Histones and histone-like DNA-binding proteins: correlations between structural differences, properties and functions

Structural studies of histones and histone-like proteins have revealed a distinction into two classes depending on whether a typical fold, characteristic for eukaryotic histones (1,2), is present or not. In this article, when the fold is present [the members of the 'histone fold family' (2) are reviewed by Reeve (14)], tentatively proposed to give the name 'compactosomes' to protein-DNA complexes that are derived from a putative solenoidal form of supercoiling (21); only a solenoidal form is able to compact the DNA substantially (10).

A further essential difference is found in the behaviour of these chromatins when they are processed for viewing in thin sections by electron microscopy. When fixed with conventional cytological fixatives, eubacterial chromatin is strongly aggregated during the dehydration steps in ethanol or acetone. No comparable artificial aggregation occurs with the chromatin of eukarya. Eubacterial chromatin is aggregation-sensitive, as has also been found with solutions of 'naked' DNA (18,19), the chromatin of dinoflagellates (9,11), the vegetative DNA of replicating bacteriophage (12) and the chromatin of mitochondria and chloroplasts (a detailed account of the relevant experiments and references has not yet been published but a manuscript is available from E. Kellenberger).

Interestingly, the chromatins of archaeal species were not aggregation-sensitive (3,4). In one case a large amount of histone-like H1a was thought to be responsible for the aggregation insensitivity; in the meantime other DNA-binding proteins — possibly of the histone fold family — were found in sufficiently high quantities to be responsible (J-F. Wen & J-Y. Li, personal communication).

The hypothesis that aggregation sensitivity is determined by a relatively small amount of bound proteins was confirmed by studying artificially assembled DNA-histone complexes with various relative amounts of histone. To be directly observable and easily manipulated under the naked eye, the experiments were designed in volumes of several μl, as described by Schreil (19). When the histone:DNA ratio was below 0.5, fixation no longer produced a gel and ultrathin sections observed by electron microscopy showed strong aggregation (16). Similar results were obtained when the histones were replaced by the histone-like protein HU from Escherichia coli. The aggregation sensitivity therefore correlates with the amount of regularly distributed DNA-bound protein in chromatin (10). A very simple test for aggregation sensitivity is available. From aldehyde-fixed material, two aliquots are prepared; one is post-fixed in uranyl acetate, the other is treated with Na2-EDTA. When the latter shows aggregation in the electron microscope, then the chromatin concerned is aggregation-sensitive due to the low amount present and/or irregularly distributed protein partners along the DNA (LP-chromatin, of which a protein:DNA ratio <0.5). When no aggregation occurs, the chromatin is aggregation-insensitive due to the sufficiently high amount and regularly distributed protein partners bound along the DNA [HP-chromatin with a protein:DNA ratio >0.5].

Conclusion

Tentatively, two main classes of chromatins can be distinguished:

(i) HP-chromatin, eukaryotic and archaeal; both are aggregation-insensitive, have histones with the typical fold and have nucleosomes that are stable enough to be isolated and further studied. Histone-like proteins, without the typical fold, are supposed to be present in addition.

(ii) LP-chromatin is found in eubacteria, dinoflagellates, vegetative DNA of replicating bacteriophages, and archaeal chromatin.
bacteriophages and mitochondria. It is aggregation-sensitive. Despite being supercoiled its putative compactosomes are extremely fragile and cannot be isolated intact. Fold-histones, i.e. DNA-binding proteins of the histone fold family, are lacking. How far histone-like proteins are involved in the formation of the putative compactosomes is still under debate.

**Perspectives**

It is not surprising that three features of DNA-binding proteins are associated: (i) presence of the histone fold, (ii) stability of nucleosomes and (iii) high relative amounts of DNA-bound proteins with correlated aggregation insensitivity. A lower relative amount of histone-like proteins without the fold leads to aggregation sensitivity and very fragile compactosomes. It is very rewarding to find that physiologically important differences, such as those described above, are in line with the evolutionary branches that repose on 16S rRNA sequences. Intensive work is needed to physiologically characterize these taxonomic differences both in greater depth and breadth. Some conspicuous exceptions need particular attention.


**rDNA spacer rearrangements and concerted evolution**

Concerted evolution has been well described in eukaryotes and is characterized by (i) the spread of mutations in repeat sequence blocks, (ii) the variation of repeat unit lengths and (iii) differences in copy number of repeat units (2). These differences tend to be least within a species (homogenized) and greater between different species. Compensatory slippage is one mechanism which has been proposed to account for these differences by the conservation of secondary structure despite the presence of sequence divergence (6).

In prokaryotes concerted evolution has recently been proposed to explain the spread between *rrn* operons of a *Bgl* site which is responsible for most of the ribotype variation in the seventh pandemic clone of *Vibrio cholerae* (8). Some sequence blocks within the 16S–23S spacer region of bacteria also appear to demonstrate concerted evolution by showing a high degree of sequence conservation between *rrn* copies of a species but no sequence homology between species (1,4,5). However, other sequence blocks are not present in all *rrn* copies of a given species (1,4,5). There are notable differences between concerted evolution in eukaryotes (2) and prokaryotes (1,4,5). In *Drosophila melanogaster* the rRNA genes are organized as 250 tandem repeats each separated by spacers which themselves contain repeat units (95, 240 and 330 bp in length) that undergo concerted evolution (2). In prokaryotes the *rrn* operons (1–10 copies) are located at different positions around a circular or linear chromosome. Concerted evolution in prokaryotes (as opposed to eukaryotes) may involve different processes in addition to homologous recombination (7) and the rearrangement of *rrn* operons (9).