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Transcriptional characteristics of in vitro assembled chromatin assayed by microinjection into *Xenopus laevis* oocytes

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Plasmid DNA was in vitro assembled into chromatin using an S-150 extract of *Xenopus laevis* oocytes. By varying the assembly temperature and DNA concentration it is possible to generate fully or partially assembled molecules. The fate of the in vitro preassembled molecules injected into *X. laevis* oocyte nuclei and their transcriptional activity were studied. Completely reconstituted molecules underwent a rearrangement of their chromatin structure after injection and showed reduced transcriptional activity compared to protein-free DNA or partially reconstituted chromatin.

Actin, α-; Oocyte injection; Chromatin reorganization

1. INTRODUCTION

Experiments on microinjection into *Xenopus* oocytes and eggs have consistently contributed towards the analysis of transcription control and chromatin formation on protein-free DNA templates [1–3]. It has been demonstrated that protein-free plasmid DNA injected into oocyte nuclei becomes organized in a nucleosomal chromatin structure, but only a very small fraction of these 'minichromosomes' is transcriptionally active whereas the majority of the molecules persists as inactive chromatin [4–6]. Recently, attention has been focussed on the interdependence of chromatin assembly with formation of stable transcription complexes using this type of in vivo gene expression assay [7,8]. Here, we used an S-150 extract prepared from *X. laevis* oocytes [9] for controlled in vitro chromatin assembly. The fate of in vitro assembled chromatin as well as the transcriptional parameters of chromatin-assembled template DNA was assayed after injection into oocyte nuclei.

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2. MATERIALS AND METHODS

The clone α3BA contains a 2.6 kb *Bam*HI/*Hin*dIII fragment of the *X. laevis* skeletal muscle α-actin gene inserted into the multiple cloning site of pUC8 [10,11]. Injection into oocytes, extraction of nucleic acids and nuclease S_1 protection assay were performed as in [11]. Injected DNA was extracted from oocytes and analyzed by Southern blot hybridization against labelled pUC8 [12]. Chromatin assembly extracts were prepared as described [9]. The assembly mixtures (6 μl final volume) contained 3 μl oocyte extract, 3 mM ATP, 3 mM $MgCl_2$, 20–100 ng DNA in small reaction tubes and were incubated at 23 or 37°C for 2–3 h.

3. RESULTS AND DISCUSSION

In a first series of experiments we studied some of the essential parameters for chromatin assembly in order to generate different types of minichromosomes. Chromatin configuration was assayed by determination of the degree of superhelicity as introduced on protein-free, relaxed circular DNA [4]. In vitro chromatin assembly was carried out at 18–23°C, the temperature optimum for *Xenopus* [13] and at 37°C, the incubation temperature for mammalian in vitro chromatin assembly assays. In contrast to our expectation, chromatin assembly using *Xenopus* oocyte extracts is more efficient at

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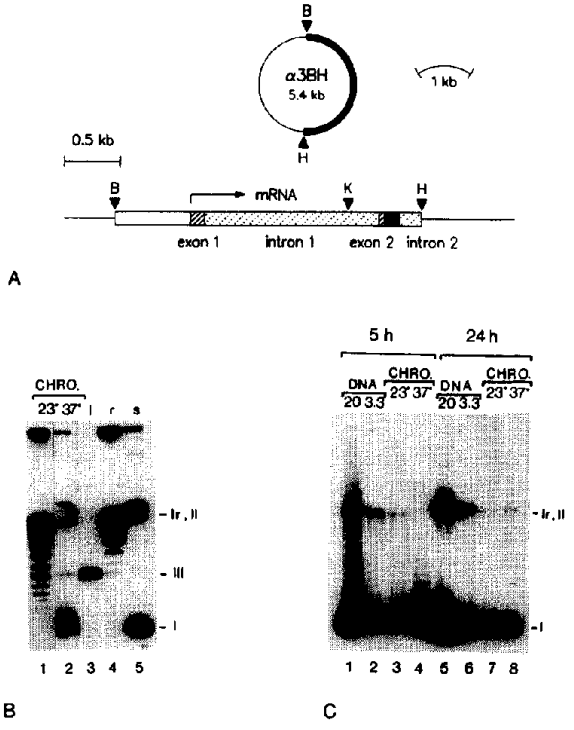


Fig.1. (A) The *X. laevis* skeletal muscle actin clone α3BH. (B) In vitro chromatin assembly of α3BH. α3BH (3.3 ng/μl) was assembled at 23°C (1) and 37°C (2) and analyzed by Southern hybridization. Control lanes: l (3), α3BH/*Bam*HI (linear DNA); r (4), α3BH incubated with DNA-topoisomerase I (relaxed DNA); s (5), untreated α3BH (supercoiled and relaxed DNA). I, supercoiled DNA; I_r, relaxed covalently closed circular DNA; II, nicked circular DNA; III, linear. (C) Fate of preassembled α3BH after injection into nuclei of *X. laevis* oocytes. 20 nl protein-free α3BH [20 ng/μl (1,5) or 3.3 ng/μl (2,6)] or in vitro assembled α3BH [(3.3 ng/μl 3,4,7,8] were injected into oocyte nuclei. The assembly temperature was either 23°C (3,7) or 37°C (4,8). After incubation for either 5 (1–4) or 24 h (5–8) the injected DNA was extracted and analyzed by hybridization.

elevated temperatures, e.g. at 25–30 or 37°C. Based on these observations it was possible by modifying the incubation conditions to generate chromatin containing defined, lower numbers of superhelical turns without changing the DNA concentration. These chromatin samples are referred to as 'partially assembled chromatin' (fig.1B). The question addressed in this study is the elucidation of whether, to some degree, the regulation of transcription of injected templates is modulated by the assembled chromatin structure. We therefore used the cloned *X. laevis* skeletal muscle α-actin gene which is transcribed when injected as protein-free DNA into oocyte nuclei, whereas the endogenous α-actin gene is silent in oocytes [11]. In vitro assembled actin gene chromatin does not show any detectable transcriptional activity in the assembly reaction mixture (not shown). This is in agreement with the observation that the assembly process in vitro prevents efficient initiation of transcription [14]. As reported in [7] injected SV40 chromatin is converted by oocyte chromatin factors into an activated form. In our study, we thus investigated the problem as to whether oocyte chromatin factors could also modulate in vitro assembled chromatin. We injected partially or fully assembled chromatin and analyzed the superhelicity 5 and 24 h after injection (fig.1). Partially assembled chromatin was found to be converted into chromatin containing a consistently higher number of superhelical turns following 5 h incubation in oocyte nuclei. After 24 h incubation, injected partially assembled chromatin was found to be converted into chromatin with full superhelicity. In contrast, fully assembled chromatin was found to be relaxed to some extent and later regeneration of superhelical turns was observed which finally resulted in chromatin with superhelicity almost identical to that of the in vitro assembled chromatin used for injection. This result indicates that a dynamic assembly/disassembly equilibrium exists in oocyte nuclei.

To examine the influence of in vitro chromatin assembly on the transcriptional activity, we injected an α-actin subclone (α3BH) into oocyte nuclei as protein-free DNA or in vitro assembled chromatin and analyzed the transcripts by the nuclease S_1 assay. Transcripts containing intron 1 should protect a 400-nucleotide band (pt). A 91-nucleotide fragment is produced when intron 1 is excised correctly (sp) (fig.2B,C). As the α3BH subclone does not contain the termination signal of the gene, a strong 320-nucleotide band appears which is caused by hybridization of read-through transcripts against the antistrand of the probe [11]. The experiment demonstrates that molecules assembled at 23°C are more efficiently transcribed than naked DNA or molecules assembled at 37°C, if small amounts of DNA are injected. Using higher DNA concentrations partially assembled chromatin and protein-free DNA are transcribed with similar efficiency, whereas the completely

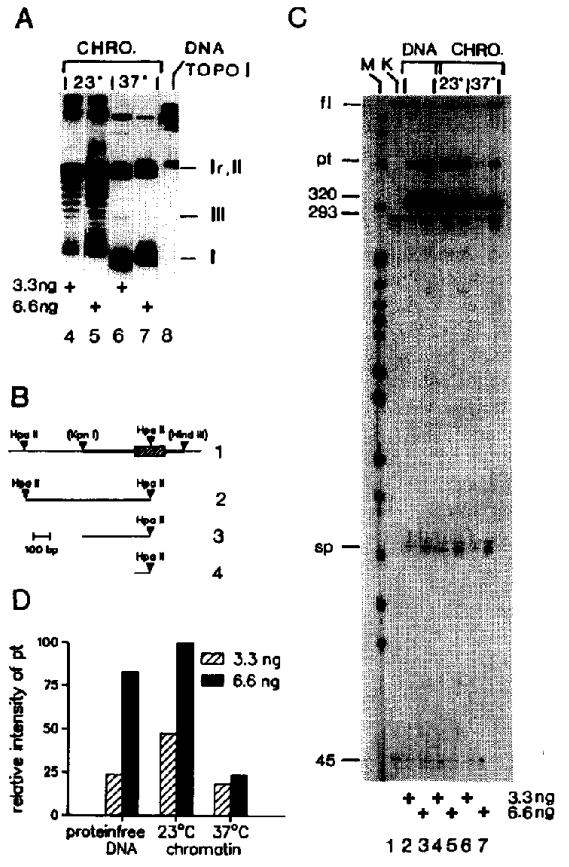


Fig.2. Transcriptional activity of fully or partially reconstituted α3BH in *X. laevis* oocytes. (A) Relaxed α3BH [3.3 (4,6) or 6.6 ng/μl (5,7)] was incubated with S-150 extract for 3 h at 23°C (4,5) and 37°C (6,7) and analyzed by Southern hybridization. Lane 8 shows the α3BH relaxed by DNA topoisomerase I. I, I_r, II and III: see fig.1. (B) Hybridization probe used for nuclease S_1 analysis: The 600 bp *Hin*dIII/*Kpn*I fragment of α3 was subcloned into pUC8 (line 1). Hatched box, 5'-untranslated leader; black box, coding sequence of exon 2; thick line, intron sequences; thin line, pUC8 sequences. The 750 bp *Hpa*II fragment (fl) was used as hybridization probe (line 2). A 400-nucleotide-long fragment (pt) is protected by unspliced transcripts (line 3) and a 91-nucleotide-long fragment (sp) by processed mRNA (line 4). (C) Nuclease S_1 analysis of α3BH transcripts. α3BH [3.3 ng/μl (2,4,6) or 6.6 ng/μl (3,5,7)] was injected into the nuclei of *X. laevis* oocytes as protein-free DNA (2,3) or in vitro at 23°C (4,5) or 37°C (6,7) assembled DNA. The oocytes were incubated for 24 h at 19°C. The RNA was hybridized against the 5'-labelled 750 bp *Hpa*II fragment (B, line 2), digested with nuclease S_1 and the protected fragments were analyzed on a 7% high-resolution polyacrylamide gel. As an internal standard we used a 293 bp *Ava*II fragment which hybridizes to endogenous rRNA and protects a 45-nucleotide fragment [17]. K, nuclease S_1 mapping of RNA from uninjected oocytes [8]; M, pBR322/*Hpa*II fragments; fl, full-length hybridization probe; pt, primary transcript (400 nucleotides); sp, spliced mRNA (91 nucleotides). (D) Relative intensity of the primary transcript. The intensity of signals corresponding to the primary transcript (pt) was measured by photometric scanning and corrected by comparison with the intensity of the 45-nucleotide band (internal standard).

reconstituted molecules are transcribed with less efficiency (fig.2D). Our findings are in accordance with the observations of Knezetic and co-workers [15,16] who used an in vitro transcription system from HeLa cells after chromatin assembly of the DNA with an S-150 *Xenopus* oocyte extract. To ensure that the amount of RNA in the different lanes was comparable, we used a 293 bp fragment which hybridizes with the endogenous rRNA and generates a 45-nucleotide fragment [17] as an internal standard. From the present experiments, the following conclusions can be drawn. The DNA/protein complexes formed during incubation of plasmid DNA with an S-150 extract of *X. laevis* oocytes are modified following injection into oocyte nuclei (fig.1). The molecules which acquire the maximal number of superhelical turns (full assembly) during incubation of extract show a reduced transcriptional activity in oocytes compared to protein-free or partially assembled DNA. This could be due to the rearrangement of the DNA/protein complexes within the nucleus where unspecifically bound proteins must be replaced. During this period the template might not be accessible to the transcription machinery. With partially reconstituted chromatin this modifying process is not required to such an extent and therefore, the injected molecules are transcribed after a shorter 'lag phase'. Another effect of the extract incubation is to minimize the disturbing effects caused by injection of protein-free DNA into oocytes.

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