Chapter 7

Interlace between Chromatin Structure, DNA Repair and Ubiquitination

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Additional information is available at the end of the chapter

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Abstract

Chromatin remodeling, ubiquitylation, and DNA damage repair may be regarded as three discrete processes, but in fact, they are three extremely important interlinked processes that are imperative for the sustenance for life. Discrepancies in one will have outcomes that will affect the other processes direly. Exogenous and endogenous factors persistently affect the DNA by inducing damage and modifications. To sustain the integrity of life, these challenges need to be combated efficiently. For the preservation of the structural and functional components of the genome, nature has allowed them to evolve numerous pathways that constantly work to repair the induced damage. This sort of response is termed as DDR (DNA damage response) that include BER and NER (base excision and nucleotide excision repair, respectively) and non-homologous end joining and homologous recombination (NHEJ & HR). Since the DNA in cells is exceedingly organized and compressed, hence any process that utilizes DNA as its substrate requires essential remodeling of the chromatin structure. The chapter emphasizes on the phenomenon of chromatin remodeling and ubiquitylation which subsequently affects the integral process of DNA damage repair.

Keywords: chromatin remodeling, ubiquitylation, DDR, NHEJ, HR, BER, NER, exogenous and endogenous factors

1. Introduction

The dynamic structure of chromatin not only aids in wrapping the entire colossal genome into the boundaries of the nucleus but also plays an imperative role in regulating the accessibility of the DNA for various processes and mechanisms like recombination, transcription, replication, and

repair. At the cytological level, the structure of nucleosome may appear inflexible; however, the repeating subunits of chromatin are highly dynamic and flexible in nature [1].

Chromatin is an intricate macromolecular structure that is basically found in cells, which consist of DNA, protein, and RNA. The protein component of chromatin is the histones, which are primarily responsible for the compaction of DNA [2]. The main functions of chromatin include packaging of DNA into a compact shape, reinforcement of the DNA molecules in such a way to allow the process of mitosis and controlling gene expression and DNA replication. The compaction of chromatin can vary depending on the type of cell and the phase of cell cycle that the cell is in. Chromatin in the nucleus can exist as euchromatin or heterochromatin. At the time of interphase cell is not dividing actively, this is euchromatin which is in less packed and compact form. DNA is usually exposed in euchromatin form and processes like replication, and transcription can take place readily. However, a small amount of chromatin exists as heterochromatin. It consists of repeating units known as nucleosomes which consist of around 150 bp of DNA wrapped around a core of octamer of histones. Core consists of two of each of the following subunits H2A, H2B, H3, and H4. The DNA is tightly packed and is not in an unwind state to facilitate the processes like replication, gene transcription, etc. During staining procedures, heterochromatin stains more darkly than euchromatin [3].

Histones play an important role in maintaining the dynamicity of the chromatin structure. Histone exchange is a process that is utilized by the cell to maintain the dynamicity and subtlety of the chromatin structure. The process involves the removal of entire nucleosome or some designated part of it which is trailed by replacement with newly synthesized histones or different components of it. This crucial mechanism of swapping is commonly known as histone turnover. Histone turnover has dominant applications in sustaining the structure composition and functions of different expanses of the genome. For instance, hyperactive exchange of histones will lead to an eventual increase in the accessibility of a specific genomic area to the different components of the cell such as the enzyme DNA polymerase II, thus facilitating and enabling the process of transcription. Nevertheless, if the components of the nucleosome are replaced with other alternatives which are not compatible with cellular processes and hinder subsequent exchange, then this will hinder the availability of DNA for important processes like transcription. A category of histones known as canonical histones can be potentially replaced by histone variants but alter both the physical and chemical structure of nucleosome ultimately direly affecting various cellular process discreetly. Factors that regulate the exchange of histones during transcription include PTMs, chromatin remodelers, and histone chaperones which work individually or in accordance with each other [1].

Histones are one of the most copiously ubiquitinated proteins. The ubiquitination of the histones plays a crucial role in many processes undergoing in the nucleus. The processes include transcription, maintenance, and regulation of the chromatin structure along with DNA repair [4]. The protein ubiquitin is involved in the process of ubiquitination. Ubiquitin is a regulatory protein weighing 8.5 kDa found in many tissues of eukaryotic organism which was discovered back in 1975 [5] by Gideon Goldstein and was further characterized and categorized

throughout the span of 1970s and 1980s [6]. It is encoded by a total of four genes in the human genome, namely UBB, UBC, UBA52, and RPS27A [7]. The addition of the regulatory protein ubiquitin to a substrate protein is known as ubiquitination. The process of ubiquitination is known to affect proteins in many ways: it usually marks them for destruction or degradation via the proteasome pathway and it is also known to change the cellular location of the proteins and sometimes promoting or inhibiting various protein interactions along with playing an important role in signal transduction and protein trafficking [8–10]. Ubiquitination involves three main steps: activation, conjugation, and ligation. These three major steps are performed by ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), and ubiquitin ligases (E3s), respectively. The result of this consecutive cascade of reactions is finally the binding of ubiquitin to lysine on the substrate protein by an isopeptide bond, cysteine residues through a thioester bond, serine and threonine residues via an ester bond, or the amino group of the protein's N-terminus via a peptide bond resulting in one of the fates mentioned above [11–13].

The process of DNA repair is closely linked to histone ubiquitination, deubiquitination, and chromatin remodeling [4, 14]. DNA repair is a systematic process by which the cells of body recognizes and consequently corrects the damage to which DNA molecules are exposed that subsequently encodes the genome of an organism. In case of the human cells, DNA damage can be caused by abnormal cell cycle, metabolic activities as well as environmental factors such as radiations especially the UV radiation. All these can cause as many as 1 million lesions and abrasions to the DNA in a single day [15]. These lesions are responsible for causing basic impairment and damage to the DNA molecule which resultantly removes or changes the ability of the cells to transcribe a gene that the affected part of the DNA encodes. Furthermore, other lesions may be able to induce destructive mutations in the genome of a cell consequently disturbing the overall survival of the progeny cells as it undergoes mitosis. Resultantly, the repair processes for DNA are continuously active because they retort to the damages in the DNA structure. When these normal repair processes of the DNA molecules fail to repair the damage and when programmed cellular death (apoptosis) does not take place, irreversible DNA occurs which include cross linkages also known as inter-stand cross links, double and single breaks in the DNA molecule which will eventually lead to the formation of malignant tumors [16, 17], or other sort of cancers according to the two-hit hypothesis. Like all other processes, DNA repair is also dependent on factors, including type and age of cell and extracellular environment of the cell. A cell that has hoarded a great amount of damaged DNA or a cell that no longer effectively repairs the damaged DNA will face one of the three possible fates:

1. An irreversible state of latency, known as senescence.
2. Suicide of the cell, also known as apoptosis.
3. Unchecked cell division leading tumor formation that can eventually lead to cancer.

Repairing of damaged DNA is vital for the maintenance of the integrity of the genome and to preserve the normal functioning of the genome [18].

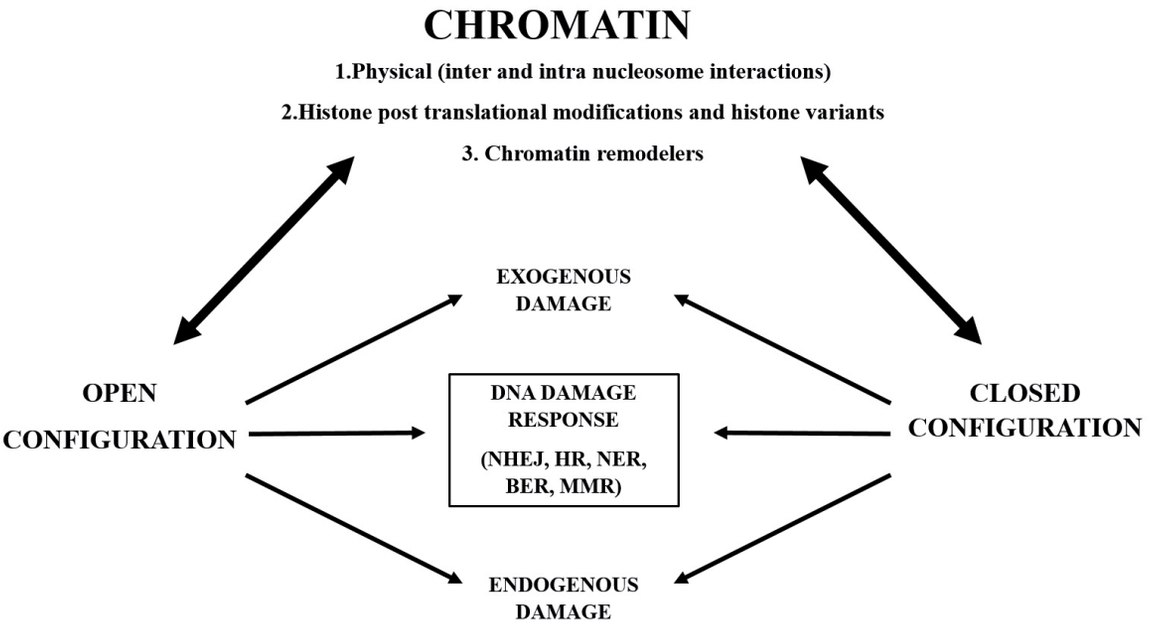

Figure 1. Interplay between chromatin rearrangement and DNA damage response, adapted from [19].

Figure 1 explains the close association between DNA damage response and repair and remodeling of the chromatin. As the subtleties of the chromatin structure play a part in maintaining the stability of the genome, intra and internucleosomal interactions along with post translational modifications of histones, histone variants, and the function of ATP-dependent chromatin remodelers all collectively play their due share in controlling and maintaining the structural assembly of the chromatin. Collectively, all these factors safeguard appropriate and suitable chromatin conformation during different stages of the diverse cell cycle and during numerous DNA templated processes. The OPEN state is vulnerable to both external and internal damage consequently leading to increased DDR. The CLOSED state on the other hand successfully overpowers the dual genomic invectives and acts as a determent to DDR. The closed state also hinders various processes such as transcription, recombination repair, etc. Hence the subtleties of the chromatin arrangement aid not only in repairing DNA lesions and damages but also in permitting access to cellular machineries to accomplish processes that depend on DNA, thus ensuring the maintaining of the steadiness of the genome [19].

In this chapter, we aim to explain the link between these three extremely important processes as current research advances have defined central roles of all the three processes, histone ubiquitination, remodeling of chromatin, and DNA repair.

2. Mechanism of gene activation and regulation

2.1. DNA repair and chromatin remodeling

Chromatin decondensation and reorganization has a crucial role in all cellular processes that use DNA as template or substrate like DNA repair mechanism, replication, and transcription. For example, base excision repair (BER) that requires the removal of altered or damaged

base relies in chromatin remodeling, similarly for the nucleotide excision repair (NER) that counteracts with helix distorting lesions caused by UV radiations [20]. NER has two modes of action which are dependent on the nature of lesion caused, the transcription coupled-NER (TC-NER) only operates in genes that are transcriptionally active where polymerase-II triggers the DNA damage response while the global genomic NER branch (GG-NER) operates when lesion is in chromatin environment but both of the pathways fill the gap by same core machinery [21, 22]. The open and compact structure of chromatin affects the activation and efficiency of DNA damage response (DDR), as it is difficult for repair proteins to reach a damaged structure in compact or highly condensed chromatin. In case of a double stranded break (DSB), chromatin relaxation along with the recruitment of break-sensing proteins at the damaged site is induced via an ATP-dependent mechanism that works independently of DDR kinases [23].

The ATP-dependent chromatin remodeling enzymes are a source of chromatin reorganization and transformations. The Snf2- or SWI/SNF enzymes, that were first discovered as chromatin remodeling enzymes during the characterization of yeast, consists of a conserved sequence of seven amino acids which is present in all eukaryotes [24, 25]. Depending on the sequence homology in the ATPase core, the Snf2 proteins have been assigned 24 subfamilies [26]. These chromatin remodeling enzymes interact with each other and induce a range of chromatin transformations such as histone octamer sliding across DNA, change in nucleosomal DNA conformation, and composition of histone octamer. DNA is tightly bound to histone octamer, which is disrupted by chromatin remodeling which is disrupted by chromatin remodeling enzymes during chromatin de-condensation and reorganization [27].

However, DDR kinase-dependent chromatin changes promote the local environment favorable for DNA repair mechanism of which the most important is regulation of nuclear organization. Studies in yeast suggest that there are repair centers for DSB repair; however, the unrepairable DSBs move towards the nuclear periphery, and these are merged into a single repair focus [28]. Moreover, increased mobility of chromatin has been observed in yeast nuclei as a consequence of DSB which increases the DNA repair efficiency, and this movement is attributed to Mec1ATR kinase, RAD51recombinase, and resection of DNA end [29, 30]. RAD51-coated DNA is efficient in finding its homologous sequence [31], thereby promoting the repair machinery to act. Double stranded breaks (DSBs) are either repaired by error-free homologous recombination (HR) that involves sister chromatids or by error-prone nonhomologous end joining (NHEJ) that involves the damage recognition by Ku70/80 that bind to damaged DNA and recruit DNA-PK, a serine/threonine protein kinase that induce conformational changes on damage site after which protein kinase ataxia-telangiectasia, mutated (ATM), and ATM and Rad3-related protein (ATR) are recruited that interact with XRCC4 and DNA ligase IV that proceed to DNA relegation as shown in **Figure 2** [22, 23, 24, 33].

The damaged region of heterochromatin moves toward the outside boundary as a consequence of heterochromatin expansion caused by the break and DDR kinases that lead to RAD51 dependent homology search [35]. The exact mechanism is still not known, but DDR kinases have been observed to modify nucleoporins that in turn breaks the interaction of chromosome and pores [36]. Another possibility is the phosphorylation of KAP1 that binds heterochromatin protein HP1 and chromatin remodeling factors like INO80 and H2A are recruited which

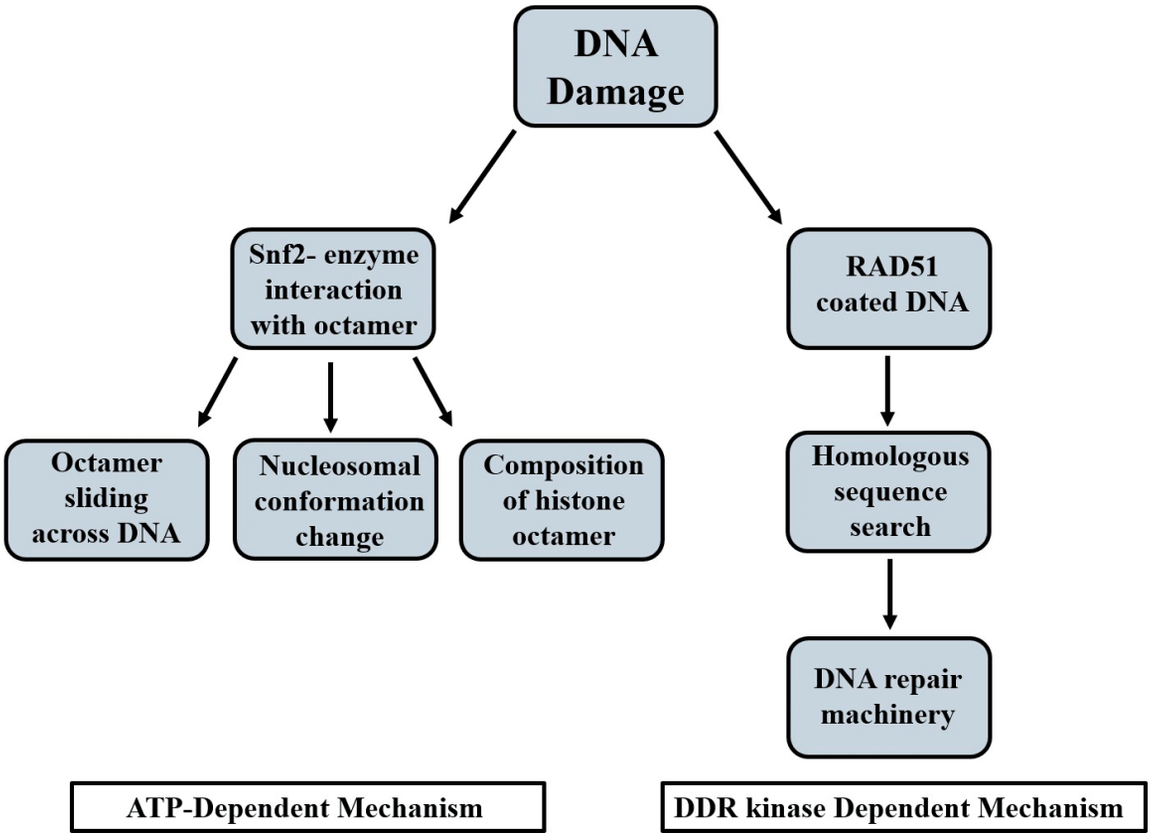

Figure 2. DSBs induces histone modification and DDR.

facilitate the mobility and repair machinery [37–39]. Damage recognition is highly dependent on chromatin conformation changes and signaling cascades based on phosphorylation, ubiquitylation, and PARylation, these pathways also facilitate DDR by halting cell cycle.

2.2. Chromatin ubiquitylation

Amongst chromatin ubiquitination, modification of H2A, core histones, H2B, H3, H4, and linker H1 are modified by ubiquitin. This modification of histone plays its vital role in transcriptional control and DDR [40]. The exact role and mechanism is still to be explored, but H2A ubiquitination has been proposed in chromatin folding [40, 41]. The ubiquitination of linker H1 occurs through TAFII250 that is a part of transcription factor TFIID [42].

2.3. DNA repair and histone ubiquitylation

Most common histone modification is histone ubiquitination that has been observed to play a vital role in DDR. Impairment of DNA repair has been identified as a major culprit as defense mechanism is evoked against cells that have cell cycle arrest, apoptosis, and DNA damage [43]. A DSB evokes the phosphorylation of H2AX at γ position and tracks the damage by ATM, ATR, and DNA-PK [44]. This phosphorylation facilitates the accumulation of Mdc1/NFBD1, RNF8, RNF168, and response regulators [45]. The K63-linked

polyubiquitination on histone H2A and H2AX is catalyzed by RNF168 and RNF8 and acts as an recognition element that in turn recruits RAP80 which consequently recruits BRCA1 [46–52]. Apart from polyubiquitination, the monoubiquitination of histones H2A, H2B, and H2AX also occurs at DNA damage site. This monoubiquitination of histones is catalyzed by RING1B/BMI1 and RNF20/RNF40, moreover, the depletion of RNF20 disrupts monoubiquitination which ultimately halts the DNA repair machinery in both HR and NHEJ pathways [53].

Histone modification at DNA damage loci is ubiquitination of H2A histone, variant H2AX, and H1 linker histone [32, 49, 54]. MDC1-dependent recruitment of E3 ligase RNF8 along with Ubc13 catalyzes K63-linked polyubiquitination of histone H1 at DSBs [55]. This ubiquitinated histone H1 mediates the recruitment of E3 ligase RNF168 and RNF8 which triggers the catalysis of histones H2A and H2AX at lysine 13–15 [49, 54, 56, 57]. These histones provoke the effector proteins BRCA1 and 53BP1 to damage site promoting homologous recombination (HR). BRAC1 through its binding partner within BRCA1-A complex RAP80 tether to histone H2A and is considered essential for HR, whereas 53BP1 is a mediator of NHEJ **Figure 3** [34, 47, 52, 58].

Ubiquitination is a prominent feature of chromatin signaling in NER, as during GG-NER E3 ligases catalyze the ubiquitination of histone H2A by UV-RING1B complex, which has DDB1, DDB2, CUL4B, and the E3 ligase RING1B as subunits that operate the early damage recognition [59]. Ubiquitylation of lysine 119 of histone H2A is catalyzed and it provides an attachment platform for H2A-ubiquitin binding protein ZRF1. CUL4B-RBX1 subunits are removed, ZFR1 thus mediating UV-DDB CUL4A complex generation at damage site, and then factor XPC is polyubiquitylated which stabilizes it at the damaged site [59, 60]. This ubiquitylation of XPC acts as a timing device for damage recognition and verification [61]. Henceforth, the ubiquitination and deubiquitination of histones mediate the DDR and compaction of chromatin [53, 62].

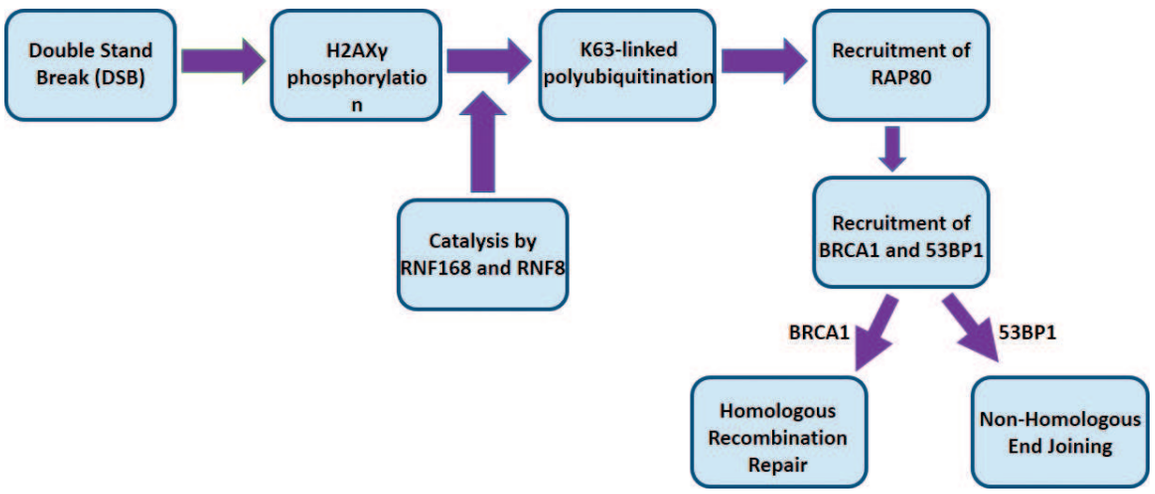

Figure 3. Chromatin remodeling and DNA repair.

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