Atypical nucleosome spacing of rat neuronal identifier elements in non-neuronal chromatin

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ABSTRACT

Rat neuronal identifier (ID) elements are located in chromatin regions that are organized in nucleosomal structures in both neuronal and non-neuronal cells. A subpopulation of ID sequences in chromatin of liver and kidney cells are relatively resistant to micrococcal nuclease digestion and are organized in nucleosomes exhibiting an atypically short repeat length. Other repetitive elements do not show this organization.

INTRODUCTION

Within the nuclei of cells, DNA is complexed with nucleosomal and non-histone proteins to form chromatin. In most tissues of rats and other higher animals, bulk DNA (which is mostly non-transcribed) is found associated with nucleosomes spaced on average about every 190-200 base pairs (1). Such DNA is coiled into supernucleosomal solenoid structures and, when isolated, is relatively resistant to experimental digestion with nucleases. The bulk DNA isolated from cortical neurons differs from that isolated from cortical glia and other tissues in that the nucleosomes are spaced on average every 160 base pairs (2). Such DNA is relatively sensitive to digestion with DNase I (3). Developmental studies have shown that chromatin isolated from rat cortical neurons undergoes a transition from the 200- to the 160-base pair repeat length by the end of the first postnatal week (4-7). The transition is not evident in bulk cerebellar chromatin which retains the 200-base pair spacing (2). The nucleosome spacing transition correlates developmentally with an increase in the complexity of brain transcripts (8) suggesting that the altered nucleosome distribution may be involved in extensive transcriptional activation of the genome in early postnatal cortical neurons. Brain is different from other tissues in that a greater portion (42%) of the genome is involved in active transcription (9,10).

The rat neuronal identifier (ID) element is repeated in the genome $1-1.5 \times 10^5$ times. It is dispersed since 43% of randomly picked genomic clones

with large (average 15 Kb) inserts contain one or more ID elements (11). Studies with nuclear RNA isolated from rat cortical neurons, cortical glia, cerebellum, whole brain, liver and kidney show that the cortical neuronal samples are highly enriched in ID content compared to the other samples, and the developmental onset of the enrichment is during the second week of postnatal development (12). Thus ID elements may be preferentially located in, but not exclusive to, genes whose expression is enriched in postnatal cortical neurons compared to other cell types. ID elements are also transcribed in a brain specific fashion by RNA polymerase III, giving rise to two small cytoplasmic RNAs (13,14). These small RNAs are especially enriched in cerebral cortex compared to cerebellum (13) and their concentration increases dramatically during the first week of postnatal development (15). Nucleotide sequence analyses of many cDNA clones of these brain-specific transcripts demonstrate extensive sequence microheterogeneity, indicating that many genomic ID elements are templates for Pol III transcription (M.A. Brow, P. Danielson, R.D. McKinnon, J. Arriza, I.R. Brown and J.G. Sutcliffe, in preparation). ID elements have the properties of transcriptional enhancers in transfection experiments, and the cellular specificity of their enhancer effect suggest it may require either Pol III transcription or the presence of the Pol III transcripts (16). Although no causal relationship has been established in vivo, these cumulative observations suggest that ID elements might serve as positive regulators for Pol II transcription of genes whose expression is elevated in postnatal cortical neurons, and that the mechanism of regulation might involve Pol III.

In general, chromatin structure renders most of the genome inert for solution reactions with trans-acting factors. However, genomic elements which prescribe changes in local chromatin structure when they bind factors cannot themselves be inert, lest such binding could not occur. Hence such genomic regulatory elements might be expected in chromatin isolated from tissues other than their tissue of action to be in a non-inert form in which they interact in a different manner with nucleosomes than does bulk DNA. For example, they could be nucleosome free or have an altered nucleosomal repeat length. Since ID elements have properties of positive effectors of gene expression and their transcription by Pol III is temporally correlated with the nucleosome spacing transition and the increase in RNA complexity, we investigated the nature of their interaction with nucleosomes in chromatin isolated from various tissues.

MATERIALS AND METHODS

Cerebral hemisphere neuronal, liver and kidney nuclei were isolated from young adult rats (17, as modified by 18) and digested for 1, 2 and 5 min at 37°C with micrococcal nuclease (Sigma) at 50 units/ml as previously described (4). Resultant nucleosomal DNA fragments were purified (4) and separated by electrophoresis on 1.5% agarose gels (8 µg DNA per lane) run in 90 mM Tris, 90 mM borate, 2 mM EDTA buffer (100 volts for 3 hours). Hae III digested ϕ X174 DNA was loaded in some gel lanes as a marker. Following staining with ethidium bromide (10 µg/ml), gels were photographed under short wavelength ultraviolet light and the size of DNA fragments in each multimer band determined. Nucleosomal repeat length was calculated using the oligomer length of the 5-mer DNA fragment minus the length of the 2-mer DNA fragment (4).

Nucleosomal DNA fragments were then blotted from the gels to BioDyne membranes and probed with ³²P-labelled ID cDNA clone p2A120 (13). Nick-translated ϕ X174 DNA was included in the hybridization reaction with the labelled ID probe. After removal of bound ID probe by boiling in 5 mM phosphate buffer, the nucleosomal DNA blot was reprobed with ³²P-labelled plasmid Ch16ACHOG49, a subclone of the insert in clone 49 containing the rodent repetitive elements B1 and B2 (19).

RESULTS

Rat cerebral hemisphere neuronal, liver, and kidney nuclei were isolated, and their chromatin was digested with micrococcal nuclease for varying times. Nucleosomal DNA fragments were then purified, separated by gel electrophoresis, and examined by ethidium bromide staining (Fig 1A) to determine the nucleosomal repeat length of bulk chromatin (Fig. 2 and Table I). This experiment revealed patterns of nucleosomal DNA fragments similar to those which we and others have previously reported (2,4-7) except that the present gel system sharpened the resolution of the nucleosomal DNA fragments. In agreement with earlier studies, DNA isolated from nuclease-digested cerebral hemisphere neuronal nuclei exhibited an atypically short nucleosomal repeat length of 160-170 base pairs while DNA from nuclease-treated liver and kidney nuclei showed typical repeat lengths of 190-200 base pairs (Table I).

To permit the determination of nucleosomal repeat lengths of chromatin regions containing ID sequences, the nucleosomal DNA fragments from the gel shown in Fig. 1A were transferred to BioDyne membranes and the resulting blot

Figure 1. Nucleosome structure of ID sequences (Panels A-C). Cerebral hemisphere neuronal (N), liver (L) and kidney (K) nuclei were digested with micrococcal nuclease as described in Methods and resultant nucleosomal DNA fragments separated by electrophoresis on 1.5% agarose gels. Numerals in the heading indicate minutes of digestion of isolated nuclei with micrococcal nuclease. The sizes of DNA standards are indicated in base pairs (Hae III digested ϕ X174 DNA). Panel A: Ethidium bromide staining pattern of nucleosomal DNA fragments. Panel B: Analysis of chromatin regions containing ID sequences. The nucleosomal DNA fragments from the gel shown in (A) were transferred to BioDyne membranes followed by probing with 32 P-labelled ID cDNA clone p2A120 (13). Panel C: Reprobing of the nucleosomal DNA blot shown in Panel B with radiolabelled plasmid Ch16ACHOG49, a subclone of the insert in clone 49 containing the rodent repetitive elements B1 and B2 (19) after removal of bound ID probe.

was probed by hybridization with an ID-containing clone labelled by nick-translation (Fig. 1B). As a non-ID control, a plasmid containing the rodent repetitive elements B1 and B2 (19) was used to reprobe the same nucleosomal DNA blot after the bound ID probe had been removed by boiling (Fig. 1C). Nucleosomal DNA ladders are observed in each lane for both probes (Fig. 1B and C), indicating that the bulk of both ID sequences and B1/B2 are located in chromatin regions which are organized in nucleosomal structures in both neuronal and non-neuronal nuclei. The monomer nucleosomal DNA is

Figure 2. Determination of nucleosomal repeat length following digestion of isolated nuclei with micrococcal nuclease for 1 min [●], 2 min [▲] and 5 min [○].

underrepresented relative to oligomeric species in both autoradiograms (Fig. 1B and C) compared to the ethidium bromide staining pattern (Fig. 1A). This is due to the fact that transfer of smaller DNA fragments to BioDyne membrane is less efficient than that of larger fragments. A greater monomer signal was observed when DBM paper was used, however resolution of nucleosomal DNA bands was much sharper on BioDyne, facilitating calculation of nucleosomal DNA repeat lengths.

At each of the three digestion time points for neuronal, liver and kidney nuclei, the B_1/B_2 nucleosomal repeat length resembled that determined for ethidium bromide-stained bulk chromatin repeat lengths (Table 1). A similar result was found for the ID repeat pattern with the exception that, at the 5

Table I Nucleosomal Repeat Lengths^a

	Neuronal			Kidney			Liver		
	1	2	5 min	1	2	5 min	1	2	5 min
Bulk Chromatin	175	170	160	198	198	196	197	191	195
ID Chromatin	166	160	160	190	191	164	195	193	159
B1/B2 Chromatin	173	169	166	184	184	181	183	184	188

^aThe mobilities of the DNA fragments in multimer bands shown in Fig. 1 were determined and sizes were estimated by reference to the mobilities of marker fragments of known lengths. Nucleosomal repeat lengths were calculated as described in Methods for bulk chromatin (Fig. 1A), ID-containing chromatin regions (Fig. 1B) and B1/B2 containing chromatin regions (Fig. 1C). Data representative of four separate experiments are shown. Variation in the repeat length calculations for the four experiments was ± 3 base pairs.

min digestion, the kidney and liver chromatin preparations show shorter repeat lengths of 164 and 159 base pairs, respectively. This is readily apparent in densitometric scans of the ID chromatin autoradiogram (Fig. 3). At the 5 min digest, peaks representing kidney and liver nucleosomal DNA bands are in phase with neuronal peaks (Panels c and d) indicating that a similar short repeat

Figure 3. Densitometric scans of the ID chromatin autoradiogram of Fig. 1B. The 2 min and 5 min lanes for neuronal (N, solid line), kidney (K, broken line) and liver (L, broken line) were scanned on a Bio-Rad model 1650 densitometer.

length is present. At the 2 min digest the neuronal peaks are clearly out of phase with kidney and liver (Panels a and b) indicating differences in repeat lengths. The phenomenon of short repeat length in a subset of nucleosomes is not due to any general effect such as nibbling of DNA, but rather is specific for ID elements, since the 5 min repeat lengths (measured from the same gel) are 196 and 195 for bulk chromatin and 181 and 188 for B1/B2 chromatin. Since all three sets of measurements were made on the same gel and since the experiment was repeated four times we are confident the differences are real. The results are not due to the presence of ID elements in more trimmable nucleosomes since repeat lengths were calculated from higher oligomers whose length is five times the average repeat length.

DISCUSSION

The present results demonstrate that ID elements in the rat genome are located in chromatin regions that are organized in nucleosomal structures in both neuronal and non-neuronal cells. A subpopulation of ID sequences in chromatin of liver and kidney cells are relatively resistant to micrococcal nuclease digestion and are organized in nucleosomes exhibiting an atypically short repeat length. This resistance to micrococcal nuclease compared to bulk chromatin probably reflects the fact that a shorter internucleosomal linker provides a smaller target for the nuclease rather than inaccessibility to nuclease (20). The observation that the effect extends at least to 5-mers indicates that the short repeat units are clustered around ID elements.

Bulk chromatin in the 160-base pair repeat form is much more accessible to DNase I in vitro than is chromatin in the 200-base pair repeat form (3). If DNase I is a representative soluble molecule that interacts with DNA, then chromatin in this short repeat form is generally more accessible to soluble factors. The finding that a subset of ID elements is packaged in a short repeat form in the chromatin of liver and kidney raises the question as to whether this subset of ID elements might also be DNase I-sensitive, in which case these elements might be available for interactions with factors in vivo and thus potentially could serve as regulatory sequences.

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