STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF CHROMATIN-RELATED PROTEINS

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In eukaryotic cells, the genetic material is packed in a highly condensed structure, known as chromatin. The formation of this hypermolecular structure buries DNA loci thus limiting their accessibility. Although this "arrangement" can be viewed as a brilliant mechanism for the accommodation of charges and mass in a tiny space, key cellular processes linked to DNA transactions or chromatin state transitions require access of the appropriate protein machinery to their substrate. Protein complexes called chromatin remodelers often mediate the rearrangement of chromatin components and the alteration of DNA loci accessibility. Many protein components of such complexes are made up from combinations of several, clearly identifiable, domains or modules, which are structurally and functionally autonomous. Among them, a protein domain known as **chromodomain**, is thought to play a central role in chromatin remodeling by interacting with chromatin components, in particular post-translationally modified histones. While the atomic resolution structure of the chromodomain of Heterochromatin Protein 1 (HP1) bound to di- and tri-methylated histone H3 revealed for the first time how a class of chromodomains recognize patterns on the protein components of chromatin, the mechanism of action of other classes of chromodomains remains poorly understood. Such chromodomains belong to Drosophila's MOF and Mi-2 proteins, the only two members of this class implicated in nucleic acid binding to date. Determination of the tertiary structure of MOF proved that it does not adopt the chromodomain fold, while its initially presumed RNA-binding has not been confirmed by more recent data. The main determinant of nucleosome recognition by the dMi-2 chromodomain, on the other hand, has

been reported to be nucleosomal DNA rather than histones. However, the structural determinants of chromodomains indicative of nucleic acid binding have not been identified to date.

To shed some light onto the mode of recognition of nucleosomal DNA by a chromodomain, we solved by multi-dimensional NMR spectroscopy the 3D structure of the chromodomain c2 of dMi-2 protein and explored its structure/function relationship. The domain was produced in bacteria and was shown to bind to dsDNA of various lengths in a sequence-independent manner **and** to histone H3. C2 folds into a compact globular domain made up of three anti-parallel β-strands and a C-terminal α-helix packed together in the canonical $\alpha+\beta$ chromodomain fold. Analysis of the electrostatic surface potential calculated for c2 reveals a large and distinct positively charged patch on one face of the domain, despite its overall acidic pI (pI=4.90). This feature is not shared by other chromodomains of known structure and function and suggests that this patch could be the site of interaction with DNA. The aromatic cage, which is crucial in histone tail binding by typical chromodomains, such as HP1 and Polycomb, is present on the surface of c2, but significantly altered. A search of the Brookhaven Protein Databank for structural homologues revealed remarkable structural similarity of c2 with both chromo and chromoshadow domains. c2 was also found to have a clear tendency for oligomerization.

Overall, the biochemical, biophysical and structural analysis of c2 showed that this chromodomain shares features of both the chromo and chromoshadow protein families and is able to interact with more than one chromatin components. The information gained from this study strongly supports the hypothesis that the chromodomain fold can be viewed as a successful structural scaffold that allows a considerable sequence variation to fulfill various functions in different molecular contexts.

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Protein stability in nanocages: a novel approach for influencing protein stability by molecular confinement.

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Confinement of a protein in a small inert space and microviscosity are known to increase its thermodynamic stability in a way similar to the mechanisms that stabilize protein fold in the cell. Here, to examine the influence of confinement on protein stability we choose four test cases of single domain proteins characterized by a wide range of melting temperatures, from approximately 73 degrees C of titin I27 to approximately 36 degrees C of yeast frataxin. All proteins are stabilized when confined in the gel, the most dramatic stabilization being that of yeast frataxin, whose melting temperature increased by almost 5 degrees C in the gel. In addition to being simple to use, this approach allows us to change the viscosity of the solvent without changing its composition or altering the structure of the proteins. The dimensions of the pores of the gels fall in the nanometer range, hence they are similar to those of the chaperone cavity. This method could therefore be used as a novel and powerful approach for protein folding studies.

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