Architectural Proteins in Plant Chromatin

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Projects
- Variability of plant HMG1 proteins
- DNA-interactions of the HMG1 proteins
- Association with chromatin
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- Architectural role in the formation of complex nucleoprotein structures
- Structure-specific recognition protein SSRP1

High mobility group (HMG) proteins are a heterogeneous class of chromosomal non-histone proteins in eukaryotic organisms. They are among the most abundant and ubiquitous non-histone proteins in the nucleus. HMG proteins appear to act as architectural components facilitating various biological processes (e.g. transcription, recombination, replication). In vertebrates, they have been subdivided into three structurally unrelated subgroups:

- HMG1/2 proteins
- HMG14/17 proteins
- HMG1/Y proteins

HMG1 proteins that have been detected in various eukaryotes contain as a distinctive feature a so-called HMG domain DNA-binding motif [see in the figure above the three-dimensional structure of the HMG domain B of the rat HMG1 protein, solved by Weir et al. (1993) EMBO J. 12, 1311-1319]. Our work is mainly focussed on the analysis of HMG1 proteins occurring in nuclei of higher plants.

Variability of plant HMG1 proteins

Higher plants express a variety of relatively abundant HMG1 proteins. We have identified five different HMG1 proteins, each from the monocotyledonous plant maize (HMGa, HMGc1/2, HMGd, HMGe), and from the dicotyledonous plant Arabidopsis thaliana (HMGα, HMGβ 1/2, HMGγ, HMGδ). These HMG1 proteins contain a single HMG domain as a common feature, but they differ markedly from each other (and from HMG1 proteins of other eukaryotes) outside this conserved DNA-binding motif. The HMG domain is flanked by a basic N-terminal domain characteristic for plant HMG1 proteins, and is linked to the acidic C-terminal domain by a short basic region. The plant HMG1 proteins are in general structurally more variable than the corresponding proteins of other eukaryotes. Furthermore, the various plant HMG1 proteins are expressed in very different amounts, and display some tissue specific variation in their relative abundance.
DNA-interactions of the HMG1 proteins

The HMG1 proteins bind in vitro with some preference for a variety of A/T-rich polymerase I and II promoter regions. The selectivity for A/T-stretches is markedly less pronounced than with HMGI/Y proteins. Maize and Arabidopsis HMG1 proteins recognise various distorted DNA structures as examined using electrophoretic mobility shift assays. They bind preferentially to supercoiled DNA, four-way junctions and DNA minicircles, when compared to linear DNA. The different plant HMG1 proteins display similar DNA-binding properties in vitro.

The DNA-bending activity of the HMG1 proteins was investigated in circularisation assays with short DNA fragments (<150 bp) in the presence of DNA ligase. All the proteins promote the formation of intramolecular ring closure of the tested DNA fragments indicating that the plant HMG1 proteins can severely bend DNA. The activity of the various HMG1 proteins differed significantly in these experiments. Furthermore, plant HMG1 proteins have the ability to introduce negative supercoils into plasmid DNA in the presence of topoisomerase I.

A series of truncated, recombinant HMGa protein constructs was used to analyse the role of the regions flanking the HMG domain in the maize HMGa protein in its DNA interactions. The basic N-terminal domain enhanced the general affinity of HMGa for linear DNA by direct contacts with DNA; conversely, the acidic C-terminal domain reduced the affinity to a comparable extent. The opposing effects of the N- and C-terminal domain appear to be balanced in HMGa, resulting in similar DNA-binding of full-length HMGa and the individual HMG domain. In HMGa (and other plant HMG1 proteins), the N- and C-terminal domains might be in direct contact 'neutralising' each other which is consistent with the DNA-binding properties of the various HMGa constructs. As observed for the binding to linear DNA, the N- and C-terminal domains modulated the activity of the protein in circularisation assays. By contrast, the structure-specific DNA-binding and the supercoiling activity were hardly influenced by the regions flanking the HMG domain, indicating that the recognition of DNA structure and supercoiling are intrinsic properties of the HMG domain.

Association with chromatin

To examine whether the various maize HMG1 proteins might be differently associated with chromatin, nuclei prepared from immature kernels were extracted with NaCl, spermine and ethidium bromide. Whereas there was no differential extraction of the maize HMG1 proteins using NaCl, significant differences could be observed with spermine and ethidium bromide. HMGa was readily released from chromatin by spermine, while HMGc and HMGd were more efficiently released by ethidium bromide. Although little is known about the specific molecular effects of ethidium bromide and polyamines on chromatin structure, the strikingly different release of plant HMG1 proteins strongly suggests that the various HMG1 proteins might interact differentially with chromatin and could be localised in different regions.

Architectural proteins of plastid nucleoids

Non-sequence-specific DNA-bending proteins such as HMG1 proteins have not only been described in eukaryotic nuclei, but also in mitochondria. In bacteria the so-called HU proteins appear to perform similar functions. Although eukaryotic HMG1 and prokaryotic HU proteins are structurally unrelated, the members of these protein families have several functional features in common (eg. non-sequence-specific binding to the minor groove of the double-helix, recognising DNA structures, DNA-bending, supercoiling). The maize HMGa protein can functionally complement the defect of the B. subtilis HU protein, termed Hbsu, in vivo. The individual HMG domain is sufficient for a full replacement of the Hbsu protein.

Little is known about functionally related proteins from plastids of plant cells. However, the plastid genome of the cryptomonad alga Cryptomonas φ encodes an HU-like protein termed HipA. The DNA-binding properties of the plastid-encoded HipA protein closely resembles the properties of bacterial HU and eukaryotic HMG1 proteins. Therefore, all ‘DNA-containing compartments’ appear to contain non-sequence-specific architectural DNA-bending proteins, indicating that these proteins are a general requirement for manipulating DNA structures in a wide variety of biological systems.

Architectural role in the formation of complex nucleoprotein structures

Many biological processes that involve regulated DNA transactions are dependent on protein-induced distortions in DNA structure brought about by DNA-bending proteins. These accessory factors (eg. HMG1, HU) bend the DNA locally, and can stimulate the assembly of complex nucleoprotein structures required for biological function. Well known examples of processes regulated by nucleoprotein complexes are bacterial site-specific recombination reactions which are stimulated by DNA-bending proteins (eg. HU, IHF). The site-specific β-recombination of B. subtilis is strictly dependent on the assistance of a DNA-bending protein such as the Hbsu protein. The maize HMG1 proteins and the Cryptomonas HipA protein can efficiently...
replace the Hbsu protein in the β-recombination reaction in vitro. However, the various HMG1 proteins promote this recombination reaction with different efficiency, suggesting that the plant HMG1 proteins could be adapted to act as architectural elements in different nucleoprotein structures in vivo.

Structure-specific recognition protein SSRP1

The structure-specific recognition protein SSRP1 (~70-85 kDa) has been identified in various eukaryotes. As characteristic feature, it displays a HMG domain DNA-binding domain at the C-terminus. SSRP1 proteins recognise certain DNA structures, and can bend DNA. They are considered to act in chromatin-remodeling, and therefore seem to be involved in transcription and replication.

The plant SSRP1 proteins exhibit some structural differences when compared to the corresponding animal and yeast proteins. We have fused the full-length and various truncated versions of maize SSRP1 to the green fluorescent protein (GFP), and expressed these GFP constructs in tobacco protoplasts. Using this method, we were able to determine a region of 20 amino acid residues that confer nuclear localisation of SSRP1. The maize SSRP1 protein is associated with chromatin and appears to be differentially expressed in the plant. Furthermore, it binds preferentially to distorted DNA when compared to linear DNA.

Our future work will concentrate on the identification of novel architectural proteins in plant chromatin. In addition, the mechanism of how these proteins work in nucleoprotein complexes will be investigated. Therefore, a variety of in vitro and in vivo approaches involving molecular biological, genetic, cytological and biochemical techniques will be employed.

Diploma Theses


Doctoral Thesis

- Christoph Ritt (1998) Struktur und Funktion von chromosomalen HMG1 Proteinen aus Mais

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