Chromatin and Aging

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Abstract Recent studies from a number of model organisms have indicated chromatin structure and its remodeling as a major contributory agent for aging. Few recent experiments also demonstrate that modulation in the chromatin modifying agents also affect the life span of an organism and even in some cases the change is inherited epigenetically to subsequent generations. Hence, in the present report we discuss the chromatin organization and its changes during aging.

1 Introduction

Aging is a complex phenomenon and is a result of many biochemical and molecular changes in the cellular level. The major aging mediated changes in a cell include: reduction in number of mitotic cells; alteration in permeability of membrane; decreased hormone and enzyme secretion; decreased immunity; decreased antioxidant defence with concomitant increase in free radical generation; increased DNA damage (Ashok and Ali 2003). There are many theories to explain aging, such as the gene regulation theory. Recently, change in chromatin structure and epigenetic alterations have also been correlated to aging (Gravina and Vijg 2010). The change in the chromatin structure during aging can be traced back to the observation that chromatin became compact in aged rats with concomitant decline in transcription (Chaturvedi and Kanungo 1985). However, progress in the area of chromatin as a mediator of aging has been relatively slow and was only dependent on few sporadic observations. Few observations of the last decade including role of histone

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deacetylases in extension of life span in few model organisms has reinforced the link of chromatin to aging.

Here, we describe age dependent changes in the chromatin structure and function. In the first section we give a brief account of the chromatin structure and organization. Then we describe different modes of chromatin remodeling such as ATP-dependent and ATP-independent (post-translational modifications of histones) remodeling. Then we describe different model organisms used for aging study. Finally we describe different changes in the chromatin organization that takes place during aging.

2 The Chromatin Organization

The amount of DNA in a eukaryotic cell is enormous (approximately two meters in case of a diploid human nucleus). Hence, in order to be packaged inside the tiny nucleus, which is approximately 10 μm in diameter, a huge level of folding is needed. To facilitate this, the eukaryotic DNA forms a large macromolecular complex called as chromatin, by associating with almost equal amount of histone and non-histone proteins (Kornberg 1974; Kornberg and Thomas 1974). The fundamental structural unit of chromatin is called nucleosome. Each nucleosome is a complex of a core histone protein octamer, around which 146/147 base pairs of DNA wraps around ∼1.7 turns and ∼54 bp (10–90 bp) remains as linker DNA. Arrays of nucleosomes appear as beads on a string structure of approximately 11 nm diameter and 5.5 nm in height (Kornberg 1974; Luger et al. 2012). These arrays of nucleosomes form the primary structure of chromatin and popularly named as 10 nm fibre.

The core histone octamer consists of histone H2A, H2B, H3 and H4, each in two copies, which are highly conserved among eukaryotes. These are lysine and arginine rich small basic proteins of molecular weight 11–22 kDa (Luger et al. 1997). The C-terminal regions of each core histone contain five α helical regions. Out of which, the central histone fold motif, comprise of α1 (9–14 a. a.), α2 (∼29 a. a.) and α3 (9–14 a. a.). This central histone fold motif is flanked on either side by two other α-helices. These α-helices are connected to each other by short β-loops. The histone fold domains interact with each other to form H2A–H2B and H3–H4 heterodimers. In physiological salt concentrations, two such H3–H4 heterodimers interact with each other by a H3:H3 interaction, to form a tetramer. Similarly a H2A–H2B dimer associates on either side of the H3–H4 tetramer via a H2B:H4 interaction (Luger et al. 1997).

Binding of the core histones to DNA is mostly achieved by weak interactions, such as electrostatic interactions, between the positively charged histone and the DNA phosphate backbone (Luger et al. 1997). The minor groove of the DNA faces towards the histones. There are about 14 such contacts per nucleosome.

The loops of adjacent histones come together and a pair of such loops forms a β-bridge, containing two arginine residues, which interact with the minor groove of

DNA. The linker DNA and the nucleosome entry and exit points are bound by H1. The nucleosome containing H1 is called as "chromatosome" which spans ∼168 bp (Fletcher and Hansen 1996).

The N-terminal tails of all the core histones, and the C-terminal tails of histone H2A are highly flexible and do not contribute much in the nucleosome formation. They emanate out of nucleosome core, are unstructured and are subject to diverse post-translational modifications (described later). These post-translational modifications act as epigenetic marks for the transcriptional activation and repression. In addition, these tails are responsible for making contact with other nucleosomes and subsequently form higher-order chromatin structure (Li and Reinberg 2011).

These 10 nm fibres undergo next level of compaction to form the 30 nm fibres, in which another six fold condensation of DNA takes place to form a secondary structure. Subsequent formation of the tertiary structure requires interaction between long-distance chromatin fibres by the involvement of histone N-terminal tails (Hansen 2002). The stabilization of higher order chromatin is achieved by the incorporation of linker histone H1 and its variants to the chromatin (Carruthers et al. 1998).

The exact structure of the 30 nm chromatin fibre still remains elusive (van Holde and Zlatanova 1995). However, to explain the conformation several models are proposed (see below). In low ionic strength, the chromatin appears as a beaded fibre, in which beads represent the nucleosomes (Olins and Wright 1973). The 10 nm fibres are wrapped in a left-handed fashion with six nucleosomes per turn to form the 30 nm chromatin structure. Two different models have been proposed to explain the 30 nm chromatin fibre structure; namely the solenoid model and the zigzag model (Robinson et al. 2006). According to solenoid model, the nucleosomes are folded to a simple helix, where the adjacent nucleosomes remain present next to each other in a chromatin fibre. In the zigzag model, the linker DNA is present in a straight path between nucleosomes (Woodcock et al. 1993). Here, two rows of nucleosomes are arranged in a zigzag fashion, due to interaction between alternative nucleosomes. This type of interaction produces a double helical structure (Bednar et al. 1998). Protein-protein cross-linking analysis revealed that from a single stack of 12 nucleosomes, two rows of six nucleosomes were produced as predicted for the zigzag model (Robinson et al. 2006).

The 300 nm fibre, which is the tertiary structure of the chromatin, is formed by folding of the 30 nm fibres. To achieve this folding, the 30 nm fibre assembles into loops, which are also associated with non-histone proteins. These loops are organized being part of scaffold proteins, which maintain its higher order structure (Woodcock and Ghosh 2010). They remain firmly attached to eukaryotic DNA even after its extraction under harsh conditions like high salt, strong detergents and polyelectrolytes. The scaffold is mainly comprised of two proteins i.e. scaffold protein 1 and 2, of M.W. 170 and 135 kDa respectively. The stretches of DNA loops that are associated with the protein scaffold are known as scaffold attachment regions (SARs). The 30 nm chromatin fibre loops are attached to the nuclear matrix via matrix attachment regions (MAR).

The 300 nm chromatin fibres further compact to form 700 nm chromatin fibre during the division phase of cell cycle. The mechanism of 700 nm fibre formation and its exact structure is still unknown. However, the metaphase chromosome is 1400 nm in diameter having two side-by-side 700 nm fibres. Altogether, it can be stated that the structure of 10 nm fibre is well understood and the structure of higher order chromatin structure still remains elusive (Woodcock and Ghosh 2010).

3   The Euchromatin and Heterochromatin: The Classical Domains of Chromatin

Historically, through staining methods, the chromatin was distinguished into a darkly stained heterochromatin and a light stained euchromatin regions. However, it is now established that these domains are differently folded (Grigoryev 2001). Some portions of the chromosomes have a dense, compact structure during interphase, in which genes are repressed, and can be stained with certain dyes. These regions are known as heterochromatin. The regions of chromatin that are relatively unfolded and permissive for gene expression are defined as euchromatin. The euchromatin and heterochromatin domains are interspersed along the chromosome and are demarcated by chromatin barriers known as boundary or insulator elements (Bi and Broach 2001). With recent studies, they have been shown to have differential pattern of post-translational modification in histones and DNA (described later).

4   Chromatin Remodeling

Chromatin becomes inhospitable to nuclear events, such as replication, transcription, recombination and repair, since the DNA remains occluded, being in the form of nucleosomes. This occlusion is reversed by chromatin remodeling. Chromatin remodeling refers to regulated alteration in the chromatin or in the nucleosome structure, making it transiently permissive/occlusive, so as to enhance/obscure binding of factors requiring DNA as template. ATP dependent chromatin remodeling factors and ATP-independent chromatin remodeling factors, with support of each other, mostly bring about the chromatin remodeling (Vaquero et al. 2003).

The ATP-dependent chromatin remodelers are multi-subunit protein complexes of >1 MDa. They utilize ATP and cause disruption in the nucleosome so that the DNA-histone contacts are loosened; mobilize the octamer in cis along a DNA sequence so that a particular DNA sequence becomes accessible; transfer the octamers in trans so that the promoters are freed to be accessed by transcription factors and RNA polymerase; cause looping in DNA; or incorporate histone variants to the nucleosomes. The above mentioned remodeling complexes mostly

contain a highly conserved ATPase subunit of helicase superfamily. Depending on the presence of other flanking domains and accessory subunits, the remodeling complexes are further divided into SWI/SNF, ISWI, CHD and INO80 families (Clapier and Cairns 2009).

4.1 SWI/SNF (Switch Defective/Sucrose Non-fermenting) Family

The SWI/SNF group of remodelers (with an approximate size of ∼2 MDa) contain an ATPases domain of the SNF2 subfamily and 8–15 other subunits (Vaquero et al. 2003). The C-terminal domain contains a bromodomain for binding to acetylated histones. The SWI/SNF complex was initially identified in yeast. Homologues of yeast *SWI2/SNF2* were subsequently identified and characterized from *Drosophila* (*brahma*), mouse (*mBrm* and *mBrg*) and human (*hBRM* and *BRG1*). Similar *BRG1* containing homologues have also been identified in rat, *Xenopus* and chicken (Panigrahi et al. 2003; Vignali et al. 2000). In yeast, there is another SWI/SNF like complex called as RSC (*R*emodels *S*tructure of *C*hromatin, size of ∼1.5 MDa) (Cairns et al. 1996). The RSC contains four homologues of yeast SWI/SNF components.

The SWI/SNF and related complexes broadly function in reorganization and positioning of nucleosomes in the promoter region for facilitating transcription factor binding. They can cause random positioning of the nucleosomes in a chromatin domain using evenly positioned nucleosomes as substrates. They are also responsible for ejection of nucleosomes and exposing a target DNA site (Dechassa et al. 2010).

4.2 ISWI (Imitation Switch) Group of Chromatin-Remodeling Complexes

Identified first in *Drosophila,* ISWI-group of remodeling factors has a helicase-ATPase domain, but they lack the bromodomain, unlike the *SWI2/SNF2*. Instead their C-terminal domain contains a SANT or a SLIDE domain (Clapier and Cairns 2009). Later the ISWI protein was found to be a principal constituent of three ATP-dependent chromatin-remodeling complexes in *Drosophila,* namely NURF (*nu*cleosome *r*emodelling *f*actor), CHRAC (*chr*omatin *a*ccessibility complex) and ACF (*A*TP-utilizing *c*hromatin assembly and remodeling *f*actor) (Vignali et al. 2000). NURF was purified from *Drosophila* embryonic extracts as an activity that stimulated transcription factor binding to chromatin promoter. Similarly, CHRAC was purified as an activity that made chromatin accessible to nucleases. ACF, on the other hand, was responsible for nucleosome assembly with the help of

histone chaperons (Vignali et al. 2000). ISWI-related complexes were also identified from yeast, *Xenopus* and human (Dirscherl and Krebs 2004). There is a considerable functional heterogeneity with the ISWI containing complexes. Except NURF, no other ISWI-complex has been shown to disrupt nucleosomes. However, they slide nucleosomes along DNA (cis-transfer) (Dirscherl and Krebs 2004).

4.3   Mi-2/CHD (Chromodomain, Helicase, DNA Binding) Chromatin-Remodeling Factors

These protein complexes were initially identified in mouse (Delmas et al. 1993). They broadly contain an N-terminal chromodomain, usually an essential domain for proteins that interact with and modify chromatin, an ATPase domain (a classical SNF2 group of helicases/ATPases), and a plant homeodomain fingers (a DNA-binding domain). Similar complexes were also identified in yeast, *Drosophila*, *Xenopus*, mouse and human (Woodage et al. 1997). In human cells *nu*cleosome *r*emodeling and *d*eacetylase complex, NuRD was identified (Xue et al. 1998). Apart from the regular components of CHD, it also includes *h*istone *deac*etylase *1 & II*. NuRD plays very significant role in cellular processes converging histone deacetylation, DNA methylation and cell-cycle regulation (Fugita et al. 2004).

4.4   INO80 (Inositol Requiring) Factors

These factors are characterized by a split ATPase domain due to a long insertion in the middle. Homologous factors of INO80 exist in yeast (INO80 and SWRI) and human (INO80, SRCAP and TIP60) (Bao and Shen 2007). They demonstrate different kinds of remodeling activities such as nucleosome repositioning, eviction, replacement and exchange of histone with histone variants, mediated by replication independent pathways. They have also role in double strand break repairs of DNA. The TIP60 complex has both histone acetylation and ATPase activity (Morrison et al. 2007).

5   ATP Independent Chromatin Remodeling: Covalent Modification of Histones

The ATP independent chromatin remodelers covalently modify the histone tails or the globular domains. Histones are one of the most conserved eukaryotic proteins and are subject to most diverse number of post-translational modifications such as:

acetylation, methylation, phosphorylation, ADP-ribosylation, ubiquitination, biotinylation, sumoylation, etc. (Vaquero et al. 2003). Altogether, they generate accessible or repressible chromosomal domains for further interaction of chromatin complexes.

5.1 Acetylation

Acetylation of histones involves transfer of an acetyl group to lysine residues, present mostly in the N-terminal tails and some core residues of histones. Acetylation of histones is a reversible process. Histone acetyl transferases (HATs) add acetyl groups while histone deacetylases (HDACs) remove it. The acetylated histones become less positive, resulting in decreased affinity for the negatively charged DNA that in turn weakens DNA binding (Hong et al. 1993). This brings about a permissive structure in the chromatin, for binding of different proteins factors to DNA (Lee et al. 1993). Transcriptional activation has been shown mostly synergistic to histone acetylation (Struhl 1998). Further, acetylated histone tails can directly recruit other chromatin interacting machineries, such as bromodomains containing factors. Hence, they act as docking sites for downstream events (Berger 2002; Zeng and Zhou 2002).

In case of vertebrates, all core histones are acetylated at multiple sites. The sites are: H2A (K5, K9); H2B (K5, K12, K15 and K20); H3 (K9, K14, K18 and K23); H4 (K5, K8, K12 and K16), except in yeast, where acetylation sites of H2A, H2B are little different from vertebrates (Roth et al. 2001). There are different HATs, with differential histone substrate specificities. The substrate specificity is accomplished by the interacting partners. The HATs can be broadly divided into type A and type B (Roth et al. 2001). The type B HATs are mostly cytoplasmic and acetylate free histones at sites which are crucial for deposition into nucleosomes. The type A HATs acetylate nucleosome bound histones and broadly belong to three families. The H3 and H4 acetylation is carried by HAT family member Gcn5, while, H4 and H2A are acetylated by the nucleosome acetyl transferase Esa1 (Suka et al. 2001). In vitro all four core histones are acetylated by p300 (Kuo et al. 1996; Ogryzko et al. 1996; Yang et al. 1996).

Histone deacetylation is carried out by histone deacetylases and it results in gene repression. All four core histones are deacetylated by Rpd3 deacetylase (Roth et al. 2001), while, H3 and H2B are deacetylated by Hda1 (Histone deacetylase 1) (Xu et al. 1998). Rpd3 and Hda1 cause deacetylation of larger regions of euchromatin (Vogelauer et al. 2000). Histone deacetylases are also responsible for deacetylation of non-histone proteins, such as p53, tubulin and various transcription factors (Jones and Baylin 2002; Timmermann et al. 2001). Depending on the functional motifs and domains, mammalian histone deacetylases are classified into Class I HDACs (1, 2, 3 and 8), Class II HDACs (4, 5, 6, 7, 9, 10) and class-III HDACs (NAD+ dependent Sir2 family) (de Ruijter et al. 2003; Grozinger et al. 2001). HDAC 11 is shared by both class-I and class-II HDACs. Histone deacetylases have role in turnover of histones, initiation of transcription and repression of active genes.

5.2   Phosphorylation

Phosphorylation of histone occurs in Ser, Thr and Tyr residues. It is also a reversible modification being regulated by kinases and phosphatases (Bannister and Kouzarides 2015). Phosphorylation of H1 and H3 has been correlated with chromosome condensation during mitosis (Hanks et al. 1983; Hirano 2000). Phosphorylation also adds a negative charge to histones. The kinases responsible for phosphorylation of histones can be divided broadly into two categories: (i) those dependent on cyclic nucleotide monophosphates for activation and (ii) those whose activity is independent of cyclic nucleotide monophosphates. However, many other functions of histone phosphorylation still remain elusive.

5.3   ADP Ribosylation

ADP-ribose molecules are transferred either to glutamic acids or arginine residues by poly (ADP-ribosyl) polymerase (Jacobson and Jacobson 1999). Though, all histones are subject to ADP-ribosylation; H2B and H1 are the most preferred substrates (Kappus et al. 1993). ADP-ribosyl transferase transfers single ADP-ribose to arginine or lysine residues of free histones in the cytoplasm. Subsequently, poly-(ADP-ribosyl) polymerase (PARP), carry out the elongation process in nucleus. PARP recognizes and binds to single or double strand DNA breaks and then exposes the DNA strand breaks to proteins involved in repair process (Le Rhun et al. 1998). The ADP-ribosylation is reversed by poly-ADP-ribose-glycohydrolase. Poly ADP-ribosylated histones are part of the relaxed chromatin. Further, it is known that by ADP-ribosylation, the H3K4me3 demethylase is excluded from the chromatin, making the chromatin domain transcriptionally active (Li et al. 2014).

5.4   Ubiquitination

Ubiquitin is a 76-amino acid protein (Conaway et al. 2002). Ubiquitination is another reversible modification, which occurs at lysines residues of histone H2A and H2B during cell cycle. H3 and H1 are also reported to be modified by the same mechanism (Belz et al. 2002). Monoubiquitination is a mark of both transcriptional activation and repression depending on the position and histone subtype. Monoubiquitination of H2BK123 in yeast is crucial for methylation of H3K4, a mark of transcriptionally active chromatin (Sun and Allis 2002). Similarly, monoubiquitination of H2AK119 is a mark if transcriptionally inactive chromatin. In vitro, polyubiquitination of histone H2A, H2B and H3 are targets for degradation (Jentsch and Schlenker 1995).

5.5 Sumoylation

Small Ubiquitin related modifiers (SUMO) is also involved in posttranslational modification of histones on lysine residues (Melchior 2000; Nathan et al. 2003; Robzyk et al. 2000; Shiio and Eisenman 2003). SUMO shares only 18 % identity with Ubiquitin group. Out of the four histones, histone H4 is efficiently modified by SUMO-1 or SUMO-3, both in vitro as well as in vivo. Acetylated histone H4 usually gets sumoylated. It leads to decreased acetylation of histone H3 due to recruitment of HDACs and increase in HP1 binding. Hence, sumoylation has a role in repressing transcribing chromatin (Girdwood et al. 2003). It has been hypothesized that probably sumoylation initiates attenuation followed by gene repression.

5.6 Lysine Propionylation and Butyrylation

Histones are subject to propionylation at K5, K8 and K12 of H4 and lysine butyrylation at K5 and K12 of histone H4 (Nathan et al. 2003). These sites are also known to be acetylated (Tanaka et al. 2004). Probably these two new types of modification create novel docking sites for downstream recruitment of other complexes which are yet to be elucidated.

5.7 Deimination

In this type of modification, an arginine or monomethyl arginine residue of the histone is converted to citruline (Cuthbert et al. 2004). In mammals, this conversion is catalysed by peptidyl deiminase 4 (PADI4) and this enzyme is known as arginine demethylase. The resultant change of the citruline instead of arginine reduces in the histones affects the positive charge of the histones and it also abolishes the respective docking site.

5.8 Methylation and Demethylation

Histone H3, H4 and H1 can be methylated on their arginine (R) and lysine (K) residues (DeLange et al. 1969; Gershey et al. 1969; Murray 1964; Patterson and Davies 1969). Depending on the number of methyl groups attached to ε amino group, lysines can be mono, di or tri methylated. Similarly, arginines can be mono or dimethylated. Lysine methylation can have mixed effects. It depends on the position of lysine residue which is methylated and the levels of methylation (mono, di or trimethylated). Methylation at K4, K36 and K79 of histone H3 are marks of

transcriptionally active chromatin, while methylation at K9, K27 of H3 and K20 of H4 are marks of transcriptionally inactive chromatin (Cao et al. 2002; Peters et al. 2003; Rice et al. 2003; Schotta et al. 2004). The lysine methylation is reversibly regulated by histone methyl transferases and demethylases. Histone methyl transferases (HMTs) methylate histone lysine and arginine residues. They have been broadly classified into three protein families: the PRMT family methylates arginine residues (Bedford and Richard 2005; Zhang and Reinberg 2001), while the SET domain containing family and the Dot1/DOTL families methylate lysine residues (Bannister et al. 2002; Kouzarides 2002; Lachner et al. 2003). The SET1 group is specific for H3K4 (Ringrose and Paro 2004; Smith et al. 2004), the SUV 39 group for H3K9 (O'Carroll et al. 2000; Rea et al. 2000; Tachibana et al. 2001; Yang et al. 2002), the SET2 group for H3K36 (Peters et al. 2001) while ASH1 shows broad specificity and methylates H3K4, H3K9 and H4K20. The EZH family methylate K27 and K9 of histone H3 (Czermin et al. 2002; Muller et al. 2002), Dot1 containing proteins (Singer et al. 1998) methylates H3K79 of the core domain (Feng et al. 2002; Lacoste et al. 2002; Ng et al. 2002; van Leeuwen et al. 2002). However, in many of these methylases, cross-reactivity is also reported.

Unlike acetylation, in methylation, there is no major change in total charge on histones. Rather specific lysine residues, after being differentially methylated (mono, di or tri), act as docking sites for further recruitment of other interacting machineries. It finally results in generation of transcriptionally permissive/occlusive chromatin template (Strahl and Allis 2000; Turner 2000). For example, the chromodomain containing protein HP1 (heterochromatin protein 1) is recruited to methylated histone H3K9 and generates a heterochromatin domain (Bannister et al. 2001; Nakayama et al. 2001). The heterochromatinization spreads by self-association of many HP1 (Nielsen et al. 2002). Antagonistically, chd1, the chromodomain containing protein (a component of SAGA and HAT complex) is recruited to methylated H3 K4, and it further recruits SAGA and HAT complexes and ultimately generates a euchromatic chromatin domain (Pray-Grant et al. 2005).

5.9 Lysine Methylation in Transcriptional Silencing

Methylation of K9 and K27 of H3 and K20 of H4 are marks of transcriptionally silenced chromatin domains (Bannister and Kouzarides 2015). Methylation of H3K9 is a deterministic mark of constitutive heterochromatin and also marks chromatin domains for transcriptional silencing. The differential role is played by the fact that euchromatic genes are regulated being dimethylated at H3K9 and the heterochromatic silencing takes place by generating trimethylated H3K9. However, few deviations are also available (Vakoc et al. 2005). Similarly, H3K27 methylation is also a mark of transcriptional silencing and X-chromosome inactivation.

There are reports that methylation of H9 of H3 (H3K9me) is a prerequisite for DNA methylation (Jackson et al. 2002; Lehnertz et al. 2003; Tamaru and Selker 2001), as binding of HP1 to H3K9me recruits DNA methyl transferases. It has been

further demonstrated that E2H2 complex, which methylates H3K27, also interacts with DNA methyl transferases (Vire et al. 2006). However, results supporting the opposite hypothesis have also been documented. There is significant reduction in H3K9 methylation marks in DNA methyl transferase deficient cells (Sarraf and Stancheva 2004).

H4K20 methylation also correlates with the condensed regions of chromatin. In vitro its methylation inhibits of H4K16 acetylation, which is a mark of transcriptionally active chromatin domain. The H4K20me is also an epigenetic mark of repression and generally goes synergistically to H3K9 methylation (Zhang and Reinberg 2001).

5.10   Lysine Methylation and Active Transcription

5.10.1   Methylation of H3K4

The complex associated with *setI* (COMPASS) in association with elongating RNA Polymerase II, methylates H3K4 (Briggs et al. 2001; Daser and Rabbitts 2004; Gerber and Shilatifard 2003; Krogan et al. 2002; Miller et al. 2001). The COMPASS of the MLL complex are classically known as generators of euchromatin marks (Roguev et al. 2001). Components of COMPASS generate trimethyl K4 of histone H3 (H3K4me3) marks (Schneider et al. 2005). Actively transcribing genes are enriched with both dimethyl and trimethyl H3K4 marks. While, the dimethyl modifications mostly restricted to the coding regions, the trimethyl modification localize specifically to the regulatory regions of genes (Bernstein et al. 2005). WDR5 and *Chd1* are recruited to the methylated H3K4, and they acetylate other lysine residues like H3K14 and K9. The K9 acetylation mark is antagonistic to K9 methylation mark and thus the respective chromatin domain remains in an activated state (Timmers and Tora 2005). Further, the WDR5 binds to H3K4me2 and is responsible for maintaining global trimethylated H3K4 condition (Wysocka et al. 2005).

5.10.2   Methylation of H3K36

Set2 was initially identified in yeast to have HMTase activity specific to H3K36 (Strahl et al. 2002). *Set2* was speculated to have role in transcriptional elongation as was reported to associate with phosphorylated RNA Polymerase II (Krogan et al. 2003). It is thus hypothesized that recruitment of *Set2* along with RNA Pol II is crucial for establishing K36 methylation on chromatin (Gerber and Shilatifard 2003, Hampsey and Reinberg 2003).

5.10.3 Methylation of H3K79

The nonset domain containing protein *dot1* (disrupter of telomeric silencing 1) methylates the core domain of nucleosomal histone H3. It is further seen that 90 % of the nucleosomal histone H3K79 remains methylated (van Leeuwen et al. 2002). The H3K79me mark prevents binding of silent domain Sir Proteins and maintains the chromatin in euchromatic state and prevents further heterochromatinization.

5.10.4 Histone Lysine Demethylation

Histone methylation was considered to be a relatively permanent modification since, the histone demethylases were not known. However, subsequently the histone demethylases have been identified and characterized, at least for quite a few methylated residues. Lysine specific demethylase-1 (LSD1) was shown to be specific for H3K4me2 (Shi et al. 2004). LSD1 is a component of Co-REST co-repressor complexes (Hakimi et al. 2002; Shi et al. 2003; You et al. 2001). Demethylation by LSD1 generates unmethylated histones, formaldehyde and H_2O_2 and in the process FAD^+ is reduced to $FADH_2$, which is then reoxidised by (O). The (O) is reduced to form H_2O_2. However, LSD1 shifts its specificity from H3K4me2 to H3K9me2, and acts as a H3K9 demethylase and transcriptional activator in the presence of Androgen receptor (AR) (Metzger et al. 2006).

Another group of proteins called Jumonji (JmjC) proteins are shown to have histone dimethylase activity (Tsukada et al. 2006). JHMD1/Fbx11 (JmjC domain containing histone demethylase1, alias Fax11) demethylates mono and di meH3K36. Similarly, JHDM2 demethylates H3K9me2 (Yamane et al. 2006). Recently JMJD 2A-2D groups are identified. They reverse trimethylation, specifically H3K9me3 and H3K36me3 (Whetstine et al. 2006). Since, H3K9me3 and H3K36me3 are total antagonists: as the former is a mark of transcriptionally inactive region and the later is a mark of region engaged in transcriptional elongation. The dual site specificity of the JMJD2A/C may suggest a coordinated role in regulating H3K9/K36 trimethylation for gene regulation (Carrozza et al. 2005).

5.10.5 Histone Arginine Methylation

Arginine methylation is mainly linked to active transcription. Protein arginine methyl transferases (PRMTs) transfers a methyl group to arginine residues (Gary and Clarke 1998). Arginine methylation occurs in R2, R17 and R26 of histone H3 and R3 of histone H4. Arginines can be mono- or dimethylated. Further, the dimethylation can be symmetric or asymmetric. The arginine methyl transferases (PRMT) which methylate Arg residues have been divided into two groups. The type-I enzyme catalyse the formation of -monomethyl and asymmetric dimethyl arginine residues, whereas the type-II enzyme catalyzes the formation of monomethyl and symmetric dimethyl arginines (Zhang and Reinberg 2001).

5.10.6 Reversal of Arginine Methylation

Monomethylated and unmethylated arginine can be converted to citrulline by arginine deiminase, PADI4 (Cuthbert et al. 2004). PADI4 can deiminate unmodified arginine and monomethyl (but not dimethyl) arginine. It is a Ca^{+2} dependent enzyme that acts as a repressor of transcription, in the *pS2* promoter. It demethylates H3 and H4 arginines (mono or unmethylate) by deimination, at positions R2, R8, R17 and R26 of histone H3 in vitro and R2, R8 and R17 in vivo. Similarly, JMJD6 demethylates H3R2me2 and H4R3me2 in vitro and in vivo (Chang et al. 2007).

5.10.7 DNA Methylation

Methylation also occurs in few nucleotides of DNA and is often synergistic or antagonistic to few histone methylations (described earlier). 5-methyl cytosine and subsequent guanine (5meCpG) dinucleotides are mostly marks of gene silencing and also confer epigenetic memory. DNA methylation is regulated by de novo DNA methyl transferases and maintenance methyl transferases (Marchal and Miotto 2015). The first group of methyl transferases, DNMT3a and DNMT3b, methylate one strand of the newly synthesized DNA and convert them to hemimethylated DNA. The hemimethylated DNA is converted to a methylated DNA by maintenance methylase, DNMT1. However, DNMT3a and DNMT3b have been shown to also act on hemimethylated DNA (Marchal and Miotto 2015). Few recent reports have also demonstrated methylation outside the CpG (Yang et al. 2013). The human genome has approximately 30 million CpG. Dense clustering of these CpG in some regions of the genome is observed and these regions are known as CpG islands. Presence of 5meCpG islands in the promoter region of a gene is a mark of silenced gene. 5meCpG in the coding region of a gene is also observed, however, its mode of regulation is yet to be properly understood (Shenker and Flanagan 2011). DNA demethylases were not known until recently and hence DNA methylation was believed to be a relatively permanent mark. Now, formation of 5-hydroxymethyl cytosine (5-hmeC) is believed to be an intermediate in DNA demethylation process (Pfaffeneder et al. 2011). Oxidation of 5-meC generates 5-hmeC and then to several intermediates which are observed (Jurkowski and Jeltsch 2011). These reactions are catalysed by TET (ten-eleven translocations) enzyme family. Embryonic stem cells have higher levels of 5-hmC and it decreases upon differentiation (Xu et al. 2011). However, few other methods are also proposed for demethylation of DNA. They include non-replacement of methylation marks in the daughter cells during division, removal of methyl group by DNA glycosylase and deamination of cytosine or oxidation.

5.10.8 The Histone Code and Cross-Talk in Histone Modification

Many of the histone modifications act as docking sites and cause further recruitment of protein complexes that bring about broadly two effects: a permissive chromatin domain for binding of factors; or an occlusive chromatin domain refractory for factor binding. Accordingly, a group of modifications coexist and work synergistically but are not compatible with others group of modifications (Jenuwein and Allis 2001). Say, for example, when H3K4 is methylated, there is synergistic acetylation of H3K14 and phosphorylation of H3S10. All are involved in transcriptional activation, and all of them are antagonistic to the inhibitory H3K9 methylation. Further, the complexity increases as lysines can be mono, di or tri methylated. Accordingly, they encode different signals for activation. Taken together, a language of histone modification can be created with the available information, which was initially framed as histone code (Strahl and Allis 2000). This code was then supported by yet another hypothesis that the simplest code can be binary (Jenuwein and Allis 2001). For example, H3K9 and H3K4 cannot be methylated simultaneously in the same chromatin domain. Likewise, H4K20 methylation and H4K16 acetylation are antagonistic. Distinct histone modifications, on one or more tails, act sequentially to form a "histone code", that is read by chromo and bromodomain containing proteins. This creates specific combinations of modifications on a local domain. All together they bring about formation of the euchromatic and heterochromatic domains of chromatin. A detailed account of the different post-translational modification and their cross-talk is represented in Fig. 1.
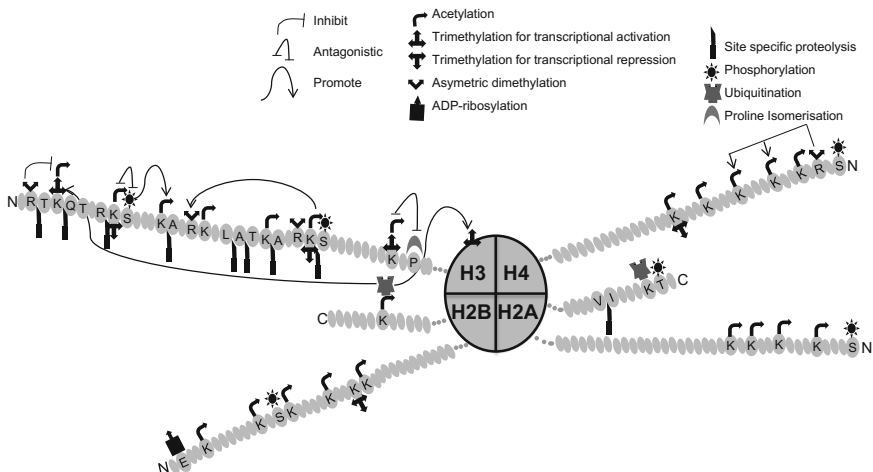
Fig. 1 Histone post-translational modification and their cross-talk: The N- and C-terminal core histone tails are represented with a. a. residues that are subject to different modifications. Here, the proteolytic cleavage is also included. Further, few deterministic cross-talks between the modified docking sites are also represented

5.10.9   The Proteolytic Processing of Histones

One permanent way of removal of the histone modification is by clipping the histone tails. Proteolysis of histones, though rare, is believed to be another mode of irreversible post-translational modification (Allis et al. 1980). The N-terminal/C-terminal tails of histones are more susceptible for the proteolysis, when histones exist in the form of chromatin. Proteolysis of histones emerged as a problem for chromatin isolators (Brandt et al. 1975). However, later the site specific histone proteolysis has been linked to aging and chromatin structure modulation (see below).

In calf thymus, chromatin-bound proteolytic activity which was specific to H2A was demonstrated (Eickbush et al. 1988). The cleaved product, which lacked 15 a. a. from the C-terminus, was named as cH2A. The cleavage site was between Val-114 and Leu-115. (Okawa et al. 2003). Two truncated forms of H2A have been purified from the nuclei of acute myeloid leukemia OCI/AML1a cells (Okawa et al. 2003). However, the enzyme catalysing this cleavage is not known. Proteolysis of histones has also been proposed during transformation of spermatids into spermatozoa (spermeogenesis), at the terminal stages of spermatogenesis (Marushige et al. 1976). During the process, chromosomal DNA becomes tightly packaged as a result of proteolytic replacement (Marushige and Marushige 1983) of somatic type histones, by protamines. The mechanism of histones displacement requires their proteolysis for removal. A protease activity associated with mouse testicular chromatin has also been observed, which might be involved in proteolytic removal of somatic histones during spermatogenesis (Faulkner and Bhatnagar 1987). Further, this specific proteolysis was absent in other tissues like thymus and liver suggesting tissue specific functional regulation.

Selective and site specific proteolysis of a specific histone subtype has also been observed during viral infection. There is histone H3 degradation in mammalian kidney cells (BHK cells) infected with Foot-and-Mouth Disease Virus (FMDV) (Grigera and Tisminetzky 1984). Further, it is demonstrated that the H3 degradation is catalysed by the FMDV 3C protease and cleavage site has been mapped between Leu-20 and Ala-21, from the N-terminal end (Falk et al. 1990). As the processed H3 lacks most of the N-terminal acetylation sites, it would shut off/reduce the host cellular transcription (Tesar and Marquardt 1990).

Proteolytic processing of histone H1 and H3 has been observed as a physiologically and developmentally regulated event in *Tetrahymena* nuclei (Allis et al. 1984). Histone H3 is cleaved at multiple site spanning between a.a. 21–27 by Cathepsin L during mouse embryonic stem cell differentiation (Duncan et al. 2008). The protease Cathepsin L is extensively characterized. The H3 cleavage by Cathepsin L is regulated by specific covalent modification code of histone H3. Recently, in chicken liver tissue, glutamate dehydrogenase was identified as a histone H3 N-terminus specific protease (Purohit et al. 2013). A yet to be identified histone H2A specific protease is also characterized from chicken liver (Panda et al. 2013). In summary, in recent years histone specific proteolytic cleavage and histone subtype non-specific degradation have also been observed simultaneously,

suggesting that histone-specific and histone non-specific proteases exist in nature and they have evolved along with histones. Hence, it is recently opined to distinguish these proteases into broadly two groups: 1. histone proteases with broad spectrum specificity mostly function for turnover of histones. 2. Histone proteases with site specificity have role in modulating chromatin structure and function by modulating epigenetic marks (Dhaenens et al. 2015).

6 Change in Chromatin During Aging

With the preliminary understanding on the chromatin organization and function, in the following section we intend to describe the age dependent change in the chromatin organization and function. Since, aging is naturally a slow process to score, at least in a number of higher organisms; simple models are developed to study aging. So in the first part of the following section, we describe different suitable model systems which are presently being studied to understand the chromatin structure and aging. In the subsequent section we will describe different changes in the chromatin organization and function that take place during aging.

7 Organismic Models for Studying Chromatin and Aging

7.1 Yeast

Due to the ease in genetic manipulation, short life cycle and conservation of mechanisms of aging across the eukaryotes, the budding yeast *Saccharomyces cerevisiae* is a growing model for aging study (Wood and Helfand 2013). One of the measurement parameters for aging is the replicative life span in budding yeast or in other words the number of daughter cells a budding mother cell produces before it reaches non-dividing stage. It has been observed convincingly that there is loss of transcriptional silencing with concomitant decrease in chromatin compaction in budding yeast during the process of aging (Smeal et al. 1996). In budding yeast, three regions of the genome remain silent: viz. the mating type switching locus (MAT locus), the ribosomal DNA locus (rDNA) and the telomere proximal regions. In aged yeast, there is loss of silencing of the MAT locus leading to co-expression of both mating type genes, causing insensitivity to pheromones and increased sterility in aged yeast. Further, there is movement of silencing proteins such as HDAC (NAD$^+$ dependent histone deacetylase) and silencing information regulator 2 (Sir2) away from the telomeric region in the aged yeast (Kennedy et al. 1997). Also, in the aged yeast, there is loss in silencing of the rDNA region with concomitant rise in recombination and formation of extra ribosomal chromosomal circles and increase in genomic instability. These events can be monitored with

limiting replicative life spans. It is further seen in yeast model that deletion of Sir2 reduces replicative life spans, while introduction of an extra copy of Sir2 gene increases the same (Etzioni et al. 1999). Hence, from these observations, it can be concluded from yeast model that chromatin of the silent regions remains in more relaxed state in aged yeast. Sir2 is known to deacetylate H4K16Ac (Imai et al. 2000). Further, there is an age dependent decrease in Sir2 proteins in yeast leading to increase in H4K16Ac mark in global level (Dang et al. 2009). The H4K16Ac is a unique epigenetic mark which inhibits formation of 30 nm fibres and subsequent folding of the chromatin into higher order. Thus, age dependent increase in H4K16Ac leads to formation of open chromatin structures globally (Shogren-Knaak et al. 2006).

There is also change in the gene expression pattern during aging in yeast (Wood and Helfand 2013). Some of the change in gene expression could be due to elevated stress responses and induction in repair genes during aging. However, a significant amount of change in the gene expression could also be due to altered chromatin structure during aging. It is observed that there is increased transcription of the core histones with aging. On the other hand, short-lived yeast mutants deficient in the ability to acetylate H3K56Ac have decreased level of core histone transcript in aged cells, which is in turn similar to that of telomerase deficient cells (Lesur and Campbell 2004). H3K56Ac is enriched in histone gene promoters for transcription. Conversely, elevation in the histone transcript levels in aged yeast does not correlate with increase in bulk histones in the aged yeast. In fact, there is decrease in bulk histones in aged yeast cells (Feser et al. 2010). This could be due to the feedback inhibition of the histone transcript translation. Further, by ChIP analysis it has been revealed that there is reduced histone occupancy in many regions of the chromatin including the telomere proximal region, the rDNA region and the MAT locus in the aged yeast (Wood and Helfand 2013). The loss of histone from the chromatin of aged yeast leads to the formation of a more open chromatin and in turn increases transcription of some genes. On the other hand, lower concentrations of the synthesized histones in the aged yeast leads to decreased pool of free histones and also alters epigenetic marks of the histones. In another line of evidences, it has been found that aged yeast cells have decreased polyamine synthesis (amines are required for cell proliferation). Yeast cells treated with spermidine showed hypoacetylation of H3K9, K14, K18 and H4K16 with concomitant decrease in HAT activity, specific for these sites and increase in replicating life span (Eisenberg et al. 2009). However, it was in contrast to the hyperacetylation of the H3K56 sites suggesting again the site specific role of diverse histone acetylations in modulation of aging in yeast model.

7.2   Drosophila

Similar to the change in the acetylation at multiple sites of histone H3 and H4, the marks of active chromatin, from aged yeast, a decline in few methylation sites (which are mark of active chromatin) have also been reported from aged

Drosophila models. There is decrease in H3K4me3 and H3K36me3 marks with age in *Drosophila* (Wood and Helfand 2013). However, there is also less enrichment in the H3K9me3, and Heterochromatin protein1, HP1 (mark of inactive chromatin) in the pericentric heterochromatin. These flies with decreased HP1 expression also exhibited increased levels of rRNA transcripts. Recently it has been reported that flies with decreased HP1 expression exhibited shorter lifespan whereas in flies where HP1 was overexpressed, there was increase in the lifespan (Larson et al. 2012).

7.3   *Caenorhabditis elegans*

In *C. elegans,* a number of histone modifications have been recently correlated with the life span of the organism. The methylation marks of K4, K9 and K27 of H3 are extensively studied in the worm (Padilla et al. 2014). It is found that disruption of the H3K4 methyl transferase complex (ASH-2), leads to increase in the lifespan of the worm, while disruption of the H3K4 demethylase (RBR-2) leads to decrease of the same. It has been further shown that this increase in life span is inherited up to certain generations (Greer et al. 2011). Similar observations are also reported for other modifications sites of histone H3. An aging dependent decline in the H3K27me3 is observed in the worm with concomitant increased activity of the respective histone demethylase (UTX-1). It has been further seen that disruption of UTX-1 results in increased levels of H3K27me3 marks in the worm genome and there is increase in the life span (Maures et al. 2011). Synergistically, disruption of the LSD1 complex that acts as a demethylase for H3K4me and H3K9me sites also results in increased life span in the worm (McColl et al. 2008). However, it is not clear from these observations, why there is an increase in life span on hypermethylation of activation marks such as H3K4 as well as repression marks such as H3K9 and H3K27. It can be suggested that probably gene specific transcription plays a more important role during aging than the global level change in the transcription of an organism which can also be tissue specific.

7.4   *Mammals*

Mouse, rats and few human diseases are also used as good models to study age related changes in chromatin. Analysis of the 30 nm fibres and *Micrococcal* nuclease (MNase) digestion of the nuclei have indicated that there is irregular positioning of the nucleosomes in the chromatin of aged fibroblasts. It suggests that there is loosening of the chromatin structure with age (Macieira-Coelho and Puvion-Dutilleul 1989). There is also decrease in heterochromatic regions like less efficient inactivation of the X-chromosomes in aged mice (Cattanach 1974).

Genetic disease such as progeria in mammals mimics the aging process and pose as a good model for studying aging (Sedivy et al. 2008). The disease is caused due to mutation in Lamin A gene which forms a part of the nuclear envelope (Oberdoerffer and Sinclair 2007). Mutation of the Lamin A gene by altered splicing, generates a truncated lamin A protein, termed as progerin (Feser and Tyler 2011). Accumulation of lamin A in the nuclear envelope causes a number of downstream effects. There is decline in methylation marks for heterochromatin, like H3K9me3 and H3K27me3 levels synergistic to decline in HP1 and EZH2 (H3K27 methyl transferase). It results in increased transcription from pericentric heterochromatin, shortening of telomeric regions and disruption of nuclear architecture. Progerin expresses at very basal level in young and old mammals. Overexpression of progerin results in all these change in the histone modifications status, described as above and disorganization of the nuclear architecture. Even, by blocking the cryptic splicing site and correcting the splicing defect, the abnormal cell can be reverted back to normal and delay aging process (Feser and Tyler 2011).

It has been shown that healthy aged cells have decreased levels of NURD complex (Meshorer and Gruenbaum 2009). Progeric cells also show decreased level of NURD complex and concomitant loss of HP1 and H3K9me3 along with severe DNA damage (Feser and Tyler 2011). Hence, these systems also pose a good model to study NURD mediated epigenetic signalling for aging.

8 Change in Chromatin Structure and Function During Aging

8.1 Change in Nuclear Architecture During Aging

As discussed earlier, in yeast, the heterochromatic regions mainly silence the mating type locus and stabilize the rRNA genes and telomere regions by preventing them from recombination. As the rDNA region is repetitive, it is also prone to recombination. In yeast, the rRNA region is stabilized and silenced by REST (regulator of nucleolar silencing and telomere exit) complex (Oberdoerffer and Sinclair 2007). This complex is a Sir2 containing complex. Sir2 is a major histone deacetylase responsible for deacetylating histone H4K16Ac. The Sir2 interacts with Sir3 and Sir4 not only in the rDNA region but also in the MAT and the telomeric regions and is regulated by them (Kaeberlein et al. 1999). In yeast, the highly repetitive rDNA region is prone to recombination. Recombination mediated excision in these regions generates extra-chromosomal circles. During subsequent cycles these extra-chromosomal circle keep on accumulating in the nucleolus and cause cell death. Hence, there is a need to silence the recombining rDNA region to increase genomic stability and delay aging, which is done by the Sir2 complex. A truncated Sir4 protein, no longer interacts with the telomeric region. Instead,

more Sir2 and Sir3 are targeted to the nucleolus. It results in extension in life span of the yeast (Straight et al. 1999).

Similar observations are also obtained from human progeroid disease, Werner Syndrome (WS) (Oberdoerffer and Sinclair 2007). In this disease, there is a mutation in the DNA helicase gene. This mutation also causes genomic instability by extra-recombination in the rDNA region and results in early aging. In other similar diseases such as HGPS (Hutchinson-Gilford Progeria Syndrome), there is a mutation in the Lamin A gene which, encodes a protein of the nuclear membrane, as described earlier (Oberdoerffer and Sinclair 2007). It disturbs nuclear membrane architecture with concomitant loss of HP1 proteins. HP1 is very much essential for maintenance of the integrity of the constitutive heterochromatic region as it binds to methylated H3K9. Loss of HP1 leads to disturbance in the heterochromatic regions. Further findings have shown that a similar splice variant of lamin A also accumulates in normal aged individuals indicating similar events in the normal aging and disease induced aging processes (Scaffidi and Misteli 2006). Similarly, in the AT (ataxia telangiectasia) disease also there is mutation in the ATM gene which is a component of the DNA repair cascade important for telomere maintenance. Defective ATM products also disturb telomere and nuclear matrix interaction and enhance aging (Greenwell et al. 1995).

Age dependent changes in the heterochromatic regions, such as centromeric region are also reported. The pericentric-heterochromatin region around the centromere becomes transcriptionally more active in aged cardiac tissue (Gaubatz and Cutler 1990). There are further supporting observations hypothesizing that change in the perinuclear architecture contributes to aging in a number of organisms (Oberdoerffer and Sinclair 2007). Few recent observations have shown that there is a notable increase in the total facultative heterochromatin domains in the senescent cells (Adams 2007). By this process there is large scale reorganization of the heterochromatic regions in an age dependent manner.

8.2 Age Dependent Change in Core Histone Expression and Deposition into Nucleosomes

An age dependent change in expression of core histone is recently reported in many organisms such as yeast and mammals. There is reduction in the core histone expression during replicative aging in yeast with concomitant decrease in histone occupancy (Hu et al. 2014). It leads to aberrant up-regulation of associated genes and also causes genomic instability. Further, in yeast models, deletion of anti-silencing function-1 and CAF-1, (the central chaperone complex required for deposition and removal of histones from chromatin) causes drastic reduction in its life span (Feser et al. 2010). It has been seen that though there is significant increase in the histone transcripts during aging, there is global decrease in the chromatin bound core histones during aging. Supply of extra core histone or ectopic expression of histone H3 and H4 and not

H2A and H2B increases the replicative life span in yeast. Hence, it is concluded that expression of H3 and H4 dimer promotes nucleosome deposition and transforms again to a more uncompact form and thereby decrease genomic instability and increase life span. Though, to the best of our knowledge, similar experimental observations are limiting from mammalian cells, it can be argued that similar process can also be applicable to mammalian systems, as similar decline in histone expression is also observed in mammalian cells too.

8.3   Change in DNA Methylation

In vertebrates, transcriptionally silent regions are generally marked by the presence of 5-methyl cytosines in the CpG dinucleotide sites (described earlier). There is reduction in the 5meC in aged human cells. Further studies have indicated that there is decrease in the 5meC in the CpGs present outside the promoter regions in different human tissues (Benayoun et al. 2015). Conversely, there is an increase in 5meC in the promoter region of aged tissues of human and mice. It is further hypothesized that the methylation in the promoter regions of development related genes probably contribute to the aging mediated mis-regulation of gene expression. Recent reports have suggested a link between human age and 5meC status of DNA (Zou et al. 2014). The stem cells (embryonic and pluripotent stem cells) are estimated to be ageless in correlation to the DNA methylation age. Similarly, sperms and ovum have younger DNA methylation ages compared to respected differentiated tissues from the same individuals. Further, a comparison of the DNA methylation status of different tissues can also be useful for predicting the health of various tissues. For example, in obese individuals, there is increased DNA methylation age of liver than other tissues of the same individual. Similarly, individuals with Down's syndromes have increased methylation age of blood cells compared to normal individuals with similar biological age.

Similar observations are also seen in model organisms for aging study. In *Drosophila,* overexpression of dDnmt2 (DNA methyl transferase) gene increases longevity, whereas deletion of the gene generates short lived flies (Benayoun et al. 2015). Although, the mechanism of methylation mediated modulation of aging is still unclear, the results obtained from *Drosophila* and human samples pose a relationship between DNA methylation and aging and suggests that DNA methylation could be used as a general biomarker for aging.

8.4   Changes in Histone Methylation

As discussed earlier, methylation of core histones at specific sites, mark either the active or repressive chromatin. Further, histone methylation is reversibly regulated by histone methyl transferases and demethylases. With aging, there is global loss of heterochromatin domains with concomitant redistribution of heterochromatin

silencing proteins (Tsurumi and Li 2012). Increase in H3K27me3 by decreasing expression of H3K27me3 demethylase results in increase in life span in *C. elegans* (Ni et al. 2012). However, the results are contradictory from different organisms. In *Drosophila*, reduction in expression of H3K27me3 methyl transferase with concomitant reduction in H3K27me3 increases life span (Ni et al. 2012). On the contrary, in muscle stem cells of old mice, there is increase in gross H3K27me3 (Baumgart et al. 2015; Liu et al. 2013). The contradictory increase or decrease in methylation status of H3K27 further suggests the fact that the methylation signals are tissue and organism dependent.

Age dependent redistribution of H3K4me (a mark of active chromatin) is also observed (Shah et al. 2013). Spreading and redistribution of H3K4me3 occurs in aging fibroblast of humans and hematopoietic stem cells of mice. Further, modulators of H3K4me also influence longevity. It is observed that knocking down the main component of the H3K4 methyl transferase complex increased lifespan in *C. elegans*. On the contrary, when the respective demethylase gene was knocked down, it resulted in shortened lifespan (Greer et al. 2011). Further, overexpression of the H3K4 demethylase in the germinal cells increased lifespan. Similar observations are also obtained from other histone H3 amino acid residues which act as site for methylation. Mutation of the H3K36 demethylase gene causes expansion in replicative age in yeast. Synergistically, a mutation the K36 residue in H3 of yeast, which makes it refractory to be methylated, shortens life span (Sen et al. 2015). Further, *in C. elegans*, knockdown of the H3K36me3 methyl transferase gene also shortens life span of the worm (Pu et al. 2015).

8.5   Changes in Histone Acetylation

The acetylation pattern of core histones has been reported to change during normal aging in a number of organisms (Benayoun et al. 2015). In yeast models, during replicative aging, there is bulk level decrease in H3K56Ac, while there is increase in H4K16Ac (Dang et al. 2009). On the contrary, there is age dependent decrease in H4K16Ac in mouse and progeroid models (Krishnan et al. 2011). In aged mice also there is a lack of transcription mediated upregulation in H4K12Ac, which is a mark of transcriptional elongation.

Concomitant to the age dependent increase in H4K16Ac, the enzyme specific for deacetylating the H4K16Ac, the Sir2 also modulates life span in yeast. Increasing dose of Sir2 (Sirtuin silent information regulator 2) or inducer of Sir2 such as Resveratrol, extends life span in yeast and other model systems (Kaeberlein et al. 1999). Similarly, orthologues of Sir2 such as SIRT6 in mice deacetylates H3K9Ac and H3K56Ac. Deficiency of SIRT6 generates progeroid like phenotypes in mice, hypothesizing that it has role in aging. On the contrary, overexpression of sirt6 increases longevity (Kanfi et al. 2012). Even senescence can be induced in these cells by exposing these cells to a HDAC inhibitor such as Trichostatin A. The exact mechanisms of modulation of aging by *Sirt* genes are unclear, but it is hypothesized that they recruit specific chromatin remodeling factors and promote genome

stability. There is also decrease in HDAC-1 expression during serial passaging of human fibroblast cells.

8.6   Change in Other Histone Modifications

Though, not very well studied, histone modifications other than DNA methylation, histone methylation and histone acetylation also have contributory effects in modulating aging like phenomenon. For example histone H2A, H2B and H4 can be modified with an O-N-acetyl-glucosamine (OGNAc). These OGNAc attached histones are enriched in gene promoters important for aging and stress responses in *C. elegans* (Benayoun et al. 2015). Further, deletion of the OGNAc depositing gene shortens the lifespan of *C. elegans;* while, deletion of the gene that removes the OGNAc mark extends the life span. Age dependent changes in the oxidative modifications of histones such as carbonylation are also reported in rat liver (Sharma et al. 2006). While, the rate of carbonylation was higher for the above mentioned histones in young rat liver, the carbomylation was significantly lower in the liver histones of aged rats. Further, dietary restriction of older rats increased carbamylation rates. A detailed change in the histone modification is represented in Table 1.

8.7   Generation of Heterochromatic Foci

Senescent cells show discrete Senescence associated heterochromatic foci (SAHF) (Kosar et al. 2011). SAHF structures are readily stained by DAPI and show resistance to be digested by nucleases, suggesting a more compact structure. SAHF structures were first identified in aged human fibroblast cells (Glauche et al. 2011). SAHF contain chromatin domains that are resonant of the constitutive heterochromatin domains, such as enrichment of H3K9me3 and HP1and hypoacetylated histones and incorporation of macroH2A. However, these foci lack linker histone H1. It is further seen that each chromosome condenses into a single SAHF. Preliminary experiments have shown that disruption of the H3K9 methyl transferase promotes tumour formation in the lymphocytes of mice. In these cells there is prevention of senescence suggesting that SAHF complex formation is crucial for occurrence of senescence (Kosar et al. 2011).

8.8   Age Dependent Change in Chromatin Remodeling
       Factors

Age dependent changes in the expression of remodeling complexes are also observed, in a number of organisms. The components of NuRD (nucleosome

Table 1 A summary of aging dependent changes in chromatin organization and function: Here, '0' represents no change, '+' represents increase and '−' represents decrease in the specific modification

Epigenetic mark	Function	Age dependent change	Deviations if any
5-meC	Transcriptional repression	Global change: 0 Local changes: + Only local level increase in some region and decrease in the other	In cellular senescence model there is global decrease and local increase
Core histone expression	Form nucleosome and are generalized repressor	−	No change in histone H3 expression in Drosophila head tissue whereas decrease seen in other models such as *C. elegans*
Macro H2A	Component of heterochromatin	+	Nil
HP1	Component of heterochromatin	−	HP1β increases in human fibroblast senescence model.
H3K9me	Transcriptional repression mark of euchromatin	+ (Human fibroblast senescence model)	Nil
H3K9me2	Transcriptional repression mark of euchromatin	− (Human fibroblast senescence model and *Drosophila*)	Nil
H3K9me3	Enriched in heterochromatin	−	Increase in *Drosophila*
H3K27me3	Transcriptional repression mark of euchromatin	−	Increase in mouse brain, muscles and HSC
H4K20me2	Genomic instability and DNA repair	+ (Human fibroblast senescence model)	Nil
H4K20me3	Enriched in pericentric heterochromatin	+	Decrease in human fibroblast senescence model
H3K4me2	Enriched in transcriptionally active gene bodies	+	Nil
H3K4me3	Enriched in transcriptionally active gene promoters	0	Minor increase in HSC, muscle stem cells and neurons of human. Decrease in head tissue of *Drosophila*.
H3K56Ac	DNA damage, replication and nucleosome assembly	− (Yeast and cellular senescence model)	Nil

(continued)

Table 1 (continued)

Epigenetic mark	Function	Age dependent change	Deviations if any
H4K16Ac	Telomere silencing and chromatin compaction	−	Increase in yeast
H4K12Ac	Mark of transcriptional elongation Enriched in active gene body	−	Nil

remodeling and deacetylase) complex are found to be down regulated in aged healthy individuals as well as in HGPS patients as studied in fibroblasts (Pegoraro et al. 2009). Further, it is also demonstrated that in *Hela* cells, RNAi mediated down-regulation of NuRD results in loss of heterochromatin (Pegoraro et al. 2009). Similarly, in yeast and *C. elegans,* deletion of ISWI (chromatin remodeling imitation switch) complex increases life span (Benayoun et al. 2015). However, a functionally active SWI/SNF is required for promoting longevity in *C. elegans*. Few other observations also state that in human adrenal cortex carcinoma derived cell lines, senescence can be induced by *BRG1*. It is further seen that *BrmI* level increases in old mice liver correlating to age dependent repression complex formation. Further, when nuclear extract of young animals were incubated with *BrmI*, it resulted in the formation of similar complexes observed in aged animals (Benayoun et al. 2015).

8.9 Age Dependent Changes in Histone Variants and Histone Exchange

In non-replicating cells such as neurons, there is replication independent enrichment of histone H3.3, a histone H3 variant of the chromatin (Pina and Suau 1987). It has been further shown in chicken and mice model that during aging there is increase in H3.3 level (Urban and Zweidler 1983). This observation also correlates well with the hypothesis that aged cells have more open chromatin and hyperacetylated chromatin. In neurons, there is formation of an open chromatin due to incorporation of H3.3. In vitro also there is increased incorporation of H3.3 in human fibroblast cell lines entering aging. The H3.3 specific chaperons also increase in aged baboons (Jeyapalan et al. 2007). The histone H2A variant H2A.Z is also correlated with aging. Knockdown of H2A.Z variant or p400 (the H2A.Z exchanger) promotes aging in human fibroblasts (Chan et al. 2005).

8.10 Age Dependent Proteolysis of Histones

Two different forms of histone H3, namely a 'slower migrating' (H3^S) and a 'faster migrating' (H3^F) have been detected in *Tetrahymena thermophila* micronuclei. Using partial proteolytic peptide mapping it was suggested that the H3^F was a proteolytically clipped product of H3^S. It was cleaved by six amino acids residues from its N-terminal end, and it was a physiologically regulated proteolytic processing event (Allis et al. 1984). It has been further shown that when macronuclei became senescent and transcription was inactivated, the N-terminal tails of the core histones were proteolytically removed (Lin et al. 1991). It has been further proposed that the macronuclei, which were transcriptionally active, did not own such a proteolytic event.

Few recent observations have demonstrated the proteolytic processing of histone H3 and histone H1 in old rat liver and chicken (Chaturvedi, M.M. group, unpublished observation) and in Japanese quail (Mahendra et al. 1999; Mahendra and Kanungo 2000; Mishra and Kanungo 1994). It has been further shown that progesterone induces the H3 cleavage in Japanese quail, suggesting that the protease specific to histone H3 may be regulated by progesterone. Shanti, 1995, observed an additional band in the histones prepared either from nuclei or purified nucleosome-core from liver of old rats (Chaturvedi, M.M. group, unpublished observation). This band migrated between histone H2A and H4. The appearance of this band had a strong correlation with decrease in the stoichiometry of the histone H3. It was speculated that this band might be a clipped product of H3, and hence named as ΔH3. However, the precursor-product relationship between H3 and ΔH3 was not established. The ΔH3 generation has also been demonstrated recently in case of old chicken liver (Purohit et al. 2012). Through N-terminal sequencing, it has been shown that the ΔH3 was an N-terminally clipped product of histone H3, being cleaved at 23 amino acids from the N-terminus. The other histone post-translational modification profile of the ΔH3 is presently being investigated by western blotting (Chaturvedi, M.M. group).

9 Conclusion and Future Prospects: Attaining Epigenetic Rejuvenation?

A number of increasing lines of evidence propose chromatin structure as a modulator of aging. There is aging dependent change in regulation of transcription and nuclear architecture. When organisms age, there is decline in maintenance of the cellular structure and function which brings about change in the chromatin structure and organization. However, the age dependent change in the chromatin structure is multifaceted and often antagonistic in different organisms. For instance, in most of the organisms, with aging there is decreased histone expression and decrease in heterochromatin abundance with concomitant formation of an open chromatin
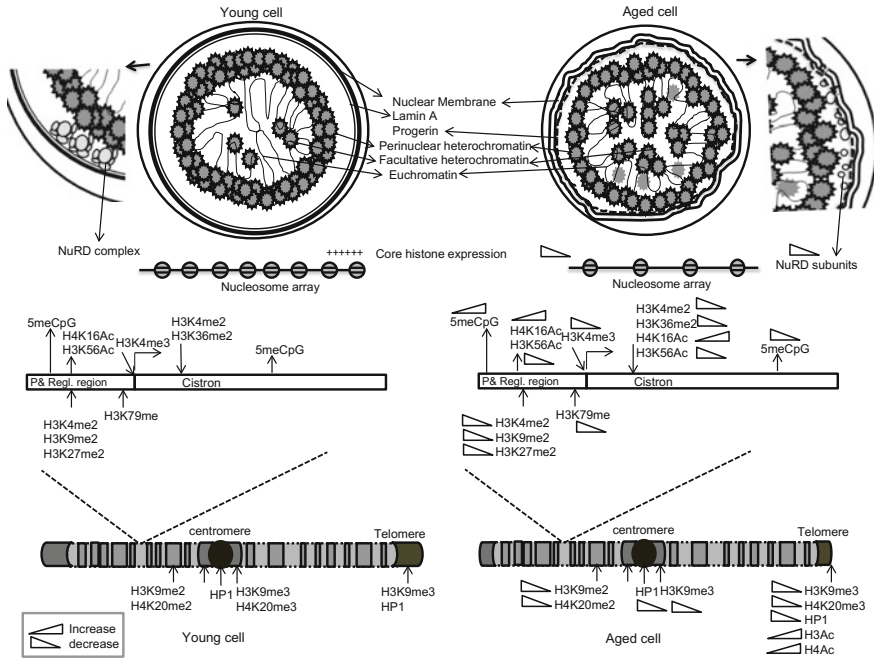
Fig. 2 Summary of the changes in the chromatin organization and function in aged cells. Normal cells have distinct perinuclear heterochromatin foci and few facultative heterochromatin loci. Aged cells on the contrary have disorganized perinuclear heterochromatin with concomitant increase in facultative heterochromatin loci. Also, in contrast to the Lamin A deposition in the nuclear membrane and interaction of NuRD complex to lamin A in young cells, there is an age dependent accumulation of progerin leading to disorganization of the nuclear architecture. In addition, there is decreased expression of NuRD complex in aged cells. Further, in aged cells, there is decreased core histone expression leading to reduced histone occupancy in the nucleosomal array. In addition to that there are a number of changes in the histone and DNA PTM, such as methylation and acetylation in the promoter and the coding region of the genes, which are indicated

structure. However, in the replicating senescence model there is age dependent accumulation of heterochromatic foci. The other examples also include the redistribution of SIR proteins in yeast during aging from the telomere and MAT locus to the rDNA regions promoting rDNA silencing and telomere shortening. At present restoring back to a youthful chromatin structure involves elevation in histone expression, reduction in acetylation of H3-N-terminal and H4K16 and inactivation of the HDAC Rpd3.

The epigenome is also influenced by environmental factors. Throughout the lifespan of an organism, the insults induced by the environment also contribute to the functioning of cells to organs. Thus it can also be proposed that the epigenomic instability induced by environment and life style also influence aging as represented by the methylation clock. It can be further stated that modulation of the epigenetic reprogramming by altering chromatin remodeling factors can revert aging. Hence,

these chromatin remodeling factors pose a good candidate for therapeutic interventions. A detailed account of the aging mediated changes is summarised in Fig. 2.

However, several questions related to aging are still unclear and require future investigation. 1. The change in chromatin modifications are the cause or the consequence of aging? 2. Could the epigenetic players be precisely modulated and could act as therapeutic targets for reverting aging? 3. Since, the epigenetic modulators are broadly global; could they be specifically managed by independent linear pathway so that when therapeutic interventions are formulated, they will have minimal side effects. We are sure, answers to these questions are crucial before formulation of any medicine/agents for epigenetic rejuvenation. Few recent reports represent that HP1β and mH2A are the prime targets in this respect. However, the concept of epigenetic rejuvenation is still in its infancy and needs manifold unravelling in near future.

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