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Most of us feel, from time to time, that other authors have not acknowledged the work of our own or other groups or have omitted to interpret important aspects of their own data. Perhaps we have observations that, although not sufficient to merit a full paper, add a further dimension to one published by others. In other instances we may have a useful piece of methodology that we would like to share.

The Editors hope that readers will take full advantage of this section and use it to raise matters that hitherto have been confined to a limited audience.

Jon Saunders, Editor-in-Chief

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 eukarya (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 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 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 μ l, 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 $\text{Na}_2\text{-EDTA}$. 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

► GUIDELINES

Communications should be in the form of letters and should be brief and to the point. A single small Table or Figure may be included, as may a limited number of references (cited in the text by numbers, and listed in alphabetical order at the end of the letter). A short title (fewer than 50 characters) should be provided.

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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 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.

(i) In mitomycin-C-induced, lysogenic *Paracoccus denitrificans* (formerly *Thiosphaera pantotropha*, but still considered as a eubacterium) some structures resembling nucleosomes were observed by electron microscopy (14). Specimen preparation by *in situ* lysis is considered to be relatively gentle (13) and could thus have preserved labile compactosomes, as is supposed to explain the chromatin threads observed by Griffith (8).

(ii) Chromatin from *Oxyrrhis marina*, a rather special dinoflagellate, seems to be aggregation-insensitive and, in contrast to others [for references see (23)], 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 dinoflagellates are so different from other flagellates that a designation as Dinokaryotes has been proposed. The aggregation sensitivity of the chromosomes of dinoflagellates 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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1. Arents, G., Burlingame, R. W., Wang, B.-C., Love, W. E. & Moudrianakis, E. N. (1991). The nucleosomal core histone octamer at 3.1 Å resolution: a tripartite protein assembly and a left-handed superhelix. *Proc Natl Acad Sci USA* 88, 10148–10152.
2. Arents, G. & Moudrianakis, E. N. (1995). The histone fold: a ubiquitous architectural motif utilized in DNA compaction and protein dimerization. *Proc Natl Acad Sci USA* 92, 11170–11174.
3. Bohrmann, B., Arnold-Schulz-Gahmen, B. & Kellenberger, E. (1990). Ultrastructural localization of the histone-like protein HTa from the archaeon *Thermoplasma acidophilum*. *J Struct Biol* 104, 112–119.
4. Bohrmann, B., Kellenberger, E., Arnold-Schulz-Gahmen, B., Sreenivas, K., Suryanarayana, T. & Reeve, J. N. (1994). Localization of histone-like proteins in thermophilic archaea by immunogold electron microscopy. *J Struct Biol* 112, 70–78.
5. Broyles, S. S. & Pettijohn, D. E. (1986). Interaction of the *Escherichia coli* HU protein with DNA. Evidence for formation of nucleosome-like structures with altered DNA helical pitch. *J Mol Biol* 187, 47–60.
6. Gao, X. P. & Li, J.-Y. (1986). Nuclear division in the marine dinoflagellate *Oxyrrhis marina*. *J Cell Sci* 85, 161–175.
7. Grayling, R. A., Sandman, K. & Reeve, J. N. (1996). Histones and chromatin structure in hyperthermophilic Archaea. *FEMS Microbiol Rev* 18, 203–213.
8. Griffith, J. D. (1976). Visualization of prokaryotic DNA in a regularly condensed chromatin-like fiber. *Proc Natl Acad Sci USA* 73, 563–567.
9. Kellenberger, E. (1988). About the organization of condensed and decondensed non-eukaryotic DNA and the concept of vegetative DNA (a critical review). *Biophys Chem* 29, 51–62.
10. Kellenberger, E. & Arnold-Schulz-Gahmen, B. (1992). Chromatins of low protein content: special features of their compaction and condensation. *FEMS Microbiol Lett* 100, 361–370.
11. Kellenberger, E., Carlemalm, E., Séchaud, J., Ryter, A. & de Haller, G. (1986). Considerations on the condensation and the degree of compactness in non-eukaryotic DNA-containing plasmas. In *Bacterial Chromatin*, pp. 11–25. Edited by C. O. Gualerzi & C. L. Pon. Heidelberg: Springer, Berlin.
12. Kellenberger, E., Ryter, A. & Séchaud, J. (1958). Electron microscopy study of DNA-containing plasmas. II. Vegetative and mature phage DNA as compared with normal bacterial nucleoids in different physiological states. *J Biophys Biochem Cytol* 4, 671–678.
13. Kellenberger, E. & Wunderli, H. (1995). Electron microscopic studies on intracellular phage development – history and perspectives. *Micron* 26, 213–245.
14. Mayer, F. & Friedrich, C. G. (1986). Higher order structural organization of the nucleoid in the thioautotrophic *Thiosphaera pantotropha*. *FEMS Microbiol Lett* 37, 109–112.
15. Oudet, P., Gross-Bellard, M. & Chambon, P. (1975). Electron microscopic and biochemical evidence that chromatin is a repeating unit. *Cell* 4, 281–300.
16. Pelzer, J., Bohrmann, B., Johansen, R., Maeder, M., Gyalog, R., Arnold-Schulz-Gahmen, B., Villiger, W. & Kellenberger, E. (1990). Structure and function of nonorthodox chromatins. I. Aggregation sensitivity of DNA is correlated to the protein content of chromosomal material. In *XIIth International Congress for Electron Microscopy*, vol. 3, pp. 120–121. Edited by L. D. Peachey & D. B. Williams. San Francisco, Seattle: San Francisco Press.
17. Reeve, J. N., Sandman, K. & Daniels, C. J. (1997). Archaeal histones, nucleosomes, and transcription initiation. *Cell* 89, 999–1002.
18. Schreil, W. H. (1961). Vergleichende Elektronenmikroskopie reiner DNS und der DNS des Bakteriennukleoids. *Experientia* 17, 391–394.
19. Schreil, W. H. (1964). Studies on the fixation of artificial and bacterial DNA plasmas for the electron microscopy of thin sections. *J Cell Biol* 22, 1–20.
20. Shioda, M., Sugimori, K. & Shiroga, T. (1989). Nucleosome-like structures associated with chromosomes of the archaeobacterium *Halobacterium salinarum*. *J Bacteriol* 171, 4514–4517.
21. Sinden, R. R. & Pettijohn, D. E. (1981). Chromosomes in living *Escherichia coli* cells are segregated into domains of supercoiling. *Proc Natl Acad Sci USA* 78, 224–228.
22. Takayanagi, S., Morimura, S., Kusaoke, H., Yokoyama, Y., Kano, K. & Shioda, M. (1992). Chromosomal structure of the halophilic archaeobacterium *Halobacterium salinarum*. *J Bacteriol* 174, 7207–7216.
23. Vernet, G., Sala-Rovira, M., Maeder, M., Jacques, F. & Herzog, M. (1990). Basic nuclear proteins of the histone-less eukaryote *Cryptosporidium parvum* (Pyrrophyta): two-dimensional electrophoresis and DNA-binding properties. *Biochim Biophys Acta* 1048, 281–289.
24. Vis, H., Mariani, M., Vorgias, C. E., Wilson, K. S., Kaptein, R. & Boelens, R. (1995). Solution structure of the HU protein from *Bacillus stearothermophilus*. *J Mol Biol* 254, 692–703.
25. Zhang, C.-Y., Zeng, C.-M. & Li, J.-Y. (1996). The comparative study on the chromosomes of *Oxyrrhis marina* and typical dinoflagellate, paying special attention to the systematic position of this organism. *Zool Res* 17, 331–336.

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 *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).