CHROMATIN STRUCTURE IN THE NUCLEI OF THE CILIATE STYLONYCHIA MYTILUS

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Received October 13,1976

Summary: Nucleosome repeat length was studied in three different nuclei of the unicellular organism Stylonychia mytilus. The transcriptionally inactive micronucleus has a nucleosome repeat of 202 base pairs, in the macronuclear anlagen and the vegetative macronucleus a repeat length of 217 resp. 220 base pairs was found.

Three different types of nuclei can be isolated from the hypotrichous ciliate Stylonychia mytilus (1, 2): The diploid micronucleus, the macronuclear anlage (developing macronucleus) which contains polytene giant chromosomes and the DNA - rich vegetative macronucleus which probably contains fragmented chromosomes (1). Whereas the macronucleus is transcriptionally active, the micronucleus is transcriptionally inactive and the macronuclear anlage makes little or no RNA (1).

The presence of histones in Stylonychia nuclei (2, 3) suggests that its DNA is organized as in other eucaryotic chromatin. In this paper we present evidence for a regular nucleosome - like organization of the chromatin which differs in nucleosome repeat length between the different nuclear types.

Material and Methods

Stylonychia mytilus (syngen I) was grown in neutral Pringsheim solution (1). Nuclei were isolated as described earlier (1, 2). Rat liver nuclei were prepared according to the method of Hewish and Burgoyne (4). Digestion of nuclei with micrococcal nuclease

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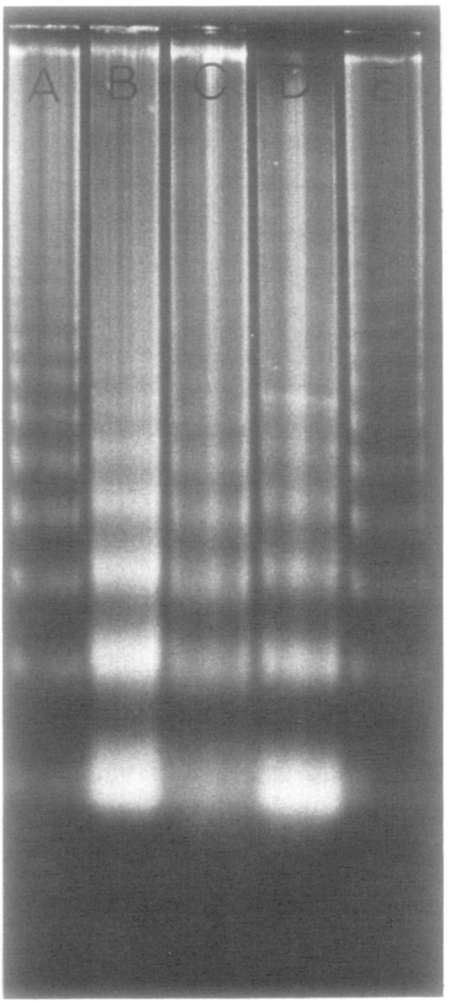

Figure 1. Electrophoresis on 1.7 % agarose gel of a short micrococcal nuclease digest of a) rat liver nuclei, b) Stylonychia macronuclear anlagen, c) Stylonychia micronuclei, d) Stylonychia macronuclei, e) rat liver nuclei. 0.2 ml of Stylonychia nuclei at 25 OD_{260}/ ml were digested for 1 min. at 37 °C with 225 units per ml of micrococcal nuclease.

was performed as described previously (5). Digested nuclei were deproteinized with SDS and chloroform - isoamylalcohol until the 280 : 260 ratio was 0.54. Electrophoretic analysis and calibration of digestion products on agarose and polyacrylamide gels with ΦX 174 - RF restriction fragments was done as described by Morris (5).

Results and Discussion

Brief digestion with micrococcal nuclease of chromatin from rat liver nuclei generates a series of DNA fragments which are approximate multiples of the nucleosome repeat length of 200

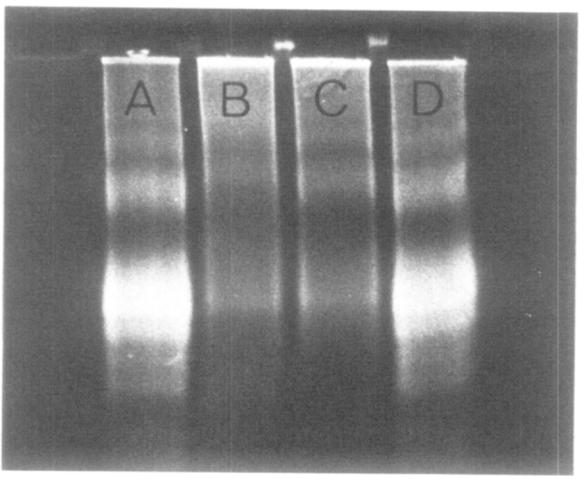

Figure 2. Electrophoresis on 6 % acrylamide gel of a long micrococcal nuclease digest of a) rat liver nuclei, b) Stylonychia macronuclei, c) Stylonychia micronuclei, d) rat liver nuclei. 0.2 ml of Stylonychia nuclei at 25 OD_{260}/ ml were digested for 15 min. at 37 °C with 225 units/ml of micrococcal nuclease.

base pairs (6). Digestion of Stylonychia nuclei gave a similar fragment pattern, indicating that the chromatin of Stylonychia is organized into nucleosomes. However, the DNA repeat length differed according to nuclear type (Fig. 1). The micronucleus had a repeat of 202 ± 3 base pairs; the repeat from the macronuclear anlage was 217 ± 4 base pairs and that from the macronucleus 220 ± 3 base pairs.

More extensive digestion of rat liver chromatin with micrococcal nuclease gives a quasi - limit digestion fragment of about 140 base pairs (7, 8) which represents the nucleosome core. Digestion of Stylonychia macro - and micronuclei under comparable conditions gave a similar fragment (Fig. 2). The size of the DNA core fragments from macronucleus and micronucleus was identical; therefore the difference in the DNA repeat length between these nuclei must result from a difference in the length of the linker DNA between nucleosome cores.

Correlative evidence suggests that the length of the DNA in the linker may be specified by histone H1. In general nuclei with less basic H1's have short repeats (5, 9, 10) and those with more basic H1's have long repeats (11, 12). In Stylonychia the amino acid composition of H1 from the different nuclei has not yet been determined; however the macronuclear H1 of the related hypotrichous ciliate Oxytricha is known to be somewhat more basic than calf thymus histone H1 (13). It could be suggested that histone H1 may be replaced by successively more basic variants during the progression from micronucleus to macronuclear anlagen to vegetative macronucleus.

Of the three types of nuclei only the vegetative macronucleus is known to be transcriptionally very active. Thus in Stylonychia as in the chicken erythrocyte (11) and in the urchin sperm (12) a change in transcriptional activity seems to be related to a change in the nucleosome repeat. This relationship has led one of us (NRM) to propose a mechanism for coarse control of transcriptional activity by H1 mediated changes in the nucleosome repeat. According to this hypothesis recognition signals (e.g. promoters) must lie between nucleosomes to be accessible to proteins (e.g. RNA polymerase). Thus changes in the nucleosome repeat length would alter the relationship of nucleosomes to recognition signals thereby altering their accessibility and modulating transcriptional activity (11). In chicken and sea urchin the inactive tissues (erythrocyte and sperm) have a longer repeat than the active tissues (liver and gastrula). In Stylonychia the inactive micronucleus has a shorter repeat than the active macronucleus. We conclude that transcriptional inactivity is not invariably associated with a long nucleosome repeat. The fact that the highly inactive macronuclear anlage has a repeat very similar

to that of the active macronucleus demonstrates that, since many factors must be involved in gene regulation, a change in nucleosome repeat need not result in a gross change in transcriptional activity.

Acknowledgement: H.J.L. thanks Dr. F.H.C. Crick for the opportunity to work in the MRC chromatin group in Cambridge. This work was supported by a short term EMBO fellowship awarded to H.J.L. and by a Senior fellowship of the Fogarty International Center of the U.S. National Institutes of Health to N.R.M.

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