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Human oocytes reprogram somatic cells to a pluripotent state

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The exchange of the oocyte's genome with the genome of a somatic cell, followed by the derivation of pluripotent stem cells, could enable the generation of specific cells affected in degenerative human diseases. Such cells, carrying the patient's genome, might be useful for cell replacement. Here we report that the development of human oocytes after genome exchange arrests at late cleavage stages in association with transcriptional abnormalities. In contrast, if the oocyte genome is not removed and the somatic cell genome is merely added, the resultant triploid cells develop to the blastocyst stage. Stem cell lines derived from these blastocysts differentiate into cell types of all three germ layers, and a pluripotent gene expression program is established on the genome derived from the somatic cell. This result demonstrates the feasibility of reprogramming human cells using oocytes and identifies removal of the oocyte genome as the primary cause of developmental failure after genome exchange.

The generation of animals by transfer of the genome from an adult cell into an unfertilized oocyte[1], and the isolation of pluripotent stem cells from human blastocysts[2], raised the prospect of generating stem cells with a patient's genome. This prospect holds much medical promise as these patient-specific stem cells could be used to generate differentiated cells for cell replacement. Unfortunately, progress towards this goal has been slowed by legal and social considerations limiting the availability of human oocytes for research. Despite these limitations, several studies were conducted[3–11], but none have achieved the derivation of a stem cell line. Thus, the question of whether human oocytes have the ability to reprogram somatic cells to a pluripotent state has remained unanswered.

Although it is now possible to induce pluripotent stem cell (iPS) formation by forced expression of transcription factors in somatic cells[12], differences between iPS- and blastocyst-derived stem cells have been reported for gene expression[13,14], DNA methylation[15,16] and differentiation potential[17]. In addition, reprogramming to iPS cells seems to compromise genomic integrity, introducing *de novo* mutations[18] and copy number variations[19,20]. Whether reprogramming using human oocytes yields pluripotent stem cells without these abnormalities remains to be determined.

Various sources have been explored, including failed fertilized oocytes[4], oocytes deemed in excess of clinical need[3,5,21], *in vitro* matured oocytes[10] and fertilized oocytes[22]. Previously, we have found that very few women agree to donate their oocytes for research without payment[11]. The majority of oocyte donors believe that payment should be provided regardless of whether the oocytes are used for research or reproductive purposes[23]. Payment for reproductive oocyte donation is common in the USA with more than 8,000 donor *in vitro* fertilization (IVF) cycles performed annually[24]. Recognizing the varying views on payments to research oocyte donors, the American Society for Reproductive Medicine and the International Society for Stem Cell Research have proposed balanced guidelines[25,26] which allow payment at the discretion of research oversight committees, which must ensure that financial considerations do not constitute an undue inducement. Following on from those guidelines, we have developed protocols that were reviewed and approved by the institutional review board and stem cell committees of Columbia University. These protocols allowed women participating in the reproductive egg donation program to select between donation for reproductive purposes and donation for research, offering equal remuneration regardless of their choice. Consequently, the decision to donate was before and independent of their decision to donate for research. Our study of 270 mature human oocytes revealed that the exchange of the oocyte genome with the genome of a somatic cell consistently leads to developmental arrest. However, when the oocyte genome is not removed, and the somatic cell genome is merely added, the activated human oocytes develop to the blastocyst stage. Human stem cells derived from these blastocysts contain both a haploid genome derived from the oocyte and a diploid somatic cell genome reprogrammed to a pluripotent state.

Results

Development fails after somatic genome replacement

In several mammalian species, somatic cell reprogramming has been achieved by replacing the oocyte genome at metaphase II (MII) of meiosis with a somatic cell nucleus (Fig. 1a). To remove the oocyte genome, we identified the location of the spindle-chromosome complex in 38/50 MII oocytes (Fig. 1b). All oocytes (43/43) survived genome removal with (31) or without (12) addition of Hoechst stain/minimal ultraviolet light exposure to verify enucleation. Oocytes lacking a genome were used for the transfer of somatic cell genomes obtained from skin cells of a male diabetic (T1D) and a healthy male adult. They were labelled with a green fluorescent protein (*GFP*) or a histone 2b (*H2B*):*GFP* transgene under the control of the ubiquitously expressed CAGGS promoter (Fig. 1c). Because Hoechst staining seemed to inhibit nuclear remodelling in rhesus oocytes[27], we monitored chromosome condensation every hour after transfer. All oocytes (35/35), whether or not they had been exposed to Hoechst, condensed the

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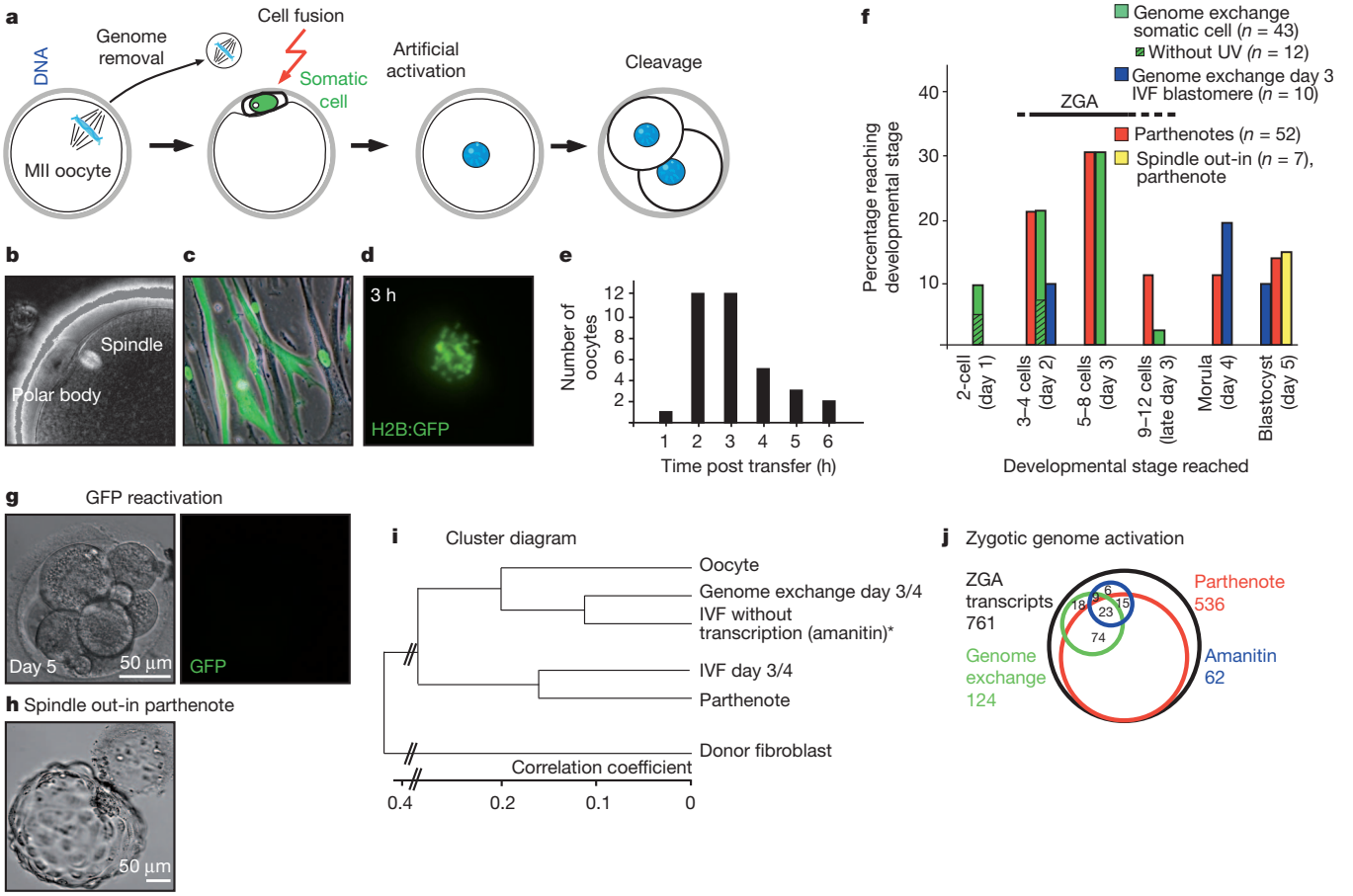

Figure 1 | Developmental and transcriptional defects after genome exchange. **a**, Schematic of genome exchange in human oocytes. **b**, Human oocyte at the MII stage, viewed by microtubule birefringence. **c**, Donor cell population marked with either H2B:GFP or GFP. **d**, Somatic chromatin 3 h after transfer. **e**, Timing of chromosome condensation. **f**, Developmental potential. Vertical axis is the percentage of activated eggs reaching specific developmental stages (horizontal axis). Days indicate the time points of normal developmental progression. UV, ultraviolet light. **g**, Arrested development after somatic genome exchange. **h**, Development after spindle removal and re-transfer. **i**, Cluster diagram of global gene expression. *from ref. 22. **j**, Venn diagram of transcripts elevated in IVF samples on day 3–4 of development (black circle) in comparison to oocytes. The overlap with parthenotes, amanitin-treated samples and genome exchange samples are shown.

somatic chromosomes (Fig. 1d, e); upon activation, 22/31 (71%) of the oocytes continued normal cleavage development. However, as we had previously observed following nuclear transfer into human zygotes[22], development arrested at a stage of 6–10 cells (Fig. 1f, g).

As a control for the quality of the oocytes, the development of *in vitro* fertilized donor oocytes was followed at the IVF clinic; 16/21 (76%) developed to the blastocyst or morula stage by day 6 of culture, indicating excellent developmental potential (Supplementary Table 1). Likewise, artificially activated oocytes developed to the morula and blastocyst stages (13/52 or 25%), well beyond the point of developmental arrest seen after genome exchange (Fig. 1f).

As a control for our experimental manipulations, we transferred H2B:GFP-labelled somatic nuclei without immediately removing the oocyte genome (Supplementary Fig. 1a). Six to eight hours after artificial activation two interphase nuclei had formed within a single cell (Supplementary Fig. 1b). The H2B:GFP-labelled genome could be distinguished from the unlabelled oocyte genome, and either of them specifically extracted using contrast optics and GFP fluorescence (Supplementary Fig. 1c, e); Hoechst staining and ultraviolet illumination was not required. Both types of cells therefore experienced the same manipulations, but differed in their ultimate genetic content, having either the oocyte genome or the somatic cell genome. Activated oocytes containing only the somatic genome formed 4–12 cells, but all arrested without reactivating the *GFP* transgene (32/32) (Supplementary Fig. 1d). In contrast, activated oocytes containing only the oocyte genome cleaved and 4/7 (57%) developed to the blastocyst stage (Supplementary Fig. 1f), allowing the generation of pluripotent parthenogenetic stem cells (Supplementary Fig. 2).

To test whether developmental potential was determined by the state of differentiation of the transferred genome, we replaced the oocyte genome with that of a blastomere. Upon activation, development to the morula and blastocyst stage occurred (Fig. 1f and Supplementary Fig. 3). Furthermore, following the removal of the oocyte's genome and subsequent retransfer into the same oocyte, parthenogenetic development to the blastocyst stage was observed (Fig. 1f, h and Supplementary Fig. 4).

Development arrests with transcriptional defects

Extensive transcriptional activity from the zygotic genome normally starts at the 4–8 cell stage[28], or on day 3 of development, coincident with the stage of developmental arrest after somatic genome transfer (Fig. 1f). To determine whether the somatic genome was being expressed, we compared the transcriptome at the 6–12 cell stage after genome exchange, to the transcriptome after artificial activation on day 3/early day 4 of development. To distinguish between expression from the transferred genome and maternal contributions to transcript abundance, we compared our data to those obtained after development of fertilized eggs from the 1-cell to the 6–8-cell stage in media containing the RNA polymerase II inhibitor alpha amanitin[22]. Using hierarchical clustering of the global gene expression patterns, we found that after genome exchange, transcript types and abundances at the 6–12-cell stage most closely resembled a state of inhibition of transcriptional activity (Fig. 1i).

We then identified transcripts that were relatively upregulated in comparison to unfertilized oocytes. Using day 3 and day 4 IVF controls, we defined 761 transcripts upregulated at zygotic genome activation (more than fivefold, $P < 0.01$). Of these 761 transcripts, only 124 (16%) were upregulated after genome exchange, and 62 (8%) were upregulated after amanitin treatment (more than fivefold, $P < 0.01$), possibly reflecting differential mRNA stability (Fig. 1j). In contrast, in parthenotes 536/761 transcripts (70%) ($P < 0.001$) were more than fivefold upregulated.

To determine if the developmental arrest correlated with continued expression of somatic cell genes, we identified 1,406 genes that were expressed at higher levels in the skin donor fibroblasts than in MII oocytes (more than tenfold, $P < 0.001$ for four biological replicates). The average transcript levels of these genes after genome transfer was 0.4-fold lower than in IVF controls. Therefore, neither the transcriptional program of a somatic cell nor that of a blastomere was being expressed. Among the few genes that were specifically elevated after genome exchange (Supplementary Table 2), one, *GADD45G*, is involved in stress-induced cell cycle arrest.

The oocyte genome rescues development

These developmental defects could be caused by an inability of the somatic cell genome to be appropriately expressed, replicated and/or segregated during cleavage development. Alternatively, molecules specific to the oocyte genome for which the somatic nucleus is unable to compensate may be removed during oocyte genome removal.

To distinguish between these possibilities, we transferred a somatic cell genome but did not remove the oocyte genome (Fig. 2a). In contrast to previous experiments, development continued to the compacted morula stage, and expression of the CAGGS:*GFP* transgene was re-initiated at the appropriate stage (35/35 cleavage stages with four or more cells). Development to the blastocyst stage was efficient (13 blastocysts of 63 transferred oocytes, or 21%), indicating that the somatic cell genome did not interfere with development to the blastocyst stage.

Derivation of pluripotent stem cells

From these blastocysts, we isolated the inner cell mass and derived two cell lines, soPS1 (for somatic cell genome, oocyte genome pluripotent stem cell 1), containing the genome of a male T1D subject, and soPS2, containing the genome of a healthy male adult. Both cell lines were triploid (Fig. 3a), containing short tandem repeat (STR) alleles consistent with the presence of a diploid somatic cell genome and the haploid genome of the oocyte (Supplementary Tables 3 and 4). soPS1 contained an additional chromosome 17 of somatic donor cell origin and a balanced translocation between chromosomes 15 and 17 (Supplementary Fig. 5a, b). At passage 23, 30% of soPS1 cells had gained additional copies of chromosome 12 and 17, chromosomal aberrations that commonly occur in pluripotent stem cell cultures because they confer a growth advantage[29]. soPS2 was karyotypically stable over more than 20 passages (Supplementary Material). During a period of 6 months, soPS1 and soPS2 completed more than 30 passages or over 100 population doublings (Supplementary Fig. 5c) without undergoing replicative crisis. Mitochondrial genomes were of oocyte donor origin without sign of heteroplasmy in either cell line (Fig. 3b and Supplementary Fig. 5d). Mitochondria transferred with the somatic nucleus may be outnumbered by the mitochondria of the oocyte, or they may be lost during cleavage development.

Both soPS cell lines expressed molecular markers characteristic of pluripotent stem cells (Fig. 3c), and when differentiated *in vitro*, or following injection into immunocompromised mice, cell types representative of all three germ layers were observed (Fig. 3d). The global gene expression profile of both soPS cell lines clustered closely with that of other pluripotent cell types, including NYSCF1, a stem cell line derived from an IVF blastocyst. The parthenogenetic stem cell line, pPS1, and iPS cell lines derived from both skin cell donors also clustered closely with soPS cells, but the somatic donor cells clustered separately (Fig. 3e). We identified 1,327 genes that were differentially expressed between soPS2 and its donor fibroblast ($P < 0.01$). Of these

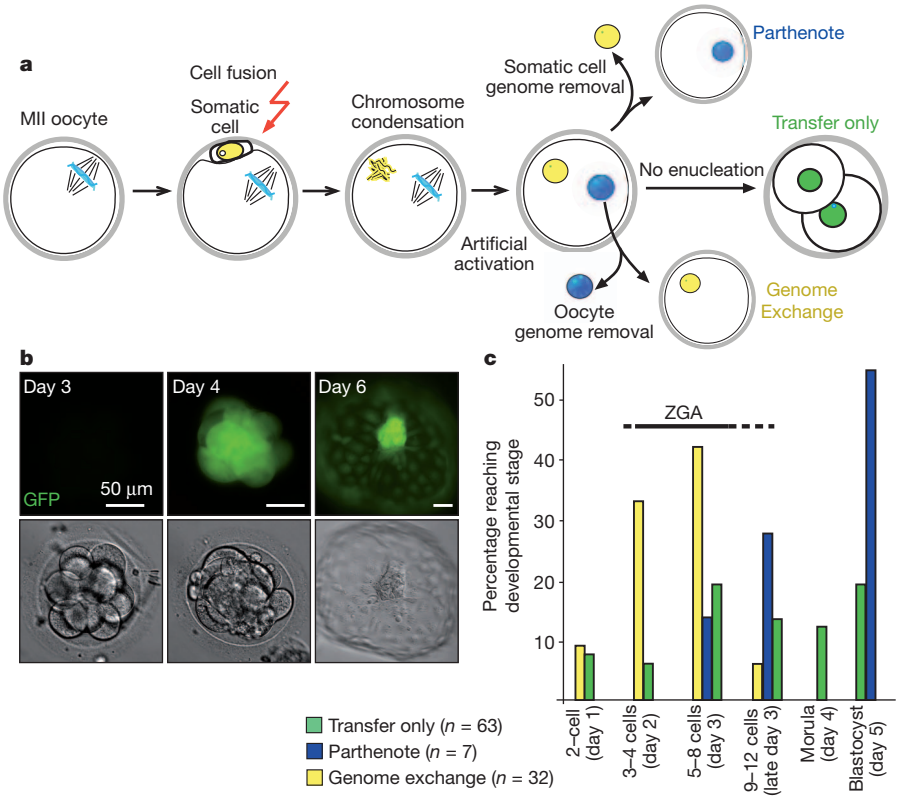

Figure 2 | Development after somatic cell genome transfer with retention of the oocyte genome. **a**, Schematic of somatic genome transfer without or with removal of either the oocyte or the somatic cell genome at the first interphase. **b**, Developmental progression 'transfer only'. Days post artificial activation are indicated. **c**, Developmental potential. Vertical axis is the percentage of activated eggs reaching specific developmental stages (horizontal axis).

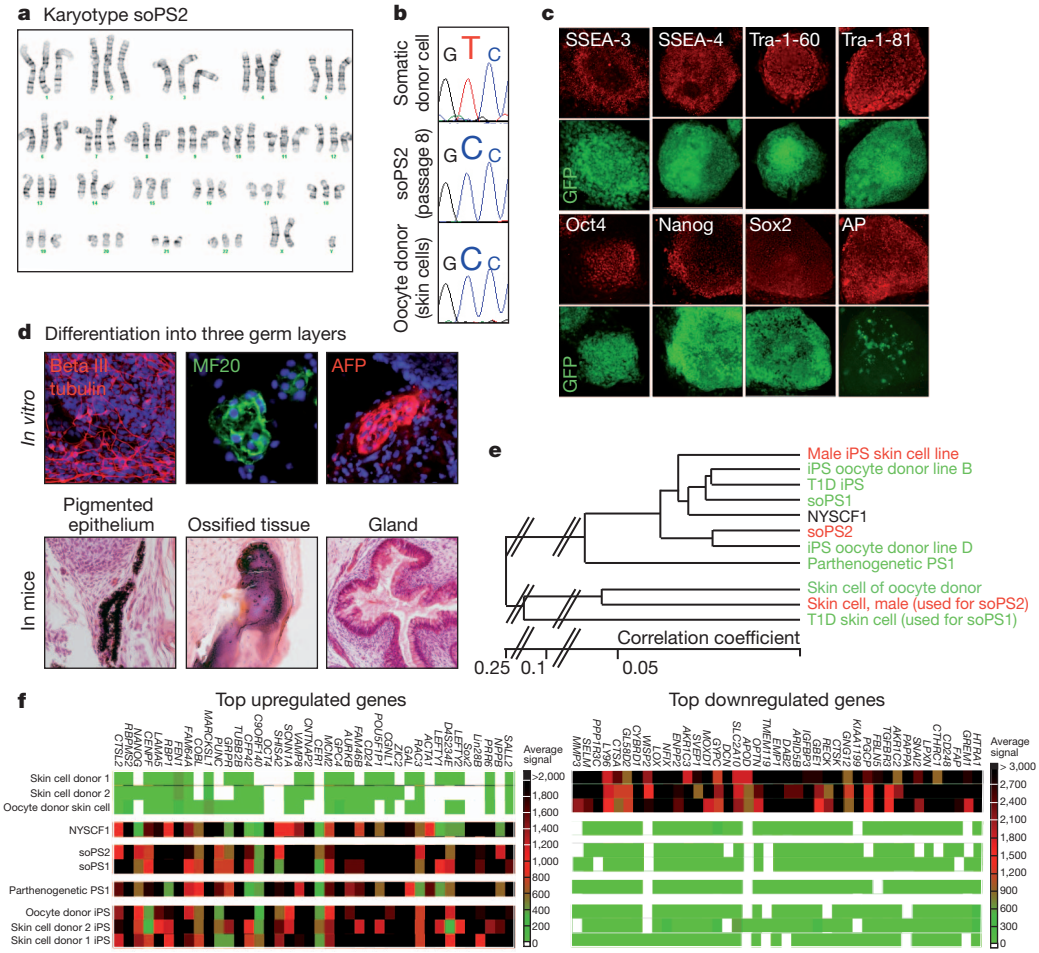

Figure 3 | soPS cells are pluripotent. **a**, Karyotype. **b**, Sequence of the mitochondrial hypervariable region I. **c**, Immunostaining for pluripotency markers. **d**, Immunostaining and histochemistry of cells/tissues upon differentiation. **e**, Cluster diagram of global gene expression analysis. Cell lines of the same colour are genetically related to either soPS1 or soPS2. **f**, Heat map of most highly up- or downregulated genes in stem cell lines in comparison to the donor fibroblasts.

1,327 transcripts, 463 were present at fivefold higher levels in the stem cells than in the fibroblasts, and 670 transcripts were decreased by a factor of five or more in soPS2. Among the genes with the most significant upregulation in soPS1 and soPS2 were genes typically expressed in pluripotent stem cells, but not in fibroblasts, such as *LIN28A*, *POU5F1*, *SOX2*, *NANOG* and *LEFTY1* (Fig. 3f). Genes that were most downregulated included those typically expressed in fibroblasts, such as fibroblast activating protein (*FAP*), pappalysin (*PAPPA*), metallopeptidase (*MMP3*), a collagen triple helix-containing protein (*CTHRC1*), and a mesoderm-specific transcription factor (*SNAI2*) (Fig. 3f). In comparison to NYSCF1, 28 genes and 24 genes were expressed at higher levels in soPS2, and soPS1 respectively (more than threefold, $P < 0.01$) (Supplementary Table 5), including neuropeptide galanin, neuronatin, *SRY*, *rex1* (also known as *ZFP42*), *NODAL*, Cerberus 1 and *LEFTY2*. Presumably, expression of these genes reflects spontaneous differentiation into various cellular lineages in soPS cultures, rather than incomplete reprogramming of the somatic cell genome. In additional comparisons we were not able to identify consistent differences between soPS cells, iPS cells, NYSCF1 and pPS1 (Supplementary Fig. 6).

Epigenetic reprogramming

Consistent with reprogramming of the somatic cell genome to a pluripotent state, methylation of DNA at the *Nanog* promoter was low (5–15%) in soPS cells and high (38–58%) in the somatic donor cells (Fig. 4a). Demethylation at the *Nanog* and *Oct4* promoters correlated with the expression of single nucleotide polymorphisms (SNPs) located in the somatic genome (Fig. 4b). Demethylation was specific and did not occur on an imprinted locus *PEG3*: two thirds of sequencing reads were methylated in soPS1, reflecting the presence of two methylated maternal alleles, one from the oocyte and one from the somatic cell, as well as a single paternal allele of somatic origin (Fig. 4c).

To determine whether reprogramming had occurred at other loci, we used a genome-wide digital allelotyping approach to distinguish gene expression from the somatic cell-derived genome and the oocyte-derived genome in soPS cells. This method is based on a library of 27,000 'padlock' probes flanking known SNPs on all 23 chromosomes of the human genome (Fig. 4d)[30]. Extension of the padlock probes by DNA polymerase captures the SNP and allows single molecule DNA sequencing. SNP capture on genomic DNA will reflect the allelic ratio of the SNP, whereas SNP capture on cDNA will reflect the transcriptional activity of an allele.

We prepared genomic DNA and cDNA from soPS cells and their corresponding somatic cells (Supplementary Fig. 7). By generating 108,982,981 sequencing reads (Supplementary Table 6), we were able to identify 787 and 483 expressed SNPs for soPS1 and soPS2, respectively, for which the oocyte donor DNA sequence differed from that of the somatic cell and for which the somatic cell was homozygous. The median allelic ratio (somatic/(oocyte plus somatic)) for the genomic DNA was 0.64 for both soPS1 and soPS2. This ratio is consistent with the inference that a diploid complement of 46,XY chromosomes originating from the somatic cell and a haploid set of 23,X chromosomes originating from the oocyte are present in the soPS cell lines.

To calculate the proportion of transcripts expressed from the somatic cell-derived genome in soPS cells (cDNA somatic/(cDNA oocyte plus cDNA somatic)), each individual SNP was normalized to the ratio

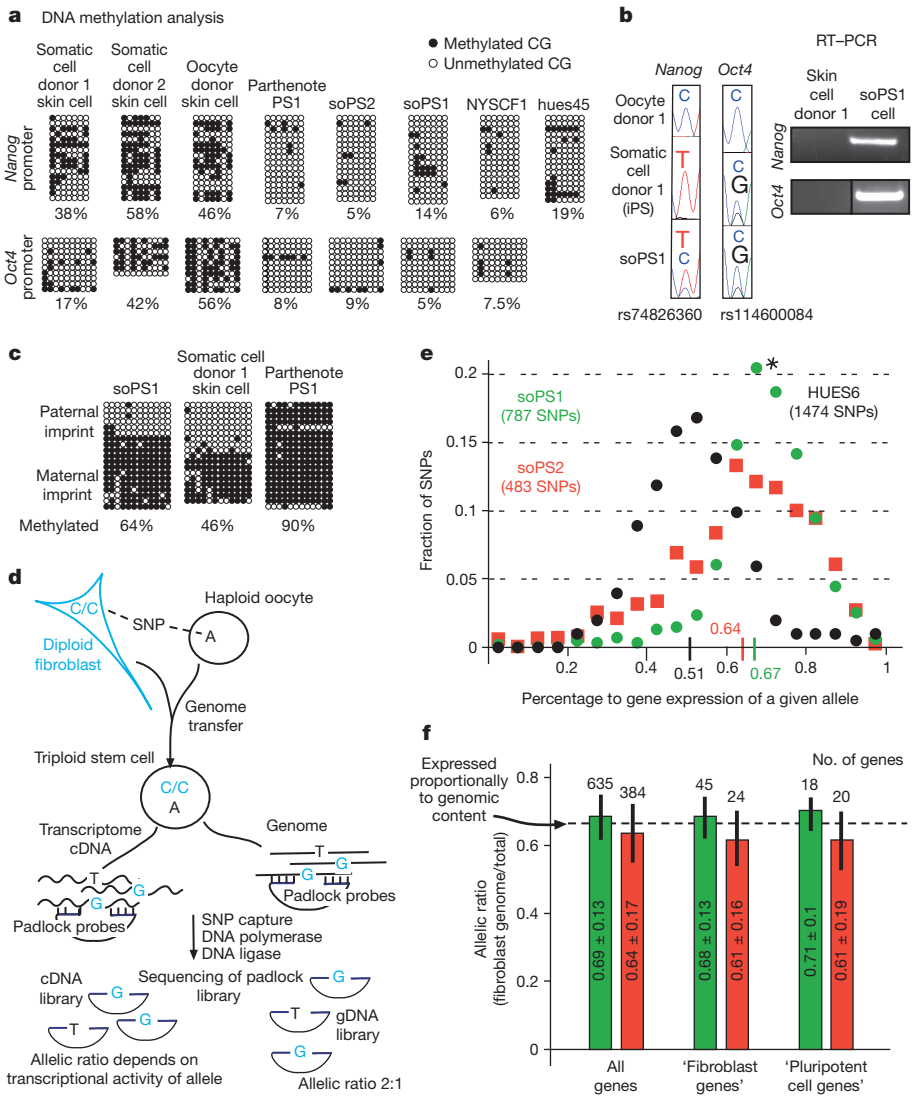

Figure 4 | Human oocytes reprogram a somatic cell to a pluripotent state. **a**, Bisulphite sequencing of the *Nanog* and *Oct4* promoters. The percentage of methylated CG is shown. **b**, PCR with reverse transcription (RT–PCR) and sequencing of expressed SNPs. **c**, Bisulphite sequencing of the differentially methylated region (DMR) of *PEG3*. **d**, Schematic of genome-wide SNP capture and allelotyping. **e**, The reprogrammed genome contributes two thirds of the transcriptome. Alleles expressed at a certain allelic ratio (binned in intervals of 0.05, horizontal axis) displayed as fraction of all captured alleles (vertical axis). **f**, No memory of somatic gene expression is observed. Mean allelic ratios and standard deviations in soPS cells for all captured SNPs represented in the Illumina array 'all genes', for SNPs in genes highly expressed in the fibroblast donor cell ('fibroblast genes'), and for SNPs in genes highly expressed in pluripotent stem cells ('pluripotent cell genes'). soPS1, green bars; soPS2, red bars. Mean ± s.d. is shown.

of the same SNP observed in genomic DNA. If a locus was expressed in proportion to its genomic content in soPS cells, the ratio of transcripts would be expected to equal 2/3 = 0.6667. Allele ratios for each SNP were binned in increments of 0.05 units, and that number of transcripts was expressed as a fraction of total SNPs captured (Fig. 4e). (For example, 162 somatic cell SNPs were expressed at a ratio of 0.65–0.7 in soPS1. As the total number of SNPs captured was 787, the fraction is 162/787 = 0.206, yielding the data point indicated by an asterisk in Fig. 4e). The median of the allelic ratio was 0.67 for soPS1 and 0.64 for soPS2, consistent with expression from the somatic genome proportional to the genomic content. The distribution of allelic ratios approximated a Gaussian curve (Shapiro–Wilk test for normality $W = 0.92$ for soPS1 and $W = 0.97$ for soPS2), indicating significant variability in the contribution of a particular allele. Such variation was not specific to soPS cells. Variability at comparable levels was also observed for allelic ratios in diploid HUES6 as well as in tetraploid HUES6–somatic cell hybrids[31] (Supplementary Fig. 8), and may be caused by polymorphisms in gene regulatory regions[30].

Our expectation was that if reprogramming in soPS cells were incomplete we would detect a bias in this distribution: genes expressed at high levels in fibroblasts, but at low levels in pluripotent stem cells, would be expressed predominantly from the fibroblast cell-derived genome in soPS cells; conversely, genes that are expressed at high levels in pluripotent stem cells, but not in fibroblasts, would be expressed predominantly from the oocyte-derived genome. Among a total of 1,019 genes represented in both gene expression array as well as in the SNP capture data, 38 (18 for soPS1 and 20 for soPS2) were upregulated at least fourfold in soPS cells ($P < 0.01$), and 69 (45 for soPS1 and 24 for soPS2) genes were expressed in the donor fibroblasts at levels fivefold or higher compared to soPS cells ($P < 0.01$). The mean allelic transcript ratio for the 'somatic cell genes' and the 'pluripotent cell genes', was identical, and did not differ from the expected allelic ratio of 0.66 nor from the allelic ratio of all captured genes (Fig. 4f and Supplementary Table 7).

Discussion

Upon replacement of the oocyte genome with that of a somatic cell, we observed developmental arrest at late cleavage stages in association with severe transcriptional abnormalities, similar to the arrest we had previously observed following somatic cell genome exchange in human zygotes[22]. These defects occurred despite the use of high quality oocytes obtained from women without history of infertility. This result is consistent with previous studies[4,9,10], but contrasts with a report of efficient development to the blastocyst stage[3]. Those authors attributed the improved developmental potential to oocyte quality. However, because the STR genotype of the blastocysts was incomplete, an alternative interpretation is that the presence of genetic material of the oocyte promoted development to the blastocyst stage. Another group has generated a single blastocyst after transfer of pluripotent stem cell genomes[5], suggesting that the developmental arrest seen with

somatic cells may not apply to pluripotent cells. Consistent with this hypothesis, we find that after transfer of blastomere or oocyte nuclei, development to the blastocyst stage occurs.

In contrast, if the somatic cell genome is merely added and the oocyte genome is not removed, development to the blastocyst stage occurs. From these blastocysts, we were able to derive triploid pluripotent stem cells containing a diploid genome complement of the somatic cell and a haploid genome complement of the oocyte.

It was previously shown that epigenetic memory is often retained in mouse iPS cells, but not in cells reprogrammed by mouse oocytes[32]. We compared gene expression from the haploid oocyte genome, which reached pluripotency through its developmental trajectory, with gene expression from the diploid somatic genome, which required a reprogramming process to establish pluripotency. Because both of these genomes are present in the same cell, they are exposed to an identical environment. Preferential expression of pluripotency genes from the oocyte genome, or preferential expression of somatic genes from the somatic cell genome, would be a strong indication of epigenetic memory. Using a genome-wide allelotyping approach and gene expression profiling, we compared the levels of expression from each allele, and did not find evidence for epigenetic memory: expression from the reprogrammed somatic genome was proportional to the genomic content and did not depend on the activity in the fibroblast donor cell.

This report demonstrates the feasibility of somatic cell reprogramming using human oocytes. With a reliable source of human oocytes, it should be possible to overcome the requirement of the oocyte genome for somatic cell reprogramming, allowing the generation of diploid pluripotent stem cells.

METHODS SUMMARY

Human oocytes were aspirated approximately 36 h after human chorionic gonadotropin application and transported to the laboratory in a portable incubator at 37 °C. The oocyte genome of the MII oocyte was identified by microtubule birefringence and/or staining in Hoechst 33342 and minimal ultraviolet illumination, whereas in activated oocytes, the oocyte genome was identified by Hoffmann modulation contrast optics only and then removed by laser-assisted micromanipulation. Somatic cells were introduced into the oocyte. Oocytes were activated in the calcium ionophore ionomycin, followed by incubation in the kinase inhibitor 6-dimethylaminopurine, thoroughly washed, and allowed to develop.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Information Illumina array data have been deposited at GEO under accession number GSE28024. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to D.E. (d.egli@nyscf.org).

METHODS

Oocyte donation. Oocyte donors of age 22–33 were recruited from the women participating in the reproductive oocyte donation program at the Center for Women's Reproductive Care (CWRC) at Columbia University P&S. These women had made a decision to enter the reproductive egg donation program, they met all criteria required for donation for reproductive purposes, and only then were presented with the option to donate oocytes for research. Both licensed medical social workers and CWRC physicians screened all women with respect to their reproductive, medical and psychosocial health. All of the women had a college degree or additional higher education, and none were financially disadvantaged. All women in the study were fully employed. During a period of 19 months, 16 women out of the 252 women enrolled in the reproductive oocyte donation program were asked if they wanted to donate oocytes to research. These women discussed the stem cell study in detail with a physician and those who chose to donate oocytes to research gave signed informed consent and initiated a standard hormone control regimen. All 16 women decided to participate in the study and gave informed consent (100% compliance). Two women did not complete the hormone treatment because of a lack of response. Two additional women donated for the study at a later time. In total, 16 women donated 270 mature MII oocytes (range of 2–26, or a mean of 16.9 oocytes per donor cycle). Payment for participation was equal to payment for women donating oocytes for reproduction at CWRC, or $8,000 (pre-tax).

Skin biopsies. Skin biopsies (3 mm) were obtained using an AcuPunch biopsy Kit (Acuderm Inc.) from the locally anesthetized (1% Lidocaine HCl, Hospira, Inc.) upper arm or the upper leg. Biopsies were cut in 10–15 smaller pieces, placed in a six-well dish around a droplet of silicon grease, covered with a glass cover slip, and allowed to grow for 3–4 weeks in medium containing DMEM, 10% FBS, 1% Anti-Anti, nucleosides, GlutaMAX, β-mercaptoethanol and nonessential amino acids (all Invitrogen). In some instances, skin biopsies were obtained from subjects also donating oocytes. The identification numbers of the skin cell donors are 1-000 (male, T1D used for generation of soPS1) and 1-016 (male, used for generation of soPS2), and 1-034 for an oocyte donor. Protocols for obtaining skin biopsies and for their use in reprogramming experiments were reviewed and approved by the institutional review board and stem cell committees of Columbia University. All subjects gave signed informed consent.

Genome transfer into human oocytes and stem cell derivation. Oocytes were transported in GMOPSplus (Vitrolife) in a portable incubator (INC-RB1, CryoLogic) at 37 °C. The oocyte genome was identified by microtubule birefringence using the Oosight imaging system, and/or staining in 2 μg ml^{-1} Hoechst 33342 and minimal ultraviolet illumination. All manipulations were done on a Nikon TE2000-U equipped with Narishige micromanipulators and a Tokai hit heating plate. Somatic cells were infected with a vesicular stomatitis virus G protein (VSVG)-pseudotyped CAGGS:GFP or CAGGS:H2B-GFP retrovirus, sorted for GFP expression with a BD FACSAriaIIu, and grown to confluence to induce cell cycle exit. A single somatic cell was inserted below the zona pellucida of the oocyte using laser-assisted zona drilling (Hamilton Thorne) and introduced into the oocyte either by two fusion pulses of 20 μs width and 1.3 kV cm^{-1} strength (LF201, NEPA Gene), in cell fusion medium 0.26 M mannitol, 0.1 mM MgSO4, 0.05% BSA, 0.5 mM HEPES, or by prior incubation of the somatic cell in inactivated Sendai virus HVJ-E (GenomeOne, Cosmo Bio), diluted with fusion buffer 1:5. For both fusion methods, the efficiency of fusion and oocyte survival was close to 100%. The first polar body was removed or ablated with two to three 500-μs laser pulses to avoid potential fusion to the oocyte. Oocytes were activated in 5 μM ionomycin (Sigma) in GMOPs plus for 5 min, followed by 4–5 h incubation in 2 mM 6-DMAP (Sigma) or until small interphase nuclei became apparent, thoroughly washed in Global medium and cultured to the blastocyst stage at 37 °C (Minc incubator, Cook), in a certified gas mixture 5% O2, 6%CO2, 89%N2 (TechAir). Some samples were harvested on day 3–5 of development for gene expression analysis. Blastocysts were used for derivation of pluripotent stem cells as described[33], with the addition of 2 μM Thiazovivin (Stemgent) and 10 μM Rock-Inhibitor Y-27632 (Stemgent) to the derivation medium. Human pluripotent stem cells were expanded manually or enzymatically and cultured under standard conditions, as previously described[34]. Human blastocysts for the derivation of NYSCF1 and human cleavage stages for gene expression analysis were thawed using the Sidney IVF thawing kit (K-SITS-5000, Cook Medical). Human blastocysts and cleavage stages were obtained from anonymous donors at CWRC under protocols reviewed and approved by the Columbia stem cell committee and the Columbia IRB. NYSCF1 characterization is described in Supplementary Fig. 9. iPS cells were generated according to published protocols using VSVG-pseudotyped retroviruses[35].

Gene expression analysis. RNA from human cleavage stages and blastocysts was isolated using a picopure RNA isolation kit (Arcturus), amplified by two rounds of T7 transcription using the total Prep RNA amplification kit (Illumina). RNA from cell lines was isolated using RNeasy Plus mini kit (Qiagen) and amplified with a single round of T7 transcription. Amplified biotin labelled RNA was hybridized to the Illumina HumanRef-8 v3 Expression BeadChips. Analysis was undertaken using GenomeStudio and Microsoft Excel programs as follows: data were normalized to the average signal. Background was subtracted. Unfertilized oocytes (two biological replicates consisting of five MII oocytes) were used as a reference point for all comparisons. Transcripts (1,345) were more than fivefold upregulated ($P < 0.01$) in IVF controls on day 3 (two biological replicates consisting of two specimens). Of these transcripts 761 were also elevated in IVF controls collected early on day 4 (two biological replicates consisting of 17 specimen, more than fivefold, $P < 0.01$). These were defined as the ZGA transcripts. We then determined how many of these ZGA transcripts were also upregulated after genome exchange (three biological replicates, nine specimens of up to 12 blastomeres), after amanitin treatment (two biological replicates consisting of two specimens) (more than fivefold, $P < 0.01$), and parthenotes (one sample consisting of four specimens). Data analysis for downregulated transcripts was done accordingly: 829 genes with transcript levels of 20% or less of those found in the oocyte were identified ($P < 0.01$). Among those 829 transcripts, we determined the number of transcripts that were also downregulated after genome exchange and after amanitin treatment ($P < 0.01$). Gene expression analysis of soPS cells was done with normalization to average and subtraction of background.

All array data and additional details on samples and analysis are available on GEO under accession number GSE28024.

Cell line analysis. For karyotype and STR analysis, live cultures were shipped to Cell Line Genetics (WI). RNA and DNA were isolated from cultures at passage 7 to 11, using QIAamp DNA Mini Kit for SNP capture. Padlock probes (27,000) were prepared to capture expressed SNPs from genomic DNA and cDNAs. The captured SNPs were quantified using single-molecule DNA sequencing according to ref. 30. Immunohistochemistry was done using primary antibodies recognizing Tra1-60 (MAB4360, Millipore), Tra1-81 (MAB5381, Millipore), SSEA-4 (MAB1435, R&D), SSEA-3 (MAB1434, R&D), Nanog (AF1997, R&D), Oct-4 (09-0023, Stemgent), Sox2 (09-0024, Stemgent), MF20 (DSHB), AFP (Dako), beta III tubulin (Sigma T2200) at dilutions of 1:500 to 1:1,000. Secondary antibodies were conjugated with Alexa Fluor (Invitrogen). Alkaline phosphatase staining was done using an alkaline phosphatase substrate kit (Vector Laboratories). Teratomas were generated by subcutaneous injection into NSG mice (Jackson laboratories) and harvested after 10–15 weeks. Animal experimentation was approved by the Columbia IACUC. For Affymetrix SNP chip analysis, gDNA was processed according to the GeneChip mapping 500K assay manual, and hybridized to a 250K Nsp Array according to the manufacturer's instructions, SNP data analysis was done using Affymetrix Genotyping Console. Bisulphite conversion of DNA was done using the EpiTect Bisulfite Kit according to the manufacturer's instructions. For bisulphite sequencing, PCR products were cloned into Topo TA vector (Invitrogen) and sequenced using M13R primer.

Primers used in this study were as follows: Primer sequence 5′ to 3′: ATTTGTTTTTTGGGTAGTTAAAGGT and CCTAAACTCCCCTTCAAAAT CTATT, bisulphite sequencing of Oct4 (ref. 36); TGG TTA GGT TGG TTT TAA ATT TTT G and AAC CCA CCC TTA TAA ATT CTC AAT TA, bisulphite sequencing of Nanog[37]; GGAAAGAAAATTTTTATAGGTAGGATAGT and AAACCCTAAACCTCCTAAACTAAATCTAA, bisulphite sequencing of PEG3 (ref. 38); GGGGTCAGTGCCTCAATAAG and TTTGGTCTCCAGGTTTCAGG, sequencing of rs7221396; CAGTTTTACCCCCTTACCTTCA and ACACATGTT GCCACCAGAGA, sequencing of rs11652263; GGGTTAGAAGCTCCTGCAAA and CTCTGGTCTGTCACCCATCA, sequencing of rs2286336; CAGGTTGACA GACATGAAATCC and CTGCTTTTTCCTGCCATTGT, sequencing of rs10521202; CACCATTAGCACCCAAAGCT and TGATTTCACGGAGGA TGGTG, sequencing of mitochondrial hypervariable region I.

Images and settings. GFP fluorescence and bright field images were aquired with a NikonTE 2000-U microscope equipped with a Nikon Digital Sight DS-Qi1Mc camera and NIS elements AR imaging program. GFP fluorescence was acquired with 1-s exposure time, no gain for all images. Bright field images were contrast-adjusted (equally across the entire image) in Adobe Photoshop. Histology analysis was done using an Olympus IX-71 microscope equipped with a U-TV0.5XC-3 colour camera. Immunofluorescence was done using Olympus DP30BW camera and Olympus imaging acquisition software. All fluorescent images are pseudo colours. Figures were assembled in Adobe Freehand MX.

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