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Identification of potential nuclear reprogramming and differentiation factors by a novel selection method for cloning chromatin-binding proteins

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Abstract

Nuclear reprogramming is critical for animal cloning and stem cell creation through nuclear transfer, which requires extensive remodeling of chromosomal architecture involving dramatic changes in chromatin-binding proteins. To understand the mechanism of nuclear reprogramming, it is critical to identify chromatin-binding factors specify the reprogramming process. In this report, we have developed a high-throughput selection method, based on T7 phage display and chromatin immunoprecipitation, to isolate chromatin-binding factors expressed in mouse embryonic stem cells using primary mouse embryonic fibroblast chromatin. Seven chromatin-binding proteins have been isolated by this method. We have also isolated several chromatin-binding proteins involved in hepatocyte differentiation. Our method provides a powerful tool to rapidly and selectively identify chromatin-binding proteins. The method can be used to study epigenetic modification of chromatin during nuclear reprogramming, cell differentiation, and transdifferentiation.

Keywords: Embryonic stem cells; Reprogramming; Epigenetics; T7 phage display; Chromatin-binding factors

Mammalian unfertilized oocytes contain factors capable of reprogramming somatic nuclei from a fully differentiated state to a toti/pluripotential state which was showed by the success of animal cloning [1–9] and nuclear transfer embryonic stem cell line establishment [10–13]. Recently, it has also been reported that embryonic stem (ES) and embryonic germ (EG) cells can induce nuclear reprogramming by hybridizing with somatic cells. The ES-somatic and EG-somatic hybrid cells exhibit the same pluripotential competence of the original ES or EG parental cells to differentiate into various tissues [14,15]. It is possible that ES and EG cells may share the same mechanism with oocytes to induce nuclear reprogramming of the somatic nucleus.

A transplanted somatic nucleus must activate stem cell-specific genes while at the same time inactivate tissue-specific genes to allow nuclear reprogramming. During this process, many proteins are specifically lost from the somatic nuclei, and others are taken up from the oocyte cytoplasm [16]. Nuclear reprogramming requires extensive remodeling of chromosomal architecture involving dramatic changes in chromatin-binding proteins. These changes alter the gene expression profile and are presumed to play a key role in the nuclear reprogramming. To understand the mechanism of nuclear reprogramming, it is important to identify and characterize such chromatin-modifying factors by cloning their encoding cDNAs. The traditional approach used to identify the chromatin-binding proteins is to isolate proteins following chromatin immunoprecipitation. Their cDNA cloning is achieved by protein purification, peptide microsequencing, and cDNA amplification with

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PCR primers designed on the basis of the amino acid sequence information. This labor-intensive strategy requires an enormous effort and can be particularly difficult if the chromatin-binding proteins are present at low cellular concentrations. Here, we have developed a novel genetic screening method to facilitate the rapid cloning of chromatin-binding proteins potentially involved in nuclear reprogramming. Our method couples the T7 phage display with chromatin immunoprecipitation and provides a fast high-throughput method.

Materials and methods

CDNA library construction. We constructed phage display cDNA libraries using the bacteriophage T7Select System. ES cells were grown on MEF feeder in Dulbecco's modified Eagle's medium (DMEM) (Gibco/BRL, NY, USA) supplied with 20% fetal calf serum, 1400 U/ml of leukemia inhibitory factor, 100 mM MEM nonessential amino acid, 0.55 mM of 2-mercaptoethanol, L-glutamine, and antibiotics (all form Gibco/BRL). Poly(A)$^+$ RNA was isolated from the ES cells by FastTrack 2.0 kit (Invitrogen, California, USA). cDNA was synthesized and modified by OrientExpress Random Primer cDNA Synthesis Kit and *Eco*RI/*Hin*dIII End Modification Kit (Novagen, Wisconsin, USA). cDNA was inserted into the *Eco*RI and *Hin*dIII digested T7Select10-3 vector (Novagen), which display products of the inserted cDNA on the surface of T7 phage particles as a fusion product with its capsid protein 10B. Phage were packaged in vitro and used to infect the host bacterial strain, BLT5615 (Novagen). The library was titrated by plaque assays and amplified prior to biopanning.

Preparation of chromatin. Chromatin was isolated from primary mouse embryonic fibroblast (MEF) of 13.5 dpc embryo from mouse strain Kunming. Cultured cells were treated with 1% formaldehyde and the crosslinking was stopped by the addition of glycine to a final concentration of 0.125 M. The cells were rinsed twice with cold phosphate-buffered saline plus protease inhibitors: 1 mM PMSF, 10 μg/ml aprotinin, and 10 μg/ml leupeptin (all from Sigma–Aldrich, St. Louis), and scraped from the dishes. The cell pellet was resuspended in cell lysis buffer (5 mM Pipes, pH 8.0, 85 mM KCl, 0.5% NP40, and protease inhibitors). After being incubated on ice for 10 min, cells were passed through a 21-gauge needle three times on ice. The nuclei were centrifuged at 5000 rpm for 5 min at 4 °C and resuspended in nuclei lysis buffer (50 mM Tris–Cl, pH 8.1, 10 mM EDTA, 1% SDS, and protease inhibitors). Following incubation on ice for 10 min, chromatin was sonicated with a 0.3 mm tip with eight bursts of 8 s at 400 V (Xinzhi JY92 II, Ningbo PLC). Chromatin was centrifuged at 14,000 rpm for 10 min at 4 °C. The supernatant was concentrated with a Miliipore centricon YM30. Chromatin was resuspended in IP dilution buffer (0.01% SDS, 1.1% Trition X-100, 1.2 mM EDTA, 16.7 mM Tris–Cl, pH 8.1, 167 mM NaCl, and protease inhibitors), snap-frozen in liquid nitrogen, and stored at −70 °C until use.

Biopanning. The 96-well ELISA plate (Nunc) was coated by 100 μl of 1:30 diluted histone H3 antibody (Cat#9712, Cell signaling, MA) at 4 °C overnight. The plate was washed three times by TBST, blocked with 3% BSA/TBS at 37 °C for 1 h, and washed three times by TBST. The plate was then incubated with 100 μl chromatin extract at 37 °C for 1 h. The plate was washed twice with RIPA buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Triton 100, 0.5% deoxycholic, 0.01% SDS, 1 mM EDTA, 50 mM PMSF, and protease inhibitor cocktail) plus 500 mM NaCl. The plate was incubated for 30 min with 1×10^9 phages at room temperature, followed by washing five times with RIPA buffer and twice with RIPA buffer plus 500 mM NaCl. Captured phages were released by incubation with elution buffer (1% SDS, 0.1 M $NaHCO_3$) for 20 min at room temperature. The eluted phages were used to infect the BLT5615 strain of *Escherichia coli* and incubated at 37 °C until extensive lysis was observed. The lysed bacteria were centrifuged at 9000 rpm for 10 min to remove bacteria debris. The supernatant was titrated and used for the next round of biopanning.

PCR amplification and sequence analysis of selected phage recombinants. To determine the efficiency of biopanning, phage lysates containing selected populations of phages or randomly picked plaques from each round of biopanning were used as templates for PCR amplification. T7 UP and T7 DOWN primer (Novagen) corresponding to the sequences in the phage vector DNA were used. PCR was performed in 20 μl of a reaction mixture containing 1× PCR buffer (10 mM KCl, 2.5 mM MgCl, and 10 mM Tris–HCl, pH 8.3), 200 μM dNTP mix, 20 pmol each of primers, and 1 U *Taq* DNA polymerase (Takara, Dalian, PLC). Amplified fragments were analyzed through 1.5% agarose gel electrophoresis, gel purified with GeneClean III kit (BIO 101, Vista, CA), and subjected to DNA sequencing. The sequences were analyzed using BLAST.

Results and discussion

Screening ES cDNA library using MEF chromatin

We chose mouse ES cells as a cell source to identify the nuclear reprogramming factors, because it was easy to obtain a large quantity of cells. We have constructed a mouse ES cell phage display cDNA library using the T7Select10-3 vector. The library contained 1.2×10^6 independent clones, as determined by the plaque assay. The cDNA insert sizes were determined by PCR amplification of 25 randomly picked plaques from the library using the T7 UP and T7 DOWN primers corresponding to the sequences in the phage vector. The size range of the cDNA clones is between 0.4 and 1.0 kb.

We used the MEF cells as the chromatin source to isolate the specific chromatin-binding proteins from the ES cell library. The MEF cells were treated with 1% formaldehyde and the chromatin was sonicated to an average length of about 750 bp. The chromatin was immobilized onto an anti-histone H3 monoclonal antibody-coated ELISA plate and confirmed by ELISA using an anti-histone H2A antibody (data not shown). Then we incubated the T7 phage display library of mouse ES cells with the chromatin-coated plate. To screen for clones specifically binding to the chromatin of MEF cells (Fig. 1), the plate was washed with RIPA buffer and specifically bound phage particles were then eluted with elution buffer (1% SDS, 0.1 M $NaHCO_3$). The eluted phages then underwent a second round of screening. Totally five rounds of screening were carried out to enrich the specific phage clones. The cDNA inserts from the eluted phages were then analyzed by PCR with primers flanking the insert site of the T7 phage DNA. The phage PCR products from the primary library, and the eluted phage from the first round appeared as a smear on agarose gel. The PCR products of the eluted phages from the second-, third-, fourth-, and fifth-round selection, however, revealed several

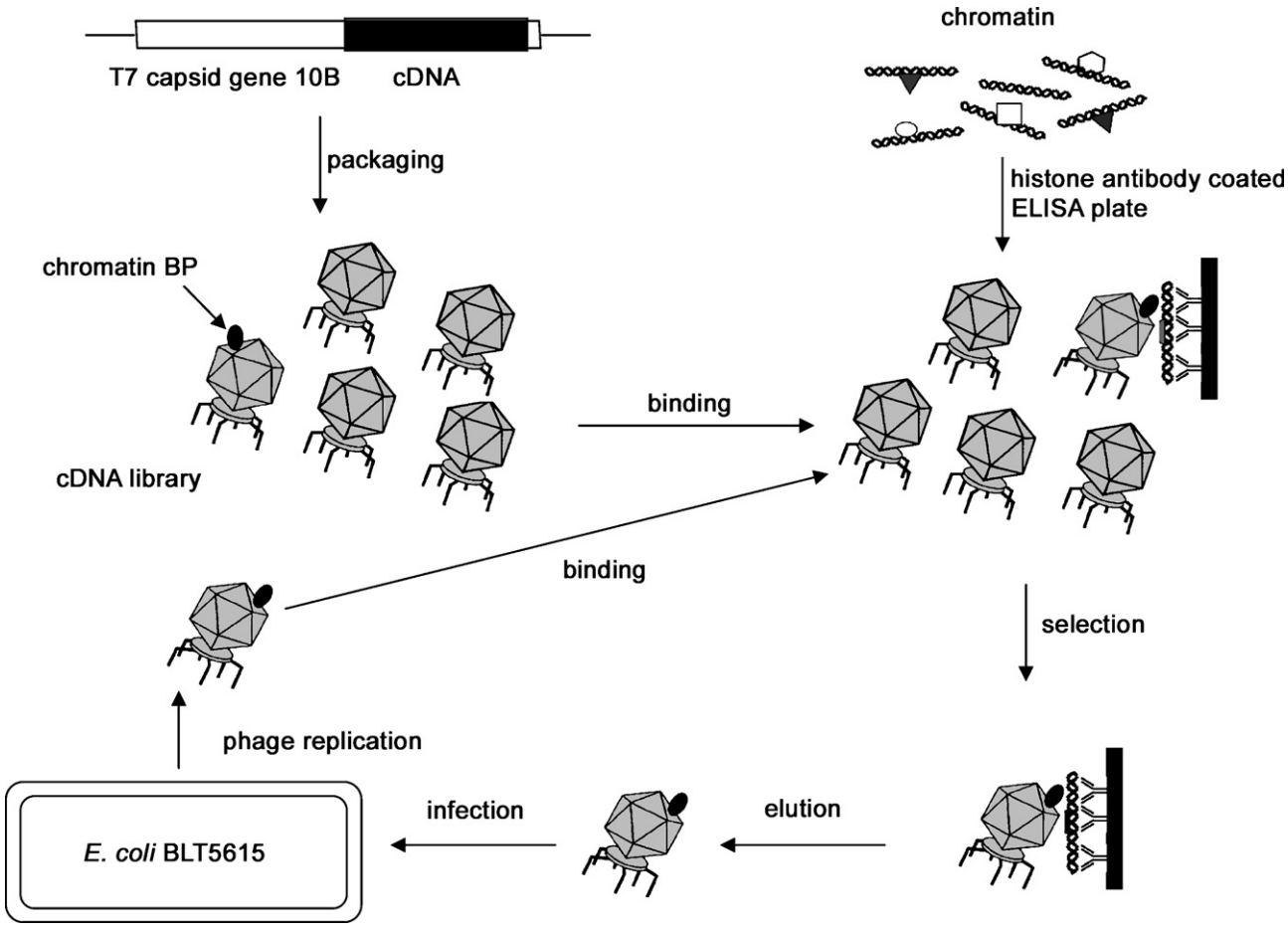

Fig. 1. Selection of chromatin-specific binding protein cDNA by using T7 phage display. Mouse ES cell cDNA was inserted into the T7 cloning vector and packaged in a phage capsid to generate recombinant phages in which proteins encoded by ES cDNA were displayed on the surface as a carboxyl-terminal fusion to the T7 capsid protein 10B. The chromatin of the differentiated cells was bound on an anti-histone H3 antibody-coated plate. Phages, which contain cDNA encoding chromatin-specific binding proteins, were captured on the chromatin-coated plate. Unbound phage was removed by washing with RIPA buffer, and then chromatin-binding phage was eluted to release from the plate by using elution buffer. After amplification, the phage progeny are subjected to additional rounds of selection.

bands between 350 and 600 bp. These results indicate that phages with chromatin-binding activity were selectively enriched by this procedure.

After five rounds of selection, the cDNA inserts from 105 individual phages were amplified by PCR and sequenced. These sequences were searched in GenBank by the Basic Local Alignment Search Tool (BLAST) program. Seven chromatin-binding proteins potentially involved in nuclear reprogramming were isolated, including makorin1, *trans*-acting transcription factor sp3, ATRX protein (putative ATPase and helicase)/X-linked nuclear protein, active L1 retrotransposon, polydom (polydomain protein), protein similar to KRAB, and Nsd1 (Table 1). To evaluate the specificity of the method, the selected phage clones were diluted with the wild type T7 phage at a ratio of $1{:}10^4$ and enriched again by the MEF chromatin-coated plates but not by plates only coated with anti-histone H3 antibody.

Potential nuclear reprogramming factors screened from ES cDNA library

Among these seven genes screened, three of them have been demonstrated to be involved in DNA methylation and histone modification, which are the most well-characterized epigenetic modifications associated with regulation of gene expression [17,18], genomic imprinting [19], and inactivation of the X chromosome [20]. We found one of the positive clones encodes the transcription factor Sp3. It has been reported that Sp3 controls the expression of the Dnmt1 [21], a DNA methyltransferase that functions to maintain appropriate patterns of DNA methylation in the genome during the cell cycle [22]. Sp3 can work either as an activator or a repressor of transcription, depending on the targeted genes [23,24]. Thus, Sp3 may be involved in nuclear reprogramming by regulating the expression of its downstream genes. The isolated Nsd1 contains several

Table 1
List of identified genes encoding MEF chromatin-specific binding proteins from ES cDNA library with homology to sequence in the public databases

Clone	Identified gene	Encoded protein (Accession number)
1	Mkrn1	Makorin1 (AF192785)
2	Sp3	*trans*-acting transcription factor sp3 (AF062567)
3	Atrx/Xnp	ATRX protein (putative ATPase and helicase)/X-linked nuclear protein (AF026032/NM_009530)
4	L1 retrotransposon ORF2	pORF2, active L1 retrotransposon (AY053455)
5	Polydom-pending	Polydom (polydomain protein) (NM_022814)
6	Similar to KRAB	Similar to Kruppel associated box (KRAB) zinc finger 1 (BC004747)
7	Nsd1	Nuclear receptor-binding SET-domain protein 1(NM_008739)

domains involved in the epigenetic control of transcription [25] including a domain that serves as a histone methyltransferase. We also isolated Atrx, a member of the SWI/SNF2 helicase/ATPase family. The SWI/SNF-like complexes have been shown to have chromatin remodeling activity. Mutations in the Atrx gene give rise to alterations in methylation sites at the highly repeated sequences, rDNA arrays, a Y-specific satellite, and subtelomeric repeats [26,27].

Other genes however, have been poorly studied, but there is some evidence suggesting that they may be involved in the process of reprogramming. One of them belongs to the active L1 retrotransposon subfamily GF [28]. The L1 retrotransposons influence the genome function in a number of ways that usually lead to major structural remodeling of the genome, often altering gene expression. A recent study has shown that the L1 family retrotransposon is actively transcribed by RNA polymerase III in the nuclei of ES and EG cells, and their hybrids with somatic cells [29]. It has been speculated that L1 functions to boost the heterochromatinization signals to inactivate the X chromosome in every female embryonic cell [30]. It is possible that the factor we isolated may be involved in the decondensation of chromatin of somatic nuclei following hybridization with ES and EG cells. Another gene Mkrn1 is a putative downstream gene of Oct-4 [31] which plays an essential role in the establishment and maintenance of toti/pluripotent cells. As a downstream factor of Oct-4, Mkrn1 may also play important roles in the establishment and maintenance of toti/pluripotent cells. We also cloned a large protein, named Polydom, containing a pentraxin domain and a von Willebrand factor type A domain, ten EGF domains, and 34 complement control protein modules [32]. The particular multidomain structure of polydom suggests an important biological role in cellular adhesion and/or in the immune system. Obviously, understanding the function of polydom in reprogramming requires further investigation.

Screening for differentiation factors from liver cDNA library

To further establish the utility of this novel method, we have attempted to isolate protein potentially involved in hepatocyte differentiation. In this case, the chromatin was isolated from the ES cells. The isolated ES chromatin was then immobilized onto an anti-histone H3 antibody-coated plate. The T7 phage library displaying proteins from a mouse liver cDNA library was screened for proteins that bind specifically to the chromatin of ES cells on the plate. After six rounds of selection, DNA inserts from 20 individual phages were amplified by PCR and sequenced. These sequences were searched in GenBank by the BLAST program. As a result, four different genes were obtained, including mouse liver fatty acid-binding protein 1, protein phosphatase 2A (PP2A), and another two not matched to any sequence in GenBank, respectively (Table 2).

PP2A is an enzyme implicated in the regulation of metabolism, transcription, RNA splicing, translation, differentiation, cell cycle, oncogenic transformation, and signal transduction. PP2A may also participate in hepatocytic differentiation [33]. PP2A can dephosphorylate the phosphorylated serine and threonine residues of some transcription factors. Very often, PP2A binds to specific regulatory proteins for its function. Identification of PP2A illustrates that our method can be used for isolating proteins that interact with the chromatin-binding proteins, and can be used for studying the specific requirement during the differentiation process from ES cells to hepatocytes.

In this study, we devised a powerful selection strategy for isolating chromatin-binding proteins, combining the T7 phage display and chromatin immunoprecipitation. A major advantage of this selection method is that it is rapid. Normally, two cycles of specific binding and selection can be performed in a single day, making it possible to isolate candidate genes within 2–3 days. This is significantly shorter than other cloning methods. The

Table 2
List of identified genes encoding ES chromatin-binding proteins from mouse liver tissue cDNA library with homology to sequence in the public databases

Clone	Identified gene	Encoded protein (Accession number)
1	Fabp1	Liver fatty acid binding protein 1 (NM_017399)
2	PPP2R3	Protein phosphatase 2 (NM_002718)
3	Unknown	Unknown (AK039001)
4	Homo sapiens, clone IMAGE:5267390, mRNA	Unknown protein (BC036664)

second benefit of this new strategy is the high-throughput selection. The high-throughput selection strategy will increase the efficiency of cloning chromatin-binding proteins. There are still a few drawbacks to be further improved. First, as the chromatin is fixed with formaldehyde, it could not properly display the dynamic process of protein–chromatin binding. Second, in the step of sonication some protein might be lost from the chromatin which might destroy the protein-binding property of the chromatin.

We have demonstrated the effectiveness of T7 phage display-based selection method as a new strategy for the isolation of chromatin-binding proteins from a cDNA library. This procedure can be used to study epigenetic modification of chromatin during differentiation and transdifferentiation. The efficiency for identification of chromatin-binding proteins will be further increased by combining this selection strategy with the microarray method.

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References

[1] I. Wilmut, A.E. Schnieke, J. McWhir, A.J. Kind, K.H. Campbell, Viable offspring derived from fetal and adult mammalian cells, Nature 385 (1997) 810–813.

[2] T. Wakayama, A.C. Perry, M. Zuccotti, K.R. Johnson, R. Yanagimachi, Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei, Nature 394 (1998) 369–374.

[3] A. Baguisi, E. Behboodi, D.T. Melican, J.S. Pollock, M.M. Destrempes, C. Cammuso, J.L. Williams, S.D. Nims, C.A. Porter, P. Midura, M.J. Palacios, S.L. Ayres, R.S. Denniston, M.L. Hayes, C.A. Ziomek, H.M. Meade, R.A. Godke, W.G. Gavin, E.W. Overstrom, Y. Echelard, Production of goats by somatic cell nuclear transfer, Nat. Biotechnol. 17 (1999) 456–461.

[4] I.A. Polejaeva, S.H. Chen, T.D. Vaught, R.L. Page, J. Mullins, S. Ball, Y. Dai, J. Boone, S. Walker, D.L. Ayares, A. Colman, K.H. Campbell, Cloned pigs produced by nuclear transfer from adult somatic cells, Nature 407 (2000) 86–90.

[5] P. Chesne, P.G. Adenot, C. Viglietta, M. Baratte, L. Boulanger, J.P. Renard, Cloned rabbits produced by nuclear transfer from adult somatic cells, Nat. Biotechnol. 20 (2002) 366–369.

[6] T. Shin, D. Kraemer, J. Pryor, L. Liu, J. Rugila, L. Howe, S. Buck, K. Murphy, L. Lyons, M. Westhusin, A cat cloned by nuclear transplantation, Nature 415 (2002) 859.

[7] G.L. Woods, K.L. White, D.K. Vanderwall, G.P. Li, K.I. Aston, T.D. Bunch, L.N. Meerdo, B.J. Pate, A mule cloned from fetal cells by nuclear transfer, Science 301 (2003) 1063.

[8] C. Galli, I. Lagutina, G. Crotti, S. Colleoni, P. Turini, N. Ponderato, R. Duchi, G. Lazzari, Pregnancy: a cloned horse born to its dam twin, Nature 424 (2003) 635.

[9] Q. Zhou, J.P. Renard, G. Le Friec, V. Brochard, N. Beaujean, Y. Cherifi, A. Fraichard, J. Cozzi, Generation of fertile cloned rats by regulating oocyte activation, Science 302 (2003) 1179.

[10] M.J. Munsie, A.E. Michalska, C.M. O'Brien, A.O. Trounson, M.F. Pera, P.S. Mountford, Isolation of pluripotent embryonic stem cells from reprogrammed adult mouse somatic cell nuclei, Curr. Biol. 10 (2000) 989–992.

[11] T. Wakayama, V. Tabar, I. Rodriguez, A.C. Perry, L. Studer, P. Mombaerts, Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer, Science 292 (2001) 740–743.

[12] Y. Chen, Z.X. He, A. Liu, K. Wang, W.W. Mao, J.X. Chu, Y. Lu, Z.F. Fang, Y.T. Shi, Q.Z. Yang, Y. Chen da, M.K. Wang, J.S. Li, S.L. Huang, X.Y. Kong, Y.Z. Shi, Z.Q. Wang, J.H. Xia, Z.G. Long, Z.G. Xue, W.X. Ding, H.Z. Sheng, Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes, Cell Res. 13 (2003) 251–263.

[13] W.S. Hwang, Y.J. Ryu, J.H. Park, E.S. Park, E.G. Lee, J.M. Koo, H.Y. Jeon, B.C. Lee, S.K. Kang, S.J. Kim, C. Ahn, J.H. Hwang, K.Y. Park, J.B. Cibelli, S.Y. Moon, Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst, Science 303 (2004) 1669–1674.

[14] M. Tada, Y. Takahama, K. Abe, N. Nakatsuji, T. Tada, Nuclear reprogramming of somatic cells by in vitro hybridization with ES cells, Curr. Biol. 11 (2001) 1553–1558.

[15] M. Tada, T. Tada, L. Lefebvre, S.C. Barton, M.A. Surani, Embryonic germ cells induce epigenetic reprogramming of somatic nucleus in hybrid cells, EMBO J. 16 (1997) 6510–6520.

[16] J.B. Gurdon, R.A. Laskey, E.M. De Robertis, G.A. Partington, Reprogramming of transplanted nuclei in amphibia, Int. Rev. Cytol. (Suppl.) (1979) 161–178.

[17] S.U. Kass, D. Pruss, A.P. Wolffe, How does DNA methylation repress transcription?, Trends Genet. 13 (1997) 444–449.

[18] A. Razin, A.D. Riggs, DNA methylation and gene function, Science 210 (1980) 604–610.

[19] M.S. Bartolomei, S.M. Tilghman, Genomic imprinting in mammals, Annu. Rev. Genet. 31 (1997) 493–525.

[20] R. Jaenisch, C. Beard, J. Lee, Y. Marahrens, B. Panning, Mammalian X chromosome inactivation, Novartis Found. Symp. 214 (1998) 200–209, see discussion 209–213, 228–232.

[21] S. Kishikawa, T. Murata, H. Kimura, K. Shiota, K.K. Yokoyama, Regulation of transcription of the Dnmt1 gene by Sp1 and Sp3 zinc finger proteins, Eur. J. Biochem. 269 (2002) 2961–2970.

[22] J.A. Yoder, R.W. Yen, P.M. Vertino, T.H. Bestor, S.B. Baylin, New 5′ regions of the murine and human genes for DNA (cytosine-5)-methyltransferase, J. Biol. Chem. 271 (1996) 31092–31097.

[23] G. Hagen, S. Muller, M. Beato, G. Suske, Sp1-mediated transcriptional activation is repressed by Sp3, EMBO J. 13 (1994) 3843–3851.

[24] S.B. Kennett, A.J. Udvadia, J.M. Horowitz, Sp3 encodes multiple proteins that differ in their capacity to stimulate or repress transcription, Nucleic Acids Res. 25 (1997) 3110–3117.

[25] R.J. Jaju, C. Fidler, O.A. Haas, A.J. Strickson, F. Watkins, K. Clark, N.C. Cross, J.F. Cheng, P.D. Aplan, L. Kearney, J. Boultwood, J.S. Wainscoat, A novel gene, NSD1, is fused to NUP98 in the t(5;11)(q35;p15.5) in de novo childhood acute myeloid leukemia, Blood 98 (2001) 1264–1267.

[26] C. Cardoso, S. Timsit, L. Villard, M. Khrestchatisky, M. Fontes, L. Colleaux, Specific interaction between the XNP/ATR-X gene product and the SET domain of the human EZH2 protein, Hum. Mol. Genet. 7 (1998) 679–684.

[27] R.J. Gibbons, T.L. McDowell, S. Raman, D.M. O'Rourke, D. Garrick, H. Ayyub, D.R. Higgs, Mutations in ATRX, encoding a SWI/SNF-like protein, cause diverse changes in the pattern of DNA methylation, Nat. Genet. 24 (2000) 368–371.
[28] J.L. Goodier, E.M. Ostertag, K. Du, H.H. Kazazian Jr., A novel active L1 retrotransposon subfamily in the mouse, Genome Res. 11 (2001) 1677–1685.
[29] T. Tada, M. Tada, Toti-/pluripotential stem cells and epigenetic modifications, Cell Struct. Funct. 26 (2001) 149–160.
[30] M.F. Lyon, X-chromosome inactivation: a repeat hypothesis, Cytogenet. Cell Genet. 80 (1998) 133–137.
[31] Z. Du, H. Cong, Z. Yao, Identification of putative downstream genes of Oct-4 by suppression-subtractive hybridization, Biochem. Biophys. Res. Commun. 282 (2001) 701–706.
[32] D. Gilges, M.A. Vinit, I. Callebaut, L. Coulombel, V. Cacheux, P.H. Romeo, I. Vigon, Polydom: a secreted protein with pentraxin, complement control protein, epidermal growth factor and von Willebrand factor A domains, Biochem. J. 352 (Pt 1) (2000) 49–59.
[33] M. Enjoji, M. Nakamuta, H. Nawata, Protein phosphatase 2A may participate in hepatocytic differentiation, In Vitro Cell Dev. Biol. Anim. 38 (2002) 5–6.