

EVOLUTIONARY DYNAMICS OF THE CENTROMERIC HISTONE CENH3

WITHIN THE TRIBE TRITICEAE

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In most species, centromere identity is defined by the presence of the centromere-specific variant of histone H3 known in plants as CENH3. Unlike canonical histone H3 with its conserved structure, CENH3 shows considerable variability across species. Most of the diploid plant species (*Arabidopsis thaliana*, maize and rice), in which the structure and copy number of CENH3 have been determined, have this gene as a single copy. However, some species in the tribe Triticeae have CENH3 in two variants. Rye (*Secale* L.) is a Triticeae genus adapted to a wider range of environmental and climatic conditions than wheat and barley. Cultivated, weedy and wild *Secale* species have different pollination systems (self-incompatible, allogamous vs self-compatible, autogamous) and life-cycle durations (perennials vs annuals). Because the CENH3 proteins and genes encoding them in *Secale* species have yet to be known, we explore the molecular structure and the evolutionary dynamics of this central component of centromere specification and function.

We have determined the molecular structure and phylogenetic relationships of the CENH3 proteins in 11 rye species and subspecies, which have diverse crossing systems and are adapted to diverse biotic and abiotic stresses. The genome of the cultured rye *Secale cereale* has been found to have two paralogous CENH3 genes, which have different intron-exon structures and transcribe into two main forms of the protein, α CENH3 and β CENH3. These forms differ by size, have different amino acid compositions and co-localize in intermingled domains in interphase and mitotic centromeres. Using a set of wheat-rye addition lines, we assigned the α CENH3 and β CENH3 forms to rye *S. cereale* chromosomes 1R and 6R. The sequence alignment of CENH3s from *Secale* species and subspecies displays a high level of similarity, despite the differences that they have in morphology, life-cycle duration and pollination systems as well as environmental and growing conditions. The CENH3 proteins are strictly controlled by genetic factors responsible for purifying selection. Diversifying selection operates at a very few sites in cross-pollinating rye species. A comparison between *Hordeum*, *Secale* and *Triticum* species demonstrates that the structure of CENH3 in the subtribes Hordeinae and Triticinae has evolved at different rates. A high similarity between rye and wheat CENH3s is indicative of the evolutionary stability and conservation of the

genetic factors that control the CENH3 structure in the subtribe Triticinae. The role of reticulate evolution as a factor stabilizing the CENH3 structure and the rate of its evolution are discussed. This research was financially supported by the Russian Fundamental Scientific Research Program (project 0310-2018-0010), the Russian Foundation for Basic Research (grant 17-04-00748a) and the Deutsche Forschungsgemeinschaft (DFG, HO 1779/15-1).