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Co-regulation of pluripotency and genetic integrity at the genomic level

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Abstract The Disposable Soma Theory holds that genetic integrity will be maintained at more pristine levels in germ cells than in somatic cells because of the unique role germ cells play in perpetuating the species. We tested the hypothesis that the same concept applies to pluripotent cells compared to differentiated cells. Analyses of transcriptome and cistrome databases, along with canonical pathway analysis and chromatin immunoprecipitation confirmed differential expression of DNA repair and cell death genes in embryonic stem cells and induced pluripotent stem cells relative to fibroblasts, and predicted extensive direct and indirect interactions between the pluripotency and genetic integrity gene networks in pluripotent cells. These data suggest that enhanced maintenance of genetic integrity is fundamentally linked to the epigenetic state of pluripotency at the genomic level. In addition, these findings demonstrate how a small number of key pluripotency factors can regulate large numbers of downstream genes in a pathway-specific manner.
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Introduction

The "Disposable Soma Theory," first described in 1977 by Kirkwood (1977) implies that because germ cells are responsible for conveying genetic information between generations, and, in so doing, maintaining the species, it is evolutionarily advantageous for these cells to expend additional energy to maintain the integrity of their genomes at more pristine levels than those in somatic cells. This theory has since been validated by a variety of studies demonstrating that germ cells maintain lower frequencies of mutations (Murphey et al., 2013; Russell et al., 1979; Russell, 2004; Walter et al., 1998), and express elevated levels of DNA repair and/or cell death activities relative to somatic cells (Coucouvanis et al., 1993; Huamani et al., 2004; Intano et al., 2001, 2002; Xu et al., 2005, 2008).

A similar argument can be posited for early embryonic cells, which give rise to entire new individuals. Available data support this contention, although this data is limited because of the difficulty in recovering sufficient numbers of pluripotent cells from early embryos to facilitate direct analyses of mutation frequencies (Murphey et al., 2009, 2013; Russell et al., 1979; Russell, 2004; Walter et al., 1998). Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) provide a surrogate for early embryonic cells, and have the advantage that they can be expanded in culture while maintaining their pluripotent status (Bradley et al., 2012; Coucouvanis et al., 1993; Huamani et al., 2004;

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Intano et al., 2001, 2002; Thomson, 1998; Xu et al., 2005, 2008).

Similar to germ cells, ESCs have been shown to carry a lower load of point mutations than that detected in differentiated cells (Cervantes et al., 2002; Momcilović et al., 2010). This lower load of point mutations during expansion in culture is likely due to increased activity of DNA repair mechanisms, which prospectively mitigate these mutations, as additional studies have shown that expression of DNA repair and cell death genes is generally elevated in pluripotent stem cells relative to differentiated cell types (Borgdorff et al., 2006; Duval et al., 2005; Fan et al., 2011; Maynard et al., 2008; Nouspikel and Hanawalt, 2000; Tichy, 2011; Tichy and Stambrook, 2008). Several of the same DNA repair pathways are reported to be elevated in germ cells and pluripotent stem cells, including base excision and mismatch repair (Intano et al., 2001; Kirkwood, 1977; Tichy et al., 2011; Tichy and Stambrook, 2008; Tomé et al., 2013), nucleotide excision repair (de Waard et al., 2008; Russell et al., 1998, 2007; Tichy and Stambrook, 2008; Van Sloun et al., 1999; Walter et al., 1998), UV and gamma radiation-induced damage repair (Momcilović et al., 2009; Van Sloun et al., 1999; Xu et al., 2008, 2012), homologous recombination and non-homologous end joining repair (Adams et al., 2010a,2010b; Fan et al., 2011; Momcilović et al., 2010; Tomé et al., 2013). Elevated cell death activity has also been observed in ESCs, particularly that related to activity of p53 (Li et al., 2012; Momcilović et al., 2011; Qin et al., 2007; Roos et al., 2007). This is likely to retroactively, rather than prospectively, mitigate the higher incidence of large scale aberrations observed in ESCs (Ben-David and Benvenisty, 2012; Ben-David et al., 2011; Draper et al., 2003; Liu et al., 2013).

Germ and somatic cells, or pluripotent and differentiated cells can be distinguished on the basis of their epigenetic states (Boland et al., 2009; Okita et al., 2007; Takahashi and Yamanaka, 2006; Yamanaka, 2012). We previously demonstrated that epigenetic reprogramming following somatic cell nuclear transfer results in enhanced genetic integrity in early embryonic cells (Murphey et al., 2009), confirming that maintenance of enhanced genetic integrity in these cells is regulated, at least in part, by epigenetic mechanisms.

Taken together, these observations suggest that enhanced genetic integrity is maintained in pluripotent cells via elevated expression of DNA repair and/or cell death genes coordinated by at least some of the same mechanisms that regulate pluripotency. To test this hypothesis, we performed a meta-analysis of transcriptome databases describing gene expression patterns in mouse and human ESCs and iPSCs compared to those in differentiated somatic cells (fibroblasts). We then examined cistrome databases describing direct or indirect regulation of these differentially expressed genetic integrity genes by pluripotency factors, or by other transcription factors which are, themselves, regulated by pluripotency factors. Our data confirm extensive differential expression of genetic integrity genes in pluripotent cell types relative to differentiated cells (fibroblasts), and predict comprehensive interactions between the pluripotency and genetic integrity gene networks that mechanistically link these functions at the genomic level.

Materials & methods

Transcriptome data mining

Data acquisition from GEO

Fig. 1 depicts a flow chart of data analysis procedures used throughout this study. The Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) was used to access raw gene expression data for human and mouse ESCs, iPSCs, and fibroblasts (human dermal fibroblasts [HDFs] for comparison with human ESCs and iPSCs, and mouse embryonic fibroblasts [MEFs] for comparison with mouse ESCs and iPSCs, respectively) including mouse transcriptome datasets from GSE15267 (Chen et al., 2010), GSE13190 (Feng et al., 2009), GSE19023 (Heng et al., 2010), GSE18286 (Ichida et al., 2009), GSE17004 (Kang et al., 2009), GSE7815 (Maherali et al., 2007), GSE7841 (Okita et al., 2007), GSE14012 (Sridharan et al., 2009), GSE5259 (Takahashi and Yamanaka, 2006), and GSE16925 (Zhao et al., 2009), all of which were derived from a minimum of two biological replicates. Human transcriptome datasets without replicates were from GSE12583 (Aasen et al., 2008), GSE16654 (Chin et al., 2009), GSE9832 (Park et al., 2008), GSE14711 (Soldner et al., 2009), and GSE9561 (Takahashi et al., 2007). Human transcriptome datasets with biological replicates used in this study were those from GSE25970 (Bock et al., 2011), the super series of GSE26451 and GSE26453 (Munoz et al., 2011), GSE13828 (Ebert et al., 2008), GSE9865 (Lowry et al., 2008), GSE12390 (Maherali et al., 2008), GSE14982 (Sun et al., 2009), and GSE15148 (Yu et al., 2009).

GenSpring GX data processing

Transcriptome data from GEO were imported into GeneSpring GX 12.0 software and normalized individually using default/recommended methods as described (Roy Choudhury et al., 2010). Probe signals for cell replicates were averaged within the software. Probes with signal intensities less than 50.0 arbitrary fluorescence units were excluded. A one-way ANOVA with a corrected p-value cutoff of 0.05 using a Benjamini and Hochberg false discovery rate multiple testing correction and Tukey's honestly significant difference test were used to identify probes with significant differential expression (Roy Choudhury et al., 2010). Gene expression differences were validated by statistical significance, and designated as differentially expressed when the fold-change was $\geq 1.5\times$.

Probe conversion and data mining

Genetic integrity and transcription factor gene lists were obtained from AmiGO (http://www.geneontology.org/) using the primary gene ontology annotation terms GO:0006281 (repair), GO:0008219 (cell death), and GO:0005667 (transcription factors). The function of each individual gene was confirmed by independent literature analysis. Gene symbols were imported into the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (Huang et al., 2008) for conversion to array probe IDs and Entrez IDs. Probe IDs were then imported into Mathematica (Wolfram Research, http://www.wolfram.com/) and matches to converted gene lists were extracted from normalized dataset files. For Entrez IDs matched to multiple probes, average fold changes were calculated in Microsoft Excel.

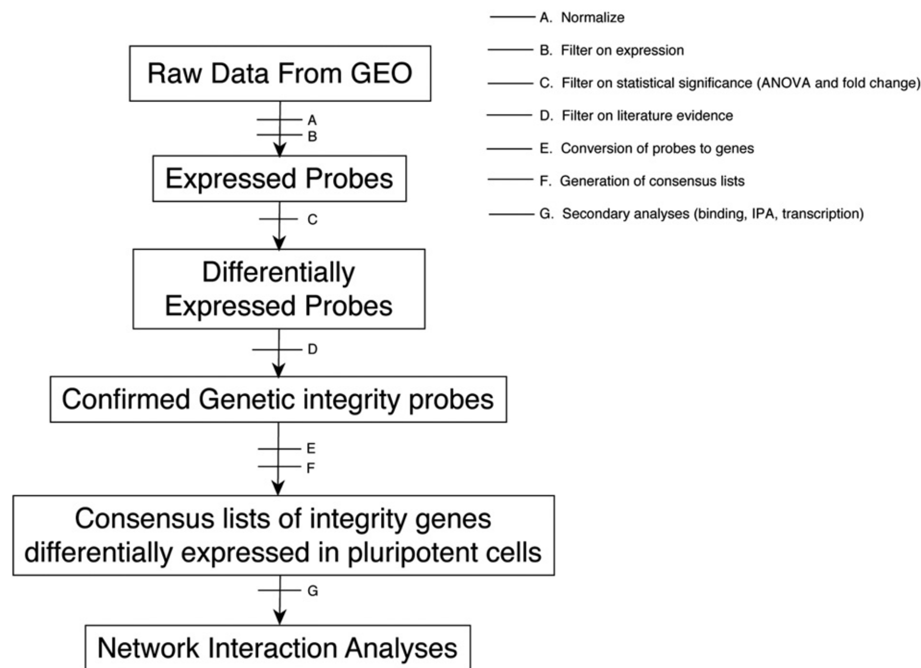


Figure 1 Pipeline for bioinformatic analysis of gene expression arrays based on filters applied. Filter A = normalization of raw data within GeneSpring (Agilent) by Robust Multi-Array analysis (RMA); filter B = identification of probes expressed with a raw intensity of ≥50 units; filter C = two-way ANOVA with Benjamini and Hochberg correction and minimum 1.5 fold change cutoff to identify differentially expressed probes in pairwise comparisons; filter D = literature-based confirmation of probes related to DNA repair and/or cell death; filter E = conversion of probes to Entrez IDs using the Database for Annotation, Visualization, and Integrated Discovery (DAVID); filter F = identification of genes differentially expressed in ≥50% of examined datasets; filter G = secondary analyses of consensus lists, including analysis with ingenuity pathway analysis (IPA) software, factor binding analysis, and matching to additional datasets.

Consensus list generation

A consensus list of genes differentially expressed in each pairwise comparison of cell types was made up of those IDs found to be differentially expressed in ≥50% of datasets examined (Supplementary Table 1).

Pathway and function analysis of differentially expressed genes

Ingenuity pathway analysis (IPA; ingenuity systems) software

Entrez IDs and average fold change data were imported into IPA software. DNA repair gene expression data was analyzed for mismatch repair, nucleotide excision repair, and double strand break repair by homologous recombination and non-homologous end joining pathways, and cell death gene expression data was analyzed for apoptosis, anoikis, and autophagy. Three data points were derived for each analysis: 1) a p-value describing the relationship of each gene list to each pathway, 2) a ratio of differentially expressed genes (from Supplementary Table 1) to total genes within each pathway, and 3) a z-score representing overall up- or down-regulation of each individual pathway.

Analysis of interactions between pluripotency and genetic integrity gene networks

First-degree interactions

Lists of genetic integrity genes directly bound by the pluripotency factors SOX2, OCT4, and/or NANOG in both human (Boyer et al., 2005) and mouse (Chen et al., 2008; Kim et al., 2008; Mathur et al., 2008; Sridharan et al., 2009) pluripotent cell types were accessed and subjected to the same list matching and extraction procedures in Mathematica as those described above for use in mining transcriptome data. For human ESCs and iPSCs, the lists of bound genetic integrity genes were derived from the single available dataset (Boyer et al., 2005), whereas for mouse ESCs and iPSCs, the lists represent those genes reported to be bound by one or more of the three pluripotency factors in two or more of the four cistrome datasets examined. Direct interactions between target genetic integrity genes or gene products and one or more of the three pluripotency factors examined were termed "first-degree interactions."

Second-degree interactions

Human and mouse ESC and iPSC transcriptome databases were mined in the same way we analyzed differential expression of genetic integrity genes as described above. Cistrome databases for these same cell types were mined to identify transcription factor genes or gene products that directly interact with one or more pluripotency factors (OCT4, SOX2, or NANOG) and were differentially expressed between pluripotent and differentiated cell types (Supplementary Table 1). The pathway builder functionalities within IPA were used to identify which of the genetic integrity genes or gene products that are differentially expressed in human or mouse pluripotent cells are regulated by one or more transcription factors encoded by genes that are also a) differentially expressed in these cell types, and b) regulated

by one or more of the three pluripotency factors investigated. These were termed "second-degree interactions" between the pluripotency and genetic integrity gene networks.

Pluripotency factor knockdown functional analyses

Transcriptome datasets from mouse ESCs following shRNA knockdowns of the pluripotency factors SOX2, OCT4, or NANOG (Ivanova et al., 2006) were mined for differential expression of genetic integrity genes as a function of reduced expression of each pluripotency factor. The transcriptome data were originally published as individual datasets describing the effects of a knockdown of each individual pluripotency factor. These three individual datasets were combined into a single, comprehensive set which was then mined for effects on expression of the genetic integrity genes that we found to be a) differentially expressed in pluripotent cells compared to differentiated cells and b) regulated directly (first-degree interaction) by the pluripotency gene network.

Experimental validation of computationally predicted interactions

Chromatin immunoprecipitation (ChIP) assays were used to validate exemplary predicted protein–DNA interactions. ESCs from C57Bl6/J mice were propagated on γ-irradiated CF-1 MEFs (Global Stem, Rockville, MD) in media consisting of 82% DMEM (Life Technologies, Carlsbad, CA), 7% heat inactivated FBS (Life Technologies), 7% ES Cult FBS (Stem Cell Technologies, Vancouver, BC), 1% penn–strep, 1% NEAA, and 1% Glutamax (all from Life Technologies), plus 0.0006% β-mercaptoethanol (Sigma-Aldrich), and 3 ng/mL leukemia inhibitory factor (Stem Cell Technologies) in six-well culture plates (Corning, Corning, NY) treated with 0.01% gelatin (Life Technologies). Cells were isolated with 0.25% trypsin (Life Technologies) and purified by MACS using SSEA-1 antibody microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) on an autoMACS Pro Separator (Miltenyi Biotec) according to the manufacturer's instructions.

ChIP was performed on purified pluripotent cells using a combination of two kits (Active Motif, Carlsbad, CA and AbCam, Cambridge, England) following the manufacturer's instructions. Briefly, cells were cross-linked with 1% formaldehyde, lysed with sodium dodecyl sulfate [1% SDS, 50 mM Tris–HCl (pH 8.1)] and 10 nM EDTA (pH 8.0) containing protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) and the DNA was sheared using a Branson Digital Sonifier as described (Yoshioka et al., 2007). Chromatin was immunoprecipitated using antibodies against OCT4, SOX2, NANOG (AbCam), or phosphorylated (ser-15) p53 (Cell Signalling Technology, Beverly, MA). Antibodies against rabbit IgG (AbCam) and histone H3 were used as negative and positive binding controls, respectively.

qPCR was carried out using SYBR premix EX Taq (Takara Bio, Otsu, Shiga, Japan) and a Chromo4 Real-Time System (Bio-Rad, Berkeley, CA) with primers listed in Supplementary Table 2. Fold enrichment calculations were based on comparisons of bound fractions to IgG isotype controls from at least three independent ChIP experiments and three qPCR experiments, with the value for IgG arbitrarily set to 1.0. Three biological replicates, each with three technical replicates were performed for ChIP–qPCR reactions.

Promoter sequences (2 kb upstream of the transcriptional start site) for ten genes were analyzed using TransFac software (http://www.gene-regulation.com/pub/databases.html) to identify putative binding sites for SOX2, NANOG, OCT4, and/or p53. Primers were designed using NCBI Primer Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) to amplify fragments spanning these putative binding sites (Supplementary Table 2).

Results

Genetic integrity genes are differentially expressed in pluripotent cells relative to differentiated cells

Our analysis of human and mouse cell gene expression datasets is shown in Fig. 2 and Supplementary Fig. 1. We found substantial differential expression of both DNA repair genes and cell death genes in both human and mouse pluripotent cells compared to differentiated cells

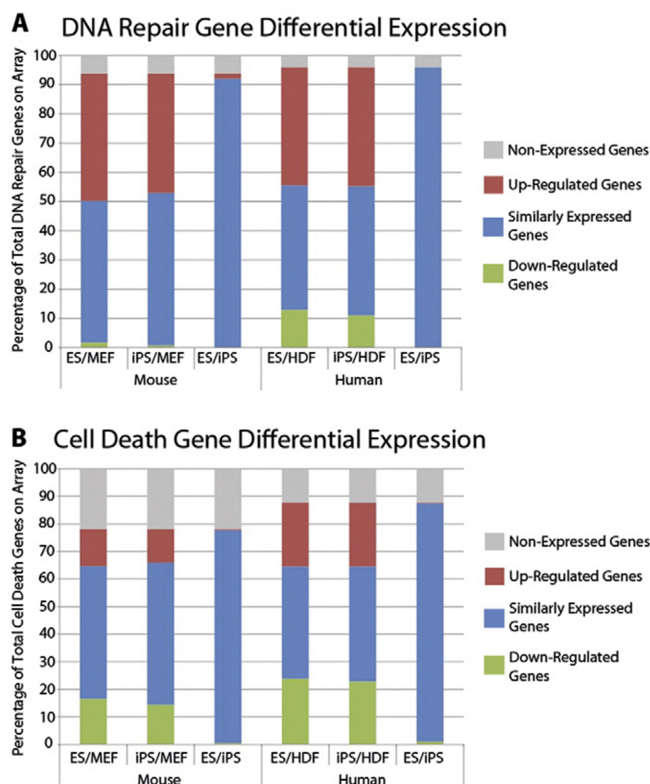
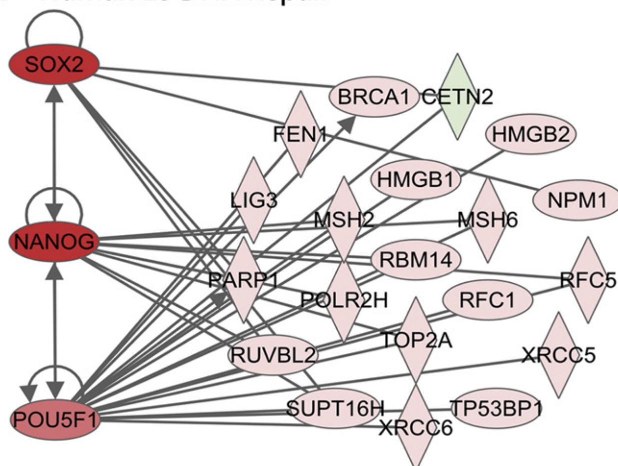


Figure 2 Differential expression of genetic integrity genes in mouse and human pluripotent cells. (A), DNA repair gene expression and (B), cell death gene expression. Percentages of genes down-regulated (green), unchanged (blue), up-regulated (red) or not expressed (gray) are shown for each pairwise cell type comparison (ES cells vs fibroblasts, iPS cells vs fibroblasts, and ES cells vs iPS cells) in each species (mouse, human).



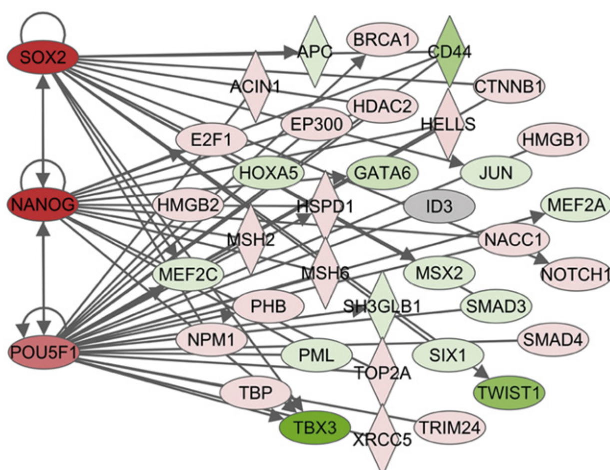


Figure 3 First-degree interactions between pluripotency and genetic integrity gene networks in human ES cells. Direct interactions between pluripotency factors and the DNA repair or cell death gene network in human ES cells are shown. (A), DNA repair-pluripotency network interactions and (B), cell death-pluripotency network interactions. Red coloring indicates up-regulation in the pluripotent cell type compared to fibroblast controls and green indicates down-regulation (darker colors indicate greater expression differentials). Shapes differentiate molecule types: ovals = transcription factors, diamonds = enzymes.

(fibroblasts) in each species (Figs. 2A, B). Expression of >90% of the DNA repair genes and ≥80% of the cell death genes represented on the human and mouse microarrays examined was detected in each pluripotent cell type. We grouped expressed genes into three categories — 1) up-regulated (≥1.5× and significantly different by ANOVA), 2) no difference (i.e. <1.5× different), or 3) down-regulated (≥1.5× and significantly different by ANOVA) in pluripotent cells compared to differentiated cells. DNA repair gene expression showed overall up- or similar regulation in pluripotent cells relative to differentiated cells, with 41–43% genes up-regulated, 44–48% genes expressed at similar levels, and only 0.7–13% genes down-regulated (Fig. 2A). Human and mouse ESCs and iPSCs showed substantial overall similarity in expression of DNA repair genes (Fig. 2A), and these minor differences likely reflect slight differences in the pluripotent state of these cells from each species (i.e. the naïve state in mouse pluripotent cells versus the primed state in human pluripotent cells) (Hassani et al., 2014). Overall, 93.8–95.1% of DNA repair genes represented on the microarrays were expressed in pluripotent cells, indicating that both the level and breadth of DNA repair gene expression are high in these cells.

Cell death genes also showed substantial differential expression in pluripotent cells compared to fibroblasts (Fig. 2B). However, in addition to 26–47% of cell death genes showing similar expression, approximately equal proportions of differentially expressed cell death genes were up-(12–23%) or down-(14–24%) regulated, respectively, in pluripotent cells relative to differentiated cells. Complete lists of consensus differentially expressed DNA repair and cell death genes are shown in Supplementary Table 1. Note that in each of the transcriptome databases we mined, fibroblasts were the only differentiated cell type examined.

Multiple DNA repair and cell death pathways are differentially expressed in pluripotent cells relative to differentiated cells

We used IPA analysis to examine differential expression of particular DNA repair or cell death pathways. We found that most DNA repair pathways, including mismatch repair (MMR), double-strand break repair by homologous recombination (DSBR-HR) and double-strand break repair by non-homologous end joining (DSBR-NHEJ), showed up-regulated expression in pluripotent cells (as defined by more genes from the relevant pathway being up-regulated than down-regulated in pluripotent cells) (Supplementary Table 3, p = 3.98E−28–2.88E−9). Nucleotide excision repair (NER) was the only DNA repair pathway for which the proportion of down-regulated genes slightly exceeded that of the up-regulated genes in pluripotent cells (Supplementary Table 3). Although it is not listed as a canonical pathway within IPA, we also examined expression of genes involved in base excision repair (BER) (Friedberg et al., 2006). Human and mouse pluripotent cells showed 35–47% of BER genes up-regulated and 0–6% down-regulated relative to differentiated cells (≥1.5× and significantly different by ANOVA). Therefore this DNA repair pathway also appears to be up-regulated in pluripotent cells. Genes associated with apoptosis were approximately equally divided into those up- or down-regulated in pluripotent cells (Supplementary Table 1). However, genes associated with intrinsically activated pro-apoptosis were particularly up-regulated in pluripotent cells, with 85% of activators of intrinsic apoptosis being up-regulated and 67% of inhibitors of intrinsic apoptosis being down-regulated in these cells.

Interactions between the pluripotency and genetic integrity gene networks

To test the hypothesis that the differential expression of DNA repair and cell death genes in pluripotent cells relative to differentiated cells is based on a mechanistic

link between the genetic integrity and pluripotency gene networks, we first investigated which differentially expressed genetic integrity genes are bound directly by one or more of the three key pluripotency factors, OCT4, SOX2 or NANOG ("first-degree" interactions). We mined published cistrome datasets from five studies examining binding of pluripotency factors genome-wide, including four datasets describing mouse pluripotent (ES and/or iPS) cells (Chen et al., 2008; Kim et al., 2008; Mathur et al., 2008; Sridharan et al., 2009) and one dataset describing human pluripotent (ES) cells (Boyer et al., 2005) (Supplementary Table 4). Overall, we found that 14–25% of DNA repair genes differentially expressed in pluripotent cells are bound by one or more of the three key pluripotency factors examined (22% in mouse ESCs, 25% in mouse iPSCs, 15% in human ESCs, and 14% in human iPSCs). Similarly we found 15–27% of differentially expressed cell death genes bound by these same pluripotency factors in the same cell types (26% in mouse ESCs, 27% in mouse iPSCs, 15% in human ESCs, and 15% in human iPSCs). We used the IPA pathway builder tool to visualize direct protein–DNA and protein–protein interactions between the pluripotency and genetic integrity networks (DNA repair and cell death). Examples of these interactions in human embryonic stem cells are shown in Fig. 3, while interactions in human iPSCs and mouse ESCs and iPSCs are shown in Supplementary Fig. 2. Arrows leading from pluripotency factors to genetic integrity genes indicate protein–DNA interactions, while blunt connection lines indicate protein–protein interactions.

We next investigated indirect, "second-degree" interactions between the pluripotency and genetic integrity gene networks involving intermediary factors that are, themselves, regulated by upstream pluripotency factors and that subsequently regulate one or more downstream genetic integrity genes. We revisited the transcriptome databases to identify genes encoding transcription factors (other than the key pluripotency factors) that are differentially expressed in pluripotent cells relative to differentiated cells (Fig. 4). This revealed that 12–21% of these transcription factor genes are up-regulated and 13–21% are down-regulated in pluripotent cells compared to differentiated cells (≥1.5× and significantly different by ANOVA).

The differentially expressed transcription factor genes were then imported into IPA to determine which of these genes or gene products are regulated by one or more of the core pluripotency factors (OCT4, SOX2 and NANOG), and which of these regulated transcription factors are, in turn, bound to one or more differentially expressed downstream DNA repair and/or cell death gene(s) in pluripotent cells. Complete lists of individual DNA repair and cell death genes bound by intermediary factors in each cell type are provided in Supplementary Table 5 and example visual representations of these second-degree interactions in human ESCs are shown in Fig. 5. Representations of similar second-degree interactions linking the pluripotency and cell death gene networks in human iPSCs and mouse ESCs and iPSCs are shown in Supplementary Figs. 3–5. Inclusion of these second-degree interactions substantially expands the extent of interactions we detected between the pluripotency and genetic integrity gene networks in pluripotent cells. Taken together, first- plus second-degree interactions account for potential direct or indirect regulation by the three pluripotency factors investigated of 21–35% of cell death genes or gene products and 22–50% of DNA repair genes or gene products differentially expressed in pluripotent cell types relative to differentiated cells.

Validation of network interactions by ChIP—qPCR

We used ChIP–qPCR to validate exemplary binding interactions between OCT4, SOX2, NANOG, and/or p53 with either downstream genetic integrity genes or downstream transcription factor genes in mouse ESCs. This allowed us to validate a set of predicted first-degree interactions between pluripotency factors and genetic integrity genes (OCT4 with *Bid*, *Dido* and

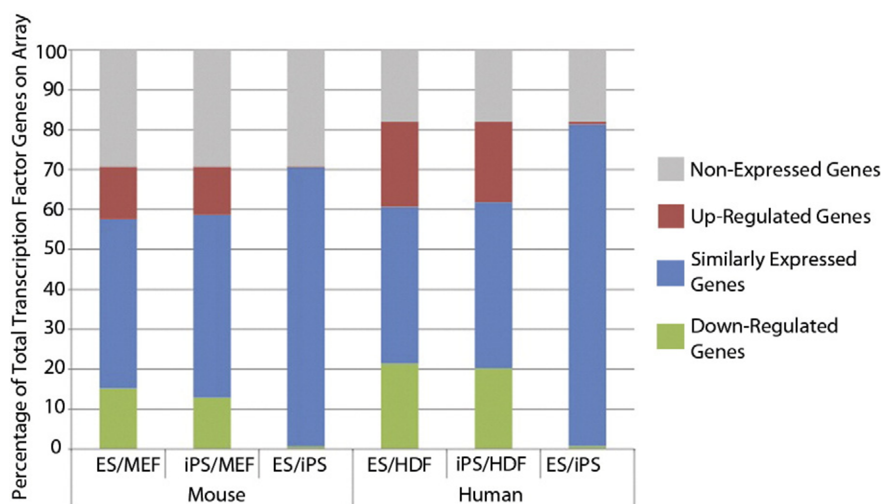


Figure 4 Differential expression of transcription factor genes in mouse and human pluripotent cell types compared to differentiated cells. Color/comparison scheme and abbreviations are as described for Fig. 2.

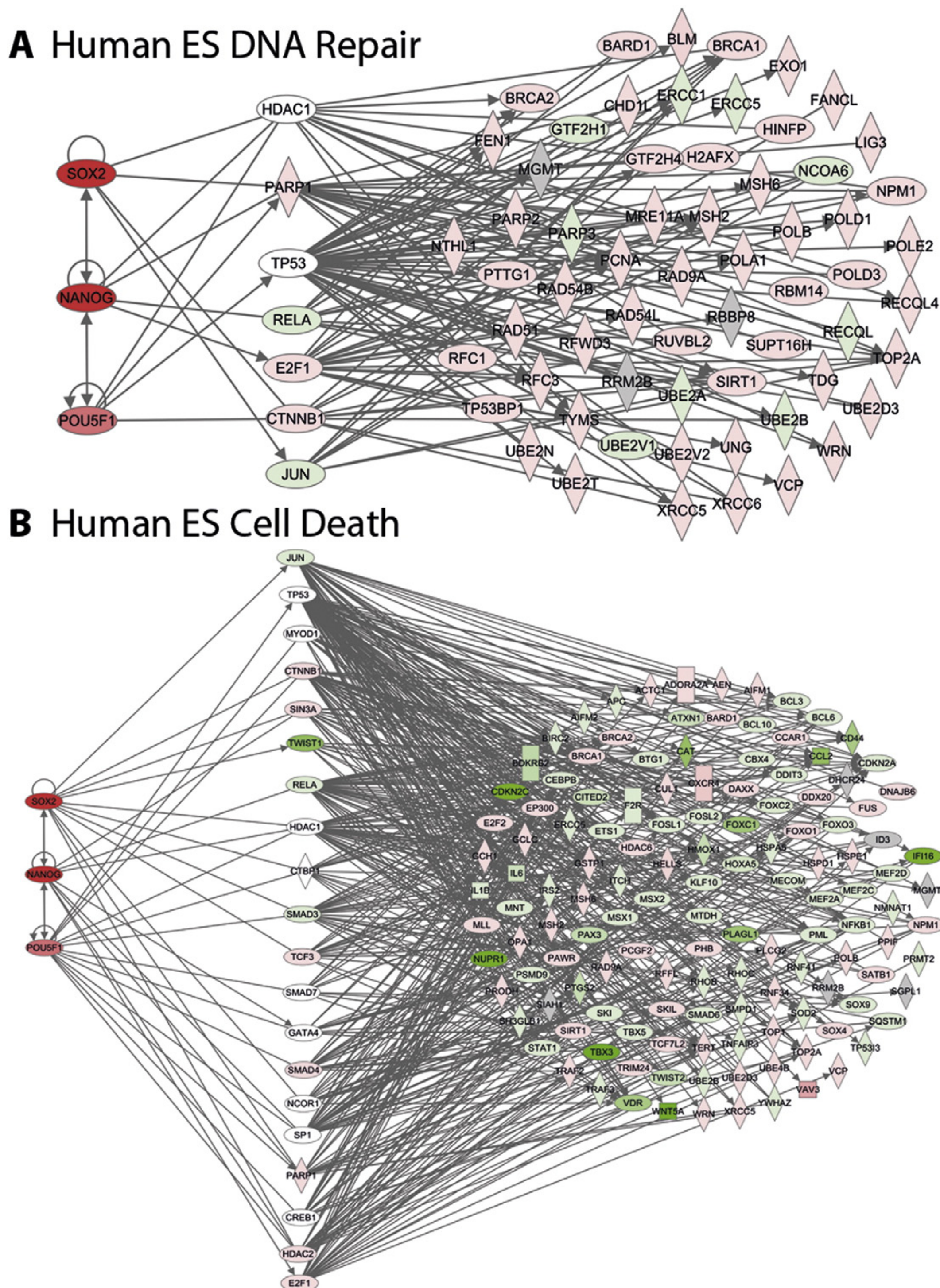


Figure 5 Second-degree interactions between pluripotency and genetic integrity gene networks in human ES cells. Indirect interactions between pluripotency factors and the DNA repair (A) and cell death (B) gene networks in human ES cells are shown. Pluripotency factors are listed on the left, intermediary regulatory factors are listed in the middle, and downstream genetic integrity genes are listed on the right in each case. Color and shape representation scheme is identical to that described in Fig. 3. Additionally, squares = growth factors.

Rad51; SOX2 with *Bid*, *Dido*, *Rad51* and *Ercc1*; and NANOG with *Dido*, *Rad51* and *Ercc1*), plus two predicted second-degree interactions (upstream binding of OCT4 to *p53* and NANOG to *e2f1*, plus downstream binding of p53 to *Mlh1* and *Rfc3*), and a combination of first- and second-degree interactions regulating *Msh2* and *Birc2* (SOX2 with *Msh2* and *Birc2*, NANOG with *Msh2* and *Birc2*, OCT4 with *Birc2*, and p53 with *Msh2* and *Birc2*). ChIP results are shown in Fig. 6.

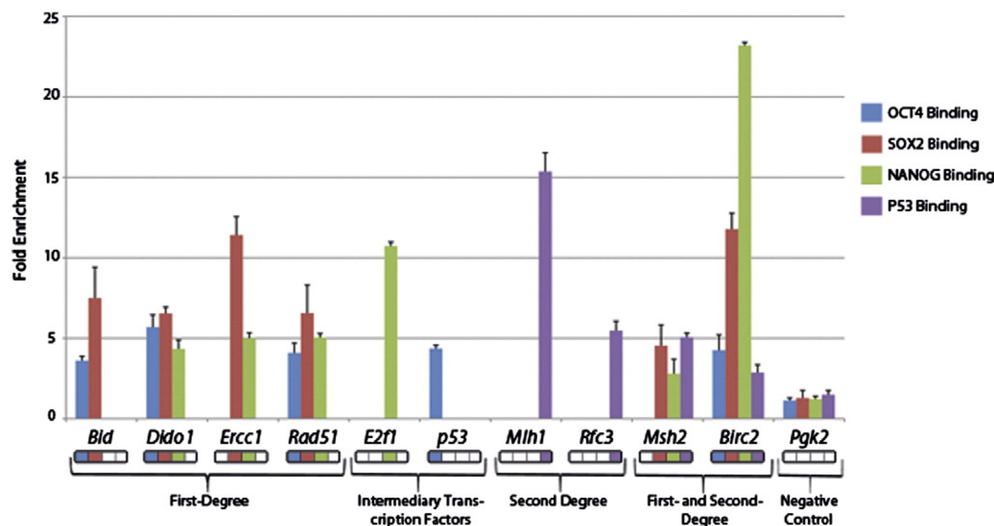


Figure 6 ChIP–qPCR validation of first- and second-degree regulation of genetic integrity genes by direct or indirect interactions with the pluripotency gene network. Exemplary ChIP–qPCR validations are shown for: i) genetic integrity genes regulated by first-degree interactions, ii) intermediary transcription factors regulated by pluripotency factors, iii) genetic integrity genes regulated by second-degree interactions, and iv) genetic integrity genes regulated by both first- and second-degree interactions. Binding to the *Pgk2* GC-box served as a negative control for non-specific binding. Boxes under gene graphs indicate genes predicted by IPA to be bound (colored boxes) or not bound (white boxes) by each corresponding factor. Values are fold enrichment relative to negative control (IgG), and error bars represent SEM based on three biological replicates each with three technical replicates for each ChIP–qPCR reaction.

Down-regulation of pluripotency factor gene expression impacts expression of genetic integrity genes

Finally, to further substantiate the existence of a mechanistic link between the pluripotency and genetic integrity networks, we mined data from a study published by Ivanova et al. (2006) in which transcriptome databases were derived from mouse ESCs following RNAi knockdowns of specific pluripotency factors (SOX2, OCT4 or NANOG). We found that approximately 13% of genetic integrity genes and 37% of cell death genes predicted to be targeted by first-degree interactions with these three pluripotency factors showed altered expression levels following knockdown of one or more pluripotency factor genes (Supplementary Table 6). Importantly, these knockdowns were each performed singly, and it is possible that many of those genetic integrity genes we suggest are targeted by first-degree interactions may be regulated by more than one factor such that there is functional redundancy in this regulatory process that might preclude down-regulation in response to a knockdown of a single factor.

Discussion

The validity of the Disposable Soma Theory (Kirkwood, 1977) is now well established for germ cells based on multiple demonstrations that these cells maintain enhanced genetic integrity relative to somatic cell types (Ehling and Neuhäuser, 1979; Russell et al., 1979; Walter et al., 1998), and that this is accomplished thru up-regulation and/or differential expression of genes in multiple DNA repair and cell death pathways (Chen et al., 1995; Intano et al., 2001, 2002; Tichy and Stambrook, 2008). Thus, it appears that enhanced maintenance of genetic integrity is a fundamental characteristic of germ cells (Murphey et al., 2013). Previous studies have also revealed enhanced maintenance of genetic integrity in pluripotent cells with particular regard to point mutations (Hong et al., 2007; Maynard et al., 2008; Momcilović et al., 2009; Van Sloun et al., 1999) and have linked this to elevated DNA repair (Hyka-Nouspikel et al., 2012) and cell death (Li et al., 2012; Momcilović et al., 2011; Qin et al., 2007; Roos et al., 2007) activities. Here we report data indicating that up (for DNA repair) or differential (for cell death) regulation of genes involved in maintenance of genetic integrity occurs in pluripotent cells relative to differentiated cells (fibroblasts) in a manner similar to that seen in germ cells and somatic cells, respectively, indicating that enhanced maintenance of genetic integrity is also a fundamental characteristic of pluripotent cells. To better understand the mechanism by which enhanced maintenance of genetic integrity is coordinately regulated with pluripotency, we investigated interactions between the gene networks that regulate each of these phenomena. Our results reveal extensive interactions between these gene networks, and suggest a mechanism by which induction and/or maintenance of pluripotency directly leads to induction and/or maintenance of enhanced genetic integrity.

Our meta-analysis of available transcriptome datasets confirmed that expression of genes encoding proteins involved in many, but not all DNA repair pathways is elevated in pluripotent cells, suggesting that pluripotent cells possess enhanced protection against a wide variety of different types of DNA damage. Nucleotide excision repair was the one DNA repair pathway that did not appear to be up-regulated at the genomic level in pluripotent cells, however this may be compensated by changes in activities of cell death pathways (see below). We also found a distinct up-regulation of intrinsically regulated, pro-apoptotic genes

in pluripotent cells, suggesting these cells are predisposed to respond to intrinsic triggers of programmed cell death. Such triggers could come from a build-up of large-scale genetic abnormalities such as polyploidy or aneuploidy (Eggan et al., 2002), or from certain types of DNA damage such as that caused by ionizing radiation (Momcilović et al., 2009; Van Sloun et al., 1999; Xu et al., 2005, 2008). Indeed, it may be that the lack of up-regulation of NER DNA repair genes in pluripotent cells facilitates preferential elimination by apoptosis of cells bearing large-scale damage or helix distorting damage. NER is the major repair pathway for ameliorating helix distorting lesions, such as photoproducts (Bradley et al., 1984; Kuper and Kisker, 2012; Murphey et al., 2009; Naegeli and Sugasawa, 2011; Thomson, 1998), suggesting that helix distorting lesions in DNA may signal a cell death response in pluripotent cells.

Taken together, these results suggest that two alternative strategies are employed by pluripotent cells to minimize accumulation of genetic defects. Elevated expression of genes associated with intrinsically regulated pro-apoptotic pathways in pluripotent cells appears to promote a retrospective elimination of cells that accumulate large-scale genetic defects, whereas elevated activity of most DNA repair pathways appears to facilitate a prospective prevention of small-scale genetic defects such as point mutations (which would normally not trigger cell death pathways unless present in abundance). Together these mechanisms appear to yield a population of pluripotent cells that displays an overall enhanced level of genetic and genomic integrity.

We next sought insight regarding the mechanism that links regulation of pluripotency with that of enhanced maintenance of genetic integrity in pluripotent cells. The discovery that fully pluripotent cells can be derived by transduction of differentiated cells starting with four (Takahashi and Yamanaka, 2006), or fewer (Huangfu et al., 2008; Kim et al., 2009), key regulatory genes suggests that small numbers of factors control entire networks of downstream genes and gene products necessary to establish the fate of a cell. We tested the hypothesis that small numbers of pluripotency factors interact with large numbers of genetic integrity genes in pluripotent cells. We found evidence for extensive interactions between the pluripotency and genetic integrity gene networks. While further research will be needed to fully interrogate the functionality of each of the molecular interactions predicted by our analysis, the predicted interactions we report can account for the potential regulation of 22–50% of DNA repair gene and 21–35% of cell death gene differential expression in pluripotent cells. Undoubtedly, the extent of interactions between these two gene networks would be even greater if additional pluripotency factors and/or higher order interactions were considered. Indeed, the involvement of intermediary regulators linking the pluripotency and genetic integrity gene networks affords potential opportunities for additional levels of coordination of expression of downstream genetic integrity pathways.

Conclusions

In summary, our data indicate the Disposable Soma Theory (Kirkwood, 1977) applies to pluripotent cells as well as to germ cells. Further, we provide insight into the mechanism by which the pluripotency gene network interacts with the genetic integrity gene network to establish and maintain enhanced genetic integrity in pluripotent cells. This, in turn, suggests that key factors might be monitored and/or manipulated to maintain optimal genetic integrity in stem cells or their differentiated derivatives intended for use in therapeutic applications.

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References

Aasen, T., Raya, A., Barrero, M.J., Garreta, E., Consiglio, A., Gonzalez, F., Vassena, R., Bilić, J., Pekarik, V., Tiscornia, G., Edel, M., Boué, S., Belmonte, J.C.I., 2008. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat. Biotechnol. 26, 1276–1284. http://dx.doi.org/10.1038/nbt.1503.

Adams, B.R., Golding, S.E., Rao, R.R., Valerie, K., 2010a. Dynamic dependence on ATR and ATM for double-strand break repair in human embryonic stem cells and neural descendants. PLoS One 5, e10001. http://dx.doi.org/10.1371/journal.pone.0010001.g006.

Adams, B.R., Hawkins, A.J., Povirk, L.F., Valerie, K., 2010b. ATM-independent, high-fidelity nonhomologous end joining predominates in human embryonic stem cells. Aging (Albany NY) 2, 582–596.

Ben-David, U., Benvenisty, N., 2012. High prevalence of evolutionarily conserved and species-specific genomic aberrations in mouse pluripotent stem cells. Stem Cells 30, 612–622. http://dx.doi.org/10.1002/stem.1057.

Ben-David, U., Mayshar, Y., Benvenisty, N., 2011. Large-scale analysis reveals acquisition of lineage-specific chromosomal aberrations in human adult stem cells. Cell Stem Cell 9, 97–102. http://dx.doi.org/10.1016/j.stem.2011.06.013.

Bock, C., Kiskinis, E., Verstappen, G., Gu, H., Boulting, G., Smith, Z.D., Ziller, M., Croft, G.F., Amoroso, M.W., Oakley, D.H., Gnirke, A., Eggan, K., Meissner, A., 2011. Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. Cell 144, 439–452. http://dx.doi.org/10.1016/j.cell.2010.12.032.

Boland, M.J., Hazen, J.L., Nazor, K.L., Rodriguez, A.R., Gifford, W., Martin, G., Kupriyanov, S., Baldwin, K.K., 2009. Adult mice generated from induced pluripotent stem cells. Nature 461, 91–94. http://dx.doi.org/10.1038/nature08310.

Borgdorff, V., Pauw, B., van Hees-Stuivenberg, S., de Wind, N., 2006. DNA mismatch repair mediates protection from mutagenesis induced by short-wave ultraviolet light. DNA Repair (Amst) 5, 1364–1372. http://dx.doi.org/10.1016/j.dnarep.2006.06.005.

Boyer, L.A., Lee, T.I., Cole, M.F., Johnstone, S.E., Levine, S.S., Zucker, J.P., Guenther, M.G., Kumar, R.M., Murray, H.L., Jenner, R.G., Gifford, D.K., Melton, D.A., Jaenisch, R., Young, R.A., 2005. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 122, 947–956. http://dx.doi.org/10.1016/j.cell.2005.08.020.

Bradley, A., Evans, M., Kaufman, M.H., Robertson, E., 1984. Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. Nature 309, 255–256.

Bradley, E., Bieberich, E., Mivechi, N., Tangpisuthipongsa, D., Wang, G., 2012. Regulation of embryonic stem cell pluripotency by heat shock protein 90. Stem Cells http://dx.doi.org/10.1002/stem.1143 (N/A–N/A).

Cervantes, R.B., Stringer, J.R., Shao, C., Tischfield, J.A., Stambrook, P.J., 2002. Embryonic stem cells and somatic cells differ in mutation frequency and type. Proc. Natl. Acad. Sci. U. S. A. 99, 3586–3590. http://dx.doi.org/10.1073/pnas.062527199.

Chen, J., Tomkinson, A.E., Ramos, W., Mackey, Z.B., Danehower, S., Walter, C.A., Schultz, R.A., Besterman, J.M., Husain, I., 1995. Mammalian DNA ligase III: molecular cloning, chromosomal localization, and expression in spermatocytes undergoing meiotic recombination. Mol. Cell. Biol. 15, 5412–5422.

Chen, X., Xu, H., Yuan, P., Fang, F., Huss, M., Vega, V.B., Wong, E., Orlov, Y.L., Zhang, W., Jiang, J., Loh, Y.-H., Yeo, H.C., Yeo, Z.X., Narang, V., Govindarajan, K.R., Leong, B., Shahab, A., Ruan, Y., Bourque, G., Sung, W.-K., Clarke, N.D., Wei, C.-L., Ng, H.-H., 2008. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell 133, 1106–1117. http://dx.doi.org/10.1016/j.cell.2008.04.043.

Chen, J., Liu, J., Han, Q., Qin, D., Xu, J., Chen, Y., Yang, J., Song, H., Yang, D., Peng, M., He, W., Li, R., Wang, H., Gan, Y., Ding, K., Zeng, L., Lai, L., Esteban, M.A., Pei, D., 2010. Towards an optimized culture medium for the generation of mouse induced pluripotent stem cells. J. Biol. Chem. 285, 31066–31072. http://dx.doi.org/10.1074/jbc.M110.139436.

Chin, M.H., Mason, M.J., Xie, W., Volinia, S., Singer, M., Peterson, C., Ambartsumyan, G., Aimiuwu, O., Richter, L., Zhang, J., Khvorostov, I., Ott, V., Grunstein, M., Lavon, N., Benvenisty, N., Croce, C.M., Clark, A.T., Baxter, T., Pyle, A.D., Teitell, M.A., Pelegrini, M., Plath, K., Lowry, W.E., 2009. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. Cell Stem Cell 5, 111–123. http://dx.doi.org/10.1016/j.stem.2009.06.008.

Coucouvanis, E.C., Sherwood, S.W., Carswell-Crumpton, C., Spack, E.G., Jones, P.P., 1993. Evidence that the mechanism of prenatal germ cell death in the mouse is apoptosis. Exp. Cell Res. 209, 238–247. http://dx.doi.org/10.1006/excr.1993.1307.

de Waard, H., Sonneveld, E., de Wit, J., Esveldt-van Lange, R., Hoeijmakers, J.H.J., Vrieling, H., van der Horst, G.T.J., 2008. Cell-type-specific consequences of nucleotide excision repair deficiencies: embryonic stem cells versus fibroblasts. DNA Repair (Amst) 7, 1659–1669. http://dx.doi.org/10.1016/j.dnarep.2008.06.009.

Draper, J.S., Smith, K., Gokhale, P., Moore, H.D., Maltby, E., Johnson, J., Meisner, L., Zwaka, T.P., Thomson, J.A., Andrews, P.W., 2003. Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. Nat. Biotechnol. 22, 53–54. http://dx.doi.org/10.1038/nbt922.

Duval, D., Trouillas, M., Thibault, C., Dembelé, D., Diemunsch, F., Reinhardt, B., Mertz, A.L., Dierich, A., Bœuf, H., 2005. Apoptosis and differentiation commitment: novel insights revealed by gene profiling studies in mouse embryonic stem cells. Cell Death Differ. 13, 564–575. http://dx.doi.org/10.1038/sj.cdd.4401789.

Ebert, A., Yu, J., Rose, F., Mattis, V., Lorson, C., 2008. Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature 457, 277–280. http://dx.doi.org/10.1038/nature07677.

Eggan, K., Rode, A., Jentsch, I., Samuel, C., Hennek, T., Tintrup, H., Zevnik, B., Erwin, J., Loring, J., Jackson-Grusby, L., Speicher, M. R., Kuehn, R., Jaenisch, R., 2002. Male and female mice derived from the same embryonic stem cell clone by tetraploid embryo complementation. Nat. Biotechnol. 20, 455–459. http://dx.doi.org/10.1038/nbt0502-455.

Ehling, U.H., Neuhäuser, A., 1979. Procarbazine-induced specific-locus mutations in male mice. Mutat. Res. 59, 245–256.

Fan, J., Robert, C., Jang, Y.-Y., Liu, H., Sharkis, S., Baylin, S.B., Rassool, F.V., 2011. Human induced pluripotent cells resemble embryonic stem cells demonstrating enhanced levels of DNA repair and efficacy of nonhomologous end-joining. Mutat. Res. 713, 8–17. http://dx.doi.org/10.1016/j.mrfmmm.2011.05.018.

Feng, B., Ng, J., Heng, J., 2009. Molecules that promote or enhance reprogramming of somatic cells to induced pluripotent stem cells. Cell Stem Cell 4 (4), 301–312.

Friedberg, E.C., Walker, G.C., Siede, W., Wood, R.D., Schultz, R.A., Ellenberger, T., 2006. DNA Repair and Mutagenesis, Second ed. ASM Press.

Hassani, S.N., Totonchi, M., Gourabi, H., Schöler, H.R., Baharvand, H., 2014. Signaling roadmap modulating naïve and primed pluripotency. Stem Cells Dev. 23, 193–208. http://dx.doi.org/10.1089/scd.2013.0368.

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., 2010. The nuclear receptor Nr5a2 can replace Oct4 in the reprogramming of murine somatic cells to pluripotent cells. Cell Stem Cell 6, 167–174. http://dx.doi.org/10.1016/j.stem.2009.12.009.

Hong, Y., Cervantes, R.B., Tichy, E., Tischfield, J.A., Stambrook, P.J., 2007. Protecting genomic integrity in somatic cells and embryonic stem cells. Mutat. Res. Fundam. Mol. Mech. Mutagen. 614, 48–55. http://dx.doi.org/10.1016/j.mrfmmm.2006.06.006.

Huamani, J., McMahan, C.A., Herbert, D.C., Reddick, R., McCarrey, J.R., MacInnes, M.I., Chen, D.J., Walter, C.A., 2004. Spontaneous mutagenesis is enhanced in apex heterozygous mice. Mol. Cell. Biol. 24, 8145–8153. http://dx.doi.org/10.1128/MCB.24.18.8145-8153.2004.

Huang, D.W., Sherman, B.T., Lempicki, R.A., 2008. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 4, 44–57. http://dx.doi.org/10.1038/nprot.2008.211.

Huangfu, D., Osafune, K., Maehr, R., Guo, W., Eijkelenboom, A., Chen, S., Muhlestein, W., Melton, D.A., 2008. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat. Biotechnol. 26, 1269–1275. http://dx.doi.org/10.1038/nbt.1502.

Hyka-Nouspikel, N., Desmarais, J., Gokhale, P.J., Jones, M., Meuth, M., Andrews, P.W., Nouspikel, T., 2012. Deficient DNA damage response and cell cycle checkpoints lead to accumulation of point mutations in human embryonic stem cells. Stem Cells 30, 1901–1910. http://dx.doi.org/10.1002/stem.1177.

Ichida, J.K., Blanchard, J., Lam, K., Son, E.Y., Chung, J.E., Egli, D., Loh, K.M., Carter, A.C., Di Giorgio, F.P., Koszka, K., Huangfu, D., Akutsu, H., Liu, D.R., Rubin, L.L., Eggan, K., 2009. A small-molecule inhibitor of Tgf-β signaling replaces Sox2 in reprogramming by inducing Nanog. Cell Stem Cell 5, 491–503. http://dx.doi.org/10.1016/j.stem.2009.09.012.

Intano, G.W., McMahan, C.A., Walter, R.B., McCarrey, J.R., Walter, C.A., 2001. Mixed spermatogenic germ cell nuclear extracts exhibit high base excision repair activity. Nucleic Acids Res. 29, 1366–1372.

Intano, G.W., McMahan, C.A., McCarrey, J.R., Walter, R.B., McKenna, A.E., Matsumoto, Y., MacInnes, M.A., Chen, D.J., Walter, C.A., 2002. Base excision repair is limited by different proteins in male germ cell nuclear extracts prepared from young and old mice. Mol. Cell. Biol. 22, 2410–2418. http://dx.doi.org/10.1128/MCB.22.7.2410-2418.2002.

Ivanova, N., Dobrin, R., Lu, R., Kotenko, I., Levorse, J., DeCoste, C., Schafer, X., Lun, Y., Lemischka, I.R., 2006. Dissecting self-renewal in stem cells with RNA interference. Nat. Cell Biol. 442, 533–538. http://dx.doi.org/10.1038/nature04915.

Kang, L., Wang, J., Zhang, Y., Kou, Z., Gao, S., 2009. iPS cells can support full-term development of tetraploid blastocyst-complemented embryos. Cell Stem Cell 5, 135–138. http://dx.doi.org/10.1016/j.stem.2009.07.001.

Kim, J., Chu, J., Shen, X., Wang, J., Orkin, S.H., 2008. An extended transcriptional network for pluripotency of embryonic stem cells. Cell 132, 1049–1061. http://dx.doi.org/10.1016/j.cell.2008.02.039.

Kim, J.B., Sebastiano, V., Wu, G., AraUzo-Bravo, M.J., Sasse, P., Gentile, L., Ko, K., Ruau, D., Ehrich, M., van den Boom, D., Meyer, J., HUbner, K., Memann, C.B., Ortmeier, C., Zenke, M., Fleischmann, B.K., Zaehres, H., Schöler, H.R., 2009. Oct4-induced pluripotency in adult neural stem cells. Cell 136, 411–419. http://dx.doi.org/10.1016/j.cell.2009.01.023.

Kirkwood, T.B., 1977. Evolution of ageing. Nature 270, 301–304.

Kuper, J., Kisker, C., 2012. Damage recognition in nucleotide excision DNA repair. Curr. Opin. Struct. Biol. 22, 88–93. http://dx.doi.org/10.1016/j.sbi.2011.12.002.

Li, M., He, Y., Dubois, W., Wu, X., Shi, J., Huang, J., 2012. Distinct regulatory mechanisms and functions for p53-activated and p53-repressed DNA damage response genes in embryonic stem cells. Mol. Cell 46, 30–42. http://dx.doi.org/10.1016/j.molcel.2012.01.020.

Liu, J.C., Guan, X., Ryan, J.A., Rivera, A.G., Mock, C., Agarwal, V., Letai, A., Lerou, P.H., Lahav, G., 2013. High mitochondrial priming sensitizes hESCs to DNA-damage-induced apoptosis. Stem Cell 13, 483–491. http://dx.doi.org/10.1016/j.stem.2013.07.018.

Lowry, W., Richter, L., Yachechko, R., 2008. Generation of human induced pluripotent stem cells from dermal fibroblasts. Proceedings of the …, pp. 1–6.

Maherali, N., Sridharan, R., Xie, W., Utikal, J., Eminli, S., Arnold, K., Stadtfeld, M., Yachechko, R., Tchieu, J., Jaenisch, R., Plath, K., Hochedlinger, K., 2007. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell 1, 55–70. http://dx.doi.org/10.1016/j.stem.2007.05.014.

Maherali, N., Ahfeldt, T., Rigamonti, A., Utikal, J., Cowan, C., Hochedlinger, K., 2008. A high-efficiency system for the generation and study of human induced pluripotent stem cells. Cell Stem Cell 3, 340–345. http://dx.doi.org/10.1016/j.stem.2008.08.003.

Mathur, D., Danford, T.W., Boyer, L.A., Young, R.A., Gifford, D.K., Jaenisch, R., 2008. Analysis of the mouse embryonic stem cell regulatory networks obtained by ChIP-chip and ChIP-PET. Genome Biol. 9, R126. http://dx.doi.org/10.1186/gb-2008-9-8-r126.

Maynard, S., Swistowska, A.M., Lee, J.W., Liu, Y., Liu, S.-T., Da Cruz, A.B., Rao, M., de Souza-Pinto, N.C., Zeng, X., Bohr, V.A., 2008. Human embryonic stem cells have enhanced repair of multiple forms of DNA damage. Stem Cells 26, 2266–2274. http://dx.doi.org/10.1634/stemcells.2007-1041.

Momcilović, O., Choi, S., Varum, S., Bakkenist, C., Schatten, G., Navara, C., 2009. Ionizing radiation induces ataxia telangiectasia mutated-dependent checkpoint signaling and G (2) but not G (1) cell cycle arrest in pluripotent human embryonic stem cells. Stem Cells 27, 1822–1835. http://dx.doi.org/10.1002/stem.123.

Momcilović, O., Knobloch, L., Fornsaglio, J., Varum, S., Easley, C., Schatten, G., 2010. DNA damage responses in human induced pluripotent stem cells and embryonic stem cells. PLoS One 5, e13410. http://dx.doi.org/10.1371/journal.pone.0013410.g007.

Momcilović, O., Navara, C., Schatten, G., 2011. Cell cycle adaptations and maintenance of genomic integrity in embryonic stem cells and induced pluripotent stem cells. Results Probl. Cell Differ. 53, 415–458. http://dx.doi.org/10.1007/978-3-642-19065-0_18.

Munoz, J., Low, T.Y., Kok, Y.J., Chin, A., Frese, C.K., Ding, V., Choo, A., Heck, A.J.R., 2011. The quantitative proteomes of human-induced pluripotent stem cells and embryonic stem cells. Mol. Syst. Biol. 7, 550. http://dx.doi.org/10.1038/msb.2011.84.

Murphey, P., Yamazaki, Y., McMahan, C.A., Walter, C.A., Yanagimachi, R., McCarrey, J.R., 2009. Epigenetic regulation of genetic integrity is reprogrammed during cloning. Proc. Natl. Acad. Sci. U. S. A. 106, 4731–4735. http://dx.doi.org/10.1073/pnas.0900687106.

Murphey, P., McLean, D.J., McMahan, C.A., Walter, C.A., McCarrey, J.R., 2013. Enhanced genetic integrity in mouse germ cells. Biol. Reprod. 88 (6), 1–8. http://dx.doi.org/10.1095/biolreprod.112.103481.

Naegeli, H., Sugasawa, K., 2011. The xeroderma pigmentosum pathway: decision tree analysis of DNA quality. DNA Repair (Amst) 10, 673–683. http://dx.doi.org/10.1016/j.dnarep.2011.04.019.

Nouspikel, T., Hanawalt, P.C., 2000. Terminally differentiated human neurons repair transcribed genes but display attenuated global DNA repair and modulation of repair gene expression. Mol. Cell. Biol. 20, 1562–1570.

Okita, K., Ichisaka, T., Yamanaka, S., 2007. Generation of germline-competent induced pluripotent stem cells. Nature 448, 313–317. http://dx.doi.org/10.1038/nature05934.

Park, I.-H., Arora, N., Huo, H., Maherali, N., Ahfeldt, T., Shimamura, A., Lensch, M.W., Cowan, C., Hochedlinger, K., Daley, G.Q., 2008. Disease-specific induced pluripotent stem cells. Cell 134, 877–886. http://dx.doi.org/10.1016/j.cell.2008.07.041.

Qin, H., Yu, T., Qing, T., Liu, Y., Zhao, Y., Cai, J., Li, J., Song, Z., Qu, X., Zhou, P., 2007. Regulation of apoptosis and differentiation by p53 in human embryonic stem cells. J. Biol. Chem. 282, 5842–5852.

Roos, W.P., Christmann, M., Fraser, S.T., Kaina, B., 2007. Mouse embryonic stem cells are hypersensitive to apoptosis triggered by the DNA damage O6-methylguanine due to high E2F1 regulated mismatch repair. Cell Death Differ. 14, 1422–1432. http://dx.doi.org/10.1038/sj.cdd.4402136.

Roy Choudhury, D., Small, C., Wang, Y., Mueller, P.R., Rebel, V.I., Griswold, M.D., McCarrey, J.R., 2010. Microarray-based analysis of cell-cycle gene expression during spermatogenesis in the mouse. Biol. Reprod. 83, 663–675. http://dx.doi.org/10.1095/biolreprod.110.084889.

Russell, L.B., 2004. Effects of male germ-cell stage on the frequency, nature, and spectrum of induced specific-locus mutations in the mouse. Genetica 122, 25–36.

Russell, L.B., Russell, W.L., Kelly, E.M., 1979. Analysis of the albino-locus region of the mouse. I. Origin and viability. Genetics 91, 127–139.

Russell, W.L., Bangham, J.W., Russell, L.B., 1998. Differential response of mouse male germ-cell stages to radiation-induced specific-locus and dominant mutations. Genetics 148, 1567–1578.

Russell, L.B., Hunsicker, P.R., Russell, W.L., 2007. Comparison of the genetic effects of equimolar doses of ENU and MNU: while the chemicals differ dramatically in their mutagenicity in stem-cell spermatogonia, both elicit very high mutation rates in differentiating spermatogonia. Mutat. Res. 616, 181–195. http://dx.doi.org/10.1016/j.mrfmmm.2006.11.003.

Soldner, F., Hockemeyer, D., Beard, C., Gao, Q., 2009. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 136, 964–977. http://dx.doi.org/10.1016/j.cell.2009.02.013.

Sridharan, R., Tchieu, J., Mason, M.J., Yachechko, R., Kuoy, E., Horvath, S., Zhou, Q., Plath, K., 2009. Role of the murine reprogramming factors in the induction of pluripotency. Cell 136, 364–377. http://dx.doi.org/10.1016/j.cell.2009.01.001.

Sun, N., Panetta, N.J., Gupta, D.M., Wilson, K.D., Lee, A., Jia, F., Hu, S., Cherry, A.M., Robbins, R.C., Longaker, M.T., Wu, J.C., 2009. Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. Proc. Natl. Acad. Sci. U. S. A. 106, 15720–15725. http://dx.doi.org/10.1073/pnas.0908450106.

Takahashi, K., Yamanaka, S., 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663–676. http://dx.doi.org/10.1016/j.cell.2006.07.024.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., Yamanaka, S., 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872. http://dx.doi.org/10.1016/j.cell.2007.11.019.

Thomson, J.A., 1998. Embryonic stem cell lines derived from human blastocysts. Science 282, 1145–1147. http://dx.doi.org/10.1126/science.282.5391.1145.

Tichy, E.D., 2011. Mechanisms maintaining genomic integrity in embryonic stem cells and induced pluripotent stem cells. Exp. Biol. Med. 236, 987–996. http://dx.doi.org/10.1258/ebm.2011.011107.

Tichy, E.D., Stambrook, P.J., 2008. DNA repair in murine embryonic stem cells and differentiated cells. Exp. Cell Res. 314, 1929–1936. http://dx.doi.org/10.1016/j.yexcr.2008.02.007.

Tichy, E.D., Liang, L., Deng, L., Tischfield, J., Schwemberger, S., Babcock, G., Stambrook, P.J., 2011. Mismatch and base excision repair proficiency in murine embryonic stem cells. DNA Repair (Amst) 10, 445–451. http://dx.doi.org/10.1016/j.dnarep.2011.01.008.

Tomé, S., Simard, J.P., Slean, M.M., Holt, I., Morris, G.E., Wojciechowicz, K., Pearson, C.E., Riele, te, H., 2013. Tissue-specific mismatch repair protein expression: MSH3 is higher than MSH6 in multiple mouse tissues. DNA Repair (Amst) 12, 46–52. http://dx.doi.org/10.1016/j.dnarep.2012.10.006.

Van Sloun, P.P., Jansen, J.G., Weeda, G., Mullenders, L.H., van Zeeland, A.A., Lohman, P.H., Vrieling, H., 1999. The role of nucleotide excision repair in protecting embryonic stem cells from genotoxic effects of UV-induced DNA damage. Nucleic Acids Res. 27, 3276–3282.

Walter, C.A., Intano, G.W., McCarrey, J.R., McMahan, C.A., Walter, R.B., 1998. Mutation frequency declines during spermatogenesis in young mice but increases in old mice. Proc. Natl. Acad. Sci. U. S. A. 95, 10015–10019.

Xu, G., Spivak, G., Mitchell, D.L., Mori, T., McCarrey, J.R., McMahan, C.A., Walter, R.B., Hanawalt, P.C., Walter, C.A., 2005. Nucleotide excision repair activity varies among murine spermatogenic cell types. Biol. Reprod. 73, 123–130. http://dx.doi.org/10.1095/biolreprod.104.039123.

Xu, G., Intano, G.W., McCarrey, J.R., Walter, R.B., Alex McMahan, C., Walter, C.A., 2008. Recovery of a low mutant frequency after ionizing radiation-induced mutagenesis during spermatogenesis. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 654, 150–157. http://dx.doi.org/10.1016/j.mrgentox.2008.05.012.

Xu, G., McMahan, C.A., Hildreth, K., Garcia, R.A., Herbert, D.C., Walter, C.A., 2012. Ionizing radiation-induced mutant frequencies increase transiently in male germ cells of older mice. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 744, 135–139. http://dx.doi.org/10.1016/j.mrgentox.2012.01.003.

Yamanaka, S., 2012. Induced pluripotent stem cells: past, present, and future. Stem Cell 10, 678–684. http://dx.doi.org/10.1016/j.stem.2012.05.005.

Yoshioka, H., Geyer, C.B., Hornecker, J.L., Patel, K.T., McCarrey, J.R., 2007. In vivo analysis of developmentally and evolutionarily dynamic protein-DNA interactions regulating transcription of the Pgk2 gene during mammalian spermatogenesis. Mol. Cell. Biol. 27, 7871–7885. http://dx.doi.org/10.1128/MCB.00990-07.

Yu, J., Hu, K., Smuga-Otto, K., Tian, S., Stewart, R., Slukvin, I.I., Thomson, J.A., 2009. Human induced pluripotent stem cells free of vector and transgene sequences. Science 324, 797–801. http://dx.doi.org/10.1126/science.1172482.

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., 2009. iPS cells produce viable mice through tetraploid complementation. Nature 461, 86–90. http://dx.doi.org/10.1038/nature08267.