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Short Communication

The Oct-1 POU Domain Directs Developmentally Regulated Nuclear Translocation in *Xenopus* Embryos

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Early embryonic development in *Xenopus* is characterized by transcriptional repression which is relieved at the mid-blastula stage. Here we show that most of the maternally inherited POU domain transcription factor Oct-1 is retained in the cytoplasm during early development, and that it gradually translocates to the nucleus around the mid-blastula transition. Overexpressed epitope-tagged Oct-1 exhibits highly similar localization properties compared to endogenous protein. The amino acid sequence that directs this developmentally regulated nuclear translocation resides in the POU domain. Our findings may suggest that cytoplasmic retention of Oct-1 facilitates or contributes to the repression of Oct-1 target genes before the mid-blastula transition.

Key words: Mid-blastula transition / Nuclear translocation / Oct-1 / POU domain.

The early embryonic development of vertebrate and many invertebrate species is characterized by a period during which the embryonic genome is transcriptionally silent. The developmental stage at which the major transcriptional activity starts ranges from the 2-cell stage in the mouse to the cycle 9–14 blastoderm stage in *Drosophila*. In nematodes and amphibians the major activation of the embryonic genome starts after 7 cell cycles (in *C. elegans*, i.e. 90–125-cells) and 12 cycles (in *Xenopus*, i.e. approximately 4000 cells) respectively (for reviews see Yasuda and Schubiger, 1992; Andéol, 1994; Nothias *et al*., 1995). In *Xenopus*, the gradual activation of the embryonic genome coincides with the acquisition of cell motility and the loss of cell cycle synchrony. These three coinciding transitions are collectively referred to as the mid-blastula transition (MBT) (Newport and Kirschner, 1982a, b).

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The timing of the MBT appeared to depend on a critical nucleus – to – cytoplasm ratio, suggesting that the exponential increase in the number of nuclei during early development titrates a component that confers pre-MBT characteristics such as transcriptional repression (Newport and Kirschner, 1982a, b). During oogenesis a stockpile of structural and regulatory factors accumulates which is to be used later on during development. As a consequence, many of these factors are present in 100 to 100000-fold excess compared to somatic cells (reviewed in Almouzni and Wolffe, 1993a). These include transcription factors which normally activate transcription, but are transcriptionally inert during the rapid cell cycling that precedes the midblastula transition. Although a repressive chromatin structure and a repressed basal transcription machinery have been implicated in repressing transcription before the MBT (Almouzni and Wolffe, 1993b, 1995; Prioleau *et al*., 1994, 1995), it is not clear how maternally inherited transcription factors are prevented from activating transcription despite the high levels present in the early embryo. These transcription factors and the RNAs encoding these regulators are produced and stored in the oocyte. During early development, the proteins present in the nucleus segregate over an increasing number of nuclei. Therefore, transcription factors which are stored in the oocyte would reach high local concentrations if translocated to the small number of nuclei in pre-MBT embryos. These high local concentrations might counteract transcriptional repression. Therefore, cytoplasmic sequestration of maternally inherited transcription factors may potentially provide a mechanism by which the transcriptional machinery is constrained *in vivo*.

We have previously studied the expression and localization of the POU domain protein Oct-1 (Veenstra *et al*., 1995), a well known transcriptional activator (for reviews see Rosenfeld, 1991; Schöler, 1991; Verrijzer and Van der Vliet, 1993; Herr and Cleary, 1995; Veenstra *et al*., 1997) which in *Xenopus* is maternally derived (Hinkley and Perry, 1992; GJCV and OHJD, unpublished results), and is highly abundant in ectodermal cell lineages during gastrulation (Veenstra *et al*., 1995). The total amount of Oct-1 protein per embryo is constant during early development (Hinkley and Perry, 1992; Hinkley *et al*., 1992).

We addressed the question whether Oct-1 would localize to the nucleus before the midblastula transition, as the maternal stockpile of Oct-1 would reach high local concentrations if translocated to the small number of nuclei in pre-MBT embryos.

The localization of endogenous and overexpressed Oct-1 in blastula and gastrula stage embryos was assessed by whole-mount immunohistochemistry of control and *Oct-1* mRNA micro-injected embryos. Oct-1 appears to be most abundant in the animal hemisphere of the blastula stage embryo (Figure 1A), concordant with the enrichment of its RNA in the animal pole in blastula stage embryos and the abundance of Oct-1 protein in ectodermal cell lineages after the MBT (Veenstra *et al*., 1995). Comparison between the immunofluorescent signal corresponding to Oct-1 and nuclear staining revealed that Oct-1 is evenly distributed over the cytoplasm and nucleus in early blastula stage embryos (stage $6^1/_2-7$), and that the protein localizes to the nucleus in gastrula stage embryos (Figure 1A and B).

We microinjected synthetic RNA encoding *Oct-1* into fertilized eggs to examine the localization of overexpressed Oct-1. The immunofluorescence signals obtained with embryos that overexpressed Oct-1 were significantly higher than the fluorescence originating from

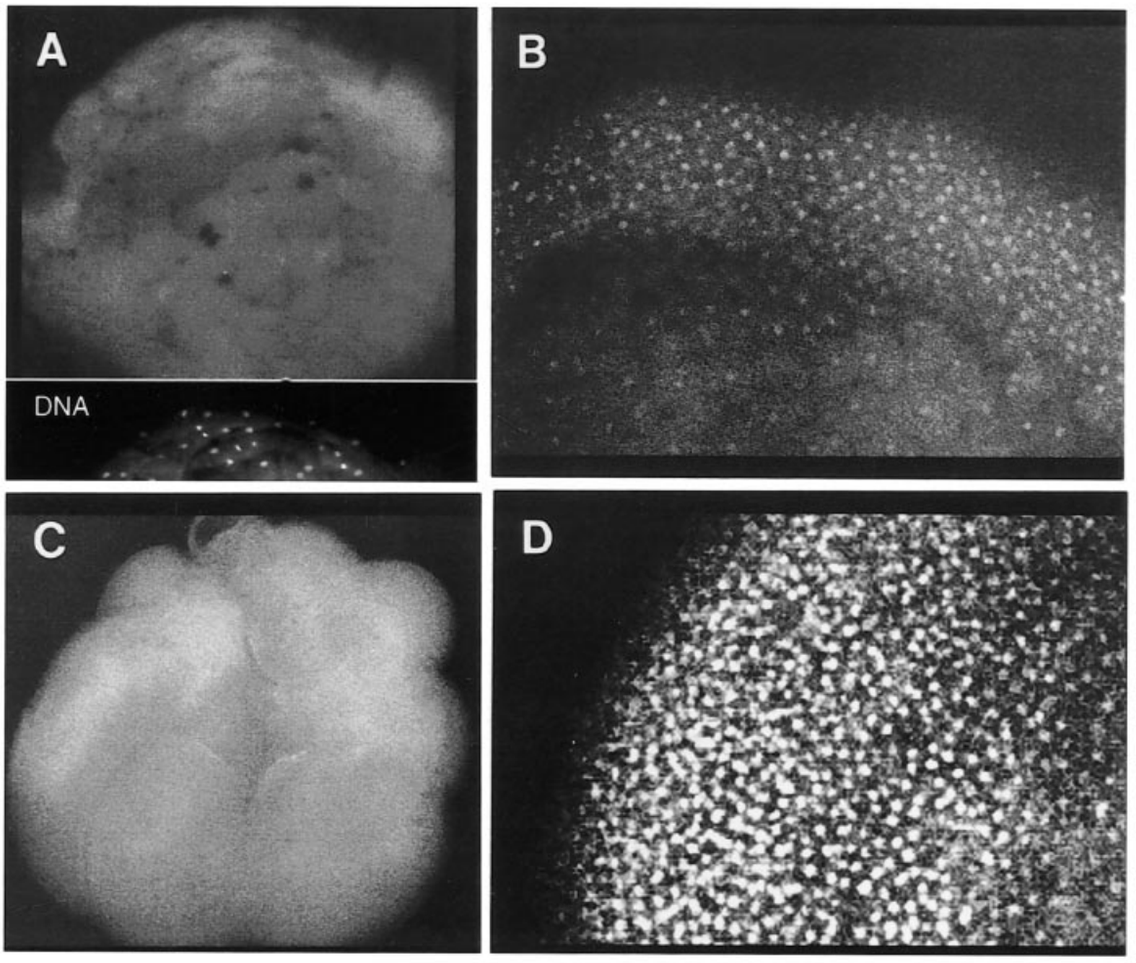

Fig. 1 Localization of the Oct-1 Transcriptional Activator during Early Embryogenesis.
(A) Endogenous Oct-1 localizes predominantly to the cytoplasm of early blastula stage (stage 7) embryos. Comparison of nuclear staining with a DNA binding dye (BOPRO3, see inset at the bottom of panel A, this shows nuclei in the animal pole region) and immunohistochemistry may suggest that blastula nuclei are not completely devoid of Oct-1. (B) Nuclear localization of endogenous Oct-1 in mid-gastrula stage (stage 11) embryo (higher magnification). (C) Cytoplasmic localization of overexpressed Oct-1 in the early blastula stage (stage $6^1/_2$) embryo. Overexpression signals in this embryo and in other embryos were significantly higher than endogenous signals. (D) Nuclear localization of overexpressed Oct-1 in the mid-gastrula stage (stage 11) embryo (higher magnification). Overexpression signals were imaged using different recording settings, allowing to distinguish between endogenous and overexpressed Oct-1.
Methods: For overexpression of full-length *Xenopus* Oct-1 in *Xenopus* embryos, capped RNA was synthesized from *Sal*I linearized pTO-NPC using an *in vitro* RNA synthesis kit (Ambion). pTO-NPC was obtained by cutting pLLB/XOct1 with *Hin*dIII and *Eco*RI, filling in the sticky ends of the 2550 bp Oct-1 fragment, and ligation of this fragment in the *Eco*RV site of pT7TS (kindly provided by Dr. P. Krieg). Albino *Xenopus* embryos, staged according to Nieuwkoop and Faber (1967), were collected and fixed as described (Veenstra *et al*., 1995). For expression of Oct-1 constructs, 0.3 fmol of the respective RNA was micro-injected into 1-cell stage embryos. Oct-1 labeling was performed as described (Veenstra *et al*., 1995). Immunohistochemistry was performed as described (Beumer *et al*., 1995) using an anti-mouse antibody conjugated to the fluorescent dye Cy5 (Jackson Immunoresearch Laboratories). Staining of nuclei was performed using the DNA binding fluorescent dye BOPRO-3 (Molecular Probes) diluted 1:1000 (stock 1 mM) in the wash buffer of the first wash following the secondary antibody incubation.

endogenous Oct-1. Like endogenous Oct-1, the overexpressed protein predominantly localizes to the cytoplasm (Figure 1C) in early blastula stage embryos (stage $6^1/_2$–7). In contrast, in gastrula stage embryos (stage 10–11), Oct-1 is present in the nucleus (Figure 1D). Oct-1 translocates to the nucleus rather gradually in time; at developmental stages close to the MBT, Oct-1 is found in the nucleus as well as in the cytoplasm (data not shown).

The subcellular localization of epitope-tagged Oct-1 deletion mutants was examined to analyze the domains involved in controlling nuclear translocation. Synthetic RNA encoding Oct-1 deletion mutants lacking either the N-terminal or the C-terminal domain, or both (i.e. the POU domain) were injected into fertilized eggs. The proteins encoded by these RNAs all localize predominantly to the cytoplasm before –, and to the nucleus after the mid-blastula transition (Figure 2 and data not shown), like full-length Oct-1 (Figure 1). This indicates that the signal(s) that confer the developmentally regulated nuclear translocation of Oct-1 map to the POU domain.

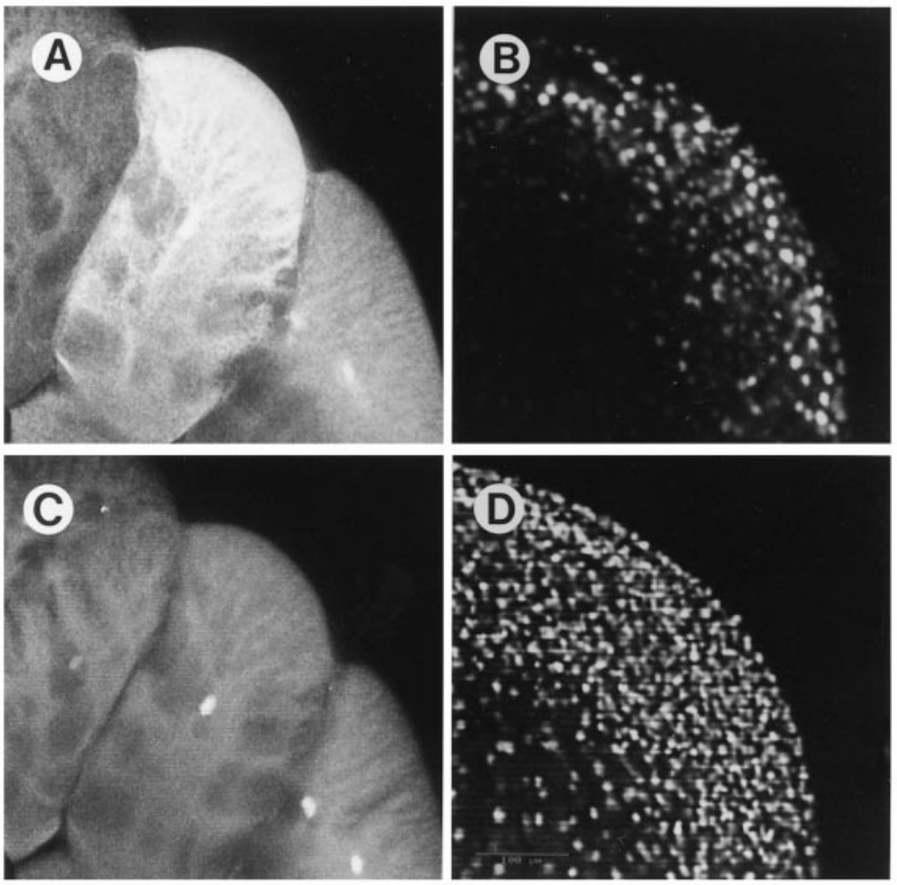

Fig. 2 The Regulatory Signal(s) for Developmentally Controlled Nuclear Translocation Reside in the POU Domain.
(A) Predominant cytoplasmic localization of epitope-tagged POU domain in the early blastula stage (stage $6^1/_2$) embryo. Some protein is also found in the nucleus. (B) Nuclear localization of epitope-tagged POU domain in a gastrula stage (stage 11) embryo. (C) Nuclei of the embryo shown in panel A, as visualized with the DNA binding fluorescent dye BOPRO-3. The cytoplasmic background staining in this panel is due to low levels of autofluorescence of yolk, as detected with the recording settings for this picture. Autofluorescence of *Xenopus* yolk decreases significantly with longer excitation wavelengths (Beumer *et al*., 1995), and is virtually absent from the Oct-1 immunohistochemistry recordings due to the use of far red dyes (Cy5). (D) Nuclei of the embryo shown in panel B, as visualized with BOPRO-3.
Methods: Myc epitope-tagged Oct-1 derived proteins were expressed in *Xenopus* embryos by micro-injection of the corresponding RNAs, which were synthesized using linearized Oct-1 expression constructs based on pCS2+MT (Rupp *et al*., 1994; Turner and Weintraub, 1994). Myc epitope-tagged proteins were detected using the 9E10 monoclonal anti-myc antibody (Santa Cruz biotechnology). Immunofluorescent signals were recorded in three dimensions using a BioRad MRC600 confocal laser scanning microscope with a 15 mW krypton-argon laser. A number of embryos were selected for quantitative analysis of the fluorescent signals obtained. For quantitation purposes, and on the basis of three dimensional information contained in multiple optical sections, the shape of animal pole cells and nuclei of early blastula embryos (such as those shown in Figure 2A and D) was assumed to be cylindrical. Using this assumption, the volume of an average blastula nucleus is approximately 7000 μm^3, as calculated from the formula Vol $\pi r^2 h$, the radius (r) and height (h) measured in optical sections to be 8 μm and 3 μm, respectively. The ratio between cytoplasm and nuclear volume is different for each cell, but can be as high as 4300 in blastula stage embryos (as for the brightly stained cell in Figure 2A), and even higher for the fused pronuclei in the fertilized egg [the egg contains approximately 0.6 μl (6.0×10^8 μm^3) of cytoplasm, the cytoplasmic-to-nuclear volume ratio approximates 86000].

Incidentally, overexpressed full-length Oct-1 and Oct-1 POU domain localize to both nucleus and cytoplasm before the MBT (Figure 2 and data not shown), a feature never observed for endogenous Oct-1. Possibly this is caused by saturation of the retention mechanism underlying the predominantly cytoplasmic localization of Oct-1 before the MBT, or by limited post-translational modification of overexpressed Oct-1. Even so, more than 90% (typically approximately 98%) of overexpressed protein is present in the cytoplasm, because of the relatively low nuclear enrichment (generally less than three-fold, Figure 2A and data not shown), and the relative volumes of nucleus and cytoplasm (ratio of cytoplasmic and nuclear volume $\gg$ 1000 see legend to Figure 2) in early embryos. The fact that a small fraction of overexpressed Oct-1 incidentally localizes to the nucleus is concordant with the observation that pre-MBT nuclei are not unambiguously devoid of endogenous Oct-1 (Figure 1A), which suggests an absence of nuclear translocation in the absence of active nuclear export before the mid-blastula transition. In contrast, Oct-1 is efficiently translocated to post-MBT nuclei.

Our results indicate that both endogenous and overexpressed Oct-1 are sequestered in the cytoplasm before the mid-blastula transition (MBT), and are translocated to the nucleus around the MBT. The regulatory signal(s) that are important for this cellular behavior reside in the POU domain. Active nuclear export of Oct-1 before the MBT is not very likely since the nuclei do not seem to be devoid of Oct-1, and moreover do incidentally contain slightly enhanced levels of overexpressed Oct-1. Even in those cases most of the protein is present in the cytoplasm.

Other proteins, including c-myc, E1a, XMyoD, CCAAT binding protein, and a zinc-finger protein referred to as xnf-7, have also been reported to be sequestered in the cytoplasm of early embryos (Dreyer, 1987; Standiford and Richter, 1992; Li *et al*., 1994; Rupp *et al*., 1994; Brewer *et al*., 1995; Lemaitre *et al*., 1995). Cytoplasmic retention before the MBT of some of the transcriptional activators is likely to be a necessity in terms of transcriptional regulation. The total amount of Oct-1 protein is more or less constant during development until late neurula stages (Hinkley and Perry, 1992; Hinkley *et al*., 1992), whereas the number of cells increases from 1 to 10^5. The Oct-1 nuclear concentration indeed decreases in most but not all cells during development from gastrula to tadpole stages (Veenstra *et al*., 1995). If the fixed amount of Oct-1 protein were to be translocated to the nucleus in a constitutive way, the local concentration reached in the single nucleus of the zygote would be unphysiologically high [approximately 200 μM as calculated from a total amount of 100 pg Oct-1 per embryo (Veenstra *et al*., 1995), and an estimated embryonic nuclear volume of 7000 μm^3, see legend to Figure 2], which might drive transcriptional regulation, despite repressing mechanisms, into nonspecific transcriptional activation. Cytoplasmic sequestration therefore probably provides a safeguard mechanism.

Further analysis is required to identify the sequence within the POU domain that confers the developmentally controlled nuclear translocation reported here. Such an analysis may shed more light on nuclear function and gene regulation in the early vertebrate embryo. Specifically, molecular dissection of the mechanisms that govern regulated nuclear translocation in the highly dynamic context of early vertebrate development may elucidate the nature of the cell biological requirements of rapid cell divisions and transcriptional repression before the MBT, and the onset of zygotic gene expression that coincides with cell cycle lengthening, the acquisition of cell motility, and morphogenesis after the mid-blastula transition.

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