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 et al. (17), acid-soluble DNA-binding proteins will be described as 'fold-histones', and as 'histone-like' only when they lack it. Fold-histones have so far been found in archaeal species (7), but not in eubacteria (24). It is suspected that they might coexist with histone-like proteins in archaeal species.

DNA complexes formed with the two kinds of protein differ in the readiness with which they can be isolated for electron microscopy and the study of their chemical composition and stoichiometry. While all attempts to extract persisting protein–DNA complexes from eubacteria have failed, they have met with success in archaeal species (20,22) to a similar extent and with even better quality than has been achieved with eukaryo (15). Takayanagi et al. (22) found that part of the isolated chromatin is in the classical 'beads on a string' form, suggesting that archaeal nucleosomes resemble eukaryotic ones in stability and structure. Eubacterial candidates, if they exist, must be extremely fragile (5); it was therefore 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 chromatin 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 HTa 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 ml, 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 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, agree so well with the evolutionary branches that reposes on compactosomes, as is supposed to explain the physiologically important exceptions need particular attention.


(iii) High relative amounts of DNA-


(i) In mitomycin-C-induced, lysogenic lysis is considered to be relatively gentle yet still considered as a feature of their compaction and condensation. FEMS Microbiol Lett71, 31–36.

(k) Chromatin from Oxyrrhis marina, a rather special dinoflagellate, seems to be aggregation-insensitive and, in contrast to others [for references see (3)], was found to contain a histone (25) and nucleosomes. At the submicroscopic level its nucleus resembles that of Euglena, not only in having histones and nucleosomes, but also in its mitotic type (6). Most dinofilagellates are so different from other flagellates that a designation as Dinokaryotes has been proposed. The aggregation sensitivity of the chromosomes of dinofilagellates in general is difficult to establish; because of their high packing density, high resolution electron microscopy on extremely thin sections is required.

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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 local 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 BglI 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).