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Genomic Integrity of Mouse Embryonic Stem Cells

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1. Introduction

Embryonic stem (ES) cells are isolated from the inner cell mass (ICM) of a blastocyst stage embryo, which consists of a layer of trophoblast cells lining the ICM and blastocoel or blastocyst cavity. The ICM and trophoblast cells give rise to the embryo proper and extra-embryonic tissue, respectively. Thirthy years ago the *in vitro* culture of mouse ES (mES) cells was first described (Evans and Kaufman, 1981; Martin, 1981) and later in 1998 also human ES (hES) cells were derived (Thomson et al. 1998). ES cells are characterized by the unique properties of unlimited self-renewal without senescence and pluripotency. The latter infers that ES cells give rise to all cell types of the body. These specific properties led to the great scientific interest in ES cell either for their potential medical applications or as models to address more fundamental questions in development.

Maintenance of the genomic integrity of ES cells is of major importance considering that these cells are the precursors of all cells making up the adult body. Any unrepaired DNA damage at the ES cell level could lead to mutations, giving rise to congenital disorders or embryonic lethality. Indeed, a lower spontaneous mutation frequency has been observed in mouse ES (mES) cells compared to somatic cells (Cervantes et al. 2002). Mutation frequencies are generally quantified using mutation reporter genes such as adenine phosphoribosyltransferase (Aprt), located on chromosome 8 or hypoxanthine-guanine phosphoribosyltransferase (Hprt), located on the X chromosome, which encode ubiquitously expressed purine salvage enzymes. Mutations at the heterozygous Aprt or hemizygous Hprt locus, leading to loss of the enzyme activity, can be detected based on the resistance of the cells to toxic purine analogs, such as 2-fluoroadenine or 2,6- diaminopurine for Aprt and 6-thioguanine, 8-azaguanine and 6-mercaptopurine for Hprt. The Aprt system allows detection of point mutations, small deletions/insertions or larger chromosomal events, such as mitotic recombination, chromosome loss and multilocus deletions, all leading to loss of heterozygosity (LOH). Hprt mutations are restricted to intragenic events and cannot be caused by large chromosomal changes such as multilocus deletions or chromosome loss. As it is X-linked and hemizygous Hprt cannot undergo mitotic recombination in XY mES cells.

The spontaneous mutation frequencies at the heterozygous Aprt locus of mES cells were shown to be markedly lower compared to somatic cells (mouse embryonic fibroblasts) i.e.

10^{-6} and 10^{-4}, respectively (Cervantes et al. 2002). Besides the 100-fold lower mutation frequency observed in mES cells, a different origin of the mutations was noted. Both for somatic and mES cells mutations were attributed to 80% of LOH and 20% of point mutations. However, in somatic cells the LOH was the result of mitotic recombination while in mES cells the cause of LOH was more diverse. Mitotic recombination, multilocus deletions and chromosome loss/nondisjunction accounted for 41%, 2% and 57% of the LOH, respectively (Cervantes et al. 2002). Moreover, no spontaneous mutations ($<10^{-8}$) were observed in the hemizygous Hprt locus of mES cells compared to $\sim10^{-5}$ in mouse embryonic fibroblasts (MEF). Although no spontaneous Hprt mutations were recorded in mES cells, these cells are able to undergo Hprt mutations as evidenced by the dose dependent increase upon treatment with alkylating agents such as ethyl methanesulfonate (EMS) and N-ethyl-N-nitrosurea (ENU) (Chen et al. 2000; Cervantes et al. 2002).

A lower mutation frequency could be the result of a lower sensitivity of the cells to a genotoxic insult, caused by better protective mechanisms such as f.e. antioxidant defences, an increased repair capacity compared to somatic cells or additional mechanisms for the prevention of mutation events (induction of apoptosis and /or differentiation). Cairns proposed in 1975 the immortal strand hypothesis as a mechanism to avoid mutations in adult stem cells. This hypothesis postulates that adult stem cells have a specific mechanism for DNA segregation where the template DNA is retained by one daughter cell, the self renewing stem cell, and the newly synthesised DNA potentially containing replication errors segregates to the differentiating daughter cell (Cairns, 2006; Rando et al. 2007; Lew et al. 2008). There is evidence supporting the immortal strand hypothesis in some cell types such as muscle stem cells (Conboy et al. 2007) and intestinal stem cells (Potten et al. 2002). There is, however, no evidence to assume that this immortal strand hypothesis is also at play in embryonic stem cells (Lansdorp, 2007).

This chapter focuses on the mechanisms responsible for the lower mutation frequency in mES cells. mES cells and somatic cells will be compared on basis of the extent of DNA damage, the cell cycle control mechanisms that are involved, the efficiency of the DNA repair and apoptosis induction. Furthermore mES cells have an additional mechanism to avoid passing on mutations to their progeny, i.e. induction of differentiation. A review of these issues in other embryonic stem cell types, more specifically hES cells and induced pluripotent stem cells, will be briefly discussed. Finally, the relation between these mES cell features and the *in vivo* situation will be described.

2. DNA damage, DNA repair mechanisms and cell cycle control

2.1 DNA damage

DNA damaging agents can arise from endogenous (e.g. reactive oxygen/nitrogen species (ROS/RNS)) or exogenous sources (alkylating agents, irradiation,…) leading to different DNA lesions, including base modifications, alkali-labile sites, single and double strand breaks, bulky adducts, intra- and inter-strand crosslinks. In this section the cell survival of mES cells and somatic cells exposed to these endogenous and exogenous genotoxicants has been evaluated.

Endogenous DNA damaging agents are ROS or RNS, which are the result of cellular metabolism. Among the ROS, hydroxyl radicals (HO·), peroxynitrite (ONO_2^-) and the

diffusible hydrogen peroxide (H_2O_2) are inducing base oxidation (Marnett, 2000). Challenging mES cells with ± 50 µM H_2O_2 showed an approximately 50% reduction of cell viability after 24h as assessed by toluidine blue staining, that stains dead cells (Guo et al. 2010). This concentration falls within the range of EC50-values (concentration at which a 50% effect is observed) in differentiated cell types (Table 1). Fifty percent toxicity was observed at a concentration of 30 µM in mouse leukemic P388 cells (Kanno et al. 2003) and at a concentration above 200 µM in two human gastric adenocarcinoma cell lines, MKN-45 and 23132/87 (Gencer et al. 2011). Based on this data no marked difference in cell survival between mES cells and somatic cell lines can be concluded.

	Cell type	EC50 (µM)	Test method	Reference
mES cells				
	ns	± 50	Toluidine blue staining	Guo et al. 2010
Somatic cells				
	P388 mouse leukemic cells	30	MTT assay	Kanno et al. 2003
	human gastric adenocarcinoma cell line MKN-45	> 200	MTT assay	Gencer et al. 2011
	human gastric adenocarcinoma cell line 23132/87	> 200	MTT assay	Gencer et al. 2011

Table 1. Comparison of the EC50 after 24h treatment of mES cells and somatic cells with H_2O_2. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; ns, not specified.

The cell survival of mES cells after an exogenous genotoxic insult compared to somatic cells has been investigated for a number of genotoxicants. Comparison with mouse embryonic fibroblasts (MEF) revealed a lower cell survival of mES cells after γ-ray irradiation, inducing oxidative lesions and DNA breaks, after mitomycin C treatment, inducing interstrand crosslinks, mono adducts and oxidative DNA damage and after UV irradiation, inducing DNA lesions with DNA helix distorting properties such as cyclobutane pyrimidine dimers and (6-4) photoproducts (van Sloun et al. 1999; de Waard 2008). Also treatment with N-methyl-N-nitro-N-nitrosoguanidine, that induces a whole range of DNA lesions including O[6]-methylguanine that leads to DNA mispairing, increased cytotoxicity in mES cells compared to Swiss Albino 3T3 mouse fibroblasts (SA 3T3 cells) (Roos et al. 2007).

However, one should not confound cell viability with sensitivity of the cells. The sensitivity of the mES cells should be related to the amount of DNA damage induced. Unfortunately, studies comparing the extent of DNA damage in mES cells with somatic cells under the same experimental conditions are scarce.

Under cell culture conditions (pO_{2gas} of 142mm Hg) Guo et al. demonstrated that 25 µM of H_2O_2 did not induce increases in relative tail length in mES cells as measured by alkaline comet assay. This assay enables the detection of single and double strand breaks as well as alkali-labile sites. The methodology is based on the migration of DNA by electrophoresis,

revealing a higher capacity for DNA migration with increased DNA strand breaks and alkali-labile sites (Box1). However, 85 and 150 µM induced a 1.3 and 1.6 fold induction of relative tail length. When combining the Comet assay with formamidopyrimidine DNA glycosylase (FPG) enzymatic treatment, which enables the detection of oxidized purines, a 1.6 and 2.3 fold induction was observed after treatment with 85 and 150 µM H_2O_2 respectively (Powers et al. 2008). In contrast, in P388 cells an increase in DNA migration of approximately 15-fold and 30-fold has been observed after 1h incubation with 30µM and 100µM H_2O_2, respectively (Kanno et al. 2003).

Combining the data on cell survival and amount of DNA damage obtained by the alkaline comet assay, one can conclude that mES cells are indeed more sensitive than differentiated or somatic cells, as fewer lesions lead to similar cell toxicity. In agreement with this, upon UV-C treatment, in mES cells only half of photoproducts (cyclobutane pyrimidine dimers and (6-4) photoproducts) are induced (Van Sloun 1999) and at the same time a higher level of cell death is observed compared to somatic cells (de Waard et al. 2008). Therefore the same amount of DNA lesions, induces a higher level of cell death in mES cells compared to somatic cells. Moreover, by using cell survival to compare the sensitivity of cell types, the sensitivity is underestimated. However, to enable a sound comparison of the sensitivity of mES cells and somatic cells, one should perform the appropriate genotoxicity assays at the appropriate dose (Box1).

Box 1: Commonly-used genotoxicity assays

Alkaline comet assay

The comet or single-cell gel electrophoresis assay was developed during the late 1970s and 1980s. The main principle of the methodology is that when single and/or double strand DNA breaks are induced, this leads to increased relaxation of the supercoiled DNA forming DNA loops. These relaxed negatively-charged DNA loops migrate to a higher extent towards the positive pole compared to supercoiled DNA during electrophoresis, resulting in the characteristic 'comet tails' (Collins et al. 2008).

Several variations on the methodology exist. The methodology that is used most commonly to date was described by Singh et al. in 1988. This comet assay, also referred to as the alkaline comet assay, introduces electrophoresis at alkaline conditions (pH > 13). The alkaline comet assay enables the detection of single (SS) and double strand (DS) DNA breaks as well as alkali-labile sites. Other variations are the neutral comet assay and the neutral comet assay with a lysis step at 50°C. Both variations are able to detect DS breaks, however the lysis at high temperature disrupts the nuclear matrix, thereby eliminating interference of SS breaks (for review Collins, 2004; Møller, 2006). The extent of DNA damage can be expressed in different ways, i.e. tail length (TL), percentage of tail DNA (%TD) or tail moment (TM). Tail moment is the TL multiplied by % TD. Several arguments are in favor of the use of % TD. De Boeck et al. (2000) demonstrated less inter-electrophoresis and inter-experimenter variability when using % TD compared to TL (De Boeck et al. 2000). Collins (2004) argues that TL can be useful at low DNA damage levels, but not at higher levels of DNA damage and that TL is more sensitive to background and threshold settings of the image analysis (Collins, 2004). Furthermore the %TD has a linear dose-response relationship with known DNA break-inducing agents (Collins, 2004; Møller, 2006).

Additional use of enzymes enables the detection of specific lesions. The most commonly used enzymes are endonuclease III (endoIII) for the detection of oxidized pyrimidines, formamidopyrimidine DNA glycosylase (FPG) and human 8-hydroxyguanine DNA glycosylase (hOGG1) for the detection of oxidized purines, T4 endonuclease V for the detection of UV-induced cyclobutane pyrimidine dimers and Alk A for the detection of 3-methyladenines. Each of these enzymes introduces a strand break at the enzyme-sensitive site (for review Collins, 2004). Smith et al. (2006) found that hOGG1 detected oxidized purines with greater specificity and sensitivity compared to endoIII and FPG. Recently, the European Comet Assay Validation Group (ECVAG) performed a study for validation of the comet assay. The inter-laboratory study retrieved dose-response relationships for oxidative DNA damage by assessment of FPG sensitive-sites in coded samples (Johansson et al. 2010; Møller et al. 2010).

It has been shown that mES have a high level of spontaneously induced DNA strand breaks as detected by alkaline comet assay. However, global chromatin decondensation seems involved rather than high levels of DNA strand break formation (Banath et al. 2009).

In vitro **micronucleus assay**

Micronuclei (MN) are small, extra-nuclear bodies, containing chromosome/chromatid fragments or entire chromosomes/chromatids. MN are formed during cell division, when during anaphase chromosome/chromatid fragments or entire chromosomes/chromatids are not pulled to the spindle poles and lag behind. These acentric fragments or entire chromosomes/chromatids are not incorporated in the two daughter nuclei when the nuclear envelope is reassembled during telophase. MN can occur spontaneously or can be mutagen-induced. Exposure to clastogen can lead to MN containing acentric chromosome or chromatid fragments through different mechanisms. Misrepair of double strand breaks, simultaneous base excision repair in close proximity and on opposite complementary DNA strands and fragmentation of nucleoplasmic bridges may lead to the formation of acentric chromosome/chromatid fragments (for review Fenech et al. 2011; Kirsch-Volders et al. 2011a, 2011b).

Exposure to aneugens leads to MN containing entire chromosomes. Several mechanisms are responsible for aneuploidy. Hypomethylation of cytosine in centromeric and pericentromeric regions lead to chromosome malsegregation/loss probably due to defects in kinetochore assembly. Defects in spindle assembly, mitotic checkpoints and centrosome amplification are also related to increased incidence of aneuploidy. Furthermore dicentric chromosomes, when the centromeres are pulled to opposite poles, can detach from the spindle during anaphase and lead to chromosome-containing MN (for review Fenech et al. 2011; Kirsch-Volders et al. 2011a).

Since cell division is a prerequisite for MN expression, identification of mitosis is crucial. Until 1985, the method was hampered by the difficulty to identify the cells that divided in culture. Fenech and Morley introduced in 1985 the cytokinesis block in the methodology. The CBMN assay is based on the addition of cytochalasin-B, an actin inhibitor and therefore an inhibitor of cytokinesis, allowing the discrimination between cells that did not divide (mononucleated cells or MONO) and cells that divided once (binucleated cells or BN) or more (multinucleated cells) during *in vitro* culture. MN in mononucleated cells can represent the background frequency of MN (the frequency of MN that was present before

treatment when considering cell lines or before *in vitro* culture when considering primary cells) or cells that did escape cytokinesis block (Kirsch-Volders and Fenech, 2001). In addition, mononucleated cells with MN can be indicative of mitotic slippage (Elhajouji et al. 1998). In the absence of a functional spindle, cells can exit mitosis without chromatid segregation and immediately proceed to the next interphase, yielding tetraploid cells. This was shown by Elhajouji et al. (1998) in lymphocytes after treatment with nocodazole (Elhajouji et al. 1998).

The number of mono-, bi- and multinucleated cells allows the calculation of the cytokinesis-block proliferation index or CBPI, a measure for cell proliferation, which is a requirement for the expression of MN. Besides MN, other biomarkers of cytogenetic damage (NPB and NBUD) as well as apoptosis and necrosis can be evaluated simultaneously. Discrimination between MN containing acentric fragments and whole chromosomes/chromatids provides useful information on the mode of action of the mutagen and hence, its classification as a clastogen or aneugen, respectively. Information on the MN content can be obtained in different ways. The use of antibodies against the kinetochore has the disadvantage that with this technique whole chromosomes with defective centromeres, and hence, absent centromeres are not detected. Furthermore, test chemicals interfering with mRNA responsible for kinetochore protein production could lead to false negatives when using antibodies against the kinetochore. The size of the MN can be indicative of its content, but is not definitive. The most commonly used method fluorescence *in situ* hybridisation (FISH) with pancentromeric or chromosome specific probes. Pancentromeric probes allow discrimination between centromere-negative MN, indicating clastogenicity, and centromere-positive MN, indicating aneugenicity. Chromosome-specific probes can additionally detect non-disjunction or unequal distribution of chromosomes in the two daughter nuclei (Elhajouji et al. 1995, 1997).

No data is available on micronucleus frequencies in mES cells, either spontaneously induced frequencies (or background levels) or frequencies induced by genotoxicants. This assay has the great advantage to discriminate chromosome breakage and chromosome loss events and could therfore greatly contribute to our understanding of DNA damage responses.

Detection of γ-H2AX

Histone protein H2AX is phosphorylated on serine 139 (γ-H2AX) at sites flanking double strand breaks. Detection of these γ-H2AX foci, using antibodies against the phosphorylated form of the protein, can be used as a measure of double strand breaks in cells. These foci can be quantified by microscopical methods or FACS analysis. It has been shown that mES cells show a high number of spontaneous γ-H2AX foci, which appears to be related to global chromatin decondensation rather than spontaneous or pre-existing DNA damage (Banath et al. 2009).

2.2 Repair mechanisms

Repair of DNA lesions is achieved through different repair mechanisms, i.e. base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and double strand break repair (DSBR) or the combinations of different repair mechanisms dependent on the

type of damage. Lesions where only one of the two DNA strands are affected are repaired by base excision repair (BER) or nucleotide excision repair (NER). BER is involved in the repair of lesions as a result of oxidative damage, alkylation, deamination and depurination/depyrimidination and single strand breaks (SSB). NER repairs lesions that impair transcription and replication by interfering with the DNA helical conformation, such as bulky adducts, intra- and interstrand crosslinks, UV induced pyrimidine dimers and photoproducts. Some oxidative lesions, such as cyclopurines are repaired by NER, either by global genome repair (GGR) recognising strand distortions or transcription coupled repair (TCR) removing lesions that block RNA polymerases. DSBR repairs double strand breaks (DSB) as well as SSB converted into DSB after replication. Double strand breaks are repaired by two main mechanisms, i.e. non-homologous end-joining (NHEJ) and homologous recombination (HR). Damage that disturbs replication can be repaired or bypassed by homologous recombination (template switching and strand displacement) or by translesional synthesis (TLS). MMR removes mispaired nucleotides as a consequence of base deamination, oxidation or methylation or replication errors (for review Hoeijmakers, 2001; Garinis et al. 2008, Decordier et al. 2010).

Base excision repair. BER consists of different steps. The first step involves recognition, base removal and incision. Damaged or incorrect bases are recognised by DNA glycosylases that remove bases through hydrolyzing the N-glycosidc bond leaving an apurinic/apyrimidinic (AP) site. Some DNA glycosylases (f.e. OGG1) have endogenous 3'-endonuclease activity leading to formation of a single strand break. In a next step polymerase β inserts the nucleotide. Depending on the state of the 5' deoxyribose phosphate (5'dRP) terminus, either short-patch BER or long-patch BER will be induced. Oxidised or reduced AP sites will undergo long-patch BER whereas unaltered AP sites will be repaired through short-patch BER. Finally ligation is performed either by ligase I, interacting with PCNA and polymerase β in long-patch repair, or ligase III, interacting with XRCC1, polymerase β and poly(ADP-ribose)polymerase-1 (PARP) for short-patch BER (Figure 1) (Christmann et al. 2003; Hegde et al. 2008).

The BER capacity in mES cells is greater compared to MEF. It has been shown that proteins involved in BER, f.e. Ape1, DNA ligase III, Parp1, Pcna, Ung2, Xrcc1, are expressed to a higher extent in mES cells. Furthermore a BER incorporation assay and a DNA incision assay have shown a higher BER activity. The latter assay revealed a six-fold greater level of incision production in mES cells compared to MEF cells (Tichy et al. 2011).

Nucleotide excision repair. The NER mechanism consists of the removal of a short stretch of DNA containing the lesion and the subsequent restoration of this lesion using the non-damaged DNA strand as a template. NER is divided into two distinct pathways, the global genome NER (GG-NER) and the transcription-coupled NER (TC-NER). GG-NER is largely transcription-independent and removes lesions in non-transcribed domains of the genome or non-transcribed strands in the transcribed domains. In contrast TC-NER removes lesions from the transcribed strand of active genes. In GG-NER, DNA damage recognition is performed by the XPC-HR23B and UV-DDB complex. TC-NER is triggered by the blockage of the RNA polymerase. In both NER pathways, the transcription factor TFIIH as well as XPA and RPA are recruited to the lesion for verification of the lesion. After dual incision around the lesion, mediated by XPF-ERCC1 and XPG, the single strand gap is filled by DNA polymerase δ, PCNA and RFC. Ligation occurs through DNA ligase III-XRCC1 activity. In

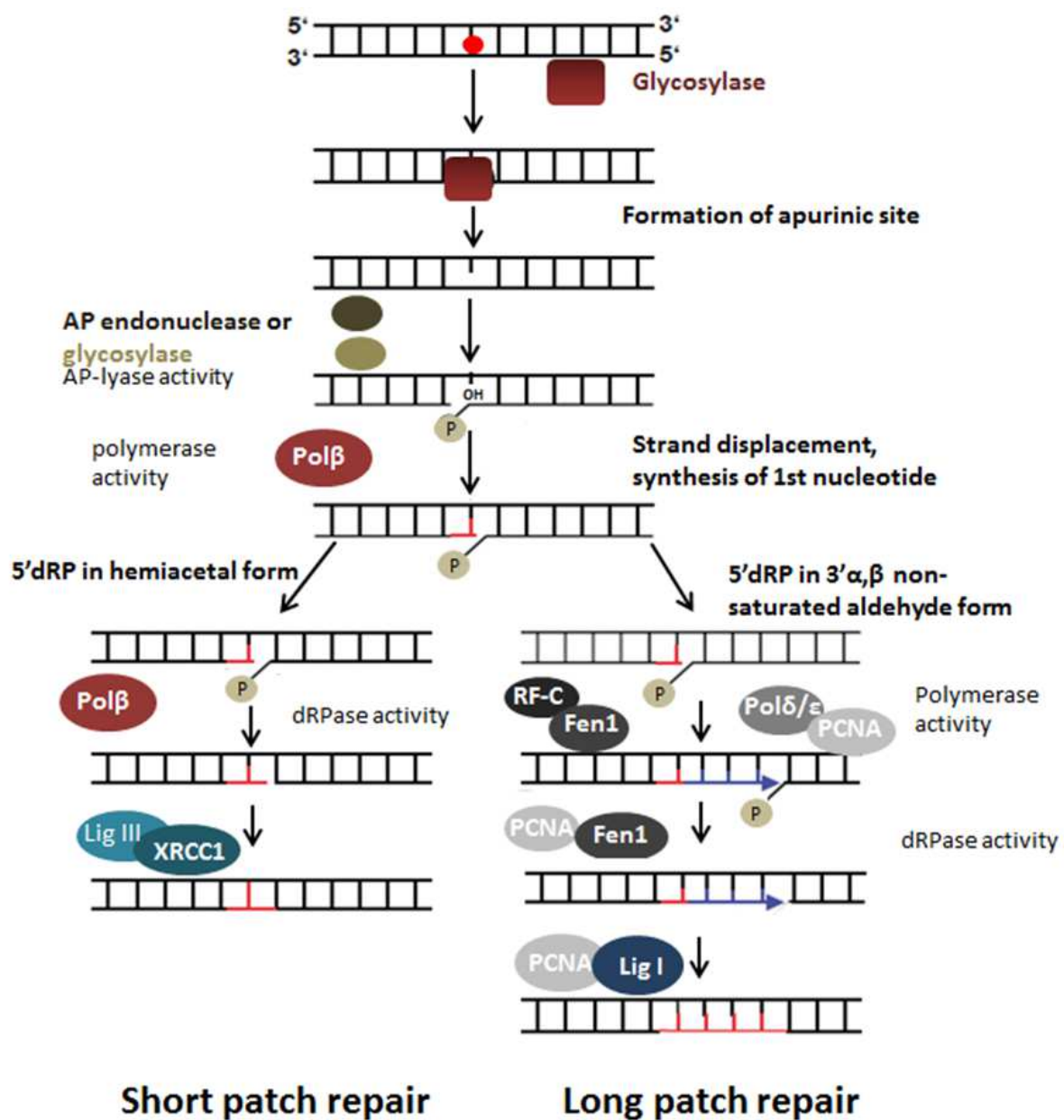dividing cells, additionally ligase I and DNA polymerase ε play a role (Figure 2) (Fousteri and Mullenders, 2008).

Fig. 1. Schematic representation of base excision repair (BER) mechanism. BER consists of two main pathways, short patch repair(left) and long patch repair (right). The first step of BER involves recognition, base removal and incision. The choice between short-patch BER or long-patch BER depends on the state of the 5' deoxyribose phosphate (5'dRP) terminus. In the final step ligation is performed (modified from Christmann et al. 2003).

Analysis of gene-specific removal of UV-C induced photolesions showed a lower NER activity in mES cells compared to MEF. In mES cells, UV-C induced cyclobutane pyrimidine dimers were not removed and (6-4) photoproducts were removed up to 30% compared to MEF that are able to remove 40-70% of (6-4) photoproducts and 80% of cyclobutane pyrimidine dimers (Van Sloun 1999). Furthermore a saturation of the NER activity was

observed already at effective dose 5J/m² of UV-C corresponding to three-fold lower dose than in MEF (Van Sloun 1999, van der Wees, 2007). The contribution of both types of NER, GGR and TCR, was investigated using ES cell lines deficient in repair specific genes (Xpa for total NER, Csb for TCR and Xpc for GGR). This study showed that GGR played a greater role in the survival of mES cells after UV radiation, although TCR is functional in mES cells (de Waard, 2008). In addition, the observation that Xpc-/- cells are hypersensitive but do not undergo apoptosis, leads to the conclusion that Xpc might play a role in both DNA damage sensing and the induction of apoptosis (de Waard et al.2008).
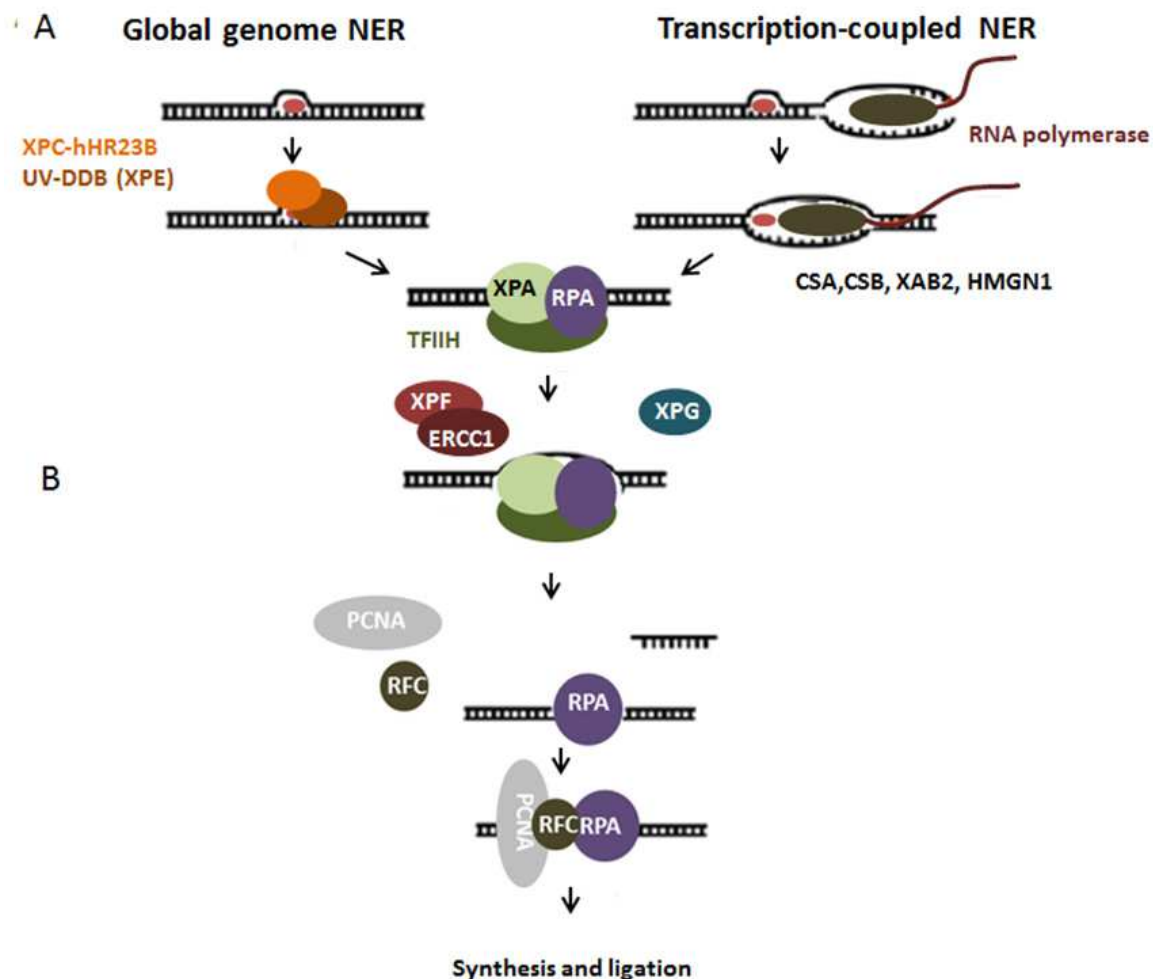
Fig. 2. The two main pathways of nucleotide excision repair (NER), global genome repair (GG-NER) and transcription-coupled repair (TC-NER) (A) and the common NER pathway. The NER mechanism consists of the removal of a short stretch of DNA containing the lesion and the subsequent restoration of this lesion using the non-damaged DNA strand as a template. GG-NER removes lesions in non-transcribed domains of the genome or non-transcribed strands in the transcribed domains; TC-NER removes lesions from the transcribed strand of active genes (modified from Fousteri and Mullenders, 2008).

Mismatch repair. Recognition of the mismatches or chemically modified bases is mediated by MSH2 and MSH6 proteins that form the MutSα complex. This complex requires phosphorylation for efficient binding to base-base and insertion/deletion mismatches.

Alternatively MSH2 can form together with MSH3 the MutSβ complex, which is able to bind to insertion/deletion mismatches. In the next step the daughter strand is identified by non-ligated single strand breaks. Two proposed models exist, i.e. the molecular switch model and the hydrolysis-driven translocation model. The former implies that MutSα-ADP binding leads to ADP-ATP transition and the formation of a hydrolysis-independent sliding clamp, followed by the binding of the MutLα complex (MLH1-MLH2). The latter model proposes that ATP hydrolysis induces translocation of MutSα along the DNA. In both cases after the association of MutSα with MutLα, excision is performed by exonuclease I and ligation by DNA polymerase δ (Figure 3) (Christmann et al. 2003).

Mismatch recognition proteins, Msh2 and Msh6, and accessory proteins, Pms2 and Mlh1, are expressed at higher level in mES cells compared to MEFs. Also mRNA levels were elevated but not to the same extent, indicating that a mechanism beyond mere higher transcription was underlying the elevated levels of MMR proteins. MMR capacity, as assessed using a MMR reporter plasmid, was shown to be 30-fold and 2-fold higher compared controls in mES cells and MEF transfected with a control vector, respectively, indicating a significantly more active repair in mES cells (Tichy et al. 2011).
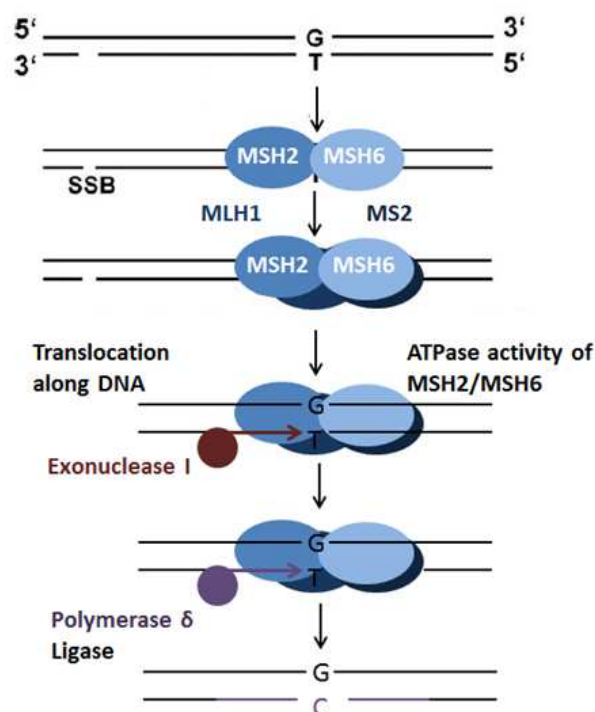

Fig. 3. Schematic representation of mismatch repair. Recognition of the mismatches or chemically modified bases is mediated by MSH2 and MSH6 proteins that form the MutSα complex. In the next step the daughter strand is identified by non-ligated single strand breaks. MutLα complex (MLH1-MLH2) associates with MutSα, excision is performed by exonuclease I and ligation by DNA polymerase δ (adapted from Christmann et al. 2003).

Double strand break repair. There are two main pathways for DSBR, error-prone non-homologous end-joining (NHEJ) and error-free homologous recombination (HR). NHEJ seems to be the predominant pathway in mammalian cells, however cell cycle phase also plays a role in the choice of pathway. NHEJ occurs in G0/G1, whereas HR occurs in late S

and G2 phase. NHEJ initiates through binding of the Ku70-Ku80 complex to damaged DNA and subsequent binding of DNA-PK leading to the formation of the DNA-PK holoenzyme. Processing of the DSB is performed by the MRE11-Rad50-NBS1 complex that has exonuclease, endonuclease and helicase activity and Artemis that acts in complex with DNA-PK. After processing, XRCC4-Ligase IV binds to the DNA end and performs ligation (Figure 4A) (Christmann et al. 2003).
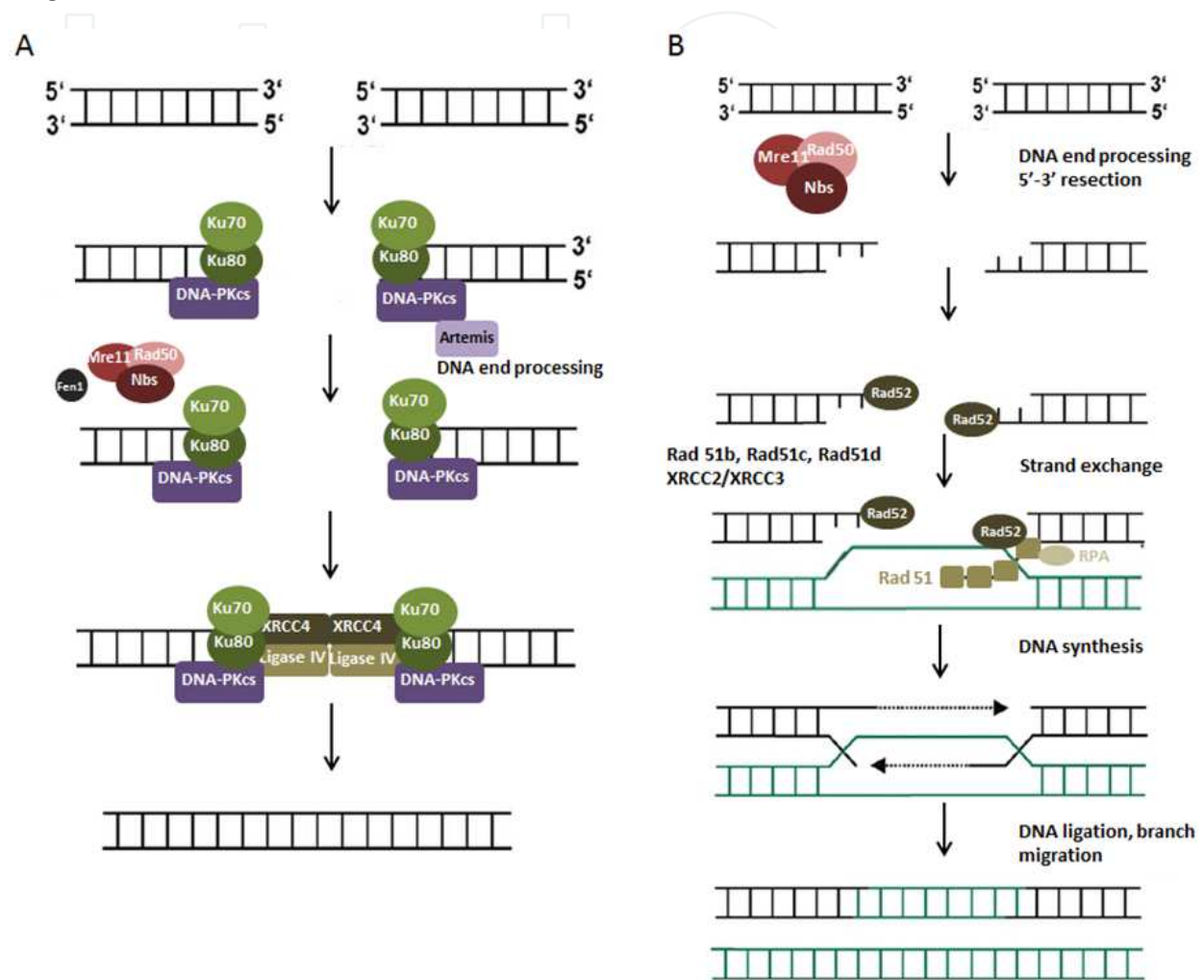

Fig. 4. Two pathways of double strand break repair: (A) non homologous end joining and (B) homologous recombination. NHEJ initiates through binding of the Ku70-Ku80 complex to damaged DNA and subsequent binding of DNA-PK leading to the formation of the DNA-PK holoenzyme. Processing of the DSB is performed by the MRE11-Rad50-NBS1 complex that has exonuclease, endonuclease and helicase activity and Artemis that acts in complex with DNA-PK. After processing, XRCC4-Ligase IV binds to the DNA end and performs ligation. HR starts with the resection of the DNA ends at the double strand breaks. This is mediated by the MRN complex (MRE11-Rad50 and NBS). The resulting single strand DNA tails are coated with RPA protein and the resulting nucleoprotein invades the complementary sequence of the sister chromatid forming heteroduplex DNA. Both Rad51 and BRCA2, involved in controlling the recombinase activity of Rad 51, are required for this process. Other proteins are also involved BRCA1, Rad52, Rad54 and Rad51 paralogues (adapted from Christmann et al. 2003).

HR starts with the resection of the DNA ends at the double strand breaks. This is mediated by the MRN complex (MRE11-Rad50 and NBS). Recruitment of this complex is promoted through binding of NBS to phosphorylated histone H2AX. The resulting single strand DNA tails are coated with RPA protein and the resulting nucleoprotein invades the complementary sequence of the sister chromatid forming heteroduplex DNA. Both Rad51 and BRCA2, involved in controlling the recombinase activity of Rad 51, are required for this process. Other proteins are also involved BRCA1, Rad52, Rad54 and Rad51 paralogues (Figure 4B) (Altieri et al. 2008).

No direct comparison of the DSBR in mES cells and somatic cells could be found in the published literature. Nonetheless, DSBR seems active in mES cells. Chuykin et al. reported induction of γ-H2AX foci after γ-irradiation (1 Gy) with a maximal number of foci obtained after 2h. Subsequently the number of foci decreased, suggesting DSBR is activated in mES (Chuykin et al. 2008).

2.3 Cell cycle control

The cell cycle of somatic cells and mES cells differs markedly both in length and cell cycle phase distribution. The mES cells are characterised by a short cell cycle of 11 to 16 hours (Orford and Scadden, 2008). Cell cycle distribution analysis showed that 10%, 75% and 15% of mES cells are respectively in G1, S and G2/M phase, indicating a very brief G1 phase (~1.5h) compared to somatic cells (~10h) (Savatier et al. 1996; Chuykin et al. 2008). In contrast, embryonic fibroblasts show a cell cycle distribution of 70%, 25%, and 5% of cells in G1, S and G2/M phase, respectively. In this section an overview and comparison of the cell cycle control pathways that are at play in mES cells and somatic cells is given.

In somatic cells, G1/S transition is mediated through the activation of Cdk4/6 and Cdk2 kinases. Upon binding to cyclin-D Cdk4/6 is activated, leading to the phosphorylation of proteins of the retinoblastoma family (pRB). This, in turn, leads to a partial inhibition of RB and the release of E2F transcription factors. The latter induces the transcription of E2F targets such as E-type cyclins. Type E-cyclins activate Cdk2 upon binding, leading to additional phosphorylation of pRB and phosphorylation of other targets important in S-phase progression. Furthermore as a consequence of the full release of E2F genes required for S-phase progression are transcribed (Figure 5A) (Wang and Blelloch, 2009). In mES cells, the G1/S transition is regulated in a different way. The Cdk4/6-Cyclin D complex is absent and the Cdk2-Cyclin E is constitutively active (Figure 5B) (Savatier et al. 1996; Wang and Blelloch, 2009).

Upon DNA damage, G1 arrest can be achieved through two main pathways in somatic cells. Double strand breaks are sensed by MRE11, a member of the MRN complex (MRE11, Nijmegen breakage syndrome and Rad50), which activates ATM. ATM autophosphorylates and phosphorylates p53 and Chk2. p53 phosphorylated by ATM and Chk2 activates the transcription of p21, that is a Cdk inhibitor, leading to G1 arrest. Active Chk2 also leads to the degradation of Cdc25 that is responsible for Cdk2 dephosphorylation necessary for G1/S phase transition (Figure 6A) (Hong and Stambrook, 2004; Stambrook, 2007). In contrast, in mES cells the G1/S checkpoint is lacking (Aladjem et al. 1998). It has been shown that in mES cells DNA damage induced by ionising irradiation does not lead to the degradation of Cdc25, as is the case in MEF. Therefore upstream events were examined,

revealing that Chk2 is localised at the centrosomes and not intranuclear like in MEF, thereby unable to phosphorylate Cdc25 (Figure 6B) (Hong and Stambrook, 2004).
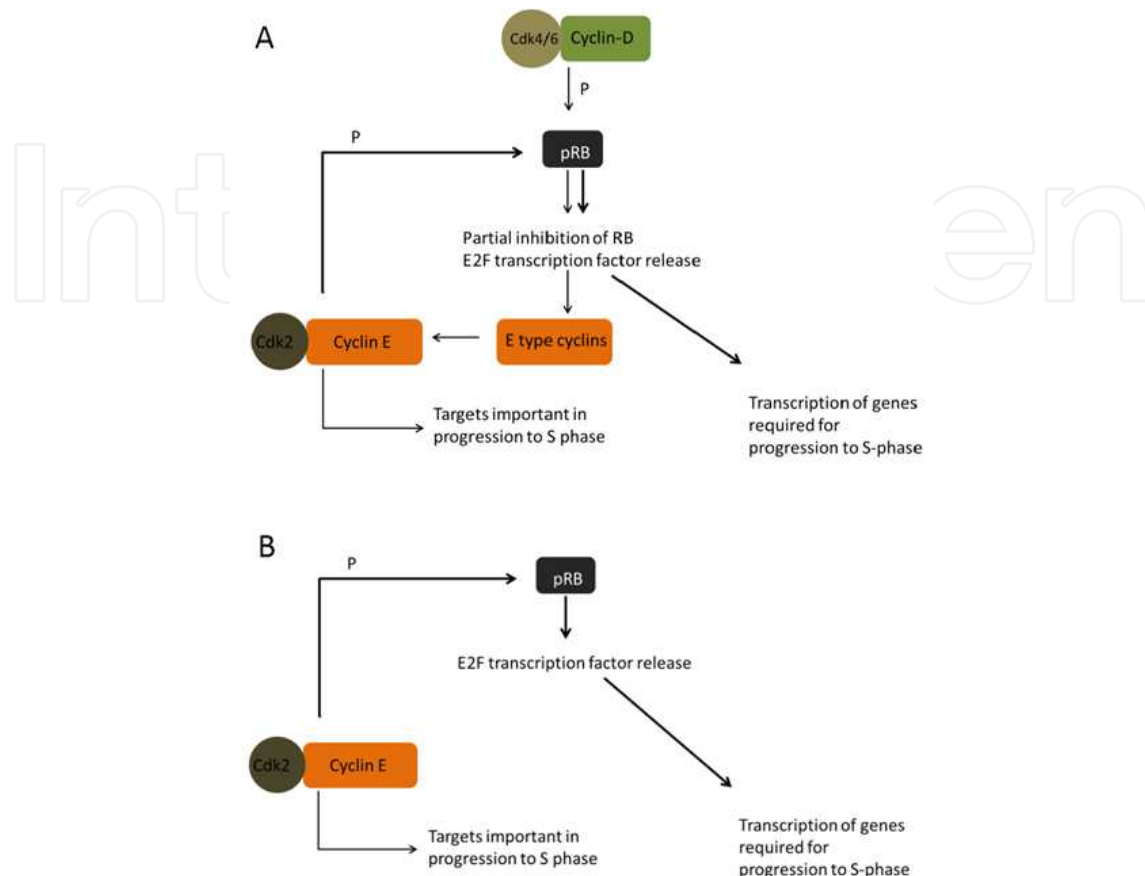

Fig. 5. Regulation of G1/S phase transition in somatic cells (A) and mES cells (B). In mES cells the Cdk4/6-Cyclin D complex is absent and the Cdk2-Cyclin E is constitutively active.

The pathway involving phosphorylation of p53 and subsequent transcription of p21 in mES cells remains unclear as data is contradictory. The amount of p53 protein is much higher in mES cells than in MEF (27-fold higher) (Sabapathy et al. 1997) or NIH3T3 cells (Solozobova et al. 2010). The higher amount of p53 proteins in mES cells was not due to a higher stability in the protein, however, both RNA content and RNA stability were increased compared to MEF cells. The cause of the higher p53 protein content was due to an enhanced translation of p53 in mES cells as well as a lower expression of miRNA 125a and miRNA125b in mES cells compared to differentiated cells (Solozobova et al. 2010).

p53 is located in the cytoplasm in undifferentiated mES cells (Solozobova et al. 2009) and is translocated to the nucleus upon challenge with IR or UV. Depending on the type of genotoxic insult the temporal pattern of p53 presence in the nucleus differs. For instance, IR induces the nuclear translocation of p53 after 1h while after 8h all p53 had disappeared from the nucleus, to reappear again in the nucleus after 24h. In contrast, upon UV light exposure p53 remained in the nucleus up till 24h (Solozobova et al. 2009). Treatment of mES cells with the antimetabolite n-phosphonacetyl-L-aspartate (PALA) leading to rNTP depletion did not induce efficient translocation of p53 to the nucleus resulting in a significant heterogeneity in PALA-treated cells (Aladjem et al. 1998).
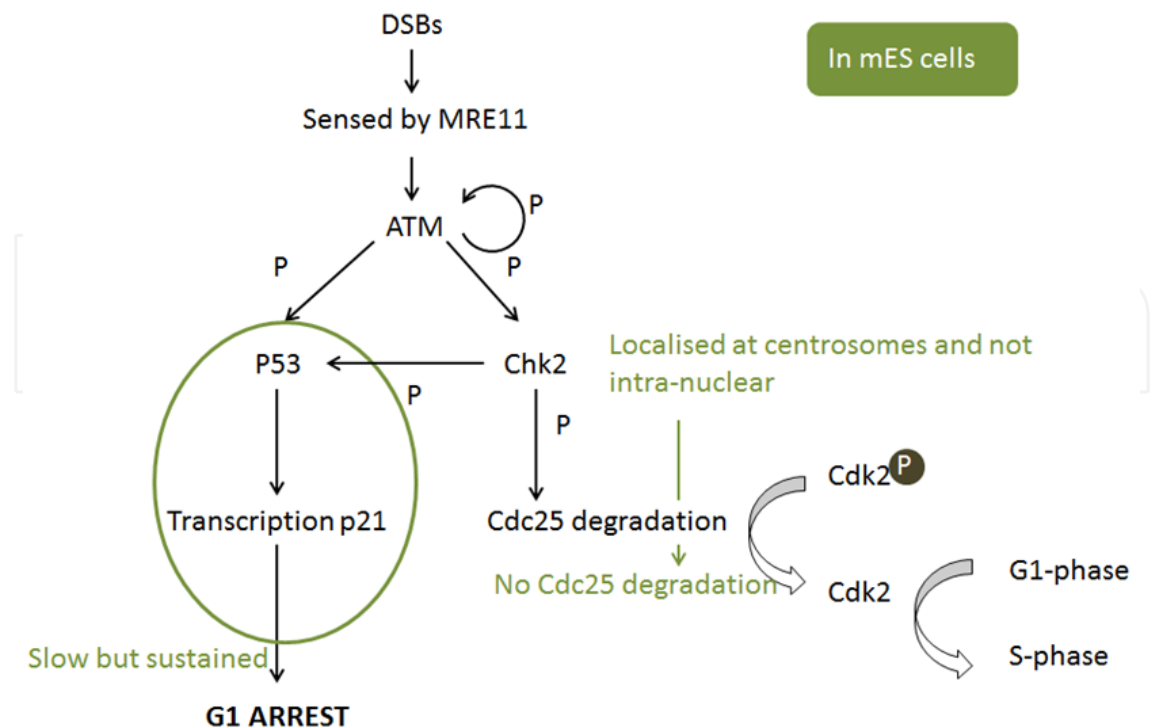
Fig. 6. Schematic representation of G1/S checkpoint in somatic cells. Differences in mES cells are depicted in green.

The transcriptional activity of p53 in mES cells is another subject of debate. On the one hand there is data supporting the transcriptional activation of p21 by p53 in mES cells. p53 activity was demonstrated using a p53-specific reporter plasmid in untreated and UV-C treated ES cells, indicating that there is a p53 baseline activity (Prost et al. 1998). Roos et al. found that upon treatment with the methylating agent N-methyl-N-nitro-N-nitrosoguanine p53 was stabilized in mES cells, which was not observed in SA 3T3 cells. Furthermore an upregulation of p21 protein was observed (Roos et al. 2007). Solozobova et al. demonstrated that p21 RNA was present and this in a comparable amount to 3T3. Furthermore p21 RNA levels further increased after ionizing radiation. However, at protein level p21 was not detectable before or after treatment with ionizing radiation, suggesting that post-transcriptional regulation plays an important role (Solozobova et al. 2009). Indeed it has been shown that micro RNAs regulate p21 expression. Members of the miR-290 cluster directly target and suppress p21, thereby modulating cell cycle progression (Wang and Blelloch, 2009).

In contrast, there is data supporting that p53 is not a transcriptional activator of p21 in mES cells. Aladjem et al. did not detect p21 expression by immunoblots, immunofluorescence or northern hybridization analysis (Aladjem et al. 1998). This is in agreement with earlier findings of Savatier et al. that did not detect p21 RNA or proteins (Savatier et al. 1996).

The G2/M checkpoint and spindle assembly checkpoint (SAC) are far less studied in mES cells. Both checkpoints are functional in mES cells (Hirao et al. 2000). It has been shown that 12h after a 10 Gy dose of γ-irradiation cells were arrested in G2-phase (Hirao et al. 2000). Furthermore at lower dose of γ-irradiation (1Gy) mES cells underwent a G2/M delay (Chuykin et al. 2008). Chk-1 is required for the initiation of the G2 arrest (Liu et al. 2000),

whereas Chk-2 has been shown to play a role in the maintenance of this arrest (Hirao et al. 2000). Treatment with nocodazole, a microtubule assembly inhibitor, induced mitotic arrest in 35% of the cells after 12h, indicating that the spindle assembly checkpoint was functional (Hirao et al. 2000).

2.4 Induction of apoptosis in mES cells

In somatic cells, p53 is stabilised and activated upon genotoxic stress which can lead to cell cycle arrest, senescence or apoptosis. Upon stress p53 activates the transcription of many genes such as p21, Mdm2, Noxa and Puma that mediate the cell cycle arrest, senescence and apoptotic processes. Apoptosis can also be induced in a p53-dependent but transcription-independent manner by targeting the mitochondria thereby inducing cytochrome-C release (Zhao and Xu, 2010).

Roos et al. demonstrated the induction of p53-dependent apoptosis through p53 transcriptional activation in mES cells. Upon induction of O^6-methylguanine by treatment with N-methyl-N-nitro-N-nitrosoguanidine, apoptosis seemed activated via the Fas death pathway as the Fas receptor, which is transcriptionally regulated by p53, was upregulated and caspase-3 and -7 were activated. The lack of cytochrome-C release and the increase of the Bcl-2/Bax fraction, indicative of protection against mitochondrial-mediated apoptosis, demonstrate the inactivity of this latter apoptotic pathway (Roos et al. 2007).

mES cells have been shown to undergo apoptosis in a p53-dependent as well as in a p53-independent way. Corbet *et al.* demonstrated that upon UV treatment the majority of the cells underwent apoptosis within 36h whereas treatment with γ-irradiation induced less than 25% apoptosis within 72h corresponding to control values. This corresponded with the induction of p53 expression that was induced 4h after UV treatment and returned to basal levels after 48h. Upon γ-irradiation no overall increase in p53 protein expression was observed. Exposing p53$^{-/-}$ mES cells to UV treatment reduced apoptosis to the background level confirming the p53 dependency. However, these p53 $^{-/-}$ cells still showed a low but significant level of apoptosis, indicative of a p53-independent pathway (Corbet et al. 1999).

Aladjem *et al.* demonstrated, using p53 $^{+/+}$ and p53 $^{-/-}$ mES cells, similar kinetics in the apoptotic response upon treatment with Adriamycin, a DNA intercalating agent and inhibitor of macromolecular biosynthesis. Together with the observation that p53 was not functioning as an efficient transcriptional activator, this indicates that mES cells undergo p53-independent apoptosis (Aladjem et al. 1998).

3. DNA damage and its role in differentiation/pluripotency

One way to deal with DNA damage for ES cells is to induce the process of differentiation in order to avoid pass mutations to their progeny. A key player in this process is p53.

It has been shown that p53 induces differentiation of mES cells through suppression of Nanog, a gene required for mES cell renewal. Four hours after DNA damage, induced by UV light or doxorubicin, an increase in the level of p53 is induced. Suppression of Nanog is mediated by binding of p53 to its promotor region, leading to the differentiation of mES cells. However, this decrease in Nanog expression was not due to the expression of Oct3/4, another marker for undifferentiated mES cells. Differentiation of mES cells into other cell

types upon DNA damage can therefore be a mechanism by which damaged mES cells are removed from the proliferative pool and are more efficiently subjected to p53-dependent apoptosis or cell cycle arrest (Lin et al. 2005).

Surprisingly, another target of p53 in mES cells, but not in mES cell-derived neural progenitor cells or in MEF, has been shown to be the Wnt pathway. Five Wnt ligands (Wnt3, Wnt3a, Wnt8a, Wnt8b and Wnt9a), five Wnt receptors (Fz1, Fz2, Fz6, Fz8 and Fz10), one component of the Lef1/Tcf complex (Lef1) and nine putative regulators or downstream targets of the Wnt signalling pathway (Ppp3cb, Nfatc1, Ccnd2, Ppard, Smad3, FosL1 and PPP2r2c) were identified as targets of p53. Lee et al. demonstrated that this induction of Wnt genes is not restricted to a genotoxic response (doxorubicin and UV treatment), but rather a general p53-mediated stress response as the Wnt gene expression was also induced after the use of a non-genotoxic p53 inducer, nutilin, and decreased after the reduction of p53 expression through siRNA treatment. Furthermore the induction of Wnt genes was not dependent of the induction of apoptosis or of the repression of Nanog. Wnt pathway activation leads to the inhibition of mES cell differentiation and promotion of cell proliferation. Conditioned medium of mES cells treated with UV light (CM with UV) was used to grow mES cells and lead to an increase in Nanog-positive (~29%) and Oct3/4-positive (~41%) cells, two markers of undifferentiated mES cells. Furthermore they demonstrated that cell proliferation was induced as the number of mES cells, grown in CM with UV, was two times higher after 7 days of culture in absence of leukemia inhibitory factor (LIF) as the number of mES cells, grown in CM without UV (Lee et al. 2010).

To explain these both seemingly contradictory observations, i.e. the induction of differentiation through the repression of Nanog and the antidifferentiation signals through Wnt pathway activation, the following model has been proposed. Upon DNA damage, p53 is activated to induce differentiation and apoptosis in order to prevent the DNA damage to be passed on to the progeny. To avoid differentiation and/or cell death of the entire mES cell population, as mES cells are hypersensitive to DNA damage, Wnt secretion by stressed mES cells inhibit differentiation and promote proliferation presumably of the other undamaged mES cells in order to maintain cell population numbers (Lee et al. 2010).

Box 2: Human embryonic stem cells and induced pluripotent stem cells

Human embryonic stem cells

hES cells have a higher repair capacity for different types of DNA damage compared to human primary fibroblasts. This has been shown for hES cells exposed to H_2O_2, UV-C, ionizing radiation and psoralen. In all cases, except one, the repair capacity in hES cells was higher. Only after UV-C treatment Hela cells, but not WI-38 and hs27 cells, demonstrated enhanced repair capacity compared to hES cells (Maynard et al. 2008). Furthermore, Maynard et al. demonstrated that although the level of 8-oxoG, a common oxidative lesion in DNA, was significantly lower in hES cells compared to WI-38 cells, this was not due to a higher activity of OGG1, a key component of BER. Other genes were shown to be upregulated in the different repair pathways: BER (Fen1, Lig3, Mpg, Nthl1 and Ung), NER (Rpa3), DSBR (Brca1 and Xrcc5) and interstrand crosslink repair (Blm, Fancg, Fancl and Wrn) (Maynard et al. 2008).

The G1/S checkpoint is lacking in mES cells. Whether the G1/S checkpoint exists in hES

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cells is a matter of debate. Data supporting the existence of the G1/S checkpoint arises from experiments using synchronized hES cells. These synchronized cells were subjected to 15 J/m² UVC light and were shown to accumulate in G1-phase (47% of hES cells compared to 19% in controls). Cdk2 was reduced in G1-arrested hES cells. Both potential pathways in which G1 arrest is induced, i.e. p53 transcriptional activation of p21 and the pathway involving Cdc25 degradation, were investigated. Barta et al. demonstrated that upon UVC treatment Cdc25 is downregulated in a dose-dependent manner. Furthermore inhibition of Chk1 and Chk2 demonstrated that both play a redundant role in the regulation of cell cycle progression of hES cells after UV treatment (Barta et al. 2010). Others state that the G1/S checkpoint is not functional in hES cells. Filion et al. came to this conclusion as they did not observe increases in G1-phase cells after treatment of hES cells with γ-irradiation. They did, however, observe G2-arrest, indicating that G2/M checkpoint is functional (Filion et al. 2009). In agreement with this, Sokolov et al. observed a G2-arrest, but no G1-arrest after treatment of hES cells with 1Gy dose of X-rays (Sokolov et al. 2011).

It has been shown that in hES cells apoptosis is not induced through p53 transcriptional activation. p53 protein levels are enhanced after UV irradiation and γ-irradiation, however the expression level of p53 target genes, mdm2, p21, bax and puma are down-regulated. p21 seems to be post-transcriptionally regulated as p21 protein levels were increased 2-fold after UV treatment. Apoptosis in hES cells is induced through the mitochondrial pathway. p53 was shown to accumulate in the mitochondrial fraction and caspase-9activity increased 3-fold upon UV treatment. Furthermore p53 knockdown rescued 40 % of hES cells from apoptosis and inhibited caspase-9 increase by 50%, indicating a p53 dependent apoptosis. The use of pifithrin-µ, a molecule specifically inhibiting binding of p53 to the mitochondria, resulted in an increased survival of hES cells, confirming the mitochondrial pathway apoptosis (Qin et al. 2007).

Induced pluripotent stem cells (iPS cells)

Induced pluripotent stem cells are somatic cells that have been reprogrammed with a set of transcription factors. Combinations of Oct4, Sox2, Klf4 and c-Myc or Oct-4, Nanog, Sox2 and Lin28 have been successful to transform somatic cells into cells with the same key features as ES cells, i.e. pluripotency, self-renewal and the expression of the pluripotency markers, Oct4, Nanog, Sox2 and SSEA-4. Some of these inducing transcription factors (e.g. c-Myc, Klf4, Oct4 and Lin28), however, have known oncogenic activity. Some studies have demonstrated a higher incidence of aneuploidy such as chromosome 12 duplications which might increase tumorigenicity (Mayshar et al. 2010; Pasi et al. 2011). Moreover, inactivation of p53 has been shown to increase reprogramming efficiency (Krizhanovsky and Lowe, 2009). Therefore assessing the genomic integrity in these iPS cells is of great importance for further development of applications (Sarig and Rotter, 2011). However, to date data on DNA damage responses in iPS cells is scarce.

After 1Gy of γ-irradiation, the induction of γ-H2AX foci, indicating the presence of double strand breaks, was observed in iPS cells. These foci returned to basal level within 6h after treatment. Repair of these double strand breaks appeared to be mediated by homologous recombination (HR), evidenced by the formation of Rad51 foci, a recombinase that is essential for HR. Moreover, the capacity of HR in iPS cells was similar to hES cells (Momcilovic et al. 2010). Upon γ-irradiation the activation of the checkpoint signalling cascade was induced as

evidenced by phosphorylation of ATM and target proteins, such as p53. However, no G1-arrest was induced after treatment of iPS cells with 1Gy of γ-irradiation. Nine hours after treatment 77% of iPS cells were arrested in G2-phase and after 24h the cell cycle distribution resembled non-irradiated cells. iPS cells detached from the substrate which is indicative of apoptosis. In support of this, an increase in cleaved caspase-3 (more pronounced in detached cells) 4h after γ-irradiation was observed (Momcilovic et al. 2010).

4. DNA damage in embryonic development

In this section, some similarities between genotoxic effects seen in mES cells and mouse embryos are highlighted, for an extensive review of all effects caused by genotoxicants in mouse embryos I refer to some excellent reviews on the effects of ionizing radiation (De Santis et al. 2007) and oxidative stress (Wells et al. 2010).

4.1 DNA damage and repair in mouse embryos

It has been well established that exposing pregnant mice to genotoxicants leads to adverse effects in their offspring. As for mES cells, assessment of the actual extent of DNA damage in mouse embryos upon genotoxic stress is, however, scarce. A summary of the findings available in literature is given in table 2. Mainly the amount or induction of γ-H2AX foci, indicating the formation and repair of double strand breaks has been investigated in these studies (Adiga et al. 2007; Yukawa et al. 2007; Luo et al. 2006). The formation and/or repair of double strand breaks is not detected in one- and two cell stage embryos after *in* or *ex utero* exposure of the embryos to ionizing radiation, and seems therefore developmental stage-dependent. One study investigated the extent of DNA migration by alkaline comet assay after exposure of pregnant mice to pyrimethamine, a dihydrofolate reductase inhibitor used for prophylaxis and treatment of malaria and toxoplasmosis. This study showed an induction of DNA strand breakage and alkali-labile sites in the embryo at day 13 of embryonic development (E13) (Tsuda et al. 1998). The presence of DNA repair pathways is essential for correct response of mouse embryos to genotoxicants, as shown by exposure of mice bearing null mutations in genes involved in DNA repair pathways to different toxicants. These experiments demonstrated a greater susceptibility of mice bearing null mutations in DNA repair-related genes. Both knockouts of OGG1, involved in base excision repair, and CSB, involved in transcription-coupled nucleotide excision repair, showed greater susceptibility to *in utero* exposure of mice to metamphetamine (Pachowski et al. 2011).

4.2 The role of p53 in the mouse embryo stress response

The role of p53 during mouse embryo development has been investigated by generation of a p53 null mutant mice and analysis of their development. Donehower et al. demonstrated that p53 null mice develop normally, but they spontaneously develop tumours, most frequently malignant lymphomas, by 6 months of age (Donehower et al. 1992). Other studies have shown developmental defects in p53 null mice at high incidence, such as exencephaly (Armstrong et al. 1995; Sah et al. 1995).

These p53 null embryos are great tools for elucidating the role of p53 in the response of embryos to DNA damage. Heyer et al. demonstrated that p53 and the upstream activator

Genotoxicant	Exposure	Analysis of embryo	Extent of DNA damage	Rer
Ionizing radiation	In utero exposure 3, 5, 10, 15 Gy of γ-rays	γ-H2AX foci in one-cell stage embryos 30 min after exposure	No detection of γ-H2AX foci after 3-10 Gy treatment, detection of γ-H2AX foci after 15 Gy treatment	13.3 blas wen pos afte
	In utero exposure 3 Gy of γ-rays	γ-H2AX foci in two-cell stage embryos 30 min after exposure	No detection of γ-H2AX foci after 3 Gy treatment	79% pos afte
	In utero exposure 3 Gy of γ-rays	γ-H2AX foci in six- eight -cell stage embryos 30 min after exposure	Detection of γ-H2AX foci after 3 Gy treatment	
	In utero exposure 3 Gy of γ-rays	γ-H2AX foci in blastocyst stage embryos 30 min after exposure	Detection of γ-H2AX foci after 3 Gy treatment	
Ionizing radiation	Ex utero exposure with 10 Gy γ-rays	γ-H2AX foci detection in embryos of different stages	No detection of γ-H2AX foci in one- or two-cell embryos 4-cell stage, morula stage and blastocyst stage embryos have a marked increased number of γ-H2AX foci compared to untreated controls	
Electromagnetic field	Ex utero exposure with 0.5mT 50Hz electromagnetic field for 24h	γ-H2AX foci in two-cell embryos γ-H2AX foci in eight-cell embryos	6.25-fold increase in γ-H2AX foci 11.9- fold increase in γ-H2AX foci	Sim afte exp
Pyrimethamine (PYR)	Oral treatment of pregnant mice with 50mg PYR/kg	E13, alkaline comet assay 6h after treatment	~18 and ~28 times higher DNA migration in head and body, respectively	
		E13, alkaline comet assay 16h after treatment	~3 and ~19 times higher DNA migration in head and body, respectively	

Table 2. Summary of studies investigating the extent of DNA damage in embryos exposed to genoto

ATM are required for the embryonic response upon relatively low dose irradiation (0.5 Gy X-rays) as apoptosis was induced in wild-type embryos but not in p53 null or Atm null embryos. Heterozygous embryos showed an intermediate apoptotic response. It should also be noted that apoptosis occurred in the embryonic region and not in the extra-embryonic region. Another study by Norimura et al. demonstrated that upon 2 Gy of X-radiation, E3.5 and E9 embryos had a differential outcome depending on their p53-status. p53 null embryos had a lower incidence of death compared to their wildtype counterparts. 44% of p53 null embryos irradiated at E3.5 died before day 9 of gestation, whereas the incidence of death was 73% in wild type embryos. E9.5 p53 null embryos exposed to 2 Gy of X-rays died during gestation with a frequency of 7% compared to 60 % for their wildtype counterparts. but the incidence of malformations was higher. However, the incidence of malformations in p53 null embryos was higher with frequencies of 22% in E3.5 and 70% in E9.5 p53 null embryos and 0% in E3.5 and 20 % in E9.5 wild type embryos. Therefore they concluded that p53 suppresses teratogenesis by removing injured cells by apoptosis (Norimura et al. 1996). Others have shown that mice lacking p53 are more sensitive to alkylating agents such as cyclophosphamide, as they demonstrated grosser limb malformations (Moallem and Hales, 1998). The presence of p53 and p53-dependent apoptosis during organogenesis is therefore required for teratogenesis suppression. Also exposure to benzo[a]pyrene leads to a 3.6-fold increase in embryo resorption or *in utero* death in p53$^{-/-}$ mice compared to their wildtype counterparts (Nicol et al. 1995).

In contrast, Pekar et al. demonstrated that the teratogenic effects of cyclophosphamide are mediated by the induction of a p53-dependent apoptotic response (Pekar et al. 2007). Therefore p53 could also act an inducer of teratogenesis. Torchinsky and Toder (2010) proposed a model to explain this duality. This model describes two pathways, one pathway where p53 acts as a teratogenesis suppressor through activation of DNA repair, cell cycle arrest and suppression of ROS formation. A second pathway where p53 acts as a teratogenesis activator through induction of apoptosis is described. However, this model does not take into account the dosage and type of stress, as well as the timing of exposure. Therefore, as for mES cells, the exact role of p53 in embryo stress responses is not elucidated yet.

5. Conclusion

Genomic integrity of the mouse embryo is crucial for correct development. The genome maintenance of embryonic stem cells was discussed here, as they are models for early development and are used for medical applications. It has been demonstrated that mES cells are more sensitive than somatic cells. However, to define the difference in sensitivity, accurate assessment of the extent of DNA damage is imperative in mES cells as well as in the mouse embryo. The *in vitro* micronucleus assay could be an excellent tool to achieve this, as it enables the detection of chromosome breakage (clastogenicity) and chromosome loss (aneuploidy). Besides difference in sensitivity to genotoxic agents, mES cells have a generally higher DNA repair capacity, contributing to the maintenance of their genomic integrity, compared to somatic cells. Furthermore the cell cycle and cell cycle control in mES cells are regulated in a distinct way with the major feature being the lack of a G1/S checkpoint. Other cell cycle checkpoints (G2/M and the spindle assembly checkpoint), however, have been far less studied. The role of p53 in cell cycle control, apoptosis and

differentiation vs. pluripotency is subject of some controversy. Because of contradictory results the exact role of p53, both in mES cells as well as in the embryo, remains unclear and deserves further attention.

6. Acknowledgment

7. References

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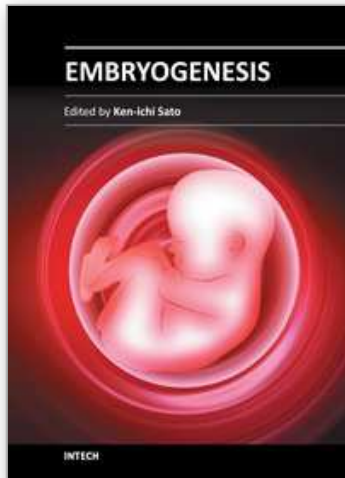
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