# A calcium-activated nuclease endogenous to *Xenopus* erythrocytes

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An endogenous nuclease is found in purified *Xenopus* erythrocyte nuclei. This enzyme is activated specifically by $Ca^{2+}$ in the presence of which chromatin is digested into discrete fragments. Both 10 bp and 200 bp ladders are detected upon electrophoresis of DNA. This endogenous enzyme therefore resembles mammalian and chicken liver endonucleases.

*Xenopus erythrocyte* *Endogenous nuclease* *$Ca^{2+}$-activated*

## 1. INTRODUCTION

In recent years, a wide variety of nucleases have been found to be useful probes for analysing the structure of chromatin. In particular, enzymes such as DNase 1 (EC 3.1.4.5) and micrococcal nuclease have been found to recognise different aspects of chromatin structure [1]. Eukaryotic cells have also been found to contain endogenous nucleases although the physiological role of these enzymes is poorly understood [2].

We have been investigating the structure of globin, tRNA and vitellogenin genes in *Xenopus* erythrocyte nuclei, to gain a better understanding of their transcriptional regulation.

Here, we report on the presence of a $Ca^{2+}$-activated endonuclease which is endogenous to *Xenopus* erythrocytes. In the presence of $Ca^{2+}$, this endonuclease cleaves chromosomal DNA into discrete fragments. The significance of this finding is discussed.

## 2. MATERIALS AND METHODS

*Xenopus* blood was collected from adult females by cardiac puncture, and the cells were suspended in cold sterile APBS (amphibian phosphate-buffered saline: 130 mM NaCl, 5 mM $MgCl_2$, 10 mM $NaPO_4$ (pH 7.4)). Erythrocytes were purified free from white blood cells by sedimentation through either a cushion of 20% Ficoll or a pre-formed self-generated gradient of Percoll (Pharmacia) with a starting density of 1.08 g/ml. Nuclei were prepared by lysing cells in buffer A (50 mM Tris–HCl (pH 7.9), 70 mM KCl, 5 mM $MgCl_2$, 10% glycerol, 1 mM DTT) containing 0.05% Triton X-100 and washing several times in the same buffer without Triton.

Endogenous nuclease activity was assayed by incubation of nuclei in buffer A containing 3 mM $Ca^{2+}$ and 25°C for a designated length of time after which the reaction was stopped with an equal volume of TEN (50 mM Tris–HCl (pH 8.0), 10 mM EDTA, 200 mM NaCl) containing 1% SDS. DNA was purified by two extractions with phenol–chloroform (25:24, v/v) and two extractions with chloroform–isoamyl alcohol (24:1, v/v) followed by ethanol precipitation. Products of digeston were electrophoresed on denaturing (7 M urea) polyacrylamide gels in a TBE buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA (pH 8.3). Gels were stained with ethidium bromide and photographed with a Polaroid CU-5 land camera.

*Abbreviations:* DTT, dithiothreiotol; SDS, sodium dodecyl sulphate; bp, base pairs; kb, kilobase; MES, 2-(N-morpholino) ethanesulphonic acid

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## 3. RESULTS

The results presented here arise from the observation that when *Xenopus* erythrocytes are autolysed in the presence of $Ca^{2+}$ the chromatin is degraded into fragments of DNA which migrate on denaturing acrylamide gels as discrete bands (fig. 1). In the presence of $Mg^{2+}$ alone, no such discrete bands are observed.

We assume that this nuclease activity is endogenous to these highly purified cells since no contaminating lymphocytes or thrombocytes can be detected after staining blood smears in May-Grunwald/Giemsa. A time course analysis of the reaction shows that the DNA is degraded progressively into nucleosomal ($200 \pm 10$ bp) and $10 \pm 0.2$ bp fragments (fig. 1). As degradation progresses, bands at 160 and 140 bp become prominent, presumably arising from core particles.

Next, the optimal concentration of $Ca^{2+}$ required to activate this nuclease was determined. A profile of the digestion products obtained when nuclei are incubated in increasing [$Ca^{2+}$] is shown in fig. 2. In the presence of $Mg^{2+}$ alone, no degradation products below 1 kb can be detected (fig. 2, lane 11). In the presence of $Ca^{2+}$ above 2 mM, however both nucleosomal (200 bp) and 10 bp fragments are produced (lanes 4–10).

The picture further shows that when nuclei from leukocytes are autodigested, only in the presence of $Ca^{2+}$ is a similar pattern of cleavage products obtained (fig. 2, lane 2). A greater extent of degradation takes place in a shorter incubation time, but this is to be expected since white blood cells are known to be rich in nucleases. The white blood cells used in this study were found to consist mainly of thrombocytes and lymphocytes. We conclude from this result that the nuclease has a strong requirement for $Ca^{2+}$ and that an endonuclease with similar if not identical properties is present in

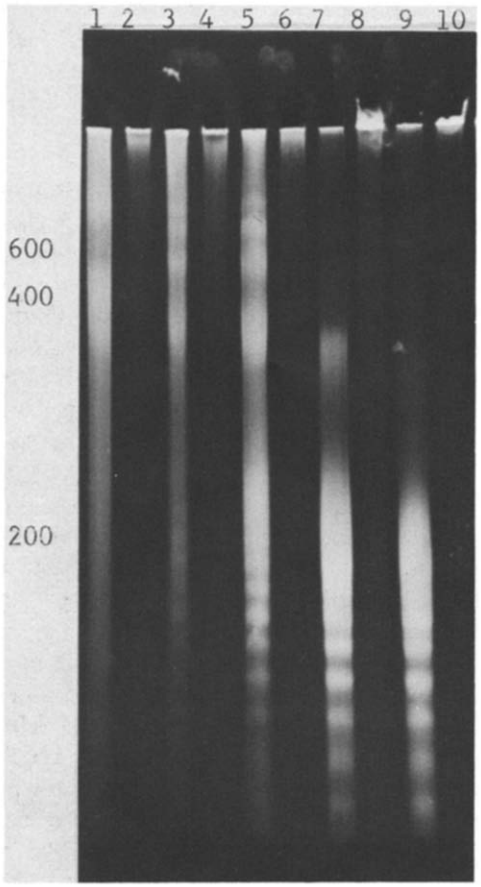


Fig. 1. Digestion of nuclear DNA by endogenous nuclease. *Xenopus* erythrocyte nuclei were incubated at 25°C in buffer A with 3 mM $Ca^{2+}$ (lanes, 1,3,5, 7), or without $Ca^{2+}$ (lanes 2,4,6,8, 10) for the following times: 0 min. (lanes 1, 2); 5 min (lanes 3, 4); 15 min (lanes 5, 6); 30 min (lanes 7, 8); 60 min (lanes 9, 10). Reactions were terminated and products analysed as in section 2.

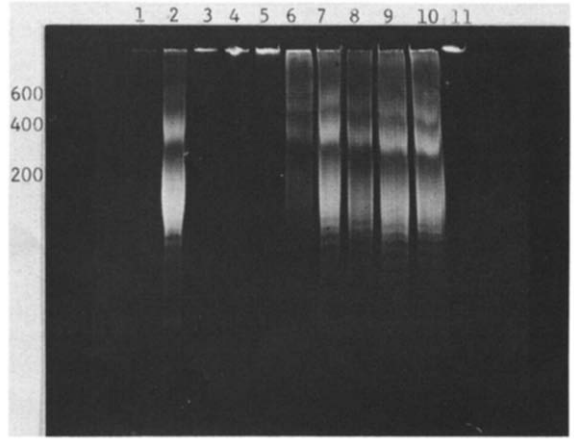


Fig. 2. Determination of optimal requirement for $Ca^{2+}$. Erythrocyte nuclei were incubated at room temperature for 40 min in buffer A containing: (lanes 4–11) 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 10.0 and 0 mM $Ca^{2+}$; lymphocyte nuclei were treated in a similar manner with 3mM $Ca^{2+}$ (lane 2) or without $Ca^{2+}$ (lane 3); lane (1) contained $M_r$ markers.

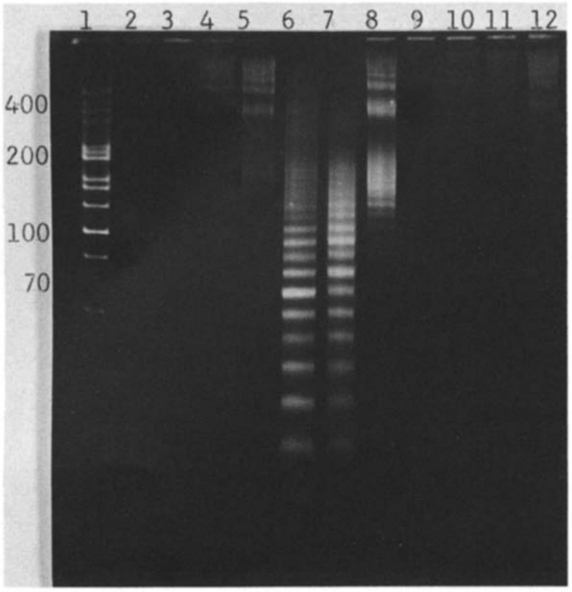


Fig. 3. Comparison of nuclease cleavage patterns. Nuclei were prepared from cultured kidney cells (lanes 2–5), erythrocytes (lanes 6–8) or hepatocytes (lanes 9–12) and autodigested in buffer A containing 3 mM $Ca^{2+}$ for 0 min (2,9), 15 min (3,10), 30 min (4,11) or 45 min (5,12). Erythrocyte nuclei were digested with 100 units DNase II [6] 10 units DNase I [7] or endogenous nuclease [8] for 40 min. All incubations were at 25°C. DNase II digestion was carried out in 20 mM MES pH 5.6, 10 mM NaCl, 1 mM $MgCl_2$. Products of digestion were analysed on 7 M urea, 6% polyacrylamide gels. Lane 1 contains an *Hinf*1 digest of pBR322.

leukocytes. The nucleolytic activity is also detectable in a kidney epithelial cell line from *Xenopus* (P. Harris, Weston Super Mare, England) and in hepatocytes (fig. 3). This figure also shows a comparison of endogenous nuclease, DNase 1 and DNase II cutting patterns. Whilst DNase II can attack DNA at half nucleosome (100 bp) intervals, endogenous nuclease gives rise to sharp bands which are multimers of 200 bp. All 3 nucleases are found to make 10 bp cuts.

## 4. DISCUSSION

Our results clearly show the presence of a calcium-dependent nuclease in *Xenopus* erythrocytes. Attempts to ascertain the location of this enzyme have revealed that it is bound very tightly to chromatin. For instance, extensive washing of nuclei in buffer A reduces but does not remove endonuclease activity. No endonuclease activity can be detected in nuclei which have been washed in buffer A containing 0.3 M NaCl. Our observations suggest that the endonuclease is endogenous to erythrocytes and binds to chromatin at low ionic strength. Similar findings were reported for chicken erythrocyte nuclei [3]. Whereas micrococcal nuclease produces a 200 bp ladder and DNase 1 makes 10 bp cuts, both types of cutting pattern are seen with endogenous enzyme (see also [31]).

This nuclease therefore appears to resemble the well known endogenous mammalian nuclease [4] and the endonuclease activity of chicken erythrocytes [3] in that the products of digestion go through the stage of discrete bands on gel electrophoresis. Many workers who have used *Xenopus* erythrocyte nuclei as a substrate for chromatin digestion have disregarded this activity. Particularly in the case of micrococcal nuclease which requires $Ca^{2+}$, the activity of the endogenous enzyme may have been a source of serious error, especially where the specificities of the introduced enzyme were being investigated. Nuclear deoxyribonucleases have been implicated in the replication of DNA [5], in the regulation of actin filament formation [6] and developmental gene rearrangement and diminution [7]. The role most likely to be played by the *Xenopus* $Ca^{2+}$-DNase is in programmed cell destruction. Activation of chromatin autodigestion late in the life span of erythrocytes may therefore be viewed as in important feature of this process.

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