BMB
reports

Translationally controlled tumor protein (TCTP) downregulates *Oct4* expression in mouse pluripotent cells

*Xiang Cheng, Junhua Li, Jie Deng, Zhenzhen Li, Shuyan Meng & Huayan Wang**

Department of Animal Biotechnology, College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, People's Republic of China

The present study aimed to investigate the function of translationally controlled tumor protein (TCTP) in the regulation of *Oct4* in mouse embryonic carcinoma P19 cells and mouse J1 embryonic stem (ES) cells. The mRNA level of endogenous TCTP in somatic cells was 2-4 folds higher than that in pluripotent P19 and J1 ES cells. Overexpression of *TCTP* in mouse pluripotent cells not only reduced the level of *Oct4* transcription, but also decreased the pluripotency of stem cells. The N-terminal end of TCTP (amino acids 1-60) played an important role in suppressing the *Oct4* promoter. Moreover, overexpression of TCTP in P19 cells suppressed the *Oct4* promoter activity in a dose- and a time-dependent manner. In addition, knockdown of *TCTP* by small interfering RNA increased the expression of *Oct4*. Our study indicates that *TCTP* downregulates the *Oct4* expression by binding the Sf1 site of *Oct4* promoter in mouse pluripotent cells. [BMB reports 2012; 45(1): 20-25]

INTRODUCTION

Octamer binding transcription factor 4 (*Oct4*), a member of the POU family, is an essential factor for maintaining the pluripotency and self-renewal of embryonic stem (ES) cells. Oct4 is also the core transcription factor being used to induce somatic cells into the induced pluripotent stem (iPS) cells (1). Oct4 is mainly expressed in the inner cell mass (ICM) of blastocyst, post-implantation embryonic epiblast and the primordial germ cells (2, 3), in which the expression of *Oct4* is precisely regulated. Two-fold upregulation or suppression of *Oct4* expression can cause ES cells to lose pluripotency and differentiate into trophectoderm or other germ layer lineages (3-5).

Translationally controlled tumor protein (TCTP, also known as tumor protein translationally-controlled I, TPT1) is highly conserved and ubiquitously expressed among eukaryotic cells and tissues. TCTP has been implicated in important cellular processes, such as cell proliferation and differentiation, cell cycle progression and apoptosis (6, 7). In addition, the *TCTP* gene contains two important motifs including the TCTP1 motif (AA.48-58) and TCTP2 motif (AA.129-151) (8).

Several proteins, such as GCNF (9), SF1 (10), LRH1 (11), Sall4 (12), and HIF-2 (13), could regulate the *Oct4* promoter region in cultured cells or in later embryonic development. Koziol *et al*. identified that *TCTP* could directly bind to the Sf1 site of *Oct4* promoter and activate *Oct4* transcription in transplanted somatic nuclei of Xenopus oocyte (14). In contrast, *TCTP* interacted with *Oct4* in the nucleus of mouse ES cells and the knockdown of *TCTP* increased *Oct4* expression (15). To further investigate *TCTP* implication in regulating *Oct4* transcription, we evaluated the effect of overexpression and knockdown of *TCTP* gene on *Oct4* expression in mouse embryonic carcinoma P19 cells and mouse J1 ES cells.

In the present study, we demonstrate that TCTP protein is present both in the nucleus and in the cytoplasm of P19 and NIH3T3 cells. TCTP overexpression suppressed the level of *Oct4* mRNA, and the knockdown of *TCTP* expression by small interfering RNA resulted in an increased level of *Oct4* expression in P19 and J1 ES cells. The N-terminal end (AA.1-60) rather than the C-terminal end (AA.61-172) of TCTP was essential for repressing the *Oct4* promoter. TCTP repressed *Oct4* by binding the Sf1 site in mouse pluripotent cells.

RESULTS

TCTP expression and subcellular localization

To verify the expression of constructs, protein extracts from P19 cells were prepared for western blot analysis at 48 h after transfection. A 45 kDa band representing the EGFP-TCTP fusion protein was observed in pEGFP-TCTP (AA.1-172) transfected cells, while a 27 kDa EGFP band was detected in cells transfected with pEGFP-C1 (Fig. 1A).In addition, the EGFP-TCTP (AA.1-172) fusion protein was distributed both in the nucleus and in the cytoplasm of P19 and NIH3T3 cells (Fig. 1B).

TCTP overexpression suppresses *Oct4* gene expression

To investigate the level of endogenous TCTP expression, we

*Corresponding author. Tel: +86-029-87080069; Fax: +86-029-87080068; E-mail: hhwang101@163.com
http://dx.doi.org/10.5483/BMBRep.2012.45.1.20

Received 20 July 2011, Revised 22 August 2011, Accepted 6 October 2011

Keywords: Gene regulation, *Oct4*, Overexpression, siRNA interference, TCTP

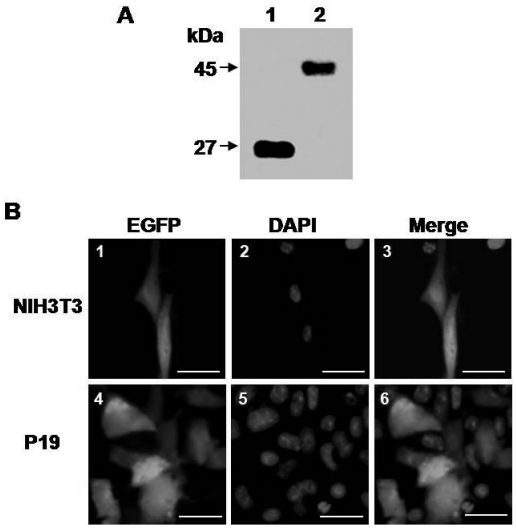

Fig. 1. TCTP expression in P19 and NIH3T3 cells. (A) The transfected P19 cell lysates were analysed by Western blotting with mouse anti-GFP antibody. Lane 1, P19 cells transfected with pEGFP-C1; Lane 2, P19 cells transfected with pEGFP-TCTP (AA.1-172). (B) The pEGFP-TCTP (AA.1-172) construct was seperately transfected into NIH3T3 and P19 cells and analyzed by the fluorescent microscopy. The EGFP-TCTP fusion protein was distributed both in the cytoplasm and in the nucleus (1 and 4), and the nuclei were stained with DAPI (2 and 5). The merge pictures (3 and 6) were also provided. Scale bar is 15 μm.

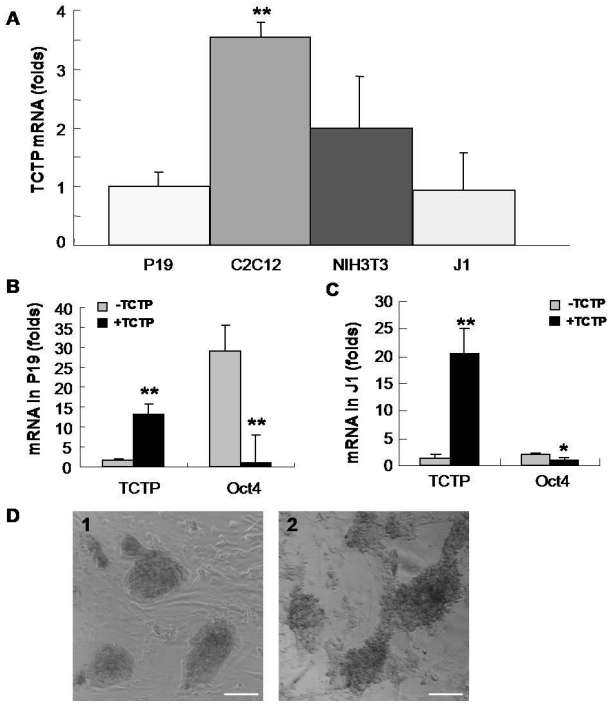

Fig. 2. TCTP overexpression downregulates the expression of Oct4. (A) The endogenous expression of TCTP in mouse P19, C2C12, NIH3T3 and J1 ES cells was determined by the real-time RT-PCR (B and C). The mRNA level of TCTP and *Oct4* were analyzed in P19 cells (B) and J1 ES cells (C). -TCTP, cells transfected with pEGFP-C1; +TCTP, cells transfected with pEGFP-TCTP (AA.1-172). *P $<$ 0.05; **P $<$ 0.01. (D) The differentiation of mouse J1 ES cells transfected with the pEGFP-TCTP (AA.1-172) construct for 72 h was evidenced by the loss of morphology of ES cells and the reduced activity of alkaline phosphotase. 1, Untransfected J1 ES cells; 2, J1 ES cells transfected with pEGFP-TCTP (AA.1-172). Scale bar is 25 μm.

compared the TCTP expression pattern in P19, C2C12, NIH3T3 and J1 ES cells by real time RT-PCR. The *TCTP* mRNA was detected in four cell lines (Fig. 2A). Moreover, the mRNA level of *TCTP* was 2-4 folds lower in pluripotent cells (P19 and J1 ES) than that in somatic cell lines (NIH3T3 and C2C12). To evaluate whether *TCTP* regulated *Oct4* expression, the pEGFP-TCTP (AA.1-172) was transfected into P19 and J1 ES cells, respectively. In (+TCTP) P19 cells transfected with pEGFP-TCTP (AA.1-172), the mRNA level of *TCTP* was significantly increased and the level of *Oct4* was significantly decreased compared to the control (−TCTP) cells transfected with pEGFP-C1 (Fig. 2B). Similar results were observed in J1 ES cells (Fig. 2C).

Since the level of *Oct4* expression is crucial to retaining the pluripotency of ES cells, we investigate whether the downregulation of *Oct4* expression due to overexpressing TCTP could induce ES cells differentiation. The differentiation of ES cells was evidenced by the loss of typical ES morphology and reduced alkaline phosphatase (AP) activity in J1 ES cells at 72 h after TCTP transfection (Fig. 2D).

To further evaluate the effect of TCTP on *Oct4* expression, we did the luciferase assay by cotransfection of pOct4-Luc with either pEGFP-TCTP (AA.1-172) or TCTP truncated constructs into P19 cells. The *Oct4* promoter activity in cells transfected with pEGFP-TCTP (AA.1-172) was 29 percent ($P < 0.05$) of that in control cells transfected with pEGFP-C1. In addition, overexpression of TCTP truncated construct pEGFP-TCTP (AA.1-60) reduced the *Oct4* promoter activity to 27 percent ($P < 0.05$) and the pEGFP-TCTP (AA.61-172) 50 percent ($P > 0.05$) of the control cells (Fig. 3A).

The previous study reported TCTP interacted with *Oct4* by binding to the Sf1 site of the *Oct4* promoter (14). To directly address the role of TCTP in *Oct4* promoter regulation, TCTP was cotransfected with either the *Oct4* report plasmid with Sf1 site or the plasmid without Sf1 site into P19 cells. Upon transfection into P19 cells, the Sf1 site deletion prevented the supression by TCTP (Fig. 3B). In addition, TCTP repressed the *Oct4* promoter activity both in a time-dependent manner (Fig. 3C) and in a dose-dependent manner (Fig. 3D). These observations further demonstrated that *TCTP* negatively regulated the expression of *Oct4*.

Knockdown of *TCTP* by siRNA upregulates the *Oct4* transcription

In light of the above observations that the exogenous *TCTP* re-

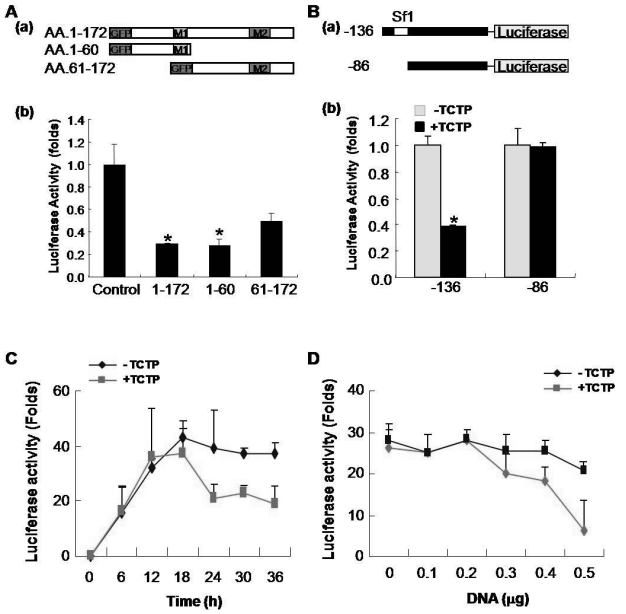

Fig. 3. Analysis of Oct4 promoter activity by the luciferase assay. (A) TCTP suppressed the *Oct4* promoter activity in P19 cells. (a) The representation of the EGFP-TCTP fusion constructs. The pEGFP-TCTP (AA.1-172) construct consists of the TCTP1 motif (M1) and TCTP2 motif (M2). The pEGFP-TCTP (AA.1-60) construct contains the TCTP1 motif, and pEGFP-TCTP (AA.61-172) construct contains the TCTP2 motif. (b) Relative *Oct4* promoter activity by cotransfecting TCTP constructs with pOct4-luc into P19 cells. Control, P19 cells transfected with pEGFP-C1. *P < 0.05. (B) TCTP represses the *Oct4* promoter through the Sf1 site. (a) The schematic representation of luciferase reporter plasmids by inserting *Oct4* promoter fragments from the 5′ nucleotide shown to nucleotide + 112 relative to the start codon into the pGL3-basic vector. (b) Relative luciferase activity of *Oct4* promoter by cotransfecting with pEGFP-TCTP (AA.1-172) into P19 cells. *P < 0.05. (C) Time-dependent effect of TCTP on *Oct4* promoter activity. The equal amount (0.4 μg) of pOct4-Luc with either pEGFP-TCTP (AA.1-172) or pEGFP-C1 was cotransfected into P19 cells. The luciferase activity was detected at interval of six hours. (D) Dose-dependent effect of TCTP on *Oct4* promoter activity. The pOct4-Luc (0.4 μg) together with either pEGFP-TCTP (AA.1-172) or pEGFP-C1 with varying concentration was cotransfected into P19 cells. The luciferase activity was detected at 24 h after the transfection. -TCTP, cells transfected with pEGFP-C1; +TCTP, cells transfected with pEGFP-TCTP (AA.1-172).

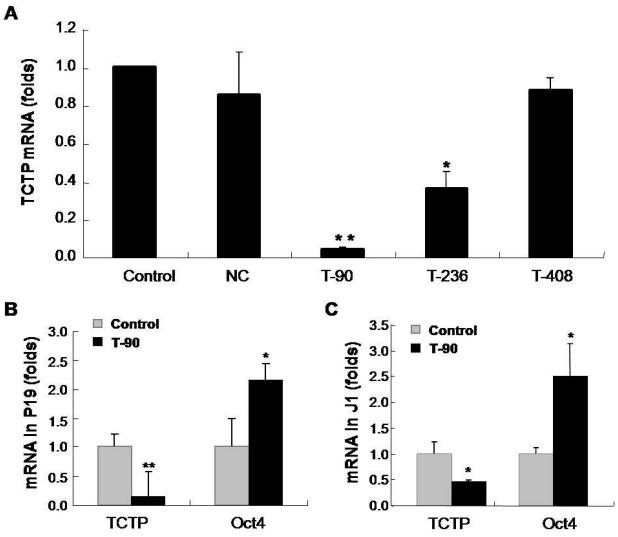

Fig. 4. siRNA decreases TCTP mRNA expression and increases the *Oct4* transcription. The siRNAs against TCTP were synthesized, including T-90, T-236, T-408 and negative control (NC). Cells were transiently transfected with different siRNAs for 48 h, and the inhibition efficiency was determined by real time RT-PCR. (A) Knockdown of TCTP in P19 cells. Control, untransfected P19 cells. (B and C) siRNA T-90 affects the expression of TCTP and *Oct4* in P19 cells and J1 ES cells. Control, cells transfected with negative control siRNA. *P < 0.05; **P < 0.01.

pressed *Oct4* expression, we doubted whether the level of *Oct4* might be elevated if the endogenous TCTP expression was knockdowned by siRNA in P19 and J1 ES cells. In the pre-experiment, three synthesized TCTP siRNA fragments (T-90, T-236 and T-408) were transfected into P19 cells to determine the efficiency of the candidate siRNAs by real time RT-PCR analysis. The highest knockdown efficiency was observed in T-90 siRNA which was used in the following experiments (Fig. 4A). In P19 cells, T-90 siRNA could significantly reduce the *TCTP* mRNA level and the knockdown of endogenous *TCTP* was associated with the two folds increase of *Oct4* mRNA transcription (Fig. 4B). The similar results were also identified in J1 ES cells (Fig. 4C).These results indicated that knockdown of the *TCTP* gene upregulated the *Oct4* transcription in mouse pluripotent cells.

DISCUSSION

Oct4 plays a crucial role in the development and is an essential factor to maintain the pluripotency of the ES cells. *Oct4* expression was regulated by cis-regulatory elements including three important elements: the distal enhancer, the proximal enhancer and the proximal promoter (16). Oct4 and Sox2 activated the Oct4 transcription by binding an Oct4/Sox2 element in the distal enhancer (17). The caudal-type homeobox transcription factor 2 (Cdx2) suppressed *Oct4* gene by binding to the distal enhancer (18). The orphan nuclear receptor liver receptor homolog 1 (LRH1) was a positive regulator of *Oct4* by binding to the proximal enhancer and proximal promoter (11).The precise level of *Oct4* was regulated by the balance between these positive and negative regulators (19). In this study we identified TCTP as a negative regulator of *Oct4* by binding the Sf1 site within the proximal promoter in mouse pluripotent cells.

The *TCTP* gene is highly conserved among eukaryotic organisms, indicating that it plays an essential role in the normal development (8). TCTP has a growth related function as the overexpression or knockdown TCTP disturbed the cell growth

(20, 21). The physiological role of TCTP epitomized that in TCTP knockout mice homozygous mutants ($TCTP^{-/-}$) were embryonic lethal and the knockout embryos suffered a high incidence of apoptosis (22). In Xenopus oocyte, it has been confirmed that TCTP has a role in transcriptional regulation of *Oct4* by directly binding to the Sf1 region, which is highly conserved between mouse and Xenopus, of *Oct4* promoter (14).

The investigation of the TCTP subcellular localization in P19 and NIH3T3 cells indicated that TCTP was located not only in the nucleus but also in the cytoplasm (Fig. 1B), which was consistent with the recent report revealing its distribution in mouse ES cells and embryonic carcinoma cells (20). In addition, the downregulation of *Oct4* by *TCTP* was confirmed through the luciferase assay by contransfecting the EGFP-TCTP fusion constructs with the *Oct4* promoter reporter plasmid pOct4-luc. Of note, the TCTP truncated construct pEGFP-TCTP (AA.1-60) containing the TCTP1 motif had a similar efficiency with the pEGFP-TCTP (AA.1-172) of repressing the *Oct4* promoter activity, which suggested that this region might contain the motif bound to the *Oct4* promoter. Furthermore, the effect of exogenous *TCTP* downregulating *Oct4* promoter activity represented a time-dependent and a dose-dependent manner in P19 cells. This regulation was due to the TCTP interacting with *Oct4* promoter by binding the Sf1 site (Fig. 3B), which was consistent with the previous report (14). Moreover, knockdown of *TCTP* by small interfering RNA upregulated *Oct4* transcription in both P19 and J1 ES cells (Fig. 4), further confirming that the *TCTP* gene downregulated the *Oct4* expression.

Our result that TCTP was a negative regulator of *Oct4* in mouse pluripotent cells was consistent with the recent reports which showed that TCTP interacted with nucleophosmin to form the complex to play the role during mitosis in mouse ES cells (20), and the knockdown of TCTP induced *Oct4* expression in mouse ES cells (15). However, our data was conflicting with the report which showed TCTP activated *Oct4* in Xenopus oocyte (14). One explanation is that amphibian oocytes and mammalian cells may have different epigenetic modification manners on *Oct4* regulation through TCTP, such as DNA methylation (14) and protein phosphorylation (23).

In summary, in this study we identified that the subcellular localization of the TCTP was present both in the nucleus and in the cytoplasm in P19 and NIH3T3 cells. Overexpression of *TCTP* gene decreased the *Oct4* expression, and the knockdown of *TCTP* by small interfering RNA molecules increased *Oct4* expression in mouse P19 and J1 ES cells. Our observation indicates that TCTP is a negative regulator of the *Oct4* gene by binding the Sf1 site in mouse pluripotent cells.

MATERIALS AND METHODS

Cell culture

The mouse embryonic carcinoma cell line P19 was cultured in α-MEM (Invitrogen) supplemented with 10% fetal bovine serum (Hyclone, USA). The mouse ES cell line J1 was maintained on the feeder layer of mouse embryonic fibroblasts in ES cell media [Dulbecco's modified eagle's medium (high glucose, USA) supplemented with 15% fetal bovine serum, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 2 mM L-glutamine, 0.1 mM β-mercaptoethanol, and 1,000 U/ml leukemia inhibitory factor (LIF, Gbico)]. The mouse C2C12 myoblasts and NIH3T3 fibroblasts were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum.

Vector constructions

The TCTP cDNA was amplified from the total RNA extracted from the P19 cells using the primers listed in the Table S1. PCR products were ligated into a pGEM-T Easy vector for sequencing. The TCTP coding sequence was subcloned into pEGFP-C1 to construct the pEGFP-TCTP (AA.1-172) vector. The pEGFP-TCTP (AA.1-172) plasmid was digested with KpnI site and the two fragments including TCTP (AA.1-60) fused with EGFP and TCTP (AA.61-172) were purified. The pEGFP-TCTP (AA.1-60) was constructed with self-ligation of the KpnI-digested pEGFP-TCTP (AA.1-172) plasmid. The TCTP fragment (AA.61-172) was ligated to KpnI digested pEGFP-C1 to construct the pEGFP-TCTP (AA.61-172). The mouse *Oct4* promoter region (−682 to +112), the region (−136 to +112) and the region (−86 to +112) were amplified from mouse liver genomic DNA and was ligated into the pGEM-T Easy vector. The mouse Oct4 promoters were subcloned into the vector pGL3-basic and positive clones were confirmed by sequencing. The mouse *Oct4* reporter vector containing the region (−682 to +112) was named as pOct4-Luc.

Quantitative real time RT-PCR

Total RNA was isolated with Trizol reagent (Invitrogen, USA) and reverse transcribed with RevertAid first-strand cDNA synthesis kit (Fermentas, Canada). Relative mRNA levels were evaluated by real-time RT-PCR carried out by using the SYBR Premix Ex Taq™ kit (TaKaRa, Japan). The β-actin was used as the internal control. Sequences of primers are listed in Table S1. All reactions were performed in triplicate, and the data were the average of three independent experiments.

Western blot and alkaline phosphatase assay

The plasmids, pEGFP-TCTP (AA.1-172) and pEGFP-C1, were transfected into P19 cells, respectively. At 48 h after the transfection, total protein was extracted from each sample. The equal amount of protein samples were separated on 12% SDS-PAGE gel and then transferred to the nitrocellulose membrane. The membrane was blocked by 5% skim milk, and then incubated with anti-GFP antibody (1 : 2,000, Abcam, USA), and followed by incubation with HRP-conjugated secondary antibody. Immunoreactive bands were detected by ECL kit (Pierce, USA). Alkaline phosphatase assay was performed based on the manufacturer's instruction (Sigma).

Transient transfection and luciferase assay

P19 cells and J1 ES cells were transfected with the different constructs using the Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen). Due to the low efficiency of transfection, J1 ES cells were transfected following the protocol described in the recent report (24). Cells were harvested at various time points after transfection, and luciferase activity was determined with the Enhanced Luciferase Assay Kit (BD Bioscience, USA) using the Centro LB960 96-well luminometer (Berthold Technologies).

siRNA interference

Based on mouse TCTP cDNA sequence, three siRNAs (T-90, T-236, T-408) of *TCTP* gene and a negative control (NC) siRNA were synthesized. The detail information of siRNA sequences is listed in Table S2. P19 cells and J1 ES cells were transfected with 50 nM of experimental siRNAs and control siRNA using the Lipofectamine 2000 reagent (Invitrogen). At 48 h after transfection, total RNA were isolated for real time RT-PCR assay described in previous section to determine the mRNA expression of *TCTP* and *Oct4* genes.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (#30871786) and the National Basic Research Program of China (973 Program) (#2009CB941002).

REFERENCES

1. Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663-676.
2. Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Scholer, H. and Smith, A. (1998) Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* **95**, 379-391.
3. Niwa, H., Miyazaki, J. and Smith, A. G. (2000) Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat. Genet* **24**, 372-376.
4. Matin, M. M., Walsh, J. R., Gokhale, P. J., Draper, J. S., Bahrami, A. R., Morton, I., Moore, H. D. and Andrews, P. W. (2004) Specific knockdown of Oct4 and beta2-microglobulin expression by RNA interference in human embryonic stem cells and embryonic carcinoma cells. *Stem Cells (Dayton, Ohio)* **22**, 659-668.
5. Shimozaki, K., Nakashima, K., Niwa, H. and Taga, T. (2003) Involvement of Oct3/4 in the enhancement of neuronal differentiation of ES cells in neurogenesis-inducing cultures. *Development* **130**, 2505-2512.
6. Bommer, U. A. and Thiele, B. J. (2004) The translationally controlled tumour protein (TCTP). *Int. J. Biochem. Cell Biol.* **36**, 379-385.
7. Susini, L., Besse, S., Duflaut, D., Lespagnol, A., Beekman, C., Fiucci, G., Atkinson, A. R., Busso, D., Poussin, P., Marine, J. C., Martinou, J. C., Cavarelli, J., Moras, D., Amson, R. and Telerman, A. (2008) TCTP protects from apoptotic cell death by antagonizing bax function. *Cell Death Differ.* **15**, 1211-1220.
8. Thaw, P., Baxter, N. J., Hounslow, A. M., Price, C., Waltho, J. P. and Craven, C. J. (2001) Structure of TCTP reveals unexpected relationship with guanine nucleotide-free chaperones. *Nat. Struct. Biol.* **8**, 701-704.
9. Hummelke, G. C. and Cooney, A. J. (2001) Germ cell nuclear factor is a transcriptional repressor essential for embryonic development. *Front Biosci.* **6**, D1186-1191.
10. Barnea, E. and Bergman, Y. (2000) Synergy of SF1 and RAR in activation of Oct-3/4 promoter. *J. Biol. Chem.* **275**, 6608-6619.
11. Gu, P. L., Goodwin, B., Chung, A. C. K., Xu, X. P., Wheeler, D. A., Price, R. R., Galardi, C., Peng, L., Latour, A. M., Koller, B. H., Gossen, J., Kliewer, S. A. and Cooney, A. J. (2005) Orphan nuclear receptor LRH-1 is required to maintain Oct4 expression at the epiblast stage of embryonic development. *Mol. Cell Biol.* **25**, 3492-3505.
12. Zhang, J., Tam, W. L., Tong, G. Q., Wu, Q., Chan, H. Y., Soh, B. S., Lou, Y., Yang, J., Ma, Y., Chai, L., Ng, H. H., Lufkin, T., Robson, P. and Lim, B. (2006) Sall4 modulates embryonic stem cell pluripotency and early embryonic development by the transcriptional regulation of Pou5f1. *Nat. Cell Biol.* **8**, 1114-1123.
13. Covello, K. L., Kehler, J., Yu, H., Gordan, J. D., Arsham, A. M., Hu, C. J., Labosky, P. A., Simon, M. C. and Keith, B. (2006) HIF-2alpha regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. *Genes Dev.* **20**, 557-570.
14. Koziol, M. J., Garrett, N. and Gurdon, J. B. (2007) Tpt1 activates transcription of Oct4 and nanog in transplanted somatic nuclei. *Curr. Biol.* **17**, 801-807.
15. Johansson, H. and Simonsson, S. (2010) Core transcription factors, Oct4, Sox2 and Nanog, individually form complexes with nucleophosmin (Npm1) to control embryonic stem (ES) cell fate determination. *Aging (Albany NY)* **2**, 815-822.
16. Yeom, Y. I., Fuhrmann, G., Ovitt, C. E., Brehm, A., Ohbo, K., Gross, M., Hubner, K. and Scholer, H. R. (1996) Germline regulatory element of Oct-4 specific for the totipotent cycle of embryonal cells. *Development* **122**, 881-894.
17. Okumura-Nakanishi, S., Saito, M., Niwa, H. and Ishikawa, F. (2005) Oct-3/4 and Sox2 regulate Oct-3/4 gene in embryonic stem cells. *J. Biol. Chem.* **280**, 5307-5317.
18. Niwa, H. T. Y., Shimosato, D., Strumpf, D., Takahashi, K., Yagi, R. and Rossant, J. (2005) Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. *Cell* **123**, 917-929.
19. Niwa, H. (2007) How is pluripotency determined and maintained? *Development* **134**, 635-646.
20. Johansson, H., Vizlin-Hodzic, D., Simonsson, T. and Simonsson, S. (2010) Translationally controlled tumor protein interacts with nucleophosmin during mitosis in ES cells. *Cell Cycle* **9**, 2160-2169.
21. Tuynder, M., Fiucci, G., Prieur, S., Lespagnol, A., Geant, A., Beaucourt, S., Duflaut, D., Besse, S., Susini, L., Cavarelli, J., Moras, D., Amson, R. and Telerman, A. (2004) Translationally controlled tumor protein is a target of tumor reversion. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 15364-15369.
22. Chen, S. H., Wu, P. S., Chou, C. H., Yan, Y. T., Liu, H., Weng, S. Y. and Yang-Yen, H. F. (2007) A knockout mouse approach

reveals that TCTP functions as an essential factor for cell proliferation and survival in a tissue- or cell type-specific manner. *Mol. Biol. Cell* **18**, 2525-2532.
23. Yarm, F. R. (2002) Plk phosphorylation regulates the microtubule-stabilizing protein TCTP. *Mol. Cell Biol.* **22**, 6209-6221.
24. Liou, J. Y., Ko, B. S. and Chang, T. C. (2010) An efficient transfection method for mouse embryonic stem cells. *Methods Mol. Biol.* **650**, 145-153.