Figures from: Przewłoka MR, Wierzbicki AT, Ślusarczyk J, Kuraś M.., Grasser KD, Stemmer C, Jerzmanowski A (2002) The 'drought-inducible' histone H1s of tobacco play no role in male sterility linked to alterations in H1 variants. *Planta* in press

Functional analysis of a new linker histone variant from tobacco

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Introduction

Histone H1 (linker histone) is a major structural component of eukaryotic chromosomes. It is present in all higher eukaryotes in quantity of average one molecule per nucleosome. While the histones H2A, H2B, H3 and H4 form an inner protein core on which DNA is wrapped around, histone H1 is located externally to the nucleosome particle DNA.

Surprisingly, given the abundance and critical location of H1 in chromatin several knock-out experiments failed to detect serious perturbations in organisms without H1 (*Tetrahymena*, yeast) or with partial disruption of H1 (mouse, *Arabidopsis*). The role of histone H1 is mysterious.

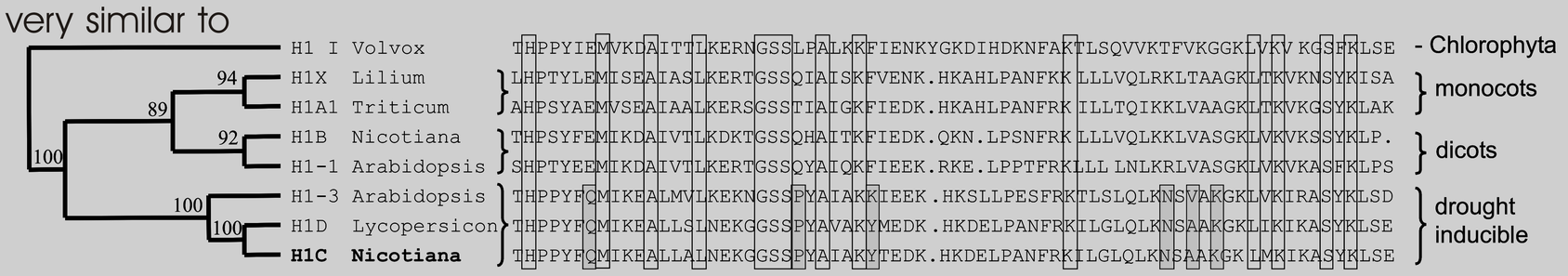
Higher eucaryotes have many nonallelic histone H1 variants. In animals some of them are specific for certain developmental stages.In plants a group of H1 variants was found to be linked to a physiological stage - the drought stress.

In the previous experiments in our laboratory the main variants of H1 (H1A and H1B) were silenced, what was accompanied by a compensatory increase in the level of the minor H1 variants (H1C, D, E and F). Native proportion of H1 variants was shown to be critical for a correct flower development and a male fertiliy.

Here we present functional analysis of H1C, a newly identified minor histone H1 variant. We determine that it belongs to a specific group of plant histone H1 variants, check if it is necessary in a course of development and try to find if its compensatory overexpession could be a direct cause of previously reported malfunction in the generative breeding.

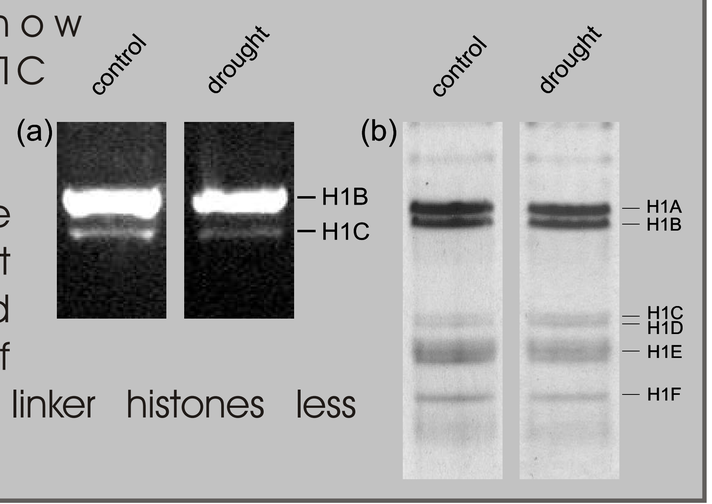
1. H1C is a homolog of drought-inducible H1s

Newly identified histone H1C was found to be very similar to variants previously known as drought-inducible. H1C groups with them in a well defined clade in the phylogenetic analysis, shares distinguishing residues and has a short, group-specific, C-terminal domain (not shown).



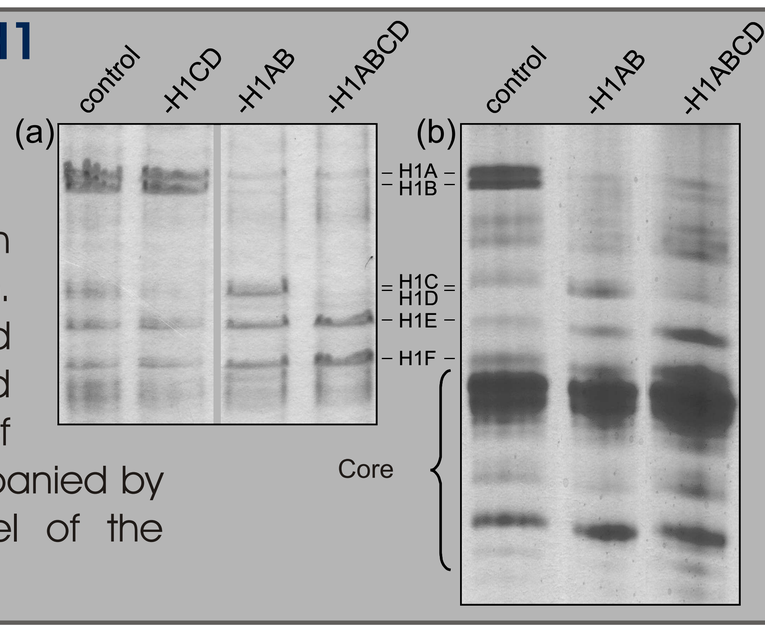
2. H1C is not induced by drought

Semi-quantitative RT-PCR (a) and protein analysis (b) failed to show induction of the H1C by drought treatment. It makes the assumptions about the role of H1C and the whole clade of 'drougt-inducible' linker histones less clear.



3. Manipulating variants of H1

To asses the role of H1C in development we applied antisense RNA strategy. We generated plants transformed with antisense H1C, antisense H1B or both. Protein analysis revealed that obtained plants were indeed devoid of desired H1 variants (a). Downregulation of some of the H1 variants was accompanied by compensatory increase in the level of the remaining variants (b).



4. H1C is dispensable for development

The plants devoid of H1C and H1D had general appearance and growth rate (not shown) as well as the flower anatomy (figure) and the course of male meiosis (not shown) the same as the controls.

The plants without H1A, B, C and D had the same abnormal phenotype as those without H1A and B, i.e. shortened stames and petals, characteristic petal-like coloring of sepals and deffects in male gametophyte. Defects in microsporogenesis concerned chromosomal abberations and asynchrony during meiosis. Both groups grew smaller and produced more lateral shoots (not shown).

Conclusions

H1C, the newly discovered variant of histone H1is very similar to linker histone variants induced by drought, but we failed to show that it is indeed induced by drought. This raises the question about the role of H1C and whole clade of 'drought inducible' H1. We checked that H1C is dispensable for normal development. We also found that silencing of H1C in plants devoid of major H1 variants introduces no additional developmental abberations nor rescues the flower phenotype. Thus H1C is not responsible for previously shown abberations in flower development in plants with silenced major H1 variants and compensatory overexpressed minor variants.

Based on analysis of 5' and 3' flanking sequences (not shown) we speculate that H1C might be regulated by sucrose concentration and be important in processes dependent on sucrose.

As change in histone H1 proportions selectively affects pollen viability, it migt be a good tool to produce male-sterile plants for hybrid seeds production.