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The Emerging Role of Centromere/Kinetochore Proteins in Cellular Senescence

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1. Introduction

Cellular senescence is an irreversible growth arrest triggered by several types of stress, including DNA damage, oxidative stress, telomere shortening, and oncogene activation (Ben-Porath & Weinberg, 2005; Collado et al., 2007; Deng et al., 2008; Hayflick & Moorhead, 1961; Serrano et al., 1997). Although how senescence is initiated remains to be determined, it has been shown to be triggered by certain defects in chromosome integrity, such as telomere shortening (Ben-Porath & Weinberg, 2005; Deng et al., 2008). In contrast to telomere shortening, the roles of which in senescence have been studied extensively, alterations in the centromere/kinetochore structure involved in senescence program remain to be elucidated. This chapter presents a discussion of the emerging roles of centromere/kinetochore proteins, particularly Centromere protein A (CENP-A, the centromere-specific variant of histone H3), in senescence.

2. Crucial roles of centromere/kinetochore proteins in mitosis

The genome of a cell is duplicated and segregated into two daughter cells during cell division (Fig. 1). Accurate chromosome segregation during cell division is essential for genome integrity and this process is mainly achieved by the structural/functional integrity of the microtubule spindle apparatus (kinetochore-microtubule interactions) and spindle assembly checkpoint (SAC) signaling (Cleveland et al., 2003; Musacchio, & Salmon, 2007; Tanaka, 2010). Spindle microtubules emanating from spindle pole bodies (centrosomes) attach to chromosomes via specialized structures called kinetochores where more than 100 proteins assemble at the centromeric region of each chromosome during mitosis. This interaction is monitored by the SAC signaling pathway to ensure high-fidelity chromosome segregation (Musacchio, & Salmon, 2007). Chromosome missegregation arising from defects in the structural integrity of the microtubule spindle apparatus and the SAC signaling pathway leads to aneuploidy, i.e., chromosome gain or loss (Compton, 2011). Aneuploidy is thought to be a major cause of congenital disorders. High rates of aneuploidy have been observed in various cancers and aneuploidy is speculated to be involved in tumorigenesis.

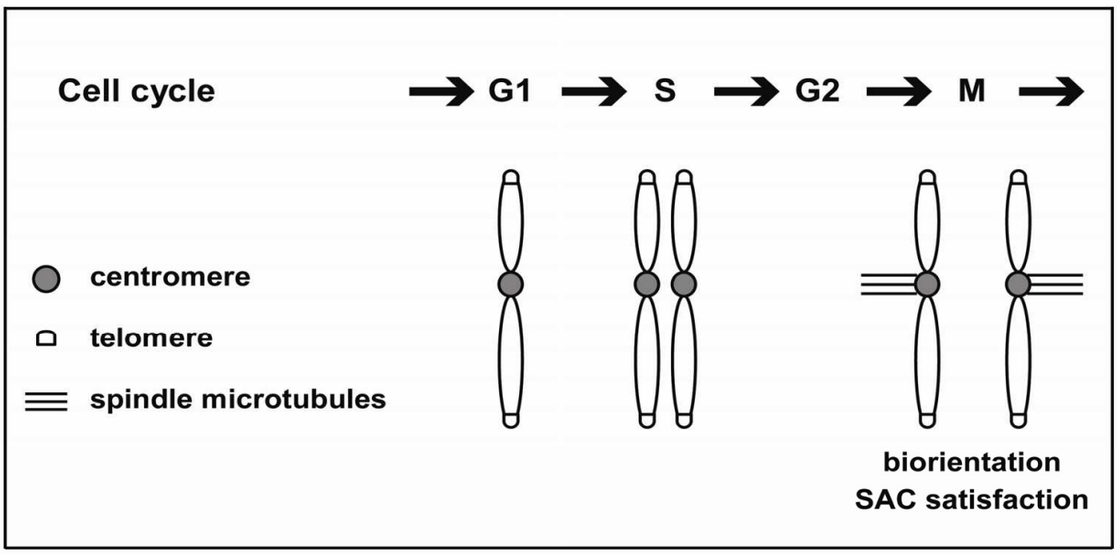

Fig. 1. Chromosome cycle and cell cycle (Adapted from Maehara, 2011)

In higher eukaryotes, the DNA sequence does not generally determine the functional centromeres except in the budding yeast *Saccharomyces cerevisiae*, in which the centromere, a 125-bp DNA element, is specified by its sequence. Centromeres in other organisms lack sequence specificity, but many of the proteins localizing at centromeres are well conserved across species (Fig. 2).

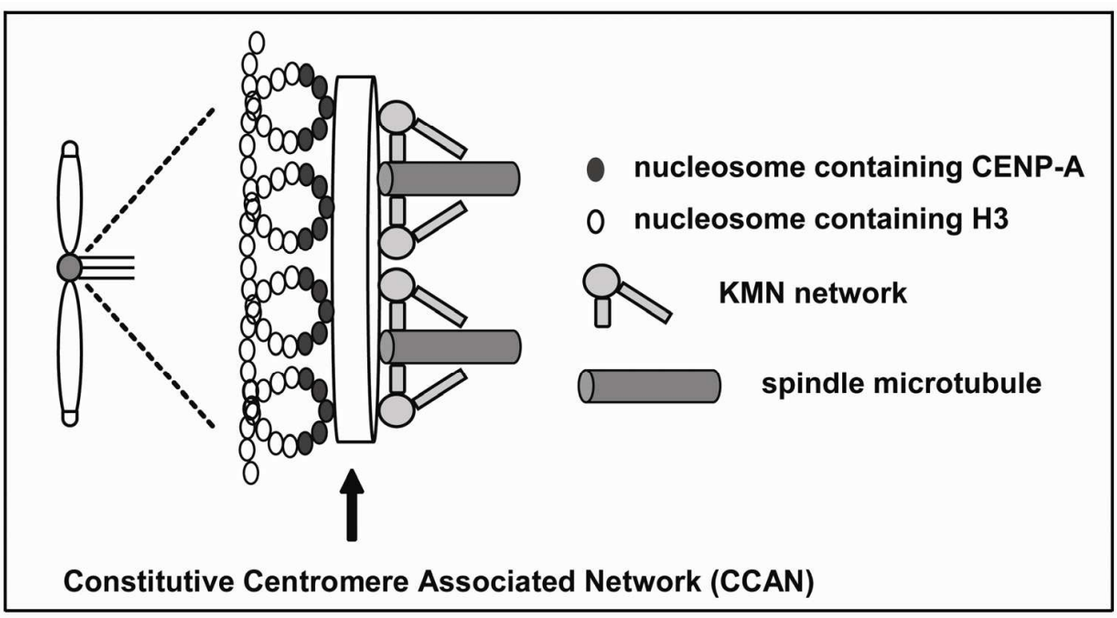

Fig. 2. Schematic representation of the kinetochore (Adapted from Maehara, 2011)

Among the numerous kinetochore-associated proteins identified to date, CENP-A represents an excellent candidate as an epigenetic marker of functional centromeres for several reasons. First, CENP-A is an evolutionarily conserved centromere-specific histone H3 variant (Blower & Karpen, 2001; Buchwitz et al, 1999; Earnshaw & Rothfield, 1985; Meluh et al., 1998; Palmer et al., 1987; Stoler et al., 1995; Takahashi et al., 2000). Canonical nucleosomes in chromosome arms consist of 146 bp of DNA wrapped around a histone octamer comprised of two subunits of each of H2A, H2B, H3, and H4. H3 is replaced with the H3 variant CENP-A at the centromeres. Second, many centromere-associated proteins are recruited to the centromere in a CENP-A-dependent manner (Foltz et al., 2006; Izuta et al., 2006; Obuse et al., 2004a, Okada et al., 2006). Third, neocentromeres, which are established as functional centromeres at ectopic chromosomal loci devoid of alpha satellite repeats, have been shown to contain CENP-A (Marshall et al., 2008). Thus, CENP-A seems to be an identifier of the functional centromere. Studies in a variety of organisms have indicated that CENP-A plays a crucial role in organizing kinetochore chromatin for precise chromosome segregation. Another conserved centromere protein, CENP-B, binds to a specific centromeric DNA sequence, the 17-bp "CENP-B box" in type I alpha satellite repeats in mammals (alphoid DNA in humans) (Earnshaw et al., 1987; Masumoto et al., 1989). CENP-B is essential for heterochromatin formation of pericentromeres and is thought to be important for the proper organization of kinetochore chromatin (Nakagawa et al., 2002; Nakano et al., 2008; Okada et al., 2007), although CENP-B is not essential for viability in higher eukaryotes (Hudson et al., 1998; Kapoor et al., 1998; Perez-Castro et al., 1998). In addition to the above proteins, several studies using proteomic approaches have identified 15 proteins known as the **C**onstitutive **C**entromere **A**ssociated **N**etwork (CCAN) (Foltz et al., 2006; Izuta et al., 2006; Okada et al., 2006). Several of these proteins have DNA binding activity or associate directly with CENP-A. The KMN network (KNL1, Mis12 complex, and Ndc80 complex) is also important as it forms the interface for kinetochore-microtubule attachment (Cheeseman et al., 2006; Obuse et al., 2004b; Ruchaud et al., 2007). SAC is a surveillance mechanism that is capable of delaying anaphase if not all chromosomes have established biorientation within the spindle (Musacchio & Salmon, 2007). It should be noted that many other centromere/kinetochore-associated proteins not mentioned in this chapter also have crucial roles in mitosis. Thus, multiple biological processes including kinetochore, microtubule functions, the mitotic spindle apparatus, and SAC signaling pathway ensure high-fidelity chromosome segregation during cell division.

3. The emerging roles of centromere/kinetochore proteins in senescence

The importance of kinetochore in regulating proper chromosome segregation has been well established. Next, I highlight some recent work on the roles of centromere/kinetochore proteins in senescence in mammals.

3.1 SAC proteins are involved in the senescence program

The SAC signaling pathway monitors the attachment of spindle microtubules to kinetochores. Core components of SAC include Mad1, Mad2, Bub1, Bub3, and BubR1, and many other proteins are also involved in this checkpoint. The onset of anaphase is triggered by activation of the anaphase-promoting complex/cyclosome (APC/C), which degrades

cyclin B and securin. SAC generates an inhibitory signal to block APC/C in the presence of unaligned chromosomes and stalls for time to establish biorientation. Mouse models have been generated with manipulation of the genes encoding SAC proteins. Complete loss of SAC proteins, including Mad2, Bub1, BubR1, Bub3, and Rae1, caused early embryonic lethality (Baker et al., 2004; Baker et al., 2006; Dobles et al., 2000; Jeganathan et al., 2007; Wang et al., 2004). These gene knockout studies revealed the essential nature of mammalian mitotic checkpoint proteins for viability. Intriguingly BubR1-insufficient (*Bub 1b*$^{H/H}$) mice, in which the levels of BubR1 are about 10% those in normal animals, develop progressive aneuploidy along with a variety of progeroid features, including short lifespan, cachectic dwarfism, lordokyphosis, cataracts, loss of subcutaneous fat, and impaired wound healing (Baker et al., 2004). Consistent with the features of premature aging of *Bub 1b*$^{H/H}$ mice, mouse embryonic fibroblasts derived from *Bub 1b*$^{H/H}$ mice show rapid senescence. Both premature aging and cellular senescence observed in *Bub 1b*$^{H/H}$ mice are attenuated by inactivation of p16, a tumor suppressor and an effector of senescence (Baker et al., 2008). In humans, biallelic mutations in *BUB1B* encoding BubR1 cause mosaic variegated aneuploidy (MVA) (Hanks et al., 2004). MVA is a rare recessive condition characterized by constitutional mosaic aneuploidy, growth retardation, microcephaly, and predisposition to cancers such as rhabdomyosarcoma, Wilms tumor, and leukemia. Although aneuploidy and cataracts are common features detected in both *Bub 1b*$^{H/H}$ mice and individuals with MVA, MVA patients do not have typical features of premature aging. The difference in phenotype between *Bub 1b*$^{H/H}$ mice and individuals with MVA may be explained by the degree of BubR1 defects. A recent study indicated that mutations in *CEP57* also cause MVA (Snape et al., 2011). CEP57 is a centrosomal protein and is involved in nucleating and stabilizing microtubules. This suggests that *BUB1B* mutations underlie only a proportion of MVA, and other genes involved in regulating chromosome segregation may cause the disease. Bub3/Rae1-haploinsufficient mice have been reported to display an array of early aging-associated phenotypes (Baker et al., 2006) and Bub1 suppression in human fibroblasts activates a p53-dependent premature senescence response (Gjoerup et al., 2007). These studies involving the manipulation of SAC genes demonstrated that low levels of several SAC proteins play crucial roles in regulating commitment to the senescent state, although it remains to be determined how individual components of this checkpoint control cell viability and cell fate.

3.2 The roles of constitutively centromere-localized proteins in senescence

In contrast to SAC proteins, which localize to the kinetochore during mitosis, CENP-A localizes to the centromere throughout cell cycle and provides a structural and functional foundation for the kinetochore. I detail the role of CENP-A in senescence.

3.2.1 CENP-A has an impact on cell proliferation

Despite extensive studies of centromere-associated proteins, it remains unclear whether these proteins are involved in the control of cell proliferation; previous studies focused on the roles of centromere proteins in chromosome segregation, and were mainly conducted in immortalized cell lines, such as HeLa (Goshima et al., 2003). With regard to CENP-A, studies in a variety of organisms have indicated that the effects of CENP-A loss on

proliferation vary widely according to the species, cell type, and methods used to delete or deplete CENP-A. *Cenpa* null mice fail to survive (Howman et al., 2000). Disruption of CID by antibody injection into *Drosophila* embryos and RNAi in cells in tissue culture exhibits a range of phenotypes affecting both cell cycle progression and mitotic chromosome segregation (Blower & Karpen, 2001). CENP-A-depleted chicken DT40 cells exhibit defects in kinetochore function and stop proliferating, although the apparent cessation of cell proliferation is caused by extensive cell death and the cells are still cycling (Régnier et al., 2005). CENP-A-depleted HeLa cells proliferate but exhibit misalignment and lagging of chromosomes during mitosis (Goshima et al., 2003). In HeLa cells, two tumor suppressor molecules, p53 and retinoblastoma protein (Rb), which have been shown to play crucial roles in cell cycle arrest in primary human cells, are inactivated due to the integration of the human papillomavirus that leads to their immortalization. Although it is essential to use primary human cells to uncover the regulatory roles of centromere proteins in cell proliferation, no such studies have yet been reported. To address whether CENP-A has an impact on cell proliferation, we examined the effects of CENP-A depletion in human primary somatic cells with functional p53 and Rb (Maehara et al., 2010). The reduction of CENP-A by retrovirally transducing CENP-A shRNA did not show growth arrest in HeLa cells, consistent with the previous results in CENP-A RNAi-mediated HeLa cells (Goshima et al., 2003). However, depletion of CENP-A in primary human TIG3 fibroblasts resulted in the immediate cessation of proliferation accompanied by increased levels of p16 and p21 expression, upregulated SAHF formation, and increased SA-β-gal activity, all of which are common markers of cellular senescence (Alcorta et al., 1996; Dimri et al., 1995; Hara et al., 1996; Narita et al., 2003; Zhang et al., 2005). Inactivation of p53 in CENP-A-depleted TIG3 cells restores proliferation leading to an increase in number of cells exhibiting aberrant chromosome behavior. These results indicate that the reduction of CENP-A drives normal human diploid fibroblasts into a senescent state in a p53-dependent manner. The senescence that arises from CENP-A depletion may be a self-defense mechanism to suppress the otherwise catastrophic impact upon genome integrity that would arise from kinetochore dysfunction following certain types of stress. It should be noted that reduction of CENP-A does not result in irreversible growth arrest in human pluripotent stem cells (Ambartsumyan et al., 2010). Ambartsumyan et al. demonstrated that CENP-A-depleted undifferentiated human pluripotent stem cells were capable of maintaining a functional centromere marks and showed no changes in morphology or proliferation rate relative to control cells, whereas CENP-A-depleted BJ fibroblasts showed arrest in G2/M and underwent apoptosis. Although the pluripotent state may cause the different phenotypes in response to CENP-A depletion, CENP-A has an impact on cell proliferation in human primary somatic cells.

3.2.2 CENP-A is downregulated in senescent human cells

Model systems with manipulation of gene expression/deletion have clearly revealed that some centromere/kinetochore-associated proteins play crucial roles in regulating commitment to the senescent state. However, the mechanisms of senescence and individual aging are presumed to be complex. To gain insights into the mechanisms that control lifespan and age-related phenotypes, Ly et al. examined mRNA abundance of more than

6000 known genes in dermal fibroblasts derived from elderly human subjects and from those with Hutchinson–Gilford Progeria Syndrome (HGPS), a rare genetic disorder characterized by accelerated aging (Ly et al., 2000). They found that genes involved in cell cycle progression, spindle assembly, and chromosome segregation, such as cyclins A, B, polo kinase, CENP-A, CENP-F, and kinesin-related proteins, were downregulated in elderly individuals and those with HGPS. We showed that CENP-A mRNA expression was reduced in both replicative and *ras*-induced senescent human TIG3 cells (Maehara et al., 2010). Another group reported a reduction in the levels of CENP-A transcripts in senescent human IMR90 fibroblasts (Narita et al., 2006). Therefore, the reduction of CENP-A mRNA levels appears to be a common feature of cellular senescence and individual aging. However, this reduction is not specific to senescence; we observed a marked reduction of CENP-A mRNA level in quiescent cells that had transiently exited from the cell cycle (Maehara et al., 2010). As CENP-A transcription is regulated by the cell cycle and occurs in G2 phase in human cells (Shelby et al., 1997), the transcription of CENP-A ceases immediately when cells are arrested regardless of whether the arrest is promoted by senescence or quiescence, even though reduction in CENP-A transcript level shows a strong association with the reduced proliferation potential of senescent cells.

In contrast to the levels of CENP-A transcript, which are reduced in both senescent and quiescent cells, CENP-A protein levels are markedly reduced in senescent cells, while quiescent cells retain similar levels of CENP-A protein to their actively growing counterparts (Maehara et al., 2010). These observations suggest that both transcriptional and posttranslational regulation are involved in the senescence-associated reduction of CENP-A protein level. CENP-A protein may be degraded via the ubiquitin – proteasome-dependent pathway in these cells. A previous study demonstrated that cullin-4A, human ring finger protein 2, and hypothetical protein FLJ23109, which have been reported or assumed to possess ubiquitin ligase activity, were coimmunoprecipitated with anti-CENP-A antibody from HeLa interphase nuclear extract (Obuse et al., 2004a). It is noteworthy that CENP-A also undergoes destruction when human cells are infected with herpes simplex virus type 1 protein ICP0 (Lomonte et al., 2001). Ubiquitin-dependent proteolysis of the yeast Cse4/CENP-A incorporated at non-centromeric regions has been reported (Collins et al., 2004). In addition to CENP-A, linker histone H1 protein level is decreased in senescent human WI38 cells, presumably because of posttranslational regulation (Funayama et al., 2006). A mitotic exit network kinase, WARTS/LATS1, was also reported to be reduced in senescent human cells (Takahashi et al., 2006). The reduction of this kinase was attenuated by addition of MG132. These results imply the presence of a senescence-associated proteolysis pathway in primary human cells. The senescence-associated proteolysis pathway may contribute to maintenance of metabolism and biosynthesis in senescent cells by recycling proteins that are no longer required for non-dividing cells and to ensure irreversible growth arrest by destruction of proteins essential for proliferation. Although the molecular mechanism of CENP-A reduction remains to be clarified, reduced levels of CENP-A protein seem to be common to cellular senescence and individual aging.

3.2.3 CENP-A reduction enhances centromeric heterochromatin formation

In our exploration of senescence-associated alterations in nuclear structure using primary human cells, we found that CENP-A levels were markedly reduced in senescent cells. In

contrast to CENP-A, the levels of the other centromere proteins, CENP-B and hMis12, increased gradually, as the cells became senescent (Maehara et al., 2010). In addition, increased HP1 proteins, which are essential components of the pericentric heterochromatin region, were enriched on centromeres alongside CENP-B. These changes in the levels of centromere proteins alter the centromere chromatin structure, and are thought to represent physiologically significant phenomena associated with cellular senescence. Forced reduction of CENP-A alters the distributions of CENP-B and HP1 proteins, which are similar to those observed in replicative and *ras*-induced senescent cells, suggesting that this centromere alteration is triggered, at least in part, by the reduction of CENP-A protein level. Recent studies have demonstrated the remarkable role of CENP-B in heterochromatin formation in the centromere. In fission yeast, the disruption of CENP-B homologs, Abp1 and Cbh1, causes a reduction of Swi6, a homolog of HP1, at centromeric chromatin and a decrease in heterochromatin-specific modifications of histone H3 (Nakagawa et al., 2002). Using human artificial chromosomes (HAC) and alpha-satellite arrays integrated into chromosomal arms as models, Okada et al. demonstrated a dual role of CENP-B in CENP-A assembly and heterochromatin formation (Okada et al., 2007). Although CENP-B is required for de novo CENP-A assembly on HAC, CENP-B enhances histone H3K9 trimethylation and DNA methylation in chromosomally integrated alphoid DNA and suppresses centromere formation. Furthermore, Nakano et al. generated HAC containing both integrated alpha satellite and tet operator (tetO) sequences and tethered tet repressor (tetR) chromatin-modifying protein fusions to the HAC centromere (Nakano et al., 2008). Stimulation of the formation of a heterochromatin state by forced binding of silencers or targeted nucleation of HP1 resulted in the inactivation of a functional HAC centromere. Depletion of dimethylated histone H3K4 (H3K4me2) by tethering the lysine-specific demethylase 1 (LSD1) causes CENP-A loss from HAC kinetochores and ultimately results in inactivation of the kinetochore (Bergmann et al., 2011). These observations suggest that inactivation of the centromere occurs through epigenetic mechanisms. Thus, the loss of CENP-A and the extended heterochromatinization mediated by CENP-B and HP1 proteins on the centromere in senescent cells are assumed to promote centromere inactivation. During senescence, primary human cells alter their centromere states from a functional centromere, which is required for faithful segregation of chromosomes, to an inactivated centromere, which is likely to contribute to the establishment of the senescent state. Further qualitative and quantitative studies are needed to understand the structural and the functional changes that occur in the centromere during the senescence process.

3.2.4 How do primary human somatic cells sense centromere/kinetochore dysfunction and undergo senescence?

Forced depletion of CENP-A induces senescence-like phenotypes in the primary cells and CENP-A appears to be actively degraded in the senescent cells. This raises the question of whether CENP-A reduction is a cause or a consequence of cellular senescence. As cellular senescence is a complex trait, it is not possible to provide a clear answer to this question. There may be a positive feedback circuit between CENP-A degradation and induction of cellular senescence during senescence. I hypothesize that primary human somatic cells possess a mechanism for monitoring centromere/kinetochore integrity, which activates the p53-dependent senescence pathway in response to centromere/kinetochore defects, such as insufficient incorporation of CENP-A at the centromere (Fig. 3).

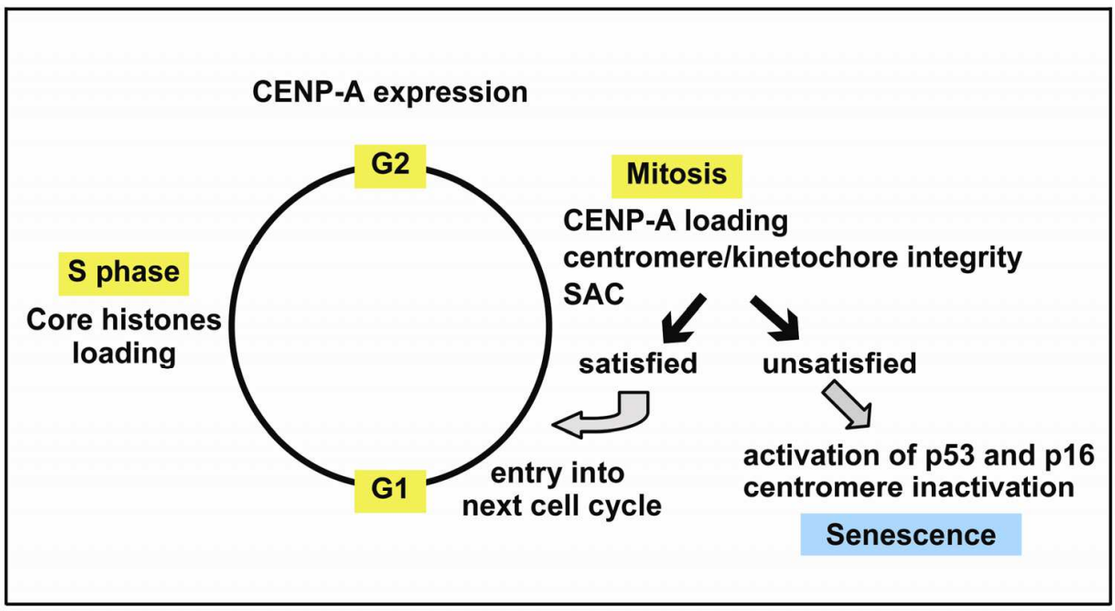

Fig. 3. A model of the roles of centromere/kinetochore proteins in senescence

How do the primary cells sense centromere/kinetochore dysfunction?

Telomere shortening triggers the DNA damage response (DDR), which is a major intrinsic factor to induce cellular senescence. Previous studies clearly demonstrated that p53 activation in oncogene-induced senescence is due to activation of the DDR (Bartkova et al., 2006; Di Micco et al., 2006; Mallette et al., 2007). We examined whether DDR plays a crucial role in activation of p53 in response to a reduction of CENP-A level. The presence of DNA damage foci (phosphorylated histone H2A.X, γ-H2AX), chk2 phosphorylated on threonine 68 and chk1 phosphorylated on serine 345, which are associated with DDR, were not detected in CENP-A-depleted-senescent cells (Maehara et al., 2010), suggesting that CENP-A depletion is not causally linked to DDR. Excess growth signals produced by oncogenes and telomere shortening seem to be sensed as DNA replication stresses, while CENP-A reduction is not. This may explain the unconventional type of senescence that does not require the activation of DNA damage signaling.

Unlike canonical core histones that are loaded into chromatin during DNA replication, newly synthesized CENP-A is incorporated into centromeric chromatin in telophase and early G1 phase (Fig. 3). Mis18 complex and HJURP/Smc3 have been implicated in the centromeric loading of CENP-A (Barnhart et al., 2011; Dunleavy et al., 2009; Foltz et al., 2009; Fujita et al., 2007; Hayashi et al, 2004). Primary cells may monitor CENP-A loading and centromere/kinetochore integrity during M and early G1 phases and immediately cease proliferation before entry into the next cell cycle in response to fatal centromere/kinetochore dysfunction under conditions in which some key centromere proteins and/or the SAC are not functioning properly. Under these conditions, senescence seems to not only prevent the cells from producing abnormal chromosomes, but also protects the organism from the potentially hazardous consequences of proliferation of cells harboring chromosomal abnormalities that arose as a consequence of defective mitosis.

4. Conclusion

Recent studies have revealed novel roles of centromere/kinetochore-associated proteins in the senescence program mainly using model systems in which target genes were manipulated. As highlighted in this chapter, while low levels of several centromere/kinetochore-associated proteins play crucial roles in regulating commitment to the senescent state, the interactions between centromere/kinetochore proteins and components of the senescence pathway remains to be determined. Further studies are required to determine the epigenetic mechanisms of centromere inactivation, particularly histone modification, and components involved in regulating the ratio of CENP-A to heterochromatin during senescence.

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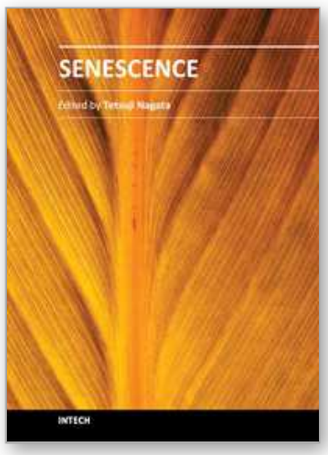

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The book "Senescence" is aimed to describe all the phenomena related to aging and senescence of all forms of life on Earth, i.e. plants, animals and the human beings. The book contains 36 carefully reviewed chapters written by different authors, aiming to describe the aging and senescent changes of living creatures, i.e. plants and animals.

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