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

Daniel J. Cooper [a], Christi A. Walter [b], John R. McCarrey [a,*]

[a] *Department of Biology, University of Texas at San Antonio, USA*
[b] *Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, USA*

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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;

* Corresponding author at: 1 UTSA Circle, Department of Biology, San Antonio, TX 78249, USA.
E-mail address: john.mccarrey@utsa.edu (J.R. McCarrey).

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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.

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