Journal of Cell Science 105, 389-395 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

Xenopus nuclear factor 7 (xnf7) possesses an NLS that functions efficiently in both oocytes and embryos

Xiaoxia Li and Laurence D. Etkin*

Department of Molecular Genetics, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas 77030, USA

*Author for correspondence

SUMMARY

***Xenopus* nuclear factor 7 (xnf7) is a nuclear phosphoprotein that is encoded by a member of a novel zinc finger gene family and likely functions as a transcription factor. It possesses a nuclear localization signal (NLS) similar to the bipartite basic NLS of nucleoplasmin, but unlike nucleoplasmin, which re-enters nuclei immediately after fertilization, xnf7 remains cytoplasmic until the mid-blastula transition (MBT). We have measured the accumulation of injected labeled xnf7 protein or protein produced from synthetic xnf7 transcripts in the oocyte nuclei (GV). The data show that the NLS of xnf7 functions efficiently in oocytes. Mutations in either of the bipartite basic domains of the xnf7 NLS inhibit nuclear accumulation, while mutations in the spacer sequences have no effect. The xnf7 NLS linked to pyruvate kinase directs the efficient accumulation of this protein into nuclei of early embryos prior to the MBT. These data suggest that retention of the xnf7 protein during development is the result of a mechanism that interferes with the xnf7 NLS function.**

Key words: nuclear translocation signals, *Xenopus laevis*, transcription, mid-blastula transition, development, embryogenesis

INTRODUCTION

It is clear that selective nuclear transport is an important strategy used by cells to regulate the function of nuclear proteins during development and cellular differentiation (Nigg et al., 1991; Schmitz et al., 1991; Silver, 1991). This was demonstrated for the distribution of several maternal proteins in *Xenopus laevis* (Dreyer et al., 1983; Dreyer, 1987; Miller et al., 1991), the *dorsal* gene product in *Drosophila* (Hunt, 1990; Roth et al., 1989; Rushlow et al., 1989; Steward, 1989; Schmitz et al., 1991), the *SWI5* gene product in yeast (Moll et al., 1991), and the transcription factor NF-kB in lymphocytes (Lenardo and Baltimore, 1989). Also, there is an indication that the function of p53, the product of a tumor suppressor gene, may be regulated by retention in the cytoplasm (Moll et al., 1992; Gannon and Lane, 1991).

Recently, we have been studying the gene *xnf*7 (*Xeno - pus* nuclear factor 7), whose protein product originates in the oocyte GV, becomes cytoplasmic at maturation, and is retained in the cytoplasm until it re-enters the embryonic nuclei at the mid-blastula (MBT) stage (Dreyer et al., 1983; Miller et al., 1989; Miller et al., 1991; Reddy et al., 1991). When xnf7 is in the cytoplasm it is hyperphosphorylated; however, it is dephosphorylated coincident with nuclear re-entry. Thus, its potential nuclear function is suppressed by its retention in the cytoplasm prior to the mid-blastula stage and its nuclear/cytoplasmic distribution may be regulated by its state of phosphorylation.

xnf7 is a member of a novel zinc finger gene family called the B box family, whose products consist mainly of oncoproteins and transcription factors (Reddy and Etkin, 1991; Reddy et al., 1992). The B-box gene family is a subgroup of a larger group of zinc finger genes called the RING finger family (Freemont et al., 1991; Haupt et al., 1991; Lovering et al., 1993). All of the B box-containing family members, in addition to the highly conserved zinc-finger domains, also possess a coiled-coil domain immediately C-terminal to the B box zinc finger domains (Reddy et al., 1992). The coiled coil domain may be involved in protein-protein interactions. Recent evidence indicates that the RING-finger (A box) domain can bind to a double-stranded oligonucleotide (Lovering et al., 1993).

Three of the six B box-containing genes, *rfp* (Takahashi et al., 1988), *PML* (de The et al., 1991; Goddard et al., 1991; Kastner et al., 1992; Kakizuka et al., 1991) and *T18* (Miki et al.,1991) have transformation potential when found as translocations in humans and mice. In all of these fusions the zinc-finger and coiled-coil domains are retained. This suggests that the B box and coiled-coil motifs in this family may play an important role in the transformation potential of these altered proteins (Reddy et al., 1992).

In addition, the conceptual xnf7 protein has an acidic region that can transactivate a reporter gene construct in a

transfection assay, three potential protein kinase phosphorylation sites, and a nuclear localization signal (NLS) (Reddy et al., 1991; Reddy and Etkin, unpublished observations). These facts suggest that the xnf7 protein may function as a transcription factor during development.

The xnf7 nuclear localization signal (NLS) is located between amino acids 106 and 120 and is very similar to the bipartite basic NLS of nucleoplasmin (Reddy et al., 1991). However, nucleoplasmin, unlike xnf7 re-enters the nuclei immediately following fertilization (Dreyer et al., 1983). Therefore, the retention of the xnf7 in the cytoplasm between oocyte maturation and the mid-blastula stage when it enters the nuclei presents somewhat of a paradox.

In the present study we analyze in detail the function of the xnf7 NLS in oocytes and developing embryos. Our data show that the bipartite basic NLS in xnf7 functions efficiently in directing protein into nuclei in both oocytes and early embryos. This suggests that retention of xnf7 in the cytoplasm prior to the MBT is the result of a process that interferes with NLS function.

MATERIALS AND METHODS

Expression vectors

A vector was constructed for synthesis of xnf7 mRNA. The vector consisted of pBS, into which a synthetic oligonucleotide containing the 5′ untranslated sequences of the *Xenopus* β-globin gene and a 12 amino acid long T7 viral coat protein tag was inserted into the *Xba*I and *Kpn*I sites. The T7 viral coat peptide permits us to distinguish the exogenous xnf7 from the endogenous protein with polyclonal antibodies. The xnf7 cDNA was cloned into the *Eco*RI site of the vector, fusing it in frame with the upstream T7 sequence. xnf7Δ145-273 and xnf7Δ280-548 were constructed by internal deletions of the xnf7 cDNA using convenient restriction sites. The wild-type NLS oligonucleotides and the mutant oligonucleotides (mutant 1, mutant 2 or mutant 3) were inserted into the vector at the *Kpn*I site, between the T7 sequence and the xnf7 cDNA fragment.

Oocyte injections and metabolic labeling of injected oocytes

Female frogs were anesthetized in 0.1% 3-aminobenzoic acid ethyl ester (Sigma), which was neutralized with sodium bicarbonate. A section of the ovaries was surgically removed, and stage VI oocytes were manually defolliculated and maintained in modified Barth's saline (MBS). Oocytes were injected with 10 ng of RNA (Etkin et al., 1984; Etkin and Maxson, 1980). Injected oocytes were incubated at 18˚C in MBS for different periods of time in the presence of [^{35}S]methionine at a final concentration of 1 mCi/ml. Usually 25-50 oocytes were injected for each sample.

Analysis of protein compartmentalization

Injected and labeled oocytes were manually dissected into GVs and cytoplasms. Protein extracts were prepared by homogenization of GVs and cytoplasm in a buffer consisting of 10 mM Tris-HCl (pH 8.0), 10 mM DDT and 5 mM EDTA followed by centrifugation. Supernatants taken from 25 GVs and cytoplasm were adjusted to a final concentration of 150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS and 10 mM Tris-HCl (pH 7.2). Antisera (1:50 of the L24 polyclonal antibody against xnf7 and 1:500 of the polyclonal antibody against the T7 coat protein) was used to immunoprecipitate the recombinant xnf7 protein. After a 1 h incubation with the antisera, 50 μl of a 50% *Staphylococcus* A slurry (Sigma) was added to absorb the immune complexes. The precipitates were washed with washing buffers A,B and C (buffer A: 1 M NaCl, 0.01 M Tris-HCl, pH 7.2, 0.1% NP40; buffer B: 0.1 M NaCl, 1 mM EDTA, 0.01 M Tris-HCl, pH7.2, 0.1% NP40, 0.3% SDS; buffer C: 0.01 M Tris-HCl, pH7.2, 0.01% NP40). The pellet was solubilized in 50 μl of 2× SDS sample buffer, separated by SDS-PAGE. Protein was detected by fluorography.

Also, we assayed for nuclear translocation by injection of radiolabeled protein into oocytes. Radiolabeled xnf7 protein was produced by injection of synthetic xnf7 mRNA into oocytes, followed by labeling for 24 h with [^{35}S]methionine. The labeled xnf7 protein was prepared by making extracts of the injected oocytes. Total labeled protein was injected into recipient oocytes and the time of entry into the GV was determined by analysis of dissected GVs and cytoplasm using immunoprecipitation with a polyclonal antibody against xnf7 (L24; Reddy et al., 1991) followed by gel electrophoresis and autoradiography.

Immunoperoxidase staining of sections

Embryos were fixed in 100% methanol at 4˚C overnight, then embedded in Paraplast Plus (Monoject) and serially sectioned into 10 μm sections. Immunoperoxidase staining was carried out according to Cornish et al. (1992). Sections were bleached in 6% hydrogen peroxide in methanol for 20-30 min. Following two 10-min Phosphate Buffered Saline (PBS) washes, non-specific staining was blocked with a 1 h incubation in a blocking buffer consisting of 10% goat serum and 3% BSA in TBS with 0.5% Tween-20 (TBST). Sections were incubated for 1 h in a 1:50 to 1:200 dilution of the L24 pAb in blocking buffer, then washed two times for 10 min each in PBS. For control sections, no primary antibody was used. The sections were treated with the secondary antibody (1:50 dilution of goat anti-rabbit conjugated to peroxidase) for 1 h and washed 2 times for 10 min each in PBS. The color reaction was carried out with 1 mg/ml DAB + 0.03% H_2O_2 in PBS for 5 min and was stopped by two 5-min washes with water. The sections were counter stained with either hematoxylin or Azur B and mounted with Permount (Fisher) or 1:1 dilution of PBS with glycerol.

Microinjection of embryos

Embryos were fertilized in vitro. Eggs were transferred into 1× modified Barth saline and microinjected according to Etkin et al. (1984; Etkin and Pearman, 1987). A 10 to 30 ng sample of RNA was injected into the equatorial region of the 1-cell embryos. One hour after microinjection, embryos were transferred back into 0.1× MBS and allowed to develop.

RESULTS

xnf7 is efficiently translocated from the cytoplasm to the nucleus in full-grown *Xenopus* oocytes

We previously determined that the xnf7 protein is retained in the cytoplasm during early development and does not re-enter the nuclei until the mid-blastula stage of development (Miller et al., 1991). A possible explanation for the slow re-entry is that the xnf7 nuclear translocation signal does not function efficiently. Therefore we investigated the nature of the NLS in xnf7. In these experiments we used a construct xnf7-8 that lacks 30 amino acids (aa 1-30) at the N terminus. We refer to this construct as the wild-type xnf7,

since it behaves in a similar manner to the endogenous protein (Miller et al., 1991).

We injected radiolabelled xnf7-8 protein into the cytoplasm of full-grown oocytes and followed the accumulation of the protein in the GV. The recipient oocytes were manually dissected into GVs and cytoplasm, and assayed for the presence of xnf7 by immunoprecipitation with the L24 antibody and autoradiography. Fig. 1A shows that the xnf7 protein was detected in the GVs of injected oocytes within 20 min following injection of the protein into the cytoplasm. The protein accumulated in a linear fashion during a 2-hour period (Fig. 1A, lanes 3-6). Densitometry measurements indicate that 47% of the injected xnf7 protein accumulated in the GV within the first 20 min interval. The slight change in mobility of xnf7-8 protein in the GV was due to a basal level of phosphorylation that occurs as the protein enters the GV (data not shown, Miller et al., 1991). This phosphorylation event does not result in the hyperphosphorylated isoforms of xnf7 produced during oocyte maturation.

We also determined the time of nuclear translocation by injecting synthetic xnf7 mRNA into oocytes and analyzing the ability of the protein product to accumulate in the GV. The construct to produce the mRNA consisted of 579 amino acids encoded by the xnf7 cDNA with the *Xenopus* globin translation leader sequence, and a 12 amino acid long T7 viral coat protein epitope tag. Injected oocytes were incubated in [^{35}S]methionine to label newly synthesized exogenous xnf7 protein. After different periods of labeling, protein from samples of GVs and cytoplasm were extracted, immunoprecipitated with the T7 antibody and analyzed by autoradiography for the presence of the exogenous xnf7 protein. Fig. 1B shows that the exogenous xnf7 protein was first detected in the GV as early as 3 hours following injection of the construct with linear accumulation in the GV at subsequent timepoints. The 2- to 3-fold increase in accumulation between the 3- and 4-hour timepoints suggests a similar translocation efficiency of the xnf7 protein in this system as was seen with injection of the labeled protein. The rate of accumulation of the exogenous xnf7 protein in the GV also was very similar to that of the endogenous protein (data not shown). These results show that the xnf7 protein possesses an efficient NLS signal capable of permitting detectable accumulation of the xnf7 protein in the GV within as little as 20 minutes.

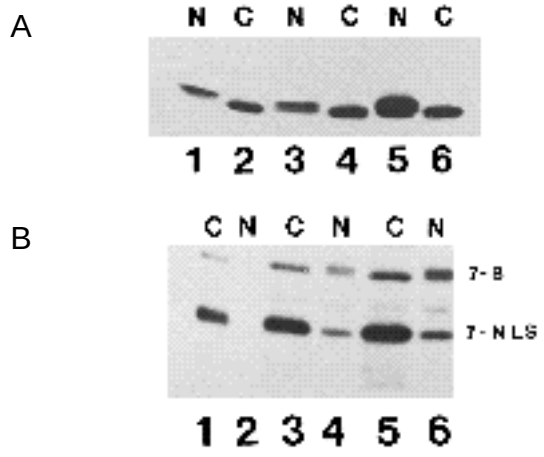

Fig. 1. Time course of translocation of the xnf7 protein from the cytoplasm to the nucleus in full-grown oocytes.
(A) [^{35}S]methionine-labeled xnf7 protein (see Materials and Methods) was injected into the cytoplasm of full-grown oocytes. Samples at different time points postinjection (20 min, lanes 1 and 2; 1 h, lanes 3 and 4; 2 h, lanes 5 and 6) were taken to analyze the GV (N) and cytoplasm (C) for the presence of the injected protein by immunoprecipitation with the L24 antibody. (B) In vitro transcribed mRNAs from constructs xnf7-8 (7-8) and xnf7NLS (7-NLS) were injected into the cytoplasm of full-grown oocytes. The injected oocytes were labeled with [^{35}S]methionine for 1 h (lanes 1 and 2), 3 h (lanes 3 and 4), or 4 h (lanes 5 and 6) and then dissected into GVs (N) and cytoplasms. (C) The labeled GVs and cytoplasms were analyzed for the presence of the exogenous xnf7 protein by immunoprecipitation with the T7 antibody. The difference in accumulation of the xnf7-8 and xnf7NLS was due to difference in the amount of mRNA injected.

Identification of the nuclear localization signal (NLS) in xnf7 protein

The conceptual protein produced by the xnf7 cDNA possesses a nuclear localization signal (NLS) similar to the bipartite signal found in nucleoplasmin. To study further the functional importance of this putative NLS sequence we deleted the DNA encoding 97 amino acids (aa 30-127) at the N terminus of the xnf7-8 cDNA that included the putative NLS (Fig. 2). This construct was called xnf7-1. xnf7-1 mRNA was injected into oocytes and the nuclear/cytoplasmic distribution of the protein analyzed. The translational efficiency and the stability of the protein from the deletion mutant was the same as the xnf7-8 construct (data not shown). In contrast to the proteins possessing the NLS, the protein produced by *xnf7-1* remained cytoplasmic even after 24 hours (Fig. 2). The above results clearly indicated that the deletion of the 97 amino acids (aa 31-127) including the putative NLS resulted in the inability of the protein to translocate into the GV.

To confirm further the function of the NLS, an oligonucleotide was made that contained the putative NLS signal and cloned into the xnf7-1 construct. This construct was designated xnf7NLS (Fig. 2). Figs 1B and 2 show that the protein produced by the xnf7NLS mRNA, like the protein from xnf7-8 mRNA, was detected in the GV within a few hours following injection of the mRNA. The results demonstrate that the NLS in xnf7 is required for nuclear translocation of the protein; however, it is formally possible that in addition to the NLS other regions of the protein may also be involved in this process.

To eliminate the involvement of other regions of xnf7 in nuclear localization we created several deletion mutants, removing amino acids 145-273 (*xnf7Δ145-273*) and 280-548 (*xnf7Δ280-548*) (Fig. 2). Proteins produced by these two deletion mutants, like the wild-type protein, were localized in the GV (Fig. 2). Since the deletions made in *xnf7NLS*, *xnf7Δ145-273* and *xnf7Δ280-548* lacked major portions of the xnf7 protein and did not effect its localization we conclude that the NLS in xnf7 can function autonomously in translocating the protein into the nucleus.

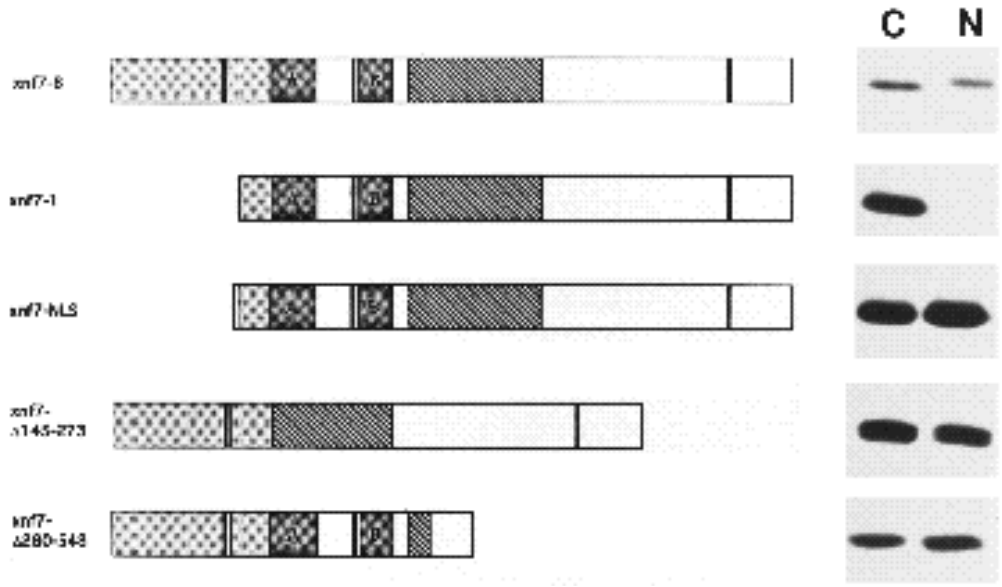

Fig. 2. Identification of the nuclear localization signal (NLS) in the xnf7 protein. In vitro transcribed mRNAs from the wild-type construct xnf7-8 and deletion mutants (*xnf7-1*, *xnf7NLS*, *xnf7Δ145-273* and *xnf7Δ280-548*) were injected into the cytoplasm of full-grown oocytes. The injected oocytes were labeled with [^{35}S]methionine for 24 h and then dissected into GVs (N) and cytoplasms (C). The labeled GVs and cytoplasms were analyzed for the presence of the exogenous xnf7 protein by immunoprecipitation with T7 antibody. The light-shaded box in the diagram is the acidic domain. The small open box in the acidic domain is the nuclear localization signal (NLS). The two dark-shaded boxes are the zinc finger domains indicated as A and B. The hatched box is the coiled coil domain.

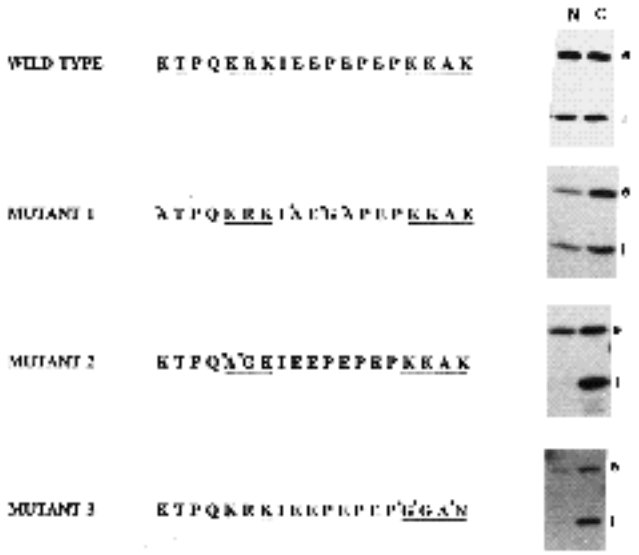

Fig. 3. Localization of the basic bipartite nuclear targeting sequences in the NLS of the xnf7 protein. In vitro transcribed mRNAs from construct xnf7NLS which contains the wild-type NLS sequences (wild type) as well as constructs containing mutated NLS sequences (mutant 1, mutant 2 and mutant 3) were injected into the cytoplasm of the full-grown oocytes. The injected oocytes were incubated for 24 h in the presence of [^{35}S]methionine and then dissected into the GVs (N) and cytoplasms (C). Both endogenous (e) and exogenous xnf7 protein from injected mRNA (i) in the samples were immunoprecipitated with L24 antibody. The two basic domains in the NLS sequences are underlined. The mutated amino acid residues are indicated by *. The double-underlined T represents a potential phosphorylation site.

Mutations in the basic bipartite region of the xnf7 NLS interfere with its function

Point mutagenesis of the NLS of nucleoplasmin has identified two clusters of basic residues that are essential in its function (Robbins et al., 1991). These two basic domains are separated by 10 intervening 'spacer' amino acids that tolerate point mutations and some insertions. Amino acids in both basic domains are necessary for nuclear targeting. By sequence comparison, it was found that the NLS signal of the xnf7 protein was very similar to the bipartite signal found in nucleoplasmin, except that the signal in the xnf7 protein has a shorter spacer consisting of nine amino acids (Fig. 3).

To investigate the function of individual amino acid residues for nuclear targeting of the xnf7 protein, we constructed several site-directed mutants using mutant oligonucleotides for the NLS sequence. These mutant NLS sequences containing oligonucleotides were cloned into the *xnf7-1* construct, which lacks a functional NLS. The mRNAs produced from these constructs were microinjected into the oocyte cytoplasm and their protein products analyzed for their nuclear or cytoplasmic distribution. Mutations in the spacer and the lysine residue in the phosphorylation site (mutant 1) did not effect the accumulation of the xnf7 protein in the GV when compared with the endogenous protein (Fig. 3). Changing the lysine and arginine residues to alanine and glycine in the first basic domain (mutant 2) resulted in the accumulation of the protein in the cytoplasm (Fig. 3). The second basic domain was mutated by replacing the three lysine residues with two glycines and one asparagine (mutant 3, Fig. 3). This protein also did not enter the GV. These results indicated that

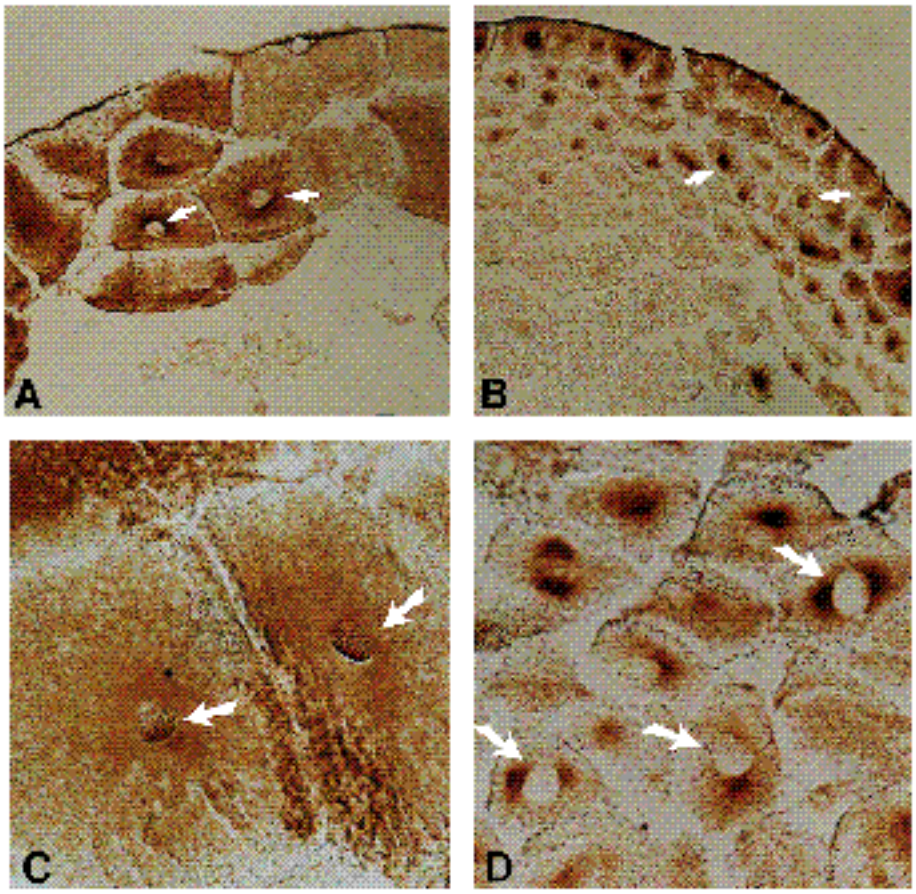

Fig. 4. xnf7 NLS linked to pyruvate kinase enters nuclei before the MBT. In vitro transcribed mRNA was injected into one-celled embryos. Injected embryos at various development stages were fixed, sectioned and treated with T7 polyclonal antibody followed by immunoperoxidase staining. (A) xnf7-8-injected embryos analyzed at stage 7. (B) xnf7-8-injected embryos analyzed at stage 9. (C) pkNLS (pyruvate kinase gene with the NLS)-injected embryos analyzed at stage 6. (D) Embryos injected with xnf7-1 analyzed at stage 9. Arrows are pointing to nuclei.

both basic domains of the NLS were necessary for the nuclear translocation of the xnf7 protein, while residues within the spacer did not effect translocation.

The NLS from xnf7 is able to direct the accumulation of pyruvate kinase protein into embryonic nuclei

The above experiments clearly showed that xnf7 protein possesses a bipartite NLS that functions efficiently in oocytes. However, previous studies indicated that in embryos the endogenous xnf7 protein remains cytoplasmic until the MBT (Miller et al., 1991). We were interested in determining if the xnf7 NLS can function efficiently in the embryo and direct the nuclear accumulation of a protein into nuclei prior to the MBT. Our strategy was to inject synthetic mRNAs into fertilized *Xenopus* eggs and analyze the nuclear accumulation of the exogenous protein by immunostaining of histological sections. To determine whether exogenous xnf7 would follow a similar time course to the endogenous xnf7 protein we injected synthetic mRNA made from the xnf7-8 construct. In order to detect this protein against the background of the endogenous xnf7 we utilized an epitope tag that consisted of 12 amino acids from the T7 viral coat protein. Injected embryos were analyzed at different stages during development using immunostaining with the T7 antibody. Fig. 4A shows that at stage 7 xnf7-8 protein was detected in the cytoplasm, while at stage 9, just following the MBT, it was detected within the nuclei (Fig. 4B). This is similar to the pattern observed for the endogenous protein and indicates that the exogenous xnf7-8 protein behaves like its endogenous counterpart.

When we linked the xnf7 NLS (pKNLS) to a cytoplasmic protein, pyruvate kinase, we found that the protein produced from the injected synthetic mRNA was detected in the nuclei as early as stage 6. This clearly demonstrates that the xnf7 NLS is capable of functioning efficiently in embryos (Fig. 4C). We also injected synthetic mRNA made from the xnf7-1 that lacked the NLS and found, as expected, that the protein did not enter the nuclei, though, it tends to accumulate in a perinuclear location (Fig. 4D).

DISCUSSION

In this report we demonstrate that xnf7 accumulates in the oocyte GVs within 20 minutes, indicating that the bipartite basic NLS functions efficiently in oocytes. Mutations in either of the bipartite basic domains inhibit nuclear translocation in oocytes, while mutations in the spacer sequences have no effect. The bipartite basic NLS found in xnf7 is similar to that found in a variety of proteins including N1/N2, nucleoplasmin, nucleolar protein 38 (No38), and p53 (Robbins et al., 1991). We also show that the xnf7 NLS linked to pyruvate kinase can efficiently direct this protein into nuclei during development.

During development several proteins, including nucleoplasmin and N1/N2, enter the embryonic nuclei immediately following fertilization, while xnf7, No 38 and a number of other proteins are retained in the cytoplasm until later stages of development (Dreyer et al., 1983; Dreyer, 1987). Recently a gene, *PwA33*, related but not homologous to *xnf7* was cloned from the urodele, *Pleurodeles waltli* (Bellini et al., 1993). The PwA33 protein also is retained in the cytoplasm until the MBT when it re-enters the nuclei. This raises the question of why these proteins are retained in the cytoplasm of embryos, since, at least in the case of xnf7, the NLS functions efficiently in oocytes. Our data indicating that the xnf7 NLS can also function efficiently in early embryos suggests that this protein is retained in the cytoplasm by a process that masks the function of the NLS.

Recently, the masking of the NLS by intramolecular interactions has been suggested in the case of the 110 kDa precursor for the p50NF-kB (Blank et al., 1991; Henkel et al., 1992). Also it is known that the dorsal gene product binds to another protein, cactus, when it is found in the cytoplasm. Therefore, it is possible that structural changes within the molecule itself or the interaction of the protein with a cytoplasmic anchor protein are potential mechanisms for the cytoplasmic retention of xnf7 during development.

The presence of the structural motifs, the nuclear localization and the similarity to other transcription factors suggest that xnf7 functions as a transcription factor. An attractive hypothesis is that xnf7 may activate specific sets of genes at the MBT following its entry into the nucleus and that its nuclear function is regulated by retention in the cytoplasm during early development.

This work was supported by grant DCB-9007410 from the National Science Foundation to L.D.E. We thank Dr Christine Dreyer, Dr Malgosia Kloc and other members of the Etkin laboratory for discussions and improvements in the manuscript.

REFERENCES

Bellini, M., LaCroix, J.-C. and Gall, J. G. (1993). A putative zinc-binding protein on lampbrush chromosome loops. *EMBO J.* **12**, 107-114.

Blank, V., Kourilsky, P. and Israel, A. (1991). Cytoplasmic retention, DNA binding and processing of the NF-kBp50 precursor are controlled by a small region in its C-terminus. *EMBO J.* **10**, 4159-4167.

Cornish, J. A., Kloc, M., Decker, G., Reddy, B. and Etkin, L. D. (1992). Xlcaax-1 is localized to the basolateral membrane of kidney tubule and other polarized epithelia during *Xenopus* development. *Dev. Biol.* **150**, 108-120.

de The, H. C., Lavau, A., Marchio, C., Chomirnnr, L., Degos and Dejean, A. (1991). The PML-RARa fusion mRNA generated by the t(15,17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell* **66**, 675-684.

Dreyer, C. (1987). Differential accumulation of oocyte nuclear proteins by embryonic nuclei of *Xenopus*. *Development* **101**, 829-846.

Dreyer, C., Wang, Ya. H., Wedlich, D. and Hansen, P. (1983). Oocyte nuclear proteins in the development of *Xenopus.* In *Current Problems in Germ Cell Differentiation* (ed. A. McLaren and C. C. Wylie), pp. 322-352. Symp. British Society for Developmental Biology. Cambridge University Press, Cambridge, UK.

Etkin, L. D. and Maxson, R. (1980). The synthesis of authentic sea urchin transcriptional and translational products by sea urchin histone genes injected into *Xenopus laevis* oocytes. *Dev. Biol.* **75**, 13-25.

Etkin, L. D. and Pearman, B. (1987). Distribution, expression, and germline transmission of exogenous DNA sequences following injection into *Xenopus laevis* eggs. *Development* **99**, 15-23.

Etkin, L. D., Pearman, B., Roberts, M. and Bektesh, S. (1984). Replication, integration, and expression exogenous DNA injected into fertilized eggs of *Xenopuslaevis*. *Differentiation* **26**, 194-202.

Freemont, P. S., Hanson, I. M. and Trowsdale, J. (1991). A novel cysteine-rich sequence motif. *Cell* **64**, 483-484.

Gannon, J. V. and Lane D. P. (1991). Protein synthesis required to anchor a mutant p53 protein which is temperature sensitive for nuclear transport. *Nature* **349**, 802-806.

Goddard, A. D., Borrow, J., Freemont, P. and Solomon, E. (1991). Characterization of a zinc finger gene disrupted by the t(15;17) in acute promyelocytic leukemia. *Science* **254**, 1371-1374.

Haupt, Y., Alexander, W. S., Barri, G., Klinken, S. P. and Adams, J. M. (1991). Novel zinc finger gene implicated as myc collaborator by retrovirally accelerated lymphogenesis in Eμ-myc transgenic mice. *Cell* **65**, 753-763.

Henkel, T., Zabel, U., vanZee, K., Muller, J., Fanning, E. and Baeuerle, P. A. (1992). Intramolecular masking of the nuclear location signal and dimerization domain in the precursor for the p50 NF-kB subunit. *Cell* **68**, 1121-1133.

Hunt, T. (1990). Cytoplasmic anchoring proteins and the control of nuclear localization. *Cell* **59**, 949-951.

Kakizuka, A., Miller, W. H., Umesono, K., Warrel, R. P. Jr, Frankel, S. R., Murty, V. V. V. S., Dimitrovsky, E. and Evans, R. (1991). *Cell* **66**, 663-674.

Kastner, P., Aymee, P. Lutz, Y., Rochetty-Egly, C., Gaub, M.-P., Durand, B., Lanotte, M., Berger, R. and Chambon, P. (1992). Structure, localization, and transcriptional properties of two classes of retinoic acid receptor a fusion proteins in acute promyelocytic leukemia (APL): structural similarities with a new family of oncoproteins. *EMBO J.* **11**, 629-642.

Lenardo, M. J. and Baltimore, D. (1989). NF-kB: A pleiotropic mediator of inducible and tissue-specific gene control. *Cell* **58**, 227-229.

Lovering, R., Hanson, I., Borden, K., Martin, S., O'Reilly, N., Evan, G., Rahman, D., Pappin, D., Trowsdale, J. and P. S. Freemont (1993). Identification and preliminary chracterization of a new protein motif related to the zinc finger. *Proc. Nat. Acad. Sci. USA* (in press).

Miki, T., Fleming, T. P., Crescenzi, M., Molloy, C., Blam, S., Reynolds, S. and Aaronson, S. (1991). Development of a highly efficient expression cDNA cloning system: Application to oncogene isolation. *Proc. Nat. Acad. Sci. USA* **88**, 5167-5171.

Miller, M., Kloc, M., Reddy, B., Eastman, E., Dreyer, C. and Etkin, L. D. (1989). Xlgv7, a maternal gene product localized in nuclei of the central nervous system in *Xenopus laevis. Genes Dev.* **3**, 572-583.

Miller, M. M., Reddy, B., Kloc, M. Li, X. X., Dreyer, C. and Etkin, L. D. (1991). The nuclear-cytoplasmic distribution of the *Xenopus* nuclear factor, Xnf7, changes during early development coincident with its state of phosphorylation. *Development* **113**, 569-575.

Moll, U. M., Riou, G. and Levine, A. (1992). Two distinct mechanisms alter p53 in breast cancer: mutation and nuclear exclusion. *Proc. Nat. Acad. Sci. USA* **89**, 7262-7266.

Moll, T., Tebb, G., Surana, U., Robitsch, H. and Nasmyth, K. (1991). The role of phosphorylation and the CDC28 protein kinase in cell-cycle regulated nuclear import of the *S. cerevisiae* transcription factor SW15. *Cell* **66**, 743-758.

Nigg, E. A., Baeuerle, P. A. and Luhrmann, R. (1991). Nuclear import-export: in search of signals and mechanisms. *Cell* **66**, 15-22.

Reddy, B. and Etkin, L. D. (1991). A unique bipartite cysteine-histidine motif defines a subfamily of potential zinc finger-proteins. *Nucl. Acids Res.* **19**, 6330.

Reddy, B., Freemont, P. S. and Etkin, L. D. (1992). A novel zinc finger coiled coil domain in a family of nuclear proteins. *Trends Biochem. Sci.* **17**, 344-345.

Reddy, B., Kloc, M. and Etkin, L. D. (1991). The cloning and characterization of a maternally expressed novel zinc finger nuclear phosphoprotein (xnf7) in *Xenopus laevis. Dev. Biol.* **148**, 107-116.

Robbins, J., Dilworth, S. M., Laskey, R. and Dingwall, C. (1991). Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: Identification of a class of bipartite nuclear targeting sequences. *Cell* **64**, 615-623.

Roth, S., Stein, D. and Nusslein-Volhard, C. (1989). A gradient of nuclear localization of dorsal protein determines dorsoventral pattern in the *Drosophila* embryo. *Cell* **59**, 1189-1202.

Rushlow, C., Han, K., Manley, J. and Levine, M. (1989). The graded distribution of the dorsal morphogen is initiated by selective nuclear transport in *Drosophila. Cell* **59**, 1165-1177.

Schmitz, M. L., Henkel, T. and Baeuerle, P. A. (1991). Proteins controlling the nuclear uptake of NF-kB, Rel, and dorsal. *Trends Cell Biol.* **1**, 130-137.

Silver, P. A. (1991). How proteins enter the nucleus. *Cell* **64**, 489-497.

Steward, R. (1989). Relocalization of the dorsal protein from the cytoplasm to nucleus correlates with its function. *Cell* **59**, 1179-1188.

Takahashi, M., Inaguma, Y., Hiai, H. and Hirose, F. (1988). Developmentally regulated expression of a human finger-containing gene encoded by the 5′ half of the ret transforming gene. *Mol. Cell. Biol.* **8**, 1853-1856.

(Received 22 January 1993 - Accepted 25 February 1993)