Xenopus laevis Oct-1 does not bind to certain histone H2B gene promoter octamer motifs for which a novel octamer-binding factor has high affinity

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ABSTRACT

Oct-1 and a second, previously unidentified octamer-binding protein (Oct-R) have been identified in extracts of *Xenopus laevis* oocytes and embryos. Oct-1 does not bind to the octamer motif associated with certain *Xenopus laevis* histone H2B gene promoters, whereas Oct-R binds well to this motif, but only in the sequence context of the H2B gene promoter.

However *Xenopus laevis* Oct-1 does not bind to the octamer motif associated with certain *Xenopus laevis* histone H2B gene promoters. We have identified a novel factor which binds well to this motif, but only in the sequence context of the histone H2B gene promoter.

INTRODUCTION

The octamer motif or its inverse complement is a conserved promoter/enhancer sequence element involved in the transcriptional regulation of a range of genes with diverse patterns of expression (1). For example, this element directs the lymphoid-specific expression of immunoglobulin genes (2, 3), the ubiquitous expression of snRNA, genes and the ubiquitous expression of histone H2B genes at S-phase of the cell-cycle (4, 5).

The mechanism by which the octamer motif is able to regulate the transcription of diversely expressed genes has been partly resolved by the discovery and cloning of two human octamer-binding transcription factors, Oct-1 and Oct-2 (6, 7, 8, 9, 10, 11). Oct-2 is predominately expressed in B-cells (Oct-2 message is also detected in neural tissue (12), and complexes of similar mobility can be seen in band shifts with extract from other mouse tissues (13)) and involved in the B-cell specific expression of immunoglobulin genes (8, 14, 15). Oct-1 is ubiquitous and necessary for the ubiquitous expression of snRNA and histone H2B genes, although it is not sufficient for the stimulation of H2B transcription at S-phase (16, 5).

Sequence comparisons of Oct-1, Oct-2, the rat Pit-1 transcription factor and the *unc*-86 gene of *C. elegans* identified a highly conserved sequence motif known as the POU-Domain (17). The POU-Domain of Oct-1 and Oct-2 is responsible for recognition of and binding to the octamer motif(18, 19). The POU-Domain contains a POU-specific box and a homeo box. The homeo box was originally identified as a sequence motif shared by *Drosophila* homeotic proteins (20).

We have previously reported the isolation of a *Xenopus laevis* cDNA homologue of Oct-1 (21). Mammalian Oct-1 has been implicated in the transcriptional regulation of histone H2B genes.

MATERIALS AND METHODS

Preparation of Oct-1 fusion protein constructs

The *Xenopus laevis* Oct-1 cDNA (21) with *Eco* RI linkers was subcloned into M13mp18 and oligonucleotide directed mutagenesis (22) used to convert the sequence AGATGC at nucleotide 196 (15 amino acids downstream of the first in frame methionine) to a *Bgl* II site (AGATCT). A fragment from the *Bgl* II site to the *Bam* HI site in the Oct-1 sequence was subcloned into pET3B (23) to create pET-2. The *Xenopus laevis* Oct-1 cDNA with *Eco* RI linkers was subcloned into pBluescript SK⁻ (Stratagene) and a fragment from the *Bam* HI site in the Oct-1 sequence to the *Bam* HI site at the 3' end of the cDNA in the pBluescript SK⁻ polylinker was subcloned into the *Bam* HI site of pET-2 to create pET-1. pET-2 was cut with *Bam* HI (at the 3' end of the cDNA insert) and *Stu* I (internal to the cDNA), the *Bam* HI end blunted with the Klenow fragment of DNA polymerase I and the ends ligated to create pET-3. A fragment from the *Bgl* II site of the mutated Oct-1 cDNA in M13mp18 to the *Eco* RI site at the 3' end of the cDNA was subcloned into pBluescript SK⁻ to create pBS-1.

The Oct-1 cDNA is in frame with gene 10 of phage T7 in pET constructs, and will produce a fusion protein in the *E. coli* strain BL21 (DE3) which expresses T7 RNA polymerase (24). The Oct-1 cDNA in pBS-1 is in frame with the beta-galactosidase gene of pBluescript SK⁻.

Cell culture

L929 mouse cells (Ltk⁻) (25) were maintained at 37°C in Glasgow modified Eagle medium (GMEM) supplemented with 10% foetal calf serum, penicillin (10μg/ml) and streptomycin (10μg/ml).

Xenopus laevis Xtc cells (26) were maintained at 25°C in GMEM NEAA supplemented as above.

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For serum starvation subconfluent Xtc cells were washed twice in GMEM and then maintained at 25°C in unsupplemented medium for 18 hours. Supplemented medium was then added back and the cells maintained at 25°C for 6 hours.

For hydroxyurea treatment the inhibitor was added to 5mM in the culture medium of subconfluent Xtc cells for 90 minutes.

Preparation of extract for band shift assays

All extracts were made in extract buffer: 50mM Tris-HCl pH8, 50mM KCl, 0.1mM EDTA, 5mM MgCl₂, 10μg/ml aprotinin, 10μg/ml leupeptin, 25% (v/v) glycerol.

(a) From *Xenopus laevis* oocytes and embryos. Oocytes and embryos were homogenised on ice in 5μl of extract buffer per oocyte/embryo, microfuged at 4°C for 10 minutes and the supernatant snap frozen in liquid nitrogen. Enucleated oocyte extract was prepared in the same way from oocytes from which the nuclei had been removed by manual dissection.

(b) From cultured cells. Subconfluent cells were washed in phosphate buffered saline (PBS) and scraped from the surface of the 75cm² flask in 10mls of ice cold PBS, pelleted and resuspended in 30μl of extract buffer per flask. The suspended cells were homogenised on ice, microfuged at 4°C for 10 minutes and the supernatant snap frozen in liquid nitrogen. When levels of binding proteins were to be compared the protein concentration of the extract was measured (Biorad protein detection kit) and equalised at 5mg/ml.

(c) From *E. coli* expressing fusion proteins. *E. coli* BL21 (DE3) carrying pET constructs and *E. coli* TG2 carrying pBS-1 were grown to A₆₀₀ = 1.5, pelleted and resuspended in 0.01 volumes of extract buffer. The suspended cells were sonicated on ice, microfuged at 4°C for 10 minutes and the supernatant snap frozen in liquid nitrogen.

Band shift assays

25μl binding reactions were carried out on ice for 20 minutes in binding buffer (45mM KCl, 15mM HEPES pH7.9, 5mM spermidine, 1mM MgCl₂, 1mM dithiothreitol, 0.5mM PMSF, 0.1mM EDTA, 7% glycerol) containing 2μg of pAT153, 1μg of salmon sperm DNA, 20ng of non-specific duplex oligonucleotide (ACAGACCGAAGCTTAGCT), 0.5ng of duplex oligonucleotide probe (25-mers, end labelled with γ-³²P-ATP and T4 polynucleotide kinase, sequences are given in legends to figures 3 and 4), up to 5μl of protein extract and (when added) 1μl of antiserum. For competition analysis the non-specific duplex oligonucleotide probe was replaced by 20ng of specific duplex oligonucleotide.

The reactions were electrophoresed on a 5% polyacrylamide gel (29:1 bis) in Tris-borate buffer (22.5mM Tris-borate pH8, 0.5mM EDTA) at 200 volts and 4°C for 2 hours. The gel was fixed, dried and exposed to X-ray film with an intensifying screen for 2—12 hours at —70°C.

Generation of anti *Xenopus laevis* Oct-1 antiserum

The pET-3 fusion protein was purified by preparative SDS-polyacrylamide gel electrophoresis for use as an antigen. *E. coli* BL21 (DE3) carrying pET-3 was grown at 37°C to A₆₀₀=0.8, at which point IPTG was added to 1mM to induce T7 RNA polymerase and growth continued for a further 3 hours. The cells were pelleted, resuspended in SDS-loading buffer, sonicated, boiled for 3 minutes and the proteins resolved on a SDS polyacrylamide gel.

The T7 gene 10—Oct-1 fusion protein was visualised by briefly staining the gel with 0.05% Coomassie blue in water. Gel slices

were ground with a pestle and mortar and, for the first injection, emulsified with Freund's complete adjuvant (27). For subsequent injections, slices were emulsified with Freund's incomplete adjuvant. Pre-immune serum was taken from a New Zealand White rabbit before the first injection. The rabbit was given 3 injections of 50—100μg of protein at 2 week intervals, and 2 weeks after the third injection immune serum was taken.

RESULTS

The *Xenopus laevis* Oct-1 cDNA is a functional octamer-binding protein

We have previously reported the nucleotide sequence of a *Xenopus laevis* homologue of the human octamer-binding transcription factor, Oct-1, isolated from a mature folliculated oocyte cDNA library (21). A *Bgl* II site was created by oligonucleotide directed mutagenesis just downstream of the first in frame methionine (figure 1) and this site was used to ligate into the *Bam* HI site of pET3B (23) or pBluescript SK⁻ to produce T7 gene 10 or beta-galactosidase fusion proteins in *E. coli*. pET-1 and pBS-1 contain Oct-1 sequence from the *Bgl* II site to the 3' end of the cDNA clone, and pET-2 and pET-3 from the *Bgl* II site to *Bam* HI and *Stu* I sites, respectively, within the Oct-1 coding sequence.
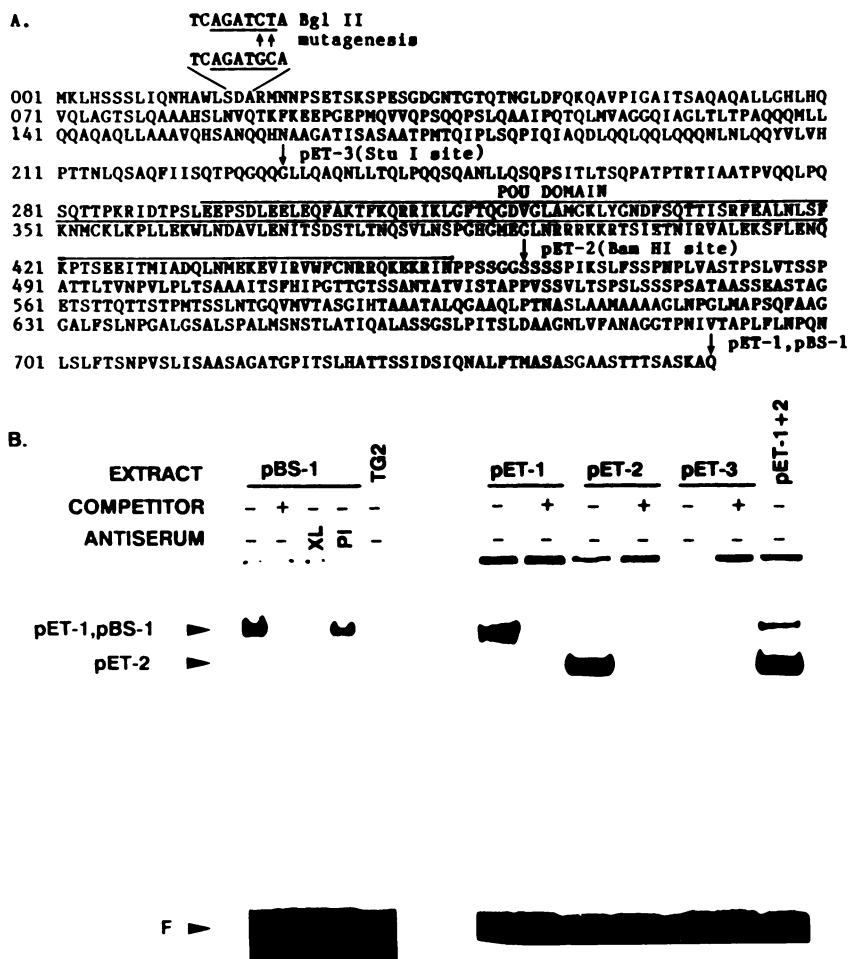
Extract from *E. coli* expressing pET-1, pET-2 and pBS-1 bind to an octamer probe in band shift assays (figure 1), and as expected the pET-2 extract gives a higher mobility shifted band, since the Oct-1 sequence contained in this construct is deleted at the C-terminal end. This binding is specific as it can be competed-out by inclusion of an excess of unlabelled probe in the binding reaction. As controls, extract from the host *E. coli* strain (TG2) of pBS-1 and from *E. coli* expressing pET-3 (which does not contain the DNA binding POU-Domain) do not bind to the octamer probe in band shift assays. An antiserum raised against the pET-3 fusion protein included in the pBS-1 band shift assay inhibits formation of the Oct-1—DNA complex, whereas pre-immune serum does not.

Functional expression of Oct-1 in *E. coli* suggests that no other factors are required for DNA binding activity. Mixing of pET-1 and pET-2 extracts in band shift assays gives two strong shifted bands of the mobility seen in unmixed extracts (figure 1). This shows that Oct-1 binds to the probe as a monomer, since if Oct-1 bound as a dimer a third band of -intermediate size corresponding to a pET-1:pET-2 heterodimer would be expected.

Oct-1 and a second octamer binding protein can be detected in *Xenopus laevis* oocyte extract

Band shift assays with an octamer probe and oocyte protein extract detect two specific bands as a result of octamer binding proteins. Specific binding can be competed-out by inclusion of an excess of unlabelled probe in the binding reaction (figure 2).

The upper specific band is of a similar mobility to the pBS-1 fusion protein band. The pBS-1 fusion protein is of similar size to the protein encoded by the Oct-1 cDNA (in pBS-1 16 amino acids of Oct-1 coding sequence are replaced by 31 amino acids of the beta-galactosidase gene of pBluescript SK⁻, resulting in a protein of 775 residues rather than 760). Formation of the upper band (and not the lower band or the non-specific binding) is inhibited by inclusion of antiserum raised against the pET-3 Oct-1 fusion protein in the binding reaction, and by inclusion of an antiserum raised against a full-length human Oct-1 fusion protein (11) in the binding reaction.

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                    TCAGATCTA Bgl II
                         ↓↓ mutagenesis
                    TCAGATGCA
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001 MKLHSSSLIQNHAWLSDARMNNPSETSKSPESGDGNTGTQTNGLDFQKQAVPIGAITSAQAQALLGHLHQ
071 VQLAGTSLQAAAHSLNVQTKFKEEPGEPMQVVQPSQQPSLQAAIPQTQLNVAGGQIAGLTLTPAQQQHLL
141 QQAQAQLLAAAVQHSANQQHNAAGATISASAATPMTQIPLSQPIQIAQDLQQLQQLQQQNLNLQQYVLVH
                  ↓ pET-3(Stu I site)
211 PTTNLQSAQFIISQTPQGQQGLLQAQNLLTQLPQQSQANLLQSQPSITLTSQPATPTRTIAATPVQQLPQ
                  POU DOMAIN
281 SQTTPKRIDTPSLEEPSDLEELEQFAKTFKQRRIKLGFTQCDVGLAMGKLYGNDFSQTTISRFEALNLSF
351 KNHCKLKPLLEKWLNDAVLENITSDSTLTMQSVLNSPGHGHEGLNRRRKRTSISTNIRVALEKSFLENQ
                  ↓ pET-2(Bam HI site)
421 KPTSEEITHIADQLNMEKEVIRVWFCNRRQKEKRIHPPSSGGSSSSPIKSLFSSPHPLVASTPSLVTSSP
491 ATTLTVNPVLPLTSAAAITSFHIPGTTGTSSANTATVISTAPPVSSVLTSPSLSSSPSATAASSEASTAG
561 ETSTTQTTSTPMTSSLNTGQVMVTASGIHTAAATALQGAAQLPTNASLAAMAAAAGLNPGLMAPSQFAAG
631 GALPSLNPGALGSALSPALMSNSTLATIQALASSGSLPITSLDAAGNLVPANAGGTPNIVTAPLFLNPQN
                  ↓ pET-1,pBS-1
701 LSLFTSNPVSLISAASAGATGPITSLHATTSSIDSIQNALFTMASASGAASTTTSASKAQ
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B.

Figure 1. Oct-1 sequence contained in fusion protein constructs and their binding to a probe containing an octamer motif analysed by band shift assays. (A) Oct-1 fusion proteins contain Oct-1 sequence extending from the *Bgl* II site made by oligonucleotide directed mutagenesis to the position on the *Xenopus laevis* Oct-1 amino acid sequence indicated by the name of the construct (pET-1, pET-2, pET-3 and pBS-1). The location of the POU (DNA binding) domain is shown. (B) Band shift assays with extract from *E. coli* expressing the fusion protein constructs indicated, and a probe containing an octamer motif (oct probe, see legend to figure 3). Competitor (an excess of unlabelled probe) and antiserum (XL = anti *Xenopus laevis* Oct-1; PI = pre-immune serum) were included in the binding reaction where indicated. TG2 extract is from the host *E. coli* strain of pBS-1. The positions of undegraded pBS-1, pET-1 and pET-2 complexes and free probe (F) are indicated.

From these results we conclude that the upper band seen in band shift assays with oocyte extract is due to binding of Oct-1. The second higher mobility band is as a result of binding of a previously unidentified octamer-binding factor, which is antigenically distinct from Oct-1. We have called this factor Oct-R (for *octamer-related*). Since in band shift assays no band corresponding to Oct-1 and Oct-R binding together is seen, binding of one factor excludes binding of the second. This implies that Oct-1 and Oct-R binding sites are the same or overlapping. As will be shown below, Oct-R binding is dependent on the octamer motif of the band shift assay probe. The name Oct-R does not imply that Oct-R is necessarily related to Oct-1, or is a member of the superfamily of POU domain proteins.

Oct-1 and Oct-R have different binding properties

In *Xenopus laevis* histone H2B gene promoters the octamer motif occurs as the core of an extended consensus sequence known as the H2B-box (figure 7). The most common octamer motif in the *Xenopus laevis* H2B-box is not the perfect octamer (ATTTGCAT) but a 7 out of 8 match (GTTTGCAT). We have tested the binding of Oct-1 and Oct-R to these octamer motifs in the context of the *Xenopus laevis* H2B-box (oct probe =

ATTTGCAT, H2B probe = GTTTGCAT in the H2B box context) and in the context of the mouse immunoglobulin heavy chain enhancer (k.oct probe = ATTTGCAT, k.H2B probe = GTTTGCAT in the heavy chain enhancer context). The results are shown in figure 3.

As shown above, the oct probe (perfect octamer in H2B box) binds both Oct-1 and Oct-R, and binding of one factor excludes binding of the second. The H2B oligo (7/8 octamer in H2B box) is a poor competitor for Oct-1 binding, but an efficient competitor for Oct-R binding (figure 3A). Correspondingly Oct-R binds well to the H2B probe and Oct-1 does not detectably bind. Oct-R binding to the H2B probe is stronger than to the oct probe (figure 3B) (with the H2B probe a faint, variable and not efficiently competable band is seen between Oct-1 and Oct-R positions. This binding is not inhibited by the anti Oct-1 antisera). The binding properties of the Oct-1 fusion protein are similar to Oct-1 in oocyte extract. The Oct-1 fusion protein binds well to the Oct probe, but does not detectably bind to the H2B probe. Oct-R binding is clearly dependent on the base of the of the octamer motif which differs between Oct and H2B probes (and thus does not bind solely to sequences flanking the octamer motif) since Oct-R binds significantly more strongly to the H2B probe than
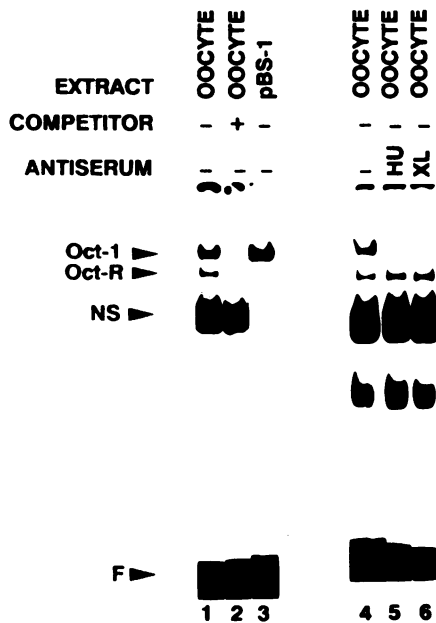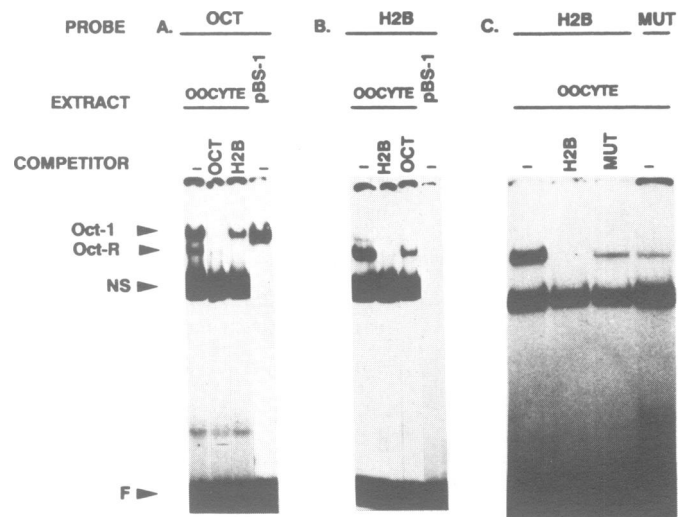
Figure 2. Band shift assays showing that oocyte extract contains Oct-1 and a second octamer-binding protein, Oct-R. The probe contains an octamer motif (oct probe, see legend to figure 3) and extract is from *Xenopus laevis* oocytes, except pBS-1 extract (from *E. coli* expressing the pBS-1 fusion protein). Assays in lanes 5 and 6 contain antiserum (XL = anti *Xenopus laevis* Oct-1; HU = anti human Oct-1). Lane 2 contains an excess of unlabelled probe in the binding reaction as competitor. The positions of Oct-1, Oct-R and non-specific complexes (NS) and free probe (F) are indicated.

Figure 3. Comparison of the binding affinities of Oct-1 and Oct-R for different octamer motifs in the sequence context of the H2B gene promoter using band shift assays. The Oct oligo contains the perfect octamer in the context of the histone H2B gene promoter (GAGCTGCCTCATTTGCATGGGAGGA). The H2B oligo contains a 7 out of 8 match octamer motif in the context of the histone H2B gene promoter (GAGCTGCCTCGTTTGCATGGGAGGA). The mut oligo is the same sequence as the H2B oligo except with two point mutations introduced into the octamer motif (GAGCTGCCTCGGTTGAATGGGAGGA). Protein extract is from *Xenopus laevis* oocytes, except pBS-1 extract (from *E. coli* expressing the pBS-1 fusion protein). A to C show band shifts using the oligos indicated as probe, and with the unlabelled oligos indicated as competitor (40 fold molar excess). The positions of Oct-1, Oct-R and non-specific complexes (NS) and free probe (F) are indicated.

the Oct probe. The further dependence of Oct-R binding on the Octamer motif was demonstrated by using the H2B probe with two point mutations in the octamer motif (mut probe, GTTTGCAT > GGTTGAAT) as probe and competitor in band shift assays (figure 3C). The mut probe binds Oct-R far less efficiently than the H2B probe. Correspondingly, the cold mut oligo is not as effficient a competitor for Oct-R binding as the H2B oligo. Some Oct-R binding to the mut probe is detectable, and this explains the significant competition seen with this oligo in large molar excess (40 times) over the H2B probe.
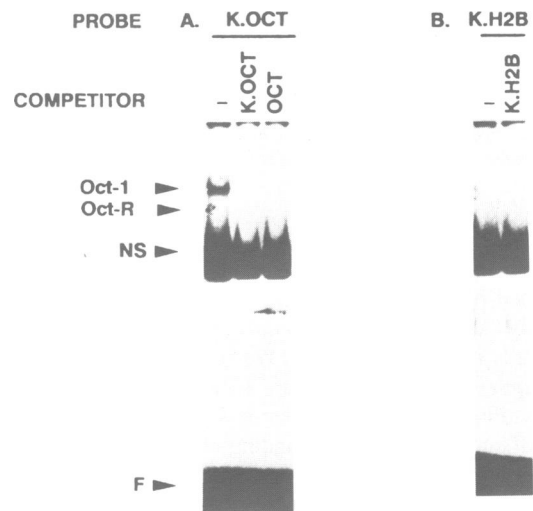
The k.oct probe (perfect octamer in heavy chain enhancer) detectably binds only Oct-1. The k.H2B probe (7/8 octamer in heavy chain enhancer) does not detectably bind Oct-1 or Oct-R (figure 4). In summary, Oct-1 binds well only to the perfect octamer motif, and binds this motif regardless of the sequence context. Oct-1 does not detectably bind to the 7/8 octamer motif most often found in *Xenopus laevis* histone H2B gene promoters. Oct-R binds well to both the perfect and 7/8 octamer motifs (significantly more strongly to the 7/8 octamer motif), but only in the sequence context of the histone H2B gene promoter. The data presented here enable us to make the above conlusions about the relative affinity of Oct-1 and Oct-R for different oligos. However, since we do not know the absolute amounts of Oct-1 and Oct-R present (but simply the amount of binding activity) it is not possible to compare the relative binding affinity of Oct-1 and Oct-R.
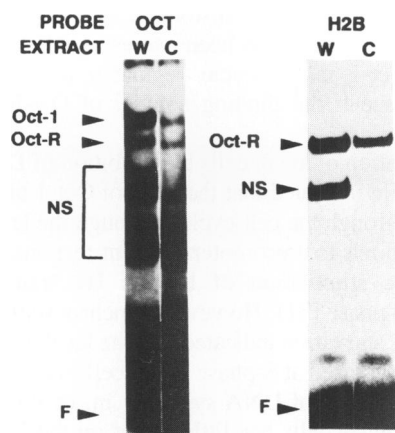
Location of Oct-1 and Oct-R in the oocyte

Band shift assays were carried out using extract prepared from whole and manually enucleated oocytes (figure 5). These assays indicate that approximately 25% of Oct-1 and 50% of Oct-R present in the oocyte is located in the cytoplasm (enucleated

Figure 4. Comparison of the binding affinities of Oct-1 and Oct-R for different octamer motifs in the sequence context of the mouse immunoglobulin heavy chain enhancer using band shift assays. The k.Oct oligo contains the perfect octamer in the context of the heavy chain enhancer (CACCTGGG-TAATTTGCATTTCTAAA). The k.H2B oligo contains a 7 out of 8 match octamer motif in the context of the heavy chain enhancer (CACCTGGG-TAGTTTGCATTTCTAAA). The sequence of the Oct oligo is given in the legend to figure 3. A and B show band shifts using the oligo indicated as probe, and with the unlabelled oligo indicated as competitor (40 fold molar excess). Protein extract is from *Xenopus laevis* oocytes. The positions of Oct-1, Oct-R and non-specific complexes (NS) and free probe (F) are indicated.

Figure 5. Location of Oct-1 and Oct-R in *Xenopus laevis* oocytes determined by band shift assays. Oct and H2B probes (see legend to figure 3) were used to maximise Oct-1 and Oct-R binding, respectively. W indicates whole oocyte extract and C indicates cytoplasmic (enucleated oocyte) extract. Extract from the equivalent of one oocyte or cytoplasm was used in each assay. The positions of Oct-1, Oct-R and non-specific complexes (NS) and free probe (F) are indicated.
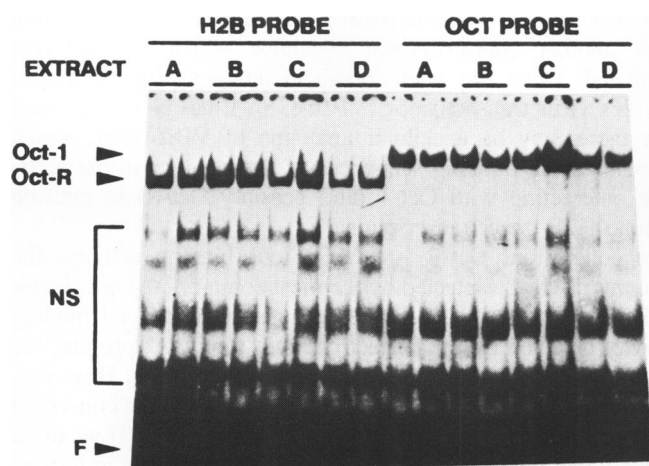
Figure 6. Band shift assays to determine the levels of Oct-1 and Oct-R in *Xenopus laevis* Xtc cells treated with hydroxyurea or serum-starved. Oct and H2B probes (see legend to figure 3) were used to maximise Oct-1 and Oct-R binding, respectively. Duplicate flasks of cells were treated, and assays with extracts from these flasks are bracketed together. Extracts A to D were from cells treated as follows: (A) Untreated growing cells (B) Treated with 5mM hydroxyurea for 90 minutes (C) Serum-starved for 18 hours and then serum added back for 6 hours (D) Serum starved for 18 hours. The positions of Oct-1, Oct-R and non-specific complexes (NS) and free probe (F) are indicated.

oocyte). A variable amount of Oct-1 and Oct-R binding activity was found in isolated nuclei (not shown). We suspect that the variation is due to leakage of these proteins from the nucleus during isolation of the nuclei.

Levels of Oct-1 and Oct-R in cells in which DNA synthesis has been inhibited and in cells which have been serum-starved

Mammalian Oct-1 has been implicated in the control of the octamer-dependent stimulation of histone H2B transcription on entry into S-phase of the cell-cycle (16). Consequently there has been interest in whether the binding activity of Oct-1 is cell-cycle regulated.

Figure 7. *Xenopus laevis* histone gene promoter H2B-boxes, with core octamer motif, aligned to each other and to oligos used as probes (see legends to figures 3 and 4) in band shift assays. The name of the *Xenopus laevis* histone gene cluster (37, 38) in which the H2B-box is found is given in the left-hand column and the name of the band shift probe in which these sequences are found in the right-hand column.

Xenopus laevis Xtc cells were treated with hydroxyurea, which inhibits DNA synthesis. The levels of Oct-1 and Oct-R (determined by band shift assays, see figure 6) show no significant change following this treatment. We have previously shown that treatment of Xtc cells with hydroxyurea causes a rapid reduction in histone mRNA levels to 25% of that seen in untreated cells (28).

Xtc cells were serum starved to block at the beginning of G1 phase of the cell-cycle, and then released from the block. The levels of Oct-1 and Oct-R (determined by band shift assays, see figure 6) show no significant change in serum starved cells relative to growing cells and adding complete medium back to starved cells resulted in no change in levels relative to starved cells.

DISCUSSION

The sequence of the *Xenopus laevis* homologue of the human octamer-binding transcription factor, Oct-1, isolated from an oocyte cDNA library shows that this protein is strongly conserved in vertebrate evolution (the predicted amino acid sequence shows 85% identity to human Oct-1). The *Xenopus laevis* Oct-1 cDNA presumably encodes a protein of similar function to human Oct-1. Synthetic message from *Xenopus laevis* Oct-1 cDNA expressed in micro-injected oocytes encodes a protein of the size predicted from the DNA sequence (not shown) and band shift assays with Oct-1 fusion proteins expressed in *E. coli* demonstrate that the cDNA encodes an octamer-binding protein. Functional expression in *E. coli* suggests that Oct-1 alone is sufficient for DNA binding, and mixing of 'full-length' and shortened Oct-1 fusion proteins in band shift assays suggests that Oct-1 binds as a monomer, since no band corresponding to a heterodimer is observed.

Band shift assays detect two octamer-binding proteins in *Xenopus laevis* oocytes. The criteria of mobility (one of the specific bands in band shift assays with oocyte extract is of similar mobility to the band produced by binding of 'full-length' Oct-1 fusion proteins) and inhibition of binding in band shift assays by two polyclonal anti Oct-1 antisera identify one of the octamer binding proteins as Oct-1. We have termed the second octamer-binding protein Oct-R (for *octamer-related*). Oct-1 and Oct-R are antigenically distinct, as revealed by inhibition of binding by polyclonal antisera raised against fusion proteins spanning the

N terminal domain of *Xenopus laevis* Oct-1, and full-length human Oct-1 (11). This means that the possibility that the higher mobility Oct-R band is a proteolytic cleavage product of Oct-1 is highly unlikely. Oct-R binding activity is also detected in a range of different extracts (oocytes, embryos, cultured Xtc cells and several adult somatic tissues tested), and the amount of Oct-R binding activity detected by the H2B probe is consistently roughly equivalent to the amount of Oct-1 binding activity detected by the Oct probe (not shown).

Oct-1 and Oct-R have different binding properties. In *Xenopus laevis* histone H2B gene promoters the octamer motif occurs as the core of a longer consensus sequence known as the H2B-box (figure 7). The most common octamer motif in the H2B-box is a 7 out of 8 match (GTTTGCAT) to the canonical octamer (ATTTGCAT). We have tested the binding of Oct-1 and Oct-R to these two octamer motifs in the context of the H2B-box and in the context of the mouse immunoglobulin heavy chain enhancer. Oct-1 binds well to the perfect octamer in either context and has low affinity for the 7/8 octamer in either context. Oct-R binds both octamer motifs strongly (the 7/8 octamer more strongly) but only in the context of the H2B-box. Little Oct-R binding to either motif in the context of the heavy enhancer is seen.

Mammalian Oct-1 has been implicated in the control of the octamer-dependent stimulation of histone H2B transcription on entry into S-phase of the cell-cycle (16). However the closely related *Xenopus laevis* homologue of Oct-1 does not bind well to the octamer motif (7/8 octamer) associated with certain *Xenopus laevis* histone H2B gene promoters. In contrast Oct-R binds well to both the perfect octamer and 7/8 octamer (more strongly) but only in the context of the H2B box. The binding properties of Oct-R suggest that it may have a role in the histone H2B gene transcription in *Xenopus laevis*, although a functional assay for the activity of this factor is clearly required. The distributions of Oct-1 and Oct-R are consistent with a potential role as ubiquitously expressed gene transcription factors, being present throughout early development and in a range of adult tissues (data not shown). Although Oct-R binding requires the sequence context of the H2B box, the binding activity is clearly dependent on the octamer component of the H2B box. The possibility that, like the octamer binding factors so far cloned, Oct-R is a member of the POU domain family of proteins warrants further investigation. Our use of the name Oct-R is not intended to imply that Oct-R must necessarily be related to other members of the family of POU domain proteins. The polyclonal antiserum raised against a full-length human Oct-1 fusion protein inhibits Oct-1, but not Oct-R binding. This antiserum cross reacts with human Oct-2 (11) and hence presumably contains antibodies against the POU domain. This may imply that Oct-R is not a POU domain protein, or if it does contain a POU domain that the POU domain is significantly diverged from the Oct-1 POU domain.

Part of the Oct-1 and Oct-R present in the oocyte is located in the cytoplasm (approximately 50% of Oct-R and 25% of Oct-1). This could be to avoid effects such as squelching (29, 30). Too much Oct-1 or Oct-R in the nucleus may interact with and hence sequester other transcription factors.

The octamer motif is required for the S-phase specific stimulation of histone H2B gene transcription (4) and extracts from HeLa cells synchronised in S-phase of the cell-cycle are able to activate transcription *in vitro*, but extracts from HeLa cells synchronised in G2 are not. An Oct-1—containing fraction from HeLa cell extract purified by affinity for the octamer DNA

sequence is able to activate histone H2B transcription *in vitro* (16). Consequently there has been interest in whether the binding activity of Oct-1 is cell-cycle regulated and we were also interested to see if the binding activity of Oct-R is cell-cycle regulated.

Synchronisation of avian cells by inhibition of DNA synthesis with aphidicolin indicated that the level of Oct-1 binding activity did not vary through the cell-cycle, although the level of another factor which binds to a promoter element responsible for the S-phase specific stimulation of histone H1 transcription was increased at S-phase (31). However, synchronisation of hamster cells by serum starvation indicated that the level of Oct-1 binding activity was increased at S-phase of the cell-cycle (32). We have found that inhibition of DNA synthesis in, or serum starvation of, *Xenopus laevis* cells has little effect on the levels of Oct-1 and Oct-R binding activity relative to that seen in growing cells. It appears that the stimulation of H2B transcription on entry into S-phase of the cell-cycle may not be a result of periodic octamer factor binding, particularly since Oct-1 is also involved in the transcription of ubiquitously expressed genes which are not cell-cycle regulated (eg. snRNA). There may be an as yet undiscovered cell-cycle regulated modification of Oct-1 (and possibly Oct-R). Oct-1 is known to be phosphorylated and O-glycosylated (33, 34). Alternatively there may be an interaction with another transcriptional regulator which is cell-cycle regulated. Oct-1 has been shown to interact with the herpes simplex virus transactivator, VP16 (35). It has been suggested that there may be a cellular analogue of VP16 (36), which recognises the flanking sequences of the octamer in the H2B-box, interacting with Oct-1 (and possibly Oct-R) to activate histone H2B gene transcription.

In conclusion, Oct-R is a ubiquitous binding activity, the amount of which (detected by the most common *Xenopus laevis* H2B box) is roughly equivalent to the amount of Oct-1 binding activity detected by the perfect octamer motif. At present, we do not know whether Oct-R is a transcription factor. However, in view of the fact that Oct-R binds specifically to the conserved H2B box promoter element (to the exclusion of Oct-1) we think it likely that this protein may have an important role in histone H2B transcriprtion in *Xenopus laevis*.

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