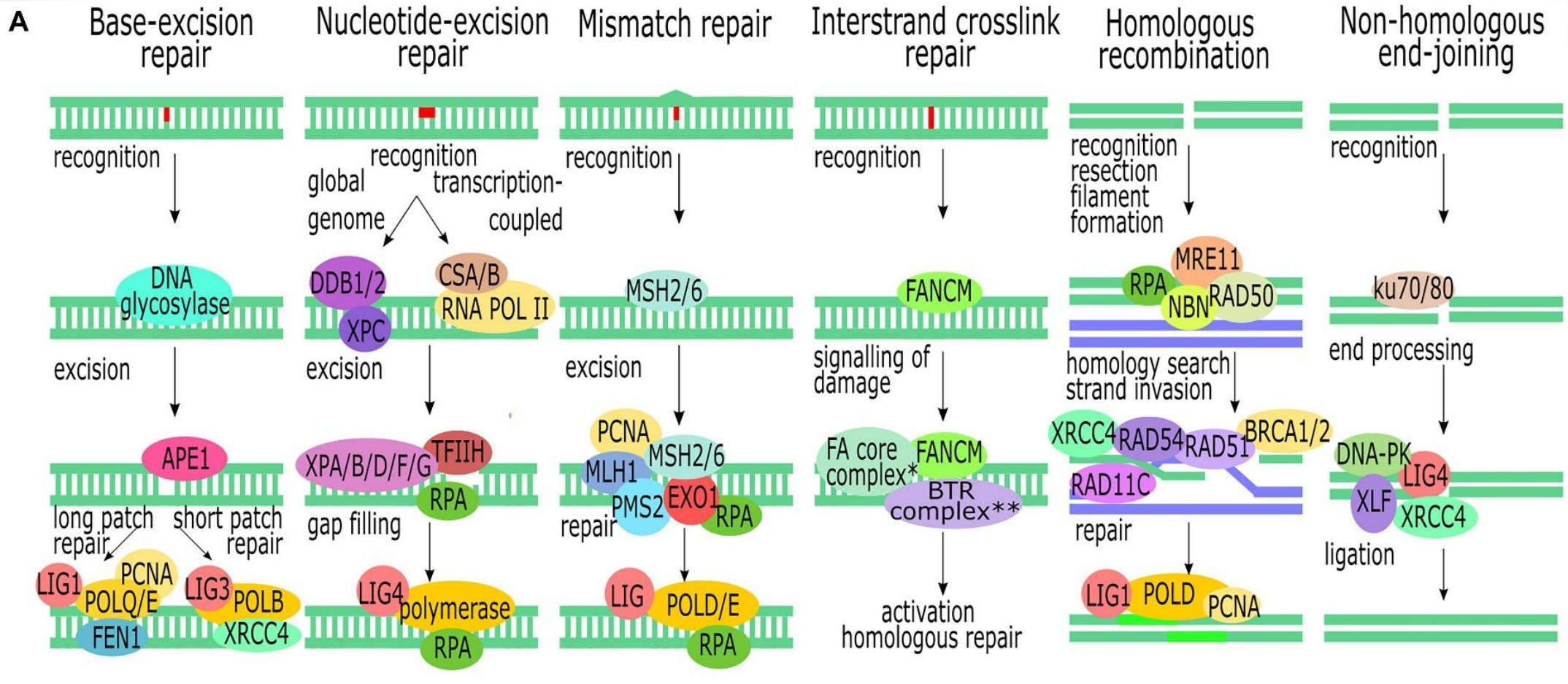


HR deficiency (BRCA mutations) increase susceptibility to single-stranded base damage

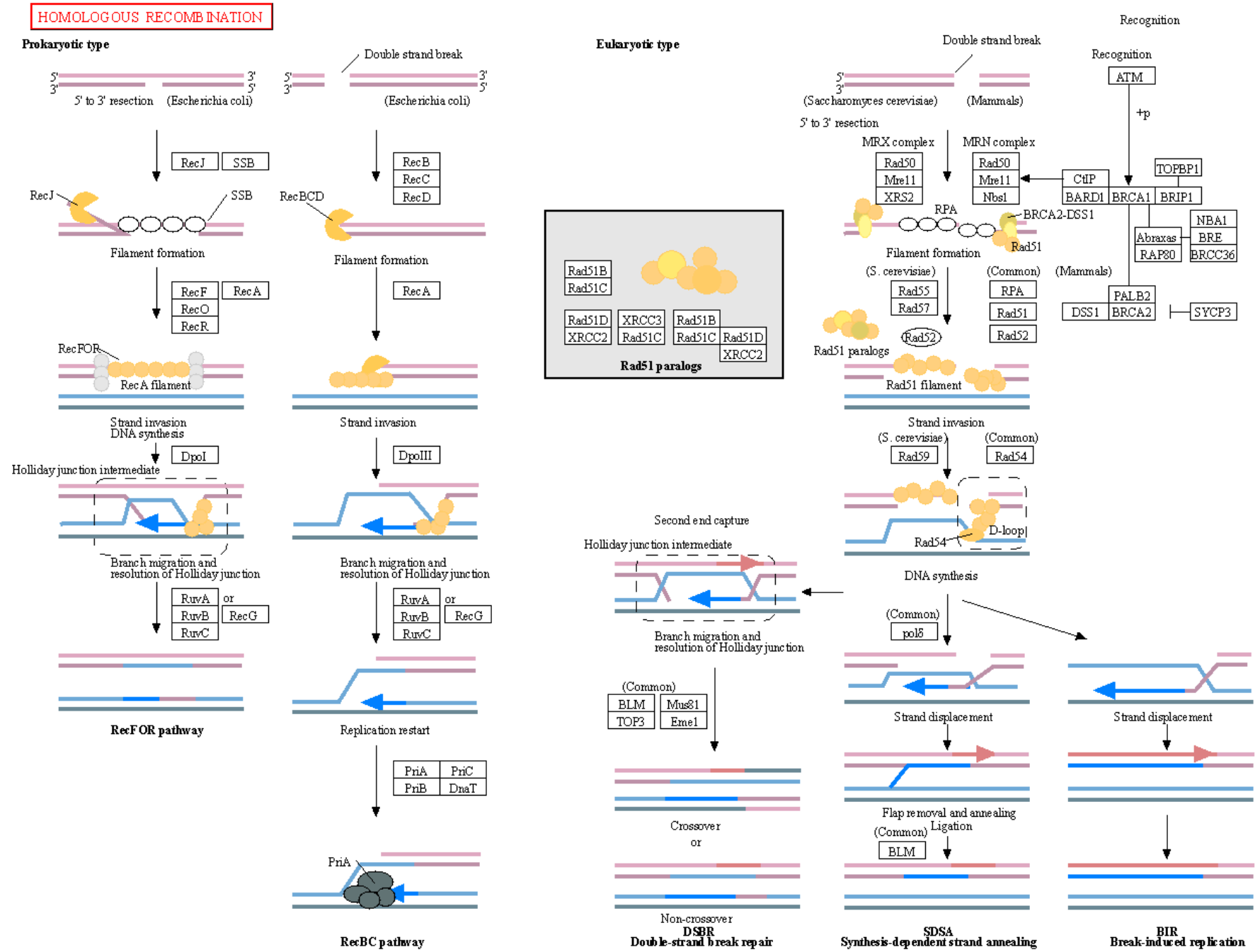
- Defects in HR stemming from BRCA mutations increase susceptibility to single-stranded base damage.

- Single-strand interruptions or adducts b**lock replication fork progression**, leading to extended fork **stalling** or collapse into a DSB–both of which require the proficiency of HR mechanisms for successful resolution [16].

DNA damage repair



- (A) Overview of the major DNA repair pathways. The Fanconi anemia core complex consists of FANCA, FANCB, FANCC, FANCD, FANCE, FANCF, FANCG, and FAAP100. The BTR complex consists of BLM, TOPOIII, RMI1, and RMI2. (B) Molecular targets within the DDR pathways and available inhibitors.


HOMOLOGOUS RECOMBINATION
Prokaryotic type
Double strand break
5' to 3' resection (Escherichia coli)
(Escherichia coli)
RecJ
SSB
RecB
RecC
RecD
Filament formation
RecBCD
RecF
RecO
RecR
RecA
RecFOR
RecA filament
Strand invasion
DNA synthesis
DpoI
DpoIII
Holliday junction intermediate
Branch migration and resolution of Holliday junction
RuvA
RuvB
RuvC
or
RecG
RecFOR pathway
Replication restart
PriA
PriB
PriC
DnaT
RecBC pathway
Eukaryotic type
Recognition
(Saccharomyces cerevisiae)
(Mammals)
5' to 3' resection
MRX complex
MRN complex
Rad50
Mre11
XRS2
Nbs1
ATM
+p
CtIP
BARD1
BRCA1
BRIP1
TOPBP1
NBA1
BRE
BRCC36
Abraxas
RAP80
PALB2
BRCA2
DSS1
SYCP3
RPA
BRCA2-DSS1
Rad51
Filament formation
(S. cerevisiae)
(Common)
(Mammals)
Rad55
Rad57
Rad52
Rad51 paralogs
Rad51 filament
Rad51B
Rad51C
Rad51D
XRCC2
XRCC3
Strand invasion
Rad59
Rad54
D-loop
DNA synthesis
Second end capture
Holliday junction intermediate
Branch migration and resolution of Holliday junction
BLM
TOP3
Mus81
Eme1
Crossover
or
Non-crossover
DSBR
Double-strand break repair
pol δ
Strand displacement
Flap removal and annealing
Ligation
SDSA
Synthesis-dependent strand annealing
BIR
Break-induced replication
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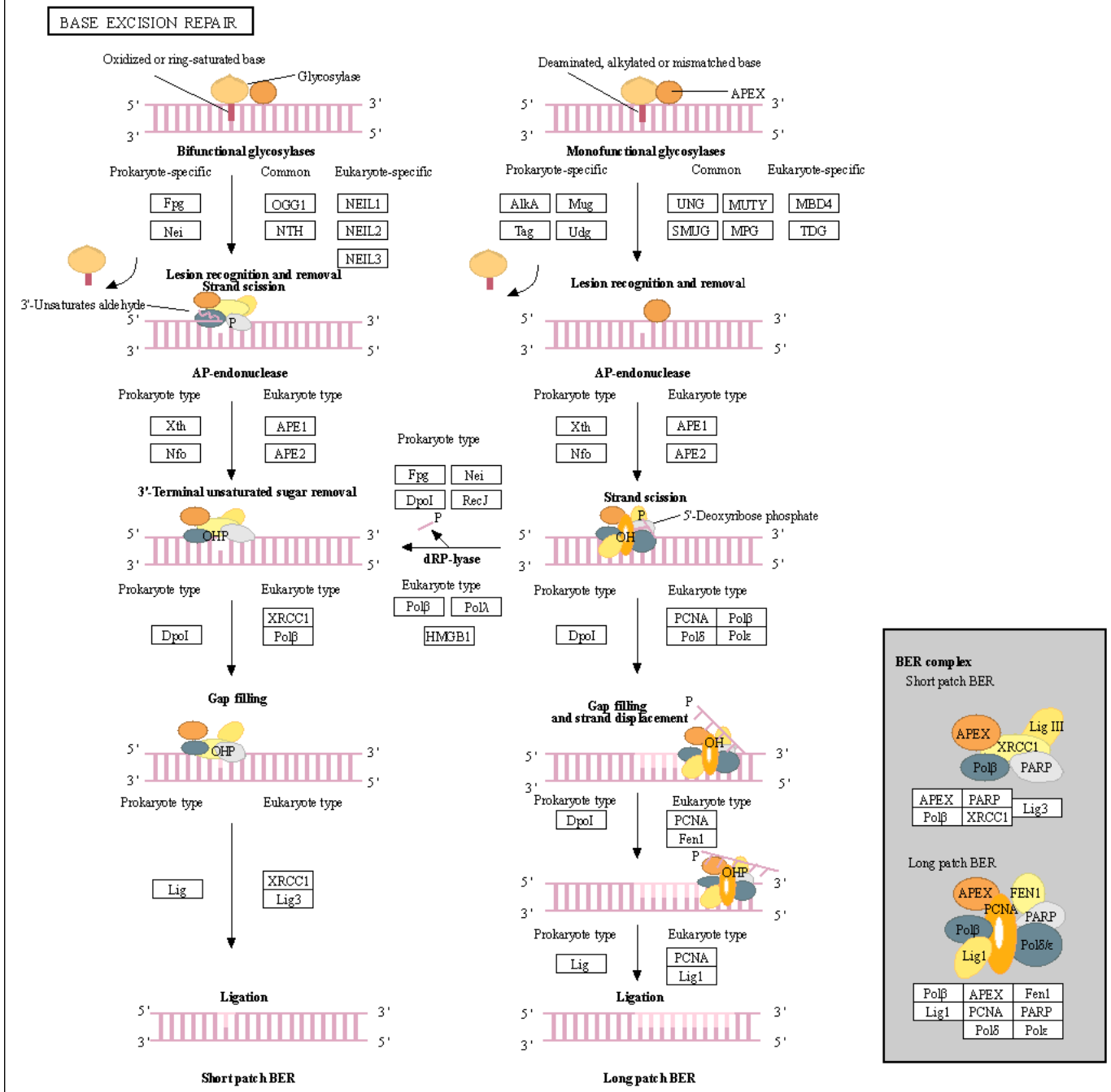
BASE EXCISION REPAIR
Oxidized or ring-saturated base
Glycosylase
Bifunctional glycosylases
Prokaryote-specific
Common
Eukaryote-specific
Fpg
Nei
OGG1
NTH
NEIL1
NEIL2
NEIL3
Lesion recognition and removal
Strand scission
3'-Unsaturates aldehyde
AP-endonuclease
Prokaryote type
Eukaryote type
Xth
Nfo
APE1
APE2
3'-Terminal unsaturated sugar removal
Prokaryote type
Fpg
Nei
DpoI
RecJ
dRP-lyase
Eukaryote type
Polβ
Polλ
HMGB1
Prokaryote type
Eukaryote type
DpoI
XRCC1
Polβ
Gap filling
Prokaryote type
Eukaryote type
Lig
XRCC1
Lig3
Ligation
Short patch BER
Deaminated, alkylated or mismatched base
APEX
Monofunctional glycosylases
Prokaryote-specific
Common
Eukaryote-specific
AlkA
Mug
Tag
Udg
UNG
MUTY
SMUG
MPG
MBD4
TDG
Lesion recognition and removal
AP-endonuclease
Prokaryote type
Eukaryote type
Xth
Nfo
APE1
APE2
Strand scission
5'-Deoxyribose phosphate
Prokaryote type
Eukaryote type
DpoI
PCNA
Polβ
Polδ
Polε
Gap filling and strand displacement
Prokaryote type
Eukaryote type
DpoI
PCNA
Fen1
Prokaryote type
Eukaryote type
Lig
PCNA
Lig1
Ligation
Long patch BER
BER complex
Short patch BER
APEX
Lig III
XRCC1
Polβ
PARP
APEX
PARP
Polβ
XRCC1
Lig3
Long patch BER
APEX
FEN1
PCNA
PARP
Polβ
Polδ/ε
Lig1
Polβ
APEX
Fen1
Lig1
PCNA
PARP
Polδ
Polε
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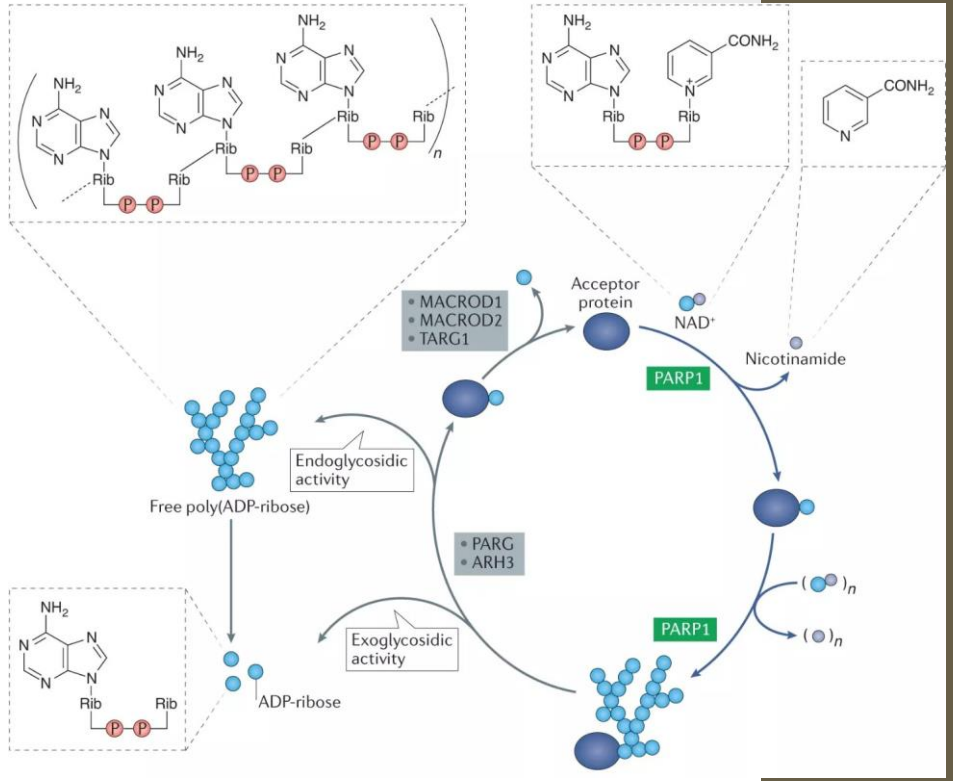
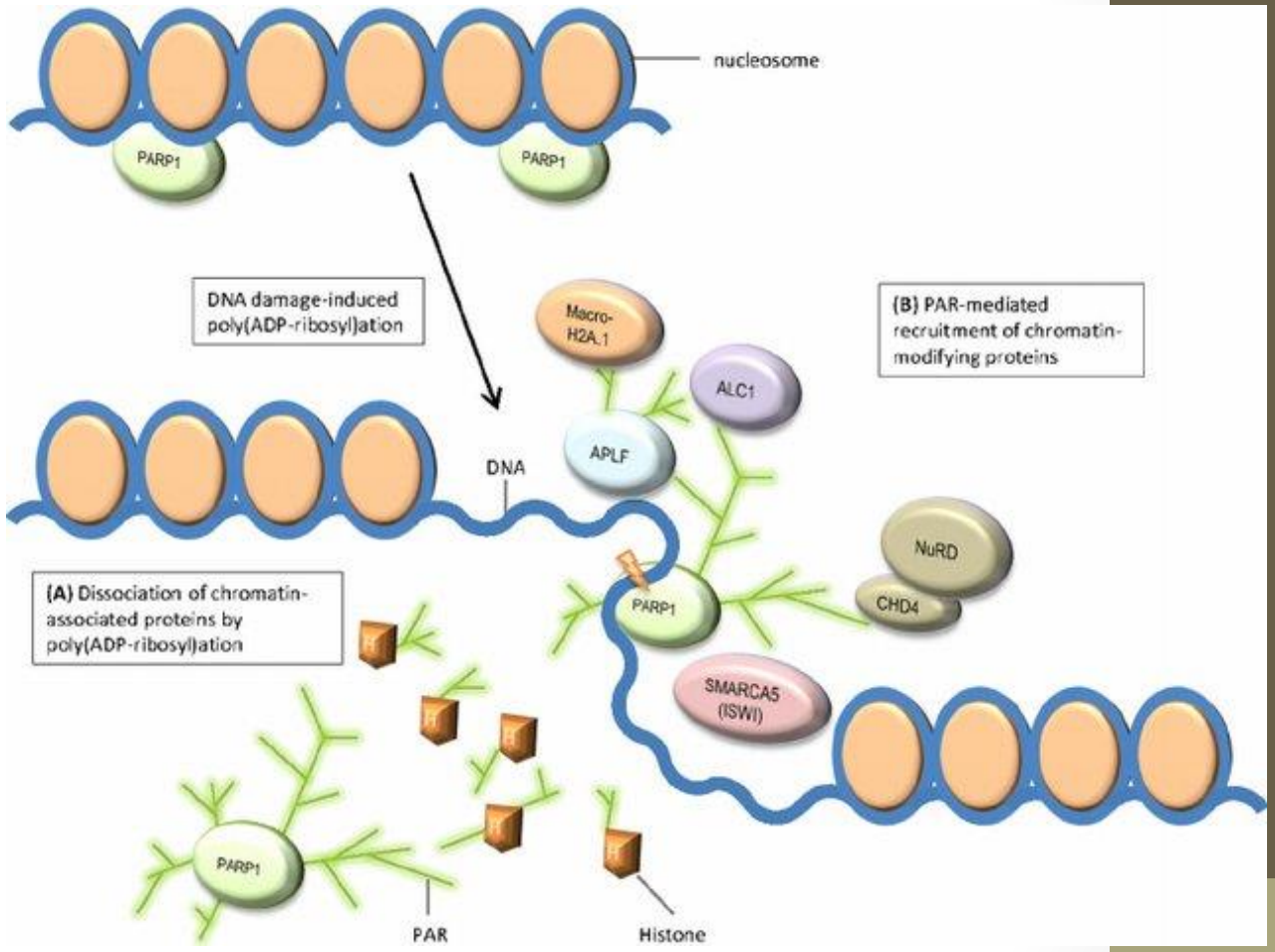
(c) Kanehisa Laboratories

Poly(ADP-ribose) polymerase (PARP)



- Poly(ADP-ribose) polymerase (PARP) proteins catalyze the transfer of an ADP (adenosine diphosphate)-ribose subunit of nicotinamide adenine dinucleotide (NAD+) onto a broad range of **proteins and themselves**, forming **poly(ADP-ribose) (PAR) polymers**. PARPs are known to function in a large range of cellular processes including **DNA repair**, **DNA replication**, **transcription** and **modulation of chromatin structure**.

- Poly-ADP-ribosylation (PARylation) is a reversible post-translational modification

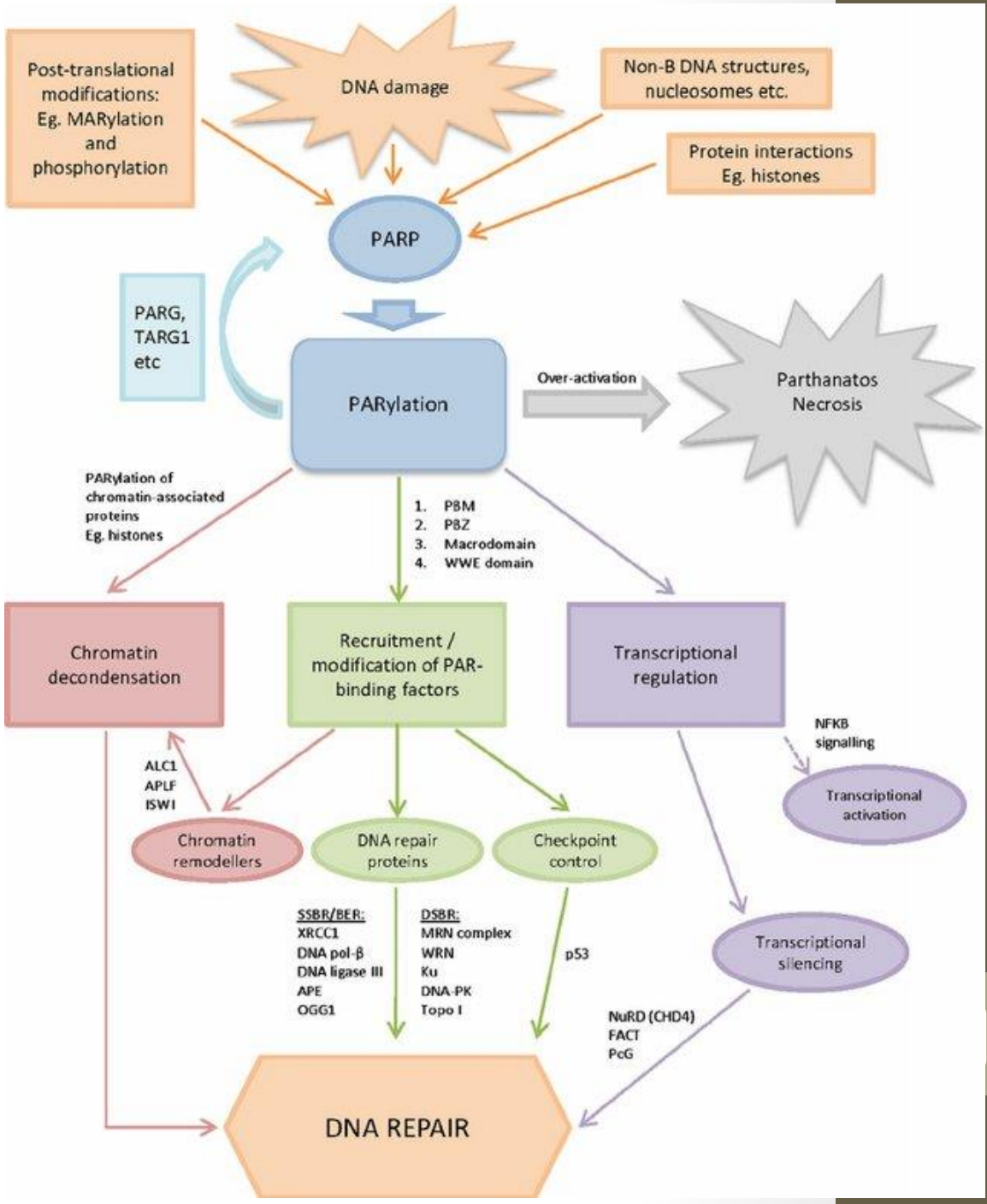


PARylation of proteins

After DNA dameg, **PARylation of histone proteins** around the damage site remodels the surrounding chromatin to allow repair.

When the PARP1 protein level is reduced, either by CRISPR knockout or small interfering RNA (siRNA) targeting, or catalytic activity is impaired, by expression of a catalytically inactive mutant or targeting by small molecule inhibitors:

- A delayed repair of SSBs is observed leading to G2/M arrest, chromosomal instability, and cytotoxicity.



PARP inhibitors

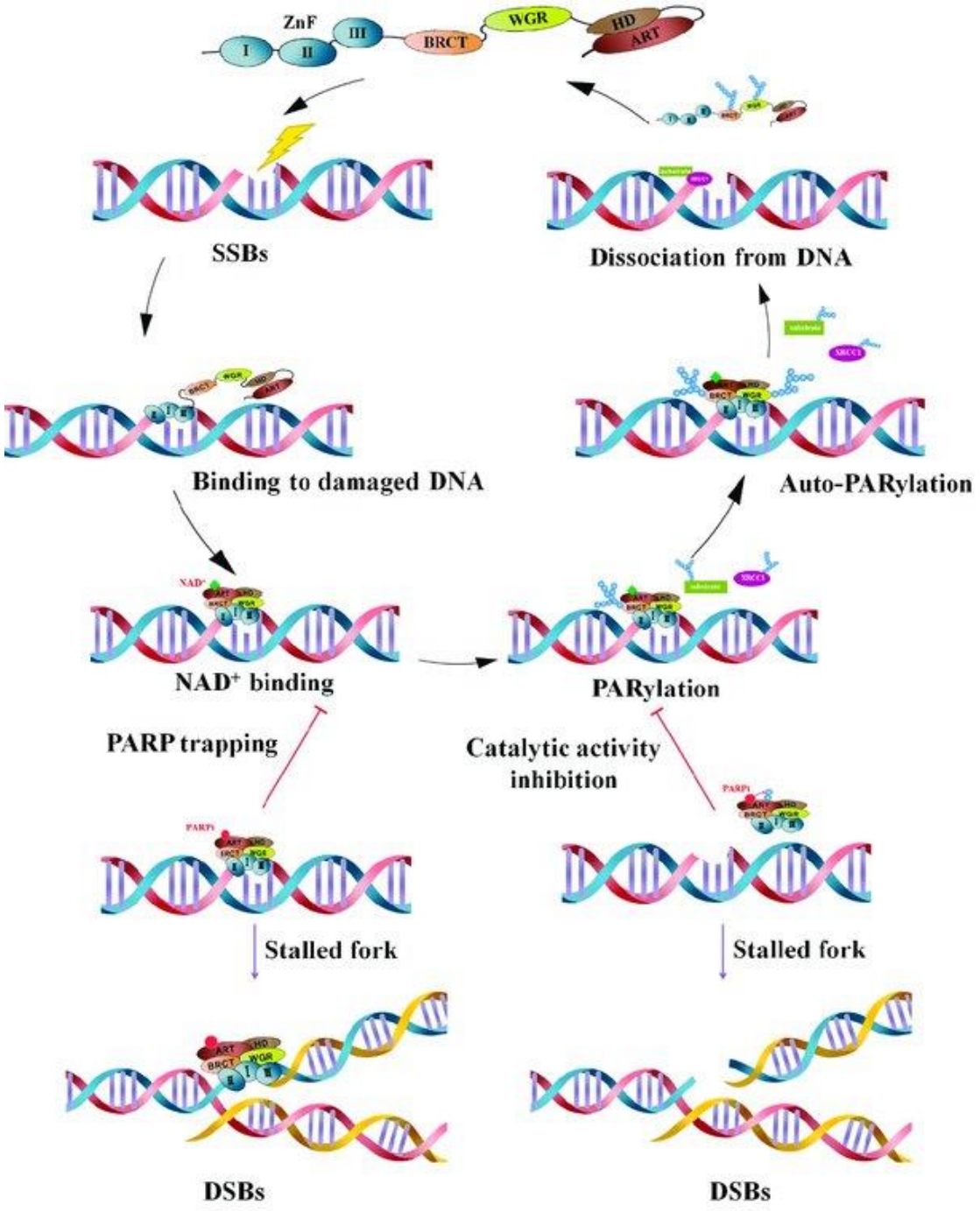
PARPi that have now received FDA approval include:

- Olaparib (AstraZeneca/Merck/KuDOS, Cambridge, UK; approved 2014)
- Rucaparib (Clovis, Boulder, CO, USA; approved 2016)
- Niraparib (GlaxoSmithKline, Brentford, UK; approved 2017)

Additional agents veliparib (AbbVie, North Chicago, IL, USA) and pamiparib (Beigene, Beijing, China) are currently being evaluated in phase III trials.

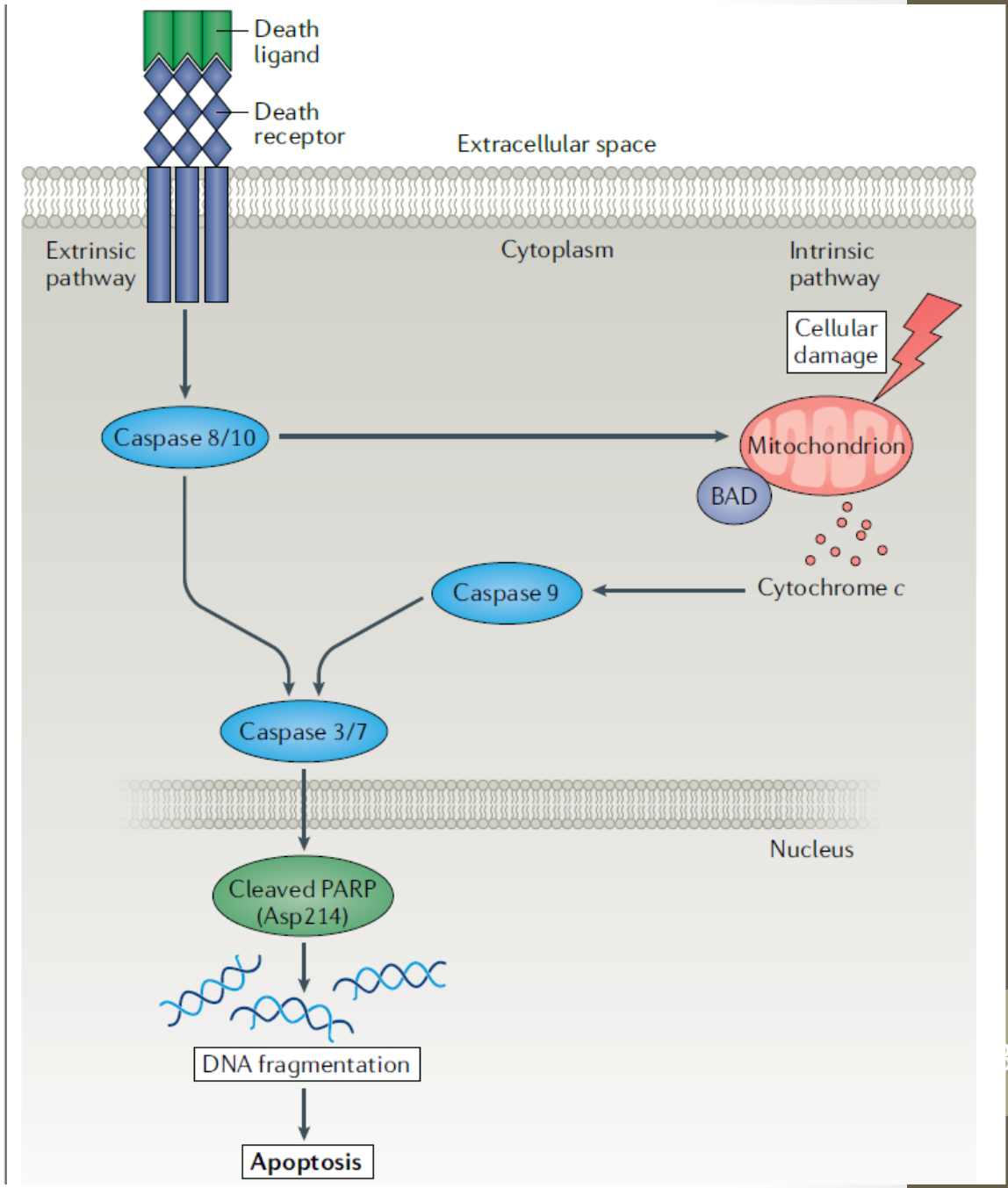
- In 2018, the **second generation PARPi talazoparib** (Pfizer, New York, NY, USA) was approved by the FDA, offering enhanced potency over its predecessors. Talazoparib possessing a trapping capacity 100-fold greater than the next most potent PARP trapper, niraparib.

- PARPi interact with the **binding site of the PARP substrate NAD+**, reducing PARylation activity.



PARP cleavage and apoptosis

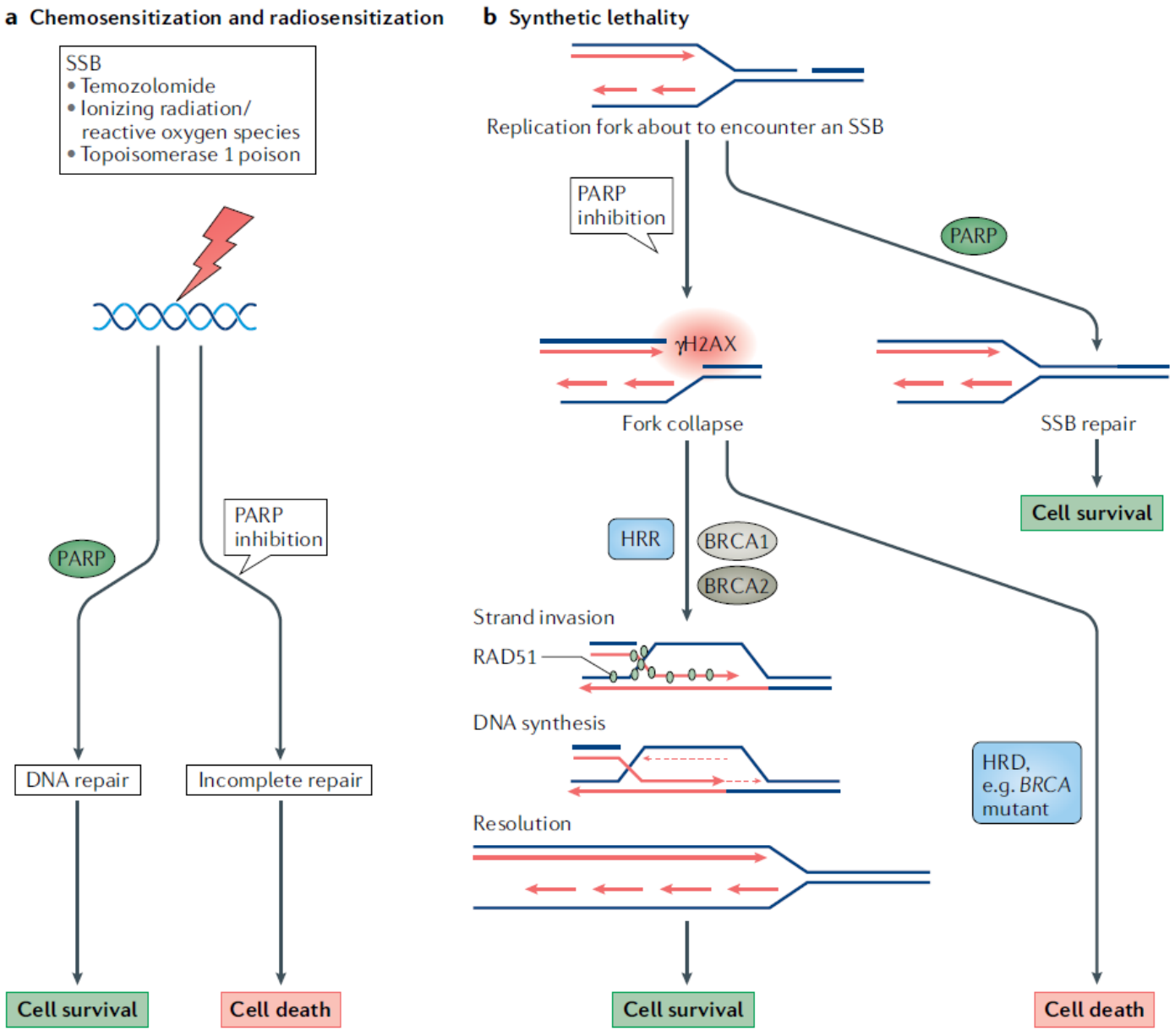
- PARP can be cleaved during apoptosis
- The cleavage of PARP by activate caspases separates the DNA- binding domain from the catalytic domain, thereby preventing PARP activation.
- Whether this is to prevent APRP from consuming NAD+ and/or limit poly(ADP- ribose) polymer formation is not clear.
- If PARP is not inactivated by cleavage, then there is a massive activation of PARP in response to DNA damage, resulting in NAD+ and ATP depletion.



PARP inhibitors: Chemosensitization and synthetic lethality

PARPi selectivity for cancer cells harboring defects in DNA double strand break (DSB) repair was first described in concurrent publications by Farmer et al. [13] and Bryant et al. [14] in 2005, with both groups reporting exquisite PARPi sensitivity in BRCA mutated tumors.

Failure of BRCA-mutant cells to repair these PARPi-induced lesions is associated with an **accumulation of cytotoxic DSB damage** and the eventual activation of apoptotic mechanisms to limit the deleterious effects of error-prone repair [13,14].



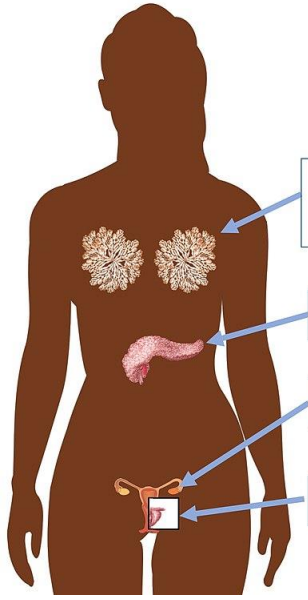
"BRCAness" or Homologous Recombination (HR) deficiency

"BRCAness": the phenotypic traits that some tumors share with cancers that have *BRCA1/2* mutations

BRCA-like tumors are particularly sensitive to

- DNA-damaging agents (e.g., platinum agents) because of inadequate *BRCA*-mediated DNA repair mechanisms (nucleotide-excision repair and homologous recombination (HR))
- Poly(ADP-ribose) polymerase inhibitors, which cause synthetic lethality in HR-deficient cells

BRCA mutations are relatively rare: How we can therapeutically use the concept of Synthetic lethality for a larger number of patients



	BRCA1	BRCA2
Breast cancer:	**50% to 65%** Males: 1.2%	**40% to 55%** Males: Up to 9%
Pancreas cancer:	1-3%	2-7%
Ovarian cancer:	**40% to 65%**	**15% to 25%**
Prostate cancer:	9%	15%

- Synthetic lethality: T**wo independently non-lethal defects** (BRCA mutation and PARP inhibition), combine to induce potent cell death.
- This powerful therapeutic strategy allows **few off-target complications**.
- However, **BRCA mutations are relatively rare**
- There is a growing push for the identification of tumors that exhibit '**BRCAness**', mimicking the **defective HR phenotype of BRCA mutation** and potentially expanding the therapeutic utility of PARPi to a larger subset of tumors [18,19].

The BRCAness phenotype has been linked to mutations in other repair factors involved in DNA damage response:

- ATM, ATR, RAD51, or the Fanconi Anemia (FA) family
- Altered gene expression by epigenetic silencing or cellular regulatory mechanisms [20].

Detecting tumor BRCAness

A number of methods have been explored to **detect tumor BRCAness and predict PARPi sensitivity**, including

- Panel sequencing for **DNA repair gene mutations**
- Repair gene **expression microarrays**
- Testing for surrogate markers of HR deficiency such as loss of heterozygosity, or functional tests of repair capacity such as RAD51 foci formation (recently reviewed in [21]).

BRCAness can be induced in HR proficient cancers

- More recently, it has become recognized that **BRCAness may be induced using therapeutic agents** that modulate a variety of molecular pathways, potentially providing a novel method for **inducing synthetic lethality** in cancers that are **otherwise HR-proficient**.

This review will focus on the therapeutic targeting of major molecular pathways that have been implicated in BRCAness, including:

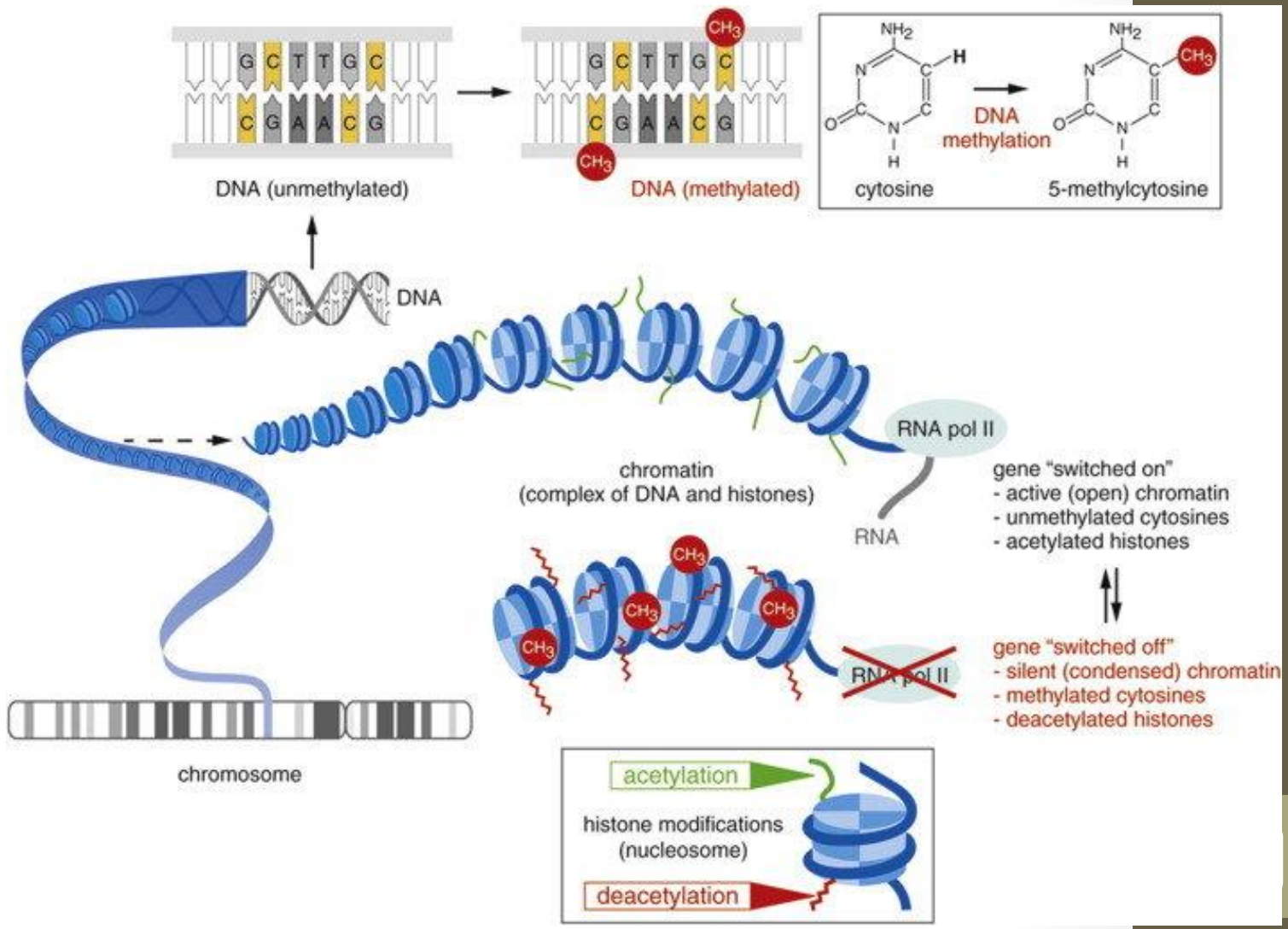
- **Epigenetic mechanisms**
- **Cell cycle checkpoints**
- **Receptor kinase activity**

Modulation of Epigenetic Pathways

Epigenetics refers to heritable factors that influence cellular phenotypes **other than DNA sequence**, including:

- DNA methylation
- Histone modification (methylation and acetylation)
- Gene silencing by non-coding RNAs

Inhibitors of the first two of these mechanisms have been linked to the induction of BRCAness.



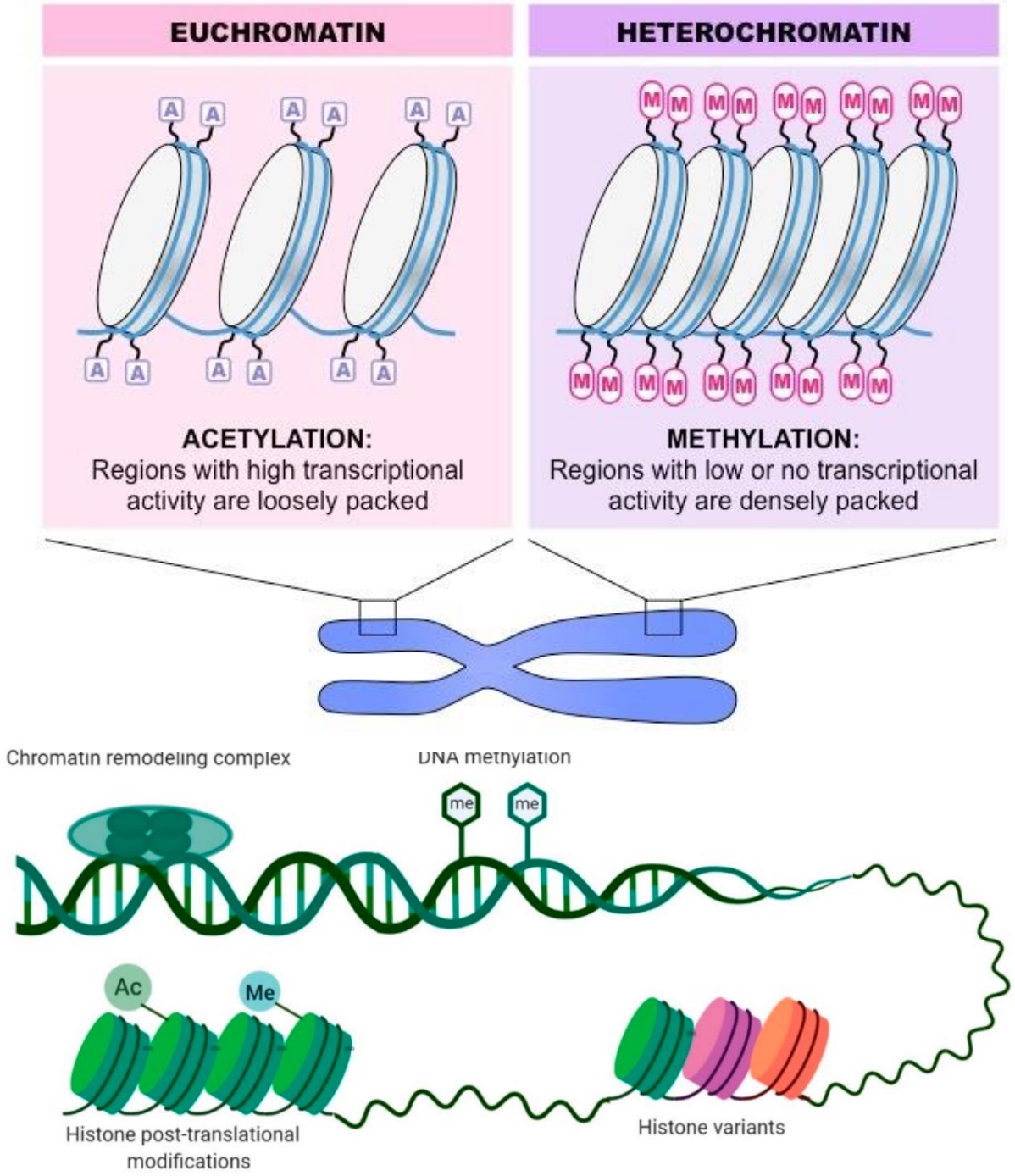
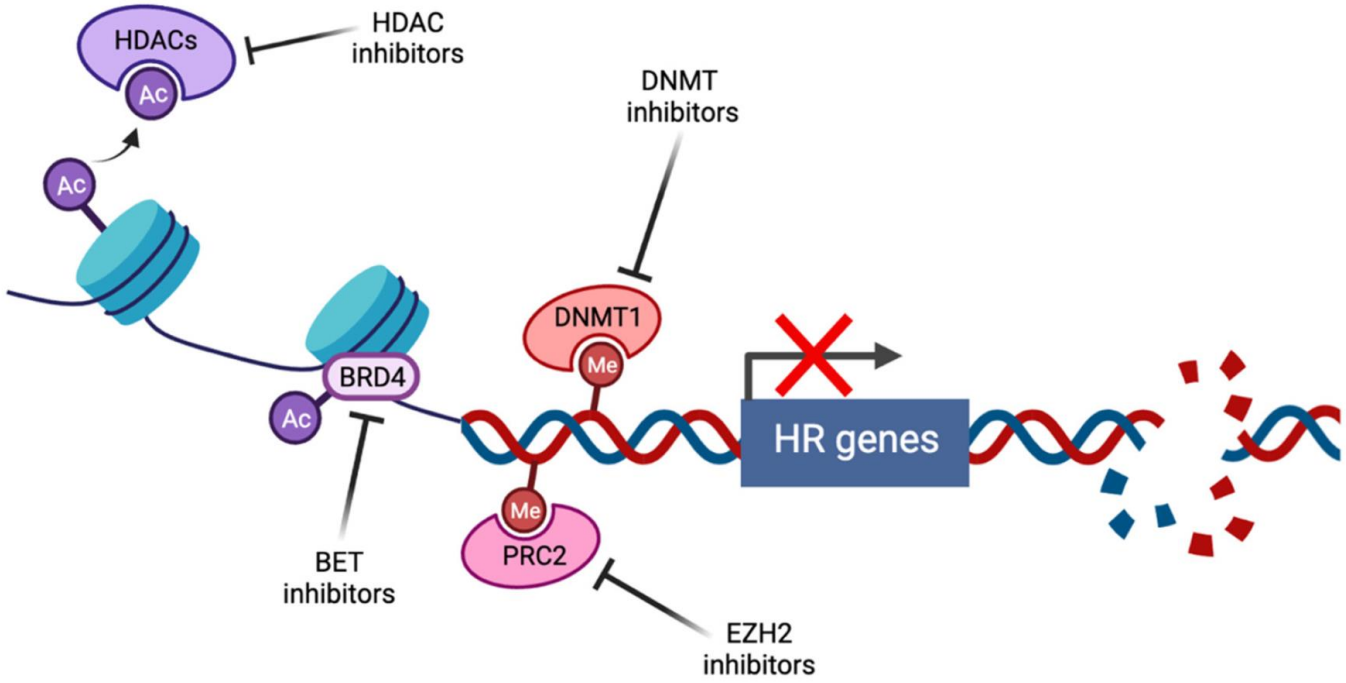

EUCHROMATIN
HETEROCHROMATIN
A
M
ACETYLATION:
Regions with high transcriptional activity are loosely packed
METHYLATION:
Regions with low or no transcriptional activity are densely packed
Chromatin remodeling complex
DNA methylation
me
Ac
Me
Histone post-translational modifications
Histone variants

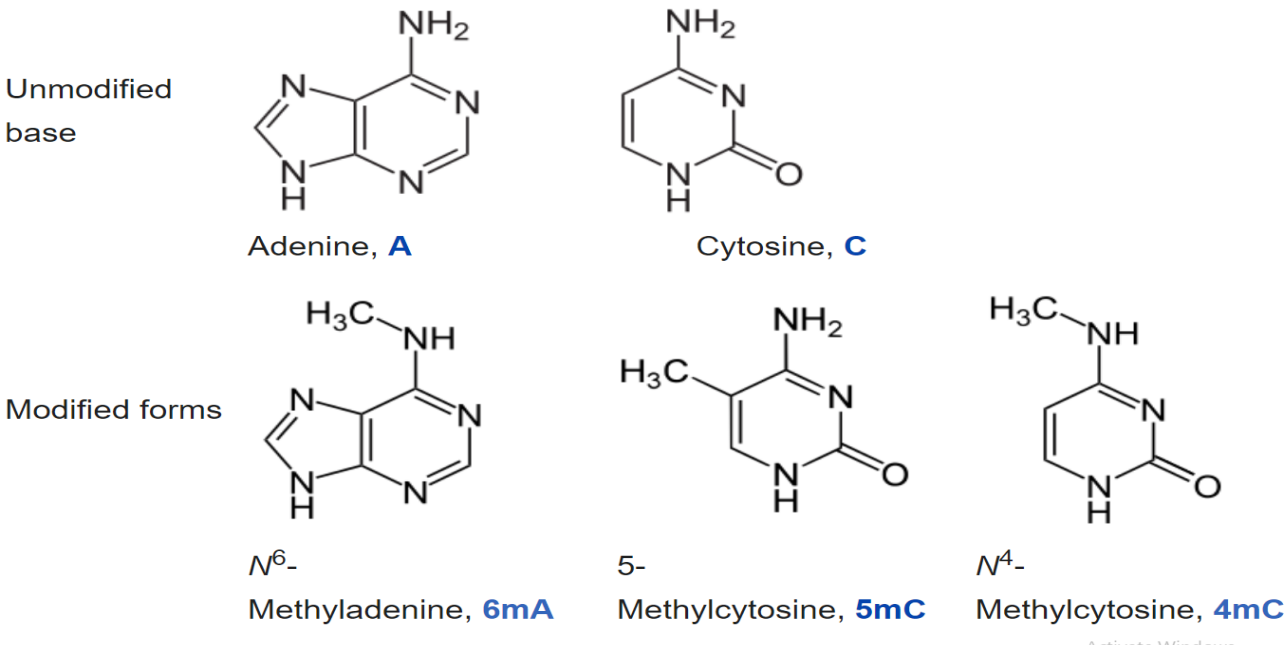
Figure 1. Induction of BRCAness by pharmacological targeting of epigenetic pathways (created with Biorender.com).



Repression of HR gene expression by:

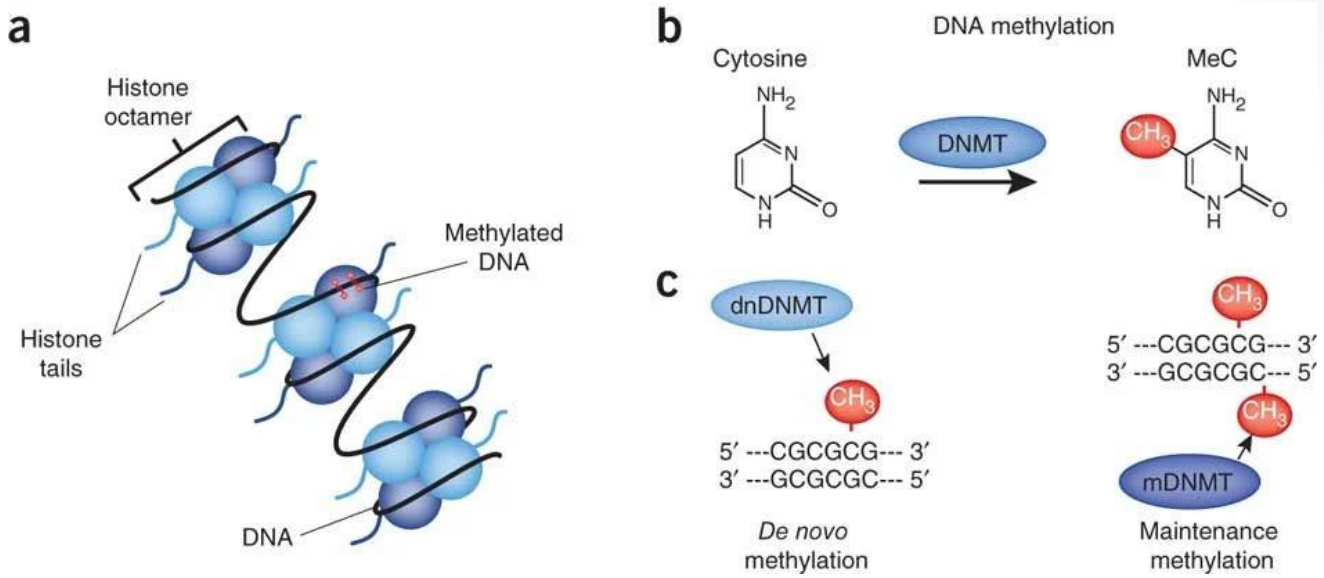
- Histone deacetylase inhibitors (HDACi)
- Inhibitors of DNA methyltransferase (DNMT)
- Inhibitors of zeste homolog 2 (EZH2)
- Inhibitors of Bromodomain and extraterminal (BET) family members such as BRD4

DNA methylation

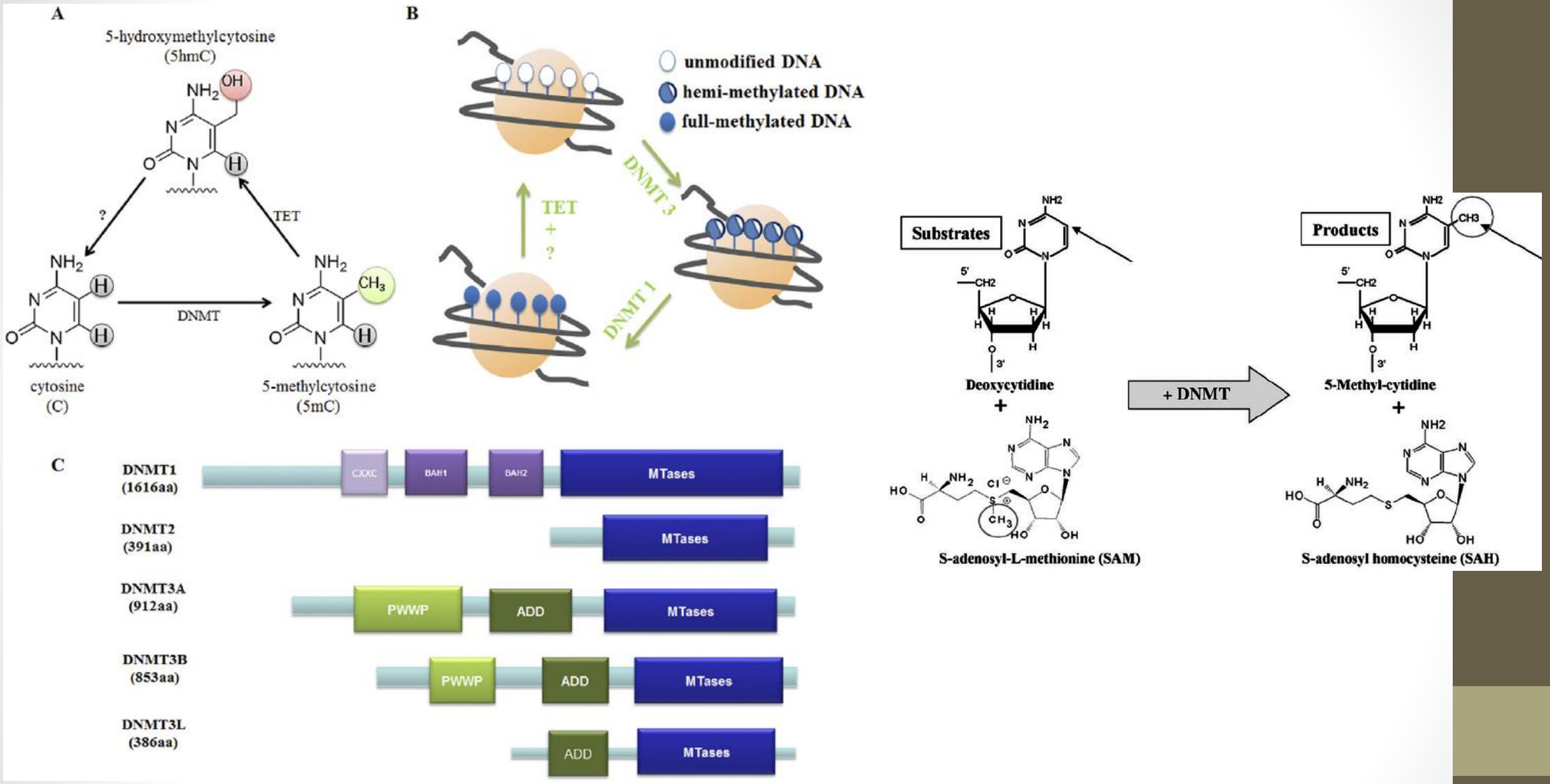


- Methylation of DNA is an **important component of mammalian epigenetic gene regulation**.
- Aberrations in methylation have been widely implicated in **cancer development**, **progression, and response to treatment** [24,25],

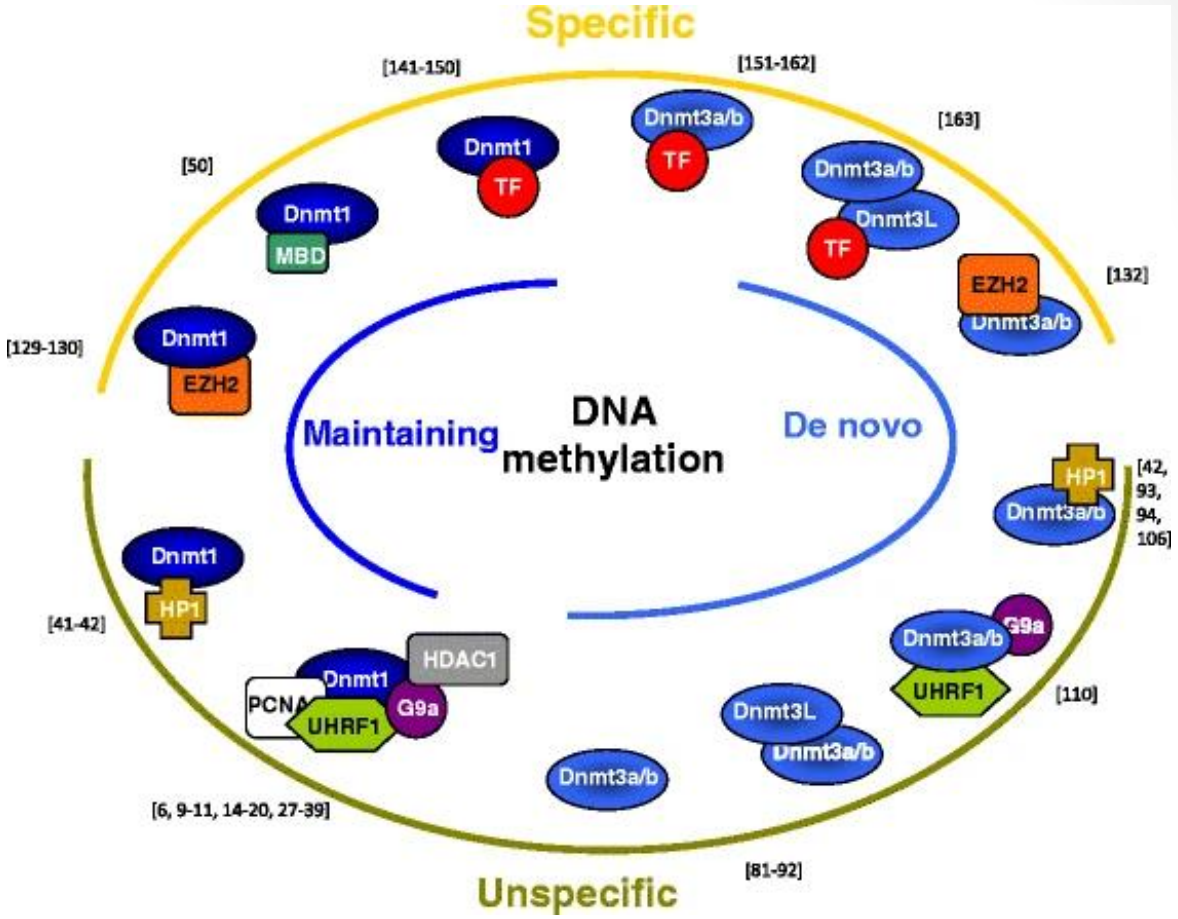
DNMT1



- DNA methyltransferases (DNMTs) are responsible for genome-wide **de novo and maintenance methylation.**
- DNMT1 transfers methyl groups to cytosine nucleotides of genomic DNA.
- It is the major enzyme responsible for maintaining methylation patterns following DNA replication and shows a preference for hemi-methylated DNA.
- Variation in this gene has been associated with cerebellar ataxia, deafness, and narcolepsy, and neuropathy.

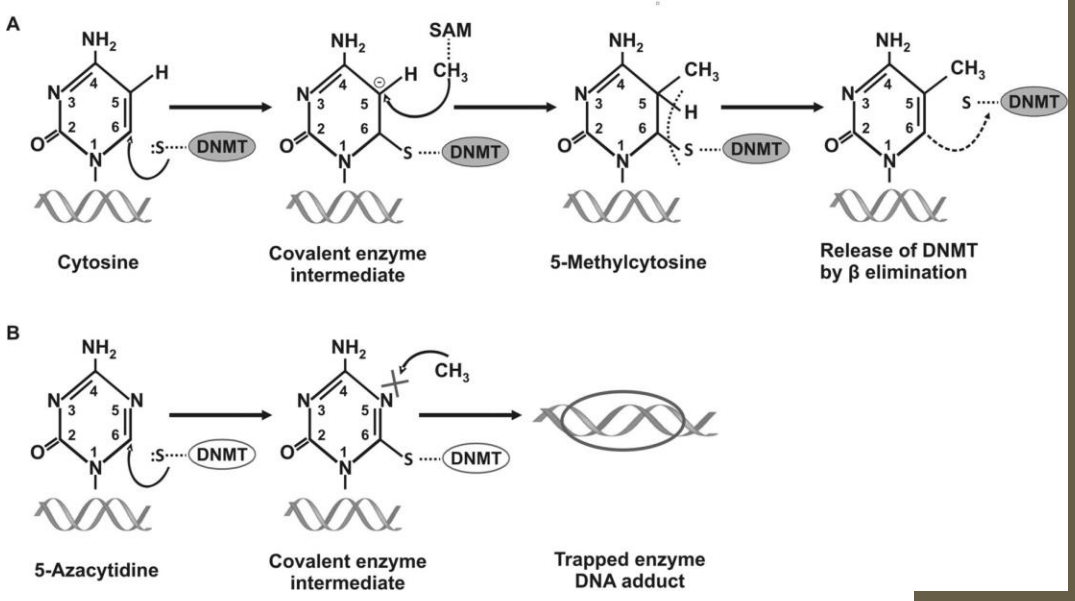

A
5-hydroxymethylcytosine
(5hmC)
?
TET
DNMT
cytosine
(C)
5-methylcytosine
(5mC)
B
unmodified DNA
hemi-methylated DNA
full-methylated DNA
DNMT 3
TET
+
?
DNMT 1
C
DNMT1
(1616aa)
CXXC
BAH1
BAH2
MTases
DNMT2
(391aa)
MTases
DNMT3A
(912aa)
PWWP
ADD
MTases
DNMT3B
(853aa)
PWWP
ADD
MTases
DNMT3L
(386aa)
ADD
MTases
Substrates
Deoxycytidine
+
S-adenosyl-L-methionine (SAM)
+ DNMT
Products
5-Methyl-cytidine
+
S-adenosyl homocysteine (SAH)

Main kinds of DNMT-including complexes.

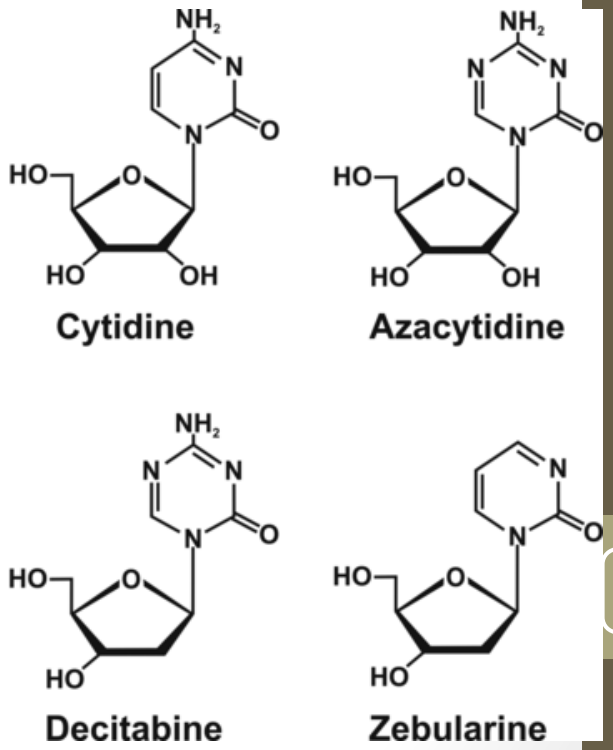


- Maintaining (mainly catalyzed by DNMT1: dark blue curve) or de novo (mainly catalyzed by DNMT3A and DNMT3B: light blue curve) DNA methylation can be processed by numerous DNMT-including complexes which are either not specific (green curve) or specific (yellow curve) of particular DNA sequences. Specific complexes included polycomb proteins or transcriptional factor (TF), whereas unspecific complexes included heterochromatin readers or replication associated proteins.

Inhibition of DNA Methylation



- DNMT inhibitors (DNMTi) including **decitabine** and **5-azacytidine** have been developed and are now FDA-approved for the treatment of cancer (myelodysplastic syndrome, etc)
- These agents are **cytosine analogs** that become incorporated into replicating DNA, where they are targeted for methylation by DNMTs.
- Due to their altered structure, they cannot be released by DNMT by -elimination, leading to the **covalent entrapment of DNMT into the DNA.**



Combination DNMTi-PARPi therapy

- There is evidence of a biological interplay between DNMT1 and PARP1, that provides a rationale for **combination DNMTi-PARPi therapy**.

- DNMT1 and PARP1 are members of a multiprotein **complex that localizes to sites of oxidative DNA damage** [30], where the presence of PARylated PARP1 inhibits methylation activity by DNMT1 [31], possibly to maintain an open chromatin structure to permit repair.

- Our group has demonstrated that combining low-dose **DNMTi treatment with the potent PARP-trapping PARPi talazoparib** enhances PARP1-DNA binding, synergistically enhancing cytotoxicity across a number of BRCA-wild type cancer types with minimal toxicity in in vivo models [32–34] or human subjects [35,36].

- Similar synergism has also been observed when talazoparib is combined with the second-generation DNMTi guadecitabine [37].

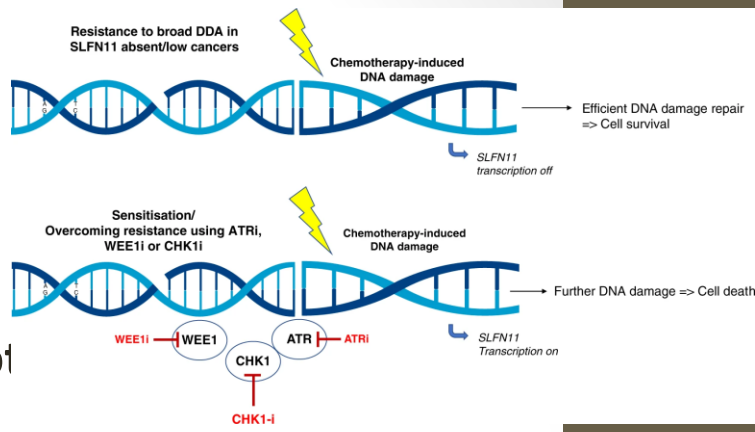
DNMTi and BRCAness phenotype

- Low doses of DNMTi are sufficient to alter methylation patterns across the genome, leading to **widespread alterations in multiple molecular pathways including the DNA damage response (DDR) and apoptosis** [40].

- Among the **myriad of pathways contributing to the Hanahan and Weinberg 'hallmarks of cancer' that are altered by DNMTi**, our recent examination of the DNA repair reactome in non-small cell lung [33], breast, and ovarian cancers [34] demonstrated a **significant reduction in DSB repair, particularly involving the FA pathway**.

- Of note was the downregulation of **FANCD2**, which is mono-ubiquitinated by other FA pathway members in response to DNA damage, leading to colocalization with BRCA1 and BRCA2 during homologous recombination repair of DSBs, and resulting in it being ascribed a role as a **BRCAness gene** [42].

- In keeping with the loss of these repair roles, **DNMTi-induced FANCD2 downregulation** was associated with a **BRCAness phenotype**, including increased replication fork stalling, DSB accumulation as measured by H2AX foci accumulation, and a reduction in RAD51-mediated DSB repair capacity.



- DNMTi can **reactivate abnormally methylated tumor suppressor gene promot**
- One emerging example is **Schlafen 11 (SLFN11),** which irreversibly inhibits replication in cells undergoing replication stress such as DNA-damaging chemotherapy [45].
- Schlafen 11 (SLFN11), a **putative DNA/RNA helicase** that is **recruited to the stressed replication fork and irreversibly triggers replication block and cell death**, has emerged as a promising predictor of sensitivity to cytotoxic chemotherapies, specifically DNA-damaging agents (DDA), such as topoisomerase (TOP) I and TOP II (irinotecan and etoposide, respectively), DNA synthesis inhibitors (e.g. gemcitabine) and DNA cross-linkers and alkylating agents (e.g. cisplatin)
- **The suppression of SLFN11 expression is observed in ~50% of cancer cell lines** and is correlated with a **resistance to DNA-damaging agents** including PARP inhibitors [47].
- SLFN11 suppression appears to be primarily epigenetic in origin, linked to promoter methylation, histone deacetylation, and PRC-mediated histone methylation [45].

These results have led to:

- A dose-finding Phase 1 trial in untreated or relapsed/refractory AML using **DNMTi decitabine and PARPi talazoparib .**

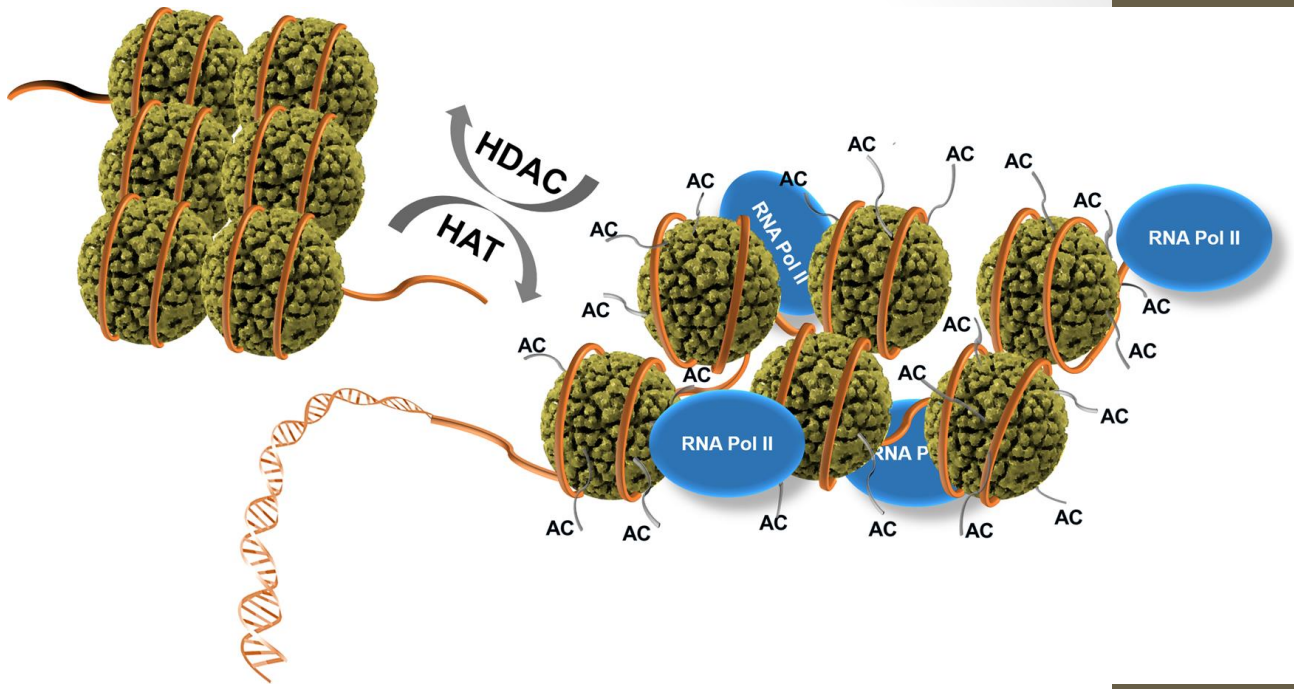
- A Phase 1 trial in BRCA-proficient breast cancer treated using oral decitabine and talazoparib (Table 1).

Table 1. Clinical trials evaluating epigenetic therapy in combination with PARPi.

ClinicalTrials.gov Identifier [86] (accessed 20 May 2022)	Phase	Epigenetic Drug	PARPi	Other Drugs	Cancer	Status
DNMT inhibitor						
NCT02878785	I/II	Decitabine	Talazoparib		Untreated or R/R [1] acute myeloid leukemia (AML)	Active, not recruiting
HDAC inhibitor						
NCT03259503	I	Vorinostat	Olaparib	Gemcitabine, busulphan, melphalan	R/R lymphoma undergoing stem cell transplant	Recruiting
NCT03742245	I	Vorinostat	Olaparib		R/R or metastatic breast	Recruiting
EZH2 inhibitor						
NCT04355858	II	SHR2554	SHR3162		Luminal advanced breast	Recruiting

[1] R/R = relapsed/refractory.

2.2. Maintenance of Chromatin Repressive States



- Histone deacetylases (HDACs) remove acetyl groups from -N-acetyl lysine residues on histones, leading to chromatin condensation and transcriptional repression.

- Abnormal acetylation resulting from **HDAC overexpression** can **downregulate the expression of various tumor suppressive mechanisms**, including:
  - Certain cyclin-dependent kinases
  - Differentiation factors
  - Proapoptotic signals

These lead to the **uncontrolled proliferation, differentiation, and survival** that is characteristic of oncogenesis and metastasis [50].

The **18 members of the HDAC family** have been classified into four groups:

- Class I
- Class IIa/b
- Class V
- Class III/sirtuins

Compounds with anti-HDAC activity are numerous, and can be divided into:

- Pan-HDAC inhibitors (HDACi), which exhibit activity against all non-sirtuin HDACs
- Selective HDACi, which target specific HDACs.

- FDA approval has been granted for the treatment of various hematological malignancies for three pan-HDACi (**vorinostat, belinostat, and panobinostat**) and one HDAC1/2-selective HDACi (**romidepsin**).

HDAC and DNA repair

- HDACi have been reported to alter DSB repair capacity [54].
- **HR proteins including BRCA1, BRCA2, and RAD51** are **suppressed by HDACi** in a variety of cancers [57,58], sensitizing to PARPi [59–65].
- Based on these results, phase I trials combining olaparib with vorinostat are underway in advanced lymphoma and breast cancer (Table 1).

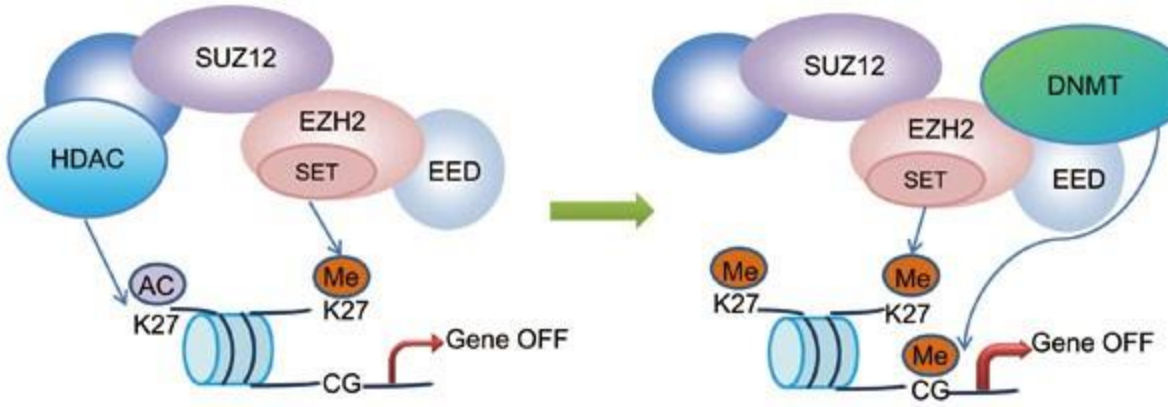
- HDACi exposure leads to **PARP1 hyperacetylation** and enrichment in chromatin that resembles **PARPi-induced PARP trapping**.

- When combined with PARPi, HDACi treatment further increases PARP trapping, synergistically sensitizing to the PARP-trapping PARPi talazoparib [66].

- Synergism has also been observed when HDACi are combined with DNMTi, specifically by enhancing the re-expression of genes silenced by abnormal promoter methylation [35,67].

- Valdez et al. have reported synergistic inhibition of AML and lymphoma cell proliferation by the triple **combination of PARPi niraparib, DNMTi decitabine, and HDACi romidepsin** or pabinostat, associated with the activation of ATM-mediated DDR, increased ROS production, and the induction of apoptosis [68].

Enhancer of zeste homolog 2 (EZH2)



- Enhancer of zeste homolog 2 (EZH2) is a **histone-lysine N-methyltransferase** enzyme (EC 2.1.1.43) that participates in **histone methylation at lysine 27** and **transcriptional repression**.
- Methylation activity of EZH2 **facilitates heterochromatin formation** thereby **silences gene function.**
- EZH2 is **the functional enzymatic component** of the **Polycomb Repressive Complex 2 (PRC2),** which is responsible for healthy embryonic development through the epigenetic maintenance of **genes responsible for regulating development and differentiation**.
- **Mutation or over-expression of EZH2** has been linked to many forms of **cancer**.
- EZH2 inhibits genes responsible for **suppressing tumor development**, and **blocking EZH2 activity may slow tumor growth.**

Polycomb Repressive Complex 2

- EZH2 is the histone methyltransferase subunit of polycomb repressive complex 2 (PRC2).

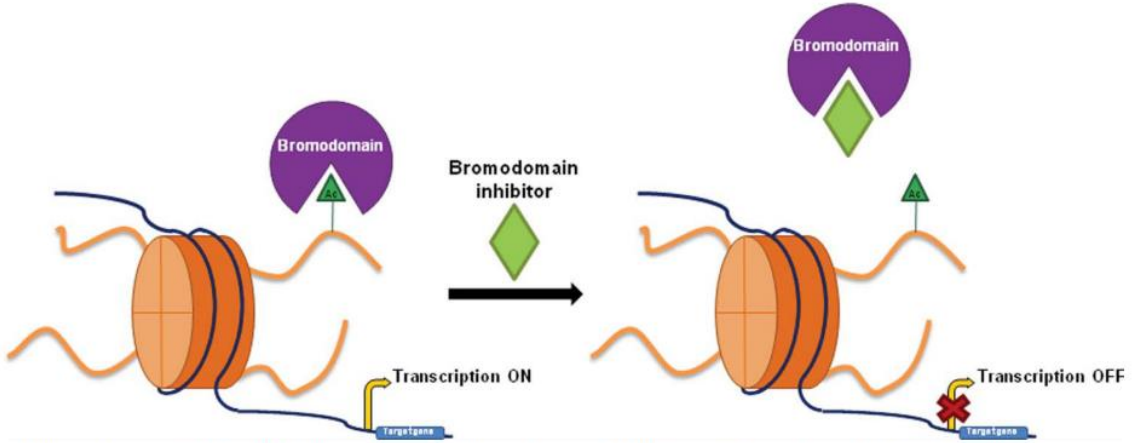
- PRC2 plays an important oncogenic role through the **modulation of the DDR** [70].

EZH2 overexpression, which is common in many cancers [71]:

- Induces the **downregulation of RAD51 homolog expression** [72]
- **Cytoplasmic BRCA1 retention** [73]
- **Impaired HR**

- PRC2 appears to play a role in the DSB repair pathway choice, being recruited to DSBs in a Ku-dependent mechanism to promote efficient NHEJ [74], and accordingly, EZH2 depletion favors HR, impairs NHEJ, and sensitizes to irradiation damage [75].

BET Proteins



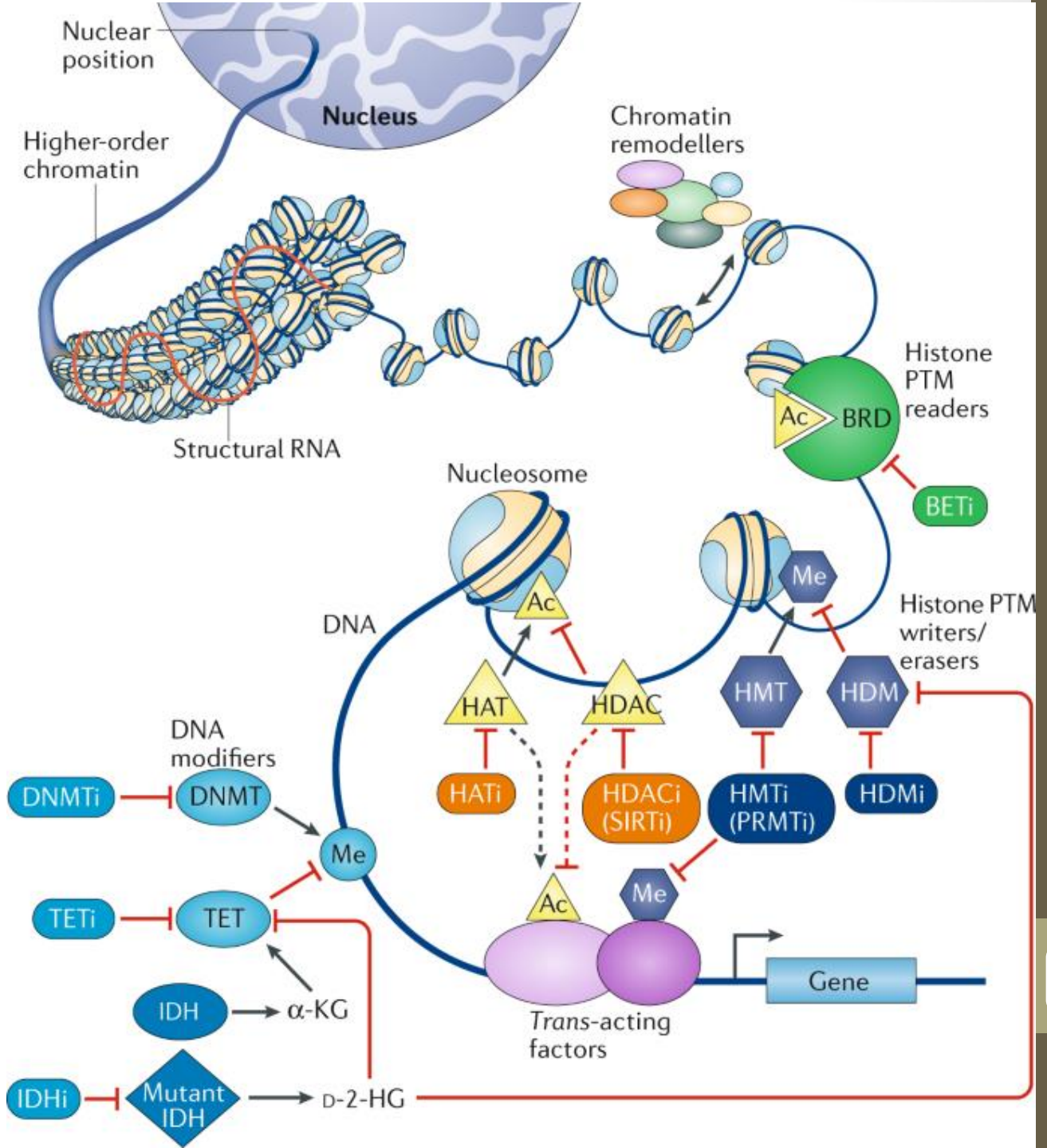
M. Pérez-Salvia and M. Esteller, "Bromodomain inhibitors and cancer therapy: From structures to applications," Epigenetics, vol. 12, no. 5, pp. 323–339, 2017, doi: 10.1080/15592294.2016.1265710.

- A **bromodomain** is an approximately 110 amino acid protein domain that **recognizes acetylated lysine residues (mainly H3 and H4)**.
- Bromodomains, as the **"readers"** of lysine acetylation. Their affinity is higher for regions where multiple acetylation sites exist in proximity.
- They recognize acetylated histones and **recruit transcription factors** (e.g., RELA) and transcription elongation complex (e.g., P-TEFb) to the chromatin, thereby promoting the phosphorylation of RNA polymerase II and subsequent transcription initiation and elongation.

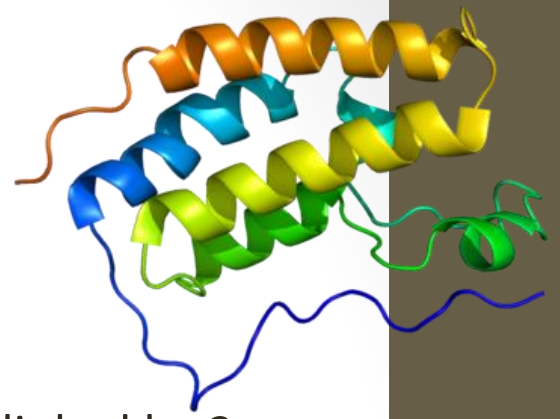
- The conserved bromodomain and extraterminal (BET) family of proteins are characterized by two tandem bromodomains that bind to activated lysine residues on target proteins. Members of this family include **BRD2, BRD3, BRD4 and BRDT**.

- BET family member BRD4 acts as a **transcriptional cofactor**, influencing the expression of a wide range of genes involved in cell fate determination.

Epigenetic modulators



BRD4 activates oncogenes



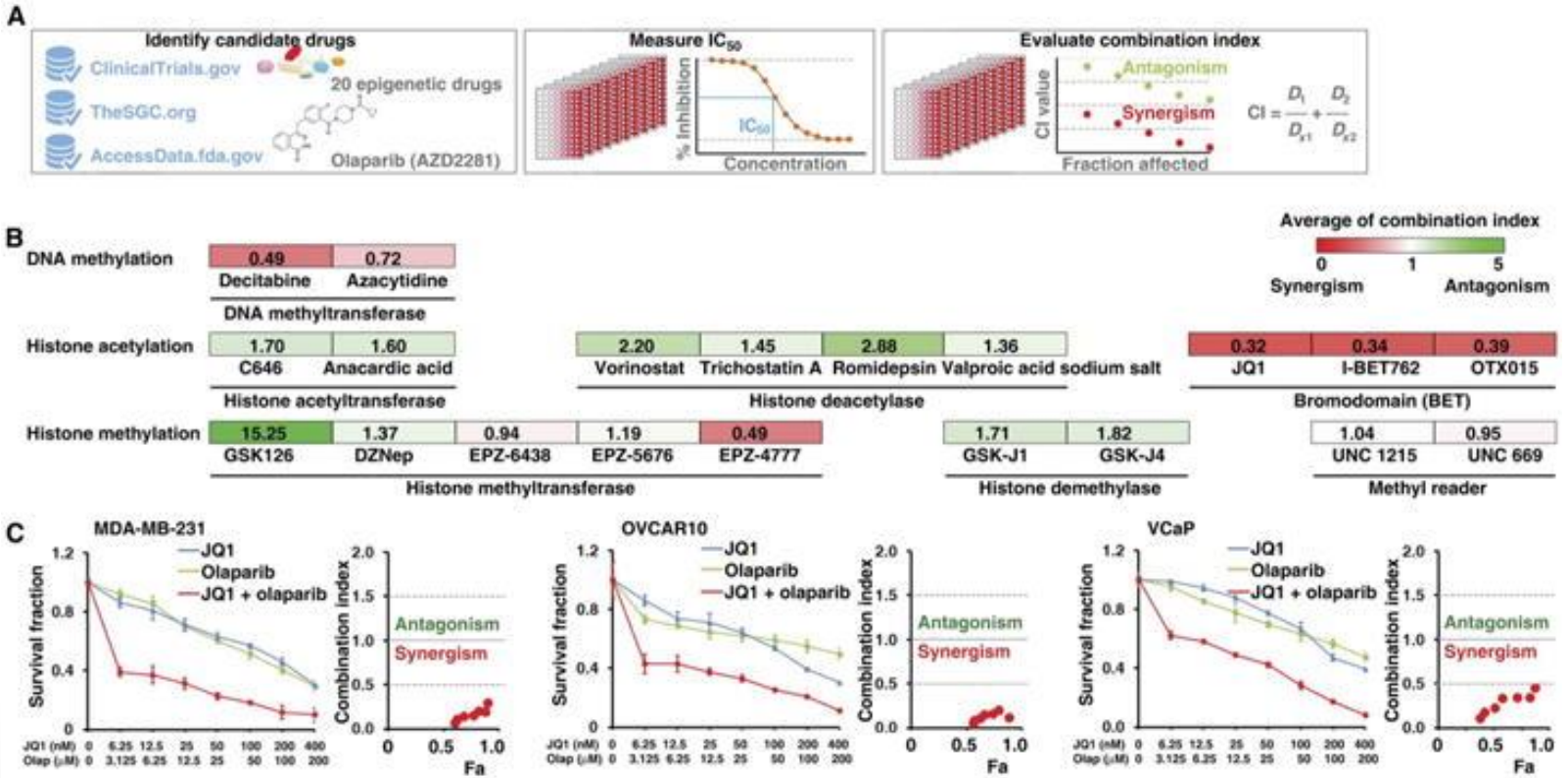
- The two bromodomains in BRD4, termed BD1 and BD2, consist of 4 alpha-helices linked by 2 loops.
- The ET domain structure is made up of 3 alpha-helices and a loop.
- The C-terminal domain of BRD4 has been implicated in promoting gene transcription through interaction with the transcription elongation factor P-TEFb and RNA polymerase II.

- In cancer, BRD4 has been implicated in the **activation of a multitude of oncogenes**, co-occupying a set of **promoter super-enhancers** associated with prominent **oncogenic drivers such as c-MYC.**

- High affinity small molecules targeting the BET bromodomains demonstrate preclinical efficacy in a wide range of cancers associated with transcriptional **suppression of key proto-oncogenes** including **c-MYC, N-MYC, FOSL1, and BLC2** (reviewed in [79].

Synergism between BET inhibitors and PARPi

- Drug combination screen testing PARPi olaparib in BRCA-wildtype triple negative breast (TNBC), ovarian, and prostate cancer in combination with 20 well-characterized epigenetic modulators across seven classes, demonstrating synergism for all tested BET inhibitors (BETi) [82].



- BETi treatment significantly enhanced PARPi-induced **DSB accumulation** independent of PARP-trapping

- Repression of BRCA1 and RAD51 transcription (suggestive of induced BRCAness)

Validation of these results in TNBC

- BETi-induced repression of BRCA1 and RAD51
- Induction of an HR defect
- Sensitivity to PARPi [83].