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The role of chromatin dynamics in the DNA damage response

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München, den 29.04.2022	*Charlotte Blessing*
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List of Abbreviations

γH2AX	Phosphorylated form of **H2A** histone family member **X**
µL	**Microl**iter
µM	**Microm**olar
3D	Three-dimensional space
53BP1	p**53**-**B**inding **P**rotein **1**
Å	**A**ngstrom
ADP	**A**denosine **di**phosphate
ALC1	**A**mplified in **L**iver **C**ancer **1**
ANOVA	**An**alysis **o**f **va**riance
AP site	**Ap**urinic/apyrimidinic site
ARH3	**A**DP-**R**ibosyl**h**ydrolase **3**
ART	**A**DP-**R**ibosyl**t**ransferase
ARTD	**A**DP-**R**ibosyl**t**ransferase **D**iphtheria Toxin-like
ATM	**A**taxia **T**elangiectasia **M**utated
ATP	**A**denosine **t**ri**p**hosphate
ATR	**A**taxia **T**elangiectasia and **R**ad3 related
BAF	**B**RG1/BRM-**A**ssociated **F**actor
BRCA	**Br**east **Ca**ncer Susceptibility Protein
BRCA1	**Br**east **Ca**ncer type **1** Susceptibility Protein
BRCA2	**Br**east **Ca**ncer type **2** Susceptibility Protein
BRCT	**BR**CA1 **C** **T**erminus
BRG1	**B**rahma-**R**elated **G**ene **1**
BRM	**BR**AH**M**A Homolog
BSA	**B**ovine **S**erum **A**lbumin
cDNA	**C**omplementary **DNA**
CENP-A	**Cen**tromere **P**rotein **A**
CHD	**C**hromodomain **H**elicase **D**NA Binding
CHD1	**C**hromodomain **H**elicase **D**NA Binding Protein **1**
CHD1L	**C**hromodomain **H**elicase **D**NA Binding Protein **1** **l**ike
CHD2	**C**hromodomain **H**elicase **D**NA Binding Protein **2**
CHD3	**C**hromodomain **H**elicase **D**NA Binding Protein **3**
CHD4	**C**hromodomain **H**elicase **D**NA Binding Protein **4**
CHD7	**C**hromodomain **H**elicase **D**NA Binding Protein **7**
ChIP-seq	**Ch**romatin **i**mmuno**p**recipitation-**seq**uencing
CHK1	**Ch**eckpoint **K**inase **1**
CHK2	**Ch**eckpoint **K**inase **2**
CRISPR	**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats
cryo-EM	**Cryo**genic **e**lectron **m**icroscopy
CSA	**C**ockayne **S**yndrome Group **A**

CSB	**C**ockayne **S**yndrome Group **B**
CtIP	**Ct**BP-**I**nteracting **P**rotein
DDR	**D**NA **d**amage **r**esponse
D-loop	**D**isplacement **loop**
DMEM	**D**ulbecco's **M**odified **E**agle **M**edium
DMSO	**Dim**ethyl **s**ulf**o**xide
DNA	**D**eoxyribo**n**ucleic **a**cid
DNA2	**DNA R**eplication Helicase/Nuclease **2**
DNA-PK	**DNA**-Dependent **P**rotein **K**inase
DNA-PKcs	**DNA**-Dependent **P**rotein **K**inase, **c**atalytic **s**ubunit
DSB	**D**ouble-**s**trand **b**reak
DTT	**Di**thio**t**hreitol
E. coli	**E**scherichia **coli**
EDTA	**E**thylene**d**iamine**t**etraacetic **a**cid
EGFP	**E**nhanced **G**reen **F**luorescent **P**rotein
ERCC1	**E**xcision **R**epair **C**ross **C**omplementing **1**
EYFP	**E**nhanced **Y**ellow **F**luorescent **P**rotein
FACS	**F**luorescent-**a**ctivated **c**ell **s**orting
FCS	**F**etal **c**alf **s**erum
FEN1	**F**lap **E**ndo**n**uclease **1**
FRAP	**F**luorescence **r**ecovery **a**fter **p**hotobleaching
FRT	**F**lippase **R**ecognition **T**arget
G1 phase	**G**rowth **1 phase**
G2 phase	**G**rowth **2 phase**
GFP	**G**reen **F**luorescent **P**rotein
h	**h**ours
H1	**H**istone **1**
H2A	**H**istone **2A**
H2AFY	macroH2A1 gene
H2AFY2	macroH2A2 gene
H2AW	**H**istone **2A** type 3
H2AX	**H2A** histone family member **X**
H2B	**H**istone **2B**
H3	**H**istone **3**
H3K9me3	Histone H3 lysine 9 trimethylation
H4	**H**istone **4**
HD domain	**H**istidine-aspartate **domain**
HDAC1	**H**istone **Deac**etylase **1**
HDAC2	**H**istone **Deac**etylase **2**
HP1	**H**eterochromatin **P**rotein **1**
HPF1	**H**istone **P**ARylation **F**actor **1**

HR	**H**omologous **r**ecombination
HRP	**H**orse**r**adish **P**eroxidase
HSS	**H**AND-**S**ANT-**S**LIDE
IgG	**I**mmuno**g**lobulin **G**
INO80	**Ino**sitol-Requiring Protein **80**
Isw1	**I**mitation **SW**itch subfamily 1
ISWI	**I**mitation **SWi**tch
ITC	**I**sothermal **t**itration **c**alorimetry
K_D	Dissociation constant
KO	**K**nock**o**ut
Ku70	Lupus **Ku** Autoantigen Protein p**70**
Ku80	Lupus **Ku** Autoantigen Protein p**80**
LIG3	DNA **Lig**ase **3**
LIG4	DNA **Lig**ase **4**
M	**M**olar
M phase	**M**itotic **phase**
m5C	**5**-**m**ethyl**c**ytosine
m6A	N^6-**m**ethyl**a**denosine
MacroD1	**Macrod**omain-Containing Protein **1**
MacroD2	**Macrod**omain-Containing Protein **2**
MDC1	**M**ediator of **D**NA Damage **C**heckpoint Protein **1**
mg	**M**illi**g**ram
mH2A	**M**acro**H2A**
mH2A1.1	**M**acro**H2A1**, isoform **1**
mH2A1.2	**M**acro**H2A1**, isoform **2**
mH2A2	**M**acro**H2A2**
min	**Min**utes
mL	**M**illi**l**iter
mM	**M**illi**m**olar
MMS	**M**ethyl**m**ethane **s**ulphonate
MRE11	**M**eiotic **Re**combination **11** Homolog A
MRN complex	**M**RE11-**R**AD50-**N**BS1 **Complex**
mRNA	**M**essenger **RNA**
NAD+	**N**icotinamide **a**denine **d**inucleotide
NBS1	**N**ijmegen **B**reakage **S**yndrome Protein **1**
NF-κB	**N**uclear **F**actor **K**appa-Light-Chain-Enhancer of Activated **B** Cells
NHEJ	**N**on-**h**omologous **e**nd **j**oining
nm	**N**ano**m**eter
nM	**N**ano**m**olar
ns	**N**on-**s**ignificant
NS3	**N**on**s**tructural **P**rotein **3**

OR	**O**dd's **r**atio
p53	Tumor Protein **p53**
PAGFP	**P**hotoactivatable **G**reen **F**luorescent **P**rotein
PAR	**P**oly-(**A**DP-**r**ibose)
PARG	**P**oly-(**A**DP-**R**ibose) **G**lycohydrolase
PARP	**P**oly-(**A**DP-**R**ibose) **P**olymerase
PARP1	**P**oly-(**A**DP-**R**ibose) **P**olymerase **1**
PARP2	**P**oly-(**A**DP-**R**ibose) **P**olymerase **2**
PARP3	**P**oly-(**A**DP-**R**ibose) **P**olymerase **3**
PARPi	**PARP i**nhibitor
PBS	**P**hosphate-**B**uffered **S**aline
PCNA	**P**roliferating **C**ell **N**uclear **A**ntigen
PCR	**P**olymerase **c**hain **r**eaction
PDB	**P**rotein **D**ata **B**ank
POL ß	DNA **Pol**ymerase **B**eta
PTEN	**P**hosphatase and **Ten**sin Homolog
PTM	**P**ost-**t**ranslational **m**odification
RAD50	**Rad**iation Sensitive Protein **50**
RAD51	**Rad**iation Sensitive Protein **51**
RecA	**Rec**ombinase **A**
REV7	**REV7** homolog
RNA	**R**ibo**n**ucleic **a**cid
RNAi	**RNA i**nterference
RNF168	**Rin**g **F**inger Protein **168**
RNF8	**Rin**g **F**inger Protein **8**
ROI	**R**egion **o**f **i**nterest
RPA	**R**eplication **P**rotein **A**
RSC	**R**emodel the **S**tructure of **C**hromatin
Rsc3	**R**emodel the **S**tructure of **C**hromatin **3**
Rsc30	**R**emodel the **S**tructure of **C**hromatin **30**
S phase	**S**ynthesis **phase**
SD	**S**tandard **d**eviation
SDS-PAGE	**S**odium **d**odecyl **s**ulphate–**p**oly**a**crylamide **g**el **e**lectrophoresis
SEM	**S**tandard **e**rror of the **m**ean
Sfh1	**S**nf **F**ive **H**omolog **1**
SHL	**S**uper**h**elical **l**ocation
siRNA	**S**mall **i**nterfering **RNA**
SLIC	**S**equence- and **L**igation-**I**ndependent **C**loning
SMARCB1	**S**WI/SNF-Related **M**atrix-Associated **A**ctin-Dependent **R**egulator Of **C**hromatin Subfamily **B** Member **1**
SnAc	**Sn**f2 **A**TP **C**oupling

Snf2	**S**ucrose **N**on**f**ermenting Protein **2**
SNF2H	**S**ucrose **N**on**f**ermenting Protein **2** **H**omolog
Snf5	**S**ucrose **N**on**f**ermenting Protein **5**
SNP	**S**ingle-**n**ucleotide **p**olymorphism
SSB	**S**ingle-**s**trand **b**reak
Sth1	**S**NF **T**wo **H**omolog **1**
SWI/SNF	**Swi**tch/**S**ucrose **N**on-**F**ermentable
SWR1	**SW**I2/SNF2-**R**elated **1**
TAD	**T**opologically **a**ssociating **d**omain
TARG1	**T**erminal **A**DP-**R**ibose Protein **G**lycohydrolase **1**
TEV	**T**obacco **E**tch **V**irus
TFIIH	**T**ranscription **F**actor **II** **H**uman
UBC13	**Ub**iquitin-**C**onjugating Enzyme E2 **13**
UV	**U**ltra**v**iolet
UV-DDB	**UV**-**D**amaged **D**NA-**B**inding Protein
WEE1	**Wee1**-Like Protein Kinase
WT	**W**ild-**t**ype
WWE	Tryptophan-tryptophan-glutamate domain
XLF	**X**RCC4-**L**ike **F**actor
XP	**X**eroderma **P**igmentosum
XPA	**X**eroderma **P**igmentosum Group **A** Protein
XPC	**X**eroderma **P**igmentosum Group **C** Protein
XPF	**X**eroderma **P**igmentosum Group **F** Protein
XPG	**X**eroderma **P**igmentosum Group **G** Protein
XRCC1	**X**-ray **R**epair **C**ross-**C**omplementing Protein **1**
XRCC4	**X**-ray **R**epair **C**ross-**C**omplementing Protein **4**

List of Publications

Blessing, C., Mandemaker, I.K., Gonzalez-Leal, C., Preisser, J., Schomburg, A., and Ladurner, A.G., 2020. The oncogenic helicase ALC1 regulates PARP inhibitor potency by trapping PARP2 at DNA breaks. Molecular Cell, 80 (5), 862–875.

Blessing, C., Knobloch, G., and Ladurner, A.G., 2020. Restraining and unleashing chromatin remodelers – structural information guides chromatin plasticity. *Current Opinion in Structural Biology*, 65, 130–138.

Blessing, C. and Ladurner, A.G., 2020. Tickling PARPs into serine action. *Nature Structural and Molecular Biology*, 27 (4), 310–312.

Kozlowski, M., Corujo, D., Hothorn, M., Guberovic, I., Mandemaker, I.K., **Blessing, C**., Sporn, J., Gutierrez-Triana, A., Smith, R., Portmann, T., Treier, M., Scheffzek, K., Huet, S., Timinszky, G., Buschbeck, M., and Ladurner, A.G., 2018. MacroH2A histone variants limit chromatin plasticity through two distinct mechanisms. *EMBO reports*, 19, e44445.

Singh, H.R., Nardozza, A.P., Möller, I.R., Knobloch, G., Kistemaker, H.A.V., Hassler, M., Harrer, N., **Blessing, C.**, Eustermann, S., Kotthoff, C., Huet, S., Mueller-Planitz, F., Filippov, D. V., Timinszky, G., Rand, K.D., and Ladurner, A.G., 2017. A poly-ADP-ribose trigger releases the auto-inhibition of a chromatin remodeling oncogene. Molecular Cell, 68 (5), 860–871.

Haag, S., Sloan, K.E., Ranjan, N., Warda, A.S., Kretschmer, J., **Blessing, C**., Hübner, B., Seikowski, J., Dennerlein, S., Rehling, P., Rodnina, M. V, Höbartner, C., and Bohnsack, M.T., 2016. NSUN3 and ABH1 modify the wobble position of mt-t RNA Met to expand codon recognition in mitochondrial translation. *The EMBO Journal*, 35 (19), 2104–2119.

1. Contributions to Publications

1.1 Publication I

Blessing, C., Mandemaker, I.K., Gonzalez-Leal, C., Preisser, J., Schomburg, A., and Ladurner, A.G., 2020. The oncogenic helicase ALC1 regulates PARP inhibitor potency by trapping PARP2 at DNA breaks. Molecular Cell, 80 (5), 862–875.

This study in *Molecular Cell* comprises my main Ph.D. project. I conceptualized the study together with Andreas Ladurner and with input from Imke Mandemaker and Adrian Schomburg. I designed, performed and analyzed all laser microirradiation experiments (Fig. 1, 3A-C, 4, 5C, 5D, 6C, 6D, 6E, S1, S2, S4, S5). For Fig. 5C and 6C, I received experimental help from Imke Mandemaker and Claudia Gonzalez-Leal.

I further designed, performed and analyzed all cell survival assays (Fig. 2A, 5A, 5B, 6A, 6F, 6G) as well as the immunofluorescence experiments in Fig. 5G, 5H, 6B, S1A, S5A. The immunofluorescence experiments in Fig. 2C, 5E and S5D-G were designed and analyzed by myself, but performed by Imke Mandemaker, Claudia Gonzalez-Leal and Julia Preißer, respectively. The cell cycle analyses in Fig. 2B were designed and performed by myself and analyzed by Imke Mandemaker.

To prepare the publication of the study, I prepared all figures and wrote the manuscript text together with Andreas Ladurner. I further handled all revision experiments and reviewers' requests to finalize the publication.

1.2 Publication II

Kozlowski, M., Corujo, D., Hothorn, M., Guberovic, I., Mandemaker, I.K., **Blessing, C.**, Sporn, J., Gutierrez-Triana, A., Smith, R., Portmann, T., Treier, M., Scheffzek, K., Huet, S., Timinszky, G., Buschbeck, M., and Ladurner, A.G., 2018. MacroH2A histone variants limit chromatin plasticity through two distinct mechanisms. *EMBO reports*, 19, e44445.

I contributed to the revision process of this manuscript, where I conducted microlaser irradiation experiments in Fig. 4C, western blot analysis and FRAP imaging in Figures EV3B and EV3C in collaboration with my colleague Imke Mandemaker. I further performed the data analysis of the laser microirradation experiments shown in Fig. 4C.

1.3 Publication III (Appendix)

Blessing, C. and Ladurner, A.G., 2020. Tickling PARPs into serine action. *Nature Structural and Molecular Biology*, 27 (4), 310–312.

For this *News & Views* article, I researched and discussed the reaction mechanism of PARP enzymes based on recent structural insight of a PARP2-HPF1 complex published in *Nature*. I wrote the manuscript text and prepared the figure with input from Andreas Ladurner.

1.4 Publication IV (Appendix)

Blessing, C., Knobloch, G., and Ladurner, A.G., 2020. Restraining and unleashing chromatin remodelers – structural information guides chromatin plasticity. *Current Opinion in Structural Biology*, 65, 130–138.

This review article summarizes and discusses the advancements made on understanding regulatory and disease mechanisms of chromatin remodeling complexes based on recently acquired structural information. I researched and discussed the topic, selected relevant "featured" publications and wrote the manuscript with input from Andreas Ladurner. I further prepared the figures with the help from Gunnar Knobloch.

2. Summary

Maintaining the integrity of genetic information is critical for cell survival. Dedicated DNA repair pathways thereby ensure the efficient removal of DNA lesions from the genome. The precise execution of these processes is highly relevant, as their dysregulation leads to a pre-deposition to genetic diseases, such as cancer. Upon DNA damage, DNA repair is accompanied by extensive re-arrangements of the chromatin environment around the lesion. However, the precise chromatin nature and the impact of different chromatin-modifying components on the DNA damage response is still poorly understood. In this thesis, I investigate the role and relevance of two chromatin modifiers at DNA lesions to drive this understanding further.

The **chromatin remodeler ALC1**, an oncogene frequently amplified in cancer, is rapidly recruited to DNA damage sites in a poly-(ADP-ribose)-dependent manner, where it relaxes local chromatin structure. However, the functional consequences of this recruitment and chromatin decompaction have remained unknown. Here, I determine the implications of ALC1-mediated chromatin remodeling in the cellular response to PARP inhibitors (PARPi), which are exploited to treat BRCA1/2-mutated cancers in the clinic. The targets of PARPi, PARP1 and PARP2, act as a first responders at DNA damage sites. Using live-cell imaging, I show that ALC1 is strictly required for the release of PARP2 from DNA breaks. This impacts the response to single-strand DNA breaks, as PARP2 trapping aberrantly retains up-stream DNA repair factors and impairs the recruitment of down-stream proteins. ALC1 deletion further potentiates PARPi-induced cancer killing through PARP2 trapping and mediates synthetic lethality upon BRCA deficiency, suggesting that the integral role of chromatin remodeling by ALC1 might enable novel cancer therapies.

The **histone variant macroH2A** is expressed in three isoforms, all of which compact chromatin. While macroH2A1.1 is closely linked to poly-(ADP-ribose) signalling, the mechanism of chromatin compaction has remained enigmatic for the other two isoforms. By measuring chromatin changes at DNA lesions, I show that the flexible linker of macroH2A compacts chromatin even when transferred to canonical H2A. This suggests a universal role of the macroH2A linker in chromatin compaction, independent of the poly-(ADP-ribose) binding status of the isoforms, and thus provides novel insights in the mechanism of macroH2A function in chromatin maintenance.

3. Zusammenfassung

Die Aufrechterhaltung der genetischen Information ist essentiell für das Überleben von Zellen. Spezielle DNA-Reparaturmechanismen sorgen daher für die effiziente Entfernung von DNA-Läsionen aus dem Genom. Die präzise Ausführung dieser Prozesse ist hochrelevant, denn eine Dysregulierung erhöht das Risiko für genetische Erkrankungen wie beispielsweise Krebs.
Bei dem Auftreten von DNA-Schäden findet zusätzlich zu dem eigentlichen Reparaturprozess eine umfangreiche Restrukturierung des Chromatins rundum die DNA-Läsion statt. Die genaue Beschaffenheit des Chromatins sowie der Einfluss verschiedener Chromatin-modifizierender Komponenten auf die DNA-Schadensantwort sind jedoch kaum verstanden. Um dieses Verständnis zu erweitern, untersuche ich in der vorliegenden Doktorarbeit die Rolle und Relevanz von zwei Chromatin-modifizierenden Proteinen an DNA-Läsionen.

Der **Chromatin Remodeler ALC1** ist ein häufig amplifiziertes Onkogen in verschiedenen Krebssorten. Es wird in Abhängigkeit der post-translationalen Modifikation poly-(ADP-ribose) schnell zu DNA-Schäden rekrutiert, wo es die Chromatinstruktur lokal öffnet. Die funktionellen Konsequenzen dieser Rekrutierung und Chromatinöffnung sind jedoch unbekannt. Hier ermittle ich den Einfluss der ALC1-basierten Chromatin-Remodellierung auf die zelluläre Antwort gegenüber PARP Inhibitoren (PARPi), welche als Krebstherapie für BRCA1/2-mutierte Krebsarten in der Klinik eingesetzt werden. Die Zielmoleküle von PARPi, PARP1 und PARP2, spielen eine kritische Rolle in der frühen DNA-Schadensantwort. Mithilfe von Live-Zell-Mikroskopieexperimenten zeige ich, dass ALC1 für das Ablösen von PARP2 von DNA-Schäden benötigt wird. Dies hat Auswirkungen auf die Reparatur von Einzelstrangbrüchen, da das „Trapping“ von PARP2 fälschlicherweise frühe DNA-Reparaturproteine am Schaden zurückhält, während es die Rekrutierung später Faktoren verringert. Die Deletion von ALC1 führt außerdem zu einer Potenzierung der PARPi-induzierten Tötung von Krebszellen durch das „Trapping“ von PARP2 und zeigt synthetische Letalität mit einer BRCA1/2-Defizienz, was dafür spricht, dass die zentrale Rolle der ALC1-basierten Chromatin Remodellierung ausgenutzt werden könnte, um neue Krebsmedikamente zu entwickeln.

Die **Histonvariante macroH2A** wird in drei Isoformen exprimiert, welche alle die Chromatinstruktur kompaktieren. Während macroH2A1.1 eng an die poly-(ADP-ribose) Signalkette gekoppelt ist, ist der Mechanismus der Chromatinkompaktierung für die anderen beiden Isoformen unbekannt geblieben. Durch das Messen der Chromatindichte an DNA-

Läsionen zeige ich, dass die flexible Linkerregion von macroH2A sogar dann Chromatin kompaktiert, wenn diese auf ein kanonisches H2A Molekül transferiert wird. Dies spricht für eine universelle Rolle des macroH2A Linkers in der Chromatinkompaktierung, unabhängig von dem Status der poly-(ADP-ribose)-Bindung der Isoformen, und gibt somit neue Einblicke in den Mechanismus von macroH2A in der Aufrechterhaltung von Chromatinstrukturen.

4. Introduction

4.1 DNA is packaged into chromatin – a dynamic entity rather than a rigid structure

The identity of different cell types, as well as the adaptation to environmental stimuli are of key importance for the survival of a multicellular organism, and require the activity of different cell programs (Yadav *et al.* 2018). Containing ~20,000 human genes as well as ~40,000 non-coding transcripts and pseudogenes, the human genome encodes the information for all cellular processes (GENCODE (version 36); Frankish *et al.* 2019). While measuring about 2 meters in length, our genetic information is found largely compacted into a structure termed chromatin in the cell nucleus (Sun *et al.* 2000).

The high degree of chromatin compaction does not only provide space-restricted storage in the nucleus, but also protects the genome from DNA damage and generates first entry points to regulate and fine-tune cellular programs, such as gene transcription, DNA replication and DNA repair (Misteli 2007, Yadav *et al.* 2018). As the integrity of genetic information is critical for cellular homeostasis, the de-regulation of such processes, e.g. by impairing access to DNA, can ultimately result in genetic alterations and disease cell states, as the formation of cancer (Hanahan and Weinberg 2011).

4.1.1 The structural organization of chromatin results in functional domains

To achieve the high degree of compaction, DNA is wrapped around histone proteins to form the basic unit of chromatin, the nucleosome (**Figure 1A**; Cutter and Hayes 2015). One nucleosome consists of 145-147 base pairs of DNA wrapped 1.65 times around a histone octamer core, which is built of two dimers of H2A-H2B histone proteins, as well as a tetramer of H3-H4 histones (Luger *et al.* 1997). Connected by small stretches of linker DNA, several nucleosomes are aligned as “beads-on-a-string” into nucleosomal arrays of 10 nm in diameter (Olins and Olins 1974).

Chromatin folding has classically been seen as a hierarchical process (**Figure 1B**), where the 10 nm fiber folds into a 30 nm secondary structure based on electrostatic contacts of nearby nucleosomes and the help of structural proteins such as the linker histone H1 (Finch and Klug 1976, Thoma *et al.* 1979). Throughout the cell cycle, the genome is further compacted into high-order chromatin folds, with the mitotic chromosome being the highest compaction state (Belmont and Bruce 1994).

Interestingly, the compaction of chromatin was already early on found not to be randomly distributed in the nucleus. The open 10 nm conformation, termed euchromatin, is preferentially localized in the inside of the nucleus, where it allows gene transcription, while compacted regions, so called heterochromatin, cluster in the nuclear periphery (Brown 1966, Weintraub and Groudine 1976). This functionally divides the nucleus into transcriptionally active and silenced regions, generating a first level of gene regulation (Misteli 2007).

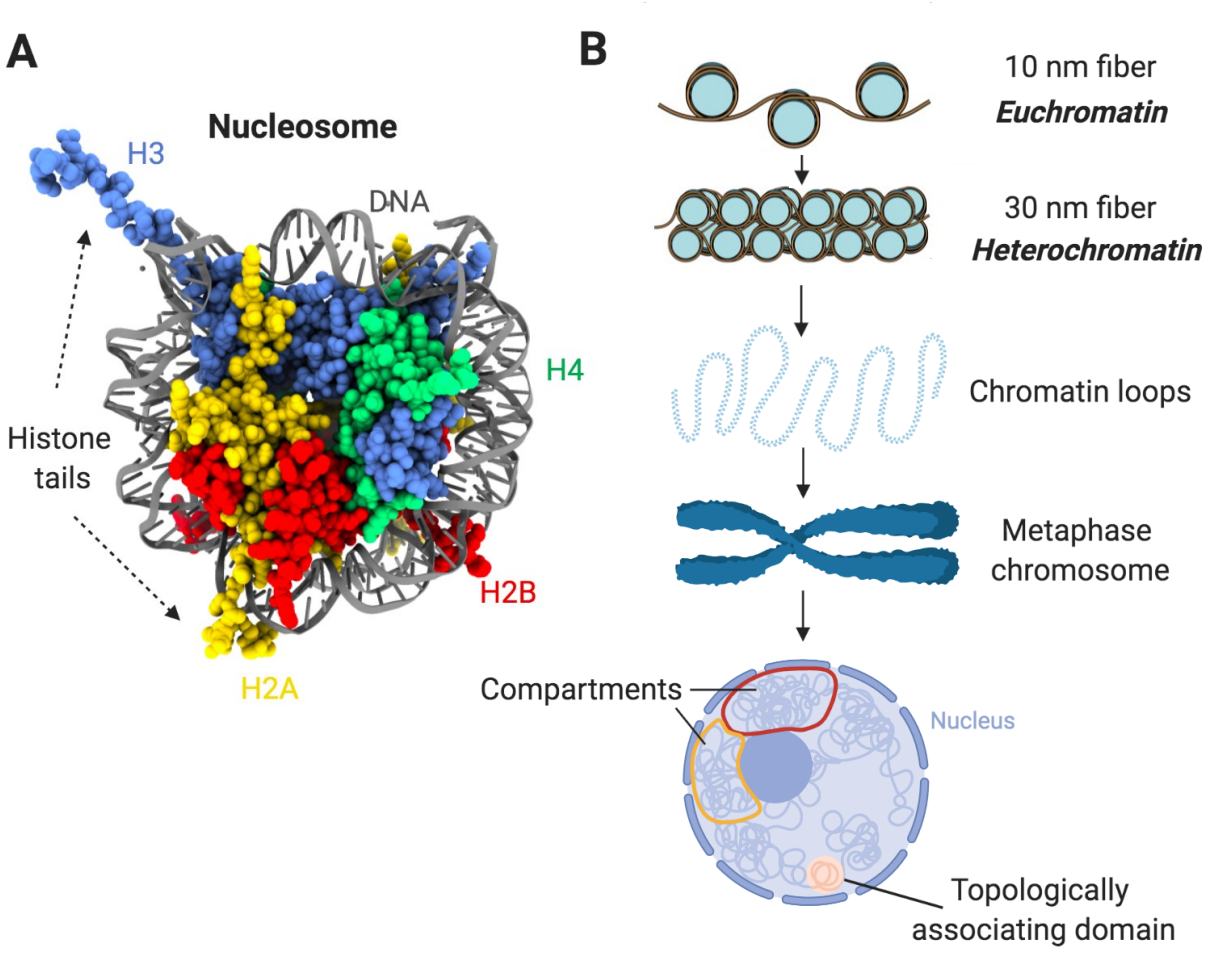

Figure 1 Chromatin structurally and functionally organizes the cell nucleus

A The high-resolution crystal structure of the nucleosome (PDB: 1AOI; Luger *et al.* 1997*)* revealed that the basic unit of chromatin consists of 146 base pairs of DNA (grey) wrapped around a core of histone proteins, built of two dimers of H2A and H2B (yellow and red), as well as a tetramer of H3 and H4 (blue and green). The N-terminal tails of the histones are extending from the core, and are thus available for protein interactions.

B The structural organization of chromatin inside the cell nucleus is dependent on its functional status and underlies sequential folding of nucleosomes into higher-order structures. Active euchromatin occurs as an open 10 nm fiber, while inactive heterochromatin is more condensed into 30 nm fibers. Long ranges of chromatin are further compacted by looping and obtain its highest compaction state as a chromosome in the metaphase of the cell cycle. The chromatin organization inside the nucleus is not random, but form functional clusters, so called topologically associating domains, as well as active and inactive chromatin compartments. The figure was created with BioRender.com with nucleosomal structures reprinted from Moraru and Schalch 2019, with permission from Portland Press.

While the occurrence and exact structures of higher-order chromatin folds in cells are still poorly understood (Moraru and Schalch 2019), newer studies could further extend the functional model of chromatin compaction (**Figure 1B**). Using next-generation sequencing techniques, researchers were able to generate high-resolution maps of 3D chromatin folding (Fraser *et al.* 2015, Kempfer and Pombo 2020). This revealed that chromatin fibers form preferential short-ranged and long-ranged interactions, clustering into so called topologically associating domains (TADs) (Dixon *et al.* 2012, Nora *et al.* 2012, Stevens *et al.* 2017). Interactions inside TADs seem to be critical for gene regulation, forming e.g. interactions between enhancer and promoter elements to enhance transcription (Lupiáñez *et al.* 2015, Schoenfelder and Fraser 2019). TADs with similar properties preferentially interact with one another, generating active, gene-rich A compartments, or inactive, gene-poor B compartments inside the nucleus (Lieberman-Aiden *et al.* 2009, Stevens *et al.* 2017), resulting in an additional functional separation of chromatin structure inside the nucleus.

4.1.2 The structure of chromatin is dynamically regulated in the cell

To form functionally diverse and convertible chromatin environments throughout different organisms, cell types, cell cycle stages and stress adaptations, the nature of chromatin is furthermore not static, but constantly adapts to environmental stimuli and changes at different positions in the genome (Seeber *et al.* 2018, Yadav *et al.* 2018, Maeshima *et al.* 2019). The human cell has therefore employed a variety of different mechanisms to extend the basic chromatin structure into the so-called epi-genome: (1) Post-translational histone modifications and (2) histone variants establish distinct chromatin signatures, that are closely linked to different functional states of the cell, while (3) chromatin remodelers directly change the access to DNA by repositioning nucleosomes (Tyagi *et al.* 2016). These mechanisms will be explained in more detail in the following sub-chapters.

The chemical modification of DNA bases, such as 5-methylcytosine (m5C), adds an additional layer of epigenetic regulation (Zhao *et al.* 2020). The dynamic introduction of this modification in the promoter regions of genes by DNA methyltransferases marks respective genes as silent and leads to more compacted chromatin regions, while demethylating these regions via oxidation pathways promotes gene transcription and an open chromatin structure (Stadler *et al.* 2011, Yin *et al.* 2017, Greenberg and Bourc'his 2019). A diverse range of chemical modifications on RNA can further diversify the genomic information post-transcriptionally (Gilbert *et al.* 2016). The methylation of adenosines in mRNA (m6A) for example modifies the

translation efficiency or the stability of the respective RNA (Wang *et al.* 2014, 2015, Roundtree *et al.* 2017). This so-called epitranscriptomic regulation of the genomic information may be tightly linked to the epigenetic status of the genome, suggesting an even broader influence of the chromatin structure on cellular processes and the functional status of the cell than previously anticipated (Tzelepis *et al.* 2019).

4.1.2.1 Histone modifications establish dynamic chromatin signatures

Histone-modifying enzymes establish post-translational modifications, such as methylation, acetylation or phosphorylation, on the N-terminal tails of histone proteins (Bannister and Kouzarides 2011). Being introduced in a highly site-specific and combinatorial manner, these modifications generate a "histone code" (**Figure 2**), which allows to dynamically fine-tune different chromatin environments through two main mechanisms (Jenuwein and Allis 2001).

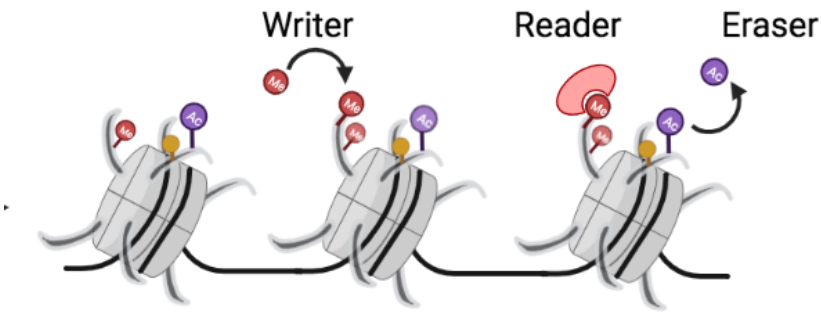

Figure 2 Histone modifications are dynamically established, read and removed

Nucleosomes can be modified in a combinatorial manner on their N-terminal tails by post-translational modifications (left). These are introduced or removed by histone modifying or demodifying enzymes, so called writers and erasers (middle, right). The dynamic action of these enzymes establishes distinct chromatin signatures, which can be recognized and processed by reader proteins (middle) and locally modulate chromatin-based processes, such as transcription. Created with BioRender.com.

The introduction of modifications can either directly change the chromatin structure through the neutralization of charge, as observed for lysine acetylation, which causes weakened histone-DNA interactions and increased DNA accessibility (Hong *et al.* 1993, Dion *et al.* 2005). Alternatively, histone modifications can modulate cellular processes through the binding of histone reader proteins (Bannister and Kouzarides 2011). Containing dedicated domains to recognize specific modifications, histone readers can "read-out" the modification to coordinate chromatin-templated mechanisms (Patel and Wang 2013). Bromodomain-containing reader proteins recognize for example acetylated lysines in histone tails (Dhalluin *et al.* 1999, Jacobson *et al.* 2000), while different forms of histone methylation are recognized by chromodomain

proteins (Bannister *et al.* 2001, Flanagan *et al.* 2005). These modifications often mark distinct regions in genes, such as promoters, and thereby differentially coordinate the transcription process by recruiting the transcription machinery or inhibitory modules (Fujisawa and Filippakopoulos 2017, Hyun *et al.* 2017). The active removal of histone modifications by histone-demodifying enzymes complements the dynamic regulation of these chromatin-templated processes, allowing the spatially and timely constricted establishment of highly specialized chromatin environments that regulate dynamic processes, such as transcription or DNA repair (Kouzarides 2007).

4.1.2.2 Histone variants mark specialized chromatin regions

The complexity of chromatin is further increased by the introduction of histone variants to mark specific chromatin regions. Having diverged in sequence and structure from canonical histones, histone variants are introduced into chromatin by specialized histone chaperones to form homotypic (two variants) or heterotypic (one variant) nucleosomes with canonical core histones (**Figure 3**; Vardabasso *et al.* 2014). The integration of histone variants can either change the chromatin structure by altering the stability of nucleosomes based on differential interactions with core histones (Bönisch and Hake 2012) or varied patterns of post-translational modifications (Corujo and Buschbeck 2018). Alternatively, histone variants can differentiate chromatin by marking specific regions in the genome (Henikoff and Smith 2015). The most prominent example is the H3 variant CENP-A, which is specifically integrated at the centromere, providing a binding platform for the kinetochore to coordinate chromosome segregation (Foltz *et al.* 2006). Other histone variants are actively involved in marking transcriptionally active genes or sites of DNA repair to recruit specified factors for the required processes (Maze *et al.* 2014). While these proteins only make up a small part of the entire histone pool, recent research demonstrates their relevance, as mutation or dysregulation of histone variant expression is negatively associated with cancer formation and progression (Martire and Banaszynski 2020).

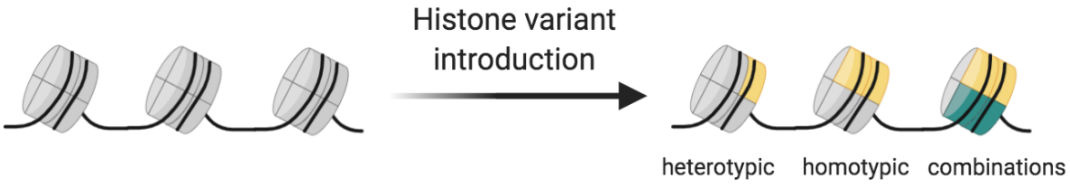

Figure 3 Histone variant incorporation diversifies chromatin

Core histones can be exchanged by histone variants to diversify the chromatin structure. Depending on the number of exchanged histones, this results in the formation of heterotypic (one variant) or homotypic nucleosomes (two variants). The combination of several histone variants within one nucleosome allows further diversification, so that specialized chromatin regions can be formed. Created with BioRender.com.

4.1.2.3 Chromatin remodelers change the access to DNA

By translocating along DNA with the help of ATP hydrolysis, chromatin remodelers can directly change the chromatin structure (**Figure 4**; Bowman and Deindl 2019). They can reposition, space or evict nucleosomes, resulting in a local compaction or opening of chromatin (Mueller-Planitz *et al.* 2013). Some remodeling complexes further have the ability to exchange histone with histone variants, and assist histone chaperones in de-novo nucleosome assembly (Clapier *et al.* 2017). Chromatin remodelers thus dynamically control the access to specific chromatin regions and induce changes in chromatin topology in a time- and space-restricted manner (Längst and Manelyte 2015).

The DNA translocation reaction is performed by a RecA-like ATPase domain, which is an integral component of each chromatin remodeling enzyme (Narlikar *et al.* 2013). Recent structural analyses have revealed that the mode of DNA translocation seems to be unified among chromatin remodelers (**Figure 5**; Bowman and Deindl 2019, Yan *et al.* 2019). Binding to the superhelical location (SHL) ±2 of the nucleosome, the ATPase domain pushes DNA along the nucleosome in one base pair steps by rotating the ATPase lobes relative to each other upon ATP hydrolysis (Li *et al.* 2019, Yan *et al.* 2019). In addition to this core remodeling reaction, chromatin remodelers contain DNA-binding and regulatory domains and subunits, often resulting in the formation of multi-subunit complexes (**Figure 5b**; Clapier *et al.* 2017). These define the specific action of distinct chromatin remodeling complexes by impacting the recruitment to genomic locations, such as regions rich in certain histone modifications, or by modulating the outcome of the nucleosome sliding reaction into e.g. the eviction of nucleosomes or the exchange of histones (Tyagi *et al.* 2016).

The interplay between the ATPase domains and regulatory domains further strictly regulates the enzymatic activity of chromatin remodeling enzymes (Clapier *et al.* 2017). When inactive, the enzymes are often found in a self-inhibited, 'gated' state, in which the two ATPase lobes are held apart from each other to prevent ATP hydrolysis (Hauk *et al.* 2010, Xia *et al.* 2016, Yan *et al.* 2016). This is achieved by the folding of regulatory domains onto the ATPase subunit, which inhibits the ATPase activity and shields the DNA- and histone binding domains from possible interaction partners (Hauk *et al.* 2010, Lehmann *et al.* 2017, Singh *et al.* 2017, Wang *et al.* 2021). Upon binding of accessory reader domains to specific DNA structures or post-translational modifications, a conformational switch is triggered in the enzyme that allows the 'opening' and activation of the chromatin remodeler in an allosteric activation mechanism (Hauk *et al.* 2010, Yan *et al.* 2016, Lehmann *et al.* 2017, Ludwigsen *et al.* 2017, Singh *et al.* 2017). Being equipped with numerous regulatory domains, multi-subunit chromatin remodeling complexes are thus highly versatile and play important roles in all chromatin-based processes, ranging from gene expression over DNA replication to DNA repair (Clapier and Cairns 2009).

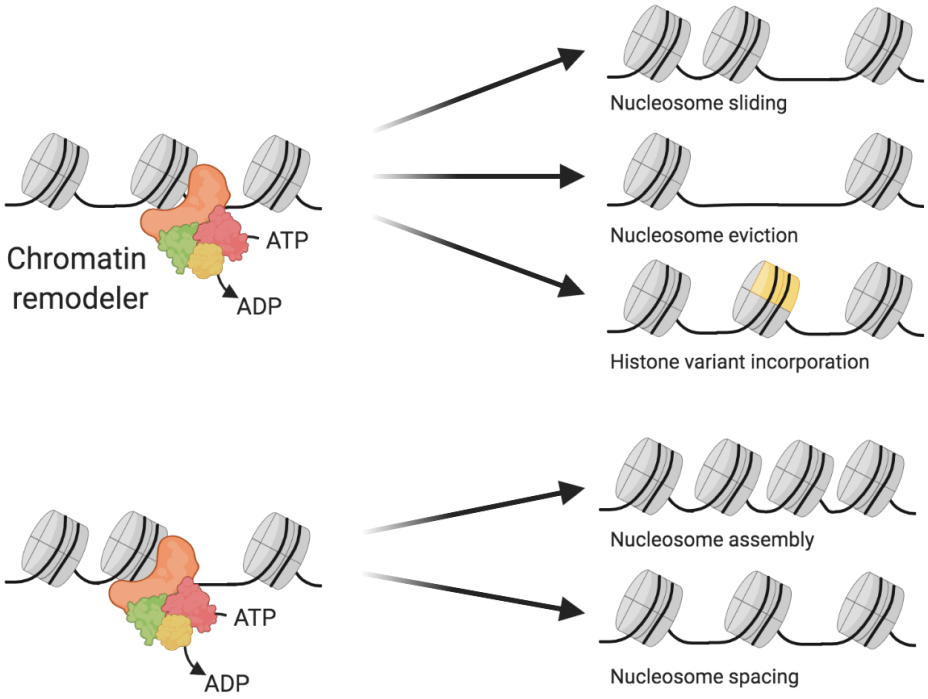

Figure 4 ATP-dependent chromatin remodelers change the accessibility of chromatin

Chromatin remodelers use the energy gained by ATP hydrolysis to increase the access to DNA by three main mechanisms (top): They can (1) slide nucleosomes to nearby positions, (2) evict nucleosomes from DNA, or (3) exchange histones within nucleosomes. Alternatively, they can also promote a more closed chromatin structure, by assembling additional nucleosomes onto chromatin or by spacing nucleosomes equally (bottom). Created with BioRender.com.

The importance of chromatin remodeling enzymes for cellular homeostasis is exemplified by the severity of diseases caused by their dysfunction. A variety of cancers and neurological disorders are caused by mutations in chromatin remodelers (Pulice and Kadoch 2016). Mutations in SWI/SNF remodeling complexes for example occur at a frequency of ~20% in human cancers, whereas the most frequently mutated tumor suppressor p53 is found mutated in 26% of cancers (Kadoch *et al.* 2013). Tumor cells can further become dependent on the function of specific chromatin remodelers upon the disruption of cellular pathways. A prominent example is the dependency of prostate cancers on the remodeler CHD1 in the absence of PTEN, which promotes cancer cell proliferation and survival through the activation of the NF-kB-signaling cascade (Zhao, Lu, *et al.* 2017). The frequency of such dysregulations has shed light on the possible therapeutic targeting of chromatin remodelers for cancer therapy (Centore *et al.* 2020). However, no effective therapy has yet reached the clinic, also because mechanistic insights into these complexes have largely been missing (Wanior *et al.* 2021). Excitingly, recent structural investigations have significantly increased our knowledge about the interaction of large chromatin remodeling complexes with nucleosomes (**Figure 5b**), allowing to more specifically dissect the regulatory mechanisms of chromatin remodelers. In a recent review for Current Opinion in Structural Biology, I summarized and discussed how these new structural insights may further guide our understanding of disease mechanisms and drug development in the near future (Blessing *et al.* 2020; see **Appendix A** of this thesis).

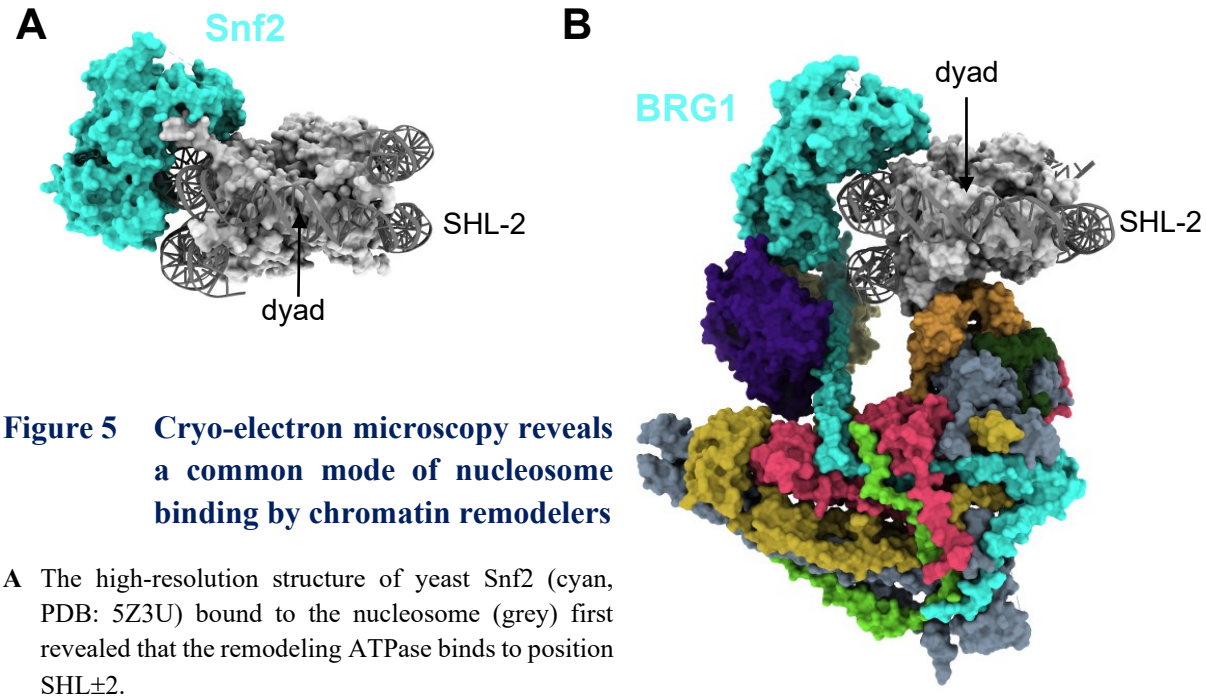

Figure 5 Cryo-electron microscopy reveals a common mode of nucleosome binding by chromatin remodelers

A The high-resolution structure of yeast Snf2 (cyan, PDB: 5Z3U) bound to the nucleosome (grey) first revealed that the remodeling ATPase binds to position SHL±2.

B The position of the ATPase and the mode of DNA translocation seem to be conversed also in multi-subunit chromatin remodeling complexes, as shown here for the human BAF complex (PDB: 6LTJ). All subunits of the complex are colored differentially, the ATPase BRG1 is labelled in cyan.

4.2 DNA repair mechanisms safeguard the genome

Although packaged into chromatin, endogenous and exogenous mutagens continuously react with DNA, causing up to 10^5 spontaneous DNA lesions per day (Hoeijmakers 2009). Damaged DNA does not only physically hinder chromatin-templated processes, such as DNA replication and gene transcription, but also harbors the risk of acquiring mutations in the DNA template, thus threatening the integrity of our genomic information (Jackson and Bartek 2009). To prevent genomic mutations and chromosomal aberrations, the cell has developed dedicated DNA repair pathways to detect and remove lesions from DNA (Chatterjee and Walker 2017). Cell cycle checkpoints further control the intactness of DNA throughout the cell cycle, while apoptotic mechanisms ensure the timely killing of a cell if the damage becomes too severe (Jackson and Bartek 2009). The cellular homeostasis critically depends on the accuracy of such mechanisms, as unrepaired, mutated DNA extensively increases the risk of dysregulating cells into disease states, with cancer being the most prominent example (Ciccia and Elledge 2010).

4.2.1 Dedicated DNA repair pathways repair distinct DNA lesions

Mutagens of various sources harm the DNA, each of them forming distinct DNA lesions (Chatterjee and Walker 2017). Endogenous reactive oxygen species or alkylating agents, such as the cancer drug methyl methane sulfonate, modify DNA bases directly (Pegg 1990, Breen and Murphy 1995). UV light from the sun results in the crosslinking of neighboring bases on the same DNA strand (Davies 1995), while crosslinking agents, such as mitomycin C, form interstrand crosslinks between the two opposite DNA strands (Clauson *et al.* 2013). DNA double-strand breaks, generated e.g. by ionizing radiation, are the most difficult to repair and thus the most harmful DNA lesions (Lomax *et al.* 2013).

To coordinate the efficient repair of the great variety of DNA lesions, dedicated DNA repair pathways are specialized on repairing different types of DNA lesions. These can be broadly divided in single- and double-strand break repair mechanisms, based on the arising strand breaks on one or both of the DNA strands (Chatterjee and Walker 2017).

4.2.1.1 Single-strand break repair

Single-strand break repair pathways repair damaged bases, single-strand breaks as well as bulky, DNA helix-distorting lesions localized on one DNA strand by base excision repair and nucleotide excision repair, respectively (Hoeijmakers 2009). These pathways share a general repair principle of three main steps: (1) recognition of the damaged DNA bases, (2) excision of the modified segment on the affected DNA strand, generating a single-strand break as an

intermediate, and (3) the final re-synthesis of the DNA on basis of the unimpaired opposite DNA strand (Lee and Kang 2019). To accommodate the efficient removal of the respective lesions, they do however differ in the repair proteins used and the specific execution of the three steps, as outlined below.

Dealing with 10,000-20,000 damaged DNA bases and single-strand breaks per day, the base excision repair pathway is one of the most prevalent DNA repair pathways in the cell (Ciccia and Elledge 2010). It is initiated by the subsequent action of DNA glycosylases and AP endonucleases (**Figure 6**), which recognize and excise the modified base and the leftover ribose moiety, respectively (Chatterjee and Walker 2017). To be able to detect the entire repertoire of base modifications, the human cell employs 11 different DNA glycosylases, each of which is responsible for the specific excision of one or few modified bases (Krokan and Bjørås 2013).

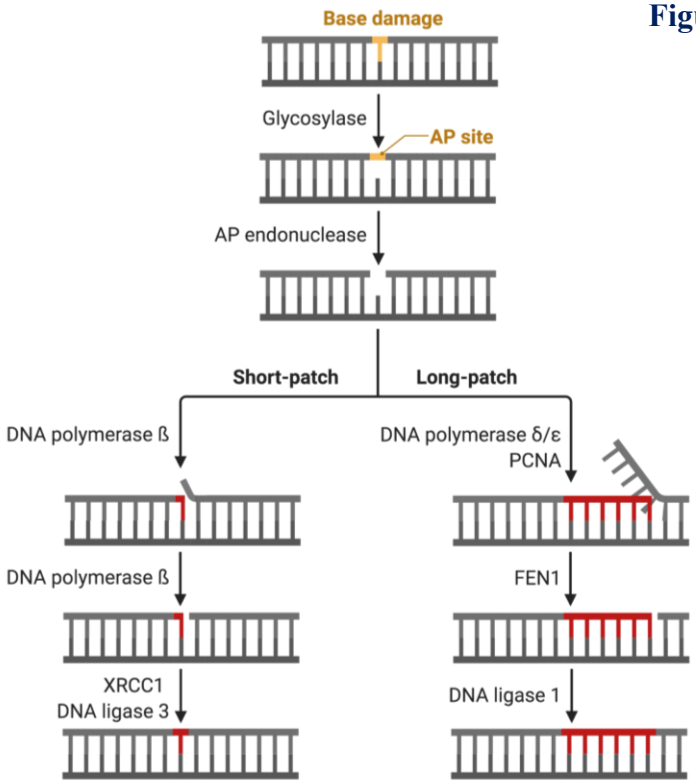

Figure 6 Base excision repair repairs base damages and single-strand breaks

Base damages are detected by specialized glycosylases, which excise the damaged base. The resulting AP site is cleaved by an AP endonuclease causing a single-strand break. This can be repaired by two main sub pathways. In the short patch pathway, DNA polymerase ß, XRCC1 and DNA ligase repair the single-strand gap. In the long-patch repair pathway, DNA polymerase δ/ε and PCNA generate an overhang, which is cleaved by FEN1 and ligated by DNA ligase 1. Created with BioRender.com.

Having generated a single-strand gap of one nucleotide by removing the damaged DNA base, the following steps of the repair pathway equal the direct repair of single-strand breaks and focus on re-synthesizing and ligating the missing nucleotide(s) with the help of the single-strand break repair factors POL ß, XRCC1 and LIG3 (Matsumoto and Kim 1995, Cappelli *et al.* 1997).

At difficult-to-repair lesions, the so-called short-patch repair may be replaced by a long-patch repair mechanism, in which the single-strand gap initiates the synthesis of more nucleotides than necessary to fill the gap, thus pushing the already existing DNA off at newly synthesized positions and generating a DNA flap, which requires processing by the flap removal enzyme FEN1 (Frosina *et al.* 1996, Klungland and Lindahl 1997). The long-patch repair mechanism is considered as a back-up mechanism for the canonical short-patch pathway at specific sub-types of DNA base lesions and in post-replicative chromatin, although the switch from short-patch to long-patch repair and thus the exact function of the repair pathway remains poorly understood (Krokan and Bjørås 2013). A well-functioning base excision repair mechanism is crucial to prevent replication defects and the occurrence of deleterious double-strand breaks in the cell (Kuzminov 2001, Dianov and Hübscher 2013). The cellular relevance becomes especially evident in mice, where the knockout of POL ß, XRCC1 or LIG3 results in embryonic lethality (Sobol *et al.* 1996, Tebbs *et al.* 2003, Puebla-Osorio *et al.* 2006).

Exposure to UV light results in the crosslinking of neighboring bases on one DNA strand, with 6-4-photoproducts and cyclopyrimidine dimers being the most prevalent types of crosslinked bases (Davies 1995). With nucleotide excision repair, eukaryotic cells have developed a specific pathway to repair these large, bulky lesions (Schärer 2013). The initiation of the repair pathway is dependent on the position of the lesion in the genome (**Figure 7**; Marteijn *et al.* 2014). In transcriptionally active regions, lesions are recognized by RNA polymerase II, which is stalled at the lesion due to steric hindrance (Mei Kwei *et al.* 2004, Brueckner *et al.* 2007). The stalled RNA polymerase is recognized by the recognition factors CSA and CSB, which activate the transcription-coupled sub-pathway of nucleotide excision repair (Xu *et al.* 2017, Lans *et al.* 2019). In contrast, lesions in transcriptionally inactive regions are unavailable to RNA polymerase II stalling, and are instead recognized based on the bending of DNA introduced by the lesion (Schärer 2013). The main recognition factor of this global-genome repair sub-pathway, XPC, detects thermodynamic instabilities of the DNA helix, which allows recognizing various types of DNA lesions that cause helix destabilization (Sugasawa *et al.* 1998, 2001). Its action is accompanied by the UV-DDB complex, which facilitates the recognition of lesions with only little helix distortion, such as cyclopyrimidine dimers (Fitch *et al.* 2003, Scrima *et al.* 2008). Lesion detection by both sub-pathways triggers the recruitment of a pre-incision complex of TFIIH, XPA and RPA, which does not only verify the existing damage, but also prepares the lesion for incision by separating the two DNA strands with the help of helicases (Li *et al.* 2015, Compe and Egly 2016). The two endonucleases XPF-ERCC1

and XPG subsequently excise about 30 nucleotides of the damaged DNA strand in the vicinity of the 5' and 3' end of the lesion (Staresincic *et al.* 2009). This dual incision event is immediately followed by re-synthesis and ligation of the DNA fragment to close the generated single-strand gap (Lehmann 2011).

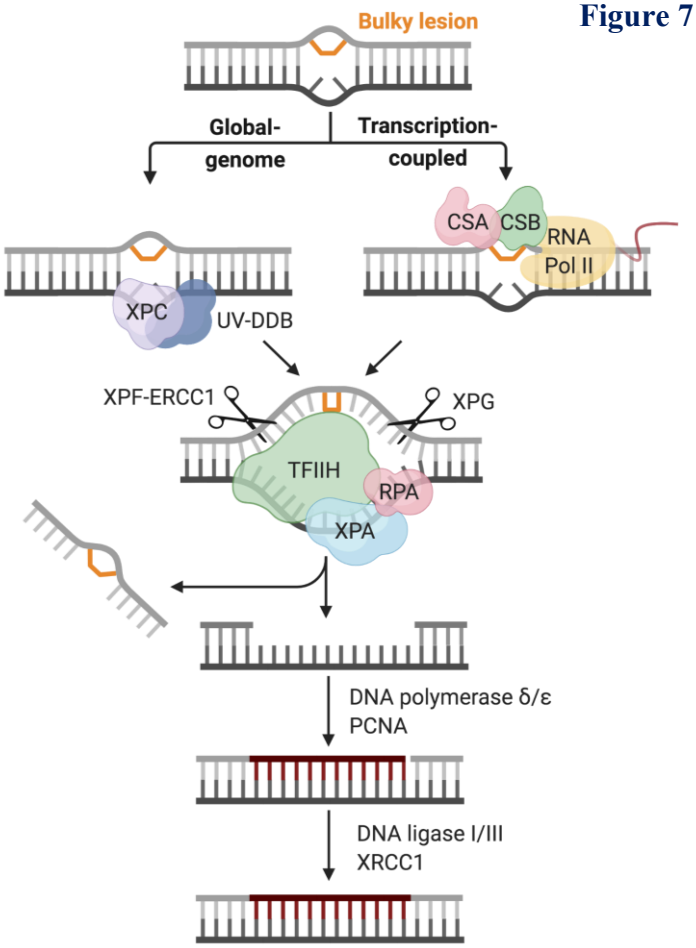

Figure 7 Nucleotide excision repair repairs lesions caused by UV-light

UV-light causes bulky lesions on one strand of the DNA, which are repaired based on their position in the genome. In global-genome repair, XPC and UV-DDB recognize the damaged bases, while CSA and CSB detect a stalled RNA polymerase II in transcription-coupled repair. The existing lesion is subsequently verified by TFIIH, XPA and RPA. This promotes the excision of the damaged piece of DNA by XPF-ERCC1 and XPG, which will be resynthesized and ligated by DNA polymerases δ/ε, PCNA, DNA ligase I/III and XRCC1. Created with BioRender.com.

The high relevance of this repair pathway for cellular homeostasis is demonstrated by the severity of syndromes developed by patients with single mutations in nucleotide excision repair proteins (Marteijn *et al.* 2014). Patients with mutations in XP proteins for example harbor a 1,000-100,000 fold increased risk of developing skin and tongue cancer upon contact with sunlight (DiGiovanna and Kraemer 2012), while mutations in proteins involved in the transcription-coupled repair pathway result in severe neurological disorders with short life expectancy, as observed in Cockayne Syndrome (Laugel 2013).

4.2.1.2 Double-strand break repair

In addition to base damages and single-strand breaks, the human cell encounters about 10 double-strand breaks per day (Lieber 2010). While occurring at a lower rate than lesions repaired by single-strand break repair pathways, DNA double-strand breaks pose a great harm for the cell, since the complete disruption of the DNA harbors the risk of loss or translocation of large chromosomal regions (Javadekar and Raghavan 2015). These breaks thus require the faithful repair with the help of two main pathways: Non-homologous end joining and homologous recombination (Ceccaldi *et al.* 2016). Their use is thereby largely dependent on the cell cycle state of the damaged cell (Karanam *et al.* 2012, Chang *et al.* 2017).

Non-homologous end joining (NHEJ) is active in all cell cycle stages and follows a similar concept as the single-strand break repair pathways: After recognition of the damage and processing of the DNA ends, the two DNA strands are re-ligated (Yang *et al.* 2016). However, due to the processing of a double-strand break, the repair proteins fuse the broken ends independently of a homologous DNA sequence that can act as a template, thus harboring the risk of acquiring mutations (Chang *et al.* 2017). NHEJ is initiated by the Ku70/Ku80 heterodimer (**Figure 8**), which serves as the main recognizer of the double-strand break (Ceccaldi *et al.* 2016). Forming a ring structure, Ku binds each of the broken DNA ends in a sequence-independent manner (Walker *et al.* 2001). This does not only restrict the movement of the double-strand breaks to prevent abnormal translocation, but also provides a scaffold for the recruitment of the serine/threonine kinase DNA-PKcs and various processing factors that cleave DNA overhangs to generate blunt-ended DNA for ligation (Chang *et al.* 2017). Although not well understood, DNA-PKcs is thought to phosphorylate various NHEJ factors and itself, which controls the recruitment of processing factors, such as Artemis, as well as the ligation step (Goodarzi *et al.* 2006, Jiang *et al.* 2015).

End processing of the double-strand breaks triggers the recruitment of XRCC4 and XLF, which first help to stabilize the alignment of the DNA strands (Hammel *et al.* 2011, Andres *et al.* 2012) and finally promote the ligation of the broken ends by LIG4 (Lu *et al.* 2007). Although the short DNA resection in the absence of a template strand harbors the risk of acquiring genomic mutations, NHEJ seems to be the dominant repair pathway due to its fast kinetics and availability throughout all cell cycle stages (Chang *et al.* 2017). Studies estimated that homologous recombination only contributes to 15-30% of repaired double-strand breaks, while the majority of lesions is repaired by non-homologous end joining (Beucher *et al.* 2009, Shibata

et al. 2011). Mutations in NHEJ proteins are extremely rare, likely due to the severity of associated phenotypes (Chang *et al.* 2017).

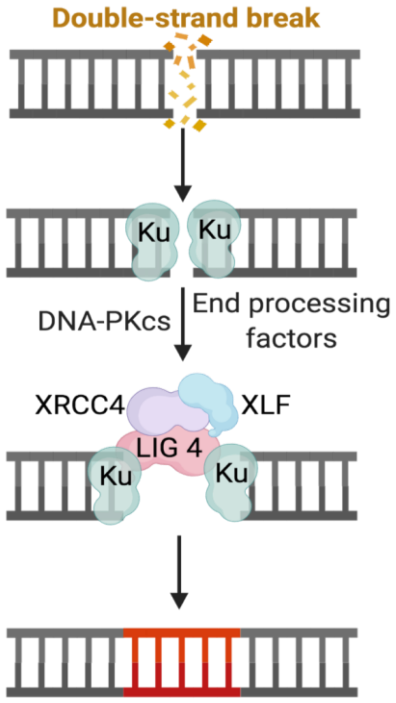

Figure 8 Non-homologous end-joining (NHEJ) reconnects double-strand breaks

NHEJ provides a fast way to repair double-strand breaks in all cell cycle phases. The Ku complex recognizes broken DNA ends, which can be further trimmed with the help of DNAPkcs and end processing factors to generate blunt double-strand ends. These can subsequently be ligated by DNA ligase 4 with the help of XRCC4 and XLF. Created with BioRender.com.

In the late S and G2 phases of the cell cycle, NHEJ is accompanied by homologous recombination (**Figure 9**; Karanam *et al.* 2012). Based on the principle of genetic recombination, the repair pathway acts with slower kinetics than NHEJ and uses homologous DNA as a template for repair, which is present during these cell cycle phases in the form of the duplicated genome (Karanam *et al.* 2012, Ceccaldi *et al.* 2016). To start the recombination process, the ends of the broken DNA strands are resected in a 5'-3' direction, a process that is initialized by CtIP and the MRE11-RAD50-NBS1 (MRN) complex, and continued by different exonucleases (Mimitou and Symington 2008, Zhu *et al.* 2008, Anand *et al.* 2016). This generates a 3' single-strand overhang of up to 4 kilobases in length, that can be used for the search of homologous sequences on the sister chromatid (Chung *et al.* 2010, Zhou *et al.* 2014). As excessive or wrongly timed end resection is highly deleterious, the initiation of the process is tightly controlled by cyclin-dependent kinases and the BRCA1 protein (Ira *et al.* 2004, Huertas *et al.* 2008, Cruz-García *et al.* 2014, Densham *et al.* 2016). By forming a nucleoprotein filament around the generated DNA single strand overhang, RAD51 then induces the homology search (Benson *et al.* 1994). This process is regulated to ensure its correct timing and precise

action, with BRCA2 and BRCA1 being the key mediators that promote RAD51 filament formation (Zhang *et al.* 2009, Jensen *et al.* 2010, Zhao, Steinfeld, *et al.* 2017, Ranjha *et al.* 2018).

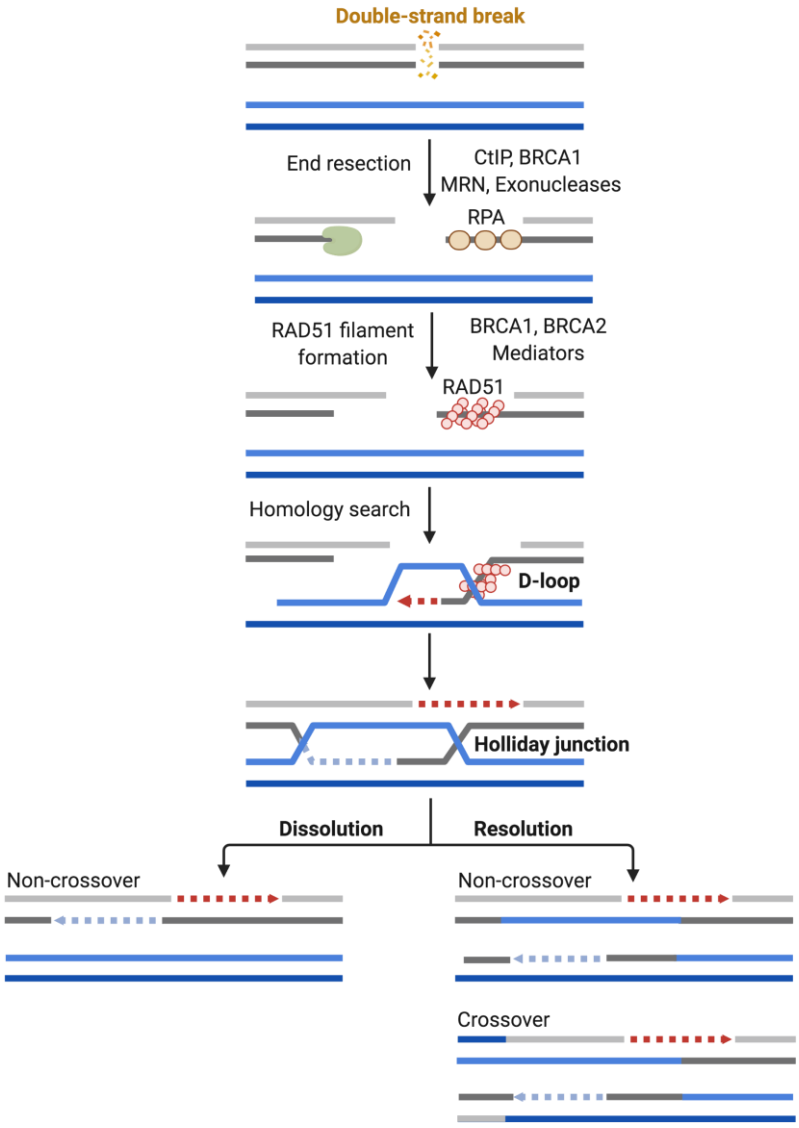

Figure 9 Homologous recombination repairs double-strand breaks with the help of homologous DNA strands

To start homologous recombination, the broken DNA ends are resected in 5'-3' direction with the help of the MRN complex and exonucleases, a process that is initiated and controlled by CtIP and BRCA1. The resected DNA strands are covered by RPA, before BRCA1, BRCA2 and other mediators initiate the RAD51 filament formation. This initiates the search for homologous sequences on the sister chromatid, resulting in the formation of a D-loop. The subsequently formed double Holliday junction can be opened by two pathways: Dissolution creates non-cross-over products, while resolution may result in both non-crossovers and crossovers of genetic information on the two chromatids. Created with BioRender.com.

Having found a homology region, the filament invades the DNA duplex of the sister chromatid and forms base pair interactions with the homologous strand, forming a so-called displacement loop (D-loop) (Baumann *et al.* 1996). The formed structure stimulates DNA synthesis, which further extends and stabilizes the D-loop structure (Wilson *et al.* 2013, Ranjha *et al.* 2018). This allows annealing of the second resected strand and re-ligation of the double-strand breaks, resulting in the formation of a double Holliday junction (Duckett *et al.* 1988).

The double Holliday junction can be either relieved by dissolution, in which the two junctions are moved towards each other and finally unraveled with the help of helicases and topoisomerases (Wu and Hickson 2003, Cejka *et al.* 2012). This process prevents the crossover of genetic information between the two DNA strands and is thus the preferred way to repair double-strand breaks (Wechsler *et al.* 2011). Double Holliday junctions that evade from dissolution can further be cleaved by nucleases in a resolution process later in the cell cycle, giving rise to both crossover and non-crossover of genetic information to the opposite DNA strand (Dehé and Gaillard 2017). The importance of homologous recombination as a repair mechanism is demonstrated best by patients with mutations in BRCA1 or BRCA2. Germline mutations of either of those genes are the most common cause of hereditary breast cancer, with up to 65% or 46% increased risk in developing breast cancer or ovarian cancer until the age of 70 (Chen 2007).

4.2.2 Efficient DNA repair requires coordinated chromatin dynamics

While many fundamental steps of the various repair pathways are well characterized, the coordination of DNA repair processes in different chromatin environments is still largely enigmatic (Polo and Almouzni 2015). In recent years, the dynamics of nucleosomes, the introduction of post-translational modifications as well as the involvement of chromatin-modifying proteins in DNA repair have therefore been under intense investigation to gain a better insight into the regulation of DNA repair processes within chromatin (House *et al.* 2014, Aleksandrov *et al.* 2020).

4.2.2.1 Post-translational modifications mark damaged DNA regions

It is now known that several post-translational modifications are introduced in the vicinity of DNA damage sites to mark the chromatin around the DNA lesion as an environment requiring immediate attention and repair (Ferrand *et al.* 2021). The most prominent modifications are (1) poly-(ADP-ribose) (PAR), mediated by the poly-(ADP-ribose) polymerases PARP1 and

PARP2, (2) the phosphorylation of the histone variant H2AX (γH2AX), mediated by the serine/threonine kinases ATM and ATR, as well as (3) the ubiquitination of histones by the E3 ubiquitin ligases RNF8 and RNF168 (Stadler and Richly 2017).

PAR is a fast and very transient modification forming long chains and branches on histones and other proteins in the vicinity of the DNA lesion (Barkauskaite *et al.* 2015). Considered as an early warning signal, the modification provides a binding platform to recruit the necessary repair proteins and chromatin modifiers (Gupte *et al.* 2017). PAR thus modulates both the outcome of the repair process as well as the chromatin environment around the lesion (Ray Chaudhuri and Nussenzweig 2017). Due to its role in all major DNA repair pathways and its potential to be exploited for cancer treatment, PAR and PAR-dependent processes have received great attention in recent years (Slade 2020), which will be highlighted in detail in the following two chapters of this introduction.

γH2AX is considered as the most prevalent marker of DNA damage sites (Bonner *et al.* 2008). While also reported in single-strand break repair pathways, its function is best described in double-strand break repair (Rogakou *et al.* 1998), where it spreads up to megabases away from the DNA lesion (Iacovoni *et al.* 2010). Together with the scaffold protein MDC1, it acts as a signal amplifier for the recruitment of several DNA repair factors, such as NBS1, 53BP1 and BRCA1, and thus promotes the initiation of double-strand break repair pathways (Celeste *et al.* 2002, Stucki *et al.* 2005). Loss of this binding platform is associated with severe genomic instability, highlighting its key role in regulating double-strand break repair (Bassing *et al.* 2002, Celeste *et al.* 2002).

γH2AX also stimulates the ubiquitination of histones, particular H2A and H1, at sites of double-strand breaks. This allows to further fine-tune the recruitment of DNA repair proteins, such as BRCA1 and 53BP1, and regulates the pathway choice of double-strand break repair (Doil *et al.* 2009, Fradet-Turcotte *et al.* 2013, Thorslund *et al.* 2015). The ubiquitination of DNA repair proteins by RNF168 and other ubiquitin ligases further triggers the proteasomal degradation and thus the timely removal of these factors from DNA lesions, adding an additional layer of coordinating the DNA damage response (Lee *et al.* 2018).

4.2.2.2 Chromatin around DNA lesions needs to be remodeled for efficient repair

Several *in vitro* studies demonstrated that nucleosomes are refractory to the initiation of DNA repair pathways (Wang *et al.* 1991, Rodriguez and Smerdon 2013), suggesting that the chromatin around the lesion needs to be heavily re-organized for efficient recognition and processing of the lesion (Polo and Almouzni 2015). Tracking the dynamics of nucleosomes at laser microirradiation sites showed that the chromatin at DNA damage sites is rapidly decondensed, which is followed by a slower recompaction phase (Kruhlak *et al.* 2006, Strickfaden *et al.* 2016). This has led to the "access-repair-restore" model of chromatin dynamics upon DNA damage (**Figure 10**), which suggests that a fast opening of chromatin around the DNA lesion provides access for DNA repair factors to bind and repair the lesion. After successful repair, the chromatin is recondensed to maintain its functional environment (Polo and Almouzni 2015).

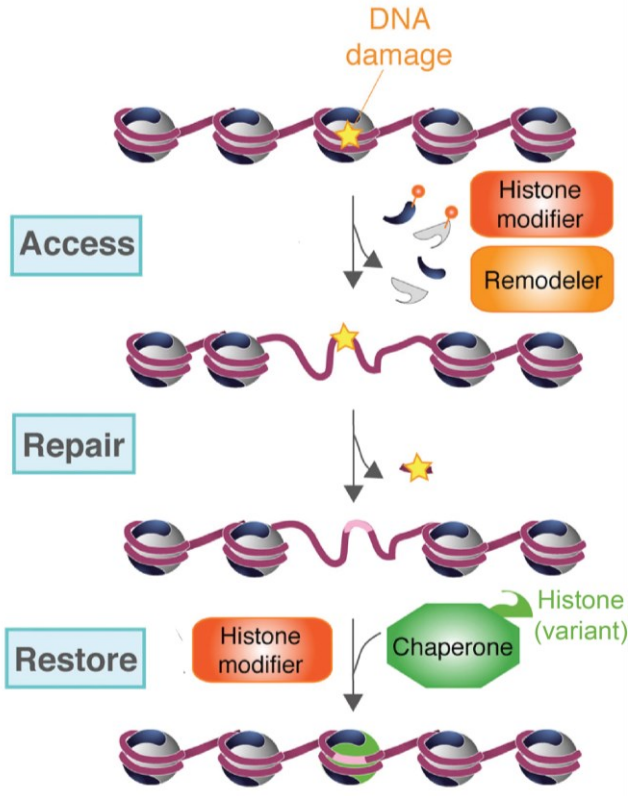

Figure 10 The access-repair-restore model outlines DNA repair in chromatin

Efficient DNA repair requires the opening of chromatin around DNA lesions with the help of histone modifiers and chromatin remodelers. This provides access for DNA repair factors for the repair of the DNA lesion. In the subsequent restoring phase, histone modifiers and histone chaperones (re)-condense the chromatin. This figure was modified from Polo and Almouzni 2015 with permission from Elsevier.

The initial decondensation phase was shown to depend largely on ATP, suggesting the involvement of chromatin remodeling enzymes in this process (Kruhlak *et al.* 2006). Indeed, various chromatin remodelers, such as ALC1 (CHD1L), BRG1, SNF2H and CHD2 were linked to the opening of chromatin upon DNA damage, many of which are recruited by the post-translational modification PAR in a direct, PAR-binding manner (Zhao *et al.* 2009, Klement *et*

al. 2014, Luijsterburg *et al.* 2016, Sellou *et al.* 2016). Interestingly, PAR itself further promotes chromatin relaxation due to the high negative charge of both the modification and DNA, which results in repulsion (Sellou *et al.* 2016). Likewise, acetylation marks on histones interrupt the close packing of nucleosomes around the DNA lesion (Bird *et al.* 2002, Ogiwara *et al.* 2011). This fast decondensation by chromatin remodeling enzymes and histone modifications is further supported by a second wave of chromatin remodelers consisting of CHD4, CHD3 and CHD7, which bind to already exposed DNA and enhance the opening of chromatin (Smith *et al.* 2018, Rother *et al.* 2020).

While the specific action of many chromatin remodelers as well as the exact impact of chromatin opening on the different repair pathways are not well understood yet, several studies suggest that chromatin opening may be integral to efficient DNA repair. The CHD remodelers CHD2 and CHD7 were for example reported to act upstream of lesion recognition in the NHEJ pathway, facilitating the engagement of both Ku70 and XRCC4 (Luijsterburg *et al.* 2016, Rother *et al.* 2020). In contrast, the SWI/SNF remodeler BRG1 acts further down-stream in nucleotide excision repair, impacting the recruitment of the down-stream factors XPG and PCNA (Zhao *et al.* 2009). This suggests that different chromatin remodelers have specific functions in DNA repair pathways. However, further insight in the remodeling reactions and impact of remodelers on the different pathways is needed to fully understand the implication of chromatin opening in the DNA damage response.

In addition to the fast, initial decompaction of chromatin, the subsequent compaction phase is significantly slower and was reported to persist for up to 24 hours (Strickfaden *et al.* 2016). In this phase, the histone variant macroH2A1 is recruited to DNA lesions, promoting the compaction through the inhibition of PARP1 (macroH2A1.1 isoform; Timinszky *et al.* 2009) or the introduction of the repressive histone mark H3K9 methylation (macroH2A1.2 isoform; Khurana *et al.* 2014). By removing acetylation marks, HDAC proteins further support the recondensation of chromatin (Rother *et al.* 2020). While this phase is significantly less well characterized than the initial chromatin opening, it also seems to be crucial for thorough DNA repair signaling (Burgess *et al.* 2014). Impairing chromatin condensation by interfering with macroH2A recruitment or histone deacetylation was for example shown to hinder the timely engagement of either the homologous recombination factor BRCA1 or NHEJ factors (Miller *et al.* 2010, Khurana *et al.* 2014). Interestingly, a recent study focused on the chromatin remodeler CHD7 further found a tight link between remodeler-mediated chromatin opening and subsequent recondensation by post-translational modifications. CHD7 first contributes to

chromatin opening in NHEJ through its remodeling activity, and subsequently recruits HDAC1/2 enzymes, which impact the recondensation phase in this repair pathway (Rother *et al.* 2020). Understanding the impact of additional chromatin compacting factors in a similar manner may help to further deduce the relevance of these chromatin re-arrangements on the various DNA repair pathways.

4.3 PARP enzymes are early signaling proteins of the DNA damage response

4.3.1 PARP enzymes generate the post-translational modification poly-(ADP-ribose)

In the intricate network of the DNA damage response, the poly-(ADP-ribose) polymerases (PARP) PARP1, PARP2 and PARP3 take over the key role to coordinate the early steps of DNA repair in a chromatin context (Ray Chaudhuri and Nussenzweig 2017). To this end, they dynamically generate the post-translational modification poly-(ADP-ribose) by transferring ADP-ribose moieties from the co-factor NAD+ onto several acceptor amino acids of target proteins, particularly onto serine, aspartate and glutamate residues (**Figure 11**; Leung 2014, Langelier *et al.* 2018)

The post-translational modification is catalyzed by the catalytic ART domain that is commonly shared by all 17 family members of PARP enzymes (Otto *et al.* 2005). The nuclear localized PARP1, PARP2 and PARP3 proteins additionally contain a WGR domain as well as zinc fingers (PARP1) or unstructured N-terminal domains (PARP2 and PARP3), which allow the enzymes to bind to a variety of DNA damages, such as nicks, single- and double-strand breaks (Langelier *et al.* 2012, 2014, Riccio *et al.* 2015, Grundy *et al.* 2016, Obaji *et al.* 2016). Binding to DNA lesions strictly regulates and activates the catalytic activity of these PARP enzymes (Langelier *et al.* 2018). While PARP3 is only capable of introducing mono-ADP-ribose, PARP1 and PARP2 can extend the initially transferred ADP-ribose residues into long chains or branches of the modification (**Figure 11**; Vyas *et al.* 2014). The proteins do not only mark histones and proteins in the vicinity with poly-(ADP-ribose), but also auto-modify themselves drastically (Messner *et al.* 2010, Daniels *et al.* 2014, Martello *et al.* 2016). PARP1 hereby provides about 90% of the PAR signal, while PARP2 only contributes a minor part (Shieh *et al.* 1998, Amé *et al.* 1999), but is suggested to introduce more branched PAR chains (Chen *et*

al. 2018). Recent *in vitro* studies further indicated that PARP1-3 can also add ADP-ribose to the 5'-phosphate of DNA (Talhaoui *et al.* 2016, Munnur and Ahel 2017, Belousova *et al.* 2018, Zarkovic *et al.* 2018); however, the occurrence and implication of this modification in cells is still unclear.

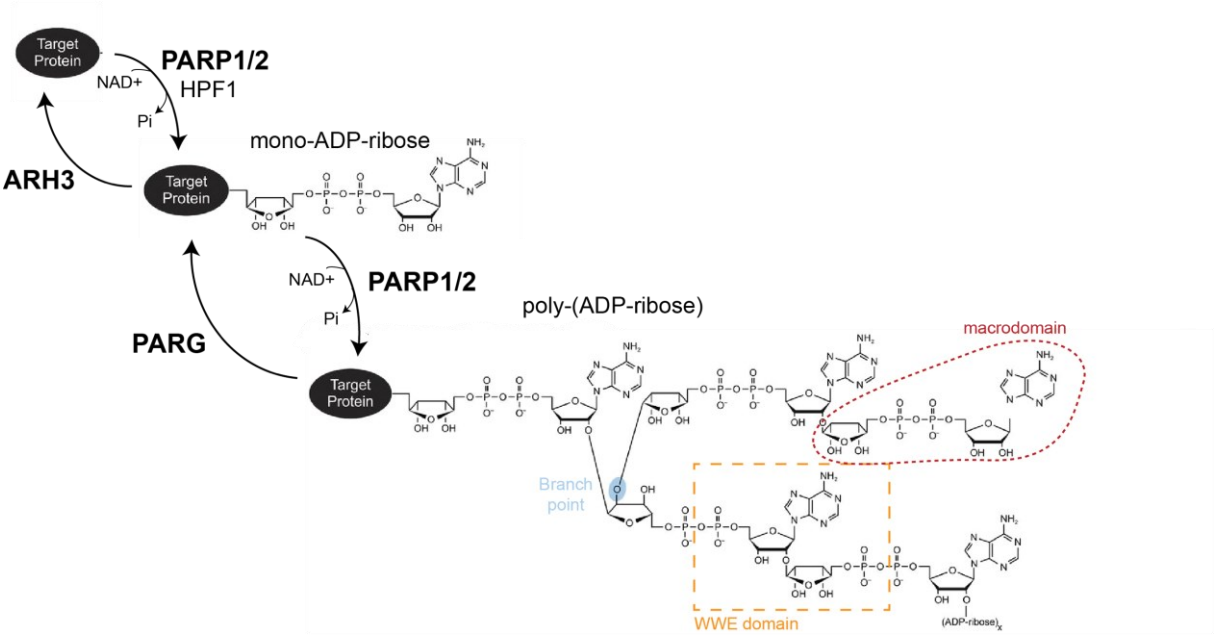

Figure 11 The post-translational modification poly-(ADP-ribose) is dynamically introduced, read and removed

The poly-(ADP-ribose) polymerases PARP1 and PARP2 use the co-factor NAD+ to establish ADP-ribose moieties on target proteins. At DNA lesions, they are further supported by HPF1 to introduce mono-ADP-ribosylation on serine residues. In a processive reaction, PARP1/2 continuously add ADP-ribose moieties onto the growing poly-(ADP-ribose) chain, which can be linear or branched. Different components of poly-(ADP-ribose) can be recognized: The macrodomain binds terminal ADP-ribose moieties, while the WWE domain detects iso-ADP-ribose linkages. PARG removes ADP-ribose from the poly-ADP-ribose chain, while ARH3 cleaves the residual mono-ADP-ribose moiety from the target protein. This figure was modified from Leung 2014 with permission from Rockefeller University press.

In addition to DNA binding, recent studies have found that PARP1 and PARP2 are further regulated by the co-factor HPF1 at DNA lesions, which complements the catalytically active site of the PARP enzymes to modify serine residues, the major target amino acid upon DNA damage (Gibbs-Seymour *et al.* 2016, Bonfiglio *et al.* 2017, Palazzo *et al.* 2018, Bilokapic *et al.* 2020, Suskiewicz *et al.* 2020). While the exact role of HPF1 in DNA repair is still poorly understood, it was shown to switch PARP1/2's activity from auto-modification to trans-ADP ribosylation of histones (Gibbs-Seymour *et al.* 2016, Palazzo *et al.* 2018), suggesting a key role

of HPF1 in regulating different PAR-dependent processes. In a recent News & Views article, I discussed the newly gained knowledge about the reaction mechanism of PARP enzymes as well as the implication of HPF1 in detail (Blessing and Ladurner 2020; see **Appendix B** of this thesis).

Poly-(ADP-ribose) is a highly dynamic modification, not only due to the processive addition of ADP-ribose to extend existing PAR chains, but also due to the active and fast removal of PAR by hydrolases (**Figure 11**; Crawford *et al.* 2018). The poly-(ADP-ribose) glycohydrolase enzyme PARG cleaves PAR chains in a processive manner, and thus balances the extent and duration of the PAR signal at DNA damage sites (Gupte *et al.* 2017). It can thereby cleave PAR chains in an endo- and exoglycosidic manner, which results in the release of free PAR chains or mono-ADP-ribose, respectively (Hatakeyama *et al.* 1986, Barkauskaite *et al.* 2013). However, PARG is not able to cleave the terminal ADP-ribose moiety from target proteins (Slade *et al.* 2011). The removal of mono-ADP-ribose therefore requires the action of additional proteins. At DNA lesions, PARG is supported by the hydrolase ARH3, which can cleave serine-linked mono-ADP-ribose residues (Fontana *et al.* 2017). ARH3 is also able to cleave poly-(ADP-ribose) chains; however, its processivity reduces with increasing length of PAR polymers (Oka *et al.* 2006, Ono *et al.* 2006), suggesting that PARG is the dominant enzyme to cleave PAR, while ARH3 cleaves the terminal mono-ADP ribose moiety. In addition to ARH3, the hydrolases MacroD1, MacroD2, and TARG1 are able to cleave mono-ADP-ribose from other target residues (Jankevicius *et al.* 2013), but their implication in cellular processes and the DNA damage response are not yet well understood.

Excessive PAR formation seems to be detrimental for the cell, as it activates parthanatos, a special caspase-independent cell death pathway, which is prevented by PARG activity (Andrabi *et al.* 2006, Yu *et al.* 2006). The deletion of PARG further results in severe genotoxicity and replication stress, and is embryonically lethal in mice, highlighting the relevance of timely PAR removal for the integrity of DNA repair and cellular homeostasis (Koh *et al.* 2004).

4.3.2 Poly-(ADP-ribose)-dependent processes modulate the outcome of DNA repair

In the DNA damage response, PARP1 and PARP2 rapidly respond to various types of DNA lesions, where they introduce PAR as a cellular warning signal (Ray Chaudhuri and Nussenzweig 2017). Not only the generated modification itself is dynamic and diverse, also its

readout is highly versatile. Several PAR-binding motifs and domains exist that can bind different sub-structures on the complex PAR chain (Teloni and Altmeyer 2016). Their distribution ranges from common to highly specialized reader domains. While the loosely defined, 20 amino acid long PAR binding motif supposedly binds PAR in a relatively unspecific manner via electrostatic interactions of basic amino acids (Pleschke *et al.* 2000, Gagné *et al.* 2008), globular PAR-binding domains interact specifically with distinct ADP-ribose moieties (**Figure 11**). Macrodomains for example bind terminal ADP-ribose moieties (Karras *et al.* 2005, Timinszky *et al.* 2009), while WWE domains recognize iso-ADP-ribose linkages within the PAR chain (Wang *et al.* 2012).

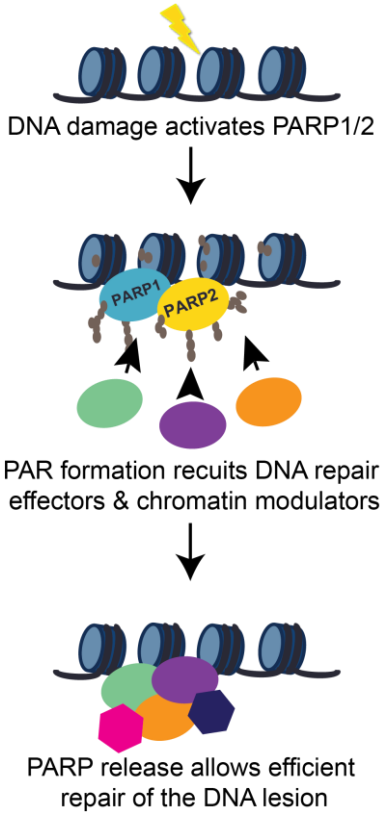

Figure 12 Poly-(ADP-ribose) recruits DNA damage response proteins to DNA lesions

The catalytic activity of PARP1/2 is activated by binding different DNA lesions. The dynamic formation of poly-(ADP-ribose) recruits the necessary DNA repair proteins as well as chromatin modulators that re-arrange the chromatin environment around the DNA lesion. The subsequent release of PARP1/2 is required to transition to later phases of the DNA process and allows the efficient repair of the lesion.

Given the recognition of DNA lesions and the rapid formation of PAR, the modification modulates early key steps of different DNA repair pathways through the PAR-dependent recruitment of integral DNA repair proteins and chromatin modulators (**Figure 12**; Ray Chaudhuri and Nussenzweig 2017). Originally, poly-(ADP-ribose) was described to be required for the efficient repair of single-strand breaks by recruiting the scaffolding protein XRCC1 via its PAR-binding BRCT domain (El-Khamisy *et al.* 2003, Fisher *et al.* 2007). By now, PAR was also shown to be implicated in nucleotide excision and double-strand break

repair (Robu *et al.* 2013, Beck *et al.* 2014). The modification ensures the efficient induction of homologous recombination through the recruitment of MRE11 and NBS1 (Haince *et al.* 2008), promotes non-homologous end joining (Luijsterburg *et al.* 2016) and further stimulates the back-up pathway alternative end-joining (Mansour *et al.* 2010). Single-strand- and double-strand break-induced PARylation seems further critical in the context of DNA replication, where PAR-dependent mechanisms stabilize stalled and collapsed replication forks and prevent the accumulation of unligated Okazaki fragments in the vicinity of replication forks (Ying *et al.* 2012, Berti *et al.* 2013, Hanzlikova *et al.* 2018). The various activities are hereby mainly attributed to PARP1 due to its large contribution to PAR formation, while the functions of PARP2 remain largely unknown (Ray Chaudhuri and Nussenzweig 2017). However, the combined deletion of PARP1 and PARP2 results in severe sensitivity to DNA-damaging agents and is embryonically lethal in mice, while the single deletions are viable, suggesting that PARP2 takes over important roles in the repair of DNA lesions in addition to PARP1 (Menissier de Murcia 2003, Ronson *et al.* 2018).

Interestingly, also many chromatin-modifying proteins are recruited to DNA lesions in a PAR-dependent manner (Ray Chaudhuri and Nussenzweig 2017). PAR can thus be seen as an intricate modulator of the DNA damage response that tightly links chromatin changes around DNA damage sites to the DNA repair process itself. WWE domains are for example found in several E3 ubiquitin ligases, which introduce ubiquitination signals at DNA lesions (Kang *et al.* 2011, Wang *et al.* 2012), while macrodomains are part of the chromatin remodeler ALC1 and the histone variant macroH2A (Ahel *et al.* 2009, Gottschalk *et al.* 2009, Timinszky *et al.* 2009), which establish the following changes in chromatin dynamics at PARylation sites.

4.3.2.1 The chromatin remodeler ALC1 relaxes chromatin by binding poly-(ADP-ribose)

The prime example of a PAR-binding protein is the macrodomain-containing chromatin remodeler ALC1, whose localization and activity are tightly controlled by its PAR-binding activity (Ahel *et al.* 2009, Gottschalk *et al.* 2009). Located at the C-terminus of the protein, the macrodomain folds back onto the N-terminally localized ATPase domains in the inactive state of the protein (**Figure 13**), thus inhibiting the chromatin remodeling activity (Lehmann *et al.* 2017, Singh *et al.* 2017). Upon DNA damage, the macrodomain rapidly binds to poly-(ADP-ribose) (Ahel *et al.* 2009, Gottschalk *et al.* 2009). This does not only recruit the remodeler to sites of PAR modification, but also allows the opening of the protein conformation, and thus the active engagement of the ATPase domains with chromatin (Lehmann *et al.* 2017, Singh *et*

al. 2017). PAR binding thus tightly constricts the chromatin remodeling activity of ALC1 to PAR activation sites, such as DNA lesions.

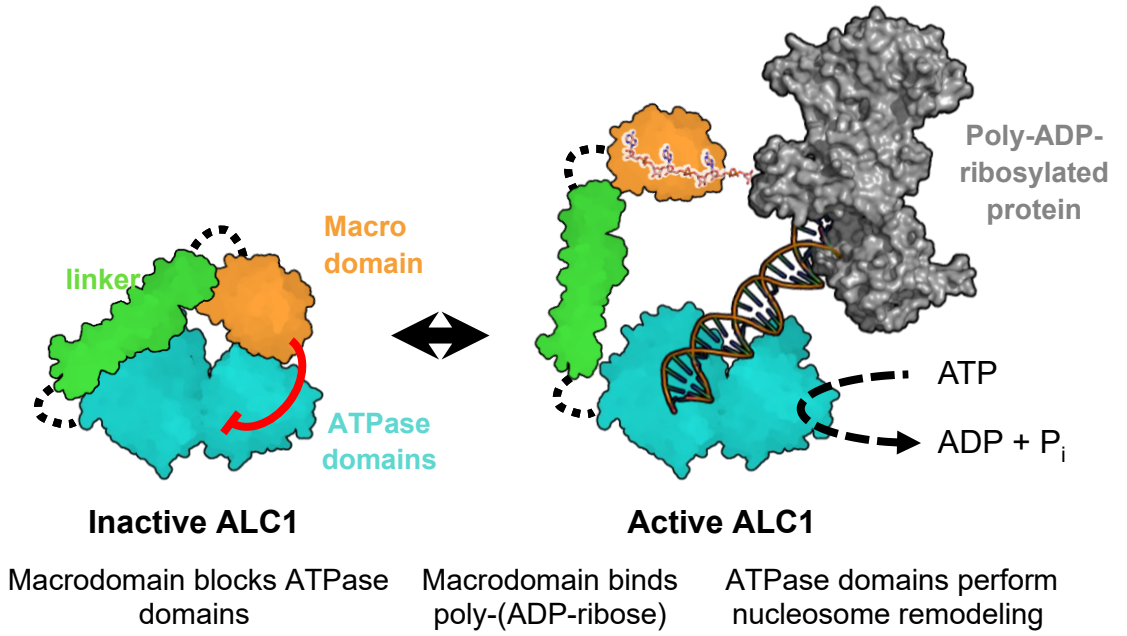

Figure 13 The ALC1 remodeling enzyme is activated by poly-(ADP-ribose)

In an inactive state, the chromatin remodeler ALC1 is found in a closed conformation, where the macrodomain folds back and inhibits the ATPase domains. Upon binding of the macrodomain to poly-(ADP-ribose), the protein conformation opens and the ATPase domains can perform ATP hydrolysis to remodel nucleosomes. The model was kindly provided by my colleague Gunnar Knobloch.

While the PAR-dependent activation mechanism of ALC1 is relatively well understood, its cellular role has remained largely enigmatic. At DNA lesions, ALC1 was shown to be one of the key contributors to the fast opening of chromatin around DNA lesions (Sellou *et al.* 2016); however, the implication of this ALC1-mediated chromatin remodeling on DNA repair and potential down-stream processes has not been unraveled yet. Interestingly, several studies suggest that ALC1-mediated chromatin remodeling may be integral to the DNA damage response, as the deletion of the chromatin remodeler rendered cells sensitive to a variety of DNA-damaging agents and replication stress (Ahel *et al.* 2009, Pines *et al.* 2012, Tsuda *et al.* 2017, Ooka *et al.* 2018). Determining the function(s) of ALC1 in DNA repair may thus help to establish the relevance of chromatin dynamics in this process.

Understanding the cellular role of ALC1 may be particularly interesting due to its tight link to the formation of cancerous states. The protein was first identified as an oncogene in hepatocellular carcinoma, located in the frequently amplified chromosomal region 1q21 (Ma *et al.* 2008), and has now also been found overexpressed in various other cancer types such as breast, lung, and colon cancer (Ji *et al.* 2013, Su, Ding, *et al.* 2014, Su, Zhao, *et al.* 2014, Wu *et al.* 2014, He *et al.* 2015). The upregulation of the oncogene ALC1 drives cancer progression and metastasis and is correlated with decreased patient survival and poorer outcome of chemotherapy (Chen *et al.* 2010, Ji *et al.* 2013, Wu *et al.* 2014); however, the underlying molecular mechanisms of de-regulation are poorly understood. Determining the cellular role of ALC1 may thus unveil molecular pathways that could be targeted for the treatment of ALC1-overexpressing cancers.

4.3.2.2 The histone variant macroH2A inhibits the activity of PARP1

Another PAR-binding protein with impact on chromatin dynamics is the histone variant macroH2A. Belonging to the H2A family of histone variants, macroH2A contains a macrodomain in addition to the classical H2A histone fold, which is connected through a flexible linker region and extends from the nucleosome core (Pehrson and Fried 1992). In total, three isoforms of the histone variant exist, which exhibit structural differences in their macrodomains, their ability to bind ADP-ribose and their tissue-specific expression (Pehrson *et al.* 1997, Chadwick and Willard 2001, Kustatscher *et al.* 2005, Sporn *et al.* 2009). The macrodomain of macroH2A1.1 was shown to efficiently bind poly-(ADP-ribose) at the terminal ADP-ribose moiety and thus "cap" growing poly-(ADP-ribose) chains (**Figure 14**; Timinszky *et al.* 2009). This hinders PARP1 to add further ADP-ribose moieties onto its chains and further protects the PAR chain from PARG-mediated cleavage (Nusinow *et al.* 2007, Ruiz *et al.* 2019). In this manner, macroH2A1.1 promotes the repair of DNA double strand breaks through regulating the poly-(ADP-ribose) response and promoting chromatin compaction (Timinszky *et al.* 2009, Ruiz *et al.* 2019). The PAR-dependent action of macroH2A1.1 further seems to regulate the transcription of target genes (Nusinow *et al.* 2007), and to control mitochondrial respiration by limiting nuclear NAD+ consumption (Marjanović *et al.* 2017).

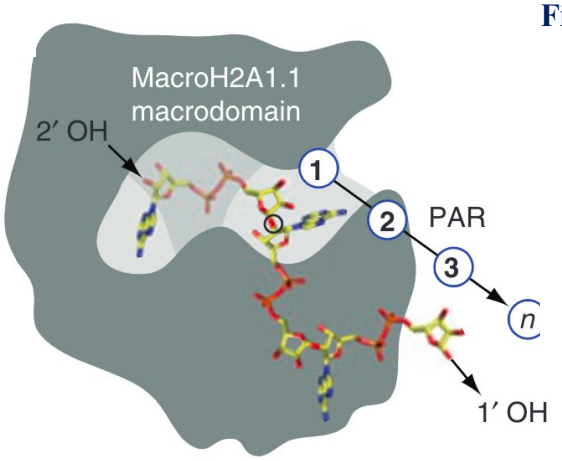

Figure 14 The macroH2A1.1 macrodomain binds terminal poly-(ADP-ribose) chains

The macrodomain of macroH2A1.1 caps the terminal ADP-ribose residues of poly-(ADP-ribose) chains, thus preventing their prolongation by PARP1. The figure was modified from Timinszky *et al.* 2009 with permission from Springer Nature.

In contrast to macroH2A1.1, the two other isoforms of macroH2A, macroH2A1.2 and macroH2A2, are incapable of binding to poly-(ADP-ribose) (Timinszky *et al.* 2009). Interestingly, these isoforms have also been described to play a role in DNA double-strand repair (macroH2A1.2) and the maintenance of compacted heterochromatin domains (macroH2A1.2 and macroH2A2); however, their exact role and function have remained largely enigmatic, as they could not be linked to PARP function and metabolism (Khurana *et al.* 2014, Douet *et al.* 2017). Establishing how the different isoforms of macroH2A form compacted chromatin domains may help to understand their contribution to chromatin-templated mechanisms, such as the compaction of chromatin at DNA lesions.

4.4 PARP inhibition – a new concept for cancer therapy

The broad implication of PARP enzymes in DNA repair processes has recently gained increasing attention, as small molecule inhibitors against PARP proteins (PARPi) were successfully developed to treat breast, ovarian, pancreatic and prostate cancers in the clinic (Mateo *et al.* 2019). The treatment with PARPi sensitizes cancer cells to DNA damage, which can be exploited in monotherapies, or in the combination with other DNA repair vulnerabilities based on the concept of synthetic lethality (Pilié *et al.* 2019) .

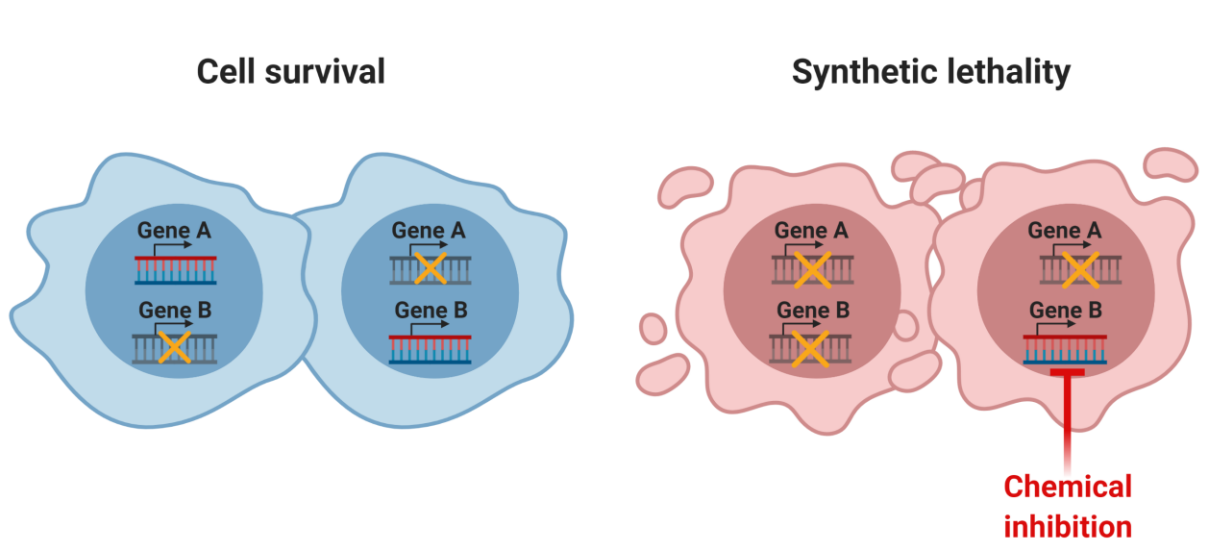

Figure 15 The combination of genetic mutations may result in synthetic lethality

The concept of synthetic lethality describes the observation that the single mutation of two genes is viable (left), while the combination of the respective mutations results in cell death (right). By chemically inducing synthetic lethality with small-molecule inhibitors against gene product B, this concept can also be exploited in cancer therapy to specifically target cancer cells harbouring a mutation in gene A. Healthy cells of the same organism should thereby not be affected, as they lack the mutation in gene A, which drives the synthetic lethal cell death. Created with BioRender.com.

Synthetic lethality describes the observation that cells only die if two genes are simultaneously impaired (**Figure 15**; O'Neil *et al.* 2017). In contrast, cells with the corresponding single genetic mutation are healthy, as they buffer the loss of the other gene (O'Neil *et al.* 2017). Originally observed in fruit flies and yeast (Wright and Dobzhansky 1946, Lucchesi 1968, Kaiser and Schekman 1990, Bender and Pringle 1991), this concept has recently been extended to the development of novel and specific cancer treatments (Hartwell *et al.* 1997, Huang *et al.* 2020). Exploiting the increasing degree of tumour genotyping in which the mutations of each tumour are identified, the specific vulnerabilities of the cancer tissue can be identified and exploited by treating the patient with small-molecule inhibitors against the synthetic lethal targets the tumour is dependent on (Huang *et al.* 2020). This does not only allow to specifically target mutated cancer cells and thus reduce side effects in healthy, non-mutated tissue, but may further enable to potentially develop treatment regiments for any available cancer mutation, also those that have been previously considered undruggable, such as tumour suppressor mutations (O'Neil *et al.* 2017).

Powerful gene editing technologies, such as RNAi, CRISPR/Cas9 or gene trap mutagenesis, have paved the way to identify synthetic lethal interactions in an unbiased manner (De La Cruz *et al.* 2015, O'Neil *et al.* 2017). Genome-wide synthetic lethality screens based on these techniques allow to identify context-specific synthetic lethal interactions as well as potential resistance markers in a multitude of cancer cell lines (McDonald *et al.* 2017, Zimmermann *et al.* 2018, Behan *et al.* 2019). Novel insights gained from these screens can serve as promising entry points for the development of a plethora of novel treatment regiments for cancer therapy, some of which are currently tested in clinical trials to broaden the indication of PARPi, the first synthetic lethal drugs that are successfully exploited in the clinic (Lord and Ashworth 2017, Huang *et al.* 2020).

4.4.1 PARP inhibitors catalytically inhibit and "trap" PARP enzymes

PARPi are analogs of the cofactor NAD+ that are designed to inhibit PARP1, and the closely related PARP2 (Ferraris 2010, Thorsell *et al.* 2017). The inhibitors thus impair the catalytic activity of PARP1/2 and the formation of PAR at DNA lesions (**Figure 16**; Menear *et al.* 2008, Jones *et al.* 2009, Shen *et al.* 2013, Slade 2020). While inhibited, PARP1/2 still recruit to DNA damage sites; however, their association with damaged chromatin is prolonged, a process that was termed "PARP trapping" (Murai *et al.* 2012, 2014).

"Trapped" PARP molecules are thought to physically block DNA lesions from efficient repair, and thus increase genome instability in cancer cells in addition to the catalytic deficiency of the enzymes (Shen *et al.* 2015). In fact, it was suggested that the degree of PARP trapping, rather than catalytic inhibition, determines the clinical potency of different PARP inhibitors (Lord and Ashworth 2017). This was based on the observation that the catalytic inhibition of PARP1 differed only 40-fold *in vitro* when inhibited by the PARPi veliparib, rucaparib, olaparib, niraparib and talazoparib, while PARP1 trapping diverged up to 10,000-fold and closely correlated with the cytotoxicity observed in PARPi-treated cells (Murai *et al.* 2014, Hopkins *et al.* 2015, 2019). Talazoparib was thereby determined as the most potent in PARP trapping, while niraparib, olaparib and rucaparib exhibited intermediate, and veliparib only mild trapping effects (Hopkins *et al.* 2015, 2019). Biophysical measurements recently suggested that the differences in PARP trapping may be due to distinct allosteric mechanisms in the PARP1 enzyme induced by different PARPi, which impact PARP1's binding and retention on DNA to varying degrees *in vitro* (Zandarashvili *et al.* 2020). However, the exact mechanism and dynamics of PARP trapping and its effects on down-stream processes in the DNA damage

response are still poorly understood. It is further not known whether and how this process is regulated intracellularly and if PARP2, despite its minor contribution to PAR formation, impacts the clinical potency of PARP inhibitors. Increasing the knowledge of the PARP trapping process may not only be of interest for basic researchers, but may also help to design more effective treatment regiments based on the unique properties of the distinct PARP inhibitors.

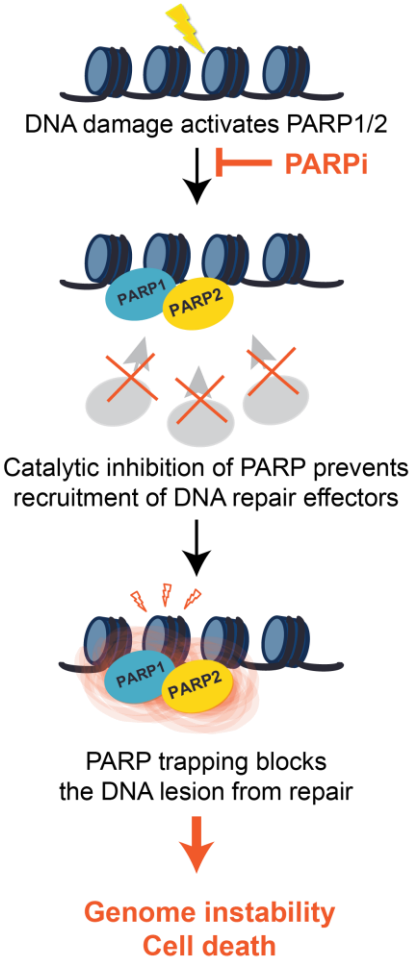

Figure 16 PARP inhibitors impair the DNA damage response

PARP inhibitors impair DNA repair processes through two main mechanisms. Firstly, they inhibit the catalytic activity of PARP1/2, which prevents the recruitment of PAR-dependent factors to DNA lesions. In addition, several PARP inhibitors restrict the release and trap PARP1/2 at DNA lesions, which physically blocks the lesion from DNA repair. Both mechanisms contribute to the accumulation of unrepaired DNA lesions and thus trigger genome instability and cell death of the treated cells.

4.4.2 PARP inhibitors can be exploited to treat cancers in the clinic

In 2005, two groups made the astonishing finding that the inhibition of PARP enzymes in BRCA-deficient cells results in massive cell death, while wild-type cells remain largely unaffected by PARP inhibitor treatment (Bryant *et al.* 2005, Farmer *et al.* 2005). This synthetic lethal interaction between PARP and BRCA1/2 paved the way for the clinical development of PARP inhibitors to treat BRCA-deficient cancers (Lord and Ashworth 2017). Due to the high frequency of BRCA mutations in ovarian cancer, several PARP inhibitors were first tested in clinical trials and approved as a maintenance therapy for this cancer type (Ledermann *et al.*

2012, Kaufman *et al.* 2015, Pujade-Lauraine *et al.* 2017). More recently, PARP inhibitor treatment was extended to BRCA1/2-deficient breast, pancreatic and prostate cancer, demonstrating that the principle of synthetic lethality between PARP and BRCA can be used for cancer therapy across different cancer types (Robson *et al.* 2017, Litton *et al.* 2018, Golan *et al.* 2019, Abida *et al.* 2020, de Bono *et al.* 2020, Hussain *et al.* 2020).

Interestingly, while performing clinical studies on ovarian cancer patients, several studies also reported increased responses in progression-free survival in cells with wildtype BRCA genes (Gelmon *et al.* 2011). This led to the additional approval of PARP inhibitors for maintenance therapy of platinum-sensitive ovarian cancer independent of its BRCA status (Mirza *et al.* 2016, Coleman *et al.* 2017). Particularly, cancers with mutations in genes encoding homologous recombination and DNA repair factors showed a high response to PARP inhibitors, suggesting that these synthetic lethality phenotypes could be used as indicators of a positive PARP inhibitor response in patients (Mirza *et al.* 2016). This also holds true for prostate cancer, where the PARP inhibitor olaparib is now approved to treat patients that either have BRCA1/2 mutations, or instead display deficiencies in one of 11 other genes of the DNA damage response, such as ATM and RAD51 (de Bono *et al.* 2020, Hussain *et al.* 2020). This increasing patient population sensitive to PARP inhibitor treatment thus makes these small molecule drugs a highly promising advancement in cancer research.

The repertoire of PARP inhibitor treatments could be further increased by combining the use of PARP inhibitors with other agents that render tumor cells susceptible to DNA damage (Yap *et al.* 2019). A prime example would be the combination of PARP inhibitors with chemotherapy to increase the extent of DNA damage in cancer cells (Lord and Ashworth 2017). However, initial clinical studies resulted in severe side effects, particularly myelosuppression, upon co-treatment (Oza *et al.* 2015). Let alone the weakly trapping, and thus least potent PARP inhibitor veliparib was found tolerable in combination with chemotherapeutic agents and is evaluated in a phase III study in this setting (Coleman *et al.* 2015).
An exciting alternative approach would be to combine PARP inhibitors with other inhibitors targeting the DNA damage response (Lord and Ashworth 2017). In fact, several preclinical studies demonstrated that a series of DNA repair proteins, as well as proteins indirectly influencing the DNA damage response, such as cell cycle and chromatin remodeling proteins, are synthetic lethal with PARP inhibitors (McCabe *et al.* 2006, Bajrami *et al.* 2014, Zimmermann *et al.* 2018). This BRCA-mimicking phenotype was termed “BRCAness”

(Ashworth and Lord 2018). Given that several inhibitors against the DNA damage response proteins, such as ATM, ATR and DNA-PK and the cell cycle proteins CHK1/2 and WEE1, are currently undergoing clinical trials, testing the combination of such inhibitors with PARP inhibitors for synthetic lethality phenotypes in cancer patients may provide novel options for treatment regiments (Cleary *et al.* 2020, Damia 2020, Ghelli Luserna Di Rorà *et al.* 2020, Lavin and Yeo 2020, Barnieh *et al.* 2021). Excitingly, the respective clinical trials, combining the PARPi olaparib or niraparib with the above-mentioned DNA repair inhibitors, have been started recently, and it will be exciting to find out whether these combinatorial treatments may provide the opportunity to increase the therapeutic window for PARPi-mediated cancer therapy (Cleary *et al.* 2020).

The combinatorial use of inhibitors could be particularly beneficial for cancers that have developed resistance against PARP inhibitors (Noordermeer and van Attikum 2019). Although PARP inhibitor treatment is generally well tolerated by patients, it ultimately results in the development of resistance mechanisms in the majority of patients after prolonged treatment (Lheureux *et al.* 2017). Often, cancer cells acquire additional mutations which can alleviate the effect of synthetic lethality by re-activating double-strand break repair or decreasing cell cycle progression (Kondrashova *et al.* 2017, Noordermeer and van Attikum 2019). Identifying new biomarkers for PARPi treatment and developing additional small-molecule inhibitors may thus also allow to counteract these resistance mechanisms and may ultimately provide alternative or combined treatment regiments to battle advanced, resistant tumors .

5. Aims of this Thesis

The poly-(ADP-ribose) polymerases 1 and 2 are critical in the early DNA damage response, as they introduce the post-translational modification poly-(ADP-ribose) (PAR) (Beck *et al.* 2014). Acting as a cellular warning signal, the modification modulates the efficient recruitment of the necessary DNA repair proteins and further coordinates the re-arrangement of chromatin around DNA lesions (Ray Chaudhuri and Nussenzweig 2017). While the distinct contributions of the core DNA repair factors are largely known, the implication of the chromatin response at DNA lesions on the outcome of the DNA damage response is still poorly understood.

Upon DNA damage, the chromatin environment is greatly re-arranged around the lesion. This process can be broadly distributed into two phases: An initial fast and transient phase of chromatin opening, that is followed by a slower phase of chromatin re-compaction (Kruhlak *et al.* 2006, Strickfaden *et al.* 2016). To understand the impact of the different phases of chromatin re-arrangements at DNA lesions, my Ph.D. research focused on two proteins that have been described to play a role in the distinct stages of the chromatin response despite sharing a PAR-binding macrodomain: the chromatin remodeler ALC1 and the histone variant macroH2A. I therefore aimed to understand their unique functions in the reorganization of chromatin.

5.1 Aim I: Determine the role of ALC1-mediated chromatin remodeling in DNA repair

The chromatin remodeler ALC1, an oncogene frequently amplified in cancer, is rapidly recruited to DNA damage sites in a poly-(ADP-ribose)-dependent manner, where it relaxes local chromatin structure (Gottschalk *et al.* 2009, Sellou *et al.* 2016). However, the functional consequences of this recruitment and chromatin decompaction on the DNA damage response have remained enigmatic.

The primary aim of my Ph.D. was therefore to establish the role of ALC1-mediated chromatin remodeling on the cellular DNA damage response. Given the clinical relevance of PARP inhibitors in treating BRCA-deficient cancers, I particularly aimed to understand if ALC1 chromatin remodeling affects the DNA damage response to PARP inhibitors. To deduce the mechanism and the consequences of chromatin remodeling by ALC1 in this process, I addressed the following research questions:

- Are ALC1-deficient cells sensitive to PARPi treatment?

- Does ALC1-mediated chromatin remodeling directly affect the recruitment or release kinetics of the PARP enzymes PARP1 and PARP2? How does this compare to PARP trapping effects induced by PARPi?
- Through which DNA repair pathway does ALC1 regulate the PARPi response and which PARP enzyme mediates these defects?
- Could a deletion or inhibition of ALC1 be exploited to treat BRCA-deficient cancers?

Answering these questions would not only allow to understand the impact of ALC1-mediated chromatin opening on the DNA damage response, but may also provide insights whether ALC1 can be exploited as a drug target to enhance or refine PARPi-based cancer therapies in the clinic.

5.2 Aim II: Identify the mode of chromatin compaction by macroH2A isoforms

In contrast to ALC1, macroH2A contributes to chromatin compaction at DNA lesions and in regions of heterochromatin (Khurana *et al.* 2014, Douet *et al.* 2017, Ruiz *et al.* 2019). While the poly-(ADP-ribose)-dependent macroH2A1.1 isoform was shown to promote chromatin compaction by limiting PARP1's activity (Timinszky *et al.* 2009), the mechanism(s) of chromatin compaction by the non-PAR-binding variants macroH2A1.2 and macroH2A have remained unclear.

Marek Kozlowski, a previous Ph.D. candidate in our research group, identified the flexible linker between the macrodomain and the core histone domain as a critical feature of all macroH2A isoforms to condense chromatin. In the context of his main study, I aimed to understand if the linker of macroH2A is necessary and sufficient for the observed chromatin compaction at DNA lesions. In particular, I addressed the question whether the linker and macrodomain of macroH2A1.1 can artificially induce chromatin compaction when transferred onto a core histone H2A protein. These experiments may thus allow to determine the universality of this newly identified mode of chromatin compaction by an unstructured linker element that is shared between all macroH2A isoforms.

6. Publications

6.1 The oncogenic helicase ALC1 regulates PARP inhibitor potency by trapping PARP2 at DNA breaks

The publication can be found here:

Blessing, C., Mandemaker, I.K., Gonzalez-Leal, C., Preisser, J., Schomburg, A., and Ladurner, A.G., 2020. The oncogenic helicase ALC1 regulates PARP inhibitor potency by trapping PARP2 at DNA breaks. Molecular Cell, 80 (5), 862–875.

DOI: 10.1016/j.molcel.2020.10.009
PMID: 33275888
URL: https://www.cell.com/molecular-cell/fulltext/S1097-2765(20)30692-4

6.2 MacroH2A histone variants limit chromatin plasticity through two distinct mechanisms

The publication can be found here:

Kozlowski, M., Corujo, D., Hothorn, M., Guberovic, I., Mandemaker, I.K., **Blessing, C**., Sporn, J., Gutierrez-Triana, A., Smith, R., Portmann, T., Treier, M., Scheffzek, K., Huet, S., Timinszky, G., Buschbeck, M., and Ladurner, A.G., 2018. MacroH2A histone variants limit chromatin plasticity through two distinct mechanisms. *EMBO reports*, 19, e44445.

DOI: 10.15252/embr.201744445
PMID: 30177554
URL: https://www.embopress.org/doi/full/10.15252/embr.201744445

7. Scientific Outlook: The chromatin remodeler ALC1 in cellular physiology and disease

In this thesis work, I established a novel role for the chromatin remodeler ALC1 in modulating the cellular response to PARP inhibitors. By regulating the release of the PARP enzyme PARP2 at DNA breaks, ALC1's chromatin remodeling activity mediates the efficient repair of single-strand breaks. ALC1 manipulation traps PARP2 at DNA lesions and thus potentiates PARPi-mediated cancer cell killing and confers synthetic lethality with BRCA1/2 deficiency. Together with three other recent, orthogonal publications (Juhász *et al.* 2020, Hewitt *et al.* 2021, Verma *et al.* 2021), these findings do not only suggest ALC1 as a promising target for the development of cancer treatment regiments, but also open up a variety of novel research questions surrounding the chromatin remodeler ALC1 and PARPi responses.

7.1 ALC1-mediated chromatin remodeling may impact replication fork stability

In our recent study, we identified the integral role of ALC1 chromatin remodeling in methylmethane sulphonate (MMS)-induced single-strand break repair (Blessing, Mandemaker, *et al.* 2020). We reasoned that this may be the underlying cause for the observed synthetic lethality with the homologous recombination proteins BRCA1 and BRCA2, as a defective single-strand break repair response renders cells more reliant on the faithful repair of double-strand breaks by homologous recombination, a feature that is abrogated in BRCA-deficient cells. A recent study by Hewitt *et al.* confirmed that toxic intermediates at single-strand break repair lesions are the underlying cause of sensitivity and synthetic lethality in ALC1-deficient cells (Hewitt *et al.* 2021). However, it has remained unclear where these lesions arise and how they are transformed into toxic double-strand breaks.

While single-strand breaks are classically thought to be turned into double-strand breaks upon replication fork stalling and collapse at these lesions, more recent studies also suggest a direct implication of PARP inhibitors and BRCA proteins in regulating the speed of DNA replication and the accumulation of replication gaps (Maya-Mendoza *et al.* 2018, Cong *et al.* 2021). Interestingly, two studies reported that single-strand breaks in ALC1-deficient cells frequently arise in the proximity of replication forks (Hewitt *et al.* 2021, Verma *et al.* 2021), raising the question whether PARP2 and ALC1 may play a direct role in DNA replication-associated processes. In fact, it was recently shown that the ligation of Okazaki fragments, a process that requires key single-strand break repair proteins, is the major source of poly-(ADP-ribose)

formation in the absence of exogenous DNA damage and that the interference with this process causes PARPi sensitivity (Hanzlikova *et al.* 2018, Cong *et al.* 2021). It would thus be exciting to investigate whether PARP trapping and ALC1-mediated chromatin remodeling are implicated in Okazaki fragment ligation and how this contributes to the PARPi response. As it is not yet clear whether PARP enzymes can directly trap at such replication intermediates, determining whether PARPi-mediated effects arise from PARP trapping and/or through effects on replication gap repair or replication fork speed may further allow to understand the mechanism of PARPi at replication forks in greater detail. This may not only unravel the underlying processes for synthetic lethality with BRCA proteins, but may also help to dissect whether single-strand breaks are cause or consequence of replication stress upon ALC1-mediated chromatin remodeling and PARP inhibition.

7.2 The mechanism of ALC1 chromatin remodeling at DNA lesions is unknown

While we have gained strong evidence that chromatin remodeling by ALC1 regulates the release and trapping of the PARP2 enzyme at DNA lesions, the underlying mechanism of interaction between the two enzymes, as well as the mode of ALC1-mediated nucleosome remodeling at DNA damage sites has remained unclear. *In vitro* interaction and nucleosome remodeling studies in the presence of ALC1 and PARP2 may allow to decipher the mode of interaction between the two proteins in greater detail. ALC1 may thereby either influence the release of PARP2 from chromatin through direct interaction between the two proteins. Alternatively, the enzyme may indirectly release PARP2 by remodeling the chromatin structure around the lesion. Recent cryo-electron microscopy studies have shown that PARP2 can bridge double-strand breaks, and potentially single-strand breaks, in between two nucleosomes (Bilokapic *et al.* 2020, Gaullier *et al.* 2020). The structures of ALC1 in complex with a single nucleosome further suggest a close engagement of the chromatin remodeler with the nucleosome core particle to perform the remodeling reaction, with interactions of the ATPase domain and the linker domain with the histone H4 tail and the acidic patch, respectively (Lehmann *et al.* 2020, Bacic *et al.* 2021, Wang *et al.* 2021). However, it remains unclear how ALC1 structurally changes the chromatin environment around DNA lesions, and whether and how the interplay with PARP2 is implicated in this process.

So far, little is known about the nature of the relaxed chromatin state around DNA lesions. Several studies have suggested that histones may be evicted and/or redistributed around DNA

lesions upon opening of chromatin (Xu *et al.* 2010, Luijsterburg *et al.* 2012, Zavala *et al.* 2014, Adam *et al.* 2016). Specifically, the eviction of the histone linker H1 has been associated with generating a loose chromatin structure that is permissive to DNA repair (Sellou *et al.* 2016, Strickfaden *et al.* 2016, Fortuny *et al.* 2021). Given that ALC1 can slide nucleosomes *in vitro* (Gottschalk *et al.* 2009) and largely contributes to the early chromatin decondensation at microirradiated DNA damage sites (Sellou *et al.* 2016), it would be exciting to investigate the mode of ALC1 chromatin remodeling at DNA lesions through ChIP-seq or super resolution microscopy of histones and chromatin-bound components. This may not only allow to decipher the mode of ALC1-mediated nucleosome remodeling at DNA lesions, but may further provide new insights in the nature of chromatin changes dependent on the type of induced DNA repair pathway.

7.3 Does ALC1 impact the trapping of PARP1 (variants)?

While we could not detect any influence of the chromatin remodeler ALC1 on PARP1, another study suggested that ALC1 regulates the release of PARP1 from DNA lesions (Juhász *et al.* 2020), while we could not detect an influence of the chromatin remodeler on PARP1. Importantly, their experimental set-up was based on a microirradiation system that preferentially induces double-strand breaks, while we used damage conditions that majorly induced single-strand lesions. It would thus be exciting to investigate whether the engagement of ALC1 with PARP enzymes is differentially regulated at different types of DNA lesions or in response to the strength or quantity of DNA damage applied.

Furthermore, PARP1 contains a widely spread single-nucleotide polymorphism (SNP) at the amino acid position 762, resulting in an amino acid change from valine to alanine (V762A; Cottet *et al.* 2000). This SNP is located in the regulatory HD domain of the PARP1 protein and results in reduced catalytic activity (Wang *et al.* 2007, Beneke *et al.* 2010, Rank *et al.* 2016). However, whether this difference in catalytic activity leads to differential cellular functions or responses to PARP inhibitors remains unknown. Interestingly, the HD domain was recently shown to regulate the degree of PARP1 trapping depending on the type of allostery induced by different PARP inhibitors in this region (Zandarashvili *et al.* 2020). This suggests that the two PARP1 variants may display different trapping behaviors upon PARPi treatment or ALC1 deletion. Using a 355 nm microlaser irradiation system, I could provide first evidence that the SNP may critically determine the cellular response to the PARPi talazoparib (**Appendix C, Figure 17**). While PARP1 A762 showed reduced maximal recruitment and slower release in comparison to the PARP1 V762 variant in untreated cells (**Figure 17A**), treatment with the

PARP inhibitors veliparib and olaparib resulted in similar degrees of trapping of either variant (**Figure 17B, C**). However, the trapping of PARP1 V762 was increased in comparison to PARP1 A762 upon talazoparib treatment (**Figure 17C**), suggesting that PARP1 V762 may induce more severe responses to talazoparib. The PARP1 variant status may thus serve as a biomarker and determine the patient's response to this PARP inhibitor. In contrast, the deletion of ALC1 did not influence the retention of either PARP1 variant (**Appendix C, Figure 18**), suggesting that ALC1 induces the release of PARP enzymes through other mechanisms or domain interactions than the regulatory HD domain, that is exploited by chemical PARP inhibitors. Hydrogen-deuterium exchange mass spectrometry experiments may allow to decipher in detail whether and in which domains ALC1 may introduce allosteric changes in the PARP proteins upon the remodeling of chromatin, and may thus serve as additional strategy to elucidate the interplay between ALC1 and the PARP enzymes in greater detail.

7.4 ALC1 inhibitors may be a compelling therapeutic strategy

The hypersensitivity of ALC1-deficient cells towards PARP inhibitors and their synthetic lethality with BRCA1/2 make ALC1 a compelling target for cancer therapy. Excitingly, a recent study using mouse xenografts showed that the deletion of ALC1 reduced the volume of BRCA-deficient tumors and prolonged their survival already in the absence of any drug (Verma *et al.* 2021). These effects were further improved upon treatment with olaparib, suggesting that the pharmacological inhibition of ALC1 could be exploited to generate alternative therapies for BRCA-deficient tumors and/or to (re-)sensitize cancer cells to PARP inhibitors.

ALC1 was also found to be synthetic lethal with several HR and DNA damage response factors, such as ATM, RAD50, RAD51, DNA2 and UBC13 (Hewitt *et al.* 2021, Verma *et al.* 2021). Given that deficiencies in homologous recombination genes are already approved as alternative biomarkers for PARPi cancer therapies in ovarian and prostate cancers (Mirza *et al.* 2016, Moore *et al.* 2019, de Bono *et al.* 2020, Hussain *et al.* 2020), these findings could form a basis to investigate the expanded use of ALC1 inhibitors in various homologous recombination- or DNA damage response signaling-deficient settings.

Verma *et al.* further found that cells depleted of the known resistance factors 53BP1, REV7 or PARG were still sensitive, albeit to a lesser degree, to PARPi treatment upon ALC1 deletion (Verma *et al.* 2021), suggesting that the targeting of ALC1 may also allow to overcome frequent resistance mechanisms of PARP inhibitors. However, more in-depth investigation on resistant

patient-derived cells or in clinical studies would be required to determine the effectiveness of ALC1 inhibition in overcoming these resistance mechanisms in cancer therapy.

Upon the development of ALC1 inhibitors, close attention should be given to the mode of action of the developed small-molecule inhibitors. In our study, the catalytic inhibition worsened the single-strand break repair response and PARP2 trapping in comparison to the deletion of the enzyme (Blessing, Mandemaker, *et al.* 2020). Identifying the mode of action of ALC1 inhibitors may thus be critical to understand the processes underlying the potential cancer treatment and to prevent unexpected side effects. As the performed synthetic lethality experiments are based on ALC1 deletions (Blessing, Mandemaker, *et al.* 2020, Juhász *et al.* 2020, Hewitt *et al.* 2021, Verma *et al.* 2021), it would be particularly important to establish potential sensitivities and mechanisms of resistance towards ALC1 inhibition by performing large-scale synthetic interaction screens in the presence of ALC1 inhibitors.

Several studies have identified ALC1 as an oncogene, that upon amplification or overexpression drives tumor progression in several cancers, such as hepatocellular carcinomas (Chen *et al.* 2010, Ji *et al.* 2013, Wu *et al.* 2014). However, the effects of ALC1 overexpression on the DNA damage response and whether and how these impact tumor progression and responses to PARP inhibitors is not well understood. One study indeed suggests that the levels of the chromatin remodeling enzyme might impact the degree of PARPi sensitivity, as the exogenous overexpression of ALC1 in WT and BRCA-depleted cells rendered cells less sensitive to olaparib treatment (Juhász *et al.* 2020). However, the implication of these findings for cancer therapy are not yet known. To establish whether ALC1 overexpression may contribute to resistance against PARPi treatment, the degree of PARPi sensitivity in dependence of ALC1 expression levels should be compared to other, known resistance mechanisms, ideally in patient-derived cells or xenograft mouse models. This may provide novel insights whether the expression level of ALC1 could be used as a biomarker for PARPi responses and may thus advance indications and treatment options for PARPi in cancer therapy.

7.5 The chromatin remodeler ALC1 may regulate transcription

Besides its newly established role in the DNA damage response, little is known about the cellular functions of the chromatin remodeler ALC1. Given its strong dependence on poly-(ADP-ribose) for the activation of its catalytic activity, it is tempting to speculate that ALC1

may support or regulate the function of PARP1/2 in other cellular processes. In addition to its key function in DNA repair, PARP1 is also involved in controlling the transcription of various genes by acting as a transcriptional co-regulator at promoter sites (Gupte *et al.* 2017). To investigate whether ALC1 may also play a role in these processes, it would be exciting to determine the degree of PARylation and the co-recruitment of ALC1 to promoters. Comparing transcriptome changes in response to PARP1 and/or ALC1 deletion may further reveal the degree of implication and regulation of ALC1-mediated transcription.

While the role of PARP1 in regulating transcription is relatively well established, little is known about the effect of PARP inhibitors on transcription. It is further not known whether PARP enzymes can be trapped at promoter sites. Interestingly, a recent study implicated that both the PARP inhibitor olaparib and the chromatin remodeler ALC1 may regulate the transcription of DNA damage signaling genes in hepatocellular carcinoma (Yang *et al.* 2021). However, their relationship and the mechanism of transcription regulation remained poorly understood. Studying the role of ALC1 and PARP inhibitors in transcriptional regulation may thus not only increase the understanding of the role and regulation of ALC1 in cellular physiology, but may also provide novel insights in the effects of PARP inhibitor treatment on cancer cells, and may thus open up new avenues of cancer therapy.

Chromatin-templated mechanisms, such as gene transcription, DNA replication and repair lie at the heart of cellular physiology. The intactness of these processes allows the faithful and coordinated action of the cell, entire organs and organisms. The chromatin remodeler ALC1 seems to be an integral component of such mechanisms. Investigating its role in different cellular processes may thus not only enhance our understanding of the regulation of chromatin-templated mechanisms, but also increase our understanding of disease mechanisms and may provide treatment options for difficult-to-treat disease phenotypes, such as cancer.

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Appendix A: Restraining and unleashing chromatin remodelers – structural information guides chromatin plasticity

The publication can be found here:

Blessing, C., Knobloch, G., and Ladurner, A.G., 2020. Restraining and unleashing chromatin remodelers – structural information guides chromatin plasticity. *Current Opinion in Structural Biology*, 65, 130–138.

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URL: http://www.sciencedirect.com/science/article/pii/S0959440X20301081

Appendix B: Tickling PARPs into serine action

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PMID: 32231290
URL: https://www.nature.com/articles/s41594-020-0412-x

Appendix C: PARP1 variants are trapped differentially by the PARPi talazoparib

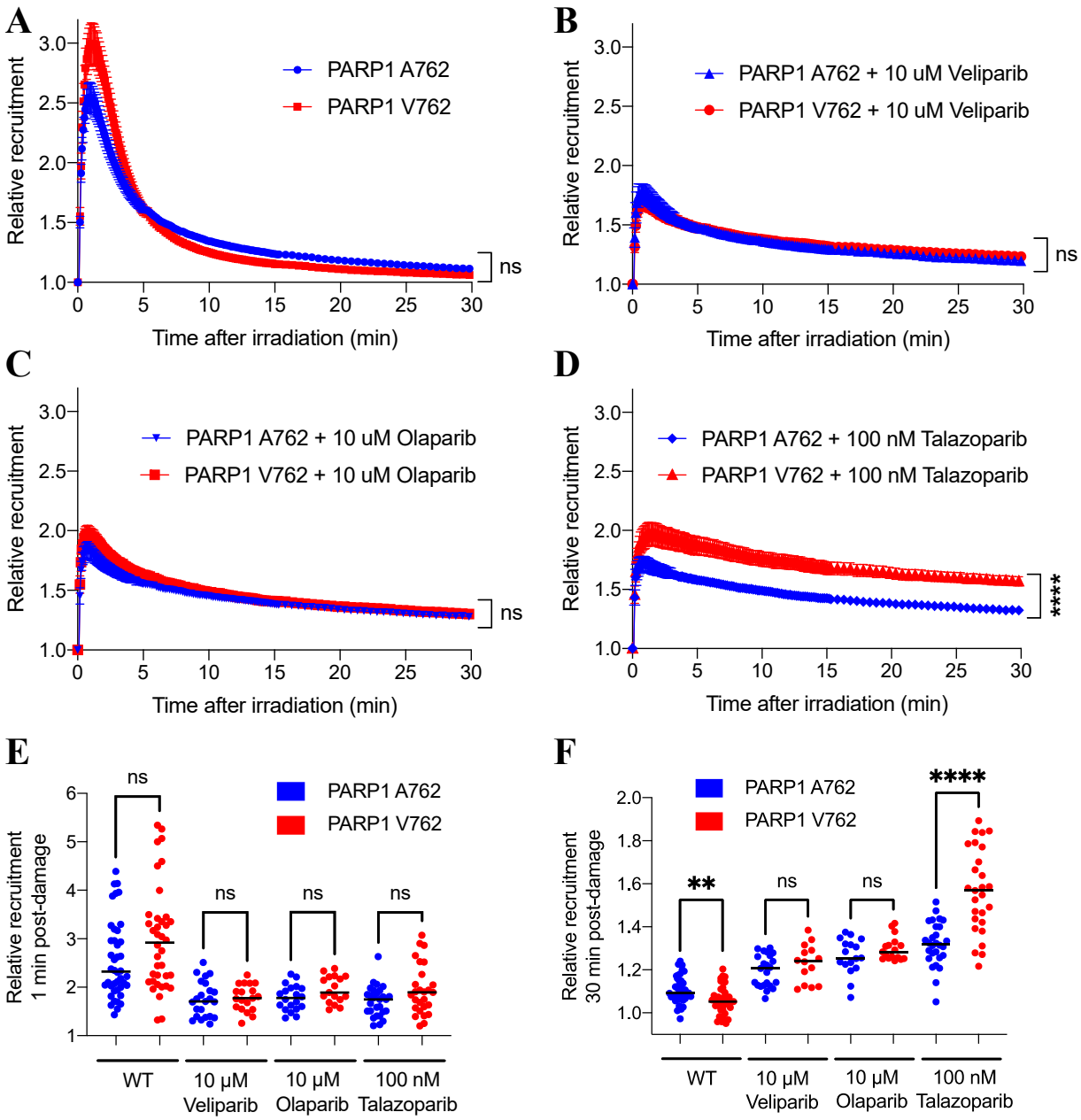

Figure 17 PARP1 variants are differentially trapped by the PARPi talazoparib

A-D Recruitment kinetics of PARP1 A762-GFP and PARP1 V762-GFP in U2OS WT cells in the absence (**A**) or presence of the PARP inhibitors veliparib (**B**), olaparib (**C**) or talazoparib (**D**). The data was collected from 15-42 individual cells in 2-4 independent experiments and was analyzed as described in Blessing *et al.* 2020 (see chapter 6.1). The graphs represent the mean ± S.E.M. normalized to pre-damage GFP intensity at microirradiation sites. Differences in curve behavior were tested by an ordinary one-way ANOVA of the area under the curve for each condition.

E, F Relative recruitment of PARP1 A762-GFP and PARP1 V762-GFP at 1 min (**E**) and 30 min (**F**) post-damage. Each data point represents a single cell, and conditions were compared with a Welch's ANOVA.

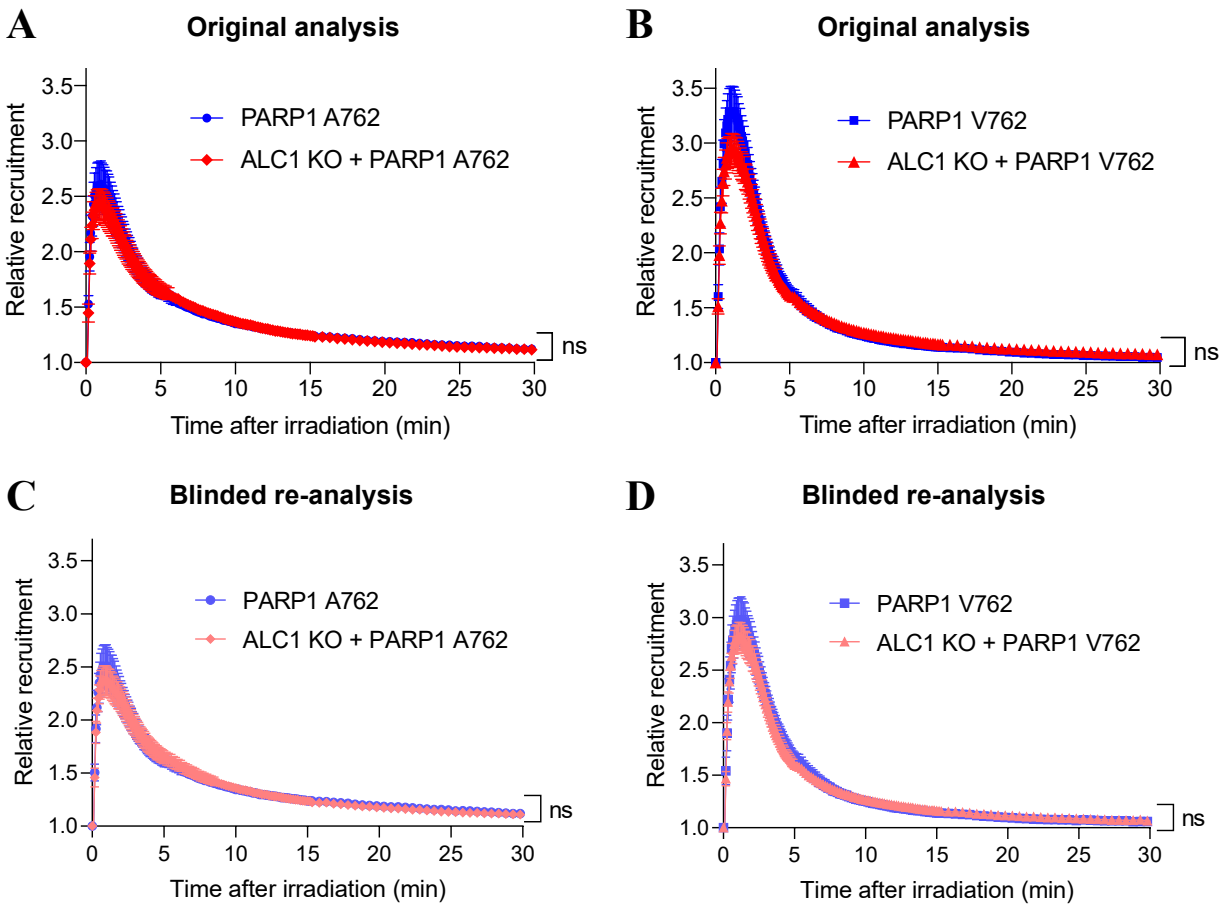

Figure 18 ALC1 does not affect the trapping of PARP1 variants at DNA lesions

A, B Recruitment kinetics of PARP1 A762-GFP (**A**) and PARP1 V762-GFP (**B**) in U2OS WT and ALC1 KO cells. The data was collected from 20-28 individual cells in 2 independent experiments and was analyzed as described in Blessing *et al.* 2020 (see chapter 6.1). The graphs represent the mean ± S.E.M normalized to pre-damage GFP intensity at microirradiation sites. Differences in curve behavior were tested by an ordinary one-way ANOVA of the area under the curve for each condition.

C, D Recruitment kinetics of PARP1 A762-GFP (**C**) and PARP1 V762-GFP (**D**) in U2OS WT and ALC1 KO cells, analyzed in a blinded manner by my colleague Tia Tyrsett Kuo as described in (**A**, **B**) to independently validate the results seen in (**A**) and (**B**).

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