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Pyruvate kinase isozyme type M2 (PKM2) interacts and cooperates with Oct-4 in regulating transcription

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Abstract

The *Oct-4* gene encodes a transcription factor that plays an important role in maintaining the pluripotent state of embryonic stem cells and may prevent expression of genes activated during differentiation. Although its role in maintaining embryonic stem cell pluripotency is well established, there is still little known about the binding partners that regulate its function. To identify proteins that control Oct-4 function, we used affinity chromatography on immobilized Oct-4 (POU) together with MALDI-TOF (matrix-assisted laser-desorption ionization-time-of-flight) MS (mass spectrometry) and isolated a novel Oct-4-interacting protein, pyruvate kinase type M2 (PKM2 or M2-PK). PKM2 is an isozyme of pyruvate kinase that is specifically expressed in proliferating cells, such as embryonic stem cells, embryonic carcinoma cells, as well as cancer cells. Oct-4 and PKM2 were co-affinity precipitated from cell extracts, and glutathione *S*-transferase pull-down assays revealed that the POU DNA binding domain of Oct-4 was required for interaction with PKM2. In addition, the C-terminal domain of PKM2 (amino acids 307–531) was involved in binding to Oct-4. Moreover, ectopic expression of the PKM2 enhanced Oct-4-mediated transcription. These observations indicate that the transactivation potential of the Oct-4 transcription factor is positively modulated by PKM2.

Keywords: Oct-4; PKM2; Embryonic stem cell; Self-renewal; Protein–protein interaction; Transactivation; Coactivator

1. Introduction

The *Oct-4* gene, also referred to as *Oct-3*, encodes a nuclear protein that belongs to a family of transcription factors containing the POU DNA binding domain (Burdon, Smith, & Savatier, 2002; Goto, Adjaye, Rodeck, & Monk, 1999; Hansis, Grifo, & Krey, 2000; Okamoto et al., 1990; Rosner et al., 1990; Scholer, Dressler, Balling, Rohdewohld, & Gruss, 1990). Members of the POU transcription factor family share a conserved DNA binding domain, the POU domain, originally identified in the transcription factors Pit-1, Oct-1, Oct-2, and Unc-86 (Herr & Cleary, 1995). Oct-4 protein is normally found in pluripotent cells including (i) unfertilized oocytes, (ii) early cleavage stage embryos, (iii) the inner cell mass of the blastocyst, (iv) pre-gastrulation embryonic ectoderm, and (v) primordial germ cells (Rosner et al., 1990; Scholer, Dressler et al., 1990; Scholer, Ruppert, Suzuki, Chowdhury, & Gruss, 1990; Yeom, Ha, Balling, Scholer, & Artzt, 1991). Its expression is down-regulated during differentiation, and knockout of *Oct-4* causes early lethality in mice due to the absence of an inner cell mass (Nichols et al., 1998). These results suggest that Oct-4 plays a pivotal role in

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mammalian development (Pesce, Wang, Wolgemuth, & Scholer, 1998) and in the self-renewal of embryonic stem (ES) cells (Niwa, Miyazaki, & Smith, 2000).

Oct-4 activates transcription via octamer motifs (5′-ATGCAAAT-3′) located proximal or distal to the transcription start sites of key genes whose expression is specific to pluripotent cells (Lamb & Rizzino, 1998; Scholer, Dressler et al., 1990; Scholer, Ruppert et al., 1990). Oct-4 binding sites have been found in various genes including *Sox-2*, *fgf-4* (fibroblast growth factor-4), *pdgfαr* (platelet-derived growth factor-α receptor), *opn* (osteopontin), *Utf1* (undifferentiated transcription factor 1), *Rex-1*, *Fbx15*, and *Nanog* (Ambrosetti, Basilico, & Dailey, 1997; Ben-Shushan, Thompson, Gudas, & Bergman, 1998; Fukushima et al., 1998; Kraft et al., 1996; Kuroda et al., 2005; Nishimoto, Fukushima, Okuda, & Muramatsu, 1999; Okuda et al., 1998; Rodda et al., 2005; Tokuzawa et al., 2003; Vigano & Staudt, 1996). In addition, Oct-4 may silence genes such as *Cdx2*, *Hand-1*, *IFN-τ* (tau interferon), and the α and β subunits of chorionic gonadotropin, expressed in the trophectoderm but not in embryos prior to blastocyst formation (Ezashi, Ghosh, & Roberts, 2001; Liu & Roberts, 1996; Liu, Leaman, Villalta, & Roberts, 1997; Niwa et al., 2000). This suggests that Oct-4 functions as a master switch during differentiation, regulating cells that have pluripotent potential or can develop such potential (Ovitt & Scholer, 1998; Pesce & Scholer, 2001).

Pyruvate kinase isozyme type M2 (PKM2 or M2-PK) is an isozyme of pyruvate kinase, a key glycolytic enzyme which is consistently altered during tumorigenesis (Mazurek, Grimm, Boschek, Vaupel, & Eigenbrodt, 2002; Mazurek, Boschek, Hugo, & Eigenbrodt, 2005). Four distinct isoforms of pyruvate kinase have been shown to occur in mammals and have been named the M1-, M2-, L-, and R-types (Imamura, Taniuchi, & Tanaka, 1972; Nakashima, Miwa, Oda, Tanaka, & Imamura, 1974; Tanaka, Harano, Sue, & Morimura, 1967). Pyruvate kinase isozymes type L (PKL) and R (PKR) are encoded by the same gene, but are under the control of different promoters (Mazurek et al., 2005; Staal & Rijksen, 1991). PKM1 and PKM2 are different splice products of the same mRNA transcript and differ by 21 amino acids (Noguchi, Inoue, & Tanaka, 1986). Expression of the pyruvate kinase isozymes is tissue-specific and developmentally regulated (Imamura & Tanaka, 1982). Different isozymes of pyruvate kinase are expressed depending upon the metabolic roles of the various cells and tissues (Mazurek et al., 2005).

PKM2 is considered to be the prototype isozyme since it is the only form detected in early fetal tissues, and is present in most adult tissues (Imamura et al., 1972; Noguchi et al., 1986). This form is progressively replaced by PKM1 in skeletal muscle, heart, and brain during development. Carcinogenesis apparently reverses this process (van Veelen, Staal, Verbiest, & Vlug, 1977). Proliferating cells, and tumor cells in particular, express PKM2. During tumor formation, a shift in pyruvate kinase isoenzyme composition always takes place in such a manner that the tissue specific isozyme, such as PKM1 in brain or PKL in the liver, disappears and PKM2 is expressed (Mazurek et al., 2005). In adenocarcinomas of the mammary gland, the content of PKM2 increases in parallel with malignancy (Bahnemann, Domingo, Eigenbrodt, & Reinacher, 1990). In addition, it seems that this variant of PKM2 is a tumor-specific oncoprotein involved in a novel metabolic strategy for energy generation during rapid cell proliferation (Guminska, Ignacak, Kedryna, & Stachurska, 1997). Upregulation of PKM2 protein correlates with an increase in pyruvate kinase mRNA and is under the control of Ras (Mazurek, Zwerschke, Jansen-Durr, & Eigenbrodt, 2001) and the transcription factors HIF-1, SP1, and SP3 (Discher, Bishopric, Wu, Peterson, & Webster, 1998; Kress et al., 1998).

To identify other factors involved in regulating the activity of the Oct-4 *in vivo*, we carried out GST pull-downs in P19 embryonic carcinoma cell lysates, followed by MALDI-TOF (matrix-assisted laser-desorption ionization-time-of-flight) MS (mass spectrometry), and identified PKM2 as a binding partner. We confirmed the interaction between Oct-4 and PKM2 *in vitro* using bacterially expressed fusion proteins and *in vivo* by affinity precipitation/Western blot analysis. Northern blot analysis showed that Oct-4 and PKM2 are co-expressed in pluripotent embryonic stem cells. In addition we found in transient transfection assays that the transcriptional activity of Oct-4 was stimulated by PKM2. Our data demonstrate that Oct-4-mediated transcriptional activity is positively regulated by PKM2.

2. Materials and methods

2.1. Materials and general methods

Restriction endonucleases, polynucleotide kinase, calf intestinal alkaline phosphatase, the Klenow fragment of DNA polymerase I, and T4 DNA ligase were purchased from New England Biolabs (Beverly, MA, USA). Plasmid DNA preparation, restriction enzyme digestion, agarose gel electrophoresis of DNA, DNA ligation, bacterial transformation, and SDS-polyacrylamide gel electrophoresis were carried out by standard methods (Sambrook & Russell, 2001). PCR

amplification products were sequenced by the chain termination method, using double-stranded DNA templates, to ensure the absence of mutations.

2.2. Isolation of Oct-4 complexes and mass spectrometry

For affinity purification of Oct-4-associated proteins, a GST-fusion Oct-4 (POU) was constructed as described below. GST pull-downs and SDS-PAGE were carried out as described previously (Lee, Rhee, Bae, Han, & Kim, 2005). The band for MALDI-TOF mass spectrometry was excised from the gel and digested with trypsin. The molecular mass of the tryptic fragments was determined as described previously (Kim, Kim, & Bahk, 2003).

2.3. Purification of six histidine-tagged Oct-4 protein

Recombinant six histidine-tagged Oct-4 proteins were purified as described (Lee et al., 2005). The elution profile of $(His)_6$-Oct-4 protein was monitored by Western blotting using monoclonal Xpress antibody (Invitrogen, CA, USA).

2.4. Plasmid construction

To construct the PKM2, total RNA was prepared from mouse ES cells and cDNA was synthesized using the Superscript™ First-strand Synthesis System (Invitrogen), as described previously (Lee et al., 2005). To construct pcDNA3-PKM2, the mouse ES cell-derived cDNAs were amplified with primer 5′-PKM2-1 [5′-GATCGAATTCATGCCGAAGCCACACAGT-3′ (an EcoRI site is underlined)] and 3′-PKM2-531 [5′-GATCCTCGAGTCAAGGTACAGGCACTAC-3′ (a XhoI site is underlined)]. The PCR product was digested with EcoRI and XhoI and cloned into the same sites of pcDNA3. To construct pCMV-Tag3B/PKM2, plasmid pcDNA3/PKM2 was digested with EcoRI and XhoI and cloned into the same site of pCMV-Tag3B (Stratagene). For pCMV-Tag2A/PKM2, plasmid pcDNA3/PKM2 was digested with BamHI and XhoI and cloned into the same site of pCMV-Tag2A (Stratagene).

pEF-BOS/GST was generated as described (Kim, Lee, Branton, & Pelletier, 1999; Sanchez et al., 1994). For pEF-BOS/GST-Oct-4, plasmid pcDNA3/Oct-4 (Lee et al., 2005) was amplified by PCR using primers 5′-mOct4-2 (5′-GATCGGATCCGCTGGACACCTGGCTTCA-3′, a BamHI site underlined) and 3′-mOct4-352 (5′-GATCGCGGCCGCTCAGTTTGAATGCATGGG-3′, a NotI site underlined), digested with BamHI and NotI, and cloned into the same sites of pEF-BOS/GST.

The construction of GST-Oct-4 (NTD), GST-Oct-4 (POU), and GST-Oct-4 (CTD) has been described (Lee et al., 2005). For GST-PKM2 (1–305), pCMV-Tag3B/PKM2 was digested with XmnI, blunted with Klenow, and digested with EcoRI. The resulting fragment was ligated into pGEX (4T-1), which had been digested with EcoRI and XhoI (rendered blunt). For GST-PKM2 (241–408), plasmid pCMV-Tag3B/PKM2 was amplified by PCR using primers 5′-PKM2-241 (5′-GATCGAATTCTTTGCATCTTTCATCCGC-3′, an EcoRI site underlined) and 3′-PKM2-408 (5′-GATCCTCGAGGGGGTCGCTGGTAATGGG-3′, a XhoI site underlined), digested with EcoRI and XhoI, and cloned into the same sites of pGEX (4T-1). For GST-PKM2 (307–531), pCMV-Tag3B/PKM2 was digested with XmnI and ApaI and blunted with Klenow. The resulting fragment was ligated into pGEX (3×) that had been digested with EcoRI (rendered blunt).

2.5. Subcellular localization of Oct-4 and PKM2

Immunocytochemical analyses were performed as previously described (Lee, Kim, Pelletier, & Kim, 2004). Briefly, COS-7 cells were plated on glass coverslips and transfected with pcDNA3/Oct-4 and pCMV-Tag2A/PKM2 plasmids. 48 h after transfection, the cells were washed in phosphate-buffered saline (PBS) and fixed for 10 min at −20 °C with acetone/methanol (1:1, v/v). To detect Oct-4 and PKM2, we used primary antibodies for Oct-4 (C-10, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and Flag tag (F7425, rabbit polyclonal, Sigma, St. Louis, MO, USA), respectively. The subcellular distribution of Oct-4 or PKM2 was examined using a confocal laser scanning microscope (LSM5 Pascal, Carl Zeiss Co. Ltd., Germany).

2.6. GST pull-down assays and co-affinity precipitation

In vitro GST pull-down assays were performed as described previously (Lee et al., 2005). For *in vivo* co-affinity precipitation experiments, 293T cells were co-transfected with 0.1 μg of pCMV-Tag2A/PKM2 together with either 2 μg of pEF-BOS/GST or pEF-Bos/GST-Oct-4 using VivaMagic Reagent (Vivagen Co. Ltd., Republic of Korea). Co-affinity precipitation assays were also performed as described (Kim et al., 1999; Lee et al., 2005), and bound proteins were detected by immunoblotting with anti-Oct-4 (C-10) or anti-Flag (M2) antibodies (Lee et al., 2005).

2.7. Cell culture, electroporation, and reporter assays

NIH3T3, P19, HeLa, COS-7, or 293T cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (Hyclone, Logan, UT, USA), penicillin, and streptomycin. Cells were transiently transfected with plasmids by electroporation using the Gene Pulser II RF module system according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA). Luciferase assays were performed with the Dual-luciferase Assay System (Promega, Madison, WI, USA). Renilla luciferase activities were used to normalize transfection efficiencies.

2.8. Northern blot analysis

For Northern blotting, total cell RNAs were prepared using Trizol reagent (Invitrogen), and aliquots (5 μg/lane) were separated on 1.5% agarose-formaldehyde gels. RNA was transferred to Hybond nylon membranes (Amersham) and cross-linked to the membranes in a GS Gene Linker UV Chamber (Bio-Rad). The gel-purified PstI and XhoI-digested DNA fragment (0.65 kb) of mouse Oct-4 cDNA was ^{32}P-labeled using a Prime-It II Random Primer Labeling kit (Stratagene). For the PKM2-specific probe, the PKM2-specific coding region (PKM2 cDNA nt 1184–1252) (de Luis & del Mazo, 1998) of pCMV-Tag2A/PKM2 was amplified with primers 5′-PKM2-1184 [5′-TCGAGGAACTCCGCCGCCTG-3′] and 3′-PKM2-1252 [5′-CCACGGCACCCACGGCGGCA-3′]. The PCR product was directly cloned into pCR2.1-TOPO Vector (Invitrogen) to generate pCR2.1-TOPO/PKM2 (1184–1252). The gel purified EcoRI-digested DNA fragment (69 bp) of pCR2.1-TOPO/PKM2 (1184–1252) was also ^{32}P-labeled using a Prime-It II Random Primer Labeling kit (Stratagene). Hybridizations, using the radiolabeled probe, were carried out overnight in ExpressHyb Solution (Clontech) at 68 °C. The blots were washed twice at 68 °C with 2× SSC/0.1% SDS, and once at 68 °C with 0.2× SSC/0.1% SDS, and radiolabeled bands were visualized by autoradiography.

3. Results

3.1. Identification of PKM2 as an Oct-4-interacting protein

To isolate potential regulators of Oct-4 protein, we used mass spectrometry to identify proteins associating with it. The POU domain of Oct-4 is believed to act as a domain for DNA binding and protein interaction (Ambrosetti et al., 1997; Butteroni, De Felici, Scholer, & Pesce, 2000; Ezashi et al., 2001; Lee et al., 2005; Zhang, Liao, Xu, & Jin, 2007). Bacterially expressed GST or GST-Oct-4 POU DNA binding domain (POU) fusion proteins immobilized on glutathione-Sepharose beads were mixed with P19 embryonic carcinoma cell extracts. After extensive washing, bound proteins were eluted with SDS loading buffer and analyzed by SDS-PAGE and Coomassie blue staining. Several proteins including proteins of ∼60 kDa (denoted by an arrow), ∼30 kDa (denoted by an arrowhead), and ∼22 kDa (denoted by an asterisk), were visible in the Coomassie blue stained gel (Fig. 1A). The 22 and 30 kDa species were present in eluates from both the GST and GST-Oct-4 (POU) affinity matrices (Fig. 1A, lanes 3 and 5) and were not observed if the P19 cell extract was omitted from the GST or GST-Oct-4 (POU) beads (Fig. 1A, lanes 2 and 4). This suggested that the 22 and 30 kDa proteins were not retained specifically by Oct-4 (POU), and these species were not further characterized. The 60 kDa protein species was observed in eluates from the GST-Oct-4 (POU) beads (Fig. 1A, lane 5), not from a column that had GST coupled to it and had been exposed to P19 cell extracts (Fig. 1A, lane 3), and not in eluates from GST-Oct-4 (POU) beads that had not been exposed to P19 cell extract (Fig. 1A, lane 4). These results indicated that it was specifically retained by and eluted from the GST-Oct-4 (POU) affinity matrix. The band was extracted from the gel matrix by trypsin digestion and analyzed by MALDI-TOF mass spectrometry; this identified peptides derived from PKM2 (Fig. 1B). In addition, there are several other bands, for example bands of MW ∼70 kDa and MW ∼45 kDa, that are specific for eluates from GST-Oct-4 (POU) beads that had been exposed to P19 cell extract (Fig. 1A, lane 5). These bands were also excised and submitted to identification. The corresponding proteins are currently under investigation.

3.2. Oct-4 interacts with PKM2 in vitro and in vivo

In vitro interaction between Oct-4 and PKM2 was investigated using GST-fusion proteins containing PKM2. As shown in Fig. 2A, approximately 10% of input Oct-4 protein from P19 embryonic carcinoma cell lysates was retained on PKM2-conjugated Sepharose beads. Bound Oct-4 protein was quantified using the ChemiDocTM XRS System (Bio-Rad). As Oct-4 did not bind to GST alone (Fig. 2A, lane 2), the interaction was considered to be specific. We conformed that similar amounts of GST and GST-

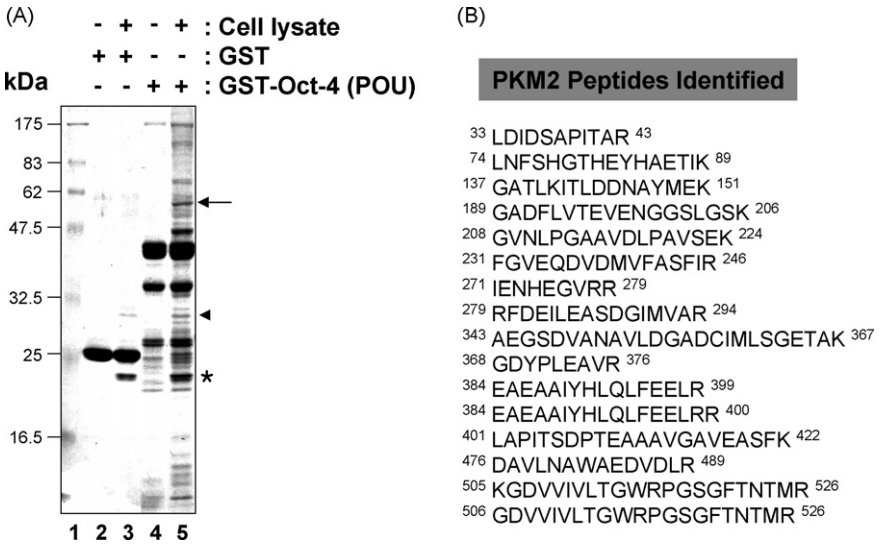

Fig. 1. Identification of PKM2 as an Oct-4-associated protein. (A) SDS-PAGE of Oct-4 complexes isolated from P19 EC cells. Total P19 EC cell lysates were incubated with either GST or a GST fusion protein containing the POU domain of Oct-4 (indicated above the panel). Complexes were resolved by 12% SDS-PAGE and stained with Coomassie brilliant blue. Molecular mass markers are shown on the left; they are derived from prestained protein standards (broad range, New England Biolabs). The band investigated in this analysis is indicated by the arrow to the right. Lane 1, size marker; lane 2, GST; lane 3, GST plus P19 cell lysate; lane 4, GST-Oct-4 (POU); lane 5, GST-Oct-4 (POU) plus P19 cell lysate. (B) Peptide sequences of PKM2 identified by MALDI-TOF analysis. The PKM2 peptides matched by MALDI-TOF are shown with amino acid numbers displayed at both ends.

PKM2 protein were used in the pull-down assays (Fig. 2B).

To determine whether the interaction between Oct-4 and PKM2 occurred *in vivo*, we performed co-affinity precipitations following transfection of 293T cells with an expression vector driving the synthesis of Oct-4. For these assays, mammalian expression vectors containing Oct-4 fused to GST [pEF-BOS/GST-Oct-4], and the GST domain alone [pEF-BOS/GST], were co-transfected with pCMV-Tag2A/PKM2 plasmid (encoding Flag-tagged PKM2) into 293T cells. The cells were lysed 48 h after transfection and the lysate was incubated with glutathione beads. Immunoblotting of the eluates with anti-Flag-antibody revealed that Flag-tagged PKM2 was co-precipitated with GST-Oct-4, but not with GST alone (Fig. 2C). These results demonstrate that Oct-4 and PKM2 associate *in vivo*.

To test the possibility that an uncharacterized eukaryotic adaptor molecule mediated the interaction between Oct-4 and PKM2, we performed the following experiment. Recombinant $(His)_6$-tagged Oct-4 protein was purified from *Escherichia coli* and used in pull-down assays with GST-PKM2 produced in *E. coli*. As shown in Fig. 2D, the bacterially produced proteins interacted, suggesting that the Oct-4–PKM2 interaction does not require an adaptor protein.

3.3. The POU DNA binding domain of Oct-4 is involved in the PKM2 interaction

To define the domain within Oct-4 required for interaction with PKM2, we performed *in vitro* GST pull-down assays with a series of Oct-4 deletion mutants. The structures of the mutants are shown schematically in Fig. 3A. As shown in Fig. 3B, PKM2 strongly bound to GST-Oct-4 (POU) (Fig. 3B, lane 4). However, GST (lane 2) and GST fusions with Oct-4 (NTD) (lane 3) and Oct-4 (CTD) (lane 5) failed to bind, indicating that the POU domain of the Oct-4 is necessary and sufficient for interaction with PKM2. Similar amounts of the GST fusion proteins were used in the pull-down assays (data not shown).

3.4. The C-terminal domain of PKM2 contains an Oct-4 interacting motif

To delineate the regions of PKM2 responsible for interaction with Oct-4, PKM2 (1–305), PKM2 (241–408), and PKM2 (307–531) were expressed as GST-fusion proteins in *E. coli* (Fig. 4A). In GST pull-down assays with GST or the GST-PKM2 truncation mutants, deletions of PKM2 containing amino acids 307–531 interacted strongly with Oct-4, whereas

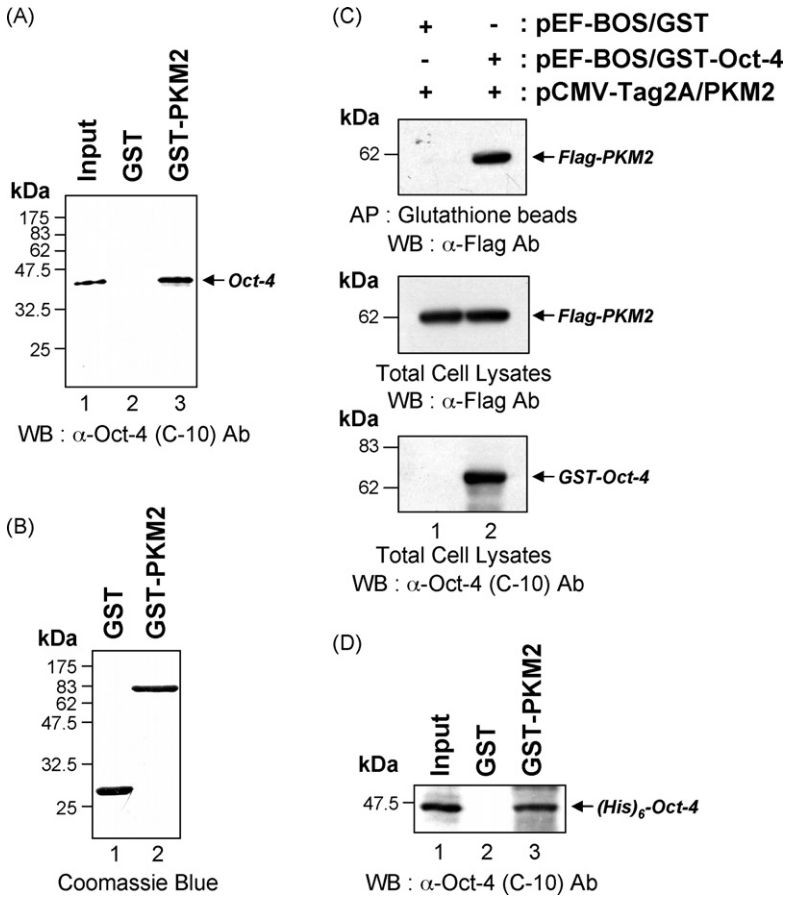

Fig. 2. Binding of Oct-4 to PKM2 *in vitro* and *in vivo*. (A) Association of Oct-4 with PKM2 *in vitro*. GST fusion protein containing PKM2 were incubated with P19 cell lysates. An aliquot of the input (5%) and the pellets from the pull-downs were analyzed on a 12% SDS-polyacrylamide gel and bound Oct-4 protein was detected with anti-Oct-4 antibody (C10; Santa Cruz Biotechnology). The identities of the GST fusion proteins are indicated above the panel, and the positions of the molecular weight markers are indicated to the left. Oct-4 is indicated by an arrow to the right. Three independent experiments gave similar results. Lane 1, 5% input; lane 2, GST alone; lane 3, GST-PKM2. WB, Western blotting; Ab, antibody. (B) Quantitation of the GST-fusion proteins used in the GST pull-down assays. The GST fusion proteins utilized in the pull-down assays were fractionated by 12% SDS-PAGE and visualized by Coomassie blue staining. The positions of the molecular weight markers are indicated to the left. Lane 1, GST alone; lane 2, GST-fusion PKM2. (C) Co-affinity purification of Oct-4 with PKM2 from transfected cells. Forty-eight hours after transfection of 293T cells with 0.1 μg of pCMV-Tag2A/PKM2 (Flag-tagged) and either 2 μg of pEF-BOS/GST or pEF-BOS/GST-Oct-4, cell extracts were prepared as described in Section 2 and affinity-precipitated with glutathione-Sepharose beads. After fractionation by SDS-polyacrylamide gel electrophoresis, the proteins were Western blotted with an anti-Flag (M2 monoclonal; Sigma) or an anti-Oct-4 (C-10) antibody. The identities of the transfected DNAs are indicated above the panel. The positions of the molecular weight markers are indicated to the left and the positions of Flag-tagged PKM2 and GST-fusion Oct-4 are indicated by the arrows to the right. Three independent experiments gave similar results. AP, affinity precipitation; Ab, antibody. (D) The interaction between Oct-4 and PKM2 is direct. Bacterially produced $(His)_6$-tagged Oct-4 protein was incubated with GST alone or GST-PKM2 at 4 °C for 1 h. After extensive washing, bound Oct-4 was assessed by 12% SDS-PAGE and Western blot analysis with an anti-Oct-4 antibody (C-10). The positions of the molecular weight markers are indicated to the left and that of $(His)_6$-tagged Oct-4 protein is indicated by the arrow to the right. Three independent experiments gave similar results. Lane 1, 5% input; lane 2, GST alone; lane 3, GST-fusion PKM2.

PKM2 (1–305) and PKM2 (241–408) did not (Fig. 4B). These results show that the C-terminal domain (amino acids 307–631) of PKM2 binds to Oct-4. We confirmed that similar amounts of the GST fusion proteins had been used in pull-down assays (data not shown).

3.5. Co-expression of Oct-4 and PKM2 in ES and EC cells

To confirm the Oct-4–PKM2 interaction *in vivo*, we performed immunofluorescence staining of the transfected cells. Consistent with a previous report (Lee et al.,

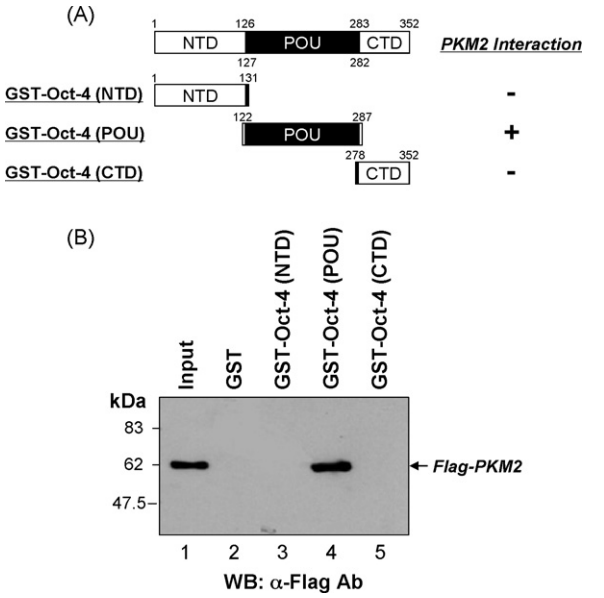

Fig. 3. Involvement of the POU DNA binding domain of Oct-4 in the interaction with PKM2. (A) Schematic representation of the GST-Oct-4 fusion proteins and their ability to bind to PKM2. Numbers refer to amino acid residues, and binding ability is indicated by + or −. (B) Strong binding of PKM2 to GST-Oct-4 (POU). Recombinant GST-Oct-4 deletion mutants were incubated with 293T cell lysates transfected with Flag-tagged PKM2. Following GST pull-down, the bound proteins were eluted with SDS loading buffer and analyzed by Western blotting with anti-Flag antibody. The positions of molecular weight markers and of Flag-tagged PKM2 are indicated. Three independent experiments gave similar results. Lane 1, 10% input; lane 2, GST alone; lane 3, GST-fusion Oct-4 (NTD); lane 4, GST-fusion Oct-4 (POU); lane 5, GST-fusion Oct-4 (CTD). WB, Western blotting; Ab, antibody.

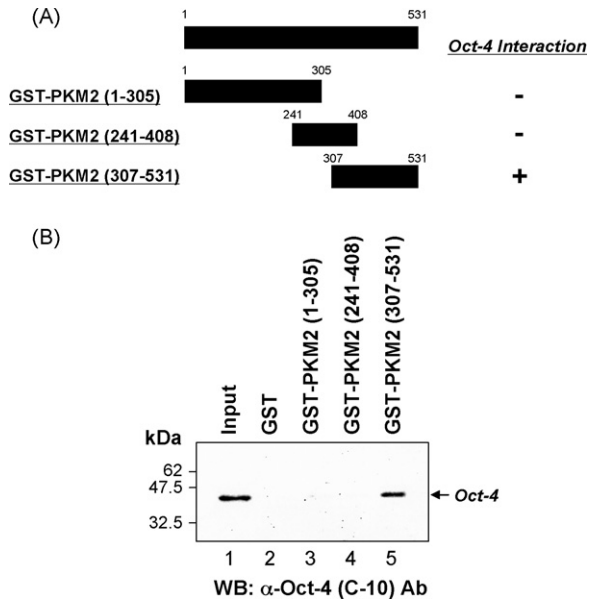

Fig. 4. Mapping the PKM2 region that interacts with Oct-4. (A) Schematic representation of the GST-PKM2 fusion proteins and their ability to bind to Oct-4. Numbers refer to amino acid residues and binding ability is indicated by + or −. (B) Binding of PKM2 (307–531) to Oct-4. Recombinant GST-PKM2 deletion mutants were incubated with P19 cell lysates. Aliquots of the input and the pellets from the GST pull-down assays were analyzed by 15% SDS-polyacrylamide gel electrophoresis, and bound Oct-4 was detected by Western blotting. The positions of molecular weight markers and of Oct-4 are indicated. Three independent experiments gave similar results. Lane 1, 10% input; lane 2, GST alone; lane 3, GST-fusion PKM2 (1–305); lane 4, GST-fusion PKM2 (241–408); lane 5, GST-fusion PKM2 (307–531). WB, Western blotting; Ab, antibody.

2005), Oct-4 protein localized to the nucleus in COS-7 cells (Fig. 5Aa). The majority of PKM2 protein localized to the cytoplasm, especially in the perinuclear region (Fig. 5Ab). However, a significant amount of PKM2 was also detected in the nucleus, as recently reported elsewhere (Stetak et al., 2007) (Fig. 5Ab). Overlay images indicated that Oct-4 and PKM2 in the nucleoplasm partially overlapped (Fig. 5Ac). Similar results were obtained in 293T cells (data not shown). Therefore, we conclude that Oct-4 and a nuclear form of PKM2 colocalize, or exist in close proximity.

We investigated the expression patterns of these two mRNAs by Northern blot analysis of total RNA from six different cell lines including embryonic stem cells. As reported previously (Okamoto et al., 1990; Scholer, Balling, Hatzopoulos, Suzuki, & Gruss, 1989; Scholer, Hatzopoulos, Balling, Suzuki, & Gruss, 1989), Oct-4 expression was detected in ES cells and EC cells, but not in NIH3T3, mouse embryonic fibroblasts (MEF), HeLa, and 293T cell lines (Fig. 5B, upper gel). The expression profile of PKM2 in these same cell lines was also assessed by Northern blotting. The PKM2 gene was expressed highly in ES and EC cells; moderately in HeLa cells; and at a very low level in NIH3T3, MEF, and 293T cells (Fig. 5B, middle gel). These results show that Oct-4 and PKM2 are expressed in ES and EC cells. Roughly equal amounts of total RNA were examined for each of the cell lines (Fig. 5B, lower gel).

3.6. PKM2 modulates Oct-4-dependent transactivation

To investigate the consequences of the Oct-4–PKM2 interaction, we measured the effect of PKM2 expression on transcriptional activation by Oct-4. This was done in COS-7 and HeLa cells by examining gene expression from a luciferase-based reporter plasmid transfected along with pcDNA3/Oct-4, with or without cotransfection of pCMV-Tag2A/PKM2. The structure of the

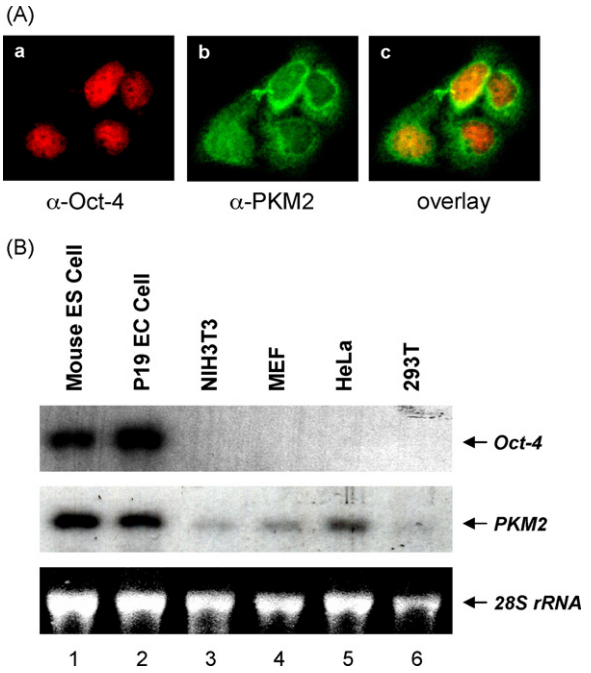

Fig. 5. Expression of Oct-4 and PKM2 in cells. (A) Subcellular localization of Oct-4 and PKM2 proteins in COS-7 cells. COS-7 cells grown on coverslips were transfected with mammalian expression vectors encoding Oct-4 or Flag-tagged PKM2 proteins. Transiently transfected cells were fixed with an acetone/methanol mixture and incubated with primary antibodies for Oct-4 (C-10) or Flag tag (F7425, rabbit polyclonal). The subcellular distribution of Oct-4 or PKM2 was examined using a confocal laser scanning microscope (LSM5 Pascal, Carl Zeiss Co. Ltd.). The merged image shows colocalization. Three independent experiments gave similar results. (B) Northern blot analysis of Oct-4 and PKM2 mRNA in mouse and human cells. Mouse ES (lane 1), P19 mouse EC (lane 2), NIH3T3 (lane 3), mouse embryonic fibroblast (lane 4), HeLa (lane 5), and 293T (lane 6) cells were harvested and total RNA prepared. Total RNA was fractionated on a 6% formaldehyde–1.5% agarose gel, transferred to a Nylon membrane, and probed with Oct-4 (upper panel), or PKM2 (middle panel) cDNAs as described in Section 2. Ethidium bromide (EtBr) staining of the agarose gel used for the Northern blotting is shown to demonstrate that approximately similar amounts of total RNA were loaded in each lane (lower panel). Arrowheads indicate the positions of migration of the respective RNAs.

reporter plasmid used is shown schematically in Fig. 6A. The introduction of Oct-4 into COS-7 cells activated transcription (up to ∼20-fold) from pOct-4 (10×) TATA luc, a reporter plasmid containing the TATA minimal promoter with 10 Oct-4 binding sites (Lee et al., 2005) driving synthesis of Renilla luciferase (Fig. 6B, left panel, lane 3). Interestingly, PKM2 augmented Oct-4-mediated transactivation up to ∼2.5-fold (Fig. 6B, left panel, lane 4). Similar results were obtained in HeLa cells (Fig. 6B, right panel). To confirm that these effects of PKM2 were not the result of increased Oct-4 protein levels, cell extracts prepared from COS-7 and HeLa cells transfected with the Oct-4 expression plasmid in the presence or absence of PKM2 expression were examined by Western blotting (Fig. 6C). Fractionation of the cell extracts and probing with anti-Oct-4 antibody demonstrated no increase in exogenously expressed Oct-4 protein in the COS-7 cells (left panels). Similar results were obtained in HeLa cells (right panels). The EGFP expression plasmid served as an internal control for monitoring transfection efficiency (bottom panels).

4. Discussion

In this work we report the identification and characterization of an Oct-4-activating protein. Identification of PKM2 as an Oct-4-interacting partner was achieved by affinity chromatography of P19 embryonic carcinoma cell extracts on immobilized Oct-4 (Fig. 1). We used POU DNA binding domain of Oct-4 as bait for affinity chromatography, because Oct-4 (POU) is believed to act as a domain for protein interaction (Lee et al., 2005). Native affinity precipitation from transiently transfected 293T cell extracts revealed association of PKM2 with Oct-4 *in vivo* (Fig. 2). In addition, GST pull-down assays revealed that the POU DNA binding domain is required for interaction with the C-terminal region of PKM2 (Figs. 3 and 4). Functionally, PKM2 enhances transcriptional activation by Oct-4 (Fig. 6).

Although it is known as a metabolic enzyme, PKM2 is able to stimulate Oct-4-mediated transcriptional activation (Fig. 6). However, this is not the first case of a metabolic enzyme involved in transcription. Like PKM2, GAPDH, was recently identified as a component of the eukaryotic transcription machinery (Zheng, Roeder, & Luo, 2003). Using an *in vitro* assay involving stimulation of Oct-1 transcription, OCA-S was chromatographically purified from a HeLa cell nuclear extract, and subsequent analysis demonstrated that GAPDH was part of the OCA-S complex implicated in regulating histone gene expression. OCA-S is a multicomponent Oct-1 coactivator that is essential for S phase-dependent histone H2B transcription. Interestingly, GAPDH binds directly to the Oct-1 transcription factor, is selectively recruited to the H2B promoter in S phase, and has an intrinsic activation domain, indicating that it interacts with an as-yet-unidentified component of the basal RNA polymerase II transcription machinery (Zheng et al., 2003). In addition, GAPDH is involved in the regulation of transactivation by the $hTAF_{II}68$-TEC oncoprotein (Kim, Lee, & Kim, 2007). This effect is dose-dependent and requires the region of GAPDH that interacts with $hTAF_{II}68$-TEC. Thus, it would be interesting to determine if PKM2 is

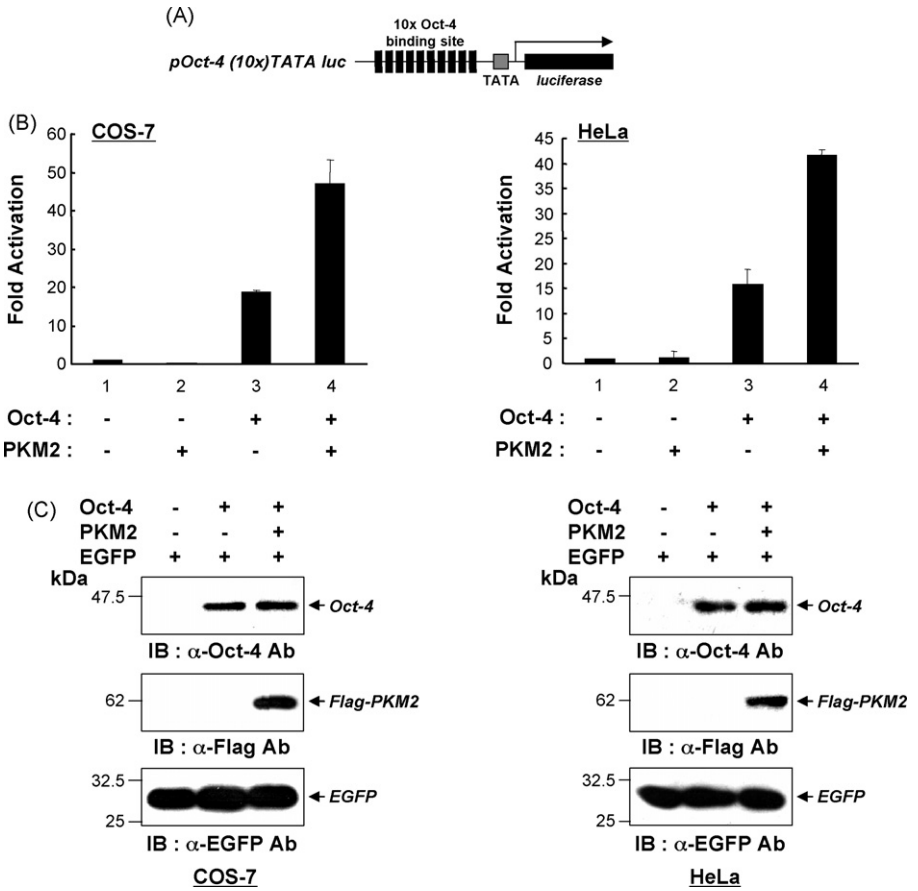

Fig. 6. PKM2 enhances Oct-4-mediated transcription. (A) Schematic representation of the reporter plasmid used in this study. The pOct-4 (10×)TATA luc reporter plasmid contains 10 copies of Oct-4 binding site upstream of a basal promoter-luciferase gene construct. The 10 copies of Oct-4 recognition sites are indicated by solid bars, the TATA box is represented by a shaded box, and the luciferase gene is indicated by a solid box. (B) Stimulation of Oct-4-mediated transactivation by PKM2 in COS-7 (left panel) and HeLa (right panel) cells. 4 μg of pcDNA3/Oct-4 (bars 3 and 4) or pcDNA3 plasmid (bars 1 and 2) were co-transfected with 4 μg of pCMV-Tag2A (bars 1 and 3) or pCMV-Tag2A/PKM2 expression plasmid (bars 2 and 4) into COS-7 (left panel) and HeLa (right panel) cells. After 48 h the cells were harvested and luciferase assays performed. The experiments were repeated three times, and the averages of two independent experiments are presented with error bars. (C) Western blot analysis of Oct-4 levels in extracts of the transfected cells to confirm that an equal amount of the exogenous Oct-4 was expressed irrespective of PKM2 overexpression. COS-7 (left panels) or HeLa (right panels) cells were transfected with expression plasmids for Oct-4 in combination with PKM2. 48 h post-transfection, the cells were harvested and lysed. A portion of each cell extract was separated by SDS-PAGE and immunoblotted with anti-Oct-4 (top panel), -PKM2 (middle panel) or -EGFP (bottom panel) antibodies as indicated. The positions of the molecular mass markers are indicated on the left-hand side, and those of Oct-4, Flag-PKM2, and EGFP are indicated by arrows on the right-hand side. The pEGFP-N1 vector was included as a control for transfection efficiency.

a component of the eukaryotic transcription machinery and interacts with other transcriptional regulators.

Oct-4 is a central mediator of the undifferentiated pluripotent state of embryonic stem cells (Pesce & Scholer, 2001). Maintaining Oct-4 activity within a certain range appears to be critical for stem cell self-renewal, with any increase or decrease triggering differentiation to endoderm/mesoderm or trophectoderm, respectively (Niwa et al., 2000). The biological activities of transcription factors including Oct-4 can be modulated by many stimuli, such as growth factors, cytokines, viruses, a wide variety of bacteria or bacterial products, and also environmental stresses. The adenovirus E1A oncoprotein binds to the POU DNA binding domain of Oct-4. Interestingly, transactivation by Oct-4 is strongly stimulated in the presence of E1A (Scholer, Ciesiolka, & Gruss, 1991). There are four well-conserved regions (CRs) in E1A (Avvakumov, Kajon, Hoeben, & Mymryk, 2004; Berk, 2005). Among them, E1A CR3 functions as a strong activation domain (AD) when tethered to a promoter by the GAL4 DNA-binding domain (Lillie & Green, 1989; Martin, Lillie, & Green, 1990). Another

oncoprotein, E7, encoded by the oncogenic human papilloma virus (HPV) type 16 also binds to the POU DNA binding domain of Oct-4 and stimulates Oct-4-mediated transactivation (Brehm et al., 1999). Since E7 is known to be involved in modulating PKM2 activity (Mazurek et al., 2001; Zwerschke et al., 1999), it is tempting to speculate that there is a linkage between the physical interaction of E7 with PKM2 and the transcriptional activity of Oct-4.

Several genes responsible for developmental alterations have been incontrovertibly linked to human cancers. There are numerous examples in which alterations in embryonic gene expression are correlated with the development of human cancers (Calvo & Drabkin, 2000). Interestingly, Oct-4 is expressed in all breast cancer cell lines and human primary breast carcinomas, but not in normal human breast tissue (Jin et al., 1999). The pyruvate kinase isoenzyme PKM2 is also expressed in proliferating cells (Eigenbrodt, Basenau, Holthusen, Mazurek, & Fischer, 1997) and is known to be associated with the metabolic shift characteristic of tumor cells (Mazurek & Eigenbrodt, 2003; Mazurek et al., 2005). It is believed that the loss of a tissue-specific PK isoenzyme, PKL (pyruvate kinase type L), in liver, or PKM1 (pyruvate kinase type M1) in brain, and the subsequent expression of PKM2 is one of the first steps in multistep carcinogenesis (Hacker, Steinberg, & Bannasch, 1998). Although it is not known if PKM2 is expressed in human breast cancer cells, it would be of interest to determine whether it can collaborate with Oct-4 to transform cells into human primary breast carcinomas.

PKM2 was present in both the cytoplasm and nucleus of COS-7 cells and the majority of PKM2 protein localized to the cytoplasm, especially to the perinuclear region (Fig. 5). Even though we found that Oct-4 and PKM2 proteins partially overlapped in the nucleoplasm, this is basically a two-dimensional observation. Because biological entities, such as Oct-4 and PKM2 proteins are distributed in a three-dimensional manner within cells, *Z*-axis analysis would provide more accurate information about the Oct-4–PKM2 interaction *in vivo*. Thus, it would be of value to image specimens in layers by moving the focal plane in precise steps with a motorized microscope z-drives or piezo-objective movers. In addition, it has been recently reported that PKM2 is translocated to the nucleus in response to TT-232 (a structural somatostatin analogue with a cyclopenta ring structure) and various apoptotic agents (Stetak et al., 2007). However, it is not clear whether PKM2 contains nuclear localization or nuclear export signals. Because Oct-4 activity is positively modulated by PKM2 (Fig. 6), it would be interesting to see whether accumulation of PKM2 in the nucleus modulates Oct-4 activity.

In conclusion, the findings of this study provide new evidence that Oct-4 function can be modulated by PKM2. The identification of both positive and negative regulators of Oct-4 activation provides tools for enumerating and elucidating the various Oct-4 signaling pathway. The isolated components can also be used as bait to identify interacting proteins in biochemical or yeast two-hybrid experiments. Targeted disruption of the corresponding genes in mice will then provide information about the physiological functions of these components and the signaling pathways mediated by them.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocel.2007.11.009.

References

Ambrosetti, D. C., Basilico, C., & Dailey, L. (1997). Synergistic activation of the fibroblast growth factor 4 enhancer by Sox2 and Oct-3 depends on protein–protein interactions facilitated by a specific spatial arrangement of factor binding sites. *Mol. Cell. Biol.*, *17*, 6321–6329.

Avvakumov, N., Kajon, A. E., Hoeben, R. C., & Mymryk, J. S. (2004). Comprehensive sequence analysis of the E1A proteins of human and simian adenoviruses. *Virology*, *329*, 477–492.

Bahnemann, R., Domingo, M., Eigenbrodt, E., & Reinacher, M. (1990). Immunohistologic photometric quantification of pyruvate kinase content of rat tumors. *Verh. Dtsch. Ges. Pathol.*, *74*, 373–375.

Ben-Shushan, E., Thompson, J. R., Gudas, L. J., & Bergman, Y. (1998). Rex-1, a gene encoding a transcription factor expressed in the early embryo, is regulated via Oct-3/4 and Oct-6 binding to an octamer site and a novel protein, Rox-1, binding to an adjacent site. *Mol. Cell. Biol.*, *18*, 1866–1878.

Berk, A. J. (2005). Recent lessons in gene expression, cell cycle control, and cell biology from adenovirus. *Oncogene*, *24*, 7673–7685.

Brehm, A., Ohbo, K., Zwerschke, W., Botquin, V., Jansen-Durr, P., & Scholer, H. R. (1999). Synergism with germ line transcription factor Oct-4: Viral oncoproteins share the ability to mimic a stem cell-specific activity. *Mol. Cell. Biol.*, *19*, 2635–2643.

Burdon, T., Smith, A., & Savatier, P. (2002). Signalling, cell cycle and pluripotency in embryonic stem cells. *Trends Cell Biol.*, *12*, 432–438.

Butteroni, C., De Felici, M., Scholer, H. R., & Pesce, M. (2000). Phage display screening reveals an association between germline-specific transcription factor Oct-4 and multiple cellular proteins. *J. Mol. Biol.*, *304*, 529–540.

Calvo, R., & Drabkin, H. A. (2000). Embryonic genes in cancer. *Ann. Oncol.*, *11*(Suppl. 3), 207–218.

de Luis, O., & del Mazo, J. (1998). Gene expression of mouse M1 and M2 pyruvate kinase isoenzymes correlates with differential poly[A] tract extension of their mRNAs during the development of spermatogenesis. *Biochim. Biophys. Acta*, *1396*, 294–305.

Discher, D. J., Bishopric, N. H., Wu, X., Peterson, C. A., & Webster, K. A. (1998). Hypoxia regulates beta-enolase and pyruvate kinase-M promoters by modulating Sp1/Sp3 binding to a conserved GC element. *J. Biol. Chem.*, *273*, 26087–26093.

Eigenbrodt, E., Basenau, D., Holthusen, S., Mazurek, S., & Fischer, G. (1997). Quantification of tumor type M2 pyruvate kinase (Tu M2-PK) in human carcinomas. *Anticancer Res.*, *17*, 3153–3156.

Ezashi, T., Ghosh, D., & Roberts, R. M. (2001). Repression of Ets-2-induced transactivation of the tau interferon promoter by Oct-4. *Mol. Cell. Biol.*, *21*, 7883–7891.

Fukushima, A., Okuda, A., Nishimoto, M., Seki, N., Hori, T. A., & Muramatsu, M. (1998). Characterization of functional domains of an embryonic stem cell coactivator UTF1 which are conserved and essential for potentiation of ATF-2 activity. *J. Biol. Chem.*, *273*, 25840–25849.

Goto, T., Adjaye, J., Rodeck, C. H., & Monk, M. (1999). Identification of genes expressed in human primordial germ cells at the time of entry of the female germ line into meiosis. *Mol. Hum. Reprod.*, *5*, 851–860.

Guminska, M., Ignacak, J., Kedryna, T., & Stachurska, M. B. (1997). Tumor-specific pyruvate kinase isoenzyme M2 involved in biochemical strategy of energy generation in neoplastic cells. *Acta Biochim. Pol.*, *44*, 711–724.

Hacker, H. J., Steinberg, P., & Bannasch, P. (1998). Pyruvate kinase isoenzyme shift from L-type to M2-type is a late event in hepatocarcinogenesis induced in rats by a choline-deficient/DL-ethionine-supplemented diet. *Carcinogenesis*, *19*, 99–107.

Hansis, C., Grifo, J. A., & Krey, L. C. (2000). Oct-4 expression in inner cell mass and trophectoderm of human blastocysts. *Mol. Hum. Reprod.*, *6*, 999–1004.

Herr, W., & Cleary, M. A. (1995). The POU domain: Versatility in transcriptional regulation by a flexible two-in-one DNA-binding domain. *Genes Dev.*, *9*, 1679–1693.

Imamura, K., & Tanaka, T. (1982). Pyruvate kinase isozymes from rat. *Methods Enzymol.*, *90*(Part E), 150–165.

Imamura, K., Taniuchi, K., & Tanaka, T. (1972). Multimolecular forms of pyruvate kinase. II. Purification of M2-type pyruvate kinase from Yoshida ascites hepatoma 130 cells and comparative studies on the enzymological and immunological properties of the three types of pyruvate kinases, L, M1, and M2. *J. Biochem. (Tokyo)*, *72*, 1001–1015.

Jin, T., Branch, D. R., Zhang, X., Qi, S., Youngson, B., & Goss, P. E. (1999). Examination of POU homeobox gene expression in human breast cancer cells. *Int. J. Cancer*, *81*, 104–112.

Kim, J., Lee, J. M., Branton, P. E., & Pelletier, J. (1999). Modification of EWS/WT1 functional properties by phosphorylation. *Proc. Natl. Acad. Sci. U.S.A.*, *96*, 14300–14305.

Kim, S. Y., Kim, Y. S., & Bahk, Y. Y. (2003). Proteome changes induced by expression of tumor suppressor PTEN. *Mol. Cells*, *15*, 396–405.

Kim, S., Lee, J., & Kim, J. (2007). Regulation of oncogenic transcription factor hTAF(II)68-TEC activity by human glyceraldehyde-3-phosphate dehydrogenase (GAPDH). *Biochem. J.*, *404*, 197–206.

Kraft, H. J., Mosselman, S., Smits, H. A., Hohenstein, P., Piek, E., Chen, Q., et al. (1996). Oct-4 regulates alternative platelet-derived growth factor alpha receptor gene promoter in human embryonal carcinoma cells. *J. Biol. Chem.*, *271*, 12873–12878.

Kress, S., Stein, A., Maurer, P., Weber, B., Reichert, J., Buchmann, A., et al. (1998). Expression of hypoxia-inducible genes in tumor cells. *J. Cancer Res. Clin. Oncol.*, *124*, 315–320.

Kuroda, T., Tada, M., Kubota, H., Kimura, H., Hatano, S. Y., Suemori, H., et al. (2005). Octamer and Sox elements are required for transcriptional cis regulation of Nanog gene expression. *Mol. Cell. Biol.*, *25*, 2475–2485.

Lamb, K. A., & Rizzino, A. (1998). Effects of differentiation on the transcriptional regulation of the FGF-4 gene: Critical roles played by a distal enhancer. *Mol. Reprod. Dev.*, *51*, 218–224.

Lee, H. J., Kim, S., Pelletier, J., & Kim, J. (2004). Stimulation of hTAFII68 (NTD)-mediated transactivation by v-Src. *FEBS Lett.*, *564*, 188–198.

Lee, J., Rhee, B. K., Bae, G. Y., Han, Y. M., & Kim, J. (2005). Stimulation of Oct-4 activity by Ewing's sarcoma protein. *Stem Cells*, *23*, 738–751.

Lillie, J. W., & Green, M. R. (1989). Transcription activation by the adenovirus E1a protein. *Nature*, *338*, 39–44.

Liu, L., & Roberts, R. M. (1996). Silencing of the gene for the beta subunit of human chorionic gonadotropin by the embryonic transcription factor Oct-3/4. *J. Biol. Chem.*, *271*, 16683–16689.

Liu, L., Leaman, D., Villalta, M., & Roberts, R. M. (1997). Silencing of the gene for the alpha-subunit of human chorionic gonadotropin by the embryonic transcription factor Oct-3/4. *Mol. Endocrinol.*, *11*, 1651–1658.

Martin, K. J., Lillie, J. W., & Green, M. R. (1990). Evidence for interaction of different eukaryotic transcriptional activators with distinct cellular targets. *Nature*, *346*, 147–152.

Mazurek, S., & Eigenbrodt, E. (2003). The tumor metabolome. *Anticancer Res.*, *23*, 1149–1154.

Mazurek, S., Zwerschke, W., Jansen-Durr, P., & Eigenbrodt, E. (2001). Metabolic cooperation between different oncogenes during cell transformation: Interaction between activated ras and HPV-16 E7. *Oncogene*, *20*, 6891–6898.

Mazurek, S., Grimm, H., Boschek, C. B., Vaupel, P., & Eigenbrodt, E. (2002). Pyruvate kinase type M2: A crossroad in the tumor metabolome. *Br. J. Nutr.*, *87*(Suppl. 1), S23–S29.

Mazurek, S., Boschek, C. B., Hugo, F., & Eigenbrodt, E. (2005). Pyruvate kinase type M2 and its role in tumor growth and spreading. *Semin. Cancer Biol.*, *15*, 300–308.

Nakashima, K., Miwa, S., Oda, S., Tanaka, T., & Imamura, K. (1974). Electrophoretic and kinetic studies of mutant erythrocyte pyruvate kinases. *Blood*, *43*, 537–548.

Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., et al. (1998). Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell*, *95*, 379–391.

Nishimoto, M., Fukushima, A., Okuda, A., & Muramatsu, M. (1999). The gene for the embryonic stem cell coactivator UTF1 carries

a regulatory element which selectively interacts with a complex composed of Oct-3/4 and Sox-2. *Mol. Cell. Biol.*, *19*, 5453–5465.

Niwa, H., Miyazaki, J., & Smith, A. G. (2000). Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat. Genet.*, *24*, 372–376.

Noguchi, T., Inoue, H., & Tanaka, T. (1986). The M1- and M2-type isozymes of rat pyruvate kinase are produced from the same gene by alternative RNA splicing. *J. Biol. Chem.*, *261*, 13807–13812.

Okamoto, K., Okazawa, H., Okuda, A., Sakai, M., Muramatsu, M., & Hamada, H. (1990). A novel octamer binding transcription factor is differentially expressed in mouse embryonic cells. *Cell*, *60*, 461–472.

Okuda, A., Fukushima, A., Nishimoto, M., Orimo, A., Yamagishi, T., Nabeshima, Y., et al. (1998). UTF1, a novel transcriptional coactivator expressed in pluripotent embryonic stem cells and extra-embryonic cells. *EMBO J.*, *17*, 2019–2032.

Ovitt, C. E., & Scholer, H. R. (1998). The molecular biology of Oct-4 in the early mouse embryo. *Mol. Hum. Reprod.*, *4*, 1021–1031.

Pesce, M., & Scholer, H. R. (2001). Oct-4: Gatekeeper in the beginnings of mammalian development. *Stem Cells*, *19*, 271–278.

Pesce, M., Wang, X., Wolgemuth, D. J., & Scholer, H. (1998). Differential expression of the Oct-4 transcription factor during mouse germ cell differentiation. *Mech. Dev.*, *71*, 89–98.

Rodda, D. J., Chew, J. L., Lim, L. H., Loh, Y. H., Wang, B., Ng, H. H., et al. (2005). Transcriptional regulation of nanog by OCT4 and SOX2. *J. Biol. Chem.*, *280*, 24731–24737.

Rosner, M. H., Vigano, M. A., Ozato, K., Timmons, P. M., Poirier, F., Rigby, P. W., et al. (1990). A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo. *Nature*, *345*, 686–692.

Sambrook, J., & Russell, D. W. (2001). *Molecular cloning: A laboratory manual (Editor ed.)*. New York: Cold Spring Harbor Laboratory Press.

Sanchez, I., Hughes, R. T., Mayer, B. J., Yee, K., Woodgett, J. R., Avruch, J., et al. (1994). Role of SAPK/ERK kinase-1 in the stress-activated pathway regulating transcription factor c-Jun. *Nature*, *372*, 794–798.

Scholer, H. R., Balling, R., Hatzopoulos, A. K., Suzuki, N., & Gruss, P. (1989). Octamer binding proteins confer transcriptional activity in early mouse embryogenesis. *EMBO J.*, *8*, 2551–2557.

Scholer, H. R., Hatzopoulos, A. K., Balling, R., Suzuki, N., & Gruss, P. (1989). A family of octamer-specific proteins present during mouse embryogenesis: Evidence for germline-specific expression of an Oct factor. *EMBO J.*, *8*, 2543–2550.

Scholer, H. R., Dressler, G. R., Balling, R., Rohdewohld, H., & Gruss, P. (1990). Oct-4: A germline-specific transcription factor mapping to the mouse t-complex. *EMBO J.*, *9*, 2185–2195.

Scholer, H. R., Ruppert, S., Suzuki, N., Chowdhury, K., & Gruss, P. (1990). New type of POU domain in germ line-specific protein Oct-4. *Nature*, *344*, 435–439.

Scholer, H. R., Ciesiolka, T., & Gruss, P. (1991). A nexus between Oct-4 and E1A: Implications for gene regulation in embryonic stem cells. *Cell*, *66*, 291–304.

Staal, G. E., & Rijksen, G. (1991). *Pyruvate kinase in selected human tumors (Editor ed.)*. San Diego: Academic Press.

Stetak, A., Veress, R., Ovadi, J., Csermely, P., Keri, G., & Ullrich, A. (2007). Nuclear translocation of the tumor marker pyruvate kinase M2 induces programmed cell death. *Cancer Res.*, *67*, 1602–1608.

Tanaka, T., Harano, Y., Sue, F., & Morimura, H. (1967). Crystallization, characterization and metabolic regulation of two types of pyruvate kinase isolated from rat tissues. *J. Biochem. (Tokyo)*, *62*, 71–91.

Tokuzawa, Y., Kaiho, E., Maruyama, M., Takahashi, K., Mitsui, K., Maeda, M., et al. (2003). Fbx15 is a novel target of Oct3/4 but is dispensable for embryonic stem cell self-renewal and mouse development. *Mol. Cell. Biol.*, *23*, 2699–2708.

van Veelen, C. W., Staal, G. E., Verbiest, H., & Vlug, A. M. (1977). Alanine inhibition of pyruvate kinase in gliomas and meningiomas. A diagnostic tool in surgery for gliomas. *Lancet*, *2*, 384–385.

Vigano, M. A., & Staudt, L. M. (1996). Transcriptional activation by Oct-3: Evidence for a specific role of the POU-specific domain in mediating functional interaction with Oct-1. *Nucleic Acids Res.*, *24*, 2112–2118.

Yeom, Y. I., Ha, H. S., Balling, R., Scholer, H. R., & Artzt, K. (1991). Structure, expression and chromosomal location of the Oct-4 gene. *Mech. Dev.*, *35*, 171–179.

Zhang, Z., Liao, B., Xu, M., & Jin, Y. (2007). Post-translational modification of POU domain transcription factor Oct-4 by SUMO-1. *FASEB J.*, *21*, 3042–3051.

Zheng, L., Roeder, R. G., & Luo, Y. (2003). S phase activation of the histone H2B promoter by OCA-S, a coactivator complex that contains GAPDH as a key component. *Cell*, *114*, 255–266.

Zwerschke, W., Mazurek, S., Massimi, P., Banks, L., Eigenbrodt, E., & Jansen-Durr, P. (1999). Modulation of type M2 pyruvate kinase activity by the human papillomavirus type 16 E7 oncoprotein. *Proc. Natl. Acad. Sci. U.S.A.*, *96*, 1291–1296.