Molecular mechanisms underlying the activation of ALC1 nucleosome remodeling

LUKA BACIC
Packaging DNA into chromatin represses essential DNA-based processes, such as transcription, DNA replication, and repair. To change the accessibility of DNA, cells have evolved a set of enzymes referred to as chromatin remodelers that act on the basic repeat unit of chromatin, the nucleosome. Chromatin remodelers are critical for normal cell physiology and development. Dysfunction or aberrant regulation of chromatin remodelers can lead to multisystem developmental disorders and cancers. DNA damage represents a major threat to eukaryotic cells. When DNA damage persists, the cell can enter programmed cell death. To avoid such a dramatic outcome, cells must rapidly recognize the DNA damage and trigger DNA repair pathways. An early event following DNA damage is the relaxation of chromatin. Chromatin relaxation depends on ATP consumption and ADP-ribosylation, where the site of DNA damage is marked with ADP-ribose units. ADP-ribose, in turn, can be recognized by the macro domain of the remodeler ALC1 (Amplified in Liver Cancer 1). ALC1 has therefore been implicated in the DNA damage response. In the absence of DNA damage, the macro domain of ALC1 is placed against its ATPase motor to inhibit its activity. However, it is unclear how ALC1, in its active state, engages the nucleosome. Moreover, the mechanism by which ALC1 is fully activated upon recruitment is poorly understood, and the impact of ALC1-catalyzed nucleosome sliding in the vicinity of a DNA damage site is unknown. This thesis investigates how ALC1 engages its substrate, the nucleosome, and how histone modifications can regulate ALC1 activity. Structural and biophysical approaches revealed an ALC1 regulatory segment that binds to the acidic patch, a prominent feature on the nucleosome surface. Further analysis showed that the interaction between ALC1 and the acidic patch is required to fully activate ALC1. Moreover, in vitro ADP-ribosylation of nucleosomes enabled us to form a stable complex of nucleosome-bound ALC1 amenable to structural determination by cryogenic electron microscopy. Our structural models visualize nucleosomal epitopes that play an important role in stimulating productive remodeling by ALC1, as confirmed by various biochemical approaches. Taken together, our data suggested a possible mechanism by which ALC1 could render DNA breaks more accessible to downstream repair factors. Since recent studies defined ALC1 as an attractive anti-cancer target, this thesis provides insights into the molecular mechanisms that regulate ALC1 activity as a potential starting point for structure-based drug development.

Keywords: ALC1, CHD1L, nucleosome, ADP-ribosylation, chromatin, remodeling, PARP1, HPF1, DNA damage

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To all my teachers
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


*These authors contributed equally.

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Contents

Introduction ................................................................................................... 11

Chromatin and its organization .................................................................... 12
   The nucleosome is the basic packaging unit of chromatin ....................................... 12
   The nucleosome acidic patch .................................................................................... 14

Chromatin remodeling complexes: classification and function .................... 16
   Remodeler subfamilies .............................................................................................. 16
   Mechanisms of remodeling ...................................................................................... 18

Regulation of remodelers .................................................................................. 21

The remodeler ALC1 ............................................................................................ 23

Paper I: Mechanistic Insights into Regulation of the ALC1 Remodeler by
   the Nucleosome Acidic Patch ............................................................................... 26

Paper II: Structure and dynamics of the chromatin remodeler ALC1 bound
   to a PARylated nucleosome .................................................................................... 29

Paper III: Asymmetric nucleosome PARylation at DNA breaks mediates
   directional nucleosome sliding by ALC1. Manuscript ........................................ 32

Future Perspectives .............................................................................................. 33

Svensk sammanfattning ....................................................................................... 34

Acknowledgments ................................................................................................. 36

References .......................................................................................................... 39
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Å</td>
<td>Ångstrom</td>
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<tr>
<td>ADP</td>
<td>Adenosine Diphosphate</td>
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<td>ALC1</td>
<td>Amplified in Liver Cancer 1</td>
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<td>AP(M)</td>
<td>Acidic Patch(-Mutated)</td>
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<td>APEX1</td>
<td>Apurinic/Apyrimidinic Endodeoxyribonuclease 1</td>
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<td>Arg</td>
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<td>Asp</td>
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<td>ATP</td>
<td>Adenosine Triphosphate</td>
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<td>BER</td>
<td>Base Excision Repair</td>
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<td>C-terminal</td>
<td>Carboxy-terminal</td>
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<td>CHD</td>
<td>Chromodomain-Helicase-DNA-binding</td>
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<td>CHD1L</td>
<td>Chromodomain-Helicase-DNA-binding protein 1-like</td>
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<td>cryo-EM</td>
<td>Cryogenic Electron Microscopy</td>
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<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<td>FRET</td>
<td>Förster Resonance Energy Transfer</td>
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<td>Glu</td>
<td>Glutamic Acid</td>
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<td>HR(D)</td>
<td>Homologous Recombination (deficient)</td>
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<td>Helicase-SANT</td>
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<td>HSS</td>
<td>HAND-SANT-SLIDE</td>
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<td>INO80</td>
<td>Inositol-requiring 80</td>
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<td>ISWI</td>
<td>Imitation Switch</td>
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<tr>
<td>LANA</td>
<td>Latency-Associated Nuclear Antigen</td>
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<td>N-terminal</td>
<td>Amino-terminal</td>
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<td>NAD⁺</td>
<td>Nicotinamide Adenine Dinucleotide</td>
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<td>PAR</td>
<td>Poly(ADP-ribose)</td>
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<td>Ser</td>
<td>Serine</td>
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<td>SF2</td>
<td>Superfamily 2</td>
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<td>SHL</td>
<td>Superhelical Location</td>
</tr>
<tr>
<td>SLIDE</td>
<td>SANT-like ISWI domain</td>
</tr>
<tr>
<td>Snf2</td>
<td>Sucrose Non-fermenting 2</td>
</tr>
<tr>
<td>WT</td>
<td>Wild-type</td>
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<td>XRCC1</td>
<td>X-Ray Repair Cross Complementing 1</td>
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Introduction

Eukaryotic cells have their DNA highly compacted into chromatin. The basic unit of chromatin compaction is a nucleosome. The nucleosome refers to a DNA segment tightly bent around the histone. Such a tight association is inherently repressive against essential cellular processes like transcription, DNA replication, and repair. In order to expose the target DNA regions to binding elements when needed, cells evolved the enzymes that can reversibly modulate the nucleosome, its position, or composition. Dynamic nucleosome repositioning and changes in nucleosome composition rely on enzymes known as chromatin remodelers. Chromatin remodelers are critical for normal cell physiology and determining and maintaining cell identity during cell development (Ho and Crabtree, 2010; Hota and Bruneau, 2016; Lessard and Crabtree, 2010). Dysfunction or aberrant regulation of chromatin remodelers can lead to developmental disorders and cancer (Clapier, 2021; Mossin et al., 2021). A detailed understanding of the biophysical and structural principles underlying the regulation and function of chromatin remodeling is, therefore, key for developing therapeutics to combat diseases such as cancer. In the following chapters, I will introduce chromatin organization and provide background information on the important family of chromatin remodeling enzymes that impinge on the chromatin landscape. Finally, of particular interest for this thesis is the chromatin remodeler ALC1 (Amplified in Liver Cancer 1), which plays a key role in DNA repair.
Chromatin and its organization

Eukaryotic cells must compact their genome to fit it into the nucleus. A fully extended human DNA could reach a length of over two meters, yet the DNA must be packaged into the nucleus with an average diameter of less than ten µm. To achieve this, linear DNA folds up together with proteins called histones into a chromatin structure (Clapier et al., 2017; Woodcock and Ghosh, 2010).

Histones can be divided into core histones and linker histones. Core histones facilitate the first level of DNA packaging into the basic unit of chromatin, the nucleosome. Linker histones with other binding factors bind to extranucleosomal linker DNA, enabling further compaction into higher-order structures (Woodcock and Ghosh, 2010). The level of compactness can influence gene expression by limiting access to the DNA for processes such as transcription. Therefore, by dynamically regulating chromatin structure, cells can establish transcriptionally more active and more silent regions. The primary substrate for controlling chromatin dynamics is the basic building unit of chromatin, the nucleosome.

The nucleosome is the basic packaging unit of chromatin

The function of chromatin is to condense and package genetic information (DNA) into the nucleus. The basic unit of DNA packaging is the nucleosome, a complex of about 150 base pairs of DNA wrapped around an octameric scaffold of core histone proteins (Davey et al., 2002; Luger et al., 1997; Richmond et al., 1984). The nucleosome structures uncovered key, previously unknown atomic details of the histone-histone and histone-DNA interactions and provided valuable information for chromatin biology (Davey et al., 2002; Luger et al., 1997; Richmond et al., 1984). A brief description of the nucleosome structure follows.

Each core histone contains a central α-helical region that forms a histone-fold motif and amino(N)- and carboxy(C)-terminal tails. The core histones are assembled into four histone-fold heterodimers (two H3-H4 and two H2A-H2B dimers). Two H3-H4 dimers bind to form a central symmetric tetramer. Each
H2A-H2B dimer attaches to each side of the H3-H4 tetramer, forming a histone octamer. The DNA wraps ~1.6 times around the histone octamer in a left-handed superhelix (Davey et al., 2002; Luger et al., 1997; Richmond et al., 1984). Contacts occur primarily between the histone-fold domains and the phosphate backbone of DNA, where the DNA major groove faces the protein surface. There are fourteen main backbone contact points. Each of the main contact points is conventionally called a superhelical location (SHL), starting from SHL 0 at the nucleosome dyad and ranging from SHL -7 to +7 for the last major grooves on each side from the dyad. The dyad is a central location and defines the pseudo-2-fold symmetry of the histone core. The prefix ‘pseudo’ refers to the flexible histone tails that protrude from the nucleosome, thereby breaking the strict symmetry. The N-terminal tails of H2A and H4 emanate from the histone-core surface outside the DNA gyres, while the N-terminal tails of H3 and H2B pass between the DNA gyres (Figure 1).

Figure 1. Nucleosome structure. Nucleosome (A) disc view and (B) side view, model derived from PDB 1KX5 (Davey et al., 2002).

Nucleosomes compact into a stable yet dynamic structure that can assemble and disassemble, allowing rapid access to the underlying genetic information during processes such as transcription, DNA replication, and repair (Clapier et al., 2017; Zhou et al., 2019). The intrinsic structural dynamics of the nucleosome manifest in rapid DNA unwrapping and rewrapping, also known as “DNA breathing” (Li et al., 2005). Moreover, nucleosomes can transiently change their composition and conformation. For example, histone tails are often targets for post-translational modifications (PTMs), including acetylation, methylation, phosphorylation, and ADP-ribosylation (Bowman and Poirier, 2015). Additionally, the canonical histones of the nucleosome can be replaced by their sequence variants (Martire and Banaszynski, 2020).
The changes in composition and conformation of nucleosomes requires different enzymes. For example, histone-modifying enzymes can change the chemical composition, while chromatin remodeling enzymes can change the architecture/location of nucleosomes. Together these enzymes dynamically modulate nucleosomal binding surfaces to mediate specific binding of various chromatin factors. A single chromatin factor can engage a nucleosome using one or more binding surfaces involving histone tails, the histone core, and/or extra-/intra-nucleosomal DNA. On a predominantly positively charged histone octamer surface, an acidic patch binding epitope formed by negatively charged side chains of H2A and H2B histones stands out, which is a topic of the next section.

The nucleosome acidic patch

The protein surface of the nucleosome is highly contoured and asymmetrically charged. A prominent feature is a cluster of acidic residues that forms a negatively-charged acidic patch (Figure 2A). Eight acidic residues contribute to the acidic patch, six from H2A (Glu56, Glu61, Glu64, Asp90, Glu91, and Glu92) and two from H2B (Glu105 and Glu113). Together, these residues form a narrow groove on the surface of the nucleosome.

Many chromatin factors interact with the acidic patch (McGinty and Tan, 2021), including a viral peptide derived from the latency-associated nuclear antigen (LANA) of Kaposi’s sarcoma-associated herpesvirus (KSHV) (Barbera et al., 2006) (Figure 2B). This peptide features an arginine ‘anchor’ residue that is critical for acidic-patch docking. Interestingly, other than the arginine, the neighboring residues do not show sequence conservation with other acidic-patch binding elements (McGinty and Tan, 2015).
Figure 2. The nucleosome acidic patch. (A) Octamer surface colored by electro-static potential (PDB: 1KX5 from Davey et al., 2002). (B) LANA viral peptide bound to the acidic patch (PDB: 1ZLA from Barbera et al., 2006).

In addition to the role as a binding epitope for chromatin enzymes, the nucleosome crystal structure from Luger and colleagues suggested that the acidic patch might participate in higher-order chromatin compaction (Davey et al., 2002; Luger et al., 1997; Richmond et al., 1984). In the context of the crystal lattice, the H4 N-terminal tail projects into the acidic cavity of the adjacent nucleosome, suggesting a role in nucleosome stacking. In conclusion, the H2A-H2B acidic patch can play roles in higher-order compaction, as well as an epitope for binding chromatin enzymes.
Chromatin represents a physical barrier that restricts access to DNA. Many DNA-based processes, such as transcription, DNA replication, recombination, and repair, require dynamic and regulated access to chromatin. To address this need, eukaryotic cells rely on chromatin remodeling complexes (remodelers) that can reversibly modulate chromatin structure. Remodelers have programmatic roles during cell development and are essential for establishing and maintaining cell pluripotency (Ho and Crabtree, 2010; Hota and Bruneau, 2016; Lessard and Crabtree, 2010). These enzymes use the energy from ATP hydrolysis to dynamically alter higher-order chromatin structure and nuclear organization. To enable this, remodelers can assemble or disassemble nucleosomes at target locations, exchange histone variants, or change the position of the histone core along DNA (a process also known as nucleosome sliding or DNA translocation) (Bartholomew, 2014).

Based on phylogenetic and functional analysis, chromatin remodelers are classified within the sucrose non-fermenting 2 (Snf2) family that, together with other families of helicase-like enzymes, belongs to the larger superfamily 2 (SF2) of RNA/DNA helicases (Flaus and Owen-Hughes, 2001; Flaus et al., 2006). Remodelers exist as single- or multi-subunit complexes, containing a catalytic ATPase subunit responsible for DNA translocation and often non-catalytic subunits typically involved in regulating remodeler activity. The importance of chromatin remodelers in biological processes is highlighted by the fact that mutations in genes encoding remodelers are often associated with various human diseases, like cancer, neurological and developmental disorders, etc. (Clapier, 2021; Kadoch and Crabtree, 2015; Lai and Wade, 2011; Li and Mills, 2014; Mossink et al., 2021; Wilson and Roberts, 2011).

Remodeler subfamilies
Chromatin remodelers are part of the sucrose non-fermenting 2 (Snf2) family of RNA/DNA helicases (Flaus et al., 2006). The Snf2 family is divided into different subfamilies of chromatin remodelers based on sequence homology and domain composition. The catalytic domain (ATPase motor) of Snf2-type enzymes features two lobes that resemble the RecA-like structure (reviewed
in Singleton et al., 2007). Within the Snf2 family, there are more than twenty subfamilies of chromatin remodelers (Flaus et al., 2006). Four well-studied subfamilies are imitation switch (ISWI), chromodomain-helicase-DNA-binding (CHD), switch/sucrose non-fermentable (SWI/SNF), and inositol-requiring 80 (INO80) (Clapier et al., 2017) (Figure 3).

![Figure 3. Schematic of remodeler classification (adapted from Flaus et al., 2006).](image)

The ISWI subfamily remodelers contain an ATPase motor and a C-terminal HAND-SANT-SLIDE (HSS) domain that binds the unmodified histone H3 tail and the linker DNA of nucleosomes. Two domains, autoinhibitory N-terminal (AutoN) and negative regulator of coupling (NegC), regulate the activity of the ATPase domain (Figure 4). Most ISWI subfamily complexes promote regular nucleosome spacing and chromatin assembly (Clapier et al., 2017).

The CHD subfamily remodelers contain the highly conserved Snf2-type ATPase domain, two tandemly arranged chromodomains at the N-terminus, and region similar to NegC and the DNA-binding domain facing the C-terminus (Figure 4). The CHD subfamily complexes can assemble nucleosomes, slide nucleosomes to expose the promoters, and exchange histone variants (Konev et al., 2007; Lusser et al., 2005; Murawska and Brehm, 2011). A remodeler of particular importance for this thesis is the ALC1 remodeler, also known as Chromodomain helicase/ATPase DNA binding protein 1-like (CHD1L) due to its similarity to the monomeric CHD subfamily remodeler CHD1. Instead of a CHD-identifiable chromodomain, ALC1 has a C-terminal macro domain that recognizes ADP-ribose as a post-translational modification (Karras et al., 2005).
The SWI/SNF subfamily remodelers, alongside the ATPase motor, feature a helicase/SANT-associated (HSA) domain and a bromodomain on the C-terminal side (Figure 4). The SWI/SNF complexes typically facilitate chromatin access by sliding or ejecting nucleosomes (Clapier, 2021).

The remodelers of the INO80 subfamily contain a variable, long insertion sequence which separates the two RecA-like lobes (Figure 4). The insertion region binds other subunits to assist the assembly of catalytically-active INO80 complexes (Ayala et al., 2018). The remodeling complexes of the INO80 subfamily have the ability to exchange histone variants as well as to regulate chromatin accessibility and nucleosome spacing (Clapier, 2021).

Figure 4. Schematic of domain organization of four well-studied remodeler sub-families (adapted from Clapier, 2021).

The interplay of catalytic and non-catalytic remodeler subunits can bring about various distinct remodeling outcomes, such as the assembly and disassembly of nucleosomes or the exchange of histone components of the nucleosome. Regardless of this rich diversity in remodeling outcomes, nucleosome sliding (translocation) is an activity shared by virtually all chromatin remodelers. Given the central importance of nucleosome sliding, its mechanism is the focus of the next section.

Mechanisms of remodeling

All Snf2-type chromatin remodelers catalyze DNA translocation through the action of a highly-conserved ATP-dependent catalytic domain. The catalytic domain consists of two distinct ATPase lobes forming together a nucleotide-
binding pocket and a nucleic acid-binding surface. Two ATPase lobes open and close depending on the occupancy of the nucleotide-binding pocket, which in turn alters interactions with the bound DNA. Alternating between open and closed states ratchets the nucleic acid past the motor domain in what is known as an inchworm-type mechanism (Lee and Yang, 2006; Singleton et al., 2007; Velankar et al., 1999).

Both RecA-like lobes bind the same DNA strand, with one lobe slightly ahead of the other, and translocate the DNA from a fixed position, superhelical location (SHL) 2 (McKnight et al., 2011; Saha et al., 2005; Schwanbeck et al., 2004; Zofall et al., 2006), which is two helical turns away from the nucleosome dyad. There are two symmetrically-related SHLs: -2 and +2. Based upon which side of the nucleosome disc the remodeler acts on, it can move the histone core back and forth along DNA.

Two notable models have been proposed to explain the mechanism of DNA translocation: loop propagation and the twist diffusion model (Figure 5). The loop propagation model suggests that the DNA loop lifts off the surface of the histone octamer and propagates around the nucleosome, locally breaking one or more minor groove contacts (Clapier and Cairns, 2009) (Figure 5A). The twist diffusion model suggests that a local change in DNA twist in a DNA segment between two minor groove contacts can absorb an additional base pair that propagates to a neighboring segment with a corkscrew shift (van Holde and Yager, 2003) (Figure 5B).
Emerging evidence supports the twist diffusion model for ATP-driven DNA translocation. The following part will focus on the experimental findings and ideas that shaped the view of how DNA translocation may be achieved. In the open (nucleotide-free and ADP-bound) state, the binding of the ATPase to the DNA strand at the SHL 2 location introduces a twist-like distortion in the DNA geometry (Bowman and Deindl, 2019; Nodelman and Bowman, 2021), which transiently accommodates one or more additional base pairs (Deindl et al., 2013; Sabantsev et al., 2019). The twist-like distortion prompts the breaks of the histone-DNA contacts that propagate around the nucleosome. As a result, the ATPase pulls in DNA from the proximal side of the nucleosome (the DNA entry side). Upon ATP binding, the ATPase lobes undergo a large rotation to a closed conformation, pushing the created DNA bulge toward the distal side of the nucleosome (the DNA exit side). Upon ATP hydrolysis and phosphate release, ATPase lobes reopen, inducing a DNA bulge again, whereby the nucleosomal DNA is distorted and primed for the next remodeling cycle. Recently, the three-color single-molecule Förster resonance energy transfer experiments have demonstrated the ATP-dependent delay in DNA movement in which the entry DNA is first pulled onto the nucleosome before the exit DNA is pushed off (Bacic et al., 2020; Sabantsev et al., 2019).
Regulation of remodelers

Chromatin remodelers act on different substrates, and their action results in a distinct remodeling outcome (Clapier et al., 2017). To achieve a remodeler-specific outcome, remodelers possess distinct accessory domains and subunits. These accessory domains and subunits can affect recruitment and, in addition, often enable sophisticated regulation of the remodeling activity. It is of great research interest to understand how these regulatory modules act together to impinge on the ATPase domains of remodeling enzymes. A few examples mentioned in the following illustrate the mechanistic roles of accessory domains in regulating the ATPase motor.

To avoid futile ATP hydrolysis in the absence of the optimal substrate, remodelers are often in an autoinhibited state. In an unproductive configuration, the ATPase lobes that are critical for ATP hydrolysis are kept in a conformation that is unlikely to be active. Examples of remodelers that are maintained in an autoinhibited state by their regulatory domains include: ISWI and SNF2h are inhibited by AutoN and NegC (Clapier and Cairns, 2012; Gamarra et al., 2018), CHD1 by chromodomains (Hauk et al., 2010), Swi2/Snf2 by the N-terminal HSA domain (Xia et al., 2016), and ALC1 by its macro domain (Lehmann et al., 2017; Singh et al., 2017; Wang et al., 2021). Some examples of how these regulatory domains release the autoinhibition of their respective ATPase motor are described further below.

Many nucleosomal and non-nucleosomal epitopes provide a platform for specificity and the regulation of catalysis by accessory subunits. For example, the interaction between the histone H4 tail basic patch and a conserved acidic cavity of the ATPase lobe triggers full remodeling activities of SNF2h, CHD1, CHD4, Snf2, and ALC1 (Armache et al., 2019; Bacic et al., 2021; Farnung et al., 2017, 2020; Liu et al., 2017).

The Sfh1 accessory subunit of the RSC remodeler interacts with the acidic patch of the nucleosome, further anchoring the remodeler onto its substrate. The disruption of this additional anchor impairs nucleosome ejection (Ye et al., 2019), suggesting that this anchor might be necessary for the ejection activity. In the case of the CHD1 N-terminal segment, its interaction with the distal acidic patch may serve as a gatekeeper that prevents other binding elements from acting on the opposite side of the nucleosome (Nodelman et al., 2022). Similar accessory domains might exist to antagonize another remodeler acting on the same nucleosome substrate. Different acidic patch recognition
modules have been identified in fine-tuning the activity of remodelers from ISWI and CHD subfamilies (Dann et al., 2017; Dao et al., 2020; Gamarra et al., 2018; Levendosky and Bowman, 2019).

Nucleosome periodicity places a role in transcription and gene expression. To space nucleosomes optimally, remodelers possess accessory subunits that can sense the linker DNA length. The HAND-SANT-SLIDE domain of the ISWI subfamily of remodelers and the DNA-binding domain of the CHD subfamily of remodelers can act as a linker DNA length sensor. In principle, sensing the length of flanking DNA can optimize nucleosome positioning relative to other DNA binding factors, for example, at promoters for the regulation of the +1 nucleosome position, the primary nucleosome inside the gene body (Clapier, 2021).

Moreover, accessory subunits can act as readers of histone post-translational modifications, potentially affecting their recruitment and regulation. Two well-studied examples of readers include bromodomains and chromodomains. Some chromodomains of the CHD subfamily of remodelers can bind methylated histones (Flanagan et al., 2005; Sims et al., 2005), while some bromodomains of the SWI/SNF subfamily of remodelers can recognize acetylated histones (Hassan et al., 2002).

Multiple binding modules usually act together to regulate the ATPase motor activity. How multistep regulation can take place will be illustrated by the example of the CHD1 remodeler. CHD1 is a monomeric remodeler with its N-terminal pair of accessory chromodomains (Delmas et al., 1993). The accessory double chromodomains of CHD1 jointly create an interaction surface for methylated histone tails. The crystal structure of the chromodomain-ATPase fragment of the S. cerevisiae CHD1 remodeler revealed an autoinhibited state (Hauk et al., 2010), where the double chromodomains block the DNA-binding element of the ATPase lobes while the interaction surface for histone methylation is exposed. Thus, the chromodomains likely negatively regulate the ATPase motor and allow activation only upon recruitment to H3-methylated nucleosomes. This form of negative allosteric regulation is unlikely to be unique to chromodomains.
The remodeler ALC1

ALC1 (Amplified in Liver Cancer 1) is an Snf2-type ATP-dependent chromatin remodeler. It consists of two RecA-like ATPase domains (C-terminal and N-terminal ATPase lobes) and a C-terminal regulatory domain called the macro domain (Figure 6). A pioneering study demonstrated that macro domains selectively interact in vitro with mono and poly(ADP-ribose) (Karras et al., 2005). Later studies confirmed this interaction in vivo (Ahel et al., 2009; Gottschalk et al., 2009; Timinszky et al., 2009).

Figure 6. Domain architecture of the human ALC1 protein.

Poly(ADP-ribose) is a post-translational modification in which the ADP-ribose unit from the NAD⁺ donor is successively transferred to an acceptor protein residue to form a polymer in a reaction called ADP-ribosylation. This reaction is catalyzed by PARP enzymes, recently organized under a new nomenclature as ADP-ribosyltransferases (ARTs) (Lüscher et al., 2021).

Early studies on the most nuclear-abundant ART member PARP1 showed that its catalytic activity is stimulated by damaged DNA, followed by the recruitment of factors involved in DNA repair (D’Amours et al., 1999; Elkamisy et al., 2003; Malanga and Althaus, 2005; Realini and Althaus, 1992). Moreover, in vivo studies associated ADP-ribosylation with chromatin relaxation (Frechette et al., 1985; Leduc et al., 1986; de Murcia et al., 1986), a response found also to be ATP-dependent (Kruhlak et al., 2006). Since ALC1 is an ATP-driven chromatin remodeler and its macro domain is a PAR binding module, it was early-on implicated in the PARP-mediated DNA damage response (Ahel et al., 2009; Gottschalk et al., 2009). Furthermore, live cell imaging studies suggested that ALC1 mediates the PARP-dependent change in chromatin accessibility (Sellou et al., 2016; Smith et al., 2018, 2019). However, the molecular mechanism of chromatin re-organization remains unclear.

Biochemical and in vivo-based experiments carried out independently by two research groups established ALC1 as a bona fide chromatin remodeling enzyme targeted to chromatin by PARP1-mediated ADP-ribosylation (Ahel et al., 2009; Gottschalk et al., 2009). In these studies, ALC1 alone exhibited a
low ATP hydrolysis rate that was only modestly increased by adding DNA or nucleosomes (Ahel et al., 2009; Gottschalk et al., 2009). Preincubation of the nucleosomes with PAPR1 and NAD$^+$ additionally stimulated ALC1 activity (Ahel et al., 2009; Gottschalk et al., 2009). The deletion of the histone H4 tail, a known epitope of remodelers, abolished the ALC1 remodeling ability (Ahel et al., 2009). Furthermore, in the ATPase-defective mutant (K77R), ALC1 exhibited prolonged retention at the site of laser-induced DNA breaks, while the macro domain fragment (annotated as C1) exhibited prolonged accumulation of the single-strand-break repair factor XRCC1 at the DNA damaged site (Ahel et al., 2009). Gottschalk and colleagues came to a similar conclusion, using ALC1 with a macro domain point mutation (D723A) that interferes with its ADP-ribose binding (Gottschalk et al., 2009). This ALC1 mutant exhibited reduced recruitment at the laser-irradiated DNA damage site. Taken together, these findings indicated that ALC1-mediated chromatin remodeling plays a role in the DNA repair process.

Since many studies have associated the ADP-ribosylation process with DNA repair and apoptosis, the ADP-ribose-mediated signaling pathway has become of pharmaceutical interest (Beneke et al., 2004; Lehtiö et al., 2013; Virág and Szabó, 2002). In particular, structural studies, in combination with elegant assay technologies, have begun to identify and develop inhibitors for writers, readers, and erasers of ADP-ribosylation (Haikarainen et al., 2017, 2018; Obaji et al., 2018, 2021; Sowa et al., 2020, 2021; Wazir et al., 2021). Such small-molecule inhibitors hold great potential for the study of ADP-ribosylation events and as a launching point for developing anti-cancer and antiviral drugs (Haikarainen et al., 2014; Nkizinkiko et al., 2018).

Not surprisingly, understanding ALC1 regulation at the molecular level has become a major focus. Two laboratories have independently described the mechanism of ALC1 autoinhibition (Lehmann et al., 2017; Singh et al., 2017). Small-angle X-ray scattering analysis (SAXS) and SAXS-based structure modeling showed that the macro domain of ALC1 is juxtaposed with the ATPase lobe, keeping the two ATPase lobes in a relative orientation unfavorable for ATP hydrolysis (Lehmann et al., 2017). In a later study, Wang and coworkers solved the crystal structure of ALC1 in an autoinhibited state, which visualized the macro domain binding to the C-terminal ATPase lobe at the atomic level. In vitro and in vivo studies agreed that the macro domain interacts with ATPase in trans, an interaction that is disrupted upon macro domain binding to PARylated PARP1 (Lehmann et al., 2017; Singh et al., 2017). Three or more ADP-ribose units are sufficient to disrupt the macro domain-ATPase interaction (Singh et al., 2017). Together, the results led to the conclusion that autoinhibition is achieved through allosteric regulation. Sequence analysis and mutagenesis studies identified possible residues of the macro domain involved in this regulatory interaction (Lehmann et al., 2017; Singh et al., 2017). Interestingly, hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) experiments in the presence and absence of
PARylated PARP1 revealed that the ALC1 undergoes extensive conformational changes upon activation (Lehmann et al., 2017; Singh et al., 2017). However, how these conformational changes upon activation of ALC1 infer its nucleosome-bound state remains unclear.

The ALC1 gene is located in the chromosomal region 1q21, commonly amplified in many cancers, including hepatocellular and breast cancers (Ma et al., 2008). In addition to gene amplification, more than 200 missense substitutions were reported in the COSMIC database (Bamford et al., 2004), including the arginine mutations at positions 857 and 860 within the macro domain, previously described to release the ALC1 autoinhibition (Lehmann et al., 2017; Singh et al., 2017). ALC1-catalyzed chromatin remodeling has been proposed to facilitate DNA repair, but the precise role of ALC1 is still unclear. ALC1 epistasis with PARP1 deficiency and the prolonged kinetics of single-strand break repair suggested the role of ALC1 in base excision repair (BER) (Tsuda et al., 2017). Moreover, ALC1 knockdown confirmed synthetic lethality in homologous recombination (HR)-deficient cells, involving ALC1 also in double-strand break repair (Hewitt et al., 2021).

Despite the notable success of PARP inhibitors (PARPi) in clinics (Curtin and Szabo, 2020), HR-deficient cancers did not respond to them, and those that did, acquired resistance to PARPi treatment (Noordermeer and van Attikum, 2019). Therefore, to completely exploit DNA repair vulnerabilities in cancer, alternative therapeutic strategies are needed. ALC1 has been proposed as a compelling therapeutic target, alone or to enhance existing anti-cancer strategies. Recently, the first compound targeting ALC1 has been developed, showing therapeutic potential against colorectal cancers (Abbott et al., 2020), potentiating the importance of structure-based drug design.

PARP1 itself is a substrate of ADP-ribosylation (Ogata et al., 1981). Alongside PARP1, its closest homolog, PARP2, is responsible for residual DNA-dependent PARP activity in PARP1-deficient cells (Amé et al., 1999). More than 300 proteins have been identified as sites of ADP-ribosylation on glutamate and aspartate (Zhang et al., 2013), as well as on serine (Leidecker et al., 2016). The mechanism of serine modification was unknown until the recent identification of the accessory subunit Histone PARylation Factor 1 (HPF1) (Bonfiglio et al., 2017). The HPF1 completes the PARP1/2 active site, changing its amino acid specificity to serine residues (Suskiewicz et al., 2020). ADP-ribosylated serines were found in the histone H3 and H2B tails of nucleosomes (Leidecker et al., 2016), suggesting that HPF1 might be a missing element for activating ALC1 in DNA damage response. In addition to that, the nucleosome structure enables a unique position of each histone copy relative to the nucleosomal two-fold symmetry. It is unclear whether asymmetry in ADP-ribosylation plays a role in ALC1-specific activity upon DNA damage response. Moreover, little is known about how ALC1-catalyzed nucleosome translocation affects nucleosome positioning in the vicinity of a DNA break.
Structural and biochemical assays previously defined the autoinhibited state of the chromatin remodeler ALC1 (Lehmann et al., 2017; Singh et al., 2017). In the absence of DNA damage, ALC1 is in an autoinhibited state, where the regulatory subunit (macro domain) of ALC1 abuts the catalytic subunit (motor domain). Upon DNA damage, poly(ADP-ribose) (PAR) chains mark the damaged site, and binding of PAR to an allosteric site within the ALC1 macro domain, recruits the remodeler and releases the autoinhibition of ALC1. This shows that ALC1 undergoes great structural changes upon activation. However, it remained unclear how ALC1, once activated, recognizes the nucleosome and how the recognition is coupled to productive remodeling.

The acidic patch, a negatively charged cavity shared by histone H2A and histone H2B (see page 14), is a unique nucleosomal epitope present on both faces of the histone core. It has been shown that the acidic patch plays an important regulatory role for several remodelers (McGinty and Tan, 2021). Hence, we investigated the importance of the nucleosomal acidic patch on ALC1 regulation.

In Paper I, we showed that ALC1 displays a modest preference to center the nucleosome, moving it towards the longer linker DNA. For such nucleosome repositioning, we showed that ALC1 critically depends on an intact acidic patch on the entry side of the nucleosome. Furthermore, data from cross-linking coupled to mass spectroscopy (XL-MS) experiments pointed to a possible interaction between histones and the linker region of ALC1. In addition, previous studies (Lehmann et al., 2017; Singh et al., 2017) observed that the ALC1 linker region plays an important role, yet its functional role was still undefined. A sequence alignment uncovered the similarity between the N-terminal part of the ALC1 linker region and the Latency-associated nuclear antigen (LANA) peptide, which is a known interactor of the nucleosome acidic patch (Barbera et al., 2006). The LANA peptide forms a hairpin that interacts with the nucleosome acidic patch predominantly through an arginine residue (arginine anchor). This arginine anchor in the LANA peptide aligns well with arginine 611 within the ALC1 linker region. The segment of the ALC1 linker that aligns with the LANA peptide (residues 604-624) was
termed the ALC1 regulatory linker segment (ALC1\textsuperscript{RLS}). Next, we showed that an ALC1\textsuperscript{RLS} synthetic peptide binds to wild-type nucleosomes but does not bind to acidic patch-mutated (APM) nucleosomes.

We then determined a cryogenic electron microscopy (cryo-EM) structure of the cross-linked complex between an ALC1\textsuperscript{RLS} peptide and a nucleosome. The structure showed that ALC1\textsuperscript{RLS} engages the nucleosome acidic patch similarly to the binding mode described for LANA (Barbera et al., 2006), however, with reverse N- to C-terminal polarity (Figure 7).

Figure 7. The ALC1\textsuperscript{RLS} peptide binds to the acidic patch via an arginine anchor (adapted from Lehmann et al., 2020).

Mutagenesis of ALC1\textsuperscript{RLS} affected both the ATPase and remodeling rates of ALC1 under saturating conditions. Additionally, \textit{in vivo} studies showed that the ALC1\textsuperscript{RLS} mutation compromises the overall extent of ALC1 recruitment to sites of DNA damage.

Therefore, we proposed the following model. Once recruited to sites of DNA damage through poly(ADP-ribose), ALC1 requires recognition of an intact acidic patch for its full activation. The binding of the regulatory motif within the ALC1 linker to the acidic patch of the nucleosome tethers the remodeler to the nucleosome and consequently couples ATP hydrolysis to nucleosome translocation (Figure 8).
Figure 8. Model for ALC1 activation through the acidic patch (adapted from Lehmann et al., 2020).
Upon DNA damage, the resulting DNA break rapidly recruits ADP-ribosyltransferases, predominantly PARP1 and PARP2. These enzymes mark the damage-proximal acceptor proteins by attaching poly(ADP-ribose) (PAR) chains onto them. Such a PAR marker triggers the DNA damage signaling pathway and stimulates the recruitment of downstream repair enzymes (Hakmé et al., 2008; Malanga and Althaus, 2005). Among the recruited repair enzymes, ALC1 has been implicated in early response to PAR by virtue of its macro domain, which is a PAR-binding module (Ahel et al., 2009; Gottschalk et al., 2009). In the absence of PAR, the macro domain of ALC1 keeps it in an autoinhibited state, and upon PAR binding to the macro domain, the inhibition is released (Lehmann et al., 2017; Singh et al., 2017). Alongside the macro domain, ALC1 comprises a motor domain that exerts chromatin remodeling activity. Chromatin re-organization was linked to PARylation (Frechette et al., 1985; Leduc et al., 1986; de Murcia et al., 1986), but the precise mechanism by which such a process happens remained unclear.

Recent studies have concluded that DNA damage-induced PARylation by PARP1/2 requires the accessory factor HPF1 (Gibbs-Seymour et al., 2016; Palazzo et al., 2018). HPF1 forms a joint catalytic site with PARP and switches the amino acid specificity (Suskiewicz et al., 2020), which also explains histone H3 and H2B tails as ADP-ribosylation sites (Leidecker et al., 2016). Thus, HPF1-dependent ADP-ribosylation of histones might be a requirement for ALC1-catalyzed response to DNA damage.

In Paper II, we found that the presence of HPF1 in the ADP-ribosylation reaction affects ALC1-nucleosome binding and ALC1-catalyzed nucleosome remodeling. The effect on binding and remodeling is due to specific modification of nucleosome components, described previously by Bonfiglio et al., 2017, which we also confirmed by Western-blot. These promising results using ADP-ribosylated nucleosomes encouraged us to pursue the structure determination of ALC1 in its active state.

We solved the structure of ALC1 bound to an ADP-ribosylated nucleosome by cryo-EM (Figure 9). The structure revealed that ALC1 shares two conserved features with other remodelers (Armache et al., 2019; Chittori et al.,
First, ALC1 tightly binds the nucleosomal DNA at the superhelical location (SHL) 2, and second, the C-terminal ATPase lobe of ALC1 interacts with the histone H4 tail. However, our map does not show clear densities that would correspond to the macro domain of ALC1 or PAR chains on the nucleosome. This is most likely due to per-particle structural variability attributable to the heterogeneous (in site, chain length, and branching) nature of the ADP-ribosylation reaction.

Figure 9. Cryo-EM structure of ALC1 bound to a PARylated nucleosome (adapted from Bacic et al., 2021).

Nevertheless, recent advances in software development (Punjani and Fleet, 2021; Zhong et al., 2021) enabled us to determine different conformational states in our cryo-EM data set. We submitted the particles from the consensus map to an analysis of heterogeneity by graph traversal algorithm implemented in cryoDRGN (Zhong et al., 2021) (Figure 10). The resulting maps showed multiple states along the graph traversal trajectory. A subset of states showed the tightly engaged ATPase domain similar to the active state (Figure 10, bottom right), and we were able to build an atomic model for this state. Other subsets indicated states with loosely bound ALC1 similar to the previously described autoinhibited state (Lehmann et al., 2017; Singh et al., 2017) (Figure 10, bottom left). Concurrently with our current study, the crystal structure of ALC1 in an autoinhibited state was published by (Wang et al., 2021), confirming the autoinhibition model described by our group and others (Lehmann et al., 2017; Singh et al., 2017) with additional atomic information on how this state is achieved. In addition, the states with a loosely bound ATPase domain...
also feature interaction with the nucleosome acidic patch that we described previously (Lehmann et al., 2020). Moreover, we could detect states that featured an additional density, most likely corresponding to the macro domain and its dynamic interaction at the location consistent with the PARylated histone H3 tail.

Figure 10. Analysis of heterogeneity of ATPase domain of ALC1 by CryoDRGN (adapted from Bacic et al., 2021).

To investigate the role of the H4 tail and acidic patch interaction in the context of ADP-ribosylated nucleosome as an ALC1 substrate, we conducted nucleosome sliding and ATP hydrolysis experiments. The observed faster sliding rate for ADP-ribosylated nucleosomes could be explained by a higher affinity of ALC1 for these nucleosomes. Both the disruption of the acidic patch and H4 tail interactions caused a decrease in ATPase activity, suggesting that these interactions regulate ALC1 remodeling primarily by stimulating its ATPase activity. Taken together, our study highlighted nucleosomal epitopes important for productive ALC1 remodeling.
Paper III: Asymmetric nucleosome PARylation at DNA breaks mediates directional nucleosome sliding by ALC1.

Manuscript

ALC1 is emerging as an important target for therapeutic intervention strategies in cancer since ALC1 inactivation exacerbates the cytotoxic effects of clinical PARP inhibitors in HRD cancer cells (Abbott et al., 2020; Blessing et al., 2020; Hewitt et al., 2021; Juhász et al., 2020; Verma et al., 2021). For this reason, there is considerable interest in the molecular analysis of ALC1 and its targeting by structure-based drug design. Our recent cryo-EM studies of ALC1 bound to an ADP-ribosylated nucleosome, enzymatically modified in vitro using PARP2 and HPF1, enabled the visualization of several intermediate states of the ALC1 ATPase motor from the recognition of the PARylated nucleosome to the tight binding and activation (Bacic et al., 2021, Paper II).

However, little is known about how histone octamer sliding by ALC1 affects nucleosome positioning in the vicinity of a DNA break. Nucleosome remodeling by ALC1 is required downstream of base excision by DNA glycosylases but upstream of APEX1, and ALC1 loss leads to single-strand gap formation and replication fork collapse (Hewitt et al., 2021). ALC1 plays a role in chromatin relaxation (Sellou et al., 2016; Smith et al., 2018, 2019), which is thought to promote DNA repair (D’Amours et al., 1999; El-Khamisy et al., 2003; Malanga and Althaus, 2005; Realini and Althaus, 1992). In our study, we leveraged a chemoenzymatic protein synthesis strategy to assemble nucleosomes with defined ADP-ribosylated H2B tails on one side only. Our data reveal that the ATPase preferentially engages one side of the nucleosome. Using single-molecule imaging strategies (Bacic et al., 2020; Deindl and Zhuang, 2012; Sabantsev et al., 2022), we demonstrated that asymmetric recruitment gives rise to directionally asymmetric ALC1-induced nucleosome sliding. Taken together, our data uncovered a possible mechanism by which ALC1 might render a DNA break accessible to downstream repair factors.
ALC1 is emerging as an important target for therapeutic intervention strategies in cancer, since ALC1 inactivation exacerbates the cytotoxic effects of clinical PARP inhibitors in HRD cancer cells (Abbott et al., 2020; Blessing et al., 2020; Hewitt et al., 2021; Juhász et al., 2020; Verma et al., 2021). For this reason, there is considerable interest in the molecular analysis of ALC1 and its targeting by structure-based drug design.

Our cryo-EM studies of ALC1 bound to a PARylated nucleosome enabled the visualization of several intermediate states of the ALC1 ATPase motor from the recognition of the PARylated nucleosome to the tight binding and activation. What is urgently needed is a high-resolution structure of ALC1 engaging with its macro domain the PAR chains of a PARylated nucleosome that most likely represents the relevant physiological substrate.

Additionally, further structural studies will be required to elucidate the manner in which emerging ALC1 inhibitors engage their target. The absence of high-resolution structural information severely hampers the improvement of inhibitor affinity and potency via medicinal chemistry. Initial lead inhibitors were recently reported that bind to ALC1 with moderate affinity and exhibit antitumor activity (Abbott et al., 2020; Prigaro et al., 2022). Although computational modeling suggested the potential binding of one such compound to an allosteric site of the ATPase, the binding mode of these early lead inhibitors is generally unknown. Moreover, at what level these current lead inhibitors impact ALC1-catalyzed remodeling is completely unknown. Single-molecule studies may provide crucial information on how ALC1 inhibitors affect the remodeling cycle. Taken together, additional structural and single-molecule scrutiny could form the basis for developing more efficient, next-generation inhibitors in combating homologous recombination-deficient cancers.

Thus far, single-molecule studies of ALC1-induced nucleosome remodeling have proven challenging. This was mostly due to the inability to generate well-behaved PARylated nucleosomes, resulting in low ALC1 binding affinity and processivity in single-molecule characterizations. The protocols established as part of this thesis will greatly facilitate future single-molecule studies of ALC1 and are expected to provide further insights into the regulation and functional outcome of ALC1-catalyzed nucleosome remodeling at DNA breaks.
Svensk sammanfattning

Eukaryota celler har sitt DNA packat inuti cellkärnan i form av kromatin. Den grundläggande enheten som styr denna packning är nukleosomen. Sådan packning av DNA möjliggör genreglering och kan även hämma livsviktiga DNA-baserade processer, såsom transkription, DNA-replikation och reparation av DNA. För att reversibelt slå gener “av” och “på” när det är nödvändigt har celler utvecklat en grupp enzymer som kallas för kromatinremodelningsenzym (kromatin remodelers). Kromatin remodelers är avgörande för normal cellfysiologi och för utvecklingen av celler. Dysfunktionell eller abnormal reglering av kromatin remodelers kan leda till utvecklingsstörningar i flera olika system och cancer.

Brott i DNA är kostsamma för eukaryota celler. Vid vissa omständigheter, exempelvis när skadorna inte lagas, kommer cellen att aktivera programmerad celldöd eller apoptos. För att undvika sådana dramatiska händelser måste celler snabbt hitta DNA-brott och påbörja lagningen av DNA. En av de första reaktionerna som startas efter att ett DNA-brott har identifierats är öppningen av kromatinet (Frechette et al. 1985). Denna process beror av konsumtionen av ATP (Kruhlak et al. 2006) och ADP-ribozylering (Sellou et al. 2016). ADP-ribosyltransferaser (ARTs), främst PARP1 och PARP2 och deras kofaktor HPF1 (Histon PARyleringsfaktor 1), är viktiga komponenter som binder enheter av ADP-ribos till acceptorproteiner vid DNA-brottet. ADP-ribos kan identifieras som en markör för DNA-brott av makromänen hos ALC1 (Amplifiered vid Levercancer 1). ALC1 utgör då en del av responsen till DNA-brott. När det inte finns några DNA-brott som måste behandlas är makromänen hos ALC1 placerad mot dess ATPas motor för att inhibera dess aktivitet (Lehmann et al. 2017; Singh et al. 2017; Wang, Chen, and Chen 2021). Däremot är det inte tydligt huruvida ALC1 interagerar med nukleosomen i sitt aktiva tillstånd. Själva mekanismen för hur ALC1 aktiveras vid rekrytering till DNA-brott är inte heller känd, vilket även gäller för rollen som ALC1 har vid lagningen av DNA-brottet.

Den här avhandlingen undersöker hur ALC1 interagerar med sitt substrat, nukleosomen, och hur modifiering av histoner kan reglera aktiviteten hos ALC1. Artikel I undersöker rollen som nukleosomens negativt laddade region har för ALC1-aktiviteten. Den negativt laddade regionen är en elektronegativ fördjupning i nukleosomstrukturen och utgör ett viktigt epitop för proteinbind-
ning och kromatindynamik. Enmolekylsstudier visade att ALC1 kräver en intakt närliggande negativt laddad region. Strukturen framtagen med kryogenisk elektronmikroskopi (kryo-EM) demontrerade att mutationer på båda sidor om bindningsstället mellan ALC1 och nukleosomen minskar aktiviteten hos ALC1. Undersökning av remodelleringsskinetiken med hjälp av enmolekylsmetod visade dessutom att interaktionen mellan ALC1 och dess negativt laddade region är nödvändig för att aktivera ALC1 fullständigt. Mutationer i det regulatoriska linkersegmentet hos ALC1 fördröjer även rekryteringskinetiken av ALC1 \textit{in vivo}.

Artikel II undersöker vilken roll som nukleosommodifieringar har på aktiviteten av ALC1. Då HPF1 har tidigare beskrivits som en essentiell kofaktor för PARP1/2 som krävs för att modifiera komponenterna i nukleosomen (Bonfiglio et al. 2017), har vår studie visat att ADP-ribosylade nukleosomer är optimala substrat för ALC1. Denna upptäckt möjligjorde strukturbestämningen av komplexet med hjälp av kryo-EM. Då vi undvek att använda en korslänkare kunde vårt kryo-EM-data användas för att visualisera flera konformationstillstånd och resultaten antydde förekomsten av flera epitop för nukleosomer. Vidare demontrerades hur viktiga dessa epitop är för effektiv ALC1-katalyserad remodellering med hjälp av biokemiska experiment.

I Artikel III (Manuskript) kunde en nyligen utvecklad kemoenzymatisk metod för positions- och längdspecifik ADP-ribosylering (Mohapatra et al. 2021) möjliggöra undersökning av den mekanistiska rollen som ALC1 har i närheten av DNA-brott. Vi fann att positionsspecifik ADP-ribosylering är avgörande för rekryteringen av ALC1 och för riktningen av dess glidning. Vårt data har framhävt rollen som ALC1 har i att göra DNA-brott mer tillgängliga för reparationsfaktorer nedströms i processen.

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A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology”.)