Research Perspective

'Relax and Repair' to restrain aging

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Nonstandard abbreviations: *DSB, double strand break; AcH4K16, acetylated lysine 16 of histone H4; IRIF, irradiation-induced foci; HR, homologous recombination; NHEJ, non-homologous end joining; HAT, histone acetyltransferase; HDAC, histone deacetylase; MEF, mouse embryonic fibroblast; HGPS, Hutchinson Gilford progeria syndrome; IR, Irradiation; AT, Ataxia-telangiectasia*
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Abstract: The maintenance of genomic integrity requires the precise identification and repair of DNA damage. Since DNA is packaged and condensed into higher order chromatin, the events associated with DNA damage recognition and repair are orchestrated within the layers of chromatin. Very similar to transcription, during DNA repair, chromatin remodelling events and histone modifications act in concert to 'open' and relax chromatin structure so that repair proteins can gain access to DNA damage sites. One such histone mark critical for maintaining chromatin structure is acetylated lysine 16 of histone H4 (AcH4K16), a modification that can disrupt higher order chromatin organization and convert it into a more 'relaxed' configuration. We have recently shown that impaired H4K16 acetylation delays the accumulation of repair proteins to double strand break (DSB) sites which results in defective genome maintenance and accelerated aging in a laminopathy-based premature aging mouse model. These results support the idea that epigenetic factors may directly contribute to genomic instability and aging by regulating the efficiency of DSB repair. In this article, the interplay between epigenetic misregulation, defective DNA repair and aging is discussed.

Organization of chromatin

The genomes of organisms are organized in the form of a fundamental structure called chromatin in which the repeating nucleosomes form the basic unit. The nucleosome consists of 147 bp of DNA wound 1.7 times around an octamer composed of the four core histones, H2A, H2B, H3 and H4. Multiple nucleosomes are further linked by DNA stretches that are occupied by linker histone H1, to form the 10-nm fibre or 'beads on a string' type of arrangement. Chromatin fibres undergo compaction through intramolecular nucleosome-nucleosome interactions to form the 30 nM chromatin fibres. At the next level of organization, chromatin is further stacked and folded to give rise to 100-400 nm interphase chromatin fibres. The DNA that is eventually folded into chromosomes has already undergone compaction by about 10,000 fold. The packaging of DNA into condensed and often inaccessible chromatin imposes a significant constraint for the efficient repair of DNA double strand breaks (DSBs). Recent efforts have been directed towards understanding how the DNA repair proteins gain access to and repair, when DNA damage is embedded within chromatin fibres.

Chromatin structure and DNA repair

Cells utilize two distinct mechanisms to modulate chromatin dynamics during DNA repair. These mechanisms include the post translational modifications of histones and ATP-dependent chromatin remodelling.

According to the 'histone code hypothesis', the biological outcome of histone modifications is manifested by providing a signalling platform for the recruitment of downstream effector and reader protein or by the physical modulation of chromatin structure [1]. Accordingly, histones within chromosomes are subjected to several forms of post translational modifications such as phosphorylation, ubiquitination, methylation, and acetylation and these modifications can either create or eliminate binding sites for non-histone proteins that mediate DNA repair or modify chromatin structure [2]. The earliest identified and one of the most important histone modifications during DNA repair is the phosphorylation of histone H2AX at the C-terminal residue corresponding to Ser139 (γ-H2AX) by the key DNA damage-responsive kinase, ATM [3]. During DSB repair, phosphorylated H2AX forms a specialised chromatin compartment capable of recruiting and retaining DNA repair factors [4]. H2AX phosphorylation spreads over a 2 Mb domain on each side of the DSB, and acts as a docking site for several DNA repair proteins such as the mediator, MDC1. Thus, H2AX phosphorylation acts as an important cue for the stable retention of DNA repair proteins which form microscopically discernible foci, called as irradiation-induced foci (IRIF) [5]. It is now recognized that apart from phosphorylation, H2AX is monoubiquitinated and later di and poly-ubiquitinated in a DNA damage-dependent manner. According to current understanding, several ubiquitin ligases including RNF8, RNF168, RNF2, Bmi1 and Herc2 are responsible for the completion ubiquitination of γ-H2AX [6]. In turn, ubiquitinated histones promote the recruitment of DNA repair proteins, Brca1and 53BP1 which directly participate in the repair of DSBs by homologous recombination (HR) or non-homologous end joining mechanisms (NHEJ). Apart from histone ubiquitination, histone methylation also plays an important role in DSB repair processes. Trimethylated H3K9 is known to be an important component of heterochromatin and the heterochromatin 1 proteins, HP1 α,β,δ, bind to trimethylated H3K9, contributing to heterochromatin maintenance. Upon DSB induction, HP1 β is phosphorylated in a casein kinase-dependent manner, which promotes HP1 β dissociation and γ -H2AX phosphorylation [7]. Following HP1 β dissociation, the MYST family histone acetyltransferase (HAT) Tip60, is recruited to trimethylated H3K9 resulting in the stimulation of its HAT activity [8]. In other examples, constitutive dimethylation of histone H3K79 by Dot1L and DNA damage-inducible H4K20 dimethylation by MMSET also provide a recruitment platform for the DSB repair protein, 53BP1 [9,10]. Thus, in general, histone methylation mainly provides binding sites for the direct recruitment for downstream repair proteins.

It is well known that histone acetylation is associated with a more 'open' configuration of DNA, because histone acetylation imparts a negative charge that causes the charge repulsion of negatively charged DNA. The acetylation of H2AX at K5 position by Tip60 regulates the ubiquitination of H2AX at K119 and enhances chromatin dynamics [11]. Tip60-TRRAP mediated histone H4 acetylation is also required for efficient recruitment of repair proteins and HR [12]. Since the requirement of Tip60-TRRAP could be overridden by forced chromatin relaxation using histone deacetylases (HDAC) inhibitors, it was proposed that the main role of histone acetylation was to improve chromatin accessibility. In a similar mechanism, Mof, another MYST family HAT, mediates H4K16 acetylation and controls higher order chromatin configuration to promote repair protein recruitment (as discussed below). Another example is the acetylation of H3K14 which increases after irradiation in a HMGN1-dependent manner. In this study, H3K14 acetylation was shown to regulate ATM activation and since the requirement for HGMN1 could be overcome by inducing chromatin relaxation using HDAC inhibitors, it was suggested that HGMN1 regulates higher order chromatin structure during DNA repair [13]. The recurring theme in these experiments that forced chromatin relaxation could bypass the requirement for histone acetylation, suggests that promoting chromatin accessibility was likely to be one of the principle role of histone acetylation in DSB repair.

The direct recruitment of ATP-dependent chromatin remodelling complexes that enzymatically modulate chromatin structure is another important mechanism in DNA repair. Chromatin remodelling complexes use ATP hydrolysis to increase accessibility of nucleosomal DNA by repositioning nucleosomes or by altering nucleosomal composition [14]. There are four main families of mammalian chromatin remodelling complexes: the SWI/SNF (switching defective/sucrose non-fermenting) family, the NuRD (nucleosome remodelling and deacetylation)/Mi-2/CHD (chromo-domain helicase DNA binding family, INO80 (inositol requiring 80) family and the ISWI (imitation-SWI) family of complexes. An idea that has emerged in recent years is that DNA damage-modified histones provide targeting sites for the recruitment of chromatin remodelling complexes. Thus, specificity is achieved by chromatin-interacting domains that bind to modified histones. In pioneering studies conducted in budding yeast, γ-H2AX was shown to provide binding sites for

the recruitment of ATP-dependent INO80, and histone acetylating NuA4 at the site of a DSB [15,16]. Likewise, both members of SWI/SNF family of ATPases, BRM and BRG1 directly interact with acetylated H3 residues in γ-H2AX containing nucleosomes [17]. This interaction increases the recruitment of the HAT, GCN5, which promotes further efficient induction of γ-H2AX after irradiation. SWI/SNF is targeted to DSBs by interaction with an early DNA damage responsive protein BRIT1/MCPH1, which then promote the recruitment of repair factors such as MDC1, Rad51, Ku70, RPA and NBS1 to DSBs [18]. The mammalian ISWI remodelling complexes containing the SNF2H or SNF2L ATPases mediate nucleosome sliding and histone replacement and are recruited to DSBs in micro-irradiation experiments [19]. SNF2H promotes DNA repair by HR and its depletion impairs RPA, Brca1 and Rad51 foci formation [20]. Mammalian INO80 chromatin remodelling complexes contain the INO80 ATPase and several other subunits which form a complex with the polycomb transcription factor YY1. INO80 is recruited to laser-generated DSBs and the loss of YYI or INO80 leads to chromosome aberrations and defective repair by HR [21]. Mammalian CHD remodelling complexes slide or eject histones and several members of this family such as the CHD1-like protein ALC1 and CHD2 have been proposed to play a role in the DSB repair by NHEJ [22,23]. Recent studies have implicated the CHD4 ATPase in multiple stages of the DDR. CHD4 is recruited to DSB lesions and its depletion led to reduction in DNA damage-induced histone ubiquitination and defective recruitment of Brca1 and RNF168 [24, 25]. The loss of CDH4 or other NuRD components also led to structural defects in the chromatin and increased accumulation of spontaneous DNA damage [26].

In other examples involving chromatin structure and DNA repair, DSB repair in the heterochromatin requires specialized mechanisms to promote access to repair proteins because heterochromatin is structurally inhibitory to DNA repair owing to its condensed structure. ATM-dependent phosphorylation of the co-repressor Kap1 was shown to allow localized and transient chromatin relaxation at regions of heterochromatin and promote the recruitment of DNA repair proteins [27]. Likewise, it has been shown that the PWWP domain-containing protein EXPAND1 accumulates in IRIF in a H2AX, MDC1, RNF8 and 53BP1-dependent manner and increases chromatin accessibility after DNA damage [28]. Thus, DSB recognition and repair are intimately associated with chromatin remodelling and histone modification events.

Laminopathy-based premature aging

Amongst the most prominent phenotypes associated with defective DSB repair in both humans and mice, is the onset of accelerated aging (progeria). In several correlative studies, γ -H2AX foci containing senescent cells increase with age in humans, mice and primates, leading to the model that inefficient DSB processing and repair can activate cellular senescence pathways and initiate premature aging [29-31]. This notion is strengthened with the observation that knockout-mice defective for DSB repair often show an accelerated aging phenotype and conversely, defective DNA repair is a common phenotype in human patients suffering from premature aging (progeria) syndromes [32-33].

In our earlier study involving human Hutchinson Gilford progeria syndrome (HGPS) patient fibroblasts, defective DNA repair and genomic instability was demonstrated [34]. Clinical manifestations in HGPS patients include accelerated aging symptoms like hair loss and greying, atherosclerosis, short stature, sculpted nose, reduced subcutaneous fat, decreased bone density and cardiovascular diseases. HGPS patients die at an average age of 13 years due to complications related to atherosclerosis. A single *de novo* point mutation of nuclear protein lamin A gene at position 1824 (C to T) in exon 11, was found to be predominantly responsible for this syndrome. Lamin A is synthesized as the precursor protein, prelamin A which is modified at its carboxyl-terminal through a series of post translational modifications. The modifications include farnesylation of the cysteine in the C-terminus CAAX (C-cysteine, A-aliphatic, X-other amino acid)-motif, followed by proteolytic cleavage of the AAX-peptide, and methylation of the farnesylated cysteine. The metalloproteinase, Zmpste24, is responsible for the sequential proteolytic cleavage of prelamin A into functional and mature lamin A. The point mutation identified from HGPS patients results in the activation of an aberrant cryptic splice site causing the deletion of a 50 amino acid region from the C-terminal end of prelamin A [35]. Since the deletion harbours a cleavage site for the enzyme Zmpste24, prelamin A cannot undergo complete processing into mature lamin A in HGPS patients, and a truncated protein that lacks amino acids 607-656 (called as progerin) accumulates in cells. Thus, the unprocessed forms of lamin A, progerin and prelamin A accumulate in HGPS and Zmpste24-null cells respectively (Figure 1).

Remarkably, human HGPS premature aging phenotypes can be recapitulated in Zmpste24-null mice indicating that the presence of 18 extra amino acid tail of prelamin