IN BRIEF

A Tale of Two CENPCs: Centromere Localization of KINETOCHORE NULL2 and CENP-C

Although we have long known the repetitive nature of the DNA in plant and animal centromeres of plant and animals and have identified hundreds of kinetochore proteins, closing the mechanistic gap between the centromere DNA and the kinetochore protein complex has remained a challenge. Much research has focused on the centromeric histone cenH3, as cenH3-containing nucleosomes may provide a mark for formation of an active centromere (reviewed in Lermontova et al., 2015). Placement of cenH3 in nucleosomes at the centromere involves initiation, deposition, and maintenance steps, and multiple factors affect cenH3 localization and formation of the active centromere. For example, the kinetochore component CENP-C interacts with cenH3 nucleosomes through its conserved CENPC motif (Kato et al., 2013) and functions in the placement of cenH3 in additional nucleosomes.

CENP-C also interacts with KINETOCHORE NULL2 (KNL2), and Lermontova et al. (2013) previously found that Arabidopsis thaliana knl2 knockout mutants showed defects in cenH3 deposition. The knl2 knockout mutants showed defects in cenH3 deposition. The knl2 knockout mutants showed defects in cenH3 deposition. The knl2 knockout mutants showed defects in cenH3 deposition.

Model for KNL2 and CENP-C localization. In interphase, KNL2 and CENP-C interact and bind to cenH3 nucleosomes, where they can license deposition of additional cenH3, thereby reinforcing the structure of the active centromere. In mitosis, long centromeric transcripts compete with the centromere DNA sequence for binding to KNL2. By contrast, short single-stranded RNAs from centromere transcripts reinforce CENP-C binding to the centromeric chromatin. (Reprinted from Sandmann et al. [2017], Figure 7.)
mitotic chromosome segregation, reduced cenH3 gene expression, and less cenH3 protein at chromocenters of meristematic nuclei. In a new study, Sandmann et al. (2017) provide further insight on the assembly and regulation of centromeric chromatin by examining KNL2 protein domains and nucleic acid binding. The authors conducted BLASTP searches and identified a CENPC-like motif in Arabidopsis KNL2, designated CENPC-k; this domain is conserved in diverse eukaryotes, including many invertebrates and vertebrates, but is absent in some KNL2 homologs, such as those in thalian mammals and Caenorhabditis elegans. KNL2 with mutations in the CENPC-k motif, or a deletion of the CENPC-k motif, fails to localize to centromeres, but KNL2 with its CENPC-k motif replaced with the CENPC motif from CENP-C can localize to centromeres. Therefore, the authors suggested that the CENPC-k motif of KNL2 is involved in binding centromeric nucleosomes, similar to the corresponding motif in CENP-C.

To test whether Arabidopsis KNL2 interacts with the centromeric DNA similar to CENP-C of mammals and other plants, the authors conducted electrophoretic mobility shift assays and showed that the C-terminal region of KNL2, which includes the CENPC-k motif, can bind the Arabidopsis centromere repeat pAL1. Competition with poly(deoxyinosinic-deoxyctydyllic) acid and tests with the centromere retroelement Athila, telomeric repeat, and the tubulin coding sequence indicated that KNL2 DNA binding does not show sequence specificity in vitro. By contrast, in vivo assays using chromatin immunoprecipitation showed that KNL2 associates preferentially with pAL1. The KNL2 protein with a mutated or deleted CENPC-k motif also showed nonspecific DNA binding in vitro. The authors suggest that in plants, DNA binding and interaction with cenH3 nucleosomes may cooperatively promote KNL2 localization to the centromere.

Transcription of centromere repeats produces full-length transcripts, which are cleaved to small RNAs. In addition to binding DNA, maize (Zea mays) CENP-C can bind mRNAs and small centromeric RNAs stabilize its binding to DNA (Du et al., 2010). To test whether Arabidopsis KNL2 behaves similarly, the authors identified a small, single-stranded RNA derived from pAL1 and found that this 23-nucleotide RNA bound to KNL2 but did not affect KNL2 binding to pAL1. By contrast, a full-length transcript of pAL1 bound to KNL2 and competed with pAL1 DNA for binding, similar to the situation for maize CENP-C.

These findings led to a model (see figure) wherein KNL2 and CENP-C bind to centromeric chromatin at interphase and initiate deposition of cenH3 into nucleosomes. During mitosis, single-stranded RNA transcripts from pAL1 compete with centromeric DNA for KNL2 binding, helping KNL2 exit the centromere, while small single-stranded RNAs stabilize the centromere binding of CENP-C. Therefore, these two proteins interact with transcripts from the centromere repeats, with differing consequences, possibly reflecting their differing functions: CENPC-C remains at the centromere during mitosis and is involved in function of the established kinetochore, but KNL2, having acted upstream of cenH3 deposition, leaves the centromere during the early stages of mitosis. Another suggestion from the authors’ model is that the nonspecific DNA binding of KNL2 and CENP-C may allow establishment of neocentromeres, an intriguing idea that awaits testing, perhaps in the authors’ next venture into the unknown territory of the centromere.

REFERENCES


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*Plant Cell* 2017;29;2-3; originally published online January 13, 2017;
DOI 10.1105/tpc.17.00035

This information is current as of July 31, 2017

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