9 Reprogramming

To date, several different approaches can be applied to the reprogramming of somatic cells to a pluripotent state [144, 145]. Briefly, a somatic cell nucleus transferred to an unfertilized egg acquires pluripotency and develops into a blastocyst, allowing cloned ES cells to be established. Alternatively, somatic cells can be reprogrammed by cell–cell hybridization (cell fusion). The disadvantages of these techniques are the destruction of the embryo (i.e. ethical problem) and the tetraploid karyotype of the fused cells (i.e. risk problem in transplantation), respectively. Because these problems can be avoided using iPS cells, most recent reprogramming studies have utilized iPS cell systems.

In iPS induction, *Oct3/4*, *Sox2*, *Klf4*, and *Myc* (4 factors) are simultaneously introduced into somatic cells, and the reprogrammed cells are selected by marker gene expression and/or morphology [146]. Retroviruses were initially used to introduce the four factors, but the resulting iPS cells showed numerous insertions in the chromosome, raising safety issues. Currently, iPS cells can be established by a DNA-free method, in which the proteins of the four factors are tagged with an arginine stretch that confers membrane permeability [147, 148]. The cell types in which successful reprogramming have been reported include fibroblasts, primary hepatocytes, and completely differentiated B lymphocytes, suggesting that all the cell types in the body can be reprogrammed using the iPS technique [149–151]. The progression of reprogramming in iPS induction is relatively slow when compared with other techniques. In cell fusion, the upregulation of *Oct3/4* in completely differentiated cells is observed within 2 days [152], whereas in the iPS process this upregulation is first detectable 16 days after induction [153].

The molecular mechanism of this reprogramming is not clear at present. The efficiency of iPS establishment is low, less than a few percent of cells treated [154], indicating that in most cells the reprogramming is aborted even in the presence of the four factors. The function of Myc for iPS induction is dispensable, although it enhances the efficiency of the iPS establishment, probably through repressing the expression of differentiated cell-specific genes while promoting binding of Oct3/4, Sox2, and Klf4 (OSK) to their target genes [75, 155]. In fact, in partially reprogrammed cells OSK does not bind to the target genes (which are thus not expressed), suggesting that the cellular environment ensuring access of these factors to the target genes is rate limiting in reprogramming [155]. In addition, administration of chemical inhibitors targeting epigenetic factors that are associated with transcriptional repression is effective for enhancing iPS cell induction. These inhibitors include BIX-01294 (G9a inhibitor) [156], AZA (5-aza-cytidine, Dnmts inhibitor) [154, 157], VPA (valproic acid, Hdac inhibitor) [158], and TSA (trichostatin A, Hdac inhibitor) [158], and such findings suggest that target accessibility accompanying the global transcriptional activation seen in ES cells is critical to the initiation of the pluripotency transcriptional network. During iPS induction, OSK activity is enhanced by the transcription factors known to co-regulate with OSK, which include Esrrb, Sall4 and Tcf3 (via Wnt signaling), since forced expression of those factors can enhance the efficiency of iPS induction [80, 142, 159–161].

Collectively, the mechanism of reprogramming in iPS induction can be hypothesized as follows. Upon introduction of the four factors, the endogenous genes for the transcription factors necessary to pluripotency are primed and gradually induced to express through the regulatory region targeted by OSK. Subsequently, the transcriptional circuit begins to self-stabilize via increasing expression of the endogenous core transcription factors, through a positive-feedback loop, while repressing the developmentally regulated genes through recruitment of epigenetic factors and various protein complexes such as Paf1C and NODE. Once the stable transcriptional network is established/self-stabilized, exogenous cDNA expression is no longer necessary, and iPS cells indistinguishable from ES cells can be obtained after selection based on the expression of endogenous *Oct3/4* and/or *Nanog*. This outcome is the result of concerted action by a group of molecules that play a central role in pluripotency.

10 Conclusion

Pluripotency of ES cells is externally regulated through several molecules, including Wnt and Lif, whose signaling pathway activates transcription factor genes such as *Klf4* and *Nanog* in the nucleus. The core transcription factors, including Oct3/4, Sox2, and Klf4 positively self-regulate while also repressing developmentally regulated genes by co-occupation with a variety of protein complexes. Introduction of *Oct3/4*, *Sox2*, and *Klf4* into the somatic cells gradually reconstitutes the above transcriptional network with the aid of Myc and epigenetic modifiers, which might allow the regulatory regions of the target genes more access to these transcription factors.

Acknowledgments I thank Dr. Mitsuhiro Endoh for his helpful suggestions. This work was supported in part by the PRESTO program of the Japan Science and Technology Agency and by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation. This work is also supported in part by the Grants-in-Aid for Scientific research from the Ministry of Health, Labor, Welfare of Japan, and by that of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1. Solter D. From teratocarcinomas to embryonic stem cells and beyond: a history of embryonic stem cell research. Nat Rev Genet. 2006;7:319–27.
2. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci USA. 1981;78:7634–8.
3. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1981;292:154–6.
4. Brons IG, Smithers LE, Trotter MW, et al. Derivation of pluripotent epiblast stem cells from mammalian embryos. Nature. 2007;448:191–5.
5. Tesar PJ, Chenoweth JG, Brook FA, et al. New cell lines from mouse epiblast share defining features with human embryonic stem cells. Nature. 2007;448:196–9.
6. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282:1145–7.
7. Guo G, Yang J, Nichols J, et al. Klf4 reverts developmentally programmed restriction of ground state pluripotency. Development. 2009;136:1063–9.
8. Chou YF, Chen HH, Eijpe M, et al. The growth factor environment defines distinct pluripotent ground states in novel blastocyst-derived stem cells. Cell. 2008;135:449–61.
9. Ogawa K, Matsui H, Ohtsuka S, Niwa H. A novel mechanism for regulating clonal propagation of mouse ES cells. Genes Cells. 2004;9:471–7.
10. Abdelalim EM, Tooyama I. BNP signaling is crucial for embryonic stem cell proliferation. PLoS ONE. 2009;4:e5341.
11. Andang M, Hjerling-Leffler J, Moliner A, et al. Histone H2AX-dependent GABA(A) receptor regulation of stem cell proliferation. Nature. 2008;451:460–4.
12. Takahashi K, Mitsui K, Yamanaka S. Role of ERas in promoting tumour-like properties in mouse embryonic stem cells. Nature. 2003;423:541–5.
13. Matoba R, Niwa H, Masui S, et al. Dissecting Oct3/4-regulated gene networks in embryonic stem cells by expression profiling. PLoS ONE. 2006;1:e26.
14. Loh YH, Zhang W, Chen X, George J, Ng HH. Jmjd1a and Jmjd2c histone H3 Lys 9 demethylases regulate self-renewal in embryonic stem cells. Genes Dev. 2007;21:2545–57.
15. Ema M, Mori D, Niwa H, et al. Kruppel-like factor 5 is essential for blastocyst development and the normal self-renewal of mouse ESCs. Cell Stem Cell. 2008;3:555–67.
16. Tanaka Y, Patestos NP, Maekawa T, Ishii S. B-*myb* is required for inner cell mass formation at an early stage of development. J Biol Chem. 1999;274:28067–70.
17. Iwai N, Kitajima K, Sakai K, Kimura T, Nakano T. Alteration of cell adhesion and cell cycle properties of ES cells by an inducible dominant interfering Myb mutant. Oncogene. 2001;20:1425–34.
18. Tarasov KV, Tarasova YS, et al. B-MYB is essential for normal cell cycle progression and chromosomal stability of embryonic stem cells. PLoS ONE. 2008;3:e2478.
19. Takahashi K, Murakami M, Yamanaka S. Role of the phosphoinositide 3-kinase pathway in mouse embryonic stem (ES) cells. Biochem Soc Trans. 2005;33:1522–5.
20. Niwa H. How is pluripotency determined and maintained? Development. 2007;134:635–46.
21. Smith AG, Heath JK, Donaldson DD, et al. Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. Nature. 1988;336:688–90.
22. Burdon T, Smith A, Savatier P. Signalling, cell cycle and pluripotency in embryonic stem cells. Trends Cell Biol. 2002; 12:432–8.
23. Cartwright P, McLean C, Sheppard A, Rivett D, Jones K, Dalton S. LIF/STAT3 controls ES cell self-renewal and pluripotency by a Myc-dependent mechanism. Development. 2005;132:885–96.
24. Niwa H, Ogawa K, Shimosato D, Adachi K. A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells. Nature. 2009;460:118–22.
25. Niwa H, Burdon T, Chambers I, Smith A. Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. Genes Dev. 1998;12:2048–60.
26. Matsuda T, Nakamura T, Nakao K, et al. STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic stem cells. EMBO J. 1999;18:4261–9.
27. Paling NR, Wheadon H, Bone HK, Welham MJ. Regulation of embryonic stem cell self-renewal by phosphoinositide 3-kinase-dependent signaling. J Biol Chem. 2004;279:48063–70.
28. Ivanova N, Dobrin R, Lu R, et al. Dissecting self-renewal in stem cells with RNA interference. Nature. 2006;442:533–8.
29. Wu MY, Hill CS. Tgf-beta superfamily signaling in embryonic development and homeostasis. Dev Cell. 2009;16:329–43.
30. Ying QL, Nichols J, Chambers I, Smith A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell. 2003;115: 281–92.
31. Ogawa K, Saito A, Matsui H, et al. Activin-nodal signaling is involved in propagation of mouse embryonic stem cells. J Cell Sci. 2007;120:55–65.
32. Toyooka Y, Shimosato D, Murakami K, Takahashi K, Niwa H. Identification and characterization of subpopulations in undifferentiated ES cell culture. Development. 2008;135:909–18.
33. Willert K, Jones KA. Wnt signaling: is the party in the nucleus? Genes Dev. 2006;20:1394–404.
34. Kielman MF, Rindapaa M, Gaspar C, et al. *Apc* modulates embryonic stem-cell differentiation by controlling the dosage of beta-catenin signaling. Nat Genet. 2002;32:594–605.
35. Hao J, Li TG, Qi X, Zhao DF, Zhao GQ. WNT/beta-catenin pathway up-regulates Stat3 and converges on LIF to prevent differentiation of mouse embryonic stem cells. Dev Biol. 2006;290:81–91.
36. Ogawa K, Nishinakamura R, Iwamatsu Y, Shimosato D, Niwa H. Synergistic action of Wnt and LIF in maintaining pluripotency of mouse ES cells. Biochem Biophys Res Commun. 2006;343:159–66.
37. Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat Med. 2004;10:55–63.
38. Ying QL, Wray J, Nichols J, et al. The ground state of embryonic stem cell self-renewal. Nature. 2008;453:519–23.
39. Kunath T, Saba-El-Leil MK, Almousailleakh M, Wray J, Meloche S, Smith A. FGF stimulation of the Erk1/2 signalling cascade triggers transition of pluripotent embryonic stem cells from self-renewal to lineage commitment. Development. 2007;134:2895–902.
40. Cheng AM, Saxton TM, Sakai R, et al. Mammalian Grb2 regulates multiple steps in embryonic development and malignant transformation. Cell. 1998;95:793–803.
41. Rosner MH, Vigano MA, Ozato K, et al. A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo. Nature. 1990;345:686–92.
42. Scholer HR, Ruppert S, Suzuki N, Chowdhury K, Gruss P. New type of POU domain in germ line-specific protein Oct-4. Nature. 1990;344:435–9.
43. Okamoto K, Okazawa H, Okuda A, Sakai M, Muramatsu M, Hamada H. A novel octamer binding transcription factor is differentially expressed in mouse embryonic cells. Cell. 1990;60:461–72.

44. Niwa H, Masui S, Chambers I, Smith AG, Miyazaki J. Phenotypic complementation establishes requirements for specific POU domain and generic transactivation function of Oct-3/4 in embryonic stem cells. Mol Cell Biol. 2002;22:1526–36.
45. Palmieri SL, Peter W, Hess H, Scholer HR. Oct-4 transcription factor is differentially expressed in the mouse embryo during establishment of the first two extraembryonic cell lineages involved in implantation. Dev Biol. 1994;166:259–67.
46. Nichols J, Zevnik B, Anastassiadis K, et al. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell. 1998;95:379–91.
47. Yeom YI, Fuhrmann G, Ovitt CE, et al. Germline regulatory element of Oct-4 specific for the totipotent cycle of embryonal cells. Development. 1996;122:881–94.
48. Okumura-Nakanishi S, Saito M, Niwa H, Ishikawa F. Oct-3/4 and Sox2 regulate *Oct-3/4* gene in embryonic stem cells. J Biol Chem. 2005;280:5307–17.
49. Gu P, Goodwin B, Chung AC, et al. Orphan nuclear receptor LRH-1 is required to maintain Oct4 expression at the epiblast stage of embryonic development. Mol Cell Biol. 2005;25:3492–505.
50. Barnea E, Bergman Y. Synergy of SF1 and RAR in activation of *Oct-3/4* promoter. J Biol Chem. 2000;275:6608–19.
51. Ben-Shushan E, Sharir H, Pikarsky E, Bergman Y. A dynamic balance between ARP-1/COUP-TFII, EAR-3/COUP-TFI, and retinoic acid receptor:retinoid X receptor heterodimers regulates *Oct-3/4* expression in embryonal carcinoma cells. Mol Cell Biol. 1995;15:1034–48.
52. Fuhrmann G, Chung AC, Jackson KJ, et al. Mouse germline restriction of *Oct4* expression by germ cell nuclear factor. Dev Cell. 2001;1:377–87.
53. Niwa H, Toyooka Y, Shimosato D, et al. Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. Cell. 2005;123:917–29.
54. Avilion AA, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell-Badge R. Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev. 2003;17:126–40.
55. Ambrosetti DC, Scholer HR, Dailey L, Basilico C. Modulation of the activity of multiple transcriptional activation domains by the DNA binding domains mediates the synergistic action of Sox2 and Oct-3 on the fibroblast growth factor-4 enhancer. J Biol Chem. 2000;275:23387–97.
56. Yuan H, Corbi N, Basilico C, Dailey L. Developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3. Genes Dev. 1995;9:2635–45.
57. Nishimoto M, Fukushima A, Okuda A, Muramatsu M. 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. 1999;19:5453–65.
58. Tomioka M, Nishimoto M, Miyagi S, et al. Identification of Sox-2 regulatory region which is under the control of Oct-3/4-Sox-2 complex. Nucleic Acids Res. 2002;30:3202–13.
59. Tokuzawa Y, Kaiho E, Maruyama M, et al. Fbx15 is a novel target of Oct3/4 but is dispensable for embryonic stem cell self-renewal and mouse development. Mol Cell Biol. 2003;23:2699–708.
60. Rodda DJ, Chew JL, Lim LH, et al. Transcriptional regulation of Nanog by OCT4 and SOX2. J Biol Chem. 2005;280:24731–7.
61. Kuroda T, Tada M, Kubota H, et al. Octamer and Sox elements are required for transcriptional *cis* regulation of *Nanog* gene expression. Mol Cell Biol. 2005;25:2475–85.
62. Nakatake Y, Fukui N, Iwamatsu Y, et al. Klf4 cooperates with Oct3/4 and Sox2 to activate the *Lefty1* core promoter in embryonic stem cells. Mol Cell Biol. 2006;26:7772–82.
63. Maruyama M, Ichisaka T, Nakagawa M, Yamanaka S. Differential roles for Sox15 and Sox2 in transcriptional control in mouse embryonic stem cells. J Biol Chem. 2005;280:24371–9.
64. Masui S, Nakatake Y, Toyooka Y, et al. Pluripotency governed by *Sox2* via regulation of *Oct3/4* expression in mouse embryonic stem cells. Nat Cell Biol. 2007;9:625–35.
65. Mitsui K, Tokuzawa Y, Itoh H, et al. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell. 2003;113:631–42.
66. Chambers I, Colby D, Robertson M, et al. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. Cell. 2003;113:643–55.
67. Wang J, Levasseur DN, Orkin SH. Requirement of Nanog dimerization for stem cell self-renewal and pluripotency. Proc Natl Acad Sci USA. 2008;105:6326–31.
68. Pan GJ, Pei DQ. Identification of two distinct transactivation domains in the pluripotency sustaining factor Nanog. Cell Res. 2003;13:499–502.
69. Chambers I, Silva J, Colby D, et al. Nanog safeguards pluripotency and mediates germline development. Nature. 2007;450:1230–4.
70. Fujikura J, Yamato E, Yonemura S, et al. Differentiation of embryonic stem cells is induced by GATA factors. Genes Dev. 2002;16:784–9.
71. Chazaud C, Yamanaka Y, Pawson T, Rossant J. Early lineage segregation between epiblast and primitive endoderm in mouse blastocysts through the Grb2-MAPK pathway. Dev Cell. 2006;10:615–24.
72. McConnell BB, Ghaleb AM, Nandan MO, Yang VW. The diverse functions of Kruppel-like factors 4 and 5 in epithelial biology and pathobiology. Bioessays. 2007;29:549–57.
73. Segre JA, Bauer C, Fuchs E. Klf4 is a transcription factor required for establishing the barrier function of the skin. Nat Genet. 1999;22:356–60.
74. Jiang J, Chan YS, Loh YH, et al. A core Klf circuitry regulates self-renewal of embryonic stem cells. Nat Cell Biol. 2008;10:353–60.
75. Nakagawa M, Koyanagi M, Tanabe K, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol. 2008;26:101–6.
76. Cole MD, Cowling VH. Transcription-independent functions of MYC: regulation of translation and DNA replication. Nat Rev Mol Cell Biol. 2008;9:810–5.
77. de Celis JF, Barrio R. Regulation and function of Spalt proteins during animal development. Int J Dev Biol. 2009;53:1385–98.
78. Elling U, Klasen C, Eisenberger T, Anlag K, Treier M. Murine inner cell mass-derived lineages depend on Sall4 function. Proc Natl Acad Sci USA. 2006;103:16319–24.
79. Sakaki-Yumoto M, Kobayashi C, Sato A, et al. The murine homolog of *SALL4*, a causative gene in Okihiro syndrome, is essential for embryonic stem cell proliferation, and cooperates with *Sall1* in anorectal, heart, brain and kidney development. Development. 2006;133:3005–13.
80. Tsubooka N, Ichisaka T, Okita K, Takahashi K, Nakagawa M, Yamanaka S. Roles of Sall4 in the generation of pluripotent stem cells from blastocysts and fibroblasts. Genes Cells. 2009;14:683–94.
81. Zhang J, Tam WL, Tong GQ, et al. Sall4 modulates embryonic stem cell pluripotency and early embryonic development by the transcriptional regulation of *Pou5f1*. Nat Cell Biol. 2006;8:1114–23.
82. Yuri S, Fujimura S, Nimura K, et al. *Sall4* is essential for stabilization, but not for pluripotency, of embryonic stem cells by repressing aberrant trophectoderm gene expression. Stem cells. 2009;27:796–805.
83. Lu J, Jeong H, Kong N, et al. Stem cell factor SALL4 represses the transcriptions of PTEN and SALL1 through an epigenetic repressor complex. PLoS ONE. 2009;4:e5577.

84. Luo J, Sladek R, Bader JA, Matthyssen A, Rossant J, Giguere V. Placental abnormalities in mouse embryos lacking the orphan nuclear receptor ERR-beta. Nature. 1997;388:778–82.
85. Hermanson O. Stem cells have different needs for REST. PLoS Biol. 2008;6:e271.
86. Singh SK, Kagalwala MN, Parker-Thornburg J, Adams H, Majumder S. REST maintains self-renewal and pluripotency of embryonic stem cells. Nature. 2008;453:223–7.
87. Jorgensen HF, Terry A, Beretta C, et al. REST selectively represses a subset of RE1-containing neuronal genes in mouse embryonic stem cells. Development. 2009;136:715–21.
88. Buckley NJ, Johnson R, Sun YM, Stanton LW. Is REST a regulator of pluripotency? Nature. 2009;457:E5–6 (discussion E7).
89. Jorgensen HF, Chen ZF, Merkenschlager M, Fisher AG. Is REST required for ESC pluripotency? Nature. 2009;457:E4–5 (discussion E7).
90. Hoppler S, Kavanagh CL. Wnt signalling: variety at the core. J Cell Sci. 2007;120:385–93.
91. Pereira L, Yi F, Merrill BJ. Repression of *Nanog* gene transcription by Tcf3 limits embryonic stem cell self-renewal. Mol Cell Biol. 2006;26:7479–91.
92. Cole MF, Johnstone SE, Newman JJ, Kagey MH, Young RA. Tcf3 is an integral component of the core regulatory circuitry of embryonic stem cells. Genes Dev. 2008;22:746–55.
93. Yi F, Pereira L, Merrill BJ. Tcf3 functions as a steady-state limiter of transcriptional programs of mouse embryonic stem cell self-renewal. Stem cells. 2008;26:1951–60.
94. Tam WL, Lim CY, Han J, et al. T cell factor 3 regulates embryonic stem cell pluripotency and self-renewal by the transcriptional control of multiple lineage pathways. Stem cells. 2008;26:2019–31.
95. Galan-Caridad JM, Harel S, Arenzana TL, et al. Zfx controls the self-renewal of embryonic and hematopoietic stem cells. Cell. 2007;129:345–57.
96. Macfarlan T, Kutney S, Altman B, Montross R, Yu J, Chakravarti D. Human THAP7 is a chromatin-associated, histone tail-binding protein that represses transcription via recruitment of HDAC3 and nuclear hormone receptor corepressor. J Biol Chem. 2005;280:7346–58.
97. Clouaire T, Roussigne M, Ecochard V, Mathe C, Amalric F, Girard JP. The THAP domain of THAP1 is a large C2CH module with zinc-dependent sequence-specific DNA-binding activity. Proc Natl Acad Sci USA. 2005;102:6907–12.
98. Dejosez M, Krumenacker JS, Zitur LJ, et al. Ronin is essential for embryogenesis and the pluripotency of mouse embryonic stem cells. Cell. 2008;133:1162–74.
99. Wysocka J, Myers MP, Laherty CD, Eisenman RN, Herr W. Human Sin3 deacetylase and trithorax-related Set1/Ash2 histone H3–K4 methyltransferase are tethered together selectively by the cell-proliferation factor HCF-1. Genes Dev. 2003;17:896–911.
100. Wang J, Rao S, Chu J, et al. A protein interaction network for pluripotency of embryonic stem cells. Nature. 2006;444:364–8.
101. Niakan KK, Davis EC, Clipsham RC, et al. Novel role for the orphan nuclear receptor Daxl in embryogenesis, different from steroidogenesis. Mol Genet Metab. 2006;88:261–71.
102. Lauberth SM, Rauchman M. A conserved 12-amino acid motif in Sall1 recruits the nucleosome remodeling and deacetylase corepressor complex. J Biol Chem. 2006;281:23922–31.
103. Kaji K, Caballero IM, MacLeod R, Nichols J, Wilson VA, Hendrich B. The NuRD component Mbd3 is required for pluripotency of embryonic stem cells. Nat Cell Biol. 2006;8:285–92.
104. Liang J, Wan M, Zhang Y, et al. Nanog and Oct4 associate with unique transcriptional repression complexes in embryonic stem cells. Nat Cell Biol. 2008;10:731–9.
105. Ding L, Paszkowski-Rogacz M, Nitzsche A, et al. A genome-scale RNAi screen for Oct4 modulators defines a role of the Paf1 complex for embryonic stem cell identity. Cell Stem Cell. 2009;4:403–15.
106. Warner MH, Roinick KL, Arndt KM. Rtf1 is a multifunctional component of the Paf1 complex that regulates gene expression by directing cotranscriptional histone modification. Mol Cell Biol. 2007;27:6103–15.
107. Fazzio TG, Huff JT, Panning B. An RNAi screen of chromatin proteins identifies Tip60–p400 as a regulator of embryonic stem cell identity. Cell. 2008;134:162–74.
108. Tyteca S, Vandromme M, Legube G, Chevillard-Briet M, Trouche D. Tip60 and p400 are both required for UV-induced apoptosis but play antagonistic roles in cell cycle progression. EMBO J. 2006;25:1680–9.
109. Ho L, Ronan JL, Wu J, et al. An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency. Proc Natl Acad Sci USA. 2009;106:5181–6.
110. Ho L, Jothi R, Ronan JL, Cui K, Zhao K, Crabtree GR. An embryonic stem cell chromatin remodeling complex, esBAF, is an essential component of the core pluripotency transcriptional network. Proc Natl Acad Sci USA. 2009;106:5187–91.
111. Kohler C, Villar CB. Programming of gene expression by polycomb group proteins. Trends Cell Biol. 2008;18:236–43.
112. Endoh M, Endo TA, Endoh T, et al. Polycomb group proteins Ring1A/B are functionally linked to the core transcriptional regulatory circuitry to maintain ES cell identity. Development. 2008;135:1513–24.
113. Boyer LA, Plath K, Zeitlinger J, et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature. 2006;441:349–53.
114. Pasini D, Bracken AP, Hansen JB, Capillo M, Helin K. The polycomb group protein Suz12 is required for embryonic stem cell differentiation. Mol Cell Biol. 2007;27:3769–79.
115. Chamberlain SJ, Yee D, Magnuson T. Polycomb repressive complex 2 is dispensable for maintenance of embryonic stem cell pluripotency. Stem cells. 2008;26:1496–505.
116. Draker R, Cheung P. Transcriptional and epigenetic functions of histone variant H2A.Z. Biochem Cell Biol. 2009;87:19–25.
117. Creyghton MP, Markoulaki S, Levine SS, et al. H2AZ is enriched at polycomb complex target genes in ES cells and is necessary for lineage commitment. Cell. 2008;135:649–61.
118. Bernstein BE, Mikkelsen TS, Xie X, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell. 2006;125:315–26.
119. Azuara V, Perry P, Sauer S, et al. Chromatin signatures of pluripotent cell lines. Nat Cell Biol. 2006;8:532–8.
120. Stock JK, Giadrossi S, Casanova M, et al. Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells. Nat Cell Biol. 2007;9:1428–35.
121. Bogdanovic O, Veenstra GJ. DNA methylation and methyl-CpG binding proteins: developmental requirements and function. Chromosome 2009;118:549–65.
122. Tsumura A, Hayakawa T, Kumaki Y, et al. Maintenance of self-renewal ability of mouse embryonic stem cells in the absence of DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b. Genes Cells. 2006;11:805–14.
123. Fouse SD, Shen Y, Pellegrini M, et al. Promoter CpG methylation contributes to ES cell gene regulation in parallel with Oct4/Nanog, PcG complex, and histone H3 K4/K27 trimethylation. Cell Stem Cell. 2008;2:160–9.
124. Meissner A, Mikkelsen TS, Gu H, et al. Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature. 2008;454:766–70.
125. Guttman M, Amit I, Garber M, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature. 2009;458:223–7.

126. Efroni S, Duttagupta R, Cheng J, et al. Global transcription in pluripotent embryonic stem cells. Cell Stem Cell. 2008;2:437–47.
127. Szutorisz H, Georgiou A, Tora L, Dillon N. The proteasome restricts permissive transcription at tissue-specific gene loci in embryonic stem cells. Cell. 2006;127:1375–88.
128. Meshorer E, Yellajoshula D, George E, Scambler PJ, Brown DT, Misteli T. Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. Dev Cell. 2006;10:105–16.
129. Gaspar-Maia A, Alajem A, Polesso F, et al. Chd1 regulates open chromatin and pluripotency of embryonic stem cells. Nature. 2009;460:863–8.
130. Wen B, Wu H, Shinkai Y, Irizarry RA, Feinberg AP. Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. Nat Genet. 2009; 41:246–50.
131. Tachibana M, Sugimoto K, Nozaki M, et al. G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. Genes Dev. 2002;16:1779–91.
132. Sampath P, Pritchard DK, Pabon L, et al. A hierarchical network controls protein translation during murine embryonic stem cell self-renewal and differentiation. Cell Stem Cell. 2008;2:448–60.
133. Tay Y, Zhang J, Thomson AM, Lim B, Rigoutsos I. MicroRNAs to *Nanog*, *Oct4* and *Sox2* coding regions modulate embryonic stem cell differentiation. Nature. 2008;455:1124–8.
134. Marson A, Levine SS, Cole MF, et al. Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells. Cell. 2008;134:521–33.
135. Bussing I, Slack FJ, Grosshans H. *let-7* microRNAs in development, stem cells and cancer. Trends Mol Med. 2008;14: 400–9.
136. Rybak A, Fuchs H, Smirnova L, et al. A feedback loop comprising *lin-28* and *let-7* controls pre-*let-7* maturation during neural stem-cell commitment. Nat Cell Biol. 2008;10:987–93.
137. Viswanathan SR, Daley GQ, Gregory RI. Selective blockade of microRNA processing by Lin28. Science. 2008;320:97–100.
138. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007;318:1917–20.
139. Terry LJ, Shows EB, Wente SR. Crossing the nuclear envelope: hierarchical regulation of nucleocytoplasmic transport. Science. 2007;318:1412–6.
140. Yasuhara N, Shibazaki N, Tanaka S, et al. Triggering neural differentiation of ES cells by subtype switching of importin-alpha. Nat Cell Biol. 2007;9:72–9.
141. Fujita J, Crane AM, Souza MK, et al. Caspase activity mediates the differentiation of embryonic stem cells. Cell Stem Cell. 2008;2:595–601.
142. Kim J, Chu J, Shen X, Wang J, Orkin SH. An extended transcriptional network for pluripotency of embryonic stem cells. Cell. 2008;132:1049–61.
143. Chen X, Xu H, Yuan P, et al. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell. 2008;133:1106–17.
144. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. Cell. 2008;132:567–82.
145. Hochedlinger K, Plath K. Epigenetic reprogramming and induced pluripotency. Development. 2009;136:509–23.
146. Feng B, Ng JH, Heng JC, Ng HH. Molecules that promote or enhance reprogramming of somatic cells to induced pluripotent stem cells. Cell Stem Cell. 2009;4:301–12.
147. Matsushita M, Tomizawa K, Moriwaki A, Li ST, Terada H, Matsui H. A high-efficiency protein transduction system demonstrating the role of PKA in long-lasting long-term potentiation. J Neurosci. 2001;21:6000–7.
148. Zhou H, Wu S, Joo JY, et al. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell. 2009;4:381–4.
149. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663–76.
150. Aoi T, Yae K, Nakagawa M, et al. Generation of pluripotent stem cells from adult mouse liver and stomach cells. Science. 2008.
151. Hanna J, Markoulaki S, Schorderet P, et al. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. Cell. 2008;133:250–64.
152. Tada M, Takahama Y, Abe K, Nakatsuji N, Tada T. Nuclear reprogramming of somatic cells by in vitro hybridization with ES cells. Curr Biol. 2001;11:1553–8.
153. Brambrink T, Foreman R, Welstead GG, et al. Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. Cell Stem Cell. 2008;2:151–9.
154. Wernig M, Lengner CJ, Hanna J, et al. A drug-inducible transgenic system for direct reprogramming of multiple somatic cell types. Nat Biotechnol. 2008;26:916–24.
155. Sridharan R, Tchieu J, Mason MJ, et al. Role of the murine reprogramming factors in the induction of pluripotency. Cell. 2009;136:364–77.
156. Shi Y, Do JT, Desponts C, Hahm HS, Scholer HR, Ding S. A combined chemical and genetic approach for the generation of induced pluripotent stem cells. Cell Stem Cell. 2008;2:525–8.
157. Mikkelsen TS, Hanna J, Zhang X, et al. Dissecting direct reprogramming through integrative genomic analysis. Nature. 2008.
158. Huangfu D, Maehr R, Guo W, et al. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol. 2008;26:795–7.
159. Yang J, Chai L, Fowles TC, et al. Genome-wide analysis reveals Sall4 to be a major regulator of pluripotency in murine-embryonic stem cells. Proc Natl Acad Sci USA. 2008;105:19756–61.
160. Feng B, Jiang J, Kraus P, et al. Reprogramming of fibroblasts into induced pluripotent stem cells with orphan nuclear receptor Esrrb. Nat Cell Biol. 2009;11:197–203.
161. Marson A, Foreman R, Chevalier B, et al. Wnt signaling promotes reprogramming of somatic cells to pluripotency. Cell Stem Cell. 2008;3:132–5.