Development 136, 2695-2703 (2009) doi:10.1242/dev.036327

Xenopus oocytes reactivate muscle gene transcription in transplanted somatic nuclei independently of myogenic factors

Adrian Biddle[1,2], Ilenia Simeoni[1] and J. B. Gurdon[1,*]

Transplantation into eggs or oocytes is an effective means of achieving the reprogramming of somatic cell nuclei. We ask here whether the provision of gene-specific transcription factors forms part of the mechanism by which a gene that is repressed in somatic cells is transcribed in oocytes. We find that M1 oocytes have an extremely strong transcription-inducing activity. They cause muscle genes of nuclei from non-muscle somatic cells, after injection into oocytes, to be transcribed to nearly the same extent as muscle genes in muscle cells. We show, surprisingly, that the myogenic factor MyoD and other known myogenic factors are not required to induce the transcription of muscle genes in a range of non-muscle somatic cell nuclei after transplantation to *Xenopus* oocytes. The overexpression of Id, a dominant-negative repressor of MyoD, prevents maternal MyoD from binding to its consensus sequences; nevertheless, muscle genes are activated in somatic nuclei to the same extent as without Id. We conclude that M1 oocytes can reprogram somatic nuclei in a different way to other experimental procedures: oocytes do not suppress the transcription of inappropriate genes and they activate a gene without the help of its known transcription factors. We suggest that these characteristics might be a special property of amphibian oocytes, and possibly of oocytes in general.

KEY WORDS: Muscle genes, Reprogramming, *Xenopus* oocytes

INTRODUCTION

When somatic cell nuclei are transplanted to enucleated eggs, they are often reprogrammed to a multipotent state, so that the nucleus of a cell that was fully differentiated rapidly regains the ability to elicit the main lineages that arise in normal development (Gurdon, 1962; Pomerantz and Blau, 2004). However, the mechanism by which the reprogramming of a nucleus takes place in eggs and oocytes is very little understood. The aim of the work described here was to investigate the role of a powerful transcription factor in this mechanism.

For the experiments described here, we have used the first meiotic (M1) prophase oocytes of Amphibia as recipients for transplanted nuclei. These oocytes do not generate new cells, but they do induce the transcription of embryo-specific genes such as *Oct4* and *Nanog* in transplanted mammalian somatic cell nuclei. Importantly, oocytes achieve this without DNA replication or protein synthesis and therefore bring about a direct switch in transcription on the same chromatin that was previously present in a somatic cell. This behaviour simplifies an analysis of the mechanism of transcriptional reprogramming and may be contrasted with that of second meiotic metaphase eggs, which also induce transcriptional switches, but only after DNA replication, cell division and protein synthesis. Gene expression during amphibian oogenesis has been reviewed by Davidson (Davidson, 1986).

To understand the role of transcription factor provision for nuclear reprogramming in *Xenopus* oocytes, we have focussed on myogenic genes. MyoD is a particularly powerful protein for activating the transcription of many muscle genes, including itself, in a range of somatic cells (Weintraub et al., 1989). *Xenopus laevis* has two *MyoD* genes, one of which (*MyoDa*) is actively transcribed in oocytes, whereas the other (*MyoDb*) is apparently not (Harvey, 1990). We have taken advantage of this situation to analyze the importance of *MyoD* and other myogenic genes in reprogramming somatic cell nuclei by oocytes. We find that oocytes strongly activate muscle gene transcription in transplanted nuclei. The reactivated transcripts are mainly unspliced transcripts, suggesting a very low efficiency of splicing activity in oocytes (Wickens and Gurdon, 1983). Quantitative analysis shows that the number of muscle gene transcripts reactivated in non-muscle nuclei transplanted to oocytes is nearly as high as in differentiated muscle cells. We unexpectedly find that muscle genes are activated in oocytes independently of known myogenic transcription factors. We conclude that nuclear reprogramming by oocytes is achieved by a mechanism very different from that of transcription factor overexpression by viruses in other reprogramming experiments (Takahashi et al., 2007; Zhou et al., 2008), and might represent a new kind of transcriptional control characteristic of some species of oocytes.

MATERIALS AND METHODS

Xenopus oocyte manipulation

After removal from the *Xenopus* ovary, oocytes were kept in bunches of ~1 cm diameter at 14°C in MBS medium [88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 10 mM Hepes pH 7.5, 0.82 mM $MgSO_4$ $7H_2O$, 0.33 mM $Ca(NO_3)_2$ $4H_2O$, 0.41 mM $CaCl_2$ $6H_2O$] containing 1 μg/ml penicillin and streptomycin. Liberase (Roche) treatment was used for the removal of follicular cell layers. A Drummond Nanoject microinjector was used for oocyte/germinal vesicle (GV) injections.

Cell culture

Embryonic stem (ES) and C3H10T1/2 cells were kindly provided by Drs A. Surani (University of Cambridge, Cambridge, UK) and H. Blau (Stanford University School of Medicine, Stanford, CA, USA), respectively. The C2C12 cell line was purchased from the American Tissue Culture Collection. G1E (for GATA-1$^-$ erythroid) cells came from the laboratory of

[1]Wellcome Trust/Cancer Research UK Gurdon Institute, Tennis Court Road, Cambridge CB2 1QN and Department of Zoology, Cambridge University, Cambridge, UK. [2]Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, 4 Newark Street, London E1 2AT, UK.

*Author for correspondence (e-mail: j.gurdon@gurdon.cam.ac.uk)

Accepted 11 June 2009

Dr Paresh Vyas (The Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK). The C3H10T1/2 and C2C12 cell lines were grown in 8 ml Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with 10% foetal bovine serum (FBS), 4 mM L-glutamine, 50 μg/ml penicillin and streptomycin. The ES cell line was grown in 10 ml Glasgow MEM (Gibco) supplemented with 20% FBS, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 50 μg/ml gentamycin, 0.1 mM β-mercaptoethanol and 1000 units/ml leukaemia inhibitory factor (LIF) (Chemicon). For differentiation of C2C12 cells into myotubes, the high-serum medium was replaced with low-serum medium (DMEM with 2% horse serum). G1E cells were cultured in IMDM medium (Gibco) containing 2% penicillin/streptomycin, 0.0125% monothioglycerol, 15% FBS, 2 units/ml erythropoietin and 50 ng/ml Kit ligand.

Nuclear permeabilisation

Cells were resuspended in SuNaSp (250 mM sucrose, 75 mM NaCl, 0.5 mM spermidine trihydrochloride, 0.15 mM spermine tetrahydrochloride) at 1×10^6 cells/ml. Hand-warmed streptolysin O (SLO) (Sigma) was added (25 μl at 20 units/μl) to 1 ml warmed cell suspension and incubated by hand for 30 seconds. SLO activity was stopped by addition of 500 μl SuNaSp-BSA (3% BSA in SuNaSp) and cells were resuspended in 100 μl SuNaSp-BSA. The level of permeabilisation by SLO was tested by mixing 1 μl cell suspension with 10 μl Trypan Blue dye with observation under a light microscope. Permeabilisation of ~85% of cells (as determined by blue staining) was considered optimal. In the case of insufficient permeabilisation, cells were rewashed in PBS and SuNaSp and then incubated in SLO for longer. After permeabilisation, cells were resuspended in 20 μl SuNaSp-BSA and kept on ice until injection into the GVs of oocytes. Before oocyte injection, practice injections were made into SuNaSp on a microscope slide to allow the number of nuclei per injection to be counted.

In vitro transcription

The Megascript SP6 Kit (Ambion Biosciences) was used to prepare mRNA as described by the manufacturer. DNase treatment and capping were as previously described (Byrne et al., 2003).

RNA extraction

RNA was extracted from *Xenopus* oocytes and mammalian cells using the RNeasy Kit (Qiagen). In the first step, 350 μl buffer RLT plus β-mercaptoethanol were added per sample, and the sample was then disrupted by vortexing for 5 minutes. The optional DNase digestion step was included to prevent carry over of contaminating genomic DNA. The RNA was eluted in 50 μl RNase-free water and used for reverse transcription.

Reverse transcription and PCR/qPCR

Reverse transcription was carried out using Superscript III reverse transcriptase (Invitrogen). A master mix was made up containing either 0.5 μl oligo(dT) (10 μM) or 0.5 μl of each reverse gene-specific primer (GSP) required for the experiment (10 μM), together with 2.5 μl 10 mM dNTP mix (Roche) and made up to 8 μl total with RNase-free water. RNA (25 μl) was added and incubated at 70°C for 10 minutes. The sample was then transferred to ice and 10 μl 10× First-Strand Synthesis Buffer (Invitrogen), 1 μl BSA (2 mg/ml), 0.5 μl RNase inhibitor (Roche) and 2.5 μl DTT (0.1 M) were added. From randomly selected samples, 5 μl was removed to act as –RT controls. Superscript III reverse transcriptase (0.5 μl) was added to each sample (except for the –RT controls). Samples were mixed and incubated at 55°C for 1 hour. Samples were then frozen at –80°C or used immediately for PCR. For semi-quantitative PCR, Hot Star Taq polymerase (Qiagen) was used. The tubes were heated to 95°C for 15 minutes to activate the Hot Star Taq polymerase, before undergoing the required number of PCR amplification cycles. A typical PCR cycle comprised dissociation at 93°C for 30 seconds, annealing at 58°C for 30 seconds and extension at 72°C for 30 seconds.

Primers (5′ to 3′; m, mouse; X, *Xenopus*) used for semi-quantitative PCR were: mMyoD forward CTCCTATGCTTTGCCTGGTC, reverse AGGAAGGAGGGCAGAGAGAC, and GSP GCCGTGAGAGTCGTCTTAACTT; mMyogenin forward CTACAGGCCTTGCTCAGCTC, reverse AGATTGTGGGCGTCTGTAGG, and GSP CAGGACAGCCCCACTTAAAA; mEmbryonic MHC (*Myh3*) forward GAAGCGTGAGGCTGAGTTCT, reverse TCTCCTTCTCCAGCTTCTGC, and GSP CTCGCTCTTCTCCTTCTCCA; mAdult MHC (*Myh1*) forward ACTGGAGGAGATCAGCGAGA, reverse TCTCCTTCTCCAGCTTCTGC, and GSP CTCGCTCTTCTCCTTCTCCA; mβ-globin (haemoglobin, beta-adult major chain) forward GCTGGTTGTCTACCCTTGGA, reverse ATCCACATGCAGCTTGTCAC, and GSP CCCAGCACAATCACGATCAT; γ-globin (haemoglobin, beta-like embryonic chain) forward TGGGAACTTGTCCTCTGCTT, reverse AGCTTGTCACAGTGCAGTTCA, and GSP GTGCAGAAAGGAGGCATAGC; mThy1 forward ACTGCCGCCATGAGAATAAC, reverse ATCCTTGGTGGTGAAGTTGG, and GSP CCCGAGACTTGAAGCTCACA; mOct4 (*Pou5f1*) forward ACCAGGCTCAGAGGTATTGG, reverse AGTTGCTTTCCACTCGTGCT, and GSP TTCTCCAACTTCACGGCATT; mNeurogenin forward CGATCCCCTTTTCTCCTTTC, reverse CCAGAATTCACTTGGGGGTA, and GSP TGCAGCAACCTAACAAGTGG; mGAD1 forward CAGCTAAGAACGGGGAGGAG, reverse CCAGGATCTGCTCCAGAGAC, and GSP CCCGTACTTCAGGGTGTCTC; mSkeletal actin forward AGACACCATGTGCGACGAAG and reverse CCGTCCCCAGAATCCAACACA; mGAPDH forward TCAACGACCCCTTCATTGAC and reverse ATGCAGGGATGATGTTCTGG; mc-Jun forward TGAAAGCTGTGTCCCCTGTC and reverse ATCACAGCACATGCCACTTC; XMyoDa forward GGTGCACGTGACTCTGCTGA and reverse AGGCGTCAGGGACTTTACTG; XMyoDb forward AGGTCCAACTGCTCCGACGGCATGAA and reverse AGGAGAGAATCCAGTTGATGGAAACA; XMRF4a forward CCAGAATGCCCAATCTCAAT, reverse GCTGGTAGAGCCGTGAAAAG, and GSP TCAGTTGGGGCAGACTTTCT; XMRF4b forward GCAGATGGATCCCCTGTCTA, reverse GGGCAGACTTTCTTTTGCAG, and GSP TCAGTTGGGGCAGACTTTCT; Xmyogenin U1 and U2 forward AGGGCTCAGGGATTGAAGAT, reverse CTTTTCAGGGCTTCAAATGC, and GSP ATACTGGATGGCGCTCCTTA; XVegT forward AGAAACTGCTGTCGGGAA and reverse CGGATCTTACACTGAGGA.

A LightCycler machine (Roche) was used for the ChIP analysis. Primers for LightCycler PCR were: Cardiac actin promoter forward CAATTGTGCTGCACCTGTCT and reverse GGATGGCCAAATAGGGATTC.

The Applied Biosystems 7300 real-time PCR system in a 96-well format with Power SYBR Green PCR Master Mix was used for the quantitative analysis. Primers for the real-time PCR were: mMyoD forward CACTCCGGGACATAGACTTGACA and reverse CGAAACACGGGTCATCATAGAA; mMyogenin forward CGTGGGCATGTAAGGTGTGTA and reverse CTGCGCTTCTCCCTCAGTGT. To obtain the number of transcripts per nucleus, we generated a standard curve using a known amount of DNA containing the MyoD or myogenin sequences. We divided the amount of DNA (in a range of 0.1 ng to 0.1 fg) by its molecular weight (420 bp × 660 g/mole). This value was multiplied by Avogadro's number (6.022×10^{23}) to obtain the absolute number of transcripts. To estimate the absolute number of MyoD or myogenin transcripts reactivated in the injected nuclei, we compared the Ct value of the injected nuclei with the Ct values of the standard curve. This value was then adjusted to the known number of nuclei injected per oocyte and the dilutions of the sample starting from the RNA extraction step. We injected 300 ES, 300 C3H10T1/2 or 500 thymocyte nuclei per oocyte. Because, in any single experimental series, it is probable that a particular oocyte will receive too few nuclei to show activation, we have duplicated part of one figure as part of another figure (see figure legends for details).

Immunohistochemistry and western blotting

For immunohistochemistry, we followed the protocol of Byrne et al. (Byrne et al., 2003). For western blots, oocytes were first lysed in a lysis buffer [137 mM NaCl, 20 mM Tris pH 8.0, 10% glycerol, 1% NP40, 2 mM EDTA, inhibitor cocktail (Roche) (from a 50× stock) and PMSF at 0.01%]. Twenty microlitres of lysis buffer was added per oocyte in each sample, or 5 μl per extracted GV. Samples were run in a 10% polyacrylamide gel. After blotting, the membrane was placed in blocking buffer containing the primary antibody and incubated overnight at 4°C with gentle shaking. After a series of washes, the membrane was placed in horseradish peroxidase-conjugated secondary antibody and developed with the ECL Plus protein detection system (Amersham).

ChIP analysis

ChIP analysis was performed according to Messenger et al. (Messenger et al., 2005). To prepare protein G beads, salmon sperm DNA solution (Sigma) was sonicated on ice for 20 seconds and then boiled for 15 minutes and cooled on ice. Magnetic protein G beads (Invitrogen) were washed three times in PBS, and then the salmon sperm DNA was added to 75 μg/ml and BSA to 200 μg/ml. PBS was added to make the bead suspension up to its original volume, and the suspension was incubated for 30 minutes at 4°C with rotation. Approximately 25 oocytes were cross-linked using 1% formaldehyde in MBS for 15 minutes at room temperature with rotation. They were then washed twice in MBS, resuspended in 100 mM Tris pH 9.4 plus 10 mM DTT, and incubated at 30°C for 15 minutes to terminate formaldehyde activity. The oocytes were resuspended in 0.5 ml lysis buffer [1% SDS, 10 mM EDTA, 50 mM Tris pH 8, protease inhibitor cocktail (Roche)] and lysed by pipetting. Samples were sonicated on ice four times for 30 seconds each at 40% amplitude and then diluted with another 0.5 ml lysis buffer. Samples were centrifuged at 13,000 rpm (15,700 ***g***) for 10 minutes at 4°C and the supernatant transferred to a new tube. The supernatant was pre-cleared with 15 μl protein G beads for 2 hours at 4°C with rotation. Beads were discarded and the sample was stored at 4°C. Three hundred microlitres of *Xenopus* MyoD antibody (Ab) was bound to 20 μl protein G beads in 900 μl RIPA buffer [1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 90 mM NaCl, 10 mM Tris pH 8, EDTA-free protease inhibitor cocktail (Roche)] at 4°C overnight with rotation. The resulting Ab-beads were washed twice in RIPA buffer and then resuspended to 20 μl in RIPA buffer. For immunoprecipitation, 100 μl pre-cleared sample, 900 μl RIPA buffer and 20 μl Ab-beads were mixed and incubated at 4°C overnight with rotation. Non-Ab beads were used in separate tubes to obtain a background signal for each sample. The Ab-beads were then removed, washed twice with wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris pH 8) and once with final wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 500 mM NaCl, 20 mM Tris pH 8). DNA was eluted from the Ab-beads by adding 450 μl elution buffer (1% SDS, 100 mM $NaHCO_3$) and rotating at room temperature for 30 minutes. The supernatant was removed to a fresh tube, 5 μl proteinase K (Roche) added and incubated at 65°C overnight with shaking to reverse the cross-linking. Samples were phenol:chloroform extracted, ethanol precipitated [with glycogen (Roche)] and resuspended in 20 μl TE buffer (10 mM Tris pH 8.0, 1 mM EDTA). DNA concentration was measured by NanoDrop (LabTech), and samples were diluted to match the concentration of the least concentrated sample. Four microlitres of each sample was used for real-time PCR analysis.

RESULTS

Xenopus oocytes contain transcripts of *MyoDa*, but not of other myogenic genes

The *Xenopus laevis* genome has two genes encoding MyoD-like proteins (Harvey, 1990; Rupp and Weintraub, 1991). The oocyte has a maternal content of MyoDa mRNA, although it is not known whether this mRNA is translated into an active protein. MyoDb, as well as other muscle regulatory factors (MRFs), are presumed not to be present in *Xenopus* oocytes as mRNA or protein. Both MyoDa and MyoDb are ubiquitously transcribed in embryos after the midblastula transition (MBT) (Rupp and Weintraub, 1991; Weintraub et al., 1991), and both are upregulated autocatalytically. In *Xenopus*, there are two copies of myogenin, called myogenin-U1 and myogenin-U2 (Charbonnier et al., 2002), and two copies of MRF4, called MRF4a and MRF4b (Della Gaspera et al., 2006). So far, only one copy of Myf5 has been identified. MyoD and Myf5 are both expressed in the presomitic mesoderm of embryos, but with different spatial expression patterns (Kablar et al., 1997). MRF4 is not expressed until stage 18, when it becomes detectable in the developing somites (Jennings, 1992). *Xenopus* myogenin is not expressed until after metamorphosis and is not required for primary myogenesis or for the formation of uninucleate muscle cells, but instead is likely to have a role in driving the terminal muscle differentiation programme, including cell fusion and adult muscle gene activation (Nicolas et al., 1998).

In *Xenopus* oocytes, the transcription of myogenic genes is not well characterised. To further investigate this matter, we looked for mRNAs encoding MyoDa, MyoDb and other MRFs in uninjected oocytes using appropriate primers. We found that although transcripts of MyoDa are clearly seen in the oocyte, there are no detectable transcripts for MyoDb, MRF4a, Myf5, myogenin-U1 or myogenin-U2, and only a very low level of MRF4b transcripts (see Fig. S1 in the supplementary material). Whereas RT-PCR for MyoDa transcripts was carried out for 35 cycles, PCR assays for all other MRFs were extended to 40 cycles, raising the sensitivity by at least 10-fold. The positive control consisted of three somites dissected from stage 18 embryos (see Fig. S1, column 2, in the supplementary material). Somites were negative for myogenin, as *Xenopus* myogenin is only expressed in the adult frog (Charbonnier et al., 2002). We conclude that in *Xenopus* oocytes, MyoDa is well transcribed, whereas MyoDb and other MRF transcripts are not.

Oocytes are permissive for ongoing transcription

We first asked whether oocytes behave like other cells that can reprogram somatic nuclei in terms of whether they suppress the transcription of genes that are expressed in the donor nuclei but not in the recipient cell. This extinction has been observed in most somatic cell hybrids (Blau et al., 1983; Terranova et al., 2006; Carlsson et al., 1974) and also in early (mammalian and amphibian) nuclear transplant embryos (Gurdon et al., 1984; Humpherys et al., 2002). To test this, we prepared donor nuclei from differentiated mouse muscle myotubes that are actively transcribing muscle genes that are not expressed in oocytes or embryos (see Fig. S2 in the supplementary material). These nuclei were transplanted into oocytes and muscle gene transcription monitored over 48 hours by RT-PCR. Because splicing is inefficient in (*Xenopus*) oocytes (Wickens and Gurdon, 1983), we assayed total transcripts (spliced and unspliced) using forward and reverse PCR primers that both prime in the same exon. We found that there is a continuing presence of both MyoD and myogenin RNA after nuclear injection (Fig. 1A). The persistence of transcripts of mouse myogenin, a type of muscle gene not expressed in *Xenopus* oocytes or embryos, argues that the oocyte does not exert any significant inhibitory effect on muscle gene transcription by transplanted nuclei in which that gene is already being actively transcribed. We also found that spliced myogenin transcripts introduced with the injected nuclei disappear within 48 hours of nuclear injection; only unspliced transcripts continue to be seen (Fig. 1A). To further support the idea that oocytes have no inhibitory effect on the transcription of donor nuclei, mouse globin-expressing cells were injected into oocytes (Fig. 1B). We found that globin transcripts continue to be present in oocytes even 72 hours after nuclear transplantation, even though oocytes completely fail to activate globin genes (see below). In conclusion, we show that oocytes do not switch off the transcription of inappropriate genes, and this supports the idea that oocytes are permissive for the ongoing transcription of differentiation genes in reprogrammed nuclei.

Myogenic gene transcripts have a short half-life in oocytes

To be sure that the RNA seen after nuclear transfer represents new transcription rather than the persistence of transcripts carried over with injected nuclei, we need to know the half-life of RNAs in oocytes. This has been determined by use of α-amanitin at a

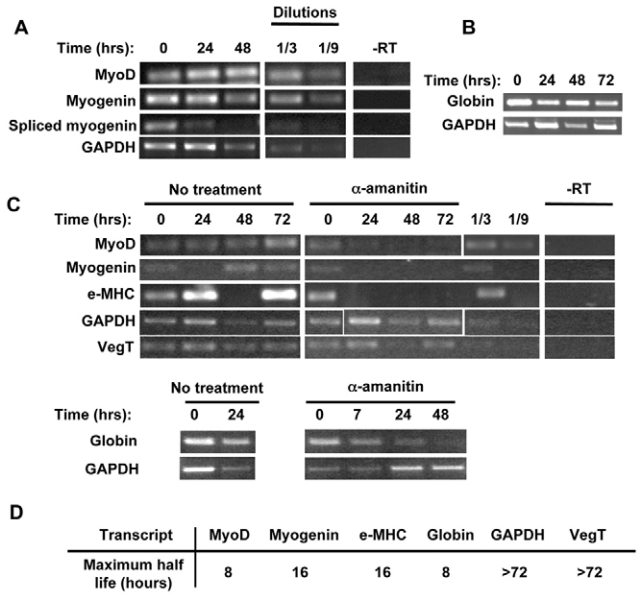

Fig. 1. Oocytes do not inhibit ongoing gene transcription in transplanted nuclei. (**A**) Transcripts from injected myotube nuclei persist but are not spliced. Nuclei of differentiated mouse muscle myotubes were permeabilised and injected into the germinal vesicles (GVs) of oocytes; samples were taken over time and analysed by semi-quantitative RT-PCR. Time is hours after nuclear injection. The dilutions are of the zero hour sample for myogenin and the 48 hour sample for MyoD. –RT, minus reverse transcriptase controls. (**B**) The nuclei of induced mouse G1E erythroblasts continue to express globin transcripts for at least 72 hours after injection into oocytes. (**C**) Half-life of muscle transcripts from injected myotube nuclei estimated using α-amanitin treatment followed by semi-quantitative RT-PCR analysis. Time points are hours after treatment with α-amanitin (time 0). *Gapdh* is a mouse housekeeping gene, and VegT is a maternal mRNA that is abundant in the *Xenopus* oocyte and is used as a control for RNA recovery. Beneath is shown an assessment of the half-life of globin transcripts from injected erythroblast nuclei after α-amanitin treatment. (**D**) Estimates of the maximum possible half-lives of transcripts based on C. To indicate that PCR products were in the linear phase, and hence to allow semi-quantitative comparison between samples, sample dilutions were added. In this and in all subsequent figures, each experiment has been repeated at least three times unless stated otherwise.

concentration known to inhibit RNA polymerase II transcription in *Xenopus* oocytes (McKnight and Gavis, 1980). We injected muscle nuclei into oocytes, then injected 100 pg α-amanitin per oocyte to suppress RNA polymerase II activity. The zero time point is the moment of α-amanitin injection.

The transcripts for GAPDH and VegT were found to be very long-lived, with half-lives of more than 72 hours. MyoD transcripts were reduced to less than one-ninth of their starting level within 24 hours, corresponding to a half-life of less than 8 hours. By a similar analysis, the half-lives of embryonic myosin heavy chain (e-MHC; Myh3) and myogenin were less than 16 hours, and globin had a half-life of 8 hours (Fig. 1C,D). Referring back to the previous section, we can now conclude that the transcripts of MyoD, myogenin and globin, which persist for over 72 hours, represent continuing transcription and not the persistence of RNA carried over with donor nuclei. We conclude that oocytes have no capacity to suppress the expression of differentiation genes. Nuclear reprogramming by oocytes does not therefore include the switch off of differentiation genes. This is a unique characteristic of *Xenopus* oocytes.

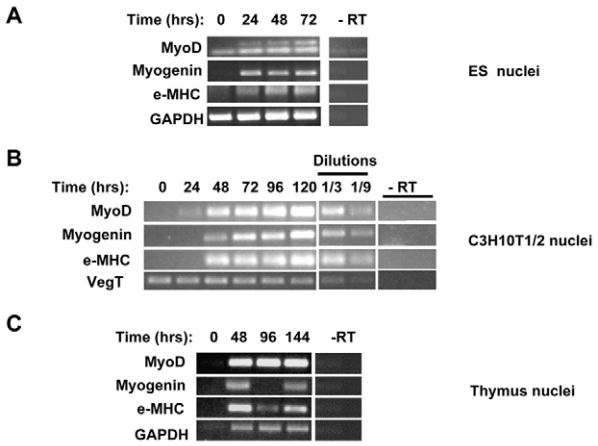

Fig. 2. Myogenic gene transcription is induced in oocytes injected with nuclei from a range of mouse cell types. The genes encoding MyoD, myogenin and embryonic myosin heavy chain (e-MHC) are reactivated in ES (**A**), C3H10T1/2 (**B**) and normal thymus (**C**) nuclei after transplantation in *Xenopus* oocytes. The 1/3 and 1/9 dilutions are of the final 120 hour time point after nuclear injection. GAPDH and VegT are used as a control for RNA recovery. The lower band in the MyoD gel of ES cell nuclei is due to a primer dimer. –RT, minus reverse transcriptase controls. Note that the image of MyoD reactivation in ES cell nuclei (top row) is a duplicate of the results shown in Fig. 7C (top row of –MyoD).

Oocytes activate the transcription of *MyoD* and other myogenic genes in injected nuclei

We next asked whether the oocyte activates myogenic genes in transplanted non-muscle nuclei. We first prepared nuclei from mouse embryonic stem (ES) cells, which have great transcriptional plasticity and might therefore be a cell type in which muscle gene transcription can be easily induced. Transcripts of mouse muscle genes in oocytes were measured by RT-PCR at several time points after nuclear injection. Fig. 2A shows the induction of the genes that encode MyoD, myogenin and e-MHC in oocytes injected with mouse ES cell nuclei. We then tested the activation of myogenic genes in the mouse C3H10T1/2 fibroblast cell line. This cell line has the ability to differentiate into myogenic, chondrogenic and osteogenic lineages. We consider these cells to be in an intermediate differentiation state because they are multipotent but do not have the plasticity of ES cells. After C3H10T1/2 nuclear injection in oocytes, we again saw the activation of the genes that encode MyoD, myogenin and e-MHC as in ES cell nuclei-injected oocytes (Fig. 2B). In order to test whether the myogenic genes are activated only when we use a pluripotent cell line like ES cells or a partially differentiated cell line like C3H10T1/2 cells, we tested the reactivation of the myogenic genes in thymocytes taken directly from adult tissue. We observed that the genes encoding MyoD, myogenin and e-MHC are also strongly activated in this case (Fig. 2C). The *Gapdh* housekeeping gene was also activated in injected thymocyte nuclei, from a low initial expression level in donor cells.

It is important to know whether the muscle gene activation we see is at a trivial 'leakage' level, or is substantial and well above the level that might be seen in non-muscle cells. A time course of gene activation in transplanted nuclei from ES, C3H10T1/2 and thymus cell nuclei showed an enormous increase in transcript levels over 2-3 days, from time zero to 48 or 72 hours (Fig. 3A-E). The increase in MyoD transcripts ranged from 50- to nearly 100-fold. Myogenin increased by 7- to 20-fold. To confirm the increases shown in Fig.

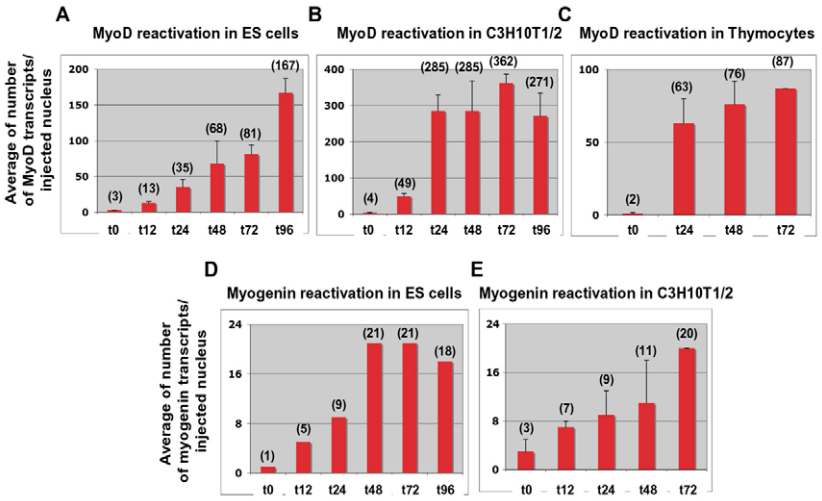

Fig. 3. Quantitative RT-PCR analysis of myogenic transcripts in injected oocytes using different donor cell types. MyoD and myogenin transcripts are reactivated in ES (**A**,**D**), C3H10T1/2 (**B**,**E**) and thymocyte (**C**) donor nuclei after transplantation in *Xenopus* oocytes. The *y*-axis shows the absolute numbers of transcripts per injected nucleus normalised against GAPDH. The *x*-axis shows the time points (hours) after nuclear injection. Each bar represents the mean value±s.d. of the number of transcripts detected. Apart from in D (where a single experiment was carried out), the value in brackets above each time point is the average from three independent experiments. For determination of the number of transcripts, see Materials and methods.

3, we made serial dilutions of injected oocyte samples frozen at 72 hours in order to determine how much dilution could be sustained while still giving a signal above the time zero level. For example, skeletal actin transcripts can be diluted over 100-fold and still give a signal above the time zero point for oocytes with thymus nuclei (Fig. 4A). Fig. 4B summarises the magnitude of transcriptional activation, using this procedure, for a range of muscle genes in the three cell types. The results are in agreement with the conclusions from Fig. 3. It is particularly interesting that oocytes activate the expression of muscle genes by approximately the same amount as they activate expression of the *Oct4* pluripotency factor. Clearly, nuclear reprogramming by the *Xenopus* oocyte does not favour factors required for the pluripotent state.

Another measure of transcriptional activation is provided by directly comparing the level of muscle gene transcripts in injected oocytes with that of differentiated muscle cells. For this purpose, we determined the abundance of MyoD and myogenin transcripts in differentiated C2C12 myotube cells with the abundance of the same transcripts in oocytes containing C3H10T1/2 nuclei at different times after injection. This comparison is based on the typical injection of 250 C3H10T1/2 nuclei per oocyte. Fig. 4C shows that both MyoD and myogenin transcripts were nearly as abundant in nuclei injected into oocytes after 2-4 days as they were in differentiated C2C12 muscle cells on a per nucleus basis. Therefore, the level of activation of muscle genes in nuclei injected into oocytes is within a few fold of muscle gene transcripts in a muscle cell. We conclude that oocytes strongly activate transcription of muscle genes in injected nuclei.

Oocytes activate transcription of all myogenic genes directly

A possible mechanism by which the *Xenopus* oocyte could induce the transcription of muscle genes in injected nuclei is by initiating the transcription and translation of MyoD, so that the resulting MyoD protein would activate the transcription of downstream genes and of itself. As this model would require the synthesis of protein after injection of nuclei into oocytes, we incubated the oocytes in cycloheximide, an inhibitor of translation. The oocytes were incubated overnight in 10 μg/ml cycloheximide, then transferred into 3 μg/ml cycloheximide just prior to injection of nuclei (Gurdon et al., 1992). Injected MyoD mRNA was used as a control for cycloheximide activity because, if the cycloheximide treatment were

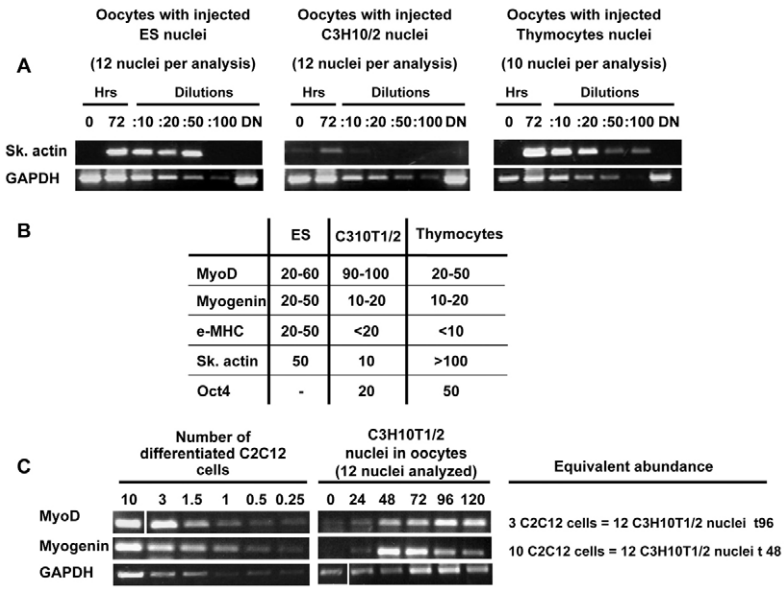

Fig. 4. Quantitation of muscle gene transcripts in injected oocytes. (**A**) Example of serial dilution of a 72 hour sample of injected *Xenopus* oocytes, analysed in this particular case for skeletal actin transcripts. For thymocyte nuclei, a 1/100 dilution still gives a signal above the zero hour value. DN, donor nuclei. (**B**) Summary of the increase in transcription for nuclei in oocytes 2-4 days after injection, based on serial dilution analysis as in A. (**C**) Transcription in nuclei as compared with whole donor cells injected into oocytes. Twelve C3H10T1/2 nuclei in oocytes at 96 hours give nearly the same MyoD signal as three donor C2C12 muscle cells. Twelve C3H10T1/2 nuclei at 48 hours give the same myogenin signal as ten C2C12 cells. These numbers have been estimated after normalisation against GAPDH. In this and in some supplementary figures, a white line is used to indicate that a band has been moved from a different gel track.

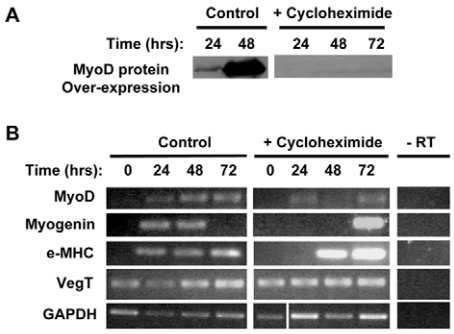

Fig. 5. Protein synthesis is not required for activation of MyoD downstream genes. (**A**) Western blot of protein produced from injected MyoD mRNA, showing that cycloheximide prevents protein production. Time points are hours after mRNA injection. (**B**) Semi quantitative RT-PCR analysis of C3H10T1/2 cell nuclei injected into control oocytes (left) or oocytes incubated in cycloheximide (right). The results show no change in activation of the genes encoding MyoD, myogenin and e-MHC in oocytes incubated in cycloheximide. Note that as not all *Xenopus* oocytes are capable of properly activating expression of all genes, some samples will occasionally be negative for expression of some genes, as is seen in this experiment (myogenin control, 72 hour time point).

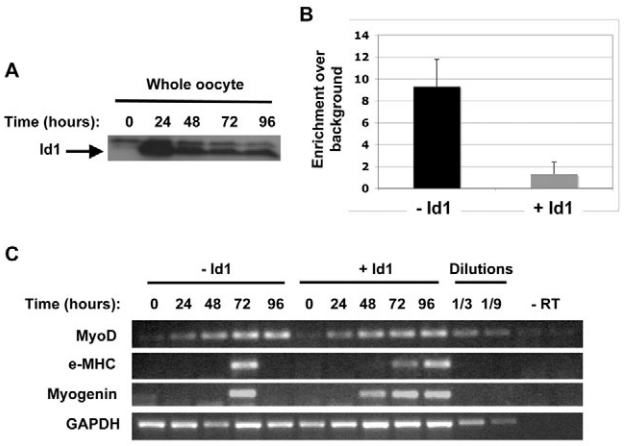

Fig. 6. Overexpressed Id1 abolishes MyoD binding. (**A**) Western blot of oocytes injected with 18 ng Id1 mRNA, using an antibody against Id1. Time points are hours after mRNA injection. All samples are from one whole oocyte. The lower band in the blot is Id1 (15 kDa). The upper band is a non-specifically interacting protein. (**B**) ChIP analysis of control oocytes (–Id1) and oocytes with overexpressed Id1 (+Id1). Cardiac actin promoter plasmid DNA was injected into the oocyte GV 24 hours after Id mRNA injection, followed by a further 24 hour incubation before fixation and ChIP analysis using an antibody against *Xenopus* MyoD. The ChIP signal is fold enrichment over background, as measured by real-time PCR on samples normalised for total DNA content. The background measurement is established by ChIP in the presence of an irrelevant antibody, and a value of one-fold enrichment corresponds to the background level. Two independent experiments were performed. (**C**) Id1 does not prevent muscle gene activation. RT-PCR analysis of oocytes injected with C3H10T1/2 cell nuclei. Control oocytes (–Id1) were not injected with mRNA. Id1-overexpressing oocytes (+Id1) were injected with 18 ng Id1 mRNA 24 hours before nuclear injection. Dilutions are of the 96 hour –Id1 sample.

effective, then no overexpressed protein would be seen in western blots. The result showed that injected MyoD mRNA is not translated in the presence of cycloheximide (Fig. 5A), indicating that cycloheximide effectively blocks protein synthesis in the oocytes. Despite this, expression of the genes that encode MyoD, myogenin and e-MHC was still activated from injected nuclei (Fig. 5B). Therefore, protein production by injected nuclei is not required for activation of muscle gene expression, thereby supporting the idea that the transcription of all tested myogenic genes is induced directly; this rules out the possibility that the necessary transcription factors are supplied by protein synthesis dependent on transplanted nuclei.

Transcriptional activation of myogenic genes does not depend on maternal MyoD content

It is possible that the transcription we observed in nuclear injections is driven by a maternal content of MyoD protein. In order to test whether the maternal MyoDa is responsible for activation of muscle gene transcription in injected nuclei, we overexpressed mouse Id1, a dominant repressor of MyoD, in oocytes prior to the injection of C3H10T1/2 cell nuclei. The Id family of proteins heterodimerises with bHLH transcription factors and prevents them from binding to their recognition sequences in gene promoters (Benezra et al., 1990). Id1 is known to bind to and sequester mouse MyoD with high affinity (Langlands et al., 1997). Western blot analysis confirmed that Id1 protein is expressed in oocytes injected with Id1 mRNA, and showed that Id1 is still detectable in the oocyte 96 hours after mRNA injection (Fig. 6A). Chromatin immunoprecipitation (ChIP) analysis with an anti-*Xenopus* MyoD antibody was used as a functional test for Id1 activity in preventing maternal MyoDa protein from binding to its consensus sequence. Plasmid DNA containing the consensus MyoD-binding sequence was injected into oocytes in the presence or absence of overexpressed Id1, and anti-MyoD ChIP used to detect whether Id1 prevents the endogenous MyoD from binding to the plasmid DNA. The results of two experiments, using real-time PCR against the consensus binding sequence on samples normalised for DNA concentration, showed that overexpressed Id1 abolishes the binding of maternal MyoDa to its consensus sequence (Fig. 6B). Furthermore, this experiment showed that there is indeed a maternal MyoD protein in the oocyte that is capable of binding its consensus sequence.

Having ascertained that overexpressed Id1 is functional in oocytes, we proceeded to overexpress Id1 in oocytes prior to injection of C3H10T1/2 cell nuclei (Fig. 6C). The overexpressed Id1 had no effect on the activation of the genes encoding MyoD, myogenin or e-MHC compared with injected control oocytes. Therefore, the sequestration of MyoD protein has no adverse effect on the activation of muscle gene expression in injected nuclei. This disproves the hypothesis that a maternal pool of MyoD protein in the oocyte is responsible for activation of muscle genes from injected nuclei. We conclude that the oocyte directly activates muscle genes in the absence of MyoD or other known maternal muscle gene-specific transcription factors. This is a unique characteristic of oocytes.

Overexpressed MyoD does not enhance the induction of its downstream genes

MyoD is a classic master transcription factor which, when overexpressed, is able to activate the transcription of other muscle genes in most cell types (Weintraub et al., 1989). Since we have shown that the oocyte does not inhibit muscle gene expression, we expected overexpressed MyoD to induce muscle gene

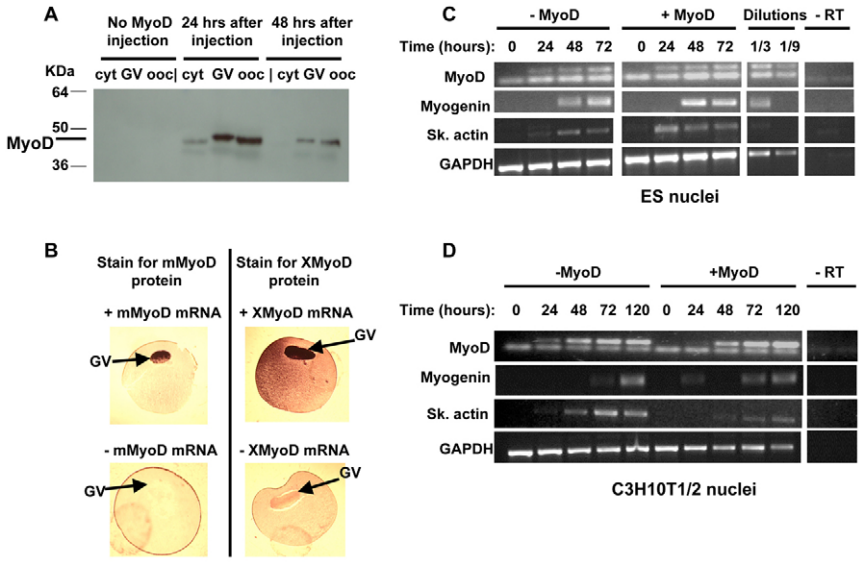

Fig. 7. Overexpression of MyoD protein in *Xenopus* oocytes. (**A**) The relative levels of MyoD protein in the cytoplasm and GV of the oocyte at intervals after 18 ng mouse MyoD mRNA injection. Each lane contains one quarter of a four-oocyte sample, and the GV and cytoplasm samples were taken from the same four oocytes. MyoD has a molecular weight of 43 kDa. GV, germinal vesicle; cyt, cytoplasm; ooc, whole oocyte. (**B**) Immunostained sections of oocytes injected with 18 ng mRNA for *Xenopus* MyoD (XMyoD) or mouse MyoD (mMyoD). (**C**) ES cell nuclei injected into oocytes transcribe MyoD, myogenin and skeletal actin mRNAs to the same extent whether or not oocytes were injected 24 hours earlier with MyoD mRNA. Note that the image of MyoD reactivation in ES cell nuclei without MyoD (–MyoD, top row) shows the same result as in Fig. 2A. (**D**) Same analysis as for C above, but with C3H10T1/2 nuclei.

expression in non-muscle nuclei injected into oocytes, if oocytes behave like somatic cells. We prepared nuclei from C3H10T1/2 and from mouse ES cells. Neither kind of cells was transcribing muscle genes at the time of nuclear transfer. Oocytes were injected with mouse MyoD mRNA, and we then waited until MyoD protein was strongly expressed in the oocyte germinal vesicle (GV) before injecting nuclei. By western analysis of isolated GVs and immunostaining of whole oocytes, we found that within 24 hours of mRNA injection, MyoD protein is highly expressed and much more concentrated in the GV than in the cytoplasm of oocytes (Fig. 7A,B). Expression of muscle genes in oocytes was measured by RT-PCR at several time points after nuclear injection. MyoD, myogenin and skeletal actin transcription was induced in both ES and C3H10T1/2 cell nuclei, even in the absence of overexpressed MyoD (Fig. 7C,D). However, overexpressed MyoD had little, if any, effect on transcription of myogenin or skeletal actin, both of which are muscle genes downstream of MyoD, in nuclei of either cell type (Fig. 7C,D). It is therefore clear that MyoD overexpression has no significant effect on the transcription of downstream muscle genes in the *Xenopus* oocyte.

It is known that the E2A enhancer binding (E) protein E12/E47 (Tcf3) heterodimerises with MyoD and augments its transcriptional transactivation activity (Lassar et al., 1991); this might be required for MyoD to have its downstream effects in oocytes. However, it has previously been shown that overexpression of E12/E47 with MyoD in the *Xenopus* oocyte does not enhance transcription from a muscle gene promoter in a plasmid reporter construct (Rashbass et al., 1992). We conclude that the *Xenopus* oocyte behaves differently from other cells because it transcribes muscle genes without a requirement for MyoD, and because it fails to enhance transcription of downstream muscle genes in response to overexpressed MyoD.

Oocytes activate genes for non-muscle lineages in C3H10T1/2 nuclei

The surprising finding that oocytes activate the MyoD and myogenin genes in injected non-muscle nuclei in the absence of endogenous or overexpressed MyoD raises the question of whether the oocyte also activates genes for other differentiation lineages. To test this, we used nuclei of C3H10T1/2 fibroblasts and thymocytes. We tested the transcription of genes of the haematopoietic lineage (embryonic γ-globin and adult β-globin) and the neural lineage [neurogenin and glutamic acid decarboxylase 1 (*Gad1*)]. This selection of genes was chosen to give a representative range of genes associated with either lineage specification or terminal differentiation for different differentiation pathways. We also tested transcription of the adult form of myosin heavy chain (a-MHC; Myh1) and the pluripotency gene *Oct4*. We injected C3H10T1/2 or thymocyte nuclei into oocytes, with no MyoD mRNA overexpression, and assayed transcripts over time by RT-PCR (Fig. 8A,B). The results showed that neural genes, including neurogenin and *Gad1*, are strongly transcribed. As expected, the pluripotency gene *Oct4* was activated, and the proto-oncogene *c-Jun*, used as a control for transcriptional activity of the oocyte, was upregulated. The amount of transcription of the neural genes was estimated as described above for myogenic genes (Fig. 4B). The increase in transcription of neurogenin and *Gad1* in oocytes injected with thymocyte nuclei was 20- to 50-fold and 50- to 100-fold respectively, and therefore seems to be substantial, as it is for the myogenic genes. Neurogenic gene transcription took place on the same time scale as for myogenic genes, that is within 48 hours of injection. However, not all genes are activated. We saw no activation of the genes encoding a-MHC, γ-globin or adult β-globin. We also injected differentiated muscle myotube nuclei and C2C12 myoblast nuclei into oocytes and found the pattern of gene activation in these nuclei to be the same as in the other types of nuclei we tested (see Fig. S3 in the supplementary material).

As a further control, we considered the possibility that cells might contain low levels of transcripts for non-MyoD myogenic genes and neural genes. These transcripts might be lost when permeabilising cells to make nuclei for injection, but then gradually replaced by nuclei in oocytes. We would therefore see an increase in transcription from 0 to 48 hours, representing the replacement of lost transcripts rather than the activation of new transcription. To test this idea, we injected whole non-permeabilised C3H10T1/2 and thymus cells, as opposed to nuclei, into oocytes, but detected no myogenic and neural transcripts at time zero or later (see Fig. S4 in the supplementary material). The appearance of myogenic and neural transcripts therefore represents a true transcriptional activation and confirms the principle that oocyte-induced changes in transplanted nuclei require access of oocyte factors to nuclei rather than to cells.

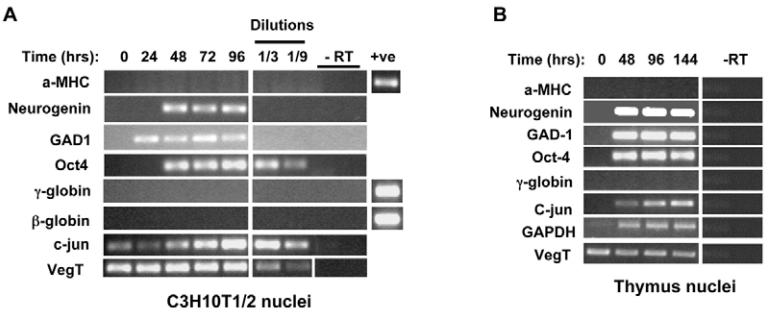

Fig. 8. Injected *Xenopus* oocytes transcribe many but not all non-muscle genes. RT-PCR analysis of gene reactivation in transplanted mouse C3H10T1/2 nuclei (**A**) and thymus nuclei (**B**). *Gad1*, *Oct4* and neurogenin represent examples of non-myogenic genes reactivated in somatic nuclei after nuclear transfer. *a-MHC*, γ-globin and β-globin are examples of genes that were not reactivated. +ve, positive control of 1000 differentiated muscle or erythroid cells. –RT, negative control without reverse transcriptase. Dilutions 1/3 and 1/9 represent samples collected 96 hours after nuclear transfer and diluted 3 and 9 times, respectively, to check for the linearity of response.

In order to test whether transcription is correctly initiated in injected nuclei, we tested for transcription of the region just upstream of the transcription start site for the MyoD and myogenin genes (see Fig. S5 in the supplementary material). We found that this upstream region is not transcribed in either gene, demonstrating that transcription is initiated from the usual transcription start site in injected nuclei.

DISCUSSION

Our results show that the transcriptional characteristics of amphibian oocytes, as revealed by somatic cell nuclear injection, are very unusual and of a kind not so far seen in any somatic cells. Instead of specifically reactivating the expression of pluripotency factors in somatic nuclei, the *Xenopus* oocyte has the special ability to strongly activate the expression of genes for multiple lineages that are inappropriate for its developmental state. The level of this expression is very large and is well beyond that which could be ascribed to low-level leaky transcription, like that which has been previously observed in ES cells (Efroni et al., 2008) and in *Xenopus* blastulae (Rupp and Weintraub, 1991; Wakefield and Gurdon, 1983). The *Xenopus* oocyte appears to be unique in activating muscle gene expression with no requirement for MyoD or for any other known MRFs. It also appears to be exceptional in activating genes for multiple differentiation lineages, as well as pluripotency genes, in a single cell. Oocytes have a very poor ability to splice intermediate transcripts and therefore to express inappropriately transcribed genes at a protein level. The strong expression of MyoDa in normal oocytes might reflect an unknown non-muscle function.

Might this exceptional property of *Xenopus* oocytes have a broader relevance to germline cells in general? There is evidence that spermatocytes also express a range of genes at much higher levels than do somatic cells (Kleene, 2001; Eddy and O'Brien, 1998). During gametogenesis, germ cells undergo a process of widespread epigenetic erasure, which is important for acquisition of a totipotent state (Surani et al., 2007). This removal of epigenetic repression might result in the inappropriate transcription of many genes, and germ cells rely heavily on post-transcriptional regulation to prevent expression of lineage-specific genes at the protein level (Kleene, 2001; Seydoux and Braun, 2006). It has been shown that mutation of post-transcriptional regulators in the *C. elegans* gonad causes oocytes to form teratomas (Ciosk et al., 2006), supporting the idea that oocytes, and possibly germ cells in general, balance wide-ranging transcription with strong regulation at the post-transcriptional level (Azuara et al., 2006; Bernstein et al., 2006). We suggest that this characteristic might be important for opening up the genome to enable transcription of all the lineages that commence in early development.

We have found that some mouse genes, including adult globin, are not activated by the *Xenopus* oocyte. However, these genes continue to be transcribed if already transcriptionally active in the injected nuclei. This shows that the oocyte does not actively repress transcription of these genes. Rather, we suggest that the oocyte might be unable to efficiently erase some repressive epigenetic modifications, such as DNA methylation, of these genes in injected nuclei. This lack of gene repression is a further unusual characteristic of oocytes. In other nuclear reprogramming experiments, including those that involve cell fusion (e.g. Terranova et al., 2006) and all somatic nuclear transfer experiments to eggs that yield normal embryos, the extinction of previously active genes is commonly observed. Two previous publications from our laboratory relate to this question. De Robertis and Gurdon (De Robertis and Gurdon, 1977) found that proteins expressed by nuclei transplanted to oocytes are extinguished. However, this analysis was at the protein level and the extinction of proteins is consistent with the presence of only unspliced transcripts. Byrne et al. (Byrne et al., 2003) described an extinction of Thy1 when mouse thymus nuclei were injected into oocytes. In more recent work (I.S., unpublished) we find a low and slowly increasing level of Thy1 expression in oocytes injected with thymus nuclei. We have not reproduced the high level of Thy1 expression described by Byrne et al. at time zero, when we now use fully permeabilised (and therefore fully mRNA-depleted) thymus nuclei.

The novel insight we have described here, that *Xenopus* oocytes reprogram muscle gene expression without the assistance of known muscle transcription factors, represents a new kind of transcriptional control and provides a basis from which the reprogramming mechanism of the oocyte might be elucidated. A reprogramming mechanism that works independently of gene-specific transcription factors might be useful for the creation of patient-specific cells for cell replacement therapy without a need for transcription factor overexpression by viruses.

Acknowledgements

We thank Drs A. Surani, H. Blau and Paresh Vyas who kindly provided the ES cells, C3H10T1/2 cells and the G1E cells, respectively. This work was supported by the Wellcome Trust and by the 'Cells into Organs' Network of Excellence of the European Union. Deposited in PMC for release after 6 months.

Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/136/16/2695/DC1

References

Azuara, V., Perry, P., Sauer, S., Spivakov, M., Jorgensen, H. F., John, R. M., Gouti, M., Casanova, M., Warnes, G., Merkenschlager, M. et al. (2006). Chromatin signatures of pluripotent cell lines. *Nat. Cell Biol.* **8**, 532-538.

Benezra, R., Davis, R. L., Lockshon, D., Turner, D. L. and Weintraub, H. (1990). The protein Id: a negative regulator of helix-loop-helix DNA binding proteins. *Cell* **61**, 49-59.

Bernstein, B. E., Mikkelsen, T. S., Xie, X., Kamal, M., Huebert, D. J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K. et al. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315-326.

Blau, H. M., Chiu, C. P. and Webster, C. (1983). Cytoplasmic activation of human nuclear genes in stable heterocaryons. *Cell* **32**, 1171-1180.

Byrne, J. A., Simonsson, S., Western, P. S. and Gurdon, J. B. (2003). Nuclei of adult mammalian somatic cells are directly reprogrammed to oct-4 stem cell gene expression by amphibian oocytes. *Curr. Biol.* **13**, 1206-1213.
Carlsson, S. A., Ringertz, N. R. and Savage, R. E. (1974). Intracellular antigen migration in interspecific myoblast heterokaryons. *Exp. Cell Res.* **84**, 255-266.
Charbonnier, F., Gaspera, B. D., Armand, A. S., Van der Laarse, W. J., Launay, T., Becker, C., Gallien, C. L. and Chanoine, C. (2002). Two myogenin-related genes are differentially expressed in Xenopus laevis myogenesis and differ in their ability to transactivate muscle structural genes. *J. Biol. Chem.* **277**, 1139-1147.
Ciosk, R., DePalma, M. and Priess, J. R. (2006). Translational regulators maintain totipotency in the Caenorhabditis elegans germline. *Science* **311**, 851-853.
Davidson, E. H. (1986). *Gene Activity in Early Development*. Academic Press: New York.
De Robertis, E. M., Partington, G. A., Longthorne, R. F. and Gurdon, J. B. (1977). Somatic nuclei in amphibian oocytes: evidence for selective gene expression. *J. Embryol. Exp. Morphol.* **40**, 199-214.
Della Gaspera, B., Sequeira, I., Charbonnier, F., Becker, C., Shi, D. L. and Chanoine, C. (2006). Spatio-temporal expression of MRF4 transcripts and protein during Xenopus laevis embryogenesis. *Dev. Dyn.* **235**, 524-529.
Eddy, E. M. and O'Brien, D. A. (1998). Gene expression during mammalian meiosis. *Curr. Top. Dev. Biol.* **37**, 141-200.
Efroni, S., Duttagupta, R., Cheng, J., Dehghani, H., Hoeppner, D. J., Dash, C., Bazett-Jones, D. P., Le Grice, S., McKay, R. D., Buetow, K. H. et al. (2008). Global transcription in pluripotent embryonic stem cells. *Cell Stem Cell* **2**, 437-447.
Gurdon, J. B. (1962). The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J. Embryol. Exp. Morphol.* **10**, 622-640.
Gurdon, J. B., Brennan, S., Fairman, S. and Mohun, T. J. (1984). Transcription of muscle-specific actin genes in early Xenopus development: nuclear transplantation and cell dissociation. *Cell* **38**, 691-700.
Gurdon, J. B., Kao, K., Kato, K. and Hopwood, N. D. (1992). Muscle gene activation in Xenopus requires intercellular communication during gastrula as well as blastula stages. *Dev.* **Suppl.** 137-142.
Harvey, R. P. (1990). The Xenopus MyoD gene: an unlocalised maternal mRNA predates lineage-restricted expression in the early embryo. *Development* **108**, 669-680.
Humpherys, D., Eggan, K., Akutsu, H., Friedman, A., Hochedlinger, K., Yanagimachi, R., Lander, E. S., Golub, T. R. and Jaenisch, R. (2002). Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei. *Proc. Natl. Acad. Sci. USA* **99**, 12889-12894.
Jennings, C. G. (1992). Expression of the myogenic gene MRF4 during Xenopus development. *Dev. Biol.* **151**, 319-332.
Kablar, B., Krastel, K., Ying, C., Asakura, A., Tapscott, S. J. and Rudnicki, M. A. (1997). MyoD and Myf-5 differentially regulate the development of limb versus trunk skeletal muscle. *Development* **124**, 4729-4738.
Kleene, K. C. (2001). A possible meiotic function of the peculiar patterns of gene expression in mammalian spermatogenic cells. *Mech. Dev.* **106**, 3-23.
Langlands, K., Yin, X., Anand, G. and Prochownik, E. V. (1997). Differential interactions of Id proteins with basic-helix-loop-helix transcription factors. *J. Biol. Chem.* **272**, 19785-19793.
Lassar, A. B., Davis, R. L., Wright, W. E., Kadesch, T., Murre, C., Voronova, A., Baltimore, D. and Weintraub, H. (1991). Functional activity of myogenic HLH proteins requires hetero-oligomerization with E12/E47-like proteins in vivo. *Cell* **66**, 305-315.
McKnight, S. L. and Gavis, E. R. (1980). Expression of the herpes thymidine kinase gene in Xenopus laevis oocytes: an assay for the study of deletion mutants constructed in vitro. *Nucleic Acids Res.* **8**, 5931-5948.
Messenger, N. J., Kabitschke, C., Andrews, R., Grimmer, D., Nunez Miguel, R., Blundell, T. L., Smith, J. C. and Wardle, F. C. (2005). Functional specificity of the Xenopus T-domain protein Brachyury is conferred by its ability to interact with Smad1. *Dev. Cell* **8**, 599-610.
Nicolas, N., Gallien, C. L. and Chanoine, C. (1998). Expression of myogenic regulatory factors during muscle development of Xenopus: myogenin mRNA accumulation is limited strictly to secondary myogenesis. *Dev. Dyn.* **213**, 309-321.
Pomerantz, J. and Blau, H. M. (2004). Nuclear reprogramming: a key to stem cell function in regenerative medicine. *Nat. Cell Biol.* **6**, 810-816.
Rashbass, J., Taylor, M. V. and Gurdon, J. B. (1992). The DNA-binding protein E12 co-operates with XMyoD in the activation of muscle-specific gene expression in Xenopus embryos. *EMBO J.* **11**, 2981-2990.
Rupp, R. A. and Weintraub, H. (1991). Ubiquitous MyoD transcription at the midblastula transition precedes induction-dependent MyoD expression in presumptive mesoderm of X. laevis. *Cell* **65**, 927-937.
Seydoux, G. and Braun, R. E. (2006). Pathway to totipotency: lessons from germ cells. *Cell* **127**, 891-904.
Surani, M. A., Hayashi, K. and Hajkova, P. (2007). Genetic and epigenetic regulators of pluripotency. *Cell* **128**, 747-762.
Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. and Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861-872.
Terranova, R., Pereira, C. F., Du Roure, C., Merkenschlager, M. and Fisher, A. G. (2006). Acquisition and extinction of gene expression programs are separable events in heterokaryon reprogramming. *J. Cell Sci.* **119**, 2065-2072.
Wakefield, L. and Gurdon, J. B. (1983). Cytoplasmic regulation of 5S RNA genes in nuclear-transplant embryos. *EMBO J.* **2**, 1613-1619.
Weintraub, H., Tapscott, S. J., Davis, R. L., Thayer, M. J., Adam, M. A., Lassar, A. B. and Miller, A. D. (1989). Activation of muscle-specific genes in pigment, nerve, fat, liver, and fibroblast cell lines by forced expression of MyoD. *Proc. Natl. Acad. Sci. USA* **86**, 5434-5438.
Weintraub, H., Davis, R., Tapscott, S., Thayer, M., Krause, M., Benezra, R., Blackwell, T. K., Turner, D., Rupp, R., Hollenberg, S. et al. (1991). The myoD gene family: nodal point during specification of the muscle cell lineage. *Science* **251**, 761-766.
Wickens, M. P. and Gurdon, J. B. (1983). Post-transcriptional processing of simian virus 40 late transcripts in injected frog oocytes. *J. Mol. Biol.* **163**, 1-26.
Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J. and Melton, D. A. (2008). In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature* **455**, 627-632.