The genome of eukaryotic organisms occurs in the cell nucleus as a complex of DNA and proteins called chromatin. Chromatin is not only a way of packaging very long DNA molecules into a small space, it also allows the genetic information encoded in the DNA to be accessed in the proper manner at the right time. The main unit of chromatin is the nucleosome, a protein complex which acts as a spool on which ~150 base pairs of DNA is wrapped. The nucleosome is an octamer comprised of two copies of each of four proteins called core histones (histones H2A, H2B, H3 and H4) around which the DNA is wound. The linker histone (histone H1) is another protein that fastens the DNA strands entering and exiting the nucleosome. The binding of H1 to nucleosomes promotes compaction of the chromatin and also decreases the availability of the information written in the DNA. The H1 molecule has a centrally-placed DNA-binding globular domain (GH1), which is highly conserved in evolution, and unstructured tails at both ends. GH1 and the tails of H1 are strongly basic.

Histone H1 is a very important player in the regulation of chromatin availability and hence the expression of genes. Linker histones in the nucleus are in a state of dynamic equilibrium between separate pools of molecules that are bound to nucleosomes or are unbound. Changing the affinity of histone H1 for the nucleosome could shift this equilibrium and disrupt the homeostasis of the cell. In both plants and animals linker histones are subject to posttranslational modifications, which usually involve the attachment of chemical groups to amino acids of the protein. Such modifications, especially in the GH1 domain, could change the affinity of H1 for the nucleosome, but this has yet to be confirmed.

Higher animals, like mammals, with mutations that decrease the abundance of linker histones by half cannot proceed past the early stages of development and die. Surprisingly, plants completely lacking linker histones are able to survive and grow. Moreover, H1-deprived plants of *Arabidopsis thaliana* (mutated in all genes encoding H1), despite gross changes in the ultrastructure of their cell nuclei, show no apparent macroscopic phenotype. This feature makes plants a good model system for studying disturbances resulting from the lack of H1. The reasons for the viability of H1-deprived plants remain unknown, although two hypotheses have been proposed: 1) plants possess other proteins which could bind nucleosomes in a similar manner to H1; 2) there are other regulatory mechanisms which compensate for disturbances caused by the lack of H1. Both hypotheses are plausible: plants could possess proteins that can substitute for histone H1 on the nucleosome and fulfill some of its functions, and there may be other regulatory mechanisms that can fill any remaining gaps in chromatin regulation. It is worth noting that, unlike animals, plants possess other proteins with a GH1 domain (like H1) but their tails are not basic and can contain other DNA-binding motifs or domains. One of these proteins could potentially substitute for histone H1 on the nucleosome.

The first goal of this project is to identify proteins that substitute for histone H1 on the nucleosome and the proteins participating in mechanisms to compensate for the lack of H1. To address this goal, chromatin isolated from control and H1-deprived plants (carrying mutations in H1-coding genes) will be sheared into short segments comprised of a few nucleosomes (oligonucleosomes) and the bound proteins will be identified, including those substituting for H1. Data collected during the identification of polynucleosome-bound proteins may also be used to characterize any posttranslational modifications of core and linker histones. We will also analyze the proteomes of whole cell nuclei from control and H1-deprived plants to detect proteins whose level is changed in the mutants. We suspect that the abundance of proteins participating in processes compensating for the lack of histone H1 will be altered in these mutants. High throughput mass spectrometry-based proteomic methods will be used to identify proteins and compare their concentrations. For protein identification we will use a ‘shot-gun’ approach where all proteins from the sample are initially digested by an endoprotease (usually trypsin). The resulting peptide mixture is separated by high-performance liquid chromatography coupled with a mass spectrometer which analyzes the masses of these peptide, fragment them and analyze the fragments. This information will be compared with a genomic database using sophisticated software for automated peptide (and protein) identification.

The second main aim of this project is to determine the impact of postrantranslational modifications on the affinity of H1 for nucleosomes. To do this we will first prepare nucleosomes by reconstitution *in vitro* from recombinant core histones and DNA produced in genetically modified bacterial cells. These nucleosomes will then be mixed with histone H1 or the GH1 and the energy released during binding will be monitored using a method called isothermal titration calorimetry (ITC). These measurements will allow us to determine the affinity of H1 (or GH1) for the nucleosome. Using this assay we will compare the nucleosome affinity of plant and animal GH1 domains with and without posttranslational modifications as well as whole plant and animal H1 proteins. GH1 domains with and without modifications will be synthesized chemically using Solid Phase Peptide Synthesis. In this method the amino acids are added one by one to build the peptide chain on a solid resin support.

We will also analyze the impact of posttranslational modification of GH1 on the structure of GH1-nucleosome complex. We will utilize cryogenic electron microscopy (Cryo-EM) and Small-angle X-ray scattering (SAXS) for structural analyzes.