Short report

Ronin influences the DNA damage response in pluripotent stem cells

Bryce A. Seifert a, Marion Dejosez b, Thomas P. Zwaka b,a

a Graduate Program in Molecular and Human Genetics at Baylor College of Medicine, Houston, TX 77030, USA
b Huffington Center for Cell-Based Research in Parkinson’s Disease, Black Family Stem Cell Institute, Department of Cell, Developmental & Regenerative Biology, Graduate School of Biomedical Sciences, New York, NY 10029, USA

A R T I C L E   I N F O

Article history:
Received 29 April 2017
Received in revised form 20 June 2017
Accepted 29 June 2017
Available online 3 July 2017

Keywords:
Ronin/Thap11
DNA repair
DNA damage sensitivity
Embryonic stem cells

A B S T R A C T

Early mammalian embryonic cells must maintain a particularly robust DNA repair system, as mutations at this developmental point have detrimental consequences for the organism. How the repair system can be tuned to fulfill such elevated requirements is largely unknown, but it may involve transcriptional regulation. Ronin (Thap11) is a transcriptional regulator responsible for vital programs in pluripotent cells. Here, we report that this protein also modulates the DNA damage response of such cells. We show that conditional Ronin knockout sensitizes embryonic stem cells (ESCs) to UV-C-induced DNA damage in association with Atr pathway activation and G2/M arrest. Ronin binds to and regulates the genes encoding several DNA repair factors, including Gtf2h4 and Rad18, providing a potential mechanism for this phenotype. Our results suggest that the unique DNA repair requirements of the early embryo are not met by a static system, but rather via highly regulated processes.

© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

The pluripotent stem cell state is maintained by a core set of transcription factors (e.g., Oct4, Sox2, and Nanog) that activate self-renewal genes and suppress lineage-specific differentiation pathways (Dejosez and Zwaka, 2012; Ng and Surani, 2011). Although pluripotency-related transcription factors are known to bind upstream of several DNA repair genes (Marson et al., 2008), the exact links that connect genomic integrity, DNA repair, and pluripotency have not yet been clearly defined. A second class of transcription factors helps maintain pluripotency by controlling general cell-vital programs that are critical for the rapid growth of pluripotent stem cells (Smith et al., 2011; Dejosez et al., 2010; Dejosez and Zwaka, 2012). Ronin (Thap11) belongs to this second class and is hence a suitable candidate for altering the DNA repair capacity of pluripotent stem cells. Ronin is a DNA-binding protein that is essential for pluripotent stem cells and is known to regulate various genes that are important for the cellular homeostasis of highly proliferative cells (Dejosez et al., 2010, 2008). Here, we provide evidence that Ronin also influences the DNA repair machinery of embryonic stem cells (ESCs). We show that Ronin regulates genes involved in the response to UV-C irradiation, and that conditional Ronin knockout increases the sensitivity of ESCs to DNA damage and activates the Atr-mediated DNA damage response. Our findings suggest that, along with lineage-specific transcription factors like Oct4 and Sox2, Ronin helps to maintain the uniquely robust genomic integrity of pluripotent stem cells.

2. Materials and methods

2.1. Cell culture

Ronin+/−, Ronin+/−; Cre-ERT2, and Ronin−/−; Cre-ERT2 mouse ESCs were derived (Dejosez et al., 2008) and cultured on 0.1% gelatin (Sigma) in DMEM + GlutaMAX (Invitrogen) supplemented with 10% FBS (Gemini), 2 mM l-glutamine (Invitrogen), 100 nM MEM non-essential amino acids (Invitrogen), 1000 U/ml LIF (Millipore), and 100 μM l-mercaptoethanol (Sigma).

2.2. DNA damage dose response curves

Cells were plated at a density of 1000 cells/20 cm², and treated for four days with ethanol (Sigma) or 0.25 μM 4-Hydroxytamoxifen (4HT) (Sigma). For γ-irradiation (γIR), the cells were irradiated in ESC medium using a 137Cs source at rates indicated. To induce DNA damage by UV-C irradiation, cells were washed twice in PBS (Invitrogen) and irradiated in the second PBS wash using a UV Stratalinker 2400 (Stratagene). Cells were then fed for an additional three days with ES cell medium supplemented with ethanol or 0.25 μM 4HT.

2.3. Serum starvation

Cells were plated in triplicate at 25,000 cells/10 cm² and fed for four days with ethanol or 0.25 μM 4HT, washed with PBS and fed for 36 or 72 h with ESC medium containing 0.1% FBS and either 0.25 μM 4HT or ethanol.

http://dx.doi.org/10.1016/j.scr.2017.06.014
1873-5061/© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
2.4. Colony and ViCell counting assays

For colony-counting assays, cells were washed in PBS, fixed in 2% PBS-buffered paraformaldehyde (Sigma), and stained with 3% Giemsa (Sigma). Colonies were counted and plotted relative to controls (mock-treated in PBS). For ViCell counting assays, cells were harvested by trypsinization and counted with a ViCell XR Cell Viability Analyzer (Beckman Coulter). Viable cells were plotted relative to untreated controls. Data indicate the mean ± s.e.m. of at least three independent experiments. Statistics analysis included a two-way ANOVA.

2.5. Neutral comet assay

Cells were plated at a density of 250,000 cells/20 cm² and fed for four days with ethanol or 0.25 μM 4HT. The medium was then changed to ES cell medium without ethanol or 4HT and the cells were γ-irradiated with 2.3 Gy. Following 4 or 6 h of recovery time, the cells were harvested and cell medium without ethanol or 4HT and the cells were

2.6. Western blots

To detect Cre-mediated Ronin knockout, cells were plated at a density of 250,000 cells/20 cm² and fed for four days with ES cell medium supplemented with ethanol or 0.25 μM 4HT. To detect protein expression after UV-C treatment, cells were plated at a density of 1.1 × 10⁶ cells/60 cm², fed as above for four days, and UV-C irradiated with 12 J/m². Cells were harvested by trypsinization, washed with PBS and whole-cell protein extracts were prepared and Western blots performed as described previously (Dejosez et al., 2010). Antibodies used were: Ronin/Thap11 (BD Biosciences), α-Tubulin (Sigma), p-Chk1 (Ser345, Cell Signaling Technology), Chk1 (Cell Signaling Technology), p-p53 (hSer15/mSer18, Abcam), p53 (Leica Biosystems), Anti-Rabbit IgG HRP Conjugate (Promega), and Anti-Mouse IgG HRP Conjugate (Promega). Signal intensities were quantified with the Image J software.

2.7. Flow cytometry of BrdU labeled cells

Cells were plated with a density of 300,000 cells/60 cm² and fed with ESC medium supplemented with ethanol or 0.25 μM 4HT for four days. The cells were washed twice with PBS and treated with 12 J/m² UV-C irradiation in the second PBS wash and allowed to recover in ESC medium for 9 h before they were pulse-labeled with BrdU as described by Savatier et al. (2002). 1–1.5 × 10⁶ cells were fixed in 70% ethanol and stained with an anti-BrdU antibody (BD Biosciences) and propidium iodide (Sigma) (Savatier et al., 2002). Cells were subjected to BrdU labeling with an LSRFortessa (BD Biosciences) and analyzed with FlowJo software (Tree Star, Inc.). Values indicate the mean ± s.e.m. of three independent experiments. Statistical analysis included a three-way ANOVA.

2.8. Bioinformatics analysis

Ronin targets with a known biological function were filtered for those falling within the GO term: Response to DNA damage stimulus (GO: 0006974) using PERL scripts. Biomial distribution analysis was conducted in Excel (Microsoft, Inc.).

2.9. Microarray

RNA was isolated with the RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions and subsequently hybridized to an Affymetrix array (Microarray Core Facility at Baylor College of Medicine). The array data were normalized and analyzed using Array Star software (DNA Star, Inc.).

2.10. ChIP-qPCR

Chromatin immunoprecipitation was conducted as described previously (Dejosez et al., 2010) with polyclonal rabbit anti-Ronin G4275 antiserum and rabbit G4275 preimmune serum. For verification of Ronin binding sites by qPCR, 2 μl of each ChIP product were used in triplicate reactions using Sybr Green PCR Master Mix (Applied Biosystems) in a final volume of 25 μl in the presence of 0.2 μM of each oligo (see Supplementary Table 1). Reactions were run on a 7900 Real-Time PCR system using standard reaction conditions (Applied Biosystems). qPCR was conducted on triplicate ChIP samples and normalized to input DNA. Values indicate the mean ± s.d.

2.11. qRT-PCR

RNA was isolated with the RNeasy Mini Kit (Qiagen) and 1 μg was reverse-transcribed into cDNA using the Improm-II Reverse Transcription System (Promega). For each qRT-PCR reaction, 2 μl of cDNA were used in triplicate reactions using Sybr Green PCR Master Mix (Applied Biosystems) in a final volume of 25 μl in the presence of 0.2 μM of each oligo (see Supplementary Table 1). Reactions were run on a 7900 Real-Time PCR system using standard reaction conditions (Applied Biosystems). Data were analyzed using 2−ΔΔCt for quantification of mRNA levels (i.e. normalization to β-Actin internal controls and subsequent normalization to EtOH-treated controls for each cell line). These normalized values for Ronin and Cre-ERT2 ESCs were then plotted relative to Ronin controls for each day of 4HT treatment. Values indicate the mean ± s.e.m. of three independent experiments. Statistical analysis included a two-way ANOVA followed by an unpaired Student’s t-test.

2.12. statistics

All statistics were conducted using SPSS software (IBM Corp.) or Excel (Microsoft Corp.)

3. Results

3.1. Ronin targets nucleotide excision repair and DNA damage genes

We examined Ronin (Dejosez et al., 2010) and Oct4 Chip-Seq data (Marson et al., 2008) from mouse ESCs to chart their DNA repair gene repertoires. We found that genes belonging to the gene ontology (GO) categories for “response to DNA damage stimulus” (GO:0006974) were enriched for the binding of Ronin (P = 0.0009) and Oct4 (P = 0.003) (Fig. 1A). Only four of these genes (Prpf19, Brc1, Rad51, and Rad18) are bound by both Ronin and Oct4, suggesting that the level of co-regulation between those two factors is low. Interestingly, the Ronin-bound genes fell into several broader DNA repair related categories, including homologous recombination repair (Brc1 and Rad51), nucleotide excision repair (Gtf2h4 and Ercc1), post-replication repair (Rad18 and Wmip1), and the DNA damage checkpoint (Claspin and Rad1). Genes in
Ronin and Hcf-1 at the promoter regions of DNA repair genes. (A) Binomial distribution analysis indicating enrichment for DNA repair genes among Ronin targets (P = 0.00086893) and Oct4 targets (P = 0.00347946). (B) ChIP-seq data indicating binding of Ronin and Hcf-1 at the promoter regions of Gtf2h4, Wnrn1, Rad18, and Ronin. (C) ChIP-qPCR analysis of Ronin-binding sites upstream of Gtf2h4, Wnrn1, Rad18, Ronin, and a non-binding site (NBS) as negative control. Values indicate the mean ± s.d. of triplicate samples.

3.2. Ronin is critical for the expression of Gtf2h4 and Rad18

To test whether the binding of Ronin to DNA repair genes is associated with their transcriptional regulation, we used Roninfl/fl control and Ronin Cre-ERT2 ESCs to conditionally delete Ronin with 4-hydroxytamoxifen (4HT). Cells were treated for four days and complete knockout of the Ronin protein was confirmed by western blot (Supplementary Fig. S1A). Using the same knockout strategy, gene expression was profiled by microarray analyses on days 1.5, 3, and 6 by comparing 4HT treated control cells with Ronin knockout cells (Fig. 2A). Our results indicated that, while overall gene expression changes were mild, some of the DNA repair genes that are bound by Ronin (Fig. 1B, C), including Gtf2h4 (P = 0.05), Rad18 and Wnrn1 were indeed downregulated upon Ronin knockout. qRT-PCR experiments validated reduced expression of Gtf2h4 and Rad18 in the absence of Ronin. In contrast, Wnrn1 was not significantly downregulated, suggesting that Ronin is not a major regulator of Wnrn1 expression (Fig. 2B). Notably, the level of Oct4 and other pluripotency related factors (Li and Belmonte, 2017) were not altered by Ronin knockout (Fig. 2B, Supplementary Table 2), indicating that the observed decrease in expression of these DNA repair genes was not a secondary consequence of differentiation.

3.3. Ronin knockout increases sensitivity to ionizing and UV-C irradiation

As Ronin knockout reduced the transcription of genes involved in nucleotide excision repair and replication fork stalling, we next investigated the effects of Ronin loss on the DNA damage response in ESCs. We used ionizing radiation (γIR) to induce double-strand breaks and UV-C irradiation to cause DNA helix-distorting lesions that arrest DNA replication (Eppink et al., 2011; Mladenov and Iliaakis, 2011) (see Supplementary Fig. S1B for experimental outline). Interestingly, Ronin loss increased sensitivity to γIR and UV-C (Fig. 3A), while DNA damage-independent serum starvation-induced cellular stress (Brooks, 1976; Joza et al., 2001) and Cre recombinase-induced genotoxic stress (Loonstra et al., 2001), had no effect on Ronin knockout cells (Supplementary Fig. S2). Consistent with this observation, the repair of the γIR induced double strand breaks measured in neutral comet assays (Banath et al., 2009) was impaired in Ronin-knockout cells compared with control cells 6 h after irradiation (Fig. 3B, C and Supplementary Fig. S3). Together, our results indicate that Ronin knockout increases the cellular sensitivity to DNA damage that induces helix-distorting lesions and double-strand breaks.

3.4. Ronin knockout increases Atr-dependent DNA damage checkpoint activation and G2/M arrest after DNA damage

Although failure of the nucleotide excision system may account for the increased DNA damage sensitivity of Ronin-knockout ESCs, we speculated that other systems could be responsible as well. It is known that stalled replication forks resulting from UV-C damage and other genotoxins activate a DNA damage checkpoint that involves Atr kinase and Chk1 (Eppink et al., 2011; Heffernan et al., 2002; Liu et al., 2000). Hence, Ronin-depleted cells could be unable to properly engage the DNA damage checkpoint, or they could fail to recover stalled replication forks (Eppink et al., 2011; Heffernan et al., 2002; Liu et al., 2000). Thus, we first assessed the extent of DNA damage checkpoint activation by examining Chk1 phosphorylation at serine 345 (Niida et al., 2007). We
found that the levels of phosphorylated Chk1 were higher in Ronin-knockout cells than in control cells beginning at 1 h after UV-C damage (Fig. 4A, Supplementary Fig. S4). To further test the contribution of stalled replication forks, we looked at another Atr target, p53, which is phosphorylated in response to stalled replication forks (Tibbetts et al., 1999). As previously reported, both phosphorylated and basal p53 protein levels increased in response to UV-C damage (Chao et al., 2000; Corbet et al., 1999). However, we found that the levels of serine 18-phosphorylated p53 did not differ between Ronin-knockout and control ESCs (Fig. 4A, Supplementary Fig. S4), likely due to the increase in basal p53 levels in both conditions. Moreover, when we examined changes in the cell cycle, we observed that UV-C-exposed Ronin-knockout ESCs were more likely to be found in G2/M and less likely to be in S phase, compared to control cells (Fig. 4B, C). Together our results indicate that Ronin knockout was associated with a sustained and elevated DNA checkpoint activation as well as an increase of cells within G2/M after UV-C damage.

4. Discussion

We herein use Ronin-knockout ESCs to show that Ronin adjusts the cellular DNA damage response of pluripotent cells to the more stringent needs of the early embryo. We report evidence suggesting that Ronin may exert its effects on the DNA repair capacity by transcriptionally regulating DNA repair genes. The important role of Ronin during early embryogenesis and the lethal phenotype associated with its knockout fit this model (Dejosez et al., 2008). DNA maintenance and repair are costly in terms of their energy requirements, biomass needs, and the number of involved proteins, which must be provided in a manner appropriate to other stringent needs of the early embryo (Dejosez et al., 2010; Vander Heiden et al., 2009). Not all cells require their DNA integrity to be so robustly maintained. For example, non-cycling cells have relatively low needs for DNA repair, and DNA damage is not as consequential. On the other hand, highly proliferative cells, such as those of the embryo, have rapid and abbreviated cell cycles that increase their sensitivity to blocked DNA replication (Bielas and Heddle, 2004; Harfouche and Martin, 2010; Mandal et al., 2011; McKinnon, 2009). This weakness reflects a finely tuned balance of DNA repair, cell cycle arrest, and apoptosis (Corbet et al., 1999; de Waard et al., 2008; Savatier et al., 2002; White and Dalton, 2005). The results of our present study suggest that Ronin is part of this fine-tuning system, as its loss is specifically associated with particular aspects of DNA damage sensitivity. Indeed, as Ronin is expressed in oocytes (Dejosez et al., 2008), our observation might extend to germ cells that have been shown to share the high levels of genomic integrity seen in ES cells (Murphey et al., 2013).

Although our results suggest that Ronin directly impacts DNA repair through the transcriptional regulation of DNA repair genes, we further sought to determine how much the canonical pluripotency factors might contribute to the transcriptional regulation of DNA repair genes. Oct4 and Sox2 are known to bind upstream of the Gtf2h4 and Rad18 genes (Marson et al., 2008), yet the levels of Oct4 and Sox2 were not affected by Ronin knockout on transcriptional level in our microarray...
This observation applies to other pluripotency related factors as well (Supplemental Table S2; Li and Belmonte, 2017). Additionally, Nanog, Suz12 and Tcf3 are also known not to bind to Gtf2h4 or Rad18 (Dejosez et al., 2010). While our results do not formally exclude the involvement of these or other factors, they support the notion that Ronin plays a decisive role in the transcriptional regulations of Rad18 and Gtf2h4. We previously proposed a model in which Ronin regulates its target genes by recruiting histone-modifying enzymes via an interaction with Hcf-1 (Dejosez et al., 2010, 2008). Because Gtf2h4 and Rad18 are bound by Ronin and downregulated upon Ronin knockout, transcriptional regulation of Gtf2h4 and Rad18 could be mediated in part through this mechanism.

Stalled replication forks resulting from UV-C damage and other genotoxins are known to activate a DNA damage checkpoint that involves Atr and Chk1 (Heffernan et al., 2002; Liu et al., 2000). The increase in phospho-Chk1 in Ronin knockout cells after UV-C damage may be explained by an increase in unrepaired damage and/or the inability of DNA replication to proceed past UV-C damage (Coin et al., 2007; Tateishi et al., 2003). Furthermore, it is known that knockout of genes in ESCs that are involved in nucleotide excision repair (e.g. Xpc) leads to slowed S phase progression and G2/M arrest after UV-C damage (de Waard et al., 2008). Those observations are in line with our results in Ronin - knockout cells, suggesting that nucleotide excision repair defects contributed to the increased Chk1 phosphorylation and G2/M accumulation observed in our present study.

Ronin belongs to a unique protein family characterized by a highly conserved THAP DNA binding domain (Roussigne et al., 2003). The Thap family proteins arose through a process called "molecular domestication," beginning from an ancient DNA transposon whose modern-day descendent is the P-element transposase (Hammer et al., 2005). As transposition of such elements involves DNA repair, we cannot exclude the possibility that Ronin in addition to its function as a transcriptional regulator (Dejosez et al., 2010; Sabogal et al., 2010) may play a more direct role in the DNA damage response (Weinert et al., 2005). Additionally, other Thap family members, including Thap5, have been suggested to play pro-apoptotic roles in the responses to UV-C irradiation and other sources of stress (Balakrishnan et al., 2011, 2009) and Thap9 was directly shown to have DNA nuclease activity, making this possibility even more likely (Majumdar et al., 2013).

5. Conclusion

In summary, we show Ronin-knockout ESCs to exhibit reduced expression of factors that respond to UV-C damage, as well as increased phospho-Chk1 and G2/M arrest. Future work is warranted to examine whether Ronin performs comparable functions in other cell types. Given the significant differences between ESCs and somatic cells in terms of their DNA repair capacities, spontaneous mutation rates, and cell cycle structures, the insights gained here may not apply universally (Tichy, 2011). However, we think that these findings could be relevant to other highly proliferative cell types, such as tumor cells.

Author contributions

BS and MD designed and performed experiments. TZ supervised all experiments and provided funding. BS, MD and TZ wrote the manuscript.

Conflict of interest

We report no conflict of interest in conducting the work within this manuscript.
Acknowledgements

This work was supported by the Huntington Foundation grant PD14-03316, and National Institutes of Health grant R01 GM077442.

Appendix A. Supplementary data

Supplementary material

References

Balakrishnan, M.P., Citeni, L., Mashak, Z., Popat, P., Alnemri, E.S., Zervos, A.S., 2009. THAP5 is a human cardiac-specific inhibitor of cell cycle that is cleaved by the proapoptotic Omi/HtrA2 protease during cell death. Am. J. Physiol. Heart Circ. Physiol. 297: H643–H653. http://dx.doi.org/10.1152/ajpheart.00234.2009.

Balakrishnan, M.P., Citeni, L., Ambrivisto, C., Goto, Y., Takata, M., Turkson, J., Li, X.S., Zervos, A.S., 2011. THAP5 is a DNA-binding transcriptional repressor that is regulated in melanoma cells during DNA damage-induced cell death. Biochem. Biophys. Res. Commun. 404:195–200. http://dx.doi.org/10.1016/j.bbrc.2010.11.092.

Banath, J.P., Banuelos, C.A., Klokov, D., MacPhail, S.M., Landesberg, P.M., Olive, P.L., 2009. Explanation for excessive DNA single-strand breaks and endogenous repair foci in pluripotent mouse embryonic stem cells. Exp. Cell Res. 315, 1505–1520.

Bielas, J.H., Heddle, J.A., 2004. Quiescent murine cells lack global genomic repair but are proficient in transcription-coupled repair. DNA Repair (Amst) 3:711–717. http://dx.doi.org/10.1016/j.dnarep.2004.02.010.

Brooks, R.F., 1976. Regulation of the fibroblast cell cycle by serum. Nature 260:248–250. http://dx.doi.org/10.1038/260248a0 (Published online: 18 March 1976).

Chin, C., Saito, S., Anderson, CW., Appella, E., Xu, Y., 2000. Phosphorylation of murine p53 at ser-18 regulates the p53 responses to DNA damage. Proc. Natl. Acad. Sci. U. S. A. 97:11936–11941. http://dx.doi.org/10.1073/pnas.220522997.

Chini, C.C.S., Chen, J., 2003. Human claspin is required for replication checkpoint control. J. Biol. Chem. 278:30057–30062.

Coint, F., Okseny, V., Egly, J.-M., 2007. Distinct roles for the XBP/p52 and XPD/p44 subcomplexes of TFIIH in damaged DNA opening during nucleotide excision repair. Mol. Cell 26:245–256. http://dx.doi.org/10.1016/j.molcel.2007.03.005.

Corbet, S.W., Clarke, A.R., Gledhill, S., Wyllie, A.H., 1999. P53-dependent and -independent pathways of homologous recombination. DNA Repair (Amst) 10:1095–1105. http://dx.doi.org/10.1016/S1567-4847(01)00015-9.

Dorn, M., Zawaka, T.P., 2012. Pluripotency and nuclear reprogramming. Annu. Rev. Biochem. 81:737–765. http://dx.doi.org/10.1146/annurev-biochem-052709-104948.

Dejosez, M., Zawaka, T.P., 2010. Ronin/Hcf-1 binds to a hyperconserved enhancer element and regulates genes involved in the growth of embryonic stem cells. Genes Dev. 24:1479–1484. http://dx.doi.org/10.1101/gad.1935210.

Eppink, B., Tafel, A.A., Hanada, K., van Drunen, E., Hickson, I.D., Essers, J., Kanar, R., 2011. The response of mammalian cells to UV-light reveals Rad54-dependent and independent pathways of homologous recombination. DNA Repair (Amst) 10:1195–1105. http://dx.doi.org/10.1016/j.dnarep.2011.08.006.

Hammer, S.E., Strehl, S., Hagemann, S., 2005. Homologs of Drosophila P transposons were mobile in zebrafish but have been domesticated in a common ancestor of chicken and human. Mol. Biol. Evol. 22:833–844. http://dx.doi.org/10.1093/molbev/msi012.

Harfouche, G., Martin, M.T., 2010. Response of normal stem cells to ionizing radiation: a crosstalk between DNA-damage, apoptosis and mutation frequency in ES cells. Oncogene 29:3951–3958. http://dx.doi.org/10.1038/onc.2010.222.

Hefferman, T.P., Simpson, D.A., Frank, A.R., Heinloth, A.N., Paules, R.S., Cordeiro-Stone, M., Kaufmann, W.K., 2002. An ATR- and Chk1-dependent S checkpoint inhibitor blocks initiation following UV- and DNA damage. Mol. Cell. Biol. 22, 8552–8561.

Ho, N., Sain, S.A., Daugas, E., Stanford, W.L., Cho, S.K., Li, C.Y., Sasaki, T., Elia, A.J., Cheng, H.Y., Ravagnan, L., et al., 2001. Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. Nature 410, 549–554.

Li, M., Belmonte, J.C., 2017. Ground rules of the pluripotency gene regulatory network. Nat. Rev. Genet. 18:180–191. http://dx.doi.org/10.1038/nrg.2016.156.

Liu, Q., Gantuku, S., Cui, X.S., Matsuoka, S., Cortez, D., Tanai, K., Luo, G., Carattini-Rivera, S., DeMayo, F., Bradley, A., Donehower, LA., Elledge, S.J., 2000. Chk1 is an essential kinase that is regulated by Atr and required for the G(2)/M DNA damage checkpoint. Genes Dev. 14, 1448–1459.

Loosntra, A., Vooijs, M., Beverloo, H.B., Allak, B.A., van Drunen, E., Kanaar, R., Berns, A., Jonkers, J., 2001. Growth inhibition and DNA damage induced by Cre recombinase in mammalian cells. Proc. Natl. Acad. Sci. U. S. A. 98:3209–9214. http://dx.doi.org/10.1073/pnas.103508798.

Majumdar, S., Singh, A., Ria, D.C., 2013. The human THAP5 gene encodes an active P-element DNA transposase. Science 339:446–448. http://dx.doi.org/10.1126/science.1217190.

Mani, P.K., Blanpain, C., Rossi, D.J., 2011. DNA damage response in adult stem cells: pathways and consequences. Nat. Rev. Mol. Cell Biol. 12:198–202. http://dx.doi.org/10.1038/nrm3060.

Marinoni, J.C., Roy, R., Vermeulen, W., Mininou, P., Lutz, Y., Weeda, G., Seroz, T., Gomez, D.M., Hoeijmakers, J.H., Egli, J.M., 1997. Cloning and characterization of p52, the...
fifth subunit of the core of the transcription/DNA repair factor TFIIH. EMBO J. 16: 1093–1102. http://dx.doi.org/10.1093/emboj/16.5.1093.

Marson, A., Levine, S.S., Cole, M.F., Frampton, C.M., Brambrink, T., Johnstone, S., Guenther, M.G., Johnston, W.K., Wernig, M., Newman, J., Calabrese, J.M., Dennis, L.M., Volkert, T.L., Gupta, S., Love, J., Hannett, N., Sharp, P.A., Bartel, D.P., Jaenisch, R., Young, R.A., 2008. Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells. Cell 134:521–533. http://dx.doi.org/10.1016/j.cell.2008.07.020.

McKinnon, P.J., 2009. DNA repair deficiency and neurological disease. Nat. Rev. Neurosci. 10:100–112. http://dx.doi.org/10.1038/nrn2559.

Mladenov, E., Iliakis, G., 2011. Induction and repair of DNA double strand breaks: the increasing spectrum of non-homologous end joining pathways. Mutat. Res. 711:61–72. http://dx.doi.org/10.1016/j.mrfmmm.2011.02.005.

Murphy, 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. http://dx.doi.org/10.1095/biolreprod.112.103481.

Ng, H.-H., Surani, M.A., 2011. The transcriptional and signalling networks of pluripotency. Nat. Cell Biol. 13:490–496. http://dx.doi.org/10.1038/ncll0511-490.

Niida, H., Katsu, Y., Banerjee, B., Hande, M.P., Nakanishi, M., 2007. Specific role of Chk1 phosphorlyiations in cell survival and checkpoint activation. Mol. Cell. Biol. 27: 2572–2581. http://dx.doi.org/10.1128/MCB.01611-06.

Olive, P.L., Banath, J.P., 1993. Detection of DNA double-strand breaks through the cell cycle after exposure to X-rays, bleomycin, etoposide and 125I-dUrd. Int. J. Radiat. Biol. 64, 349–358.

Roussigné, M., Kossida, S., Lavigne, A.-C., Clouaire, T., Ecochard, V., Glories, A., Amalric, F., Girard, J.-P., 2003. The THAP domain: a novel protein motif with similarity to the DNA-binding domain of P element transposase. Trends Biochem. Sci. 28:66–69. http://dx.doi.org/10.1016/S0968-0004(02)00011-3.

Sabogal, A., Lyubimov, A.Y., Corn, J.E., Berger, J.M., Rio, D.C., 2010. THAP proteins target specific DNA sites through bipartite recognition of adjacent major and minor grooves. Nat. Struct. Mol. Biol. 17:117–123. http://dx.doi.org/10.1038/nstrb.1742.

Savatier, P., Lapillonne, H., Jirmanova, L., Vitelli, L., Samarut, J., 2002. Analysis of the cell cycle in mouse embryonic stem cells. Methods Mol. Biol. 185, 27–33.

Smith, K.N., Lim, J.-M., Wells, L., Dalton, S., 2011. Myc orchestrates a regulatory network required for the establishment and maintenance of pluripotency. Cell Cycle 10, 592–597.

Tateishi, S., Miyaizaki, J.-L., Fujimoto, S., Inoue, H., Yamaizumi, M., 2003. Enhanced genomic instability and defective postreplication repair in RAD18 knockout mouse embryonic stem cells. Mol. Cell. Biol. 23, 474–481.

Tibbetts, R.S., Brumbaugh, K.M., Williams, J.M., Sarkaria, J.N., Ciby, W.A., Shiie, S.Y., Taya, Y., Prives, C., Abraham, R.T., 1999. A role for ATR in the DNA damage-induced phosphorylation of p53. Genes Dev. 13, 152–157.

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

Vander Heiden, M.G., Cantley, L.C., Thompson, C.B., 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324:1029–1033. http://dx.doi.org/10.1126/science.1160809.

de Waard, H., Sonneveld, E., de Wit, J., Esvedt-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.

Weinert, B.T., Min, B., Rio, D.C., 2005. P element excision and repair by non-homologous end joining occurs in both G1 and G2 of the cell cycle. DNA Repair (Amst) 4: 171–181. http://dx.doi.org/10.1016/j.dnarep.2004.09.004.

White, J., Dalton, S., 2005. Cell cycle control of embryonic stem cells. Stem Cell Rev. 1: 131–138. http://dx.doi.org/10.1385/SCR:1:2:131.

Yoshimura, A., Seki, M., Kanamoto, M., Tateishi, S., Tsurimoto, T., Tada, S., Enomoto, T., 2002. Physical and functional interaction between WRNIP1 and RAD18. Genes Genet. Syst. 84, 171–178.