Generation of Transgenic Rats through Induced Pluripotent Stem Cells

Received for publication, June 13, 2013, and in revised form, August 6, 2013. Published, JBC Papers in Press, August 7, 2013, DOI 10.1074/jbc.M113.492900

Ming-Gui Jiang†§1, Tianda Li†§1, Chunjing Feng†§1, Rui Fu§, Yan Yuan**, Quan Zhou†*, Xin Li†‡, ‡¶, Haifeng Wan§, Liu Wang§, Wei Li§, Yamei Xiao§, Xiao-Yang Zhao‡¶, and Qi Zhou†‡

From the †State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China, the §College of Life Sciences, Hunan Normal University, Changsha, Hunan 410081, China, the ‡Key Laboratory of Translational Stem Cell Research, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China, the ‡¶Graduate School of the Chinese Academy of Sciences, Beijing 100039, China, the ‡¶‡State Key Laboratory of Reproductive Medicine, Department of Histology and Embryology, Nanjing Medical University, Nanjing, Jiangsu 210000, China, and the ‡¶‡¶College of Life Sciences, Northeast Agricultural University of China, Harbin 150030, China

Background: Rat induced pluripotent stem cells (riPSCs) failed to produce transgenic rats. Results: We found that an optimized induction medium improved the efficiency of iPSC generation from rat somatic cells. The riPSCs could successfully generate transgenic rats.

Conclusion: We could generate high quality riPSCs that could be used to produce transgenic rats.

Significance: RiPSCs can be used as a novel tool in genetic and genomic studies of the rat.

The rat is an important animal model for human disease research. Using inhibitors of glycogen synthase kinase 3 and MAPK signaling pathways, rat embryonic stem cells and rat induced pluripotent stem cells (riPSCs) have been derived. However, unlike rat embryonic stem cells, germ line competent riPSCs have only been derived from Wistar rats at low efficiency. Here, we found that an optimized induction medium containing knock-out serum replacement and vitamin C improved the rate and efficiency of riPSC generation from Dark Agouti rat fibroblasts and Sertoli cells. RiPSCs maintained an undifferentiated status for >30 passages and could differentiate into various cell types including germ cells when injected into rat blastocysts. Moreover, transgenic riPSCs could be generated through the PiggyBac transposon, which could be used to generate transgenic rats through germ line transmission. RiPSCs can be used as a novel tool in genetic and genomic studies of the rat.

Somatic cells can be reprogrammed into pluripotent stem cells by forced expression of the four Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc) (1). Induced pluripotent stem cells (iPSCs)4 can differentiate into multiple kinds of functional somatic cells and generate all-iPSC mice by tetraploid complementation (2). They are a promising resource for cell replacement therapy with lower or no immune rejection after transplantation (3, 4). iPSC technology can also be successfully used for research of specific disease models (5–8). However, the efficiency of iPSC generation is extremely low and accompanied by wide variation of the quality of iPSC colonies, which may hinder iPSC applications.

The rat is used as an important model of human diseases, especially those of the neural and cardiac systems, because of similar physiological features (9). In addition, the efficiency of small organ transplantation in mice is extremely low, which hinders regenerative medicine research in mice. When using rat iPSCs (riPSCs) to generate large organs, testing the function of the transplanted organ in the rat will be easier and more consistent. However, unlike in the mouse that can be used to generate transgenic animals with embryonic stem (ES) cells and iPSCs, the use of pluripotent stem cells to generate transgenic rats has only been successful using rat ES cells. riPSCs can be generated from the somatic cells of Wistar, Dark Agouti (DA), Fischer 344 (F344) and Brown Norway rats, which differentiate into the three germ layers in teratomas and chimeric rats (10, 11). However, only Wistar riPSCs can contribute to the germ line of chimeric rats (12). Whether live rats with germ line transmission can be obtained from iPSCs of strains other than the Wistar rat is unclear. Moreover, similar to ES cells, whether riPSCs can be used to generate transgenic rats is unknown (13, 14).

Chemicals and induction medium have been reported to improve mouse and human iPSC generation (15–17), but few studies have focused on riPSCs. Previous reports have shown that knock-out serum replacement (KOSR) and vitamin C (Vc) can improve mouse iPSC generation. On the other hand, stage-specific embryonic antigen 1; SSLP, simple sequence length polymorphism; tg, transgenic; Vc, vitamin C.
whether combined use of KOSR and Vc can facilitate riPSC generation is unclear. Here, we optimized an induction system that combined KOSR and Vc to improve riPSC generation from both DA rat fibroblasts and Sertoli cells. The riPSCs expressed pluripotency markers and could differentiate into various cell types including germ cells. We also generated transgenic rats using these riPSCs, which provides a new approach to generate rat disease models.

**EXPERIMENTAL PROCEDURES**

**Animals**—DA, Sprague-Dawley (SD) and F344 rats and CF-1 mice were purchased from Vital River Laboratories, Beijing, China, and maintained in a specific pathogen-free environment. All experiments were performed in accordance with the Beijing Animal Protection Laws of China.

**Cell Culture**—Rat embryonic fibroblasts (REFs) derived from an embryonic day (E)14.5 DA rat fetus were cultured with DMEM supplemented with 10% fetal bovine serum (FBS), non-essential amino acids, L-glutamine, and penicillin-streptomycin. riPSCs were cultured in 6-well plates on feeder cells (mitomycin-C-treated mouse embryonic fibroblasts) and in N2B27 medium supplemented with rat recombinant leukemia inhibitory factor (LIF) and 2i (3 μM GSK3 inhibitor CHIR99021 and 1 μM MEK inhibitor PD0325901). Vc (10 μg/ml) and 10% KOSR were added to the medium as indicated. riPSCs were subcultured every 2–3 days.

**Infection of REFs**—The lentiviral vector 4F2A (Addgene) was used to reprogram REFs. At 48 h after transfection of 293 cells, supernatants containing virus particles were harvested, filtered, and subjected to ultracentrifugation for concentration.

REFs were plated at 2 × 10^5 cells/35-mm culture dish at 1 day prior to infection. Doxycycline (2 μg/ml) and Polybrene (4 μg/ml) were added on the first day of infection (day 0), and rLIF was added to the medium on day 1. Infected REFs were seeded onto a feeder layer in N2B27 medium containing doxycycline, rLIF, Vc, and 10% KOSR on day 2. On day 3.5, 2i was added to the medium, and ES cell-like colonies appeared from day 4.5. Colonies with a typical dome shape were picked up and transferred to 4-well plates after approximately 4 weeks and fixed in 4% paraformaldehyde for paraffin embedding and hematoxylin and eosin staining following standard procedures.

**Blastocyst Injection**—Ten to 15 riPSCs cells were injected into blastocysts collected from the uterus of 4.5 days postcoitum female F344 rats followed by transfer into the uterine horns of E3.5 pseudopregnant female SD rats. Chimeric rats and germ line-transmitted rats were identified by coat color. Red fluorescence protein (RFP) was detected by an IVIS Spectrum system (PerkinElmer Life Sciences).

**Simple Sequence Length Polymorphism (SSLP) Analysis**—SSLP analysis was used to determine rat strains. DNA samples were extracted from riPSCs and the tails of littermates and chimeric rats. Tail DNA from DA, F344, and SD rats was used for the controls. The primer sequences are described in The National Bio Resource Project for the Rat in Japan.

**Electroporation**—Approximately 1 × 10^6 riPSCs were electroporated with 4.5 μg of PiggyBac vector (PiggyBac plasmid with an EF1α promoter-driven DsRed-IRE5-neo cassette) and 1.5 μg of PBase vector (PiggyBac integrase) according to the manufacturer’s instructions. Electroporated cells were cultured in N2B27 medium (without antibiotics) containing 5% KOSR and 200 μg/ml G418. Neomycin-resistant colonies exhibiting RFP fluorescence were picked up and transferred to 4-well plates after 7 days of selection.

**Transgenic Rats Generated from riPSCs**

The genital ridge from E15.5 fetuses were fixed in 4% paraformaldehyde overnight and embedded in paraffin. Fixed sections were permeabilized with 0.5% Triton X-100 in PBS at room temperature for 30 min and then incubated with 2% BSA/PBS (w/v) for 1 h at room temperature for blocking. Sections were incubated with a...
primary rabbit anti-DDX4/mouse vasa homolog antibody (1:500; Abcam) overnight at 4 °C and then an Alexa Fluor 488-conjugated donkey anti-rabbit IgG (1:400; Jackson Laboratory) for 1 h at room temperature. Hoechst 33342 was used for nuclear staining. Sections were observed under a confocal laser scanning microscope.

**FIGURE 1.** KOSR and Vc improve riPSC generation. A, scheme for riPSC generation with the optimized reprogramming system. B, Morphology of REFs before viral infection. Scale bar represents 100 μm. C, riPSCs generated with the optimized induction system at day 5.5 postinfection. Scale bar represents 100 μm. D, AP staining of colonies obtained from the KOSR-Vc group (upper) and control (lower) at days 3.5, 5.5, and 7.5 postinfection. Insets show the thumbnail of a region in the relative tissue culture plate. E, statistical analysis of the number of AP-positive colonies shown in D. F, Flow cytometric analysis of SSEA-1-positive cells among transfected REFs. Error bars represent the S.D. (n = 3), and p < 0.001, t test. G, Immunofluorescence staining of pluripotency markers SSEA-1 (red) and Esrrb (red) in KOSR-Vc and control groups at day 5.5 postinfection. DNA (blue) was stained with Hoechst 33342. Scale bars represent 50 μm. H, Q-PCR analysis of the expression levels of Nanog and Esrrb at days 1.5, 3.5, and 5.5 postinfection. DA5-3 is a rat ES cell control with germ line competency. Expression values are relative to β-actin gene expression set as 1. Error bars represent the S.D. (n = 3).
RESULTS

**KOSR and Vc Improve the Generation of riPSCs**—To test the combinatorial effects of KOSR and Vc on riPSC induction, DA REFs were transfected with the four Yamanaka factors to generate riPSCs (Fig. 1, A and B). At 36 h postinfection, REFs with marked morphological changes were subcultured onto dishes containing mouse embryonic fibroblast feeder cells and cultured with riPSC induction medium supplemented with or without KOSR and Vc (KOSR-Vc group versus control group). ES cell-like colonies appeared at day 4.5 postinfection, and colonies with a typical stem cell morphology were picked up at 5.5 days in the KOSR-Vc group (Fig. 1C). AP staining was performed to detect the positive number of primary riPSC colonies. The results showed that the number of AP-positive colonies was significantly higher in the KOSR-Vc group compared to the control group.
nies in the KOSR-Vc group was almost 12-fold (459 versus 42) higher than that in the control without KOSR and Vc at day 5.5, and 8-fold higher (988 versus 124) than that in the control at day 7.5 postinfection (Fig. 1, D and E). We performed live staining of SSEA-1 to determine the percentage of SSEA-1-positive cells among induced cells by flow cytometry. The percentage of SSEA-1-positive cells in the KOSR-Vc group was approximately 6-fold more than that in the control group at day 5.5 postinfection (3.45% versus 0.55%) (Fig. 1F). In addition, pluripotency markers SSEA-1 and Esrrb detected by immunofluorescence showed strong expression in the KOSR-Vc group, whereas weak staining was observed in the control group at day 5.5 postinfection (Fig. 1G). Q-PCR analysis of Esrrb and Nanog expression showed 4- and 6-fold higher expression in the KOSR-Vc group than that in the control group at day 5.5, respectively (Fig. 1, H and I). These data suggest that KOSR and Vc improves riPSC generation. To date, this result is the most rapid reprogramming of riPSCs with very high efficiency.
Characterization of riPSCs—We picked up a total of nine colonies in three repeated experiments at day 5.5 postinfection and established seven stable riPSC lines (seven of nine, 77.8%) that were expanded for >30 passages. Two riPSC lines, riPS-1 and riPS-2, were randomly selected to perform subsequent experiments. We also generated four cell lines from eight riPSC colonies (riPS-c1, riPS-c2, riPS-c3, and riPS-c4; four of eight, 50%) at day 8.5 postinfection from the control group without KOSR and Vc. A rat ES cell line (DA5-3), which had previously been established, was used as a control. We selected six riPSC lines as positive (Fig. 2) and established seven stable riPSC lines (seven of nine, 77.8%) in three repeated experiments at day 5.5 postinfection from the control group without KOSR and Vc. A rat ES cell line (DA5-3), which had previously been established, was used as a control.

Next, we examined the differentiation capacity of riPSCs, allowing riPSCs to differentiate as embryoid bodies and evaluated the expression of differentiation marker genes by RT-PCR (Fig. 4A). We detected the expression of marker genes of the three germ layers, such as Nestin and Pax6 (ectoderm), Gata4 and Sox17 (primitive endoderm), and Flk1 (mesoderm), whereas only slight residual expression of Oct4 similar to that in the DA ES cell control was detected at day 8 of differentiation (Fig. 4B).

We also performed a more stringent pluripotency assay by examining chimeric rats. riPSCs from the riPS1–6 cell line (DA background and Agouti coat color) were injected into F344 blastocysts (white coat color) and then transferred to SD pseudopregnant females to generate chimeras. A total of 27 chimeras were obtained among 132 live pups born (Table 1). Most of the chimeric rats had a high percentage of riPSCs contribution as determined by coat color chimerism (>85%, Fig. 4D). The SSLP assay confirmed the riPSC contribution (Fig. 4E). As shown in Table 1, four riPSC lines (riPS-c1, riPS-c2, riPS-c3, and riPS-c4) from the control group were also injected into F344 blastocysts under the same condition, and 14 chimeras were obtained among 47 live pups born.

We next explored whether these riPSCs could contribute to the germ line. Ten chimeras from four cell lines (riPS-1, riPS-3, riPS-4, and riPS-5) and six chimeras (2, 3, 2, 3 chimeras, respectively), among which six showed a high level of chimerism, were mated with SD rats (white coat color), resulting in seven germ line transmission rats originating from three riPSC lines (riPS-1, riPS-3, and riPS-5) (Fig. 4F and Table 1). However, six chimeras from control riPSCs (riPS-c1, riPS-c3, and riPS-c4) only produced one germ line transmission rat (riPS-c1) (Table 1). The presence of transgenic Yamanaka factors in the DA genome would allow for the generation of transgenic rats from riPSCs for lineage tracing and functional studies.

### Transgenic Rats Generated from riPSCs

| Cell line | Passage no. | No. of injected blastocyst | Pups | Chimeras (survival) | Germ line transmission |
|-----------|-------------|----------------------------|------|---------------------|-----------------------|
| riPS-1    | 3           | 2                          | 24   | 9                   | 3                     |
| riPS-2    | 10          | 40                         | 10   | 8                   | NTa                   |
| riPS-3    | 13, 17      | 194                        | 96   | 4                   | 1                     |
| riPS-4    | 11–31       | 33                         | 4    | 1                   | NT                    |
| riPS-5    | 12, 17      | 61                         | 35   | 4                   | NT                    |
| riPS-6    | 13, 18      | 41                         | 10   | 3                   | NT                    |
| riPS-c1   | 15          | 64                         | 15   | 4                   | NT                    |
| riPS-c2   | 19, 26      | 63                         | 15   | 4                   | NT                    |
| riPS-c3   | 19–28       | 242                        | 44   | 13                  | NT                    |
| riPS-c4   | 20          | 71                         | 13   | 4                   | NT                    |
| riPS-tg-1 | 13–28       | 242                        | 44   | 13                  | NT                    |
| riPS-tg-2 | 19          | 71                         | 13   | 4                   | NT                    |

Note: NT: not tested.

### Table 1

Generation of chimeras and germ line-competent offspring from riPSCs

riPS-1–6 were riPSC lines generated with the optimized induction system using KOSR and Vc. riPS-c1–c4 were riPSC lines generated with the traditional induction system without KOSR and Vc.
was confirmed by genomic PCR and showed that
riPSCs contributed to the germ line. Moreover, germ line trans-
mission rats (DA/H11003/SD background) produced healthy proge-
nies (Fig. 4, H and I). In summary, these data showed that the
riPSCs were pluripotent stem cells, especially the iPSCs
induced with KOSR and Vc, which showed a robust potential to
contribute to the germ line.

Transgenic riPSCs Produce Transgenic Rats through Germ
Line Transmission—To explore the feasibility of using
riPSCs to generate transgenic rats, RFP and neo genes
driven by the EF1α promoter through PiggyBac transposons
were electroporated into two riPSC lines (riPS-1 and riPS-2)
to generate RFP transgenic riPSCs (Fig. 5A). After G418
selection for 1 week, two surviving clones expressing RFP

FIGURE 5. Germ line transmission of transgenic riPSCs. A, schematic of fluorescently labeled vector (the two-component transposon PiggyBac systems) including PB transpose (PBase, helper) and transposon (PB, donor). B, fluorescence detection of transgenic riPSCs (riPS-tg-1, P25). Scale bar represents 100 μm. C, E15.5 chimeric fetus formed by injection of riPS-tg-1 cells into SD diploid blastocysts. RFP fluorescence indicated the contribution of riPS-tg-1 cells (left). A nonchimeric fetus without RFP was used as the control (right). D, genome PCR analysis of various organs from the fluorescently labeled transgenic chimeras from C. E, fluorescence detection of gonads (male, upper; female, lower). RFP fluorescence indicated the contribution of riPS-tg-1 cells to the gonads (left). A nonchimeric fetus without RFP was used as the control (right). F, immunofluorescence staining of the genital ridge. Mouse vasa homolog- and RFP-double positive cells were observed in the germ line. Scale bar represents 50 μm. G, fluorescence detection of a chimeric pup (left) generated from riPS-tg-1 cells and a wild-type SD pup (right). H, fluorescence detection of transgenic rats (left, Agouti coat color) and an RFP-negative littermate (right, white coat color), which were generated by mating an RFP-positive chima with a wild-type SD rat. I, genome PCR analysis of transgenic rats and littermates. J, morphology of ear fibroblast from fluorescently labeled transgenic rat. Scale bar represents 100 μm. K, morphology of riPSCs from J generated at day 5 without virus infection. Scale bar represents 100 μm. L, AP staining of riPSC from K. Scale bar represents 100 μm. M, karyotype of riPSCs from K (P5, 2N = 42). Scale bar represents 10 μm.
Transgenic Rats Generated from riPSCs

with control iPSCs, the iPSCs generated with KOSR and Vc contributed more efficiently to the germ line, indicating that KOSR and Vc also improve the quality of riPSCs by reducing the variation of colonies. Using this robust reprogramming system, we can conveniently derive riPSCs with other backgrounds.

This is the first report demonstrating that riPSCs can produce transgenic rats. This approach will be useful to study the mechanisms of diseases in rat models. However, the virus integration may have some negative effects. Generation of integration-free riPSCs from rat somatic cells and subsequent production of genetically modified rats with these riPSCs still require further study. This novel approach may improve the application of rat disease models and regenerative medicine in the future.

Acknowledgments—We thank all of the members of Dr. Zhou’s and Dr. Zhao’s laboratory for discussions and the Medical and Health Analysis Center Molecular Imaging Laboratory, Peking University, for the help with animal fluorescence images experiment.

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. Zhao, X. Y., Li, W., Lv, Z., Liu, L., Tong, M., Hai, T., Hao, J., Guo, C. L., Ma, Q. W., Wang, L., Zeng, F., and Zhou, Q. (2009) iPSC cells produce viable mice through tetraploid complementation. Nature 461, 86–90
3. Zhao, T., Zhang, Z. N., Rong, Z., and Xu, Y. (2011) Immunogenicity of induced pluripotent stem cells. Nature 474, 212–215
4. Araki, R., Uda, M., Hoki, Y., Sunayama, M., Nakamura, M., Ando, S., Sugiuira, M., Ideno, H., Shimada, A., Nifujii, A., and Abe, M. (2013) Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. Nature 494, 100–104
5. Takahashi, K., Okita, K., Nakagawa, M., and Yamanaka, S. (2007) Induction of pluripotent stem cells from fibroblast cultures. Nat. Protoc. 2, 3081–3089
6. Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., Nie, J., Jonsdottir, G. A., Ruotti, V., Stewart, R., Skulv, I. L., and Thomson, J. A. (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318, 1917–1920
7. Wernig, M., Zhao, J. P., Pruszak, J., Hedlund, E., Fu, D., Soldner, F., Broccoli, V., Constantine-Paton, M., Iascon, O., and Jaenisch, R. (2008) Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson’s disease. Proc. Natl. Acad. Sci. U.S.A. 105, 5856–5861
8. Wu, G., Liu, N., Rettelmeyer, I., Sharma, A. D., Sodda, M., Zaehres, H., Bleidissel, M., Greber, B., Gentile, L., Han, D. W., Rudolph, C., Steinemann, D., Schambach, A., Ott, M., Scholer, H. R., and Cantz, T. (2011) Generation of healthy mice from gene-correlated disease-specific induced pluripotent stem cells. PLoS Biol. 9, e1001099
9. Jacob, H. J., and Kwitek, A. E. (2002) Rat genetics: attaching physiology and pharmacology to the genome. Nat. Rev. Genet. 3, 33–42
10. Liao, J., Cui, C., Chen, S., Ren, J., Chen, J., Gao, Y., Li, H., Jia, N., Cheng, L., Xiao, H., and Xiao, L. (2009) Generation of induced pluripotent stem cell lines from adult rat cells. Cell Stem Cell 4, 11–15
11. Li, W., Wei, W., Zhu, S., Zhu, J., Shi, Y., Lin, T., Hao, E., Hayek, A., Deng, H., and Ding, S. (2009) Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. Cell Stem Cell 4, 16–19
12. Hamanaka, S., Yamaguchi, T., Kobayashi, T., Kato-Itoh, M., Yamazaki, S., Sato, H., Umino, A., Wakiyama, Y., Arai, M., Sanbo, H., Hirabayashi, M., and Nakauchi, H. (2011) Generation of germ line–competent rat induced
pluripotent stem cells. *PloS One* **6**, e22008
13. Tong, C., Li, P., Wu, N. L., Yan, Y., and Ying, Q. L. (2010) Production of p53 gene knockout rats by homologous recombination in embryonic stem cells. *Nature* **467**, 211–213
14. Kawamata, M., and Ochiya, T. (2010) Generation of genetically modified rats from embryonic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 14223–14228
15. Shi, Y., Do, J. T., Desponts, C., Hahm, H. S., Schöler, H. R., and Ding, S. (2008) A combined chemical and genetic approach for the generation of induced pluripotent stem cells. *Cell Stem Cell* **2**, 525–528
16. Esteban, M. A., Wang, T., Qin, B., Yang, J., Qin, D., Cai, J., Li, W., Weng, Z., Chen, J., Ni, S., Chen, K., Li, Y., Liu, X., Xu, J., Zhang, S., Li, F., He, W., Labuda, K., Song, Y., Peterbauer, A., Wolbank, S., Redl, H., Zhong, M., Cai, D., Zeng, L., and Pei, D. (2010) Vitamin C enhances the generation of mouse and human induced pluripotent stem cells. *Cell Stem Cell* **6**, 71–79
17. Zhao, X. Y., Li, W., Lv, Z., Liu, L., Tong, M., Hai, T., Hao, J., Guo, C. L., Wang, X., Wang, L., Zeng, F., and Zhou, Q. (2010) Efficient and rapid generation of induced pluripotent stem cells using an alternative culture medium. *Cell Res.* **20**, 383–386
18. Zhao, X., Lv, Z., Liu, L., Wang, L., Tong, M., and Zhou, Q. (2010) Derivation of embryonic stem cells from Brown Norway rats blastocysts. *J. Genet. Genomics* **37**, 467–473
19. Li, W., Shuai, L., Wan, H., Dong, M., Wang, M., Sang, L., Feng, C., Luo, G. Z., Li, T., Li, X., Wang, L., Zheng, Q. Y., Sheng, C., Wu, H. J., Liu, Z., Liu, L., Wang, L., Wang, X. J., Zhao, X. Y., and Zhou, Q. (2012) Androgenetic haploid embryonic stem cells produce live transgenic mice. *Nature* **490**, 407–411