Conservation of CENH3 Interaction Partners in Plants

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Abstract

The loading and maintenance of centromeric histone 3 (CENH3) at the centromere are critical processes ensuring appropriate kinetochore establishment and equivalent segregation of the homologous chromosomes during cell division. CENH3 loss of function is lethal whereas mutations in the histone fold domain are tolerated and lead to chromosome instability and chromosome elimination in embryos derived from crosses with wild type pollen. A wide range of proteins in yeast and animals has been reported to interact with CENH3. The histone fold domain interacting proteins are potentially alternative targets for the engineering of haploid inducer lines, which may be important when CENH3 mutations are not well supported by a given crop. Here, we provide an overview of the corresponding plant orthologs or functional analogs of CENH3 interacting proteins. We also list putative CENH3 phosphorylation and ubiquitination posttranslational modifications that are also candidate targets for modulating chromosome stability and inheritance.

Key words: CENH3, centromere, protein interaction, post translational modification, chromosome, haploid induction
CENH3 as a core component of centromeres

The histone H3 variant CENH3 is a component of the centromeric nucleosomes in eukaryotes (McKinley and Cheeseman, 2016). The role of CENH3 in nucleosome formation is conserved in yeast, mammals and plants, but compared to other histones, its amino acid sequence is poorly conserved (Drinnenberg et al., 2016) and specific names were given: CENTROMERE PROTEIN A (CENPA) in mammals, CHROMOSOME SEGREGATION 4 (CSE4) in Schizosaccharomyces pombe (Shrestha et al., 2017). In A. thaliana it was previously named HRT12 (Talbert et al., 2002) but in more recent papers it is now named as CENH3. For clarity we use in this review the common name CENH3 to discuss general properties and extend it with the specific name in superscript when addressing species-specific features.

CENH3 loading onto the centromeres is of key importance for the ensuing establishment of the kinetochore (McKinely and Cheeseman, 2016; Sandmann et al., 2017) and to ensure the fidelity of chromosome segregation during mitosis (Shrestha et al., 2017). Specialized histone chaperones selectively bind centromeric histone and mediate the assembly of the centromeric nucleosomes (Zasadzińska and Foltz, 2017). The loading of CENH3\textsuperscript{CENPA} onto centromeres takes place during the G1 phase of the cell cycle when it complexes with histone H4 and nucleophosmin, and assembles the centromeric nucleosomes with the help of the chaperone HOLLIDAY JUNCTION RECOGNITION PROTEIN (HJURP) (Foltz et al., 2009; Dunleavy et al., 2009). CENH3\textsuperscript{CENPA} nucleosome assembly depends on a protein complex consisting of Mis18α, Mis18β, and KINETOCHORE NULL 2 (KNL2\textsuperscript{M18BP1}), recruiting HJURP to the centromeres (Foltz et al., 2009; Dunleavy et al., 2009). The Mis18-KNL2\textsuperscript{M18BP1} complex does however not directly interact with CENH3\textsuperscript{CENPA} (Hayashi et al., 2004; Fujita et al., 2007). While KNL2\textsuperscript{M18BP1} mediates the recruitment of Mis18 proteins to the centromere (Fujita et al., 2007), Mis18 proteins restrict the deposition of CENH3\textsuperscript{CENPA} to the centromeres (Nardi et al., 2016).

The histone fold domain (HFD) of CENH3\textsuperscript{CENPA} contains a centromere-targeting domain (CATD) that is responsible for binding HJURP (Foltz et al., 2009). In yeast, HJURP\textsuperscript{SCM3} and the CENH3\textsuperscript{CENPI} histone chaperone NASP\textsuperscript{SIM3} are involved in centromeric nucleosome assembly (Dunleavy et al., 2007; Pidoux et al., 2009). An orthologue of NASP identified in Arabidopsis thaliana shows H3 chaperone activity (Maksimov et al., 2016). NASP also binds CENH3 and NASP down regulation impairs the loading of CENH3 at the centromeres (Le Goff et al., 2019). An HJURP-like CENH3-selective chaperon has hitherto not been identified in plants.

CENH3 is assembled into nucleosome complexes with Histone 2A, Histone 2B and Histone 4, substituting the canonical histone H3 complex (Ramachandran and Henikoff, 2016). As in most eukaryotes, the plant centromeres are defined by the occurrence of arrays of CENH3 nucleosomes mixed with arrays of H3 nucleosomes (Panchenko et al., 2011). Most of the centromeric histone interacting proteins described in yeast and animals have not been identified in plants (Drinnenberg et al., 2016) and for many candidate CENH3 interacting proteins experimental evidence for their role in CENH3 loading is lacking (Lermontova et al., 2015). In addition to chaperones and other CENH3 interacting proteins orchestrating its deposition, there is mounting evidence for RNA transcribed from centromeric repeat sequences in specifying the centromeric chromatin (Talbert and Henikoff, 2018). Transcripts originating from the centromeric region are associated with the loading of centromere nucleosomes and the stabilization of kinetochore proteins (Talbert and Henikoff, 2018). As neither the centromere sequence nor the CENH3s amino acid sequence are strictly conserved...
(Drinnenberg et al., 2016) and even divergent CENH3s are interchangeable between some plant species (Maheshwari et al., 2017), epigenetic factors including DNA methylation and chromatin modification are put forward as the determining regulators of CENH3 loading and maintenance.

The fidelity of chromosome segregation is impaired in animals and yeast cells by mutations that affect CENH3 loading and stability (Chen et al., 2000; Pidoux et al., 2003; Tanaka et al., 2009; Ranjitkar et al., 2010; Au et al., 2013; Shrestha et al., 2017). Loading of CENH3 to the centromeric DNA mainly depends on the C-terminally positioned HFD of CENH3 rather than its variable N-terminal tail (Sullivan et al., 1994). However, a higher incidence of chromosome missegregation has been shown in yeast carrying mutations in the N-terminal tail of CENH3 CSE4 (Chen et al., 2000) that is not directly associated with the loading of CENH3 to the centromeres (Ravi et al., 2010). Conversely, more stable association of the CENH3 CSE4 with the centromeres via reduced ubiquitination at the N-terminal tail also leads to defects in chromosome segregation (Au et al., 2013). Loading of the appropriate CENH3 amount (Regnier et al., 2005; Au et al., 2008; Shrestha et al., 2017) and/or tight regulation of the dynamics of CENH3 centromere interaction (Ohzeki et al., 2016; Bui et al., 2017) is therefore critical for ensuring kinetochore function and faithful segregation of the chromosomes.

Strict regulation of CENH3 labeling on centromeres also plays a vital role in chromosome segregation in plants. Mitotic division rate is reduced in CENH3 targeting RNAi lines whereas chromosome segregation problems were recorded in meiotic cells (Lermontova et al., 2011). More recent findings from maize demonstrate the vital importance of strict regulation of CENH3 abundance. Overexpression of CENH3 results in lethality in maize callus whereas GFP-CENH3 or CENH3-YFP overexpression is tolerated (Feng et al., 2019). Moreover, N-terminal tail and C-terminal HFD maintain their significance in chromosome segregation in plants. Both GFP-CENH3 and CENH3-YFP overexpression lines exhibit reduced deposition of the fusion proteins to maize centromeres (Feng et al., 2019). C-terminal GFP or YFP fusions of CENH3 cannot fully function in maize and A.thaliana somatic cells (De Storme et al., 2016; Feng et al., 2019) and several mutations in HFD reportedly cause chromosome elimination (Karimi-Ashtiyani et al., 2015; Kuppu et al., 2015). N-terminal tail modifications on the other hand result in chromosome elimination in plants (Ravi and Chan, 2010; Kelliher et al., 2016).

**Haploid induction through impaired CENH3 functioning**

Selection and fixation of desired traits is central to crop breeding. To breed a wide collection of vigorously growing hybrids, doubled haploids are created carrying two identical genome copies of the haploid parent (Maluszynski, 2003). These doubled haploids are crossed to generate new potential elite hybrids. In A.thaliana, the expression of CENH3 variant with the GFP tagged N-terminal tail of Histone 3.3 (H3.3) fused to the HFD of CENH3, referred to as *tailswap*, expressed in the CENH3 knockout mutant *cenh3-1*, produces 25-45% haploids upon crossing with wild type (Ravi and Chan, 2010). The expression of N-terminal GFP-CENH3 fusion protein in the *cenh3-1* mutant background also results in ~5% maternal haploid induction capacity (Ravi and Chan, 2010). Thus one might conclude that the N-tail of CENH3 has an important role in haploid induction. Specific mutations in the C-terminal HFD of CENH3 however, also evoke chromosome elimination. Depending on the mutation, the efficacy was around 1-2 % and around 10%, conferring the HFD domain some importance (Karimi-Ashtiyani et al., 2015; Kuppu et al., 2015). The expression of a similar CENH3-tailswap construct in maize was shown to induce the formation of haploid progeny and suggest it is a conserved mechanism that can be applied in other crops (Kelliher et al., 2016). CenH3-mutation and –
modification-based haploid induction strategies in plants are reviewed in more detail in Britt and Kuppu, 2016; Wang and Dawe, 2018; Wang et al., 2019.

CENH3 in species hybridization

The role of the centromere specific histone mark CENH3 in securing fidelity of chromosome segregation surfaces during species hybridization. The high accessibility of many flower structures allows for cross-pollination and requires the plant sexual reproduction system to establish multiple layers of hybridization barriers, one of which is inter-chromosome incompatibility mediated by the CENH3-centromere interaction (Tan et al., 2015). Additionally, barley doubled haploids have been produced with a strategy called “Bulbosum method” based on interspecific crosses starting with pollination of *Hordeum vulgare* (cultivated barley) with *Hordeum bulbosum* (bulbous barley grass) (Houben et al., 2011). In support of a role of CENH3 in rescinding hybridization events, interspecific crosses between *Hordeum vulgare* x *Hordeum bulbosum* result in paternal chromosome elimination during early embryogenesis following the loss of CENH3 from the centromeres of the paternal chromosomes (Sanei et al., 2011). The capacity to eliminate foreign chromosomes is transferable as expression of a CENH3 orthologous sequence derived from a different species such as maize in *A. thaliana* shows chromosome elimination when crossed with pollen carrying the original CENH3 locus (Maheshwari et al., 2015). This inability to transmit chromosomes loaded with ectopic CENH3 upon crosses with wild type indicates that the native CENH3-centromere interaction harbors species-specific characteristics. Thus the chromosome elimination is based on the incongruence of the different centromere–CENH3 interactions.

Conserved putative CENH3 interaction partners

Several candidate proteins interacting with the centromere have been reported, which are potentially involved in controlling the CENH3-centromere specificity. One of the well-studied examples is KNL2. KNL2 is required for CENH3/CENPA incorporation into chromatin, and CENH3/CENPA and KNL2 coordinately regulate chromosome condensation, kinetochore assembly, and chromosome segregation (Maddox et al., 2007). A homolog of KNL2 has been identified in *A. thaliana* (Lermontova et al., 2013). KNL2 knockout mutants display varying defects in organ development and leaf shape, and show reduced fertility. These defects are attributed to alterations in chromosome structure and dynamics during cell division (Lermontova et al., 2013). KNL2 contains a CENPC conserved motif (CENPC-k) that is required for centromeric localization (Sandmann et al., 2017) and specific mutations in the CENPC-k motif lead to the production of haploid progeny upon crossing with WT pollen. These properties indicate that KNL2 is critical in establishing the CENH3-centromere interaction. In line with its role in controlling CENH3 abundance at the centromere, mutations in the CENPC-k motif of KNL2 lead to the production of haploid progeny (Lermontova, 2019).

By screening the literature reporting CENH3/CENPA/CNP1/CSE4 candidate interacting proteins described for human CENH3/CENPA, budding yeast CENH3/CNP1, and fission yeast CENH3/CSE4, we generated a list of 78 putative orthologs or functional homologs in *A. thaliana*, *Z. mays* and *O. sativa* (Table 1). Histones were excluded from the selection because they are not directly involved in the regulation of CENH3 loading and maintenance at the centromeres. Affinity purification experiments, immunopurification coupled with Western blot or mass spectrometry, yeast-two hybrid, FRET, conditional growth arrest experiments and data showing that misexpression changes the abundance of CENH3/CENPA/CNP1/CSE4 at the centromeres, were all considered as indications for interactions with CENH3, either direct or indirect, for example as a part of a protein complex. Candidate plant homologs were identified using
reciprocal BLAST searches and the “HomoloGene” software (shown in Bold in Table 1). Candidate plant sequences were either previously reported as functional analogs (underlined in Table1) or no records were found (no markup, Table 1).

Plant orthologs of known interaction partners of CENH3\textsuperscript{CENPA/CNP1/CSE} are considered here as “putative conserved interaction partners of CENH3”. In order to find protein homologs in plants reciprocal protein blasts of human and yeast to plant sequences were performed. The selected candidate sequences were used to perform a literature survey. For the CENH3-interacting proteins HJURP, CENPI, CENPT, CENPM and CENPP, sequence homology searches did not result in the identification of putative orthologs, indicating poor sequence conservation across species or that plants do not harbor a counterpart. The previous reports suggesting rapid evolution of centromere associated/kinetochore related proteins, corroborates with an apparent lack of sequence conservation (Drinnenberg et al., 2016).

**Candidate CENH3 interacting proteins with functions related to growth and development**

The candidate plant orthologs and functional homologs listed in Table 1 have been assigned functions related to different aspects of plant development. The Arabidopsis MIS12 (Sato et al., 2005), MSI1 (Hennig et al., 2005), and CUL1 (Shen et al., 2002) for instance play a critical role in embryo development. Chromosome instability can cause arrests in embryonic development in plants. Therefore, it is also assumed that mutations in CENH3 interaction partners responsible from CENH3 deposition, incorporation and maintenance cause defects in embryo development. A candidate CENH3 interacting protein required for embryogenesis is MULTICOPY SUPPRESSOR OF IRA 1 (MSI1). MSI1 and MSI1–Like (MSIL) proteins are components of different protein complexes, including the Polycomb Repressive Complex 2 (PRC2) and B-type histone acetyltransferase complexes involved in chromatin remodeling, and pRB (retinoblastoma tumor suppressor protein) that controls the cell cycle and developmental processes (Hennig et al., 2005). MSI1 functions in seed development through interaction with retinoblastoma protein and the CULLIN4-DDB complex, controlling parental gene imprinting and a member of the MEDEA (MEA)/ FERTILIZATION-INDEPENDENT ENDOSPERM (FIE)/FERTILIZATION- INDEPENDENT SEED2 (FIS) polycomb group complex (Köhler et al., 2003; Dumbliauskas et al., 2011; Jullien et al., 2008). MSI1 (Hennig et al., 2005) and CULLIN1 (CUL1) (Shen et al., 2002) play a role in postembryonic development and null mutants are embryo lethal, in agreement with a critical role in cell division and development. The plant MSIL protein family (5 in Arabidopsis, AtMSI1-5, and 3 in rice, OsRBAP1-3) is larger and more diverse than in fungi, insects and vertebrates (Yang et al., 2013). While the function of AtMSI2 and 3 are unknown, AtMSI4/FVE regulate flowering time by repressing FLC expression through a histone de- acetylation mechanism (Ausin et al., 2004) and play a role in cold stress (Kim et al., 2004). In addition to MSIL proteins and CUL1, centromere localized plant MIS12 was shown to be essential for embryogenesis (Sato et al., 2005). The role of these candidate CENH3 interacting proteins in early stage of development suggests a critical role in mitosis, which is in line with the embryo lethal phenotype of CENH3 knockout plants (Ravi and Chan 2010) and the root developmental defects reported in plants expressing recombinant CENH3 (Wijnker et al., 2014).
Candidate CENH3 interacting proteins with functions related to histone chaperones

Nucleosome assembly is mediated by conserved histone chaperones, classified into families based on the founding member genes NAP, CAF1, SPT6, SSRP1, ASF1, HIRA, NASP, and FACT (Tripathi et al., 2015). For several members of these protein families, interaction with CENH3/CENPA/CNP1/CSE4 has been demonstrated in human and yeast (Table 1). Evidence in plants is largely missing and only indirect indications for a role in CENH3 chaperone function is available. For instance, the interaction of NASP with both CENH3 and H3.1/H3.3 has been demonstrated (Le Goff et al., 2019). HFDs of H3s and CENH3 show 50 to 60% sequence similarity within the same species (Talbert and Henikoff, 2010). Considering that HFD plays the role in chromatin targeting, the chaperoning function of NAP, CAF1, ASF1 and HIRA might also be conserved in CENH3 targeting in plants.

In the context of genome elimination, HIRA is a promising candidate for engineering. HIRA activity is specifically impaired in the Drosophila mutant sésame (ssm), causing a unique maternal zygote effect in preventing the formation of the DNA replication-competent male pronucleus, which results in the development of haploid embryo’s carrying only maternal chromosomes (Loppin et al., 2005). In vertebrates, HIRA is critically involved in nucleosome assembly of the H3.3 histone variant independent of DNA synthesis (Tagami et al., 2004). The replacement of sperm chromosomal proteins by maternally provided histones, is impaired in sésame in agreement with the histone chaperone protein function of HIRA (Loppin et al., 2005). While the A. thaliana HIRA protein interacts with H3.3, a knock out mutant displays only a mild growth phenotype and does not affect sexual reproduction and embryogenesis, suggesting that plant HIRA has diversified to function during sporophytic development (Nie et al., 2014). A weak sexual reproduction phenotype was however reported for a hira transposon mutant (same as in the study by Nie et al., 2014) and combined with the fas1-4 mutation, the double mutant did not produce viable pollen (Duc et al., 2015).

ASYMMETRIC LEAVES 2 (AS2) has been shown to repress the meristem development gene KNOTTED1-like homeobox (KNOX) during organogenesis through the interaction with histone chaperone HIRA (Guo et al., 2008). In view of the role of HIRA controlling the expression of KNOX genes through binding with the transcription factors AS1 and AS2 (Guo et al., 2008), it seems that HIRA plays a complex function in cell growth and development. It is currently not clear how this is linked with H3.3 nucleosome assembly.

Candidate CENH3 interacting proteins with functions related to DNA modification and DNA damage

A possible role of CENH3 in DNA damage response in mammals has been proposed based on the observation that CENH3/CENPA and other centromeric proteins are recruited to double strand breaks (Zeitlin et al., 2009). CENH3 also accumulates at neocentromeres that are formed at DNA breakpoints (Hasson et al., 2011) and in conditions causing genomic rearrangements such as in wide species crosses (Cuacos et al., 2015), suggesting that CENH3 functioning is somehow associated with DNA damage. In CENH3-based-haploid induction in plants, the selective loss of chromosomes is accompanied with major chromosome rearrangements relying on the DNA repair enzyme DNA ligase 4 (Tan et al., 2015). Some chromosome fragments are transmitted to the next generation and are reintegrated into the genome by DNA damage repair mechanism (Comai and Tan 2019). Whether CENH3 is linked with the unknown mechanism behind the activation of DNA damage response pathway following the chromosome elimination remains to be tested.
Genome instability upon UV induced double strand breaks triggers the highly conserved DAMAGE DNA BINDING (DDB1) proteins DDB1A and DDB1B to form a complex with CULLIN4 (CUL4) (Molinier et al., 2008; Ganpudi and Schroeder, 2013). The loss of DDB1B results in embryo lethality, indicating that these regulators are also important for basic functions in the absence of stress (Bernhardt et al., 2010). DDB1A physically interacts with MSI1 thereby regulating the PRC2 complex that controls imprinting and endosperm development (Dumbliauskas et al., 2011). In plants, a link between CENH3 in DNA damage response pathways has so far not been reported. The fact that CENH3 interacting animal and yeast proteins involved in DNA damage response are conserved in plants, calls for investigating a presumptive role of CENH3 in the CUL4, DDB1A or DDB1B and MSI1 controlled DNA damage response.

Post-translational modifications of CENH3

Chromatin displays local DNA and histone modification patterns shaping the structural organization and stability of protein-nucleosome-DNA interactions. The histones are subjected to a variety of posttranslational modifications (PTMs) including addition of methyl, acetyl, ubiquitin, phosphoryl and ADP-riboyl groups that influence the interaction with axillary factors, many of which are regulating gene expression (Rothenbort and Strahl, 2014). CENH3 PTM serves other functions such as the maintenance of centromeric nucleosomes (Niikura et al., 2015). An alignment of CENH3 from *S. cerevisiae*, *H. sapiens*, *A. thaliana*, *Z. mays* and *O. sativa* reveals multiple candidate PTM sites in plants, many of which have been reported to undergo ubiquitination, acetylation, phosphorylation and methylation (Figure 1).

The HFD of CENH3<sup>CENPA</sup> contains an acetylated or ubiquitinated lysine residue (CENPA-K124) that is conserved in the 5 aligned centromeric histone sequences (Bui et al., 2012; Niikura et al., 2015). Ubiquitination at that position in human cells depends on COPPS8, a gene conserved in plants (Table 1) and functions in ubiquitin mediated protein degradation as a component of COP9 signalosome (Schwechheimer and Isono, 2010). Plant development is orchestrated via components of COP9 signalosome by controlling of proteolysis in adjacent developmental stage (Qin et al., 2020). As an important element of cell division, CENH3 deposition and maintenance at the centromeres also can be regulated as a part of COP9 signalosome. Such regulation would give plants flexibility to cease or proceed with cell division to fulfill the requirement of different developmental stages.

Ubiquitination of CENH3 plays an important role in the stability of incorporated CENH3<sup>CSE4</sup> at the centromeres in yeast (Hewawasam et al., 2010; Au et al., 2013) and CENH3<sup>CENPA</sup> deposition in animal cells (Niikura et al., 2015), albeit that some modifications are dispensable for the long-term function and identity of the centromeres (Fachinetti et al., 2017). The ubiquitination-dependent proteolytic degradation of CENH3<sup>CSE4</sup> is clearly established in yeast. In *S. cerevisiae*, PSH1 is an E3 ubiquitin ligase controlling the stability and localization of CENH3<sup>CSE4</sup> by targeting the C terminus for ubiquitination, and is required for chromosome segregation (Hewawasam et al., 2010). An analogous function of PSH1 is executed by the *A. thaliana* ORTH/VIM proteins that function redundantly as ubiquitin ligases and regulate epigenetic silencing by modulating DNA methylation and histone modification (Woo et al., 2007; Kraft et al., 2008; Kim et al., 2014). VIM1 interacts with CENH3 in vivo in *A. thaliana*, and is required for maintenance of centromere DNA methylation and proper interphase centromere organization (Woo et al., 2008).
Several phosphorylation sites have been identified in CENH3\textsuperscript{CENPA} of which S7 is phosphorylated by Aurora kinase, and plays an unexpected role in cytokinesis (Zeitlin \textit{et al.}, 2001). Cell cycle dependent phosphorylation of CENH3\textsuperscript{CENPA} is mediated by cyclinE1/CDK2 at S18 (Takada \textit{et al.}, 2017). In maize CENH3s is also phosphorylated in a cell cycle dependent fashion at position S50 (Zhang \textit{et al.}, 2005). A recent study shows that Aurora3 phosphorylates Arabidopsis CENH3 at the position serine 65 (Demidov \textit{et al.}, 2019). Phosphorylation of S65 of CENH3 occurs in different developmental stages of Arabidopsis yet this PTM is mainly linked with floral meristem development. Further studies are required to determine what function phosphorylation of CENH3 plays in cell division.

Poly(ADP-ribose) polymerases (PARP) are responsible for ADP-ribosylation of CENH3\textsuperscript{CENPA} (Saxena \textit{et al.}, 2002) and are conserved in plants (\textit{A. thaliana} PARP1:At2g31320, \textit{O. sativa} PARP1:Os07g0413700, \textit{Z.mays} PARP1:Zm00001d005168). PARP was shown to bind the 180 bp centromeric repeat sequence from Arabidopsis suggesting that it may be independently targeted to the centromeres (Babiychuk \textit{et al.}, 2001). PARP plays a role in the DNA damage response and hence its association with CENH3 should be seen in the context of stress and UV DNA damage.

**Conclusion**

In view of the role of recombinant CENH3 in chromosome elimination and the development of methods to generate haploids for plant breeding, we point out the importance of identifying CENH3 interaction partners. A list of putative orthologs of animal and yeast CENH3 binding proteins is presented that serves as a starting point for further research. CENH3 interacting proteins are involved in a variety of biological pathways and many are putatively involved in chemically modifying CENH3. The conservation of these genes suggests that plant CENH3 undergo similar post translation modifications. Whether any of these modifications are involved in chromosome elimination remains to be discovered.
Figure 1: Model organism CENH3 amino acid sequence and reported PTMs

*A.thaliana, O.sativa, Z.mays, S.cerevisiae and H.sapiens* CENH3 sequences are shown with the existing identified post-translational modifications (me: methylation, ac: acetylation, ub: ubiquitination, ph: phosphorylation) on *S.cerevisiae, H.sapiens, Z.mays* (Zm) and *A.thaliana* (At) CENH3. PTMs listed here are reported in Zeitlin et al., 2001 (CENPA-S7ph); Zhang et al., 2005 (ZmCENH3-S50ph); Hewawasam et al., 2010 (CSE4-K4ub, CSE4-K131ub, CSE4-K155ub, CSE4-K163ub, CSE4-K172ub); Samel et al., 2012 (CSE4-R37me1/2); Bui et al., 2012 (CENPA-K124ac); Bailey et al., 2013 (CENPA-G2me3, CENPA-S17ph, CENPA-S19ph); Boeckmann et al., 2013 (CSE4-K49ac, CSE4-S22ph, CSE4-K33ph, CSE4-S40ph, CSE4-S105ph); Niikura et al., 2015 (CENPA-K124ub); Yu et al., 2015 (CENPA-S68ph); Mishra et al., 2019 (CSE4-S9ph, CSE4-S10ph, CSE4-S14ph, CSE4-S16ph, CSE4-S17, CSE4-S154ph); Demidov et al., 2019 (AtCENH3-S68ph)
Table 1. Putative conserved interaction partners of CENH3 in *A. thaliana, O. sativa* and *Z. mays*.

| S. pombe | *A. thaliana* | *O. sativa* | *Z. mays* | References |
|----------|--------------|-------------|-----------|------------|
| Ams2     | Gata5:At5g66320* | Gata6:Os04g0539500 | Gata3:Zm00001d017409 | Takayama *et al.*, 2016; Chen *et al.*, 2003 |
|          | Gata6:At3g51080 | Gata6:Zm00001d025953 |           |            |
|          | Gata7:At4g36240 |           |           |            |
| Hos2     | Hda9:At3g44680 | Hda9:Os04g0409600 | Hda102:Zm00001d003813 | Kobayashi *et al.*, 2007 |
| Mis16    | Msi1:At5g58230** | Msi1:Os03g0640100 | Msi1:Zm00001d033248 | Hayashi *et al.*, 2004 |
| Pob3     | Ssrp1:At3g28730 | Ssrp1LA:Os01g0184900 | Nfd110:Zm00001d008847 | Choi *et al.*, 2012 |
|          |              | Ssrp1LB:Os01g0184900 |           |            |
| Pst2     | Snl5:At1g59890 | Snl3L3:Os01g0109700 | Snl3L3:Zm00001d040123 | Choi *et al.*, 2012; Bowen *et al.*, 2010 |
|          | Snl6:At1g10450*** |           |           |            |
| Rpt3     | Rpt3:At5g58290 | Rpt3:Os02g0325100 | Zm00001d015886 | Kitagawa *et al.*, 2014 |
| Sim3     | Nasp:At4g37210 | Os07G0122400 | Zm00001d007972 | Dunleavy *et al.*, 2007; Pidoux *et al.*, 2003, Le Goff *et al.*, 2019 |
| Spt16    | Spt16:At4g10710 | Spt16:Os04g0321600 | Spt16:Zm00014a035465 | Choi *et al.*, 2012 |
| Spt6     | Gtb1:At1g65440 | Spt6:Os05g0494900 | Spt6:Zm00001d038570 | Choi *et al.*, 2012 |
|          | Spt6:At1g63210 |           |           |            |

*H. sapiens*

| AurkA    | Aur1:At4g32830 | Os01g0191800 | Zm00001d039498 | Kunitoku *et al.*, 2003; Slattery *et al.*, 2008 |
|          | Aur2:At12g25880 |           | Zm00001d008815 |            |
| Protein | A. thaliana | O. sativa | Z. mays |
|---------|-------------|-----------|---------|
| AurkB   | At2g45490   | Os03g0765000 | Zm00001d034166 |
| Bmi-1   | Drip1:At1g06770 | Drip2:Os12g0600200 | Drip2:Zm00001d033322 |
|         | Drip2:At2g30580 |            | Zm00001d041405 |
|         |              |            | Zm00001d030985 |
| CenpC   | At1g15660   | Os01g0617700 | Zm00001d044220 |
| CenpU   | At5g24630   | Os02g0147700 | Zm00001d003685 |
| Cops8   | At1g14110   | Os04g0428900 | Zm00001d034361 |
| Cul4-A  | At5g46210   | Os03g0786800 | Zm00001d034361 |
| Ddb1    | At4g05420   | Os05g0592400 | Zm00001d039165 |
|         | At4g21100   |            | Obuse et al., 2004 |
| Ssrp1   | At3g28730   |            |         |
| Ssrp1   | Os01g0184900 |            |         |
|         | Nfd110:Zm00001d008847 |         | Foltz et al., 2006; Okada et al., 2009 |

**Table 1.** (continued) Putative conserved interaction partners of CENH3 in *A. thaliana*, *O. sativa* and *Z. mays*.

**H. sapiens**

| Protein | A. thaliana | O. sativa | Z. mays |
|---------|-------------|-----------|---------|
| Ssrp1   | At3g28730   |           |         |

**S. cerevisiae**

| Protein | A. thaliana | O. sativa | Z. mays |
|---------|-------------|-----------|---------|
| Protein | GenBank Accession | GenBank Accession | GenBank Accession | Reference(s) |
|---------|------------------|------------------|------------------|--------------|
| Cdc53   | Cul1:At4g02570   | Cul1:Os01g0369200| Cul1:Zm00001d010858| Cheng et al., 2016 |
| Doa1    | At3g18860        | Os07g0123700     | Zm00001d018724     | Cheng et al., 2016; Au et al., 2013 |
| Fun30   | Chr19:At2g0290   | Os04g0566100     | Chr19:Zm00001d002656 | Durand-Dubief et al., 2012; Narlikar et al., 2013 |
| Gcn5    | Gcn5:At3g54610   | Gcn5:Os10g0415900| Hag101:Zm00001d014175 | Vernarecci et al., 2008; Pandey et al., 2002 |
| Hir1    | Hira:At3g44530   | Os09g0567700     | Hira:Zm00001d019789 | Sharp et al., 2002; Duc et al., 2015 |
| Mcm21   | CenpO:At5g10710  | Os04g0284100     | CenpO:Zm00001d032978 | Samel et al., 2012; Ranjitkar et al., 2010 |
| Mif2    | CenpC:At1g15660  | CenpCA:Os01g0617700 | CenpC:Zm00001d044220 | Ranjitkar et al., 2010; Collins et al., 2005; Pinsky et al., 2003; Shibata and Murata, 2004 |
| Mtw1    | Mis12:At5g35520  | Mis12:Os02g0620100| Mis12:Zm00001d001797 | Samel et al., 2012; Collins et al., 2005; Pinsky et al., 2003; Sato et al., 2005 |
| Ndc80   | Ndc80:At3g54630  | Os08g0468400     | Zm00001d032029     | Boeckmann et al., 2013; Collins et al., 2005; Shin et al., 2018 |
| Pat1    | Pat1:At4g14990   | Pat1:Os01g0769000| Pat1:Zm00001d038671 | Mishra et al., 2015; Kuromori and Yamamoto, 2000 |
| Pat1    | Pat1:At1g79090   | Pat1:Os02g0517300| Pat1:Zm00001d043329 | |
| Pat1    | Pat1:At3g22270   |                 |                  |             |
| Psh1    | Orth1:At5g39550  | Orth2:Os05g0102600| Zm00001d011108     | Samel et al., 2017; Deyter et al., 2017; Ranjitkar et al., 2010; |
| Orth2   | At1g57820        |                 |                  |             |
| Orth5   | At1g66050        |                 | Zm00001d035764     | Kim et al., 2014 |
| Sgo1    | Sgo1:At3g10440   | Sgo1:Os02g0799100| Sgo1:Zm00001d019148 | Buehl et al., 2018; Mishra et al., 2018; Zamariola et al., 2013 |
| Sgo2    | At5g04320        |                 |                  |             |
| Gene   | Chromosome/Locus | Chromosome/Locus | Chromosome/Locus | References |
|--------|-----------------|-----------------|-----------------|------------|
| Siz1   | Siz1:At5g60410  | Os05g0125000    | Siz1:Zm00001d010974 | Ohkuni et al., 2016; Catala et al., 2007 |
| Siz2   | Siz1:At5g60410  |                 |                 |            |
| Spt16  | Spt16:At4g10710 | Spt16:Os04g0321600 | Spt16:Zm000014035465 | Ranjitkar et al., 2010 |
| Sth1   | Chr12:At3g06010 | Os05g0144300    | Zm00001d006798   | Ranjitkar et al., 2010; Hsu et al., 2003 |
|        | Chr23:At5g19310 |                 |                 |            |
| Ubp8   | Ubp22:At5g10790 | **Upb22:Os04g0647300** |             | Canzonetta et al., 2016 |
| Ubr2   | Prt6:At5g02310  | Prt6:Os01g0148000 | Zm00001d039860   | Samel et al., 2017 |
|        |                 | Prt6:Os01g0148050 |                 |            |

*no markup: genes identified via reciprocal Blasts from Human or Yeast to Arabidopsis/Rice/Maize (no references found).

**bold: genes identified through the software program Homologene.

***underlined: genes identified via reciprocal Blasts from Human or Yeast to Arabidopsis/Rice/Maize and supported by previous reports (the relevant references are underlined).
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Figure 1