Journal of proteome research

ARTICLE

pubs.acs.org/jpr

Proteomic Analysis of Mouse Oocytes Reveals 28 Candidate Factors of the "Reprogrammome"

Martin J. Pfeiffer,[†,‡] Marcin Siatkowski,[†,§,∥] Yogesh Paudel,[∥] Sebastian T. Balbach,[‡] Nicole Baeumer,[⊥] Nicola Crosetto,[#] Hannes C. A. Drexler,[¶] Georg Fuellen,[*,§,∥] and Michele Boiani[*,‡]

[‡]Max-Planck Institute for Molecular Biomedicine, Mouse Embryology Laboratory, Röntgenstrasse 20, D-48149 Münster, Germany

[¶]Max-Planck Institute for Molecular Biomedicine, Bioanalytical Mass Spectrometry Facility, Röntgenstrasse 20, D-48149 Münster, Germany

[§]DZNE, German Center for Neurodegenerative Disorders, Gehlsheimer Strasse 20, D-18147 Rostock, Germany

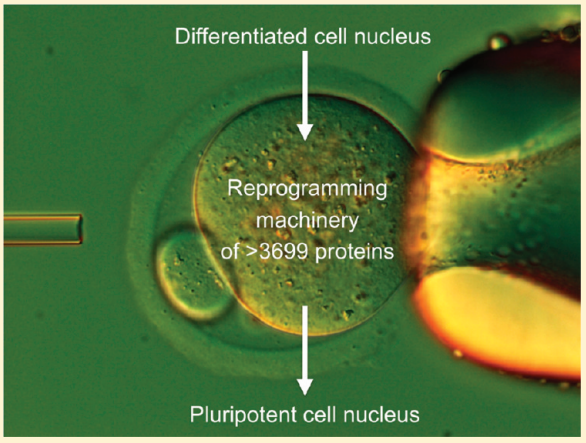
[∥]Institute for Biostatistics and Informatics in Medicine and Ageing Research, University of Rostock, Ernst Heydemann-Strasse 8, D-18057 Rostock, Germany

[⊥]Arrows Biomedical GmbH, Gievenbecker Weg 11, D-48149 Münster, Germany

[#]Institute of Biochemistry II, Goethe University Medical School, Theodor-Stern-Kai Strasse 7, D-60590 Frankfurt am Main, Germany

Supporting Information

ABSTRACT: The oocyte is the only cell of the body that can reprogram transplanted somatic nuclei and sets the gold standard for all reprogramming methods. Therefore, an in-depth characterization of its proteome holds promise to advance our understanding of reprogramming and germ cell biology. To date, limitations on oocyte numbers and proteomic technology have impeded this task, and the search for reprogramming factors has been conducted in embryonic stem (ES) cells instead. Here, we present the proteome of metaphase II mouse oocytes to a depth of 3699 proteins, which substantially extends the number of proteins identified until now in mouse oocytes and is comparable by size to the proteome of undifferentiated mouse ES cells. Twenty-eight oocyte proteins, also detected in ES cells, match the criteria of our multilevel approach to screen for reprogramming factors, namely nuclear localization, chromatin modification, and catalytic activity. Our oocyte proteome catalog thus advances the definition of the "reprogrammome", the set of molecules—proteins, RNAs, lipids, and small molecules—that enable reprogramming.



KEYWORDS: induced pluripotency, Nanog, oocyte, proteome, proteomics, reprogramming factor, SCNT, stem cell

INTRODUCTION

Mature oocytes and undifferentiated embryonic stem (ES) cells contain reprogramming factors (proteins, RNAs, lipids, small molecules) that enable these cells to swiftly reprogram a somatic nucleus to pluripotency after somatic cell nuclear transfer (SCNT) or cell fusion, respectively.[1−3] Somatic cells can also be reprogrammed by forced expression of a combination of protein transcription factors for at least 50 cell cycles,[2] producing induced pluripotent stem (iPS) cells.[4] These three reprogramming methods share with each other features such as DNA demethylation of pluripotency gene promoters and reactivation of *Oct4*, *Nanog*, and other genes. While the list of pluripotency-associated genes continues to grow,[4,5] the list of known (i.e., identified) reprogramming factors remains short even with the most recent new entries.[6] Despite the advance introduced by iPS cell technology, it is still not clear whether upstream reprogramming events and molecular processes are shared even just partially between the different reprogramming platforms.

Oocyte-mediated reprogramming outperforms the iPS cell approach not only in speed but also in terms of reprogramming rates and quality,[7] as measured by the generation of pluripotent stem cells that can give rise to live births after tetraploid embryo complementation.[8,9] Hanna and colleagues therefore proposed that the mechanism of pluripotency induction is active and directed in oocytes (and also in ES cells, when performing reprogramming via cell fusion), as opposed to passive and stochastic in iPS cells.[2] This proposition finds support in the recent study of Ono and colleagues, who inhibited the HDAC class IIb activity in

Received: July 9, 2010

 © XXXX American Chemical Society dx.doi.org/10.1021/pr100706k | *J. Proteome Res.* XXXX, XXX, 000–000

nucleus-transplanted mouse oocytes and achieved higher rates of reprogramming.[10,11] Identifying the reprogramming factors of the oocyte should therefore serve as the prime source of information to make the reprogramming process in general more robust, complete and reliable.[2]

By definition, an active reprogramming mechanism entails catalytic activity such as DNA nucleotide modification, base excision repair or histone deacetylation. This is not the case for the iPS cell factors, which are transcription factors binding to DNA but lacking enzymatic activity. Although certain transcription factors have been ascribed reprogramming ability, it is possible that they simply bind to DNA in a sequence-specific manner when the cell cycle offers them a window of opportunity and then recruit other factors that carry out the actual reprogramming. Nontranscription factor proteins, as the main catalytic and regulatory components of cells, hold the greatest promise to be the active reprogrammers. Searching the oocyte for these proteins is highly desirable since the oocyte is the only cell of the adult body endowed with a natural reprogramming ability. The analytical sensitivity of proteomic assays was so far not high enough for the relatively small numbers of oocytes available in mammals for research. For these primarily technical reasons, the proteome of oocytes has not been resolved to a depth comparable to that of ES cells.[12] Since ES cells can be easily obtained in larger quantities than oocytes, proteomic-based approaches using pluripotent ES cells have thus proven useful to identify proteins that enhance the reprogramming efficiency in iPS cell derivation, such as Smarca4 and Smarcc1.[13]

Early attempts to provide a comprehensive protein catalog of oocytes and early embryos were based on two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), resulting in thousands of spots from which a handful of proteins could be identified.[14,15] With the rise of modern mass spectrometry (MS), some of the technical limitations of 2D-PAGE (e.g., identification of proteins by spot position) have been overcome, although the identification of all proteins within a specimen remains unaccomplished, particularly when the specimen amount is minute and of high complexity, which is the case with mammalian oocytes. Still, liquid chromatography coupled to mass spectrometry (LC−MS) is the method best suited to gain an overview of the proteome of a cell.

LC−MS was first used in reprogramming studies by Novak and colleagues, who attempted to identify proteins in bovine oocytes that bind to the nuclei of permeabilized epithelial cells.[16] Although no such proteins were detected, this study pioneered the field and paved the way for LC−MS applications on mouse oocytes. Ma et al.,[17] Zhang et al.,[18] Yurttas et al.[19] and Wang et al.[20] analyzed the proteome of metaphase II mouse oocytes by LC−MS leading to the identification of a range of oocyte proteins: 380 (in 80 μg total protein), 625 (2700 oocytes), 185 (500 oocytes) and 2973 (7000 oocytes), respectively. While this depth is remarkable for mammalian oocytes, the count still lies far from the 5111 proteins identified in mouse ES cells.[12] However, as the sensitivity of mass spectrometry and the ability to analyze collected spectra is improving, more information can be gained from minute specimens such as mammalian oocytes.

Here we analyzed the proteome of metaphase II B6C3F1 mouse oocytes and undifferentiated mouse ES cells using LC−MS in order to approximate the molecular definition and thereby our understanding of what we call the *reprogrammome*, namely the set of proteins, RNAs, lipids and small molecules that enable reprogramming. Since proteins are synthesized in the cytoplasm, and oocytes and ES cells have markedly different sizes as well as nucleus-cytoplasmic ratios, comparing their protein amounts would primarily reflect these physical features rather than reprogramming ability. Therefore, as a first step toward pinpointing oocyte proteins that initiate reprogramming, we screened our catalog of 3699 proteins for members that are also present in undifferentiated ES cells regardless of the amount and that feature three of the defining properties of reprogramming factors: nuclear localization, chromatin modification and catalytic activity. Of 28 proteins that fulfill these criteria, 17 proteins have been reported to show increasing levels of coding mRNA in mouse fibroblasts during transition to iPS cells.[21] In sum, the present catalog of 3699 oocyte proteins and its subset of 28 candidate reprogramming factors advance the definition of the *reprogrammome* and provide a basis to further explore and understand the mechanisms of reprogramming for the benefit of all reprogramming platforms.

EXPERIMENTAL METHODS

Mice

Six- to eight-week-old B6C3F1 (C57Bl/6J × C3H/HeN) mice were used as oocyte and cumulus cell donors. Nanog-GFP transgenic mice were from RIKEN Institute, Tsukuba, Japan [Strain name STOCK Tg(Nanog-GFP,Puro)1Yam, RBRC no. RBRC02290]. All mice were primed with 10 IU each pregnant mare's serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) injected intraperitoneally 48 h apart at 5 pm and sacrificed by cervical dislocation 14 h after hCG to collect the cumulus-oocyte complexes (COC) from the oviducts. Mice were maintained and used for experiments according to the ethical permit issued by the Landesamt für Natur, Umwelt und Verbraucherschutz (LANUV) of the state of North Rhine-Westphalia, Germany.

Somatic Cell Nuclear Transfer (NT) and in vitro Culture

Within 30 min of COC collection, surrounding cumulus cells were removed from metaphase II oocytes using hyaluronidase (50 U/mL), and oocytes were put in culture. Micromanipulations were performed as described.[22] Briefly, the chromosomal spindle was removed by gentle suction in a piezo-operated microcapillary needle (12 μm inner diameter) in the presence of cytochalasin B (1 μg/mL). The ooplasts were transplanted with single cumulus cell nuclei by injection with a piezo-operated microcapillary needle (7 μm inner diameter) in the presence of 1% polyvinylpyrrolidone 40 kDa. The nucleus-transplanted ooplasts were parthenogenetically activated in Ca-free α-MEM supplemented with 10 mM $SrCl_2$ and 5 μg/mL cytochalasin B. All micromanipulations were conducted in HCZB medium (with 5.6 mM glucose) under Nomarski optics at 30 °C room temperature. Recovery from micromanipulation was allowed in α-MEM medium (ooplasts) or in α-MEM and HCZB medium mixed 1:1 (nucleus-transplanted ooplasts) for 1 h.

In vitro culture of cloned embryos was performed in α-MEM medium as previously described.[22] α-MEM medium was purchased (Sigma #M4526) and supplemented with bovine serum albumine (BSA, 2 mg/mL; Probumin #81-068-3, Millipore) and gentamicin sulfate (50 μg/mL). Embryos were cultured to blastocyst stage in 500 μL α-MEM in Nunc 4-well plates. When required, the medium was supplemented with 10 μg/mL cycloheximide (CHX). Treatment with CHX started immediately after enucleation or after chemical activation of nucleus-transplanted oocytes, and lasted for 96 h (chronological blastocyst stage).

dx.doi.org/10.1021/pr100706k |J. Proteome Res. XXXX, XXX, 000–000

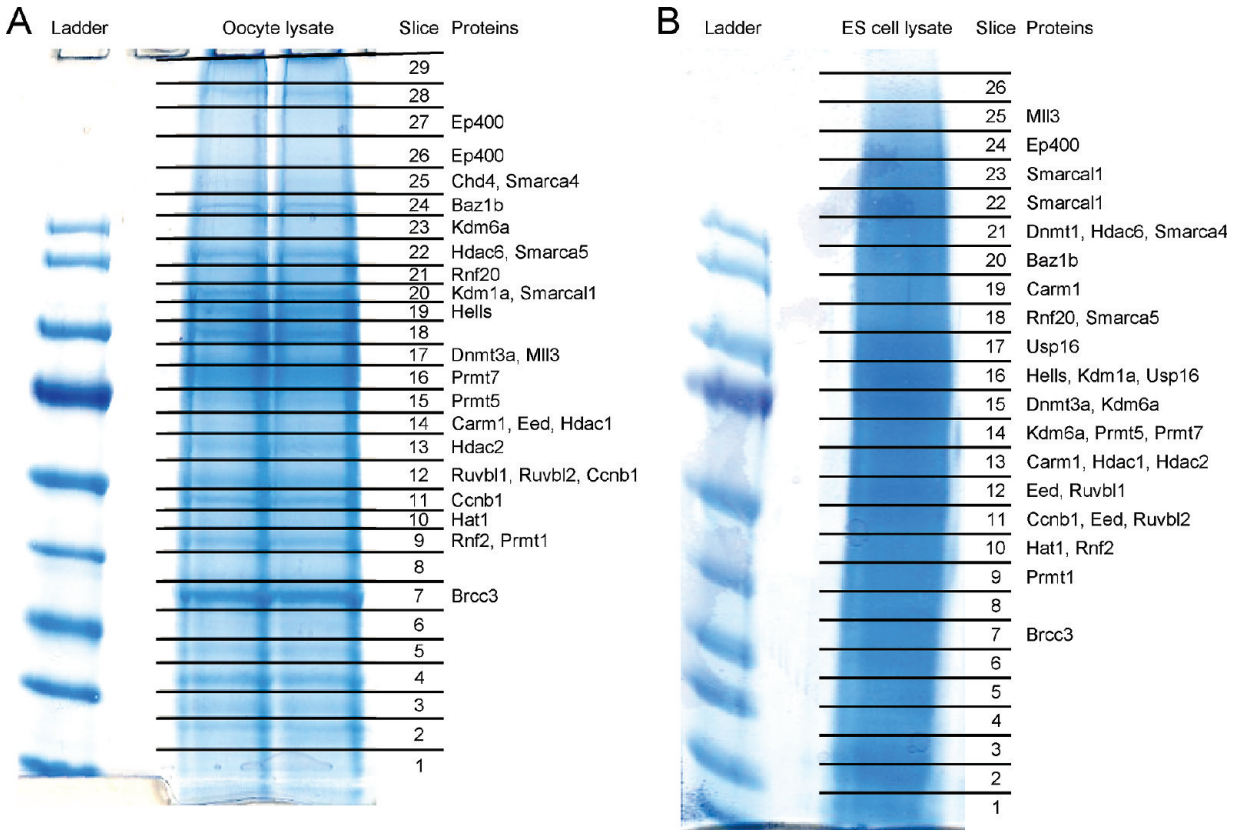


Figure 1. Colloidal Coomassie stained SDS-polyacrylamide gel. The lysate of 1884 metaphase II mouse oocytes was separated by SDS PAGE (distributed between two lanes), stained and cut into 29 slices (black lines) (left, panel A). The lysate of undifferentiated mouse ES cells was loaded (100 μg/lane) on the gel and separated as described (right, panel B). In both gels, the slices are indicated in which the candidate reprogramming factors were detected (as a peak) after subjecting the slices to in-gel trypsin digestion and subsequent nanoLC−MS/MS mass spectrometry. In case of factors lacking a peak (e.g., Dnmt1 and Usp16, detected in most slices), no slice is indicated.

This time span was chosen since the *Nanog* gene would not normally be expressed until the 8-cell stage ($\approx$ 55 h). In addition, CHX treatment was also applied to a third group of cloned embryos for only 6 h during activation.

Oocyte and ES Cell Sample Preparation and Processing for Mass Spectrometry

Sample Preparation. One-thousand eight-hundred eighty-four metaphase II oocytes were denuded using warm Tyrode's acid solution and collected in SDS lysis buffer (4% SDS, 100 mM Tris/HCl pH 7.5, 0.1 M DTT, total volume 70 μL). The sample was sonicated using a Bioruptor sonication system (5 cycles of 30 s on and 30 s off at high setting), to ensure total lysis and to shear DNA. ES cells from one 10 cm plate were subjected to double sedimentation and collected in SDS lysis buffer as described. Following addition of 20 μL LDS sample buffer and heating, oocyte lysate proteins were size fractionated by 1D gel electrophoresis in two neighboring lanes of a 4−20% NUPAGE gel (Invitrogen) and stained with Colloidal Blue staining Kit (Invitrogen). Lanes containing protein were sliced into 29 (oocytes) and 27 (ES cells) pieces and processed for GeLC−MS/MS. Briefly, proteins within each gel piece were subjected to reduction (10 mM DTT, 45 min at 56 °C) and alkylation (JAA, 30 min, RT, in the dark) followed by Trypsin cleavage (Promega) for 16 h at 37 °C. Peptides were then extracted from the gel pieces as described[23] and desalted as well as concentrated by Stage Tips.[24]

LC−MS/MS and Data Analysis. Each fraction, which represented the peptide content of two neighboring gel pieces (Figure 1), was analyzed by Reversed phase Chromatography using a EasyLC nanoflow system (Proxeon) that was online coupled via in-house packed fused silica capillary column emitters (length 15 cm; ID 75 μm; resin ReproSil-Pur C18-AQ, 3 μm) and a nanoelectrospray source (Proxeon) to an LTQ Orbitrap Velos mass spectrometer (Thermo Scientific). Peptides were eluted from the C18 column by applying a linear gradient from 5−35% buffer B (80% acetonitril, 0.5% acetic acid) over 120 min followed by a gradient from 35−98% over 15 min. The mass spectrometer was operated in the positive ion mode (source voltage 2.2 kV), automatically switching in a data-dependent fashion between survey scans in the mass range of m/z 300−1650 and MS/MS acquisition. Collision induced MS/MS spectra from the 15 most intense ion peaks in the MS were collected (Target Value of the Orbitrap survey scan: 1000000; resolution R = 60 000; Lockmass set to 445.120025). Raw data files were then processed by MaxQuant software (v 1.0.12.36) in conjunction with Mascot database searches.[25] Data were searched against the International Protein Index sequence database (mouse IPI, version 3.60) concatenated with reversed sequence versions of all entries. The parameter settings were: Trypsin as digesting enzyme, a minimum length of 6 amino acids, a maximum of 2 missed cleavages, carbamidomethylation at cysteine residues set as fixed and oxidation at methionine residues as well as acetylation at the protein N-termini as variable modifications. The maximum allowed mass deviation was 7 ppm for MS and 0.5 Da

dx.doi.org/10.1021/pr100706k |*J. Proteome Res.* XXXX, XXX, 000–000

for MS/MS scans. Protein groups were regarded as being unequivocally identified with a false discovery rate (FDR) set to 1% for all protein and peptide identifications when there were at least 2 matching peptides, one of which being unique to the protein group. Mass spectrometry data was also analyzed using the SEQUEST search algorithm and Proteome Discoverer software (Thermo Scientific).

Transcriptome

We obtained microarray data so that we could compare the mouse oocyte proteome and transcriptome. Two pools of 20 zona-enclosed B6C3F1 oocytes each were subjected to total RNA extraction followed by preamplification, reverse transcription, labeling, and hybridization as described next.

RNA (samples in 300 μL RLT buffer with 1% β-mercaptoethanol) was isolated using RNeasy Micro Kit as described by the manufacturer (Qiagen, no DNase treatment). An examination of total RNA on an Agilent 2100 Bioanalyzer and RNA Pico 6000 Lab-Chip kit confirmed the extraction of high-quality RNA, which was then prepared for gene expression profiling.

Arcturus RiboAmp HS Plus Amplification Kit (MDS Analytical Technologies GmbH, Germany) was used to amplify total RNA (two rounds of amplification) according to manufacturer's instructions. After the second round of amplification, amplified RNA was eluted with 15 μL RE buffer. Concentration of amplified RNA was measured using Agilent Bioanalyzer 2100 and RNA 6000 Lab-Chip kit.

Three micrograms of amplified RNA were labeled with Cy3 using Arcturus Turbo Labeling CY3 Kit (MDS Analytical Technologies GmbH, Germany). Concentration and frequency of incorporation (FOI) were measured using the NanoPhotometer (Implen, Munich, Germany).

Fragmentation (1650 ng Cy3-labeled amplified RNA) and hybridization were performed following the hybridization procedure recommended by the array manufacturer (Agilent Technologies), with a modification given by the manufacturer of the Turbo Labeling CY3 Kit. Microarray wash and detection of the labeled RNA on GeneChips were performed according to the instructions of Agilent Technologies. Gene expression profiling was performed using Agilent's Whole Mouse Genome Oligo Microarrays (4 × 44k, each array with 41 174 features). Array image acquisition and feature extraction was performed using the Agilent G2505B Microarray Scanner and Feature Extraction software version 9.5 (Agilent Technologies).

Real-time RT-PCR

For quantitative RT-PCR analyses, total RNA was extracted from pools of embryos using the ZR-RNA MicroPrep kit (Zymo Research #R1060) with an on-column DNA digestion using the RNase Free DNase Set (Qiagen #79254). Complementary DNA synthesis was performed using the High Capacity cDNA Reverse Transcription Kit (Invitrogen #4368813) according to manufacturer's instruction. Transcript levels were determined using the ABI PRISM Sequence Detection System 7900 (Applied BioSystems) in connection with the Power SYBR Green PCR Master Mix in 20 μL reaction volumes in triplicate. Running conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, 95 °C for 10 s and 60 °C for 1 min for a total of 40 cycles; dissociation step: 95 °C for 15 s, 60 °C for 15 s and 95 °C for 15 s. Correct amplification was verified by examining melting curves of PCR products. *Ct* values were obtained with SDS 2.2 (Applied Biosystems), with a threshold of 0.2 and baseline 3−15. Expression levels of different transcripts were normalized to the housekeeping genes *gapdh* and β-actin within the log−linear phase of the amplification curve using the $\Delta\Delta Ct$ method. Primer sequences (5′-3′) were as follows: Gapdh forward CCAATGTGTCCGTCGTGGAT; Gapdh reverse TGCCTGCTTCACCACCTTCT; Actb forward ACTGCCGCATCCTCTTCCTC; Actb reverse CCGCTCGTTGCCAATAGTGA; Nanog forward GAACGGCCAGCCTTGGAAT; Nanog reverse GCAACTGTACGTAAGGCTGCAGAA.

Immunofluorescence

Oocytes, embryos and ES cells were fixed in 4% PFA in PBS for 20 min, permeabilized in 0.01% Triton-X100 in PBS for 10 min and then blocked for at least 1 h in PBS with 0.1% Tween 20, 2% BSA, 2% glycine. Working concentrations of the primary antibodies were obtained in blocking solution (rabbit-anti-Baz1b, Sigma W3516, 30 μg/mL; rabbit-anti-PRMT7, Santa Cruz sc-98882, 1 μg/mL; goat-anti-Ruvbl2, Santa Cruz sc-34751, 0.3 μg/mL) and applied to cells at 4 °C overnight. The cells were then washed two times for 5 min in 0.1% Tween 20 in PBS and incubated with the secondary antibodies for 2 h (goat-antirabbit-IgG-Alexa 647, goat-antirabbit-IgG-Alexa 568, donkey-antigoat-IgG-Alexa 647, all diluted to 1 μg/mL in blocking solution). After two washes in 0.1% Tween 20 in PBS, the cells were counterstained with 1 μM YO-PRO-1 (Molecular Probes, Invitrogen) for 10 min and imaged on a confocal microscope (UltraView RS3, Perkin-Elmer). Captured images were analyzed using the software ImageJ.

Database Search and Bioinformatics

Analysis and preprocessing of data were performed with Bioconductor software[26] using the R statistical computing and graphics environment.[27] First, transcriptome data were analyzed by the Agilent Feature Extraction software version 9.5. All Agilent microarray probe sets were mapped to PubMed ENTREZ, which was used as the common point of reference. Mapping was accomplished using the mgug4122a.db R package version 2.4.5. Positive hybridization for both samples was taken as evidence that the mRNA corresponding to the probe was present. By this criterion the data set was reduced from 27 032 (flag filtering) to 15 476 probe sets (double positive), and the gene hits in the transcriptome were screened to eliminate duplicates and multiple probes. Thus, of the initial 16 157 genes (corresponding to 27032 probe sets), 10 833 genes (corresponding to 15 476 probe sets) were retained for further analysis.

Next, we processed the proteome data set provided by the MaxQuant software. Frequent new releases of the IPI (International Protein Index)[28] implied that an update of the MaxQuant annotation was required, so we utilized supporting history files obtained from the IPI Web site (ftp://ftp.ebi.ac.uk/pub/databases/IPI/current) to upgrade to version 3.76. Based on the ipi.MOUSE.xrefs.gz file downloaded from IPI data repository, two lists of proteins represented by PubMed ENTREZ identifiers were established from MaxQuant proteome data sets, which yielded 3574 proteins from the initial 3699 proteins detected in oocyte and 4588 proteins from the initial 4723 proteins detected in ES cell. MaxQuant creates protein groups if the identified peptide set of one protein was equal to or contained in another protein's peptide set. Protein groups were processed by taking the IPI identifier for which the highest peptide count was measured. When the same highest peptide count was obtained for two proteins in a protein group, we considered this a tie, and we arbitrarily took the first protein to not have to discard such hits. Such a tie may happen in case of isoforms (splice variants), but it may also happen in case of close paralogs, where no unique peptides could be detected that reflect the difference between the paralogs.

dx.doi.org/10.1021/pr100706k |*J. Proteome Res.* XXXX, XXX, 000–000

Table 1. Candidate Reprogramming Factors Shared between Oocytes and ES Cells and Filtered by the GO Criteria Nucleus (CC), Chromatin Modification (BP) and Catalytic Activity (MF)

factor[a]	level in 8-cell stage blastomeres compared to oocytes[53]	level during iPS cell formation[21]	function
Baz1b	→	n.d.	Component of the WICH complex (WSTF-ISWI ATP-dependent chromatin-remodelling complex). Regulates H2A.X DNA damage response via tyrosine kinase activity.[72]
Brcc3	→	↑	Deubiquitylating enzyme targeted to γH2A.X-dependent K6- and K63-linked ubiquitin polymers at double-strand breaks. Required for cell cycle checkpoint and repair responses to ionizing radiation.[73]
Carm1	→	n.d.	Histone H3R17 methylase. Enhances transcriptional activation by nuclear receptors through interactions with the coactivators p160 and CBP.[74]
Ccnb1	→	↑	Cyclin B1 drives mitosis and meiosis, e.g. in mouse oocytes.[75]
Chd4	→	n.d.	Part of the nucleosome remodeling deacetylase (NuRD) complex.[76] Involved in p53 deacetylation.[77] Planarian (*Schmidtea mediterranea*) homologue is required for regeneration.[78]
Dnmt1	↓	↑	Maintenance cytosine methyltransferase for inheritance of methylation imprints.[79] Somatic isoform implicated in improper reprogramming after cloning.[80]
Dnmt3a	↓	↑	De novo DNA methyltransferase required for the establishment of methylation imprints in oocytes.[81] Involved in methylation of Oct4 and Nanog promoters during cell differentiation.[82]
Eed	→	↑	Member of the Eed/Ezh2 Polycomb repressive complex 2, which is required for silencing of HOX genes during embryonic development.[83] and functions as a H3K27-specific methyltransferase[84] and histone deacetylatase.[85]
Ep400	→	n.d.	Component of the Tip60-Ep400 histone acetyltransferase and nucleosome remodeling complex. Regulates cell cycle progression and DNA damage-induced apoptosis.[86] Tip60-Ep400 integrates signals from Nanog and H3K4me3 to regulate ES cell identity.[87]
Hat1	→	↑	Acetylates newly synthesized histone H4 in the cytoplasm.[88] Recruited to DNA double-strand breaks.[89]
Hdac1[b]	↑	n.d.	Histone deacetylase leading to transcriptional repression. Inhibition improves efficiency of cloning by nuclear transfer[90] and direct reprogramming.[91]
Hdac2	→	↑	Histone deacetylase leading to transcriptional repression. Inhibition improves efficiency of cloning by nuclear transfer[90] and direct reprogramming[91]
Hdac6	→	↑	Lysine deacetylation of α-tubulin and HSP90, ubiquitin-binding activity.[92]
Hells	→	↑	Member of SNF2 family, participates in SWI/SNF chromatin-remodeling complexes.[93] Epigenetic regulator of heterochromatin.[94]
Kdm1a (Aof2)[c]	→	↑	Demethylates mono- and dimethylated H3K4, a post-translational modification associated with gene activation.[95]
Kdm6a (Utx)[d]	↓	n.d.	H3K27-specific demethylase that enables activation of genes involved in animal body patterning.[96]
Mll3	→	n.d.	H3K4-specific methyltransferase, required for H3K4 trimethylation, expression of p53 target genes[97] and regulation of Hox genes.[98]
Prmt1	↑	↑	Protein arginine methyltransferase in mammalian cells, functions as H4-specific histone methyltransferase and regulates STAT1 signaling.[99,100]
Prmt5	↑	↑	Symmetrically dimethylates H3R8 and H4R3 for gene repression.[101]
Prmt7	↓	↑	Symmetrical dimethylation of H4R3,[102] suggested role in male germline imprinted gene methylation.[103]
Rnf2	→	↑	Ubiquitylation of H2A linked to repression of transcriptional initiation.[104]
Rnf20	→	n.d.	E3 ubiquitin ligase; leads to H2B monoubiquitylation, higher levels of methylation at H3 lysines 4 and 79, and stimulation of HOX gene expression.[105]
Ruvbl1, Ruvbl2	↑	↑	Regulation of transcription, DNA damage response, snoRNP assembly, cellular transformation by c-myc and β-catenin function, cancer, apoptosis, mitosis and development.[106]
Smarca4	↑	n.d.	Member of the BAF chromatin remodeling complex, required for zygotic genome activation,[60] regulates self-renewal in ES cells,[107] and facilitates iPS cell formation.[13]
Smarca5[e]	→	n.d.	Part of the nucleolar remodeling complex (NoRC), which regulates the epigenetic state of rRNA genes.[108] Null mutants die at the peri-implantation stage and no ES cells can be derived.[109]
Smarcal1	↓	↑	Involved in S-phase DNA damage response and replication fork stabilization.[110]
Usp16	→	n.d.	H2A deubiquitylating enzyme, involved in cell cycle progression gene expression[111] and reversal of gene silencing.[112]

[a] Additional 45 proteins featuring GO terms nucleus, chromatin remodeling and catalytic activity were detected either in the oocyte proteome or in the ES cell proteome but not in both. These proteins are: Kdm1b, Kdm2a, Kdm2b and Kdm6b in oocytes; Atm, Cenpv, Chd1, Chd7, Chd8, Chd9, Crebbp, Dmap1, Dnmt3b, Ehmt2, Ep300, Eya3, Ezh2, Hdac3, Hltf, Jarid2, Jmjd1c, Jmjd6, Kdm3a, Kdm3b, Kdm5b, Kdm5c, Mta2, Myst1, Myst2, Myst4, Nsd1, Pkn1, Prmt6, Rnf40, Setdb1, Sirt1, Smarca1, Suz12, Taf1, Tlk1, Tlk2, Trrap, Ube2b, Usp22 and Uty in ES cells. [b] Tie with LOC100048437 at first (peptide count) level in oocyte proteome. [c] Tie with LOC100046934 at first (peptide count) level in oocyte proteome and in ES cell proteome. [d] Tie with Uty at first (peptide count) level in ES cell proteome. [e] Tie with Vmn2r-ps14, Gm13034 at second level (mapping IPI→Entrez) in oocyte proteome and in ES cell proteome.

dx.doi.org/10.1021/pr100706k |J. Proteome Res. XXXX, XXX, 000–000

A tie may also happen if a protein (referred to by a specific IPI identifier) is mapped to more than one gene locus. The disadvantage of arbitrarily selecting one of the hits is that for the subsequent Gene Ontology analysis, we may have considered the wrong protein in case of paralogs. However, since close paralogs usually have similar function and feature a closely similar GO annotation, this disadvantage is outweighed by the advantage of a more exhaustive GO analysis. As the GO analysis of the oocyte and ES cell proteomes was repeated with and without considering the ties, the analysis resulted in the same top-20 categories (not shown). In the detailed analysis of the reprogramming factors, all ties are explicitly noted (Table 1).

To identify potentially interesting gene sets, hypergeometric testing was carried out for GSEA (Gene Set Enrichment Analysis).[29] Testing was performed with a p value cutoff at 0.01 with Bonferroni multiple testing correction. Gene Ontology Biological Process (GO BP) over-representation was analyzed with the R package GOstats version 2.16.0[30] which is part of the Bioconductor package.[26] The proteome data sets of both oocytes and ES cells were compared against the proteome reference set of all mouse ENTREZ identifiers with existing IPI identifier (26463 proteins). The proteome reference set was also used for the proteome shared between oocytes and ES cells. Due to the high number of proteins in the lists being analyzed for over-representation, it was necessary to consider GO categories where the number of genes is sufficient for finding general trends, as proposed by Hahne and colleagues.[31] Therefore we considered only GO categories with more than 30 genes.

RESULTS

Depth, Coverage and Purity of Metaphase II Mouse Oocyte Proteomics

In order to comparatively analyze the mouse oocyte proteome, 1884 metaphase II B6C3F1 oocytes (free of cumulus cells and zona pellucida) and undifferentiated mouse ES cells (100 μg) were lysed and the proteins were resolved by 1D-SDS-PAGE. The two polyacrylamide gels of oocytes and ES cells were cut into 29 and 27 slices, respectively (Figure 1). Each slice was subjected to trypsin digestion and analyzed by LC−MS using an LTQ Orbitrap XL Velos mass spectrometer, processing the data in MaxQuant and Mascot Server software for protein identification. We identified 3699 and 4723 protein groups (MaxQuant created *protein groups* if the identified peptide set of one protein was equal to or contained in another protein's peptide set; see Experimental Methods, Database Search and Bioinformatics) in oocytes and ES cells, respectively, at a false discovery rate of 1% and with at least one unique and one additional peptide per protein. Mass errors of peptide identification show that identification was reliable (Supplementary Figure 1). The complete lists of peptide and protein identifications of oocytes and ES cells are available as Supporting Information (Supplementary Tables 1, 1b and 2, 2b). Corresponding mRNAs for 2842 of the 3699 protein groups were detected in the transcriptome of B6C3F1 metaphase II oocytes using an Agilent microarray. This data set contains transcripts of 16 157 genes and is available as Supporting Information (Supplementary Table 3). For the remaining 857 protein groups no corresponding RNA was detected; of these, 496 have a probe in the Agilent microarray, 236 do not have a probe, and 125 have no ENTREZ identifier.

To assess the quality of the oocyte's protein data set, we checked it for the presence of oocyte-specific proteins reported in the literature, as well as for the absence of cumulus cell-specific proteins. Of 68 proteins reported in oocytes,[18,19,32−38] 46 were

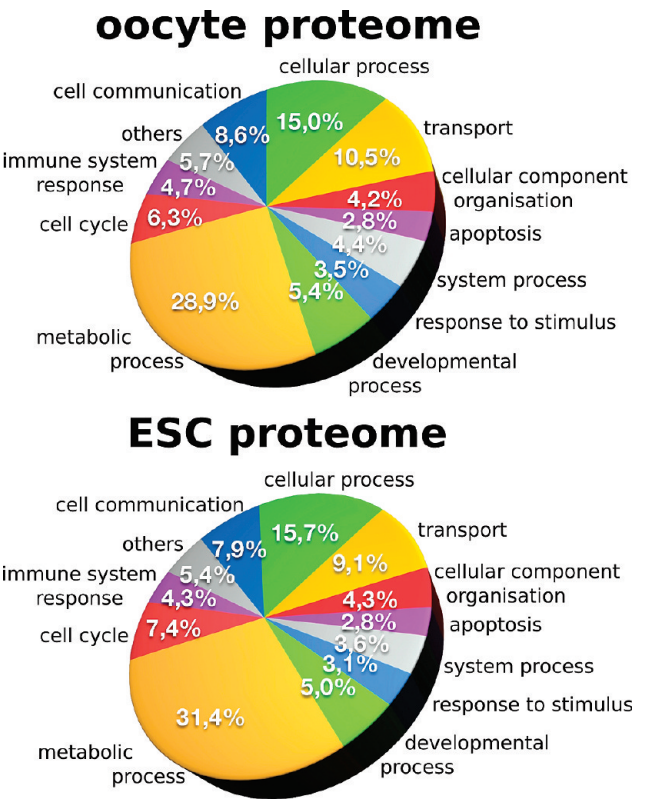


Figure 2. Pie charts describing proteins detected in oocytes (top) and in ES cells (bottom) and grouped according to the main GO biological process categories they belong to (a detailed GO analysis for biological process can be found in Supplementary Table 6, Supporting Information).

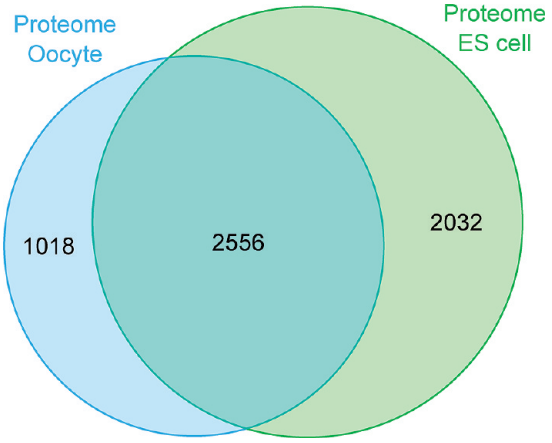


Figure 3. Venn representation of the overlap between oocyte proteome and ES cell proteome (raw data can be found in Supplementary Tables 1 and 2, Supporting Information).

detected in our proteome (Supplementary Table 4, Supporting Information). The transcription factors Oct4, Sox2, c-Myc and Klf4 were neither detected in our oocyte extracts nor in other oocyte studies[17,18] including the most recent one by Wang and colleagues[20] while we detected Oct4 and Sox2 in ES cell extracts (Supplementary Table 2, Supporting Information). Proteins considered specific for cumulus cells[39] (Supplementary Table 5, Supporting Information) were mostly undetected, indicating that oocyte processing for LC−MS was conducted properly.

dx.doi.org/10.1021/pr100706k |J. Proteome Res. XXXX, XXX, 000–000

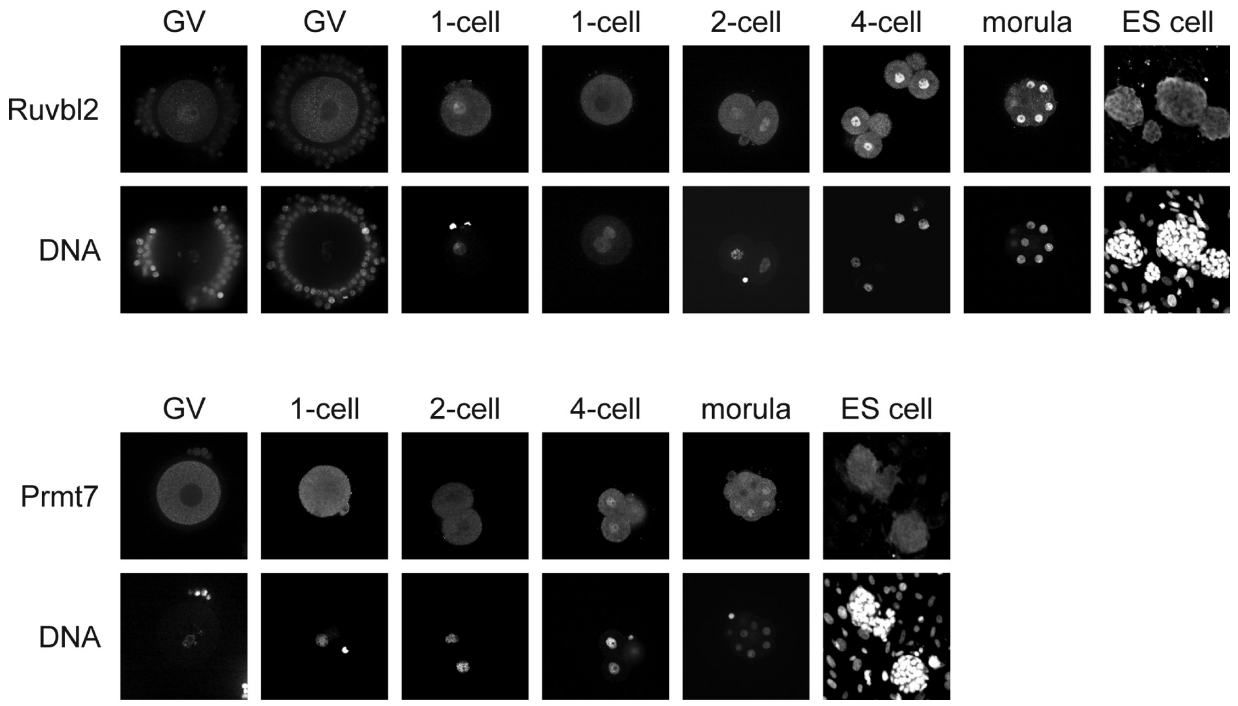


Figure 4. Confocal immunofluorescence images of Prmt7 and Ruvbl2 in mouse oocytes, embryonic cleavage stages, and ES cells. GV, germinal vesicle. Original magnification: 40×.

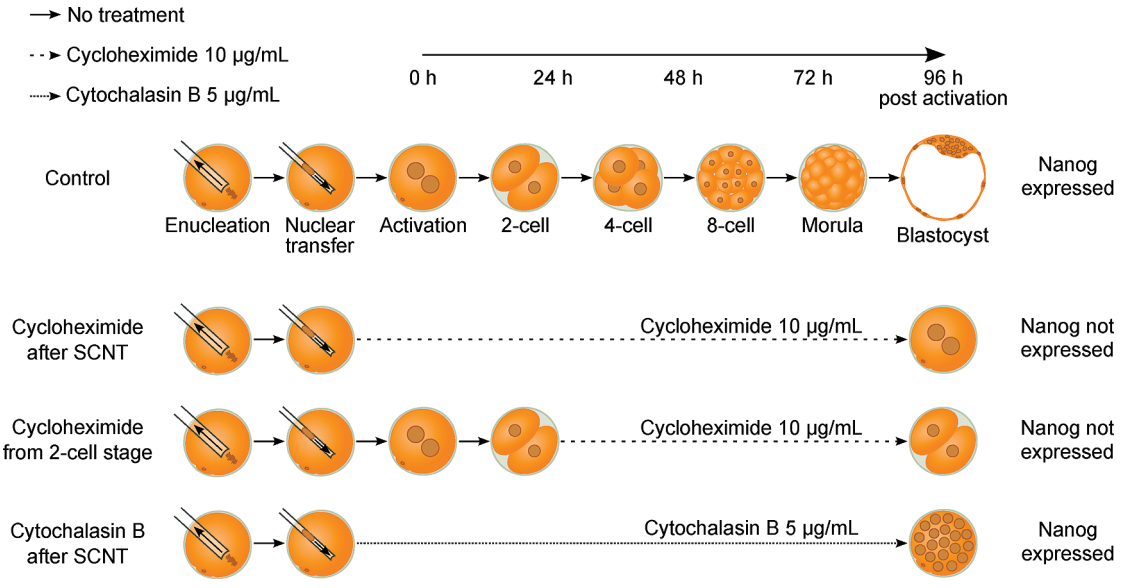


Figure 5. Streamline of the cycloheximide and cytochalasin B experiments.

However, a significant number of peptides belonging to the cumulus cell protein Pentraxin 3 were identified. This finding may be explained by a previously undetected expression of Pentraxin 3 in mouse oocytes or by translocation from cumulus cells via intercytoplasmic bridges.

Gene Ontology (GO) Analysis and Intersection of the Proteomes of Metaphase II Mouse Oocytes and Undifferentiated Mouse ES Cells

While the role of the four transcription factors Oct4, Sox2, c-Myc and Klf4 in iPS cell reprogramming is well documented, transcription factor-mediated reprogramming in iPS cells takes weeks.[2] By contrast, oocyte- as well as ES cell-mediated reprogramming takes as few as two cell cycles,[2] making it likely that oocytes and ES cells share additional (and probably active) reprogramming agents. Therefore we sought to compare the proteomes of mouse oocytes and mouse ES cells in order to find shared elements of their reprogramming feature. To this end, we filtered the two sets of proteomic data by the same criteria (see Experimental Methods, Database Search and Bioinformatics). As a result, 3574 protein IDs were retained from the initial 3699 identified in oocytes (−3.4%), and 4588 protein IDs were retained

dx.doi.org/10.1021/pr100706k |J. Proteome Res. XXXX, XXX, 000–000

Table 2. Raw Quantitative RT-PCR Results of the Cycloheximide (CHX) Experiments Provided as Ct Values[a]

	untreated Ct	CHX from 1-cell Ct	CHX from 2-cell Ct
SCNT embryos			
Nanog	29.2	38.3	38.6
Combined reference (*Gapdh* and *β*-Actin)	25.9	30.0	34.8
F embryos			
Nanog	26.6	39.8	37.9
Combined reference (*Gapdh* and *β*-Actin)	23.0	29.2	35.3

[a] Ct, threshold cycle in which the real-time PCR signal rises above the background; SCNT, somatic cell nuclear transfer; F, fertilized. All samples contained 10 embryos.

from 4723 identified in ES cells (−2.9%). We categorized the proteins of the filtered data sets in a similar way as the PANTHER classification system[40] by assigning them to the main GO terms of the biological process (BP) hierarchy (Figure 2). This overview suggests that if differences exist between the reprogramming machineries of mouse oocytes and ES cells, such differences may not be extensive after all. On this basis we analyzed the intersection of the oocyte and ES cell proteomes, exposing 2556 proteins that were detected in both oocytes and undifferentiated ES cells (Figure 3). This shared proteome was enriched for 197 GO BP terms (Supplementary Table 6, 6D, Supporting Information)

GO Filtering of the Shared Proteomes of Metaphase II Oocyte and Undifferentiated ES Cell Points to Candidate Reprogramming Factors

We screened the shared 2556 proteins for putative reprogramming factors. We considered that proteins with active reprogramming ability should be localized in the nucleus, have chromatin as substrate, and act catalytically, so they would not be consumed, since the oocyte has been proposed to hold enough reprogramming factors for up to 100 nuclei.[41] Therefore we filtered the shared proteins for the GO terms nucleus (cellular component), chromatin modification (biological process) and catalytic activity (molecular function). Of these 2556 proteins, 28 fulfill the filtering criteria: Baz1b, Brcc3, Carm1, Ccnb1, Chd4, Dnmt1, Dnmt3a, Eed, Ep400, Hat1, Hdac1, Hdac2, Hdac6, Hells, Kdm1a (Lsd1), Kdm6a (Utx), Mll3, Prmt1, Prmt5, Prmt7, Rnf2, Rnf20, Ruvbl1, Ruvbl2, Smarca4 (Brg1), Smarca5, Smarcal1, Usp16 (Table 1). Additional 45 proteins matching the three criteria were detected either in the oocyte proteome or in the ES cell proteome, but not in both (footnote to Table 1), therefore they were not pursued further. Coding mRNAs for all but one (Hdac2) of the 28 proteins were detected in our Agilent transcriptome (Supplementary Table 3, Supporting Information). We compared our list of 28 candidates with gene expression microarray of MEF cells undergoing reprogramming to iPS cells,[21] and found that 17 showed increasing levels (Table 1).

Searching the EMBL-EBI gene expression atlas[42] (http://www.ebi.ac.uk/gxa) we found that all of the 28 factors are expressed (mRNA) not just in oocytes and undifferentiated ES cells but also in a variety of other murine cells and tissues, particularly in the brain. It should be noted that also the four transcription factors Oct4, Sox2, c-Myc and Klf4, which induce pluripotency in somatic cells, are expressed in a variety of tissues including brain.[43,44] Reprogramming factors are therefore characteristic or even specific, but not exclusive for oocytes and ES cells. Next we selected two of the 28 proteins in order to confirm their actual presence and localization in mouse oocytes, early embryos and ES cells by in situ immunofluorescence. Because metaphase II oocytes lack a nuclear envelope, and therefore the nuclear localization feature of the candidates would be blind to our immunofluorescence assay, germinal vesicle (GV) stage oocytes were analyzed in place of metaphase II oocytes. We chose Ruvbl2 and Prmt7, which fall within the 30th percentile of the set of 4523 genes upregulated during mouse iPS cell formation,[21] and rank 10th and 12th, respectively, among our 28 factors; in an independent data set of genes upregulated during mouse iPS cell formation, they rank even higher (Keisuke Kaji, personal communication). The peptide sequences and MS/MS spectra of Ruvbl2, Prmt7 and also of Smarca4 are available as Supporting Information (Supplementary Figure 2, Supplementary Table 7). Immunofluorescence demonstrates that Ruvbl2 and Prmt7 are indeed present in GV oocytes, early embryos and ES cells but not in the fibroblasts used as feeders for ES cells, however they were not always exclusively localized in the nucleus (Figure 4). In particular, Ruvbl2 was detected either inside or outside of the nucleus at the GV and pronuclear (1-cell) stage.

Metaphase II Oocyte Proteome may not be Sufficient for Reprogramming

Although the 3699 oocyte proteins detected in this study expand substantially the catalog of proteins of mouse oocytes, it only accounts for a fraction of the oocyte transcriptome. Some of the oocyte proteins were not detected due to technical limitations associated with small sample amounts of oocytes, sample preparation (e.g., separation of the complex peptide mixture obtained from a whole cell extract), and insufficient sensitivity of LC−MS/MS measurements. However, as many mRNAs of the oocyte have been reported to indeed be translationally silent at the metaphase II stage,[45] failed detection of protein products of many mRNAs may be a genuine result. Certain maternal mRNAs are stockpiled in the ooplasm and are allocated for later translation during embryo cleavage.[46]

To explore if additional factors translated from maternal mRNAs upon oocyte activation are important for reprogramming, we designed an experiment in which the mRNA appearance of a key pluripotency-associated factor was tested in the absence of translation. Nucleus-transplanted oocytes were activated (i.e., chemically induced to enter the cell cycle; see Experimental Methods) and then treated with cycloheximide (CHX), an inhibitor of eukaryotic peptidyl-transferase, which is necessary for translation of mRNA into protein (Figure 5). Reprogramming was assessed by quantifying the mRNA level of the *Nanog* gene, whose product is essential for pluripotency while being absent in metaphase II oocytes.

Treatment with CHX from the time of SCNT until the chronological blastocyst stage, when the pluripotency marker gene *Nanog* is expressed in untreated embryos, allowed for at least partial reprogramming as shown by pronuclear formation, but the pluripotency gene *Nanog* was not induced as measured by

dx.doi.org/10.1021/pr100706k |J. Proteome Res. XXXX, XXX, 000–000

qRT-PCR (Table 2). Control fertilized embryos showed the same behavior as cloned embryos after treatment with CHX. Unlike the CHX treatment, administration of cytochalasin B (CYB; an inhibitor of actin polymerization and thereby cytokinesis) to 1-cell embryos until the chronological blastocyst stage allowed *Nanog* gene expression in both types of embryos, as measured by fluorescent Nanog-GFP; these CYB-treated embryos were arrested at the 1-cell stage and multinucleated because their cell cycle progressed in the nucleus while the cytoplasm could not divide. Our data indicate that *Nanog* gene induction was prevented in both cloned and fertilized embryos by the inhibition of mRNA translation.

DISCUSSION

Despite clear hints of the presence of active reprogramming factors in the oocyte, no definitive candidates have been found so far. Among proteins, transcription factors are currently considered the prime candidates.[47,48] Transcription factors have the ability to simultaneously interact with specific DNA sequences as well as with a defined set of proteins, thus guiding other proteins to these chromatin regions to exert their activities, a view that also found support in the recent study of Koche et al. who found that early reprogramming events largely depend on areas of preexisting, accessible chromatin.[49] Access to the substrate may therefore also be facilitated in particular phases of the cell cycle, such as the S-phase, owing to the open chromatin. However, we postulate that the mere presence of guiding factors and favorable conditions cannot account for the swift pace and unparalleled extent of reprogramming afforded by the oocyte. Indeed, Tani and colleagues, for example, showed that the reprogramming ability of bovine oocytes correlated with the presence of phosphorylated translationally controlled tumor protein 1 (Tpt1), a protein involved in the regulation of spindle function and anaphase progression during the cell cycle but lacking transcriptional function.[50,51] In line with the emerging role of nontranscription factor proteins in nuclear reprogramming, Singhal and colleagues showed that two enzymes, Smarca4 and Smarcc1, could account for the ability of certain ES cell protein fractions to enhance reprogramming in iPS derivation.[13]

Driven by the assumption that proteins featuring nuclear localization, chromatin remodeling and catalytic activity are among the main functional and active promoters of oocyte-mediated nuclear reprogramming, we resolved the proteome of metaphase II B6C3F1 mouse oocytes to an unprecedented depth of 3699 proteins. While there are oocytic proteins still undetected, our data set extends the catalogued mouse oocyte proteome from 185−2973 (refs 17−20) to 3699 proteins. Compared to other proteomic studies, our study has detected many proteins that were previously undetected, such as Nlrp5/Mater (not detected in refs 17,18) and Smarca4/Brg1, Zar1, Dppa3/Stella, Ctcf, Pms2 (not detected in ref 19), while the four iPS factors Oct4, Sox2, c-Myc and Klf4 remained undetected in our as well as in previous studies (refs 17−20). Notably, in our study 496 oocyte proteins were found that lacked corresponding mRNA in the transcriptome although the probes were present in the Agilent microarray.

There are several possible reasons why certain oocyte factors remain undetected even at a depth of 3699 proteins. Even though extremely high, the sensitivity of LC−MS/MS does not ensure detection of proteins of very low relative abundance. In ES cell lysates prepared according to the same protocol we could identify Sox2 and Oct4 with 4 and 11 different peptides, respectively. This indicates that these proteins are of higher relative abundance in ES cells than in oocytes, a fact that is easily explained by the higher nucleus/cytoplasm ratio of ES cells compared to oocytes. Moreover, a plethora of known and unknown post-translational modifications, such as phosphorylation and ubiquitylation, shift the molecular weight of a protein and its peptide fragments, and prevent their assignment. Additionally, inconsistencies of the genetic annotations may account for a certain number of false negatives.

Since oocytes and ES cells share the ability to swiftly reprogram a somatic nucleus to pluripotency after somatic cell nuclear transfer (SCNT) or cell fusion, respectively, we compared the two proteomes for differences and similarities. GO analysis for biological process reveals that the ES cell proteome features more transcription-related terms compared to the oocyte proteome (Supplementary Table 6, Supporting Information), although this finding may be biased in part by the very different nucleocytoplasmic ratios of oocytes and mouse ES cells. Pending a quantitative validation, our analysis is thus concordant with independent reports that GO terms associated with transcription are under-represented in the mouse oocyte proteome[18] and over-represented in the ES cell transcriptome.[52] While acknowledging these possible differences, we are more interested in the common features of oocyte- and ES cell-mediated reprogramming, because both cell types feature rapid reprogramming as opposed to the slow course of direct reprogramming enacted by the transcription factors Oct4, Sox2, c-Myc and Klf4 in iPS cells. The intersection of the oocyte and ES cell proteomes showed an overlap of 2556 proteins, which accounts for the majority of the proteins detected and is consistent with the similar distributions of the two protein sets along main GO biological process categories (Figures 2 and 3). This intersection exceeds by 1 order of magnitude the previously reported overlap of 256[17] and 371[18] proteins, and is almost as big as the whole oocyte proteome of Wang and colleagues.[20] Among the 2556 proteins that are shared by oocytes and ES cells, 28 feature the GO terms nucleus (cellular component), catalytic activity (molecular function), and chromatin modification (biological process) (see Table 1). The mRNA levels of 17 of the 28 factors (Brcc3, Ccnb1, Dnmt1, Dnmt3a, Eed, Hat1, Hdac2, Hdac6, Hells, Kdm1a, Prmt1, Prmt5, Prmt7, Rnf2, Ruvbl1, Ruvbl2, Smarcal1) were upregulated during the conversion of mouse fibroblasts to iPS cells.[21] In particular, mRNA levels of 4 of the 17 factors (Prmt1, Prmt5, Ruvbl1, Ruvbl2) were upregulated also during embryo cleavage as measured by microarray or RNA seq. analysis at the 8-cell stage[53] or 4-cell stage,[70] respectively. Comparison of our 28 candidate factors with the ES cell factors that were identified to be overrepresented as proteins in a reprogramming-competent nuclear fraction of ES cells versus MEFs by Singhal and colleagues[13] identified only 6 overlaps, namely Ccnb1, Chd4, Hdac1, Mll3, Kdm6a and Smarca4. Transcript levels of *Hdac1* and *Smarca4* peak around the 8-cell stage while *Ccnb1*, *Chd4*, *Mll3* and *Kdm6a* show an expression peak at the 1-cell stage.[53] These 10 factors (Ccnb1, Chd4, Hdac1, Mll3, Kdm6a, Prmt1, Prmt5, Ruvbl1, Ruvbl2, Smarca4), whose increased abundance is common to early embryos, iPS cells and the reprogrammingcompetent fraction of ES cells, are of particular interest as reprogramming is thought to predominantly take place within the first cell cycles after SCNT.

Among the 28 factors, we verified the presence of Prmt7 and Ruvbl2 protein in mouse oocytes, early embryos and undifferentiated ES cells. Prmt7 is a protein arginine methyltransferase that catalyzes monomethylation and symmetric dimethylation of arginine depending on substrate concentration.[54] Ruvbl2 is an

ATP-dependent DNA helicase, which is linked to DNA damage repair as well as the control of transcription.[55] Interestingly, overexpression of Ruvbl2 leads to increased cell proliferation in early *Xenopus* development,[56] whereas Ruvbl2 and Pontin52 act in an antagonistic manner in β-catenin signaling.[57] Immunofluorescence confirmed that Prmt7 and Ruvbl2 were indeed present in mouse GV oocytes, early embryos and undifferentiated ES cells, although they were not always exclusively nuclear; sometimes the signal was higher in the cytoplasm than in the nucleus (Figure 4). In fact, the GO annotation *nucleus* is not exclusive—it does not imply that the factor is present solely in the nucleus. We note that mRNAs encoding proteins potentially associated with reprogramming, that is, Prmt7 and Ruvbl2, were not found exclusively in oocytes, embryos and ES cells, but also in several tissues of the adult animal.[58] In fact, transcripts of the reprogramming transcription factors *Oct4*, *c-Myc*, *Klf4*[44] and *Sox2*[43] can also be found in unsuspected locations. This suggests that the function of the individual factors is not limited to reprogramming and that only a very specific combination of factors reprograms to pluripotency in the context of the oocyte.

Our list of 28 proteins includes well-established pluripotency-associated factors, such as the chromatin remodeling ATPase Smarca4 (Brg1). Depletion of Brg1 has been shown to inhibit the reprogramming capacity in *Xenopus* egg extracts[59] and to arrest development at the 2-cell stage in mice,[60] while its overexpression is associated with extended reprogramming activity.[59] Our microarray and proteomics data again identified Brg1 mRNA and protein in metaphase oocytes and our GO filtering criteria put the factor into the shortlist of putative reprogramming factors, thereby confirming the validity of the approach. In contrast, the transcription factors Oct4, Sox2, c-Myc and Klf4 were not detected, neither in our oocyte extracts nor in other oocyte studies,[17,18] including the most recent one by Wang and colleagues,[20] while Oct4 and Sox2 were detected in ES cell extracts. However, these transcription factors also would not fulfill two of the three stringent postulated search criteria for reprogramming factors, since they lack catalytic activity and chromatin modification function. Transcription factors, albeit present in the extracts only at low concentrations, are nevertheless not generally precluded from being detected in our LC−MS/MS approach, since the transcription factor Sall4 was detected, among others, in our oocyte extracts as both mRNA and protein. Sall4 is required for early embryonic development, is an integral part of the transcriptional network in ES cells and can enhance somatic cell reprogramming after fusion.[61,62] While transcription factors may well be underrepresented in oocyte proteomic studies to date simply due to the fact that these proteins are not present at sufficiently high enough relative concentrations in oocytes, it is worth noting that also immunoblot-based studies have led to contrasting results on the expression of Oct4 in oocytes; specifically, Liu and Keefe did not detect Oct4 in mouse oocytes,[63] whereas Palmieri and colleagues reported its presence.[64] We detected Oct4 in metaphase II mouse oocytes by immunoblotting (data not shown) and most recently also in pronuclei by immunofluorescence.[65] While we detected Oct4 in ES cells by LC−MS/MS, in oocytes the Oct4 protein may also carry a unique set of post-translational modification that hinder the reliable assignment of its peptide fragments.

Recently Egli and colleagues reported that not only metaphase II oocytes but also fertilized oocytes (zygotes) in M-phase can reprogram somatic nuclei.[66] Therefore we considered that the relevant factors may be present in the metaphase II oocyte only as mRNAs that are translated upon oocyte maturation or activation.[50,67,68] To test the hypothesis that reprogramming may require translation of specific maternal mRNAs, SCNT-derived mouse embryos were treated with the protein synthesis inhibitor cycloheximide (CHX) continuously from the 1- or 2-cell stage, followed by qRT-PCR analysis of gene expression at the chronological blastocyst stage. Bhutani and colleagues showed that reprogramming in heterokaryons between human fibroblasts and mouse ES cells takes place in the absence of cell division or DNA replication, as measured by expression of human *Oct4* and *Nanog* genes.[69] We chose Nanog as a reprogramming marker because this gene is expressed in pluripotent ES cells but not in somatic cells, and because Nanog mRNA and protein are absent in mature oocytes used as recipients for SCNT (unlike the products of *Oct4*, *Sox2* and *Klf4* (ref 70). After CHX treatment, *Nanog* mRNA was not detected ($Ct > 35$). On the contrary, SCNT-derived mouse embryos expressed Nanog-GFP when continuously exposed to cytochalasin B (CYB), which prevents cytokinesis but not cell cycling.[71] As expected, embryos treated with cytochalasin B contained multinucleated cytoplasms, indicating that cytokinesis but not cell cycling was inhibited. However, fertilized control embryos showed the same behavior as the cloned embryos upon treatment with CHX and CYB. While the failed activation of *Nanog* in fertilized oocytes suggests that our experimental treatment was not specific for the study of reprogramming, it may also indicate that metaphase II and fertilized oocytes share that part of the reprogramming machinery that is sensitive to CHX.

In summary, with the deepest mouse oocyte proteome to date and 28 candidate reprogramming factors, 17 of which showing upregulated transcript levels during mouse iPS cell formation,[21] we provide a basis to further explore and understand the mechanisms of active reprogramming achieved by the oocyte for the benefit of all reprogramming platforms. Future studies aimed at further characterizing the oocyte's reprogramming machinery will have to include the pronuclear stage, in which not only the reprogramming activities are segregated in the pronucleus but also the oocyte set of proteins has changed due to degradation and de novo translation in the context of an active cell cycle. The study of Wang et al.[20] is a step in the right direction, although we would prefer the parthenote to the zygote, so as to not introduce paternal factors in our study. We propose that at least some of the identified 28 factors could confer speed, effectiveness and specificity on the processes that take place during transcription factor-induced reprogramming, which otherwise relies on the window of opportunity offered by the cell cycle and on endogenous components that may be present in insufficient amounts or not at all in a given cell type. To test our proposal, we will have to see if oocytes can still support cloned embryo development when candidate maternal proteins have been depleted prior to SCNT (loss of function) or if iPS cells can be induced at higher rates when the oocyte proteins are coexpressed in precursor somatic cells along with the four factors Oct4, Sox2, c-Myc and Klf4 (gain of function).

■ ASSOCIATED CONTENT

Supporting Information

Supplemental Table 1. List of oocyte proteins identified by MaxQuant in combination with Mascot Server database search. Supplemental Table 1b. List of oocyte peptides identified by MaxQuant in combination with Mascot Server database search. Supplemental Table 2. List of ES cell proteins identified by MaxQuant in combination with Mascot Server database search. Supplemental Table 2b. List of ES cell peptides identified by

dx.doi.org/10.1021/pr100706k |J. Proteome Res. XXXX, XXX, 000–000

MaxQuant in combination with Mascot Server database search. Supplemental Table 3. Output list of transcripts of the Agilent microarray analysis software. Supplemental Table 4. Catalog of oocyte factors with relevant literature references. Supplemental Table 5. Purity of the oocyte proteome as inferred from presence/absence of cumulus cell-specific factors. Supplemental Table 6. The 20 most enriched GO BP terms in the whole and shared proteomes of oocytes and ES cells. The full lists of enriched GO BP terms are provided in daughter Tables 6B, 6C and 6D. Supplemental Table 7. Peptides differing in sequence that were identified for the proteins proposed to be reprogramming factors (Prmt7, Ruvbl2, Smarca4). Only peptides with a Mascot Score >25 are listed. MS/MS spectra for the top scoring peptides of each protein are provided in supplementary Figure 1 as representative examples. Supplemental Figure 1. Frequency distribution histograms of the mass errors, with which peptides were identified (A. Oocyte; B. ES cell). Supplemental Figure 2. MS/MS spectra for Prmt7, Ruvbl2 and Smarca4. Note the nearly complete Y-series of fragment ions for all three peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Michele Boiani: mboiani@mpi-muenster.mpg.de. Georg Fuellen: fuellen@alum.mit.edu.

Author Contributions

†These authors contributed equally to this work.

ACKNOWLEDGMENT

This study was supported by the special priority program (Schwerpunktprogramm) no. 1356 of the Deutsche Forschungsgemeinschaft (grants BO2540/3-1 and FU583/2-1) and by the Max Planck Society. We thank Oliver Sedelmeier for the immunofluorescence confocal images (Figure 4) and Amy Pavlak for editorial assistance with earlier versions of this manuscript.

REFERENCES

(1) Han, D. W.; Do, J. T.; Gentile, L.; Stehling, M.; Lee, H. T.; Scholer, H. R. Pluripotential reprogramming of the somatic genome in hybrid cells occurs with the first cell cycle. *Stem Cells* **2008**, *26*, 445–454.

(2) Hanna, J.; Saha, K.; Pando, B.; van Zon, J.; Lengner, C. J.; Creyghton, M. P.; van Oudenaarden, A.; Jaenisch, R. Direct cell reprogramming is a stochastic process amenable to acceleration. *Nature* **2009**, *462*, 595–601.

(3) Inoue, K.; Kohda, T.; Sugimoto, M.; Sado, T.; Ogonuki, N.; Matoba, S.; Shiura, H.; Ikeda, R.; Mochida, K.; Fujii, T.; Sawai, K.; Otte, A. P.; Tian, X. C.; Yang, X.; Ishino, F.; Abe, K.; Ogura, A. Impeding Xist expression from the active X chromosome improves mouse somatic cell nuclear transfer. *Science* **2010**, *330*, 496–499.

(4) Takahashi, K.; Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **2006**, *126*, 663–676.

(5) Som, A.; Harder, C.; Greber, B.; Siatkowski, M.; Paudel, Y.; Warsow, G.; Cap, C.; Scholer, H.; Fuellen, G. The PluriNetWork: an electronic representation of the network underlying pluripotency in mouse, and its applications. *PLoS ONE* **2010**, *5*, e15165.

(6) Heng, J.-C. D.; Feng, B.; Han, J.; Jiang, J.; Kraus, P.; Ng, J.-H.; Orlov, Y. L.; Huss, M.; Yang, L.; Lufkin, T.; Lim, B.; Ng, H.-H. The nuclear receptor Nr5a2 can replace Oct4 in the reprogramming of murine somatic cells to pluripotent cells. *Cell Stem Cell* **2010**, *6*, 167–174.

(7) Boiani, M.; Gentile, L.; Gambles, V. V.; Cavaleri, F.; Redi, C. A.; Scholer, H. R. Variable reprogramming of the pluripotent stem cell marker Oct4 in mouse clones: distinct developmental potentials in different culture environments. *Stem Cells* **2005**, *23*, 1089–1104.

(8) Kang, L.; Wang, J.; Zhang, Y.; Kou, Z.; Gao, S. iPS cells can support full-term development of tetraploid blastocyst-complemented embryos. *Cell Stem Cell* **2009**, *5*, 135–138.

(9) 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.; Zhou, Q. iPS cells produce viable mice through tetraploid complementation. *Nature* **2009**, *461*, 86–90.

(10) Ono, T.; Li, C.; Mizutani, E.; Terashita, Y.; Yamagata, K.; Wakayama, T. Inhibition of Class IIb Histone Deacetylase Significantly Improves Cloning Efficiency in Mice. *Biol. Reprod.* **2010**, *83*, 929–937.

(11) Boiani, M. Is the Perfect Mouse Cloning Experiment in Sight?. *Biol. Reprod.* **2010**, *83*, 887–889.

(12) Graumann, J.; Hubner, N. C.; Kim, J. B.; Ko, K.; Moser, M.; Kumar, C.; Cox, J.; Schöler, H.; Mann, M. Stable isotope labeling by amino acids in cell culture (SILAC) and proteome quantitation of mouse embryonic stem cells to a depth of 5,111 proteins. *Mol. Cell. Proteomics* **2008**, *7*, 672–683.

(13) Singhal, N.; Graumann, J.; Wu, G.; Araúzo-Bravo, M. J.; Han, D. W.; Greber, B.; Gentile, L.; Mann, M.; Schöler, H. R. Chromatin-Remodeling Components of the BAF Complex Facilitate Reprogramming. *Cell* **2010**, *141*, 943–955.

(14) Latham, K. E.; Garrels, J. I.; Chang, C.; Solter, D. Analysis of embryonic mouse development: construction of a high-resolution, two-dimensional gel protein database. *Appl. Theor. Electrophor.* **1992**, *2*, 163–170.

(15) Petzoldt, U.; Hoppe, P. C.; Illmensee, K. Protein synthesis in enucleated fertilized and unfertilized mouse eggs. *Dev. Genes Evol.* **1980**, *189*, 215–219.

(16) Novak, S.; Paradis, F.; Savard, C.; Tremblay, K.; Sirard, M.-A. Identification of porcine oocyte proteins that are associated with somatic cell nuclei after co-incubation. *Biol. Reprod.* **2004**, *71*, 1279–1289.

(17) Ma, M.; Guo, X.; Wang, F.; Zhao, C.; Liu, Z.; Shi, Z.; Wang, Y.; Zhang, P.; Zhang, K.; Wang, N.; Lin, M.; Zhou, Z.; Liu, J.; Li, Q.; Wang, L.; Huo, R.; Sha, J.; Zhou, Q. Protein expression profile of the mouse metaphase-II oocyte. *J. Proteome Res.* **2008**, *7*, 4821–4830.

(18) Zhang, P.; Ni, X.; Guo, Y.; Guo, X.; Wang, Y.; Zhou, Z.; Huo, R.; Sha, J. Proteomic-based identification of maternal proteins in mature mouse oocytes. *BMC Genomics* **2009**, *10*, 348.

(19) Yurttas, P.; Morency, E.; Coonrod, S. A. Use of proteomics to identify highly abundant maternal factors that drive the egg-to-embryo transition. *Reproduction* **2010**, *139*, 809–823.

(20) Wang, S.; Kou, Z.; Jing, Z.; Zhang, Y.; Guo, X.; Dong, M.; Wilmut, I.; Gao, S. Proteome of mouse oocytes at different developmental stages. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 17639–17644.

(21) Samavarchi-Tehrani, P.; Golipour, A.; David, L.; Sung, H. K.; Beyer, T. A.; Datti, A.; Woltjen, K.; Nagy, A.; Wrana, J. L. Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming. *Cell Stem Cell* **2010**, *7*, 64–77.

(22) Balbach, S. T.; Jauch, A.; Böhm-Steuer, B.; Cavaleri, F. M.; Han, Y.-M.; Boiani, M. Chromosome stability differs in cloned mouse embryos and derivative ES cells. *Dev. Biol.* **2007**, *308*, 309–321.

(23) Shevchenko, A.; Tomas, H.; Havlis, J.; Olsen, J. V.; Mann, M. In-gel digestion for mass spectrometric characterization of proteins and proteomes. *Nat. Protoc.* **2006**, *1*, 2856–2860.

(24) Rappsilber, J.; Ishihama, Y.; Mann, M. Stop and go extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. *Anal. Chem.* **2003**, *75*, 663–670.

(25) Cox, J.; Mann, M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol.* **2008**, *26*, 1367–1372.

(26) Gentleman, R. C.; Carey, V. J.; Bates, D. M.; Bolstad, B.; Dettling, M.; Dudoit, S.; Ellis, B.; Gautier, L.; Ge, Y.; Gentry, J.; Hornik, K.; Hothorn, T.; Huber, W.; Iacus, S.; Irizarry, R.; Leisch, F.; Li, C.; Maechler, M.; Rossini, A. J.; Sawitzki, G.; Smith, C.; Smyth, G.; Tierney, L.; Yang, J. Y.; Zhang, J. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.* **2004**, *5*, R80.

(27) R Development Core Team *R: A language and environment for statistical computing*; R Foundation for Statistical Computing: Vienna, Austria, 2009.

(28) Kersey, P. J.; Duarte, J.; Williams, A.; Karavidopoulou, Y.; Birney, E.; Apweiler, R. The International Protein Index: an integrated database for proteomics experiments. *Proteomics* **2004**, *4*, 1985–1988.

(29) Subramanian, A.; Tamayo, P.; Mootha, V. K.; Mukherjee, S.; Ebert, B. L.; Gillette, M. A.; Paulovich, A.; Pomeroy, S. L.; Golub, T. R.; Lander, E. S.; Mesirov, J. P. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 15545–15550.

(30) Falcon, S.; Gentleman, R. Using GOstats to test gene lists for GO term association. *Bioinformatics* **2007**, *23*, 257–258.

(31) Hahne, F.; Huber, W.; Gentleman, R.; Falcon, S. Gene Set Enrichment Analysis. In *Bioconductor case studies*; Springer: New York, 2008; p 198.

(32) Andreu-Vieyra, C.; Lin, Y. N.; Matzuk, M. M. Mining the oocyte transcriptome. *Trends Endocrinol. Metab.* **2006**, *17*, 136–143.

(33) Choi, Y.; Ballow, D. J.; Xin, Y.; Rajkovic, A. Lim homeobox gene, lhx8, is essential for mouse oocyte differentiation and survival. *Biol. Reprod.* **2008**, *79*, 442–449.

(34) Li, L.; Zheng, P.; Dean, J. Maternal control of early mouse development. *Development* **2010**, *137*, 859–870.

(35) Minami, N.; Suzuki, T.; Tsukamoto, S. Zygotic gene activation and maternal factors in mammals. *J. Reprod. Dev.* **2007**, *53*, 707–715.

(36) Oliveri, R. S.; Kalisz, M.; Schjerling, C. K.; Andersen, C. Y.; Borup, R.; Byskov, A. G. Evaluation in mammalian oocytes of gene transcripts linked to epigenetic reprogramming. *Reproduction* **2007**, *134*, 549–558.

(37) Surani, M. A.; Hayashi, K.; Hajkova, P. Genetic and epigenetic regulators of pluripotency. *Cell* **2007**, *128*, 747–762.

(38) Vallee, M.; Gravel, C.; Palin, M. F.; Reghenas, H.; Stothard, P.; Wishart, D. S.; Sirard, M. A. Identification of novel and known oocyte-specific genes using complementary DNA subtraction and microarray analysis in three different species. *Biol. Reprod.* **2005**, *73*, 63–71.

(39) Hernandez-Gonzalez, I.; Gonzalez-Robayna, I.; Shimada, M.; Wayne, C. M.; Ochsner, S. A.; White, L.; Richards, J. S. Gene expression profiles of cumulus cell oocyte complexes during ovulation reveal cumulus cells express neuronal and immune-related genes: does this expand their role in the ovulation process?. *Mol. Endocrinol.* **2006**, *20*, 1300–1321.

(40) Thomas, P. D.; Campbell, M. J.; Kejariwal, A.; Mi, H.; Karlak, B.; Daverman, R.; Diemer, K.; Muruganujan, A.; Narechania, A. PANTHER: a library of protein families and subfamilies indexed by function. *Genome Res.* **2003**, *13*, 2129–2141.

(41) Miyamoto, K.; Tsukiyama, T.; Yang, Y.; Li, N.; Minami, N.; Yamada, M.; Imai, H. Cell-free extracts from mammalian oocytes partially induce nuclear reprogramming in somatic cells. *Biol. Reprod.* **2009**, *80*, 935–943.

(42) Parkinson, H.; Kapushesky, M.; Kolesnikov, N.; Rustici, G.; Shojatalab, M.; Abeygunawardena, N.; Berube, H.; Dylag, M.; Emam, I.; Farne, A.; Holloway, E.; Lukk, M.; Malone, J.; Mani, R.; Pilicheva, E.; Rayner, T. F.; Rezwan, F.; Sharma, A.; Williams, E.; Bradley, X. Z.; Adamusiak, T.; Brandizi, M.; Burdett, T.; Coulson, R.; Krestyaninova, M.; Kurnosov, P.; Maguire, E.; Neogi, S. G.; Rocca-Serra, P.; Sansone, S. A.; Sklyar, N.; Zhao, M.; Sarkans, U.; Brazma, A. ArrayExpress update--from an archive of functional genomics experiments to the atlas of gene expression. *Nucleic Acids Res.* **2009**, *37*, D868–872.

(43) Rinn, J. L.; Rozowsky, J. S.; Laurenzi, I. J.; Petersen, P. H.; Zou, K.; Zhong, W.; Gerstein, M.; Snyder, M. Major molecular differences between mammalian sexes are involved in drug metabolism and renal function. *Dev. Cell* **2004**, *6*, 791–800.

(44) Zapala, M. A.; Hovatta, I.; Ellison, J. A.; Wodicka, L.; Del Rio, J. A.; Tennant, R.; Tynan, W.; Broide, R. S.; Helton, R.; Stoveken, B. S.; Winrow, C.; Lockhart, D. J.; Reilly, J. F.; Young, W. G.; Bloom, F. E.; Barlow, C. Adult mouse brain gene expression patterns bear an embryologic imprint. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 10357–10362.

(45) Bettegowda, A.; Smith, G. W. Mechanisms of maternal mRNA regulation: implications for mammalian early embryonic development. *Front. Biosci.* **2007**, *12*, 3713–3726.

(46) Wang, K.; Sun, F.; Sheng, H. Z. Regulated expression of TAF1 in 1-cell mouse embryos. *Zygote* **2006**, *14*, 209–215.

(47) Yamanaka, S. Pluripotency and nuclear reprogramming. *Philos. Trans. R. Soc. Lond., B: Biol. Sci.* **2008**, *363*, 2079–2087.

(48) Ralston, A.; Rossant, J. The genetics of induced pluripotency. *Reproduction* **2010**, *139*, 35–44.

(49) Koche, R. P.; Smith, Z. D.; Adli, M.; Gu, H.; Ku, M.; Gnirke, A.; Bernstein, B. E.; Meissner, A. Reprogramming factor expression initiates widespread targeted chromatin remodeling. *Cell Stem Cell* **2011**, *8*, 96–105.

(50) Tani, T.; Shimada, H.; Kato, Y.; Tsunoda, Y. Bovine oocytes with the potential to reprogram somatic cell nuclei have a unique 23-kDa protein, phosphorylated transcriptionally controlled tumor protein (TCTP). *Cloning Stem Cells* **2007**, *9*, 267–280.

(51) Yarm, F. R. Plk phosphorylation regulates the microtubule-stabilizing protein TCTP. *Mol. Cell. Biol.* **2002**, *22*, 6209–6221.

(52) Tang, F.; Barbacioru, C.; Bao, S.; Lee, C.; Nordman, E.; Wang, X.; Lao, K.; Surani, M. A. Tracing the derivation of embryonic stem cells from the inner cell mass by single-cell RNA-Seq analysis. *Cell Stem Cell* **2010**, *6*, 468–478.

(53) Zeng, F.; Baldwin, D. A.; Schultz, R. M. Transcript profiling during preimplantation mouse development. *Dev. Biol.* **2004**, *272*, 483–496.

(54) Lee, J. H.; Cook, J. R.; Yang, Z. H.; Mirochnitchenko, O.; Gunderson, S. I.; Felix, A. M.; Herth, N.; Hoffmann, R.; Pestka, S. PRMT7, a new protein arginine methyltransferase that synthesizes symmetric dimethylarginine. *J. Biol. Chem.* **2005**, *280*, 3656–3664.

(55) Gallant, P. Control of transcription by Pontin and Reptin. *Trends Cell Biol.* **2007**, *17*, 187–192.

(56) Etard, C.; Gradl, D.; Kunz, M.; Eilers, M.; Wedlich, D. Pontin and Reptin regulate cell proliferation in early Xenopus embryos in collaboration with c-Myc and Miz-1. *Mech. Dev.* **2005**, *122*, 545–556.

(57) Bauer, A.; Chauvet, S.; Huber, O.; Usseglio, F.; Rothbacher, U.; Aragnol, D.; Kemler, R.; Pradel, J. Pontin52 and reptin52 function as antagonistic regulators of beta-catenin signalling activity. *EMBO J.* **2000**, *19*, 6121–6130.

(58) van Arensbergen, J.; Garcia-Hurtado, J.; Moran, I.; Maestro, M. A.; Xu, X.; Van de Casteele, M.; Skoudy, A. L.; Palassini, M.; Heimberg, H.; Ferrer, J. Derepression of Polycomb targets during pancreatic organogenesis allows insulin-producing beta-cells to adopt a neural gene activity program. *Genome Res.* **2010**, *20*, 722–732.

(59) Hansis, C.; Barreto, G.; Maltry, N.; Niehrs, C. Nuclear reprogramming of human somatic cells by xenopus egg extract requires BRG1. *Curr. Biol.* **2004**, *14*, 1475–1480.

(60) Bultman, S. J.; Gebuhr, T. C.; Pan, H.; Svoboda, P.; Schultz, R. M.; Magnuson, T. Maternal BRG1 regulates zygotic genome activation in the mouse. *Genes Dev.* **2006**, *20*, 1744–1754.

(61) Lim, C. Y.; Tam, W.-L.; Zhang, J.; Ang, H. S.; Jia, H.; Lipovich, L.; Ng, H.-H.; Wei, C.-L.; Sung, W. K.; Robson, P.; Yang, H.; Lim, B. Sall4 regulates distinct transcription circuitries in different blastocyst-derived stem cell lineages. *Cell Stem Cell* **2008**, *3*, 543–554.

(62) Wong, C. C.; Gaspar-Maia, A.; Ramalho-Santos, M.; Reijo Pera, R. A. High-efficiency stem cell fusion-mediated assay reveals Sall4 as an enhancer of reprogramming. *PLoS ONE* **2008**, *3*, e1955.

(63) Liu, L.; Czerwiec, E.; Keefe, D. L. Effect of ploidy and parental genome composition on expression of Oct-4 protein in mouse embryos. *Gene Expr. Patterns* **2004**, *4*, 433–441.

(64) Palmieri, S. L.; Peter, W.; Hess, H.; Schöler, H. R. 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*, 259–267.

(65) Esteves, T. C.; Balbach, S. T.; Pfeiffer, M. J.; Arauzo-Bravo, M. J.; Klein, D. C.; Sinn, M.; Boiani, M., Somatic cell nuclear reprogramming of mouse oocytes endures beyond reproductive decline. *Aging Cell.* **2011**, *10*, 80−95.

(66) Egli, D.; Eggan, K. Recipient cell nuclear factors are required for reprogramming by nuclear transfer. *Development* **2010**, *137*, 1953–1963.

(67) Arumugam, K.; Wang, Y.; Hardy, L. L.; MacNicol, M. C.; MacNicol, A. M. Enforcing temporal control of maternal mRNA translation during oocyte cell-cycle progression. *EMBO J.* **2010**, *29*, 387–397.

(68) Huarte, J.; Belin, D.; Vassalli, A.; Strickland, S.; Vassalli, J. D. Meiotic maturation of mouse oocytes triggers the translation and polyadenylation of dormant tissue-type plasminogen activator mRNA. *Genes Dev.* **1987**, *1*, 1201–1211.

(69) Bhutani, N.; Brady, J. J.; Damian, M.; Sacco, A.; Corbel, S. Y.; Blau, H. M. Reprogramming towards pluripotency requires AID-dependent DNA demethylation. *Nature* **2010**, *463*, 1042–1047.

(70) Tang, F.; Barbacioru, C.; Wang, Y.; Nordman, E.; Lee, C.; Xu, N.; Wang, X.; Bodeau, J.; Tuch, B. B.; Siddiqui, A.; Lao, K.; Surani, M. A. mRNA-Seq whole-transcriptome analysis of a single cell. *Nat. Methods* **2009**, *6*, 377–382.

(71) Petzoldt, U. Regulation of stage-specific gene expression during early mouse development: effect of cytochalasin B and aphidicolin on stage-specific protein synthesis in mouse eggs. *Cell Differ.* **1984**, *15*, 163–167.

(72) Xiao, A.; Li, H.; Shechter, D.; Ahn, S. H.; Fabrizio, L. A.; Erdjument-Bromage, H.; Ishibe-Murakami, S.; Wang, B.; Tempst, P.; Hofmann, K.; Patel, D. J.; Elledge, S. J.; Allis, C. D. WSTF regulates the H2A.X DNA damage response via a novel tyrosine kinase activity. *Nature* **2009**, *457*, 57–62.

(73) Sobhian, B.; Shao, G.; Lilli, D. R.; Culhane, A. C.; Moreau, L. A.; Xia, B.; Livingston, D. M.; Greenberg, R. A. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. *Science* **2007**, *316*, 1198–1202.

(74) Miao, F.; Li, S.; Chavez, V.; Lanting, L.; Natarajan, R. Coactivator-associated arginine methyltransferase-1 enhances nuclear factor-kappaB-mediated gene transcription through methylation of histone H3 at arginine 17. *Mol. Endocrinol.* **2006**, *20*, 1562–1573.

(75) Marangos, P.; Carroll, J. The dynamics of cyclin B1 distribution during meiosis I in mouse oocytes. *Reproduction* **2004**, *128*, 153–162.

(76) Zhang, Y.; LeRoy, G.; Seelig, H. P.; Lane, W. S.; Reinberg, D. The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities. *Cell* **1998**, *95*, 279–289.

(77) Luo, J.; Su, F.; Chen, D.; Shiloh, A.; Gu, W. Deacetylation of p53 modulates its effect on cell growth and apoptosis. *Nature* **2000**, *408*, 377–381.

(78) Scimone, M. L.; Meisel, J.; Reddien, P. W. The Mi-2-like Smed-CHD4 gene is required for stem cell differentiation in the planarian Schmidtea mediterranea. *Development* **2010**, *137*, 1231–1241.

(79) Cirio, M. C.; Ratnam, S.; Ding, F.; Reinhart, B.; Navara, C.; Chaillet, J. R. Preimplantation expression of the somatic form of Dnmt1 suggests a role in the inheritance of genomic imprints. *BMC Dev. Biol.* **2008**, *8*, 9.

(80) Adams, A. M.; Pratt, S. L.; Stice, S. L. Knockdown of the Dnmt1s transcript using small interfering RNA in primary murine and bovine fibroblast cells. *Mol. Reprod. Dev.* **2005**, *72*, 311–319.

(81) Hata, K.; Okano, M.; Lei, H.; Li, E. Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. *Development* **2002**, *129*, 1983–1993.

(82) Li, J. Y.; Pu, M. T.; Hirasawa, R.; Li, B. Z.; Huang, Y. N.; Zeng, R.; Jing, N. H.; Chen, T.; Li, E.; Sasaki, H.; Xu, G. L. Synergistic function of DNA methyltransferases Dnmt3a and Dnmt3b in the methylation of Oct4 and Nanog. *Mol. Cell. Biol.* **2007**, *27*, 8748–8759.

(83) Bracken, A. P.; Pasini, D.; Capra, M.; Prosperini, E.; Colli, E.; Helin, K. EZH2 is downstream of the pRB-E2F pathway, essential for proliferation and amplified in cancer. *EMBO J.* **2003**, *22*, 5323–5335.

(84) Cao, R.; Wang, H.; He, J.; Erdjument-Bromage, H.; Tempst, P.; Zhang, Y. Role of hPHF1 in H3K27 methylation and Hox gene silencing. *Mol. Cell. Biol.* **2008**, *28*, 1862–1872.

(85) Tie, F.; Furuyama, T.; Prasad-Sinha, J.; Jane, E.; Harte, P. J. The Drosophila Polycomb Group proteins ESC and E(Z) are present in a complex containing the histone-binding protein p55 and the histone deacetylase RPD3. *Development* **2001**, *128*, 275–286.

(86) 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*, 1680–1689.

(87) Fazzio, T. G.; Huff, J. T.; Panning, B. An RNAi screen of chromatin proteins identifies Tip60-p400 as a regulator of embryonic stem cell identity. *Cell* **2008**, *134*, 162–174.

(88) Parthun, M. R. Hat1: the emerging cellular roles of a type B histone acetyltransferase. *Oncogene* **2007**, *26*, 5319–5328.

(89) Qin, S.; Parthun, M. R. Recruitment of the type B histone acetyltransferase Hat1p to chromatin is linked to DNA double-strand breaks. *Mol. Cell. Biol.* **2006**, *26*, 3649–3658.

(90) Bui, H.-T.; Wakayama, S.; Kishigami, S.; Park, K.-K.; Kim, J.-H.; Van Thuan, N.; Wakayama, T. Effect of Trichostatin A on Chromatin Remodeling, Histone Modifications, DNA Replication, and Transcriptional Activity in Cloned Mouse Embryos. *Biol. Reprod.* **2010**, *83*, 454–463.

(91) Kaichi, S.; Hasegawa, K.; Takaya, T.; Yokoo, N.; Mima, T.; Kawamura, T.; Morimoto, T.; Ono, K.; Baba, S.; Doi, H.; Yamanaka, S.; Nakahata, T.; Heike, T. Cell line-dependent differentiation of induced pluripotent stem cells into cardiomyocytes in mice. *Cardiovasc. Res.* **2010**, *88*, 314–323.

(92) Boyault, C.; Sadoul, K.; Pabion, M.; Khochbin, S. HDAC6, at the crossroads between cytoskeleton and cell signaling by acetylation and ubiquitination. *Oncogene* **2007**, *26*, 5468–5476.

(93) Eisen, J. A.; Sweder, K. S.; Hanawalt, P. C. Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Res.* **1995**, *23*, 2715–2723.

(94) Muegge, K. Lsh, a guardian of heterochromatin at repeat elements. *Biochem. Cell Biol.* **2005**, *83*, 548–554.

(95) Forneris, F.; Battaglioli, E.; Mattevi, A.; Binda, C. New roles of flavoproteins in molecular cell biology: histone demethylase LSD1 and chromatin. *FEBS J.* **2009**, *276*, 4304–4312.

(96) Swigut, T.; Wysocka, J. H3K27 demethylases, at long last. *Cell* **2007**, *131*, 29–32.

(97) Kim, D. H.; Lee, J.; Lee, B.; Lee, J. W. ASCOM controls farnesoid X receptor transactivation through its associated histone H3 lysine 4 methyltransferase activity. *Mol. Endocrinol.* **2009**, *23*, 1556–1562.

(98) Ansari, K. I.; Kasiri, S.; Hussain, I.; Mandal, S. S. Mixed lineage leukemia histone methylases play critical roles in estrogen-mediated regulation of HOXC13. *FEBS J.* **2009**, *276*, 7400–7411.

(99) Pawlak, M. R.; Banik-Maiti, S.; Pietenpol, J. A.; Ruley, H. E. Protein arginine methyltransferase I: substrate specificity and role in hnRNP assembly. *J. Cell. Biochem.* **2002**, *87*, 394–407.

(100) Weber, S.; Maass, F.; Schuemann, M.; Krause, E.; Suske, G.; Bauer, U. M. PRMT1-mediated arginine methylation of PIAS1 regulates STAT1 signaling. *Genes Dev.* **2009**, *23*, 118–132.

(101) Wysocka, J.; Allis, C. D.; Coonrod, S. Histone arginine methylation and its dynamic regulation. *Front. Biosci.* **2006**, *11*, 344–355.

(102) Lee, J. H.; Skalnik, D. G. CpG-binding protein (CXXC finger protein 1) is a component of the mammalian Set1 histone H3-Lys4 methyltransferase complex, the analogue of the yeast Set1/COMPASS complex. *J. Biol. Chem.* **2005**, *280*, 41725–41731.

(103) Jelinic, P.; Stehle, J. C.; Shaw, P. The testis-specific factor CTCFL cooperates with the protein methyltransferase PRMT7 in H19 imprinting control region methylation. *PLoS Biol.* **2006**, *4*, 355.

(104) Vidal, M. Role of polycomb proteins Ring1A and Ring1B in the epigenetic regulation of gene expression. *Int. J. Dev. Biol.* **2009**, *53*, 355–370.

(105) Zhu, B.; Zheng, Y.; Pham, A. D.; Mandal, S. S.; Erdjument-Bromage, H.; Tempst, P.; Reinberg, D. Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. *Mol. Cell* **2005**, *20*, 601–611.

(106) Jha, S.; Dutta, A. RVB1/RVB2: running rings around molecular biology. *Mol. Cell* **2009**, *34*, 521–533.

(107) Kidder, B. L.; Palmer, S.; Knott, J. G. SWI/SNF-Brg1 regulates self-renewal and occupies core pluripotency-related genes in embryonic stem cells. *Stem Cells* **2009**, *27*, 317–328.

(108) Santoro, R.; Li, J.; Grummt, I. The nucleolar remodeling complex NoRC mediates heterochromatin formation and silencing of ribosomal gene transcription. *Nat. Genet.* **2002**, *32*, 393–396.

(109) Stopka, T.; Skoultchi, A. I. The ISWI ATPase Snf2h is required for early mouse development. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 14097–14102.

(110) Postow, L.; Woo, E. M.; Chait, B. T.; Funabiki, H. Identification of SMARCAL1 as a component of the DNA damage response. *J. Biol. Chem.* **2009**, *284*, 35951–35961.

(111) Joo, H. Y.; Zhai, L.; Yang, C.; Nie, S.; Erdjument-Bromage, H.; Tempst, P.; Chang, C.; Wang, H. Regulation of cell cycle progression and gene expression by H2A deubiquitination. *Nature* **2007**, *449*, 1068–1072.

(112) Shanbhag, N. M.; Rafalska-Metcalf, I. U.; Balane-Bolivar, C.; Janicki, S. M.; Greenberg, R. A. ATM-dependent chromatin changes silence transcription in cis to DNA double-strand breaks. *Cell* **2010**, *141*, 970–981.