DNA DAMAGE RESPONSE

CHDI PROJECT 26.1

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INTRODUCTION

DNA Damage Repair is a well characterized cellular stress response mechanism with multiple pathways (base excision, transcription coupled nucleotide excision, inter-strand cross linking, etc.) involved in maintaining the integrity of the genome. Oxidation, radiation and normal biological function can all contribute to DNA damage. Several working hypotheses of the mechanism of Huntington's Disease involve DNA Damage response and repair as a component, including Somatic Expansion of the CAG Repeat and Reactive Oxygen Species (ROS) DNA Damage Monitoring and Repair by P53. In this work we have explored the state of the scientific literature to identify and articulate the genes and pathways involved in DNA Damage Response and Repair with a special emphasis on the effect in Huntington's and other trinucleotide repeat diseases such as spinocerebellar ataxia, myotonic dystrophy 1 and Friedreich ataxia[1]. In this report we detail the methodologies and results of Clarivate Analytics review of the scientific literature to establish the relevant genes and mechanisms of DNA Damage Repair through a focused search of terms, reviewing the scientific literature and development of custom pathway maps of major mechanisms of DNA Damage Repair.

METHODS

Each of the several mechanisms of DNA Damage Response were explored using the Clarivate Analytics Knowledge Modeling Framework (Figure 1).

Figure 1: Clarivate Analytics Knowledge Modeling framework. A) search for relevant articles in B) several literature databases; C) assess the identified articles by reviewing the title and abstract for relevancy.

Briefly, a collection of search terms was generated and tested against several sources of scientific literature to generate a reasonably sized list of primary scientific publications and reviews. Each publication was manually curated to generate lists of genes and interactions specific to the mechanism of interest in each stage of project execution. These lists were used to create pathway maps in the Clarivate Analytics Pathway Map Creator tool. The resultant pathway maps present detailed molecular interactions detailing the step wise processes of DNA damage repair.

The pathway maps are interactively linked to underlying metadata including literature citations, experimental methods and editorial notes through Clarivate Analytics MetaCore™ web interface, the content of which was also exported into a tabular format for network visualization in tools such as Cytoscape[2]. Briefly, the node pairs of all interactions in each pathway map were extracted and converted to MetaBase network objects, maintaining their orientation as source or target interaction nodes. The list of source and target nodes was used to perform a first degree network search for all interactions one step away from the source and target nodes in the MetaBase network. The interactions

specific to the pathway map were identified as the intersection of interactions to the source or target nodes that were contained in *both* sets of first degree interactions maintaining correct directionality. The interaction metadata was extracted by SQL query against MetaBaseTM to identify NCBI gene symbols and IDs, Pubmed literature identifiers[3] and Clarivate Analytics editorial notes for each interaction. Metadata for an interaction was aggregated for each interaction ID.

RESULTS

Here we detail the output and findings of each of the DNA Damage Response mechanisms in detail.

BASE EXCISION REPAIR

Base excision repair (BER) is a repair system resulting from damaged bases in DNA that are subsequently removed and repaired. Two complementary pathways exist in BER, the long-patch and short-patch pathways that repair >2 bases and single base repairs respectively. Direct damage is recognized by glycosylase enzymes, which is followed by the excision of the phosphodiester bond by the AP endonuclease (APE1) at the 5' side of the damaged site leaving a gap. The small gap left in the DNA helix is filled in by the sequential action of DNA polymerase and DNA ligase, using the undamaged strand as a template. The dysfunction or mis-regulation of the DNA damage repair mechanisms has been associated with human diseases including cancers and neurodegenerative diseases such as Huntington's disease and Alzheimer's disease. It has been hypothesized that these DNA lesions affect cellular homeostasis and mitochondrial or cellular functions.

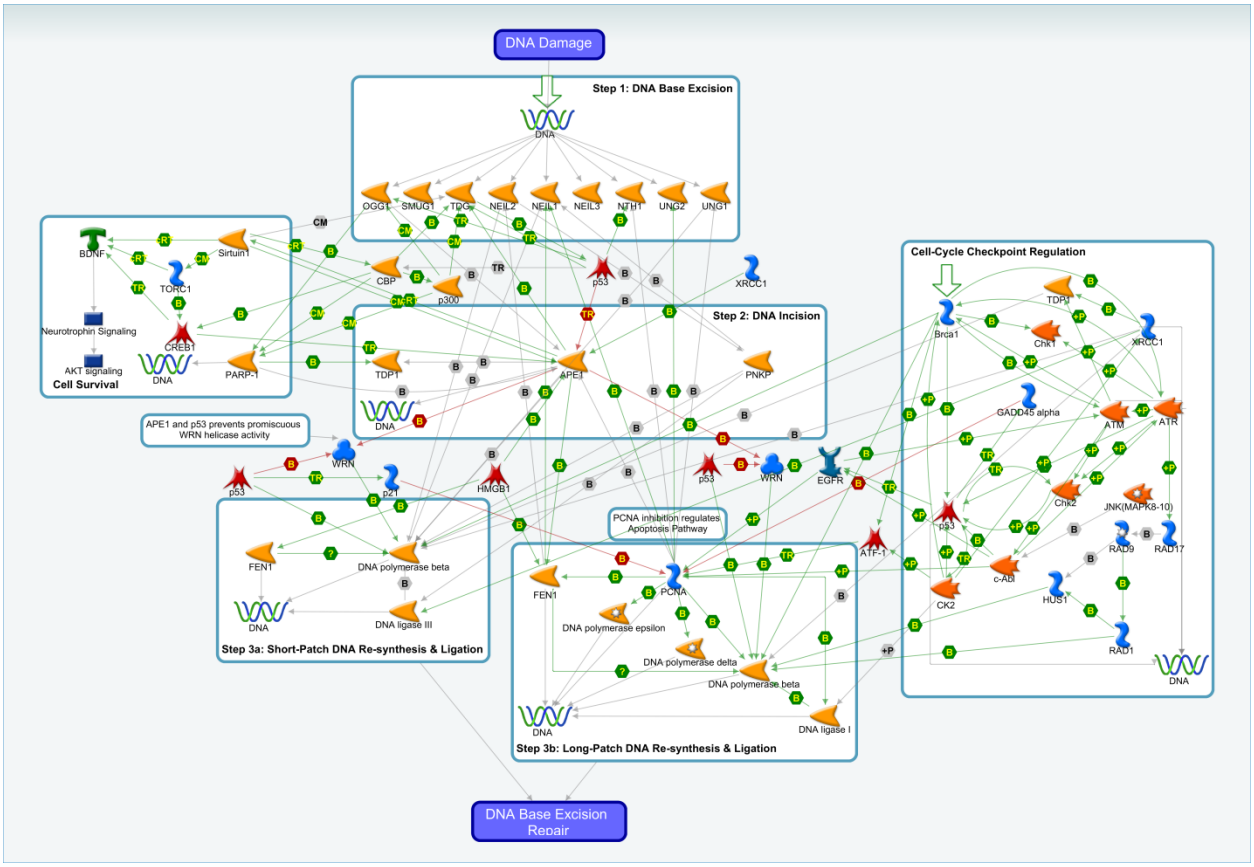

Figure 2: DNA Base Excision Repair Pathway

Step 1: DNA Base Repair Initiation and Excision

The initiation of the base excision repair (BER) pathway occurs when DNA glycosylases locate covalently modified bases within the DNA helix that causes the excision of the damaged base from an abasic site. There are DNA glycosylases that recognize oxidized/reduced bases, deaminated bases and mismatches. The formation of 8-oxoguanine (8-oxoG) from the oxidation of the guanine residue in DNA initiates the BER pathway due to the interaction between the OGG1 glycosylase and the DNA lesion (10706276; 11927259; 22837659). OGG1 expression has been shown to be up-regulated in Parkinson's disease (15841414; 10589547) and Alzheimer's disease (24121118). Polymorphs of OGG1 have been found in Alzheimer's disease with the A53T and A288V variants demonstrating a significant reduction in the catalytic activity and the A53T variant having decreased substrate binding, whereas the A288V polymorphism has reduced AP lyase activity (23684897). OGG1 is implicated in the removal of 8-oxoG lesions in CAG hairpin loops (24423876). The damage to uracil bases is recognized by the uracil-DNA glycosylase SMUG1 (12820976) which has been shown to have a high affinity for the abasic DNA uracil lesion (10074426). Moreover Uracil-DNA N-glycosylase (UNG) removes uracil from DNA (26457437; 10393198). UNG has been shown to be down-regulated in Alzheimer's disease patients (17704129). Thymine DNA glycosylase (TDG) binds to and removes both thymine and uracil moieties in damaged DNA (8127859; 18512959; 26932196). Cytosine and 8-oxoG lesions are bound and repaired by NEIL glycosylases (NEIL1-3) (12097317; 16129732; 26751644; 20185759). NEIL glycosylases and OGG1 have been shown to be inhibited by the transition metals iron and copper (20622253), which is significant as these ions have been shown to be dysregulated in neurodegenerative diseases. The NTH1 glycosylase also binds and excises 8-oxoG lesions (11328882; 9705289). The loss of p53 (growth suppressor protein) results in a decrease in OGG1 repair activity as p53 acts as a positive transcriptional regulator of OGG1 (16293709; 21605570). p53 has been shown to be up-regulated in Alzheimer's disease (17399897; 9125193; 15548589) which has been posited to result in increased levels of neuronal apoptosis. It has also been reported that p53 interacts with HTT (huntingtin) *in vitro*, and it was posited that the expanded repeat in HTT causes aberrant transcriptional regulation through interaction with p53, which may result in neuronal dysfunction and cell death in HD (10823891; 16278683; 21465263). p53 has a similar effect on the regulation of TDG. It has been shown that p53 binds to a domain of the TDG promoter containing two p53 consensus response elements (p53RE) to activate its transcription (23165212) while TDG promotes p53 activity (18951877). CBP/p300 bind each other (15695103; 17970752) and then form a physical and functional complex with TDG (11864601; 17060459) while acetylating the TDG glycosylase. Moreover, p300 acetylates OGG1 which increases OGG1 activity *in vivo* (16478987; 25660075). DNA pol β interacts with many of the glycosylases, including NEIL1 (15260972), NEIL2 (16982218) and UNG1 (10629618) suggesting coordination of NEIL1-initiated repair. PCNA has also been shown to interact with other BER glycosylases including NEIL1 (18032376; 26134572) and PCNA enhances NEIL1 loading onto the DNA substrate. PCNA directly binds to NTH1 (15358233), UNG1 and UNG2 (20967232; 18562313; 22521144; 15479784) but its effect has not been determined.

Step 2: DNA Incision

Following the glycosylase catalyzed lyase reaction to remove the base and form the apurinic/apyrimidinic (AP) site, an endonuclease is recruited to the site of the lesion. The 3'-sugar residue is removed by an AP endonuclease to form a gap in the DNA strand to prime for DNA repair synthesis. In BER, the primary endonuclease is APE1, which binds to the DNA lesion (10667800). It has been hypothesized that structural distortions that result from the APE1-DNA interaction causes insertion of specific APE1 amino

acid residues into the abasic DNA void in the course of stabilization of the precatalytic complex (22243137). The first step in APE1 binding to DNA substrate is the formation of non-specific contacts between the enzyme binding surface and DNA, followed by the insertion of the APE1 amino acid residues into the duplex, which induces DNA flipping-out and formation of specific contacts between the enzyme active site and the outward turned 5'-phosphate-2'-deoxyribose residue (27099790). The direct interaction between APE1 and the DNA duplex results in conformational changes in APE1, resulting in APE1 relaxing its grip on the DNA incision section to initiate the handoff to the downstream BER enzyme (20377204). APE1 expression has been shown to be lower in Alzheimer's disease models (24121118). Direct interactions between APE1-DNA Polβ, APE1-TDP1, and APE1-PARP1 (26020771) pairs have been detected (26013813), along with PARP1-TDP1 (26181362; 24493735). APE1 stimulates TDG by disrupting the product complex rather than merely depleting (endonucleolytically) the APE1-DNA interaction (18805789), while TDG bound to DNA regulates recruitment of repair endonuclease APE1 to the site of the lesion (11864601) with direct interaction between TDG-APE1 observed (9867812). p53 can transcriptionally activate gene expression by binding DNA in response to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. p53 significantly decreases the activity of APE1 (23846616; 18208837), which has been posited to play a role in the regulation of the p53-mediated cell-cycle arrest/apoptosis pathway. The polynucleotide kinase (PNKP), which ensures that DNA termini are compatible with extension and ligation by either removing 3'-phosphates from, or by phosphorylating 5'-hydroxyl groups on the ribose sugar of the DNA backbone, interacts with the NEIL glycosylases (26134572; 16982218) and BER proteins, such as DNA pol β and DNA Ligase III, later in the repair *(see Step 3: Short-Patch DNA Re-synthesis & Ligation.)*.

Step 3a: Short-Patch DNA Re-synthesis & Ligation

p53 stabilizes the APE1-DNA pol β complex on the DNA abasic site (9207062; 11179235) which stimulates the binding between p53 to the DNA lesion (15674341; 9119221), and moreover stimulates BER. DNA pol β has been shown to be down-regulated in Alzheimer's disease models and patients (24121118; 17065437). The Werner protein (WRN) possesses 3'-5' exonuclease and ATP-dependent helicase activities and is able to unwind single strand break BER intermediates. APE1 inhibits WRN helicase activity, likely through binding of APE1 to nicked apurinic/apyrimidinic sites. This would suggest APE1 prevents the promiscuous unwinding of BER intermediates to promote BER (15385537). The physical interaction between WRN and DNA pol β stimulates DNA pol β strand displacement DNA synthesis (12665521; 17173071), and WRN exonuclease activity can act cooperatively with DNA pol β (16449207). A specific inhibitory interaction between p53 and WRN is observed and regulates the functional state of p53 (10506209; 10364153). This binding causes the inhibition of WRN helicase activity (12080066; 15735006; 11427532; 18982914), and similar to APE1-WRN it prevents promiscuous DNA unwinding. Overexpression of WRN leads to augmented p53-dependent transcriptional activity and induction of p21 protein expression (10506209; 11280729) which can regulate cell-cycle pathways. PCNA and p21 form a complex (8101826) to regulate both cell-cycle arrest/apoptosis and DNA repair pathways (18782865; 16551699).

BRCA1 and DNA pol β were found to interact in immunoprecipitation assays (23826138) with DNA pol β expression required for BRCA1 recruitment, suggesting a partnership between these repair factors in DNA damage repair. p53 causes the activation of DNA pol β by binding (11850801; 11179235, 12189182), and p53 binds to and stimulates APE by facilitating loading of DNA pol β onto DNA AP sites. PNKP binds DNA pol β (11163244). DNA Ligase III (LIG3), which catalyzes the joining of DNA ends, is

recruited to the site of DNA damage by DNA pol β (16060670), but a direct interaction between LIG3 and DNA pol β has not been definitively determined (15141024; 16060670).

Step 3b: Long-Patch DNA Re-synthesis & Ligation

Certain proteins are expressed and activated to a greater degree in Long-Patch base excision repair compared to Short-Patch BER. These include proliferating cell nuclear antigen (PCNA) which interacts with DNA pol β to form a complex (12063248; 21245343). The complex is loaded into DNA replication forks and mediates DNA replication in postmitotic neurons (25184665). The tumor suppressor p53 acts as a transcriptional inducer of PCNA (16980608; 26677001) and binds/activates Gadd45α (9566896; 8226988; 1423616) by transcriptional regulation. Gadd45α directly binds with and inhibits PCNA and this interaction has been posited to impede negative growth control (10828065; 7973727) as decreased PCNA expression may result in apoptosis (11682006; 21253613). Gadd45α regulates DNA repair by participating in cell growth arrest, growth suppression, and apoptosis. Gadd45α expression is induced in AD (9848094) and in AD fly models (25252831) through interaction with p53. As well as binding to DNA pol β, PCNA also binds to DNA polymerase epsilon (DNA pol ε) and DNA polymerase delta (DNA pol δ). PCNA is a cofactor of DNA pol δ, which consists of the catalytic subunit, p125, and additional subunits p50, p68 and p12 all of which are also stimulated by PCNA (12403614; 10480866; 11986310). PCNA also activates DNA pol ε (20227374; 9878404; 7827047). PCNA directly interacts with and activates DNA Ligase I (LIG1) (22918593; 10959839; 14729473; 9371766). The direct binding between PCNA and LIG1 results in a negative feedback and causes the inhibition of PCNA (10559261; 9603940). It is proposed that after Okazaki fragment DNA synthesis is completed by a PCNA-DNA pol δ complex. DNA pol δ is released, allowing DNA ligase I to bind to PCNA at the nick between adjacent Okazaki fragments and catalyze phosphodiester bond formation. EGFR phosphorylates PCNA (25825764; 25907674; 17115032; 22692198; 17115032).

Flap structure-specific endonuclease 1 (FEN1) removes 5' overhanging flaps in DNA repair and processes the 5' ends of Okazaki fragments in lagging strand DNA synthesis. This protein has been shown to bind to PCNA with high affinity, and PCNA activates FEN1 recruitment to the site of DNA damage (10899134; 21383776; 9305916). FEN1 regulation has been implicated in trinucleotide repeat disorders, whereby low expression of FEN1 is associated with an increase in somatic CAG repeat expansion in the striatum. Weaker FEN1 cleavage activity results in accumulation of CAG repeat hairpins in the tissue, thus leading to CAG repeat expansion (19997493; 26160176; 15082797). FEN1 stimulates LP-BER DNA synthesis activity of DNA pol β and causes the displacement of DNA pol β from the DNA lesion (10660619; 15561706; 11440997) which was also reported in trinucleotide repeat expansion (19674974). FEN1 has been shown to bind and activate NEIL1 glycosylase (18662981, 26134572). NEIL1 can also participate in strand displacement repair synthesis (long patch repair-BER) mediated by FEN-1 and stimulated by PCNA. FEN1 also binds to NEIL2 (26134572). The p53 tumor suppressor protein has been shown to activate FEN1 by transcriptional regulation in UV-induced DNA damage repair pathways (16103874). FEN1 physically interacts with APE1 (11601988) and APE1 stimulates the removal of the displaced flap by FEN1 (12200445). The high-mobility group box 1 protein (HMGB1) acts as a co-factor to both FEN1 and APE1 (17803946) and HMGB1 has been posited to modulate the propensity of a given tissue for somatic CAG and other TNR expansion (19997493; 8913860; 8972860). FEN1 activates the recruitment of LIG1 to the site of DNA damage (19596905; 16079237) in conjunction with PCNA, and the C terminus of the FEN1 helps coordinate the entry of DNA ligase during Okazaki fragment maturation.

Other interactions involving LIG1in LP-BER include DNA pol β, which forms a stable complex (9685411; 8663274). LIG1 inhibits strand-displacement synthesis of DNA pol β (12000832). The interaction between LIG1 and APE1 results in the stimulation and inhibition of LIG1 by APE1 to prevent the futile cleavage and ligation cycle between these two proteins (12200445; 11641413).

Cell-Cycle Checkpoint Regulation

The regulation of the cell cycle (BRCA1-ATM-ATR-CHK1-CHK2) arrest pathway involved in DNA damage repair is in part mediated by the activation of tyrosyl-DNA phosphodiesterase 1 (TDP1). TDP1 has been implicated in the removal of glycolate from single-stranded DNA containing 3-prime phosphoglycolate by hydrolyzing the phosphodiester bond at a DNA 3' end linked to a tyrosyl moiety, suggesting a role in repair of free-radical mediated DNA double-strand breaks. TDP1 is post-translationally phosphorylated by ATM (19851285; 19851285), and this phosphorylation promotes cell survival and DNA repair. TDP1 has been shown to associate with DNA pol β (23042675; 24183900) and also immunoprecipitates with XRCC1 (13679147) forming a covalent complex. XRCC1 acts as a scaffolding protein that interacts with multiple repair enzymes, which allows these repair enzymes to then carry out their enzymatic steps in repairing DNA. Chk2 formed a complex with XRCC1 and phosphorylates XRCC1 (18971944). XRCC1 interacts with DNA pol β *in vivo*, *in vitro* and in human cells (8948628). This interaction causes the suppression of strand displacement by DNA pol β, allowing for more efficient ligation after filling of a single base patch (8978692). DNA pol β then promotes the recruitment of DNA ligase III-XRCC1 complex (8948628; 16060670; 8264637; 8532526).

BRCA1 is a tumor suppressor involved in cell cycle arrest, apoptosis, transcriptional regulation and DNA damage repair. The pathological accumulation of the Amyloid-β (Aβ) depletes neuronal BRCA1, which may contribute to cognitive deficits in AD (26615780). Moreover BRCA1 has been shown to be localized with Tau neurofibrillary tangles, which is a pathological hallmark of AD (17505559). *In vitro* and *in vivo* analysis demonstrates that BRCA1 and p53 bind (14978302; 9926942) which promotes p53 activation. BRCA1 is phosphorylated by the checkpoint kinases ATR (11114888) and ATM (10866324; 12024016). This phosphorylation is also dependent upon CHK1 and CHK2 (12427729), as BRCA1 activates CHK1 (11836499; 11836499) and CHK2 activates BRCA1 via phosphorylation (15131084; 16675955; 18797466). This in turn causes ATR-CHK2 signaling in p53 activation for DNA damage response (18162465; 10673500) through ATR-dependent phosphorylation of ATM which activates ATM phosphorylation of Chk2 (17124492). PolyQ repeats have been shown to induce ATM/ATR-dependent DNA damage response through accumulation of reactive oxygen species (12915485).

RAD9 checkpoint clamp component A (RAD9) is a cell cycle checkpoint protein required for cell cycle arrest and DNA damage repair that possesses 3' to 5' exonuclease activity, which may contribute to its role in sensing and repairing DNA damage. RAD9 forms a checkpoint protein complex with RAD1 and HUS1 and recruited by checkpoint protein RAD17 to the sites of DNA damage (21659603; 25091155), which is phosphorylated by ATR (11799063; 19020305). The RAD9-RAD1-HUS1 interactions form the 9-1-1 complex (19446481; 26021743; 20505337; 26088138). The 9-1-1 complex interacts with DNA pol β and has a stimulatory effect on DNA pol β activity (15314187; 17426133).

Specific physical association between the BRCA1 RING domain and ATF1 and the expression of BRCA1 stimulates the activity of ATF1 (10945975), which is involved in the stress response. ATF1 acts as a positive transcriptional regulator of PCNA (9883884; 9114015; 7479004; 7910946), CK2 (casein kinase II) contributes to cell survival by cooperating with other survival-promoting pathways, and has been shown to phosphorylate ATF1 and activate ATF1 transcription (9685505; 8600455). p53 binds to CK2 and results

in the down-regulation of CK2 (11180407; 10214938; 9180277) which regulates cell apoptosis pathway, whereas CK2 phosphorylates and activates p53-binding to damaged DNA (10214938; 14640983; 9501176). CK2 also phosphorylates BRCA1 (10403822); DNA Ligase I (11331287; 10523317), and XRCC1 (21840775; 20471329). The imbalance in non-phosphorylated and phosphorylated BRCA1 has been posited to modulate the DNA damage response in HD (22580959). Moreover, CK2 has been observed to be increased in the hippocampus and temporal cortex of AD patients compared to non-demented controls and interacts with Aβ (26732432; 8287280), and has been shown to be up-regulated in HD models (17971125).

Cell Survival

Brain-derived neurotrophic factor (BDNF) promotes the cell survival against oxidative DNA damage-induced death by nhancing DNA repair. BDNF has been shown to be reduced in Huntington's disease models (10825501; 22179319; 17959817) and its rescue results in decreased levels of neurodegeneration (18086127; 21048129). BDNF enhances BER through transcriptional activation of cyclic AMP response element-binding protein (CREB1) which, in turn, transcriptionally activates apurinic/apyrimidinic endonuclease 1 (APE1) (24114393). CBP acts as a coactivator of CREB1 through binding (7913207; 8413673; 9413984) and increases its activity. The ability of BDNF to activate CREB and upregulate APE1 expression is abolished by shRNA of TrkB as well as inhibitors of TrkB, PI3 kinase, and Akt kinase (24114393). Elevated levels of CREB1 have been shown in Huntington's disease models, and that CREB functionality is lost during the early stages of cell stress that contributes to the pathogenic process (14749423; 19632326). APE1 is a co-transcriptional regulator of the SIRTUIN1 (SIRT1) protein. SIRT1 associates with APE1 and regulates the acetylation state of APE1 (19934257; 22918947), and this association is increased with genotoxic stress (19934257). In turn APE1 stimulates SIRT1 activity (24356447). This covalent modification of APE1 by SIRT1 promotes binding of APE1 to the BER protein X-ray cross-complementing-1 (XRCC1) (19934257). SIRT1 is a co-transcriptional regulator of CBP through direct binding (22826441; 22826441). SIRT1 has been shown to be upregulated in AD and Amyloid Lateral Sclerosis models (17581637) which is posited to promote neuronal survival mechanisms and has been shown to be impaired in Huntington's disease models (26815359; 24436303; 22227661; 22179319; 22179316). SIRT1 co-transcriptionally activates BDNF (22179316; 22179319) further enhancing BER. The neuroprotective effect of SIRT1 requires the presence of CREB-regulated transcription coactivator 1 (TORC1), a brain-specific modulator of CREB. The activity of SIRT1 in the deacetylatation and activation of TORC1 promotes TORC1 interaction with CREB. Moreover, TORC1 and SIRT1 act as co-transcriptional regulators of BDNF (22179316). PARP-1 binds to DNA through nonionic interactions (14567702; 23333033; 24588584) which conformationally activates PARP-1. PARP1-DNA interaction facilitates survival of proliferating cells under conditions of DNA damage, probably through its contribution to DNA base excision repair. PARP-1 is acetylated by CBP/p300 (16204234; 15607978). PARP-1 levels have been shown to be high in Alzheimer's disease models (27234294; 25274115; 27034851) and posited that the overactivation of PARP-1 can result in energy depletion and necrosis in response to high levels of DNA damage. However PARP-1 has been shown to be down-regulated in AD mice models (24121118). SIRT1 also enhances TDG glycosylase activity by deacetylation of acetylated TDG through covalent modification (23952905).

The proto-oncoprotein, c-Abl, is a member of the non-receptor tyrosine kinases and has a pro-apoptotic role and is posited to act in the decision as to whether to activate the proapoptotic pathway when the DNA damage is too severe to be repaired. Alzheimer's and Parkinson's diseases patients have shown c-Abl activation, and overexpression in adult mouse neurons results in neurodegeneration and neuroinflammation (15474370; 21728062). The growth suppressor c-Abl increases the DNA binding activity of p53 (17339230; 15661746; 18490454; 15865930) and c-Abl interacts with p53 in response to

DNA damage (10629029). c-Abl phosphorylates ATR and causes its positive activation (20798688; 21151157) as well as catalyzes the phosphorylation of EGFR (18721752; 16943190) promoting an increase in cell-surface expression of the EGFR. c-Abl interacts with JNK1(MAPK8-10), and JNK1 may play an anti-apoptotic role (10022809; 19052872; 15696159). GADD45α has been associated with growth suppression, and GADD45α binds to and inhibits PCNA related negative growth control and inhibits apoptotic cell death (10828065; 7973727; 7784094). Exposure of neurons to Aβ has been shown to induce the up-regulation of GADD54 (11470486; 10448433) indicating DNA damage during early in Aβ cytotoxicity. p53 acts as a transcriptional regulator for a large subset of proteins involved in MMR, including: physical interaction and inhibition of BRCA1 transcription (14710355; 9482880; 25650659), actively binds and down-regulates HMGB1 (11748221; 15170359; 12534345; 11748232), induces FEN-1 transcription (16103874), activates DNA Ligase 1 transcription (10722849; 21332394), activates PCNA transcription (8570655; 8524244; 9651310; 15777783; 12947108), increasing the secretion of EGFR and stimulates the EGFR signaling pathway (20190820; 26799187; 18359760; 9285564), and positively regulates GADD45α (8226988; 10749144; 9566896). The epidermal growth factor receptor (EGFR) directly binds to ATM and causes its phosphorylation and regulation (25601159; 26825989; 11466608), and depletion of endogenous EGFR impairs ATM-mediated DNA repair. EGFR has been suggested to be one of the most significant AD risk genes, as Aβ oligomers can induce the activation of the EGFR (23019586), and over-expression of EGFR may trigger oxidative neuronal injury (10854274). PolyQ peptides have also been shown to cause the constitutive activation of EGFR and potently inhibit EGFR-mediated ERK activation in fly glial cells and posited that disruption of EGFR signaling and ensuing glial cell dysfunction could play a direct role in the pathogenesis of HD and other polyQ diseases in humans (15677486).

NUCLEOTIDE EXCISION REPAIR

Nucleotide Excision Repair (NER) removes helix-distorting DNA lesions and structures from the genome. The serial steps in NER are similar in organisms from unicellular bacteria to complex mammals, and involve (*a*) recognition of lesions, adducts or structures that disrupt the DNA double helix, (*b*) removal of a short oligonucleotide containing the DNA lesion, (*c*) synthesis of a repair patch copying the opposite undamaged strand, and (*d*) ligation to restore the DNA to its original form. Two sub-pathways exist in NER: (*i*) Transcription-coupled NER (TC-NER) is dedicated to the removal of lesions from the template DNA strands of actively transcribed genes, and (*ii*) Global Genome NER (GG-NER), detects and repairs bulky damages in the entire genome, including in the untranscribed regions and silent chromatin.

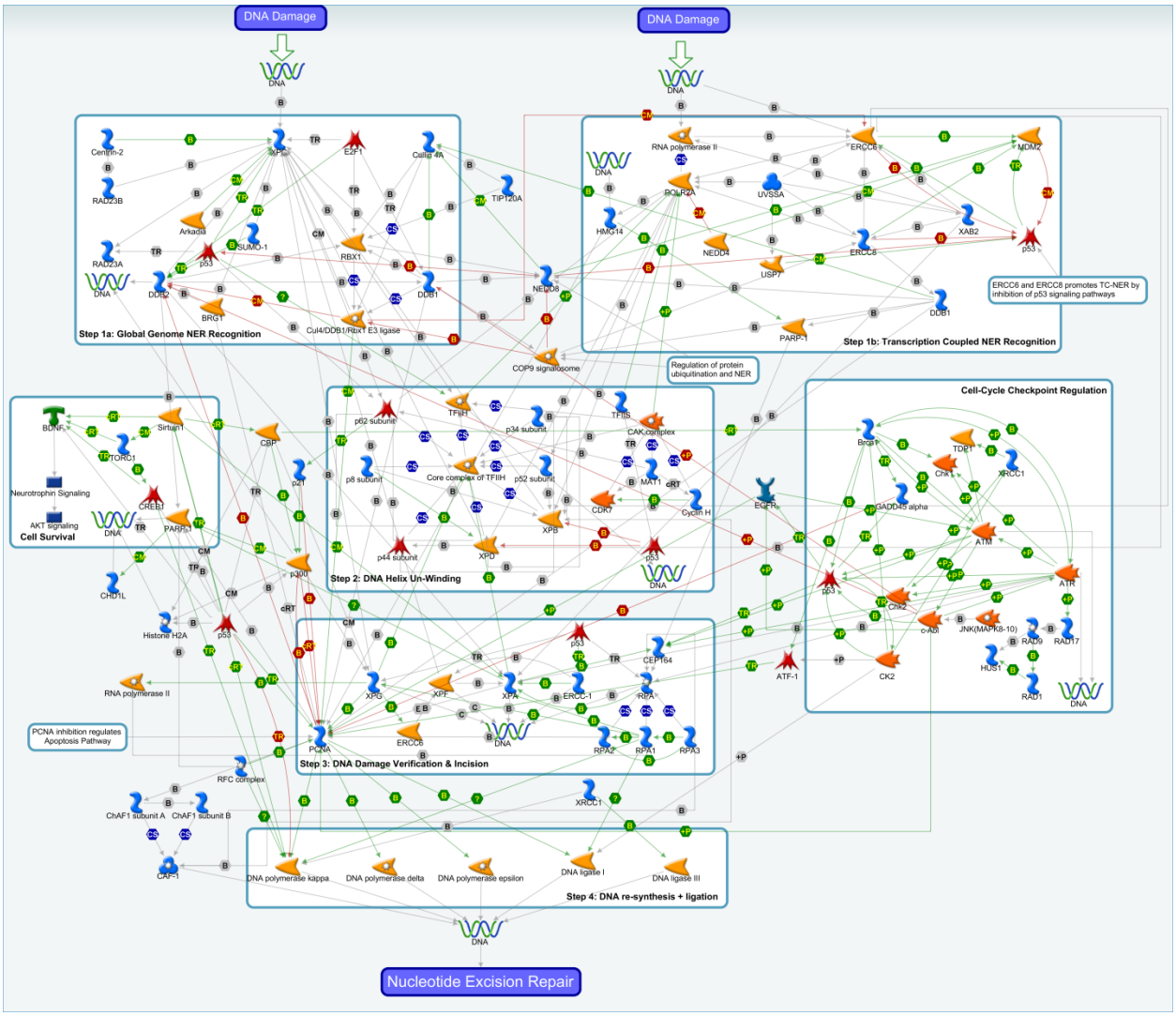

Figure 3: Nucleotide Excision Repair Mechanism

Step 1a: Global Genome Nucleotide Excision Repair Recognition

In GG-NER, a helix distorting lesion or structure is recognized by XPC complexed with RAD23B and centrin 2 (CEN2). The recognition of the DNA lesion is performed by the XPC-RAD23B complex (10873465; 16460043; 8692695; 8756644; 9372923; 15885096; 9164480) and/or XPC-RAD23A (9372924; 9164480) as both RAD23A and RAD23B stimulate the activity of XPC. In the presence of the Aβ peptide, XPC is up-regulated at the mRNA level and NER capacity is also increased following oxidative stress (26263968). Both RAD23A and RAD23B have been shown to accumulate in neuronal inclusions in brain material from HD and PD patients (16860562). CEN2 has a structural function in the centrosome and has posited to have a fundamental role in the structure and function of the microtubule-organizing center, possibly being required for the proper duplication and segregation of the centrosome. CEN2 stimulates the binding and increases the yield of DNA adducts with XPC-RAD23B during protein recognition of bulky damages in DNA (22809153). Inhibition of XPC expression correlates with decreased CEN2 transcript and protein, indicating that XPC is required for the fine tuning of CEN2 gene

expression (21676658). CEN2 directly binds and activates XPC through both electrostatic and apolar inter-molecular interactions through the burial of apolar bulky side-chains into the hydrophobic pocket of CEN2 (17897675; 16533048; 12890685). The interaction between XPC-CEN2 results in the stabilization of XPC, thus stimulating XPC activity (12890685; 15964821). The XPC-RAD23B-CEN2 complex melts the DNA around the lesion and recruits the multiprotein complex TFIIH.

Damaged DNA binding protein 2 (DDB2) directly binds to damaged DNA (16951172) when certain DNA lesions do not significantly destabilize the DNA duplex. In complex with damaged DNA binding protein 1 (DDB1), it creates a kink that is recognized by XPC. DDB2 is stabilized by PARP-1 binding (23319653; 23045548; 22492724). DDB1 and DDB2 are part of the CUL4A-RBX1 ubiquitin ligase complex that ubiquitinates DDB2, whereby DDB2 and DDB1 co-immunoprecipitate with CUL4A and RBX1 (18593899; 18936169; 16951172). The cullin associated and neddylation dissociated 1(TIP120A) is an essential regulator of Cullin-RING ubiquitin ligases, and binds to un-neddylated Cullin 4A (17079684; 21249194; 16964240) and RBX1 (15537541; 12504026; 12609982; 12504025). TIP120A binding to cullin-RING Ub-ligase has been posited to result in the negative regulation of the CUL4A ligase activity through sequestration of cullin proteins and preventing auto-ubiquitination of substrate receptors (12504026; 15448697). DDB2 itself is subject to ubiquitination, which results in degradation by the 26S proteasome (17363340; 25628365). Poly-ubiquitination of XPC is performed by the DDB1-DDB2-CUL4A-RBX1 E3 ligase complex and has been posited to be performed by CUL4A component of the complex (25628365). Degradation of DDB2 is believed to be a factor in the recruitment of XPC to the DNA lesion (16527807; 15811626; 11564859; 12034848).

XPC is covalently modified through the binding of SUMO-1 and DDB2 for UV-induced XPC modification, which is believed to stabilize XPC (16030353; 15882621; 17693435; 26151477). SUMO-1 has also been shown to be responsible for the SUMOylation of DDB2 that facilitates the repair of cyclobutane pyrimidine dimers from global genome damage (23860269). Moreover RNF111/Arkadia (Arkadia), which is a SUMO-targeted ubiquitin ligase, promotes ubiquitination of SUMOylated XPC that participates in the recruitment of XPC to UV-damaged DNA (23751493). SUMO-1 has been shown to covalently modify a range of neurodegenerative proteins, including huntingtin (15064418), which has been posited to reduce huntingtin aggregation but may also stabilize toxic huntingtin species.

E2F-1 is shown to accumulate at sites of DNA damage and the E2F-1 protein transcriptionally regulates DDB1 (11564859; 9418871; 10373543), DDB2 (11564859; 10373543; 17173070), RBX1 (11245432), and XPC (19376752; 20413589) all of which are involved in GG-NER. Post-mortem brain samples from Huntington's patients show an increase in the expression of E2F-1 in comparison with control samples (18768156). Moreover, E2F-1 has been shown to up-regulate Huntington's disease associated micro RNA's (22581158) as has been observed in Alzheimer's disease (24027266). Neurons lacking the E2F-1 transcriptional regulator show significant protection from Aβ induced cell death (10766769), while Aβ deposition may trigger E2F-1-mediated neuronal apoptosis as E2F1 is up-regulated in Alzheimer's disease-like neurodegeneration in Down's syndrome (11423103). DDB2 is ubiquitinated targeting it for proteasomal degradation as a GG-NER control mechanism (25628365; 16713579).

The tumor suppressor protein, p53, is also an important transcriptional regulator in the GG-NER subpathway. p53 transcriptionally activates DDB2 (11971958; 14560002; 16140933; 16260627; 18922183) in humans but not in mice, and activates and promotes transcriptional expression of XPC (16413492; 9892649; 26029824). The chromatin remodeling factor BRG1 has been posited to play a role in the recruitment of XPC to the site of DNA damage (19901545). BRG1 is also required for XPC foci formation. BRG1 interacts with XPC within chromatin (19901545; 19740755) and is recruited to UV-damaged sites in a DDB2- and XPC-dependent manner (19901545; 19740755). It was shown that loss

of BRG1 increased damaged and dying neurons associated with oxidative stress in BRG1 knockout mice (26133793) and may play a critical role in neuronal growth. PARP-1 has been posited to play a role in the DDB2 and DDB1 lesion recognition through the stabilization of DDB2 and the recruitment of the chromatin remodeler ALC1 (CHD1L) (23319653; 23045548). DDB1 (16964240; 25675097) and DDB2 co-localize with PCNA and p21 at local UV-induced DNA-damage sites. DDB2-PCNA association is required for DDB2 proteolytic degradation (24200966) and this interaction influences cell cycle progression (26697842). DDB2 has been posited to be up-regulated in Amyloid Precursor Protein (APP) expressing cells as a result of Aβ-dependent activation of these DDB2 following H2O2 treatment (26263968). Moreover, this demonstrates that Aβ secretion and oxidative stress leads to the over-expression of DDB2.

Step 1b: Transcription Coupled Nucleotide Excision Repair Recognition

TC-NER is activated by stopped RNA polymerase II (RNAP II) at the damaged sites of an actively transcribing gene. RNAP II produces messenger RNA during transcription of protein-coding genes in all eukaryotic cells. RNAP II recognizes a DNA lesion located in the transcribed strand as it transcribes a gene, which causes the RNAP II to stall or arrest. RNAP II physically interacts with the DNA lesion (11087726, 26789250; 16407975). CSB/ERCC6 (ERCC6) interacts with RNAP II engaged in ternary complexes containing DNA and nascent RNA (9372911), and functions as a DNA-activated ATPase, and hydrolyses the ATP beta-gamma phosphoanhydride bond required for the formation of a stable RNAP II-ERCC6-DNA-RNA complex (9372911; 9312053; 16246722). ERCC6 does not act as a helicase nor does it dissociate stalled RNA polymerase II. It has been posited to be involved in a coupling mechanism in humans (8999876; 15226310), as ERCC6 transiently interacts with the transcription machinery and upon RNAP II arrest. The ERCC6 binding interactions are prolonged, acting to monitor the progression of transcription by regularly probing elongation complexes. Other transcription-coupled factors bind to ERCC6 during the recognition step of TC-NER, including CSA/ERCC8 (ERCC8). ERCC6, ERCC8, MDM2, and p53 form a complex (22032989) which has been posited to regulate beneficial and detrimental effects of p53 activity upon cellular stress. ERCC6 and ERCC8 physically interact (16916636; 20541997; 22032989), and ERCC6 binds and activates MDM2 (22032989; 10698517), and this interaction has been posited to play a role in the UV induced apoptotic pathway. Mutations of the ERCC6 and ERCC8 genes are the predominant cause of the Cockayne syndrome, a rare fatal autosomal recessive neurodegenerative disorder, and the ERCC6 gene mutation is present in approximately 65% of cases (25251875; 25376329; 19309286). ERCC6 has been posited to protect CAG repeats from expansion by either active reduction of the tract length during parent-child transmission or by antagonizing the action of the base excision repair protein, OGG1, which tends to promote expansion in somatic cells (21566259). The regulation of MDM2 expression is through a feedback loop with p53 (22032989), whereby p53 transcriptionally up-regulates MDM2 (23800173; 12138177; 20679392; 10618704), which in turn inhibits p53 by mono-ubiquitination (22679490; 26132471; 19450511; 20679392) thus regulating cell fate. MDM2 levels have been shown to be decreased under conditions of cellular stress and this is thought to contribute to the hyper-phosphorylation of Tau in AD (20181016). Furthermore, Aβ treatment of primary cortical neurons led to caspase-dependent MDM degradation (26477779). Elevated levels of cellular p53 were detected in expanded CAG RNA-expressing cells (22847428) and huntingtin (21465263). The direct binding of ERCC6 and p53 is inversely related with ERCC6 facilitating the association of p53 with DNA when p53 concentrations are low, and p53 preventing ERCC6 binding to nucleosomes when p53 concentrations are high (22383384; 21852235; 22032989; 20470425). ERCC8 binds and inhibits the activity of p53 (22032989; 12888115) which regulates the repair of the DNA lesion and the restoration of transcription. Therefore, both ERCC6 and ERCC8 promote transcription-coupled excision repair by inhibiting p53 induced apoptosis signaling pathways.

The UV-stimulated scaffold protein A (UVSSA) is required for stabilization of ERCC6 (22466612; 22466610; 22902626) and knockdown of UVSSA results in TC-NER deficiencies (22466611). This stabilization of ERCC6 is the result of UVSSA recruitment of the ubiquitin-specific peptidase 7 (USP7) protein (22466611; 27129218), resulting in a critical regulatory mechanism of TC-NER in restoring gene expression. The interaction between USP7 and UVSSA cooperate to protect ERCC6 from UV-induced degradation via the ubiquitin-proteasome pathway in TC-NER. This was demonstrated by induced depletion of USP7 by siRNA causing a reduced recovery of RNA synthesis deficiency and decreased levels of ERCC6 (22466611; 22621766; 22466612). At later stages of the repair process, ERCC6 is degraded by by the E3 ubiquitin ligase complex (Cul4/DDB/ROC1 E3 ligase) (16751180). ERCC8 co-immunoprecipitates with the UVSSA-USP7 complex and is recruited to RNAP II (22466612; 22466611; 22466610). This recruitment to RNAP II-ERRC6 is facilitated by ERCC8 (22466612; 22902626). USP7 covalently modifies the transcription regulator p53, removing ubiquitin to regulate p53 turnover and regulated p53-dependent cell cycle arrest and apoptosis (14506283; 12507430; 11923872; 17525743).

The high-mobility group (nonhistone chromosomal) protein 14 (HMG14) binds nucleosomal DNA and is associated with transcriptionally active chromatin and is posited to help maintain an open chromatin configuration around transcription ready genes. HMG14 co-immunoprecipitates with RNAP II upon UV-induced damage (16916636). HMG14 has been shown to induce changes in chromatin structure and histone modifications, and is overexpressed in people affected by Down's syndrome (22009741). The HMG proteins have been implicated in neurodegenerative disorders and have been shown to bind to Aβ, cause the stabilization of Aβ oligomers, and are implicated in AD (12565837; 22645697). The knockdown of the XPA-binding protein 2, (XAB2), with small interfering RNA results in hypersensitivity to killing by UV light and a decreased recovery of RNA synthesis after UV irradiation (17981804). XAB2 has been shown to co-immunoprecipitate with ERCC6 (22466610; 10944529; 16916636; 18166977), ERCC8 (16916636; 18166977), UVSSA (10944529; 22466610), and RNAP II (10944529; 17981804). ERCC8 forms a complex with DDB1 (22118460; 18794354; 16916636) that is posited to result in ubiquitin ligase activity.

Regulation of GG-NER and TC-NER

The regulation of the ERRC8 and DDB2 ligase activity in TC-NER and GG-NER, respectively, is controlled by the COP9 signalosome (CSN) (12732143). CSN acts as a regulator of E3 ubiquitin ligases and has been shown to suppress ubiquitination of Cul4/DDB1/Rbx1 E3 ligase (17363340; 27029275; 16005295). Therefore CSN regulates NER protein degradation and moreover positively regulates DNA lesion repair while repressing apoptosis. CSN binds to DDB1 (16949367) and ERCC8 (22118460), suggesting that CSN plays a role in both GG-NER and TC-NER. CSN has been shown as a regulator of dendritic arborization, which has been shown to modulate and affect memory, synaptic plasticity, behavior, and learning (19855832; 19859546). In GG-NER, CSN rapidly dissociates from the DDB2 complex after UV irradiation. After DNA repair has occurred, CSN is posited to re-associate with the DDB2 complex. In contrast, CSN rapidly associates with the CSA complex after UV irradiation (22118460) presumably leading to suppression of the ubiquitin ligase activity, suggesting that CSN plays a central role in GG-NER and TC-NER.

Neural precursor cell expressed, developmentally down-regulated 8 (NEDD8) is a ubiquitin-like protein that partly mediates cell cycle control and embryogenesis. The attachment of NEDD8 to cullins causes their associated E3 ubiquitin ligase activity, promoting polyubiquitination and proteasomal degradation of cyclins and other regulatory proteins (25833379). NEDD8 covalently modifies Cullin 4A causing its activation (10597293; 9694792; 20682250), which in turn activates the Cullin 4A ubiquitin ligase activity (21487042; 17439941). The ubiquitin ligase activity is then further regulated by CSN (27029275). RBX1, ring-box 1 protein, has been posited to play a role in the ubiquitination of Cullin A4 by NEDD8 (24949976),

as RBX1 and NEDD8 interact in the Cul4/DDB1/Rbx1 E3 ligase complex (11961546; 12565873). NEDD8 also interacts with DDB1, which is part of the Cul4/DDB1/Rbx1 E3 ligase complex (18247557; 16964240; 21145461). Both DDB1 and DDB2 have been shown to be negatively regulated by their phosphorylation by C-Abl (12107171; 16713579). NEDD8 binds and activates MDM2, which has been posited to increase MDM2 stability (19784069; 16980297; 15242646) and promote ubiquitin-mediated inhibition of p53 (15242638) and regulation of the NER and cell cycle/apoptosis pathways. NEDD8 has been implicated in Huntington's disease in rescuing neurons from huntingtin (23525043), and found to be localized in Lewy bodies in PD and neurofibrillary tangles/senile plaques in AD (12533840; 15634231). The neural precursor cell expressed, developmentally down-regulated 4, E3 ubiquitin protein ligase (NEDD4) functions in the ubiquitin proteasome system of protein degradation. It has been shown that NEDD4 is recruited to the RNA polymerase II subunit POLR2A as a result of transcriptional arrest at DNA lesions and causes the ubiquitination of RNAP II (17996703; 9305852; 10490634) and causes RNAP II transcriptional arrest and subsequently leads to RNAP II degradation. NEDD4 has been shown to promote ubiquitination of MDM2, which in turn causes the stabilization of MDM2 (24413081). This ubiquitination of MDM2 has been shown to play a role in the MDM2-p53 feedback loop, whereby NEDD4 knockdown in p53-bearing cells increases basal p53 levels and activity in an MDM2-dependent manner, causing stronger p53 responses to DNA damage and resulting in p53-dependent growth inhibition compared to corresponding NEDD4-1-proficient control cells (24413081; 26250624; 27000207). NEDD4 has been implicated in the synaptic alterations induced by Aβ (26843640). Overexpressed NEDD4 in the Drosophila brain prevent the α-synuclein-induced locomotor defect whereas reduction in endogenous NEDD4 by RNAi leads to worsening motor function and increased dopaminergic neuron loss (24388974; 24831002).

Histones are highly alkaline proteins that package and order the DNA into chromatin in eukaryotic cells. The covalent modification of histones has been implicated in regulating NER, whereby the hyper-acetylation of histones enhances NER following UV irradiation (7142158; 2738057). p300/CBP acts as a histone acetyltransferase (8945521), and has been implicated in chromatin relaxation prior to and after lesion detection, repair synthesis, and chromatin restoration after NER (21217779; 18263614). UV-induced chromatin relaxation is achieved by p53-mediated histone acetylation and p53 binds to and recruits p300 to sites of NER (12574133; 11511360), which facilitates CBP/p300 induced histone acetylation (21315607; 11511360; 8945521; 12665567; 11134336; 17320507) and regulates histone H2A (16751067; 24268990; 19287485). The depletion of CBP has been linked to HTT -induced neurotoxicity both in cellular models and transgenic mouse models (16766198; 15994095). It has been proposed that CBP is sequestered in the protein aggregates of mutant HTT observed in the brain tissue of patients and in most experimental models of the disease (11264541; 10823891; 22116937; 16525063). CBP has also been shown to be dysregulated in AD, whereby activation of amyloid precursor protein-dependent signaling reduces CBP levels in primary neuronal cultures (14657026) and Aβ impairs CBP activity (11278679; 25888034). The polyglutamine-containing domain of HTT inhibits the enzymatic activity of both p300 and CBP (11607033). p53 also binds to histone H2A (18097557) and has been posited to play a role in the histone acetylation. Histone modifications are altered in HD, and mono-ubiquitination of histone H2A may not function at the level of the individual gene but may rather influence transcription through global chromatin structure (25062675). H2A histone family members are specifically overexpressed in the blood and frontal cortex of patients with HD compared with controls (21969577).

Step 2: DNA Helix Un-Winding

Transcription factor II H (TFIIH) is a basal transcription complex involved in transcription regulation. TFIIH is a multiprotein complex containing the core complex proteins (TFIIH p62 subunit, TFIIH p44 subunit,

TFIIH p52 subunit, TFIIH p8 subunit, TFIIH p34 subunit, XPD, and XPB) and the CAK (cyclin-activating kinase) complex (MAT1, CDK7, and Cyclin H). TFIIH is the only general transcription factor with known enzymatic activities, including DNA-dependent ATPase, ATP-dependent DNA helicase, and CTD (carboxy-terminal repeat domain of the largest RNA polymerase II subunit) kinase activities. TFIIH also regulates the transition from transcription initiation to elongation, functions as an essential component in nucleotide excision repair and has been implicated in mammalian cell cycle progression. The main function of TFIIH in NER is to open the DNA around the lesion and thereby allow the excision of the damaged oligonucleotide and its replacement by a new DNA fragment. Mutations in these subunits are associated with three genetic disorders: xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD) (10064601; 26149386; 11062469; 10660593).

In GG-NER, the TFIIH complex is recruited to the DNA lesion through the binding of the TFIIH p62 subunit to XPC (26278177; 26909369; 26029824). Additionally XPC associates with XPB and stimulates the XPB ATPase activity to initiate damaged DNA opening (18809580; 10734143) through the 3´ - 5´ DNA helicase activity of XPB. p53 binds to THIIF p62 subunit (26029824) and diphosphorylation of p53 leads to a significant enhancement in p53 binding to THIIF p62 subunit (16793543; 18160537). THIIF p62 subunit has been shown to co-precipitate with XPB and XPD both *in vitro* and *in vivo* (8652557; 15220921; 11259578; 8152490). The XPD helicase associates with XPC (18277385) and XPD catalyzes the 5´ - 3´ DNA helicase activity. The tumor suppressor protein p53 has been shown to inhibit both helicases XPB and XPD, but does not inhibit their ATPase activities or transcriptional activities (7663514), this is posited to act as a failsafe mechanism to prevent promiscuous DNA unwinding. Patients carrying mutations in the XPB helicase subunit display the combined cancer and developmental-progeroid disorder xeroderma pigmentosum / Cockayne syndrome (19114557).

Within the THIIF complex, the THIIF p52 subunit interacts with XPB and stimulates its ATPase activity (17466626) by stabilizing XPB. Drosophila models show that point mutations introduced in the THIIF p52 subunit destabilizes the interaction between THIIF p52 subunit and XPB, compromising the assembly of the THIIF complex (17339330). THIIF p34 subunit has been suggested to play a role in splicing mechanisms in the first step of mRNA splicing (23147676) and has been shown to interact with the THIIF p44 subunit and XPD in the TFIIH complex (25013903; 8652557). The THIIF p44 subunit also interacts with XPD and stimulates its ATPase and 5´ - 3´ helicase activities (25268380; 25681444; 9771713; 10924514). The TFB5 (THIIF p8 subunit) subunit interacts with and stimulates the XPB ATPase activity to trigger DNA opening during DNA repair and is implicated in regulating cellular levels of TFIIH (16669699; 19172752; 24127601). It is posited that the THIIF p8 subunit has a stabilizing function and protects TFIIH from degradation (15220921). The THIIF p8 subunit also binds with and increases the activity of XPD (16427011) and its overexpression in trichothiodystrophy cells counteracts the detrimental effect of XPD mutations by restoring the cellular TFIIH concentration (16427011). THIIF p8 subunit is thought to act in conjunction with XPB as an ATP-driven motor that supplies the energy that is required to reorganize the intermediate DNA repair complex and thereby supports the repositioning of XPC–RAD23B and the unwinding of DNA by XPD (14981083).

The cyclin-dependent kinase (CDK)-activating kinase) (CAK) subcomplex of TFIIH has been implicated in cell cycle control, is responsible for activating phosphorylation of several kinases and is thought to serve as a direct link between the regulation of transcription and the cell cycle. Cyclin H binds to and increases the activity of CDK7 (11319144; 8521393). CDK7 catalyzes the carboxyl-terminal domain (CTD) kinase activity. CDK7 also interacts with MAT1 (7588631; 8521393; 8521818) to form the CAK complex. CDK7 is significantly elevated in susceptible hippocampal neurons of Alzheimer disease patients in comparison with age-matched controls (11124424) and has been implicated in CAG instability (25026993). MAT1

acts as a co-transcriptional regulator of cyclin H (23765726; 20231280). The CAK component, MAT1, is rapidly recruited to UV-induced DNA damage sites and co-localizes THIIF p62 subunit (23083890). The release of the CAK complex from TFIIH has been posited to initiate the incision/excision of the damaged oligonucleotide in NER (18614043; 20543986) and thereby regulate the helicase activities of the TFIIH complex (23083890).

In TC-NER, TFIIH catalyzes the formation of the open complex and phosphorylates POLR2A (10385623; 9852112; 21326911), which has been posited to result in a conformation change in RNAP II leading, to the formation of a transcription initiation complex. This phosphorylation of POLR2A is believed to be mediated by the CAK complex, CDK7 kinase subunit (24855060; 10886368), and to participate in damage-induced RNAP II degradation (20543986). The p52 subunit has been shown to interact with RNAP II (24134817; 9822615), along with the THIIF p62 subunit (9774388). The two helicases, XPD (23446344; 18510924) and XPB (23446344; 22751016; 16916636), interact with POLR2A to form part of the pre-incision complex. In HD transgenic mice models POLR2A expression has been measured up-regulated at later time points in the mouse model (20089533). ERCC6 binds to XPB (16916636; 23637612) and recruits it as part of the DNA repair mechanism. Additionally, ERCC6 binds to XPD (20541997). TFIIS binds to and stimulates RNAP II activity to hydrolyze the transcript to create a new RNA 3´ end for transcription, has been posited to have an anti-arrest function and to induce transcript cleavage by RNAPII (12646562; 9765293; 9305922). However, knockdown of TFIIS does not readily affect the cellular UV toxicity under the applied conditions suggesting no or only a minor role of TFIIS in counteracting the cytotoxic effects of UV-C exposure (20729154), but instead has been posited to regulate the phosphorylation of RNAP. The transcription of TFIIS is regulated by p53 (27005522).

Step 3: DNA Damage Verification and Incision

Xeroderma pigmentosum complementation group A (XPA) has been shown to directly bind to damaged DNA (8218288; 10373492; 9592168), and has been posited to act as the last recognition step of damage during the repair mechanism. Its involvement in NER has been suggested as a scaffold by interacting with both damaged DNA and several NER proteins (25056193). It has been posited that XPA acts to verify the presence of a lesion (9734359; 8538652) and recruits the structure specific endonuclease ERCC1-XPF heterodimer (19940136; 7876263), which incises the damaged strand of DNA 5´ to a lesion. XPA directly binds to the ERCC1-XPF complex (8197174; 7598728; 7891694; 8972858; 15358100; 17948053) and plays a role in the localization or loading of an incision complex composed of ERCC1-XPF (22547097). XPA mutants are UV-sensitive and have a reduced capacity to repair UV-induced DNA damage, while *C.elegans* containing mutants have shortened lifespan (22091407). Moreover, XPA deficiency does not substantially affect TNR instability, however it dramatically reduces CAG repeat instability in the neuronal tissues: striatum, hippocampus and cerebral cortex (21926083). ERCC1 deficient mice models have been shown to have impaired NER and develop age-dependent motor neuron abnormalities (20602234). Moreover, ERCC1 deficiencies impact the dopaminergic system and are associated with human PD pathology (27210754). Ercc1 deficient mice exhibit an age-dependent decrease in neuronal plasticity and progressive neuronal pathology (21880916). DDB2 complex-mediated ubiquitination plays an important role in recruiting XPA to damaged sites (20368362; 19056823; 11278856) and mutation in XPA result in DDB failing to stimulate the excision of cyclobutane pyrimidine dimer DNA damage lesions (19056823). DDB2 has also been posited to assists in the recognition of damage through the efficient recruitment of XPA (11278856). Finally, the binding between DDB2 and XPA has been hypothesized to be important in clinical cases through studies of mutant XPA *in silico* data (24063568).

As well as being a general transcription factor in NER, TFIIH has also been shown to be required for the transcription-independent excision reaction. XPA specifically binds to TFIIH and is essential for affecting the excision reaction (9287294; 9698541; 7876263; 15358100). The XPA DNA damage verification is in part regulated by sirtuin 1 (SIRT1), which is part of the cell survival pathways. SIRT1 binds to and deacetylates XPA and is required for optimal NER pathway. It has been shown that if SIRT1 expression is increased it results in a decrease in acetylated XPA, and inhibition of SIRT1 abrogated XPA binding to DNA lesions (20670893; 26317794). It has been suggested that ATR is required for the UV-induced nuclear focus formation of XPA, and that the ATR checkpoint pathway may modulate NER activity through the regulation of XPA redistribution in human cells upon UV irradiation (16862173; 16540648; 19586908; 22174788; 23178497). Moreover, the ATR checkpoint pathway can modulate the cellular activity of NER through phosphorylation of XPA (16540648; 19586908). Replication protein A (RPA), in particular the RPA1 subunit, has been shown to bind and increase the activity of XPA (7876167; 7665601; 8972858; 7565690; 9535929; 10828957; 15358100) and was suggested to influence the stabilization of the XPA-DNA interaction as mutant RPA that is defective in its interaction with XPA fails to stabilize XPA-damaged DNA complex (10828957). The proliferating cell nuclear antigen (PCNA) has also been shown to be an essential interaction partner to XPA in NER (23152873; 8814338), as XPA(-/-) cells complemented with mutant XPA incapable of binding PCNA had increased UV sensitivity and reduced repair of cyclobutane pyrimidine dimers lesions (23152873). PARP1 activation following UVR exposure promotes association between PARP1 and XPA (23038248; 24953096; 26880244), and inhibition of PARP1 activity decreases UVR-stimulated XPA chromatin association and mutational analysis shows that mutant PARP1 lowers DNA binding affinity of XPA. XPA also binds to XAB2 (10944529) and CEP164 and this interaction has been shown to be enhanced during UV irradiation (19197159). XPA binding to CEP164 mediates localization of CEP164 at sites of DNA damage. Furthermore, CEP164 is phosphorylated upon replication stress, ultraviolet radiation (UV) and ionizing radiation by ATR and ATM (18283122). CEP164 has been shown to co-localize with p53 (19197159), which acts as a transcriptional regulator (23651856). Finally, analysis of CEP164 knockdown cells demonstrates a critical role of CEP164 in G2/M checkpoint and nuclear divisions.

XPG is a latent endonuclease with a structural and catalytic role in NER. XPG is recruited to the damage region to complete the formation of a stable pre-incision complex and fulfills a structural role in stabilizing the pre-incision complex generating an open-stable complex. Dual incisions of the lesion at the 5´ and 3´ end are performed by XPF and XPG, respectively (9351836; 10026181). Defects in XPG can cause either the cancer-prone condition xeroderma pigmentosum (XP) alone, XP combined with the severe neurodevelopmental disorder Cockayne Syndrome (CS) or the infantile lethal cerebro-oculo-facio-skeletal (COFS) syndrome, characterized by dramatic growth failure, progressive neurodevelopmental abnormalities and greatly reduced life expectancy (25299392). The recruitment of XPG to the DNA lesion has been posited to be mediated by TFIIH, as XPG physically interacts with the core TFIIH protein complex (15572672;17000769; 11259578; 8652557; 17466625; 11141066). Moreover, it has been shown that XPG acts to stabilize the TFIIH complex as mutations in XPG found in XP-G/CS patient cells that prevent the association with TFIIH also resulted in the dissociation of CAK and XPD from the core TFIIH (17466625). In the absence of ATP TFIIH has been reported to inhibit the nuclease activity of both XPG and ERCC1-XPF (11141066), suggesting that TFIIH regulates the endonuclease activities of these proteins. XPG has a part in transcription-coupled NER as XPG has been shown to interact and bind with RNAP II, whereby bound RNAP II blocks bubble incision by XPG, but an ATP hydrolysis-dependent process involving TFIIH creates access to the junction allowing XPG incision (16246722; 16118227; 12110180; 16407975). XPG also physically interacts with ERCC6 and increases its activity (8652557; 16916636). XPG has been posited to play a role in global genome NER as XPG probably binds to XPC, and in yeast models it was shown that XPG/RAD2 displaces XPC/RAD4 from the repair complex which may be mediated by TFIIH (18277385; 23295669; 11259578). The interaction between p300 and XPG is

regulated by p21. Human fibroblasts lacking p21 show abnormal accumulation of XPG at DNA damage sites. As such, p300/CBP may facilitate NER by acetylating not only histones but also the core NER factor XPG (22954786), and p300/CBP has been shown to bind to and covalently modify XPG. This acetylation of XPG has been shown to be in part mediated by PCNA (22954786), and this binding between XPG and PCNA (16189514; 24674623; 9305916), also protects PCNA from p21 induced inhibition (18079701), and XPG-PCNA may also regulate cell cycle arrest pathways (24674623). Mutations in p21 have been implicated in modulating Alzheimer's disease and Parkinson's disease (25625488; 23306186). The ratio of p21/p21(thr145) in peripheral blood lymphocytes have been posited as potential biomarkers in Alzheimer's disease (22503900). An N-terminal fragment of HTT has been shown to repress the transcription of p21 (10823891). p21 binds to HTT *in vivo* and *in vitro*, and p21 overexpression enhances the aggregation of mutant HTT (18065495). p53 has been posited to act as a transcriptional factor for XPG as a putative binding site between the two has been identified (20018659; 20233728; 22848513). The endonuclease activity of XPG has been posited to be in part mediated by RPA as a strong DNA interaction domain of RPA is positioned at the 5' side of its binding region while a weak DNA-binding domain resides at the 3' side (9716411), this polarity appears crucial for positioning of the excision repair nucleases XPG and ERCC1-XPF on the DNA. XPG and RPA interact to enhance the endonuclease activity of XPG (9716411; 8626644; 7700386).

ERCC1-XPF nuclease nicks DNA specifically at junctions between double-stranded and single-stranded DNA, when the single-strand is oriented 5' to 3' away from a junction. Nuclease activity resides entirely on the XPF module (11953324) while the ERCC1 is responsible for binding both the *ss/ds*DNA Y junction and XPA (16338413; 16076955; 17948053). XPF binds to ERCC1 (16076955; 15932882; 16338413), and stabilizes the endonuclease subunits. XPF has also been shown to physically interact with ERCC6 (20541997) and co-localizes with XPC after UV induced DNA damage (19279666). ERCC1 are transcriptionally regulated by both CBP (25314079) and p53 (15220520; 11571296).

The replication protein A2 (RPA) acts as a ssDNA-sensing ubiquitin ligase during the DNA damage response driven by ATR (24332808; 20616048). Defective RPA mutants inhibit the activation of ATR kinase in DNA damage response demonstrating that RPA acts as an activator of ATR kinase. ATR-mediated suppression of dormant origins shields active forks against irreversible breakage by preventing exhaustion of nuclear RPA (24267891). RPA has been shown to greatly enhance the activity of LIG1 (11698410) and is posited to increase the catalytic rate of LIG1, but not improve substrate binding. RPA has been shown to interact with PCNA (12171929; 11254741; 20227374), PARP1 (21945626; 26456830; 22246237) and RNAP II (8609996; 16407975) whereby RPA localizes to regions of active transcription, which is strongly correlated with active RNAP II transcription but independent of replication (22055186). RPA expression levels have been shown to be elevated in Huntington's disease (24191263). RPA has also been shown to associate with DNA polymerase κ (20227374); and in cooperation with PCNA, RFC stimulates re-synthesis (11784855).

PCNA acts as a clamp to load DNA polymerases to the DNA lesion while RFC complex acts as a clamp loader, specifically binding to PCNA (25450506; 14530260; 11121020; 10051561) to load PCNA onto the DNA (16980295; 9822671) with polymerase κ (11784855; 18162470; 20227374). It was postulated that reversible ubiquitination of PCNA can prevent spurious translesion synthesis polymerases, including DNA Pol κ recruitment and regulate replication fork speed to ensure the maintenance of genome integrity (22157819).

Cul4/DDB1/RBX E3 ligase causes the mono-ubiquitination of PCNA in response to DNA damage, which has been posited to result in a critical posttranslational modification essential for DNA repair by translesion DNA synthesis (20129063). HMGN1 is a PCNA interacting protein that enhances the binding

of PCNA to chromatin (22393258). BRG1 co-localizes with replication factors at sites of DNA replication on extended chromatin fibers. BRG1 co-localizes even more strongly with PCNA, and this co-localization was highly enriched at sites of DNA replication (20571081). PCNA and p21 form a complex (8101826) which regulates both cell-cycle arrest/apoptosis and DNA repair pathways (18782865; 16551699). p21 co-localizes with both p300 and PCNA at UV-damaged sites. Loss of p21, or its inability to bind PCNA, results in a prolonged binding to chromatin and an increased association of p300 with PCNA in UV-irradiated cells. The histone acetyltransferase (HAT) activity of p300 is reduced after DNA damage and p21 is required during DNA repair to regulate p300 HAT activity by disrupting its interaction with PCNA (18263614). The tumor suppressor p53 acts as a transcriptional inducer of PCNA (16980608; 26677001), and binds/activates Gadd45α (9566896; 8226988; 1423616) by transcriptional regulation. Gadd45α directly binds with and inhibits PCNA, and this interaction has been posited to serve to impede negative growth control (10828065; 7973727) as decreased PCNA expression may result in apoptosis (11682006; 21253613). PARP-1 regulates the function of PCNA through direct binding (12930846; 9649317; 18093328; 20303835). RFC specifically binds to PCNA (25450506; 14530260; 11121020; 10051561) and RFC loads PCNA onto the DNA (16980295; 9822671). PCNA expression levels have been shown to be elevated in Huntington's disease (24191263). PCNA also binds to DNA polymerase epsilon (DNA pol ε) and DNA polymerase delta (DNA pol δ). PCNA is a cofactor of DNA pol δ and DNA pol ε consists of the catalytic subunit, p125, and the p50 subunit along with p68 and p12, and this complex is stimulated by PCNA (12403614; 10480866; 11986310). PCNA also activates DNA pol ε (20227374; 9878404; 7827047). PCNA directly interacts with and activates DNA Ligase I (LIG1) (22918593; 10959839; 14729473; 9371766). The direct binding between PCNA and LIG1 results in a negative feedback and causes the inhibition of PCNA (10559261; 9603940). It is proposed that after Okazaki fragment DNA synthesis is completed by a PCNA-DNA pol δ complex, DNA pol δ is released allowing DNA ligase I to bind to PCNA at the nick between adjacent Okazaki fragments and catalyze phosphodiester bond formation. EGFR phosphorylates PCNA (25825764; 25907674; 17115032; 22692198; 17115032).

Step 4: DNA re-synthesis & ligation

PCNA recruits DNA polymerase δ and ε to initiate the re-synthesis of the strands, while DNA Ligase I (LIG-1) is recruited to the site of the DNA damage to seal the nick of the DNA strand completing the excision repair process. LIG-1 has been shown to be highly expressed in the cerebellum of HD patients and mice models compared to the striatum, which the investigators posited was a reason why the cerebellum may not be as affected in the disease (24191263). LIG-1 has also been shown to play a role in CTG repeat maintenance in the maternal germline, being involved in mediating CTG expansions and in the avoidance of maternal CTG contractions (21378394; 19628465). PCNA directly binds to and activates LIG-1 (22918593; 10959839; 9371766; 11331287; 15502161; 9649448), DNA pol δ (25813050; 23610416; 12403614) that is responsible for synthesis of the lagging strand and DNA pol ε (20227374; 11741962; 9878404) which is responsible for synthesis of the leading strand. Increased repeat instability was linked to LIG-1 overexpression and expression of a mutant LIG-1 incapable of interacting with PCNA (19628465), suggesting that disruption of LIG-1 PCNA interaction influences trinucleotide repeat instability. CK2 phosphorylates LIG-1 (11331287; 10523317). XRCC1 interaction with LIG3 plays a central role in ligation of NER-induced breaks and repair of UV lesions in quiescent cells and XRCC1-LIG3 and DNA polymerase δ co-localize and interact with NER components in a UV- and incision-dependent manner throughout the cell cycle (17643379). Moreover, in contrast, LIG-1 and DNA polymerase ε are recruited to UV-damage sites only in proliferating cells (17643379).

DNA polymerase κ is recruited to repair sites by ubiquitinated PCNA, XRCC1 and DNA polymerase δ by the replication factor RFC (20227374). PCNA has been shown to effect DNA polymerase κ (POLκ) correct nucleotide incorporation in the presence of RPA and RFC by reducing the apparent K_m (11784855;

18162470; 20227374). POLκ is positively transcriptionally regulated by CREB1 (15753290; 17099721), and SIRT1 acts as a co-transcriptional regulator of POLκ (18987333). Moreover, POLκ is negatively regulated by p53 (15202001) and loss of p53 function may in part contribute to the POLκ up-regulation. POLκ binds with mono-ubiquitinated PCNA, and has been posited to help POLκ to form nuclear foci after UV radiation (11784855; 18162470; 20227374). Importantly, both RPA (20227374; 11784855) and RFC (11784855) have been implicated in this recruitment of POLκ to the DNA site for re-synthesis.

After DNA re-synthesis the CAF-1 complex mediates chromatin assembly to the newly synthesized DNA. The CHAF1 subunit A interacts directly with PCNA (19059499), whereas both the TFIIH subunit XPB (19059499) and RPA (19059499; 10648606; 15805117) have also been implicated in the process. Reduced levels of CAF-1 due to oxidative stress have been shown to impair DNA repair, however aberrant expression of CAF-1 has not been found in brains of patients with AD, but the decreased CAF-1 is considered specific for Down's syndrome.

Cell-Cycle Checkpoint Regulation

Downstream of the regulation of the cell cycle (BRCA1-ATM-ATR-CHK1-CHK2) arrest pathway involved in DNA damage repair, is the activation of tyrosyl-DNA phosphodiesterase 1 (TDP1). TDP1 has been implicated in the removal of glycolate from single-stranded DNA containing 3-prime phosphoglycolate by hydrolyzing the phosphodiester bond at a DNA 3' end linked to a tyrosyl moiety, suggesting a role in repair of free-radical mediated DNA double-strand breaks. TDP1 is post-translationally phosphorylated by ATM (19851285; 19851285), and this phosphorylation promotes cell survival and DNA repair. TDP1 has been shown to immunoprecipitate with XRCC1 (13679147) as a covalent complex. Mutations in TDP-1 are associated with Spinocerebellar ataxia with axonal neuropathy (16775218; 19211312), but wild-type TDP-1 has been implicated in trinucleotide repeat transcription-induced repeat instability (21628532). XRCC1 acts as a scaffolding protein that interacts with multiple repair enzymes, which allows these repair enzymes to then carry out their enzymatic steps in repairing DNA. CHK2 can form a complex with XRCC1 or phosphorylated XRCC1 (18971944), with this modification affecting XRCC1 interactions with other downstream MMR proteins including PCNA, LIG1, and LIG3. These phosphorylation driven interactions stabilize XRCC1 as a scaffold protein and modulating DNA repair capacity (18971944; 23868975; 20471329). PolyQ repeats have been shown to induce ATM/ATR-dependent DNA damage response through accumulation of reactive oxygen species (12915485), and ATM may modulate the cytotoxicity of Aβ in Alzheimer's disease (14980204), as Aβ-induced apoptosis was significantly reduced in ATM knockout cell cultures. Moreover, ATR is induced during oxidative stress, and phosphorylated ATR accumulates in HD patients (12915485; 23602907)

The pathological accumulation of the Amyloid-β (Aβ) depletes neuronal BRCA1 which may contribute to cognitive deficits in AD (26615780); moreover, BRCA1 has been shown to be localized with Tau neurofibrillary tangles, which is a pathological hallmark of AD (17505559). *In vitro* and *in vivo* analysis demonstrates that BRCA1 and p53 bind each other (14978302; 9926942) which promotes p53 activation. BRCA1 promotes cell survival by directly regulating the DNA damage tolerance pathway through interaction and activation of CHK1 (23901102; 11836499) to regulate the G2/M checkpoint and DNA stability (11836499; 11836499). CHK2 activates BRCA1 via phosphorylation (15131084; 16675955; 18797466) in turn inducing ATR-CHK2 signaling through p53 activation of DNA damage response (18162465; 10673500) through ATR-dependent phosphorylation of ATM and subsequent ATM phosphorylation of CHK2 (17124492). BRCA1 positively regulates the transcription of GADD45α (10367887; 10962562; 11777930). This phosphorylation is also dependent upon CHK1 and CHK2

(12427729). BRCA1 is phosphorylated by the checkpoint kinases ATR (11114888) and ATM (10866324; 12024016; 11016625; 10550055; 23939379; 10866324).

The ATR serine/threonine kinase (ATR) participates in the kinase cascade that is activated in response to replication blocks and participates in cell-cycle arrest. ATR phosphorylates and interacts with ATM (17124492; 15322239; 18583959) and knockdown of ATM and ATR enhances cell death (23960094). ATR also actively phosphorylates CHK1 (21680713; 23667469; 23960094; 15110787) and CHK2 (23960094; 15367709; 12773400). This phosphorylation causes cell-cycle arrest, preventing the cells from entering the cell cycle to allow time for DNA damage to be repaired.

The ATM serine/threonine kinase (ATM) is a cell cycle checkpoint kinase that phosphorylates and functions as a regulator of a wide variety of downstream proteins including CHK1 (15322239; 19285939; 19530246; 18508566), CHK2 (19285939; 15322239; 15269203; 19530246; 18508566) and c-Abl (20798688; 9168117; 9168116). Both ATR (11358490; 22713662; 25336189; 22556425) and ATM (11358490; 9733514; 15159397; 10608806) phosphorylate p53, thus activating p53. The tumor suppressor protein p53 is partly responsible for transcriptional activation and p53 induces cell cycle arrest, apoptosis, senescence, DNA repair and changes in metabolism. PolyQ repeats have been shown to induce ATM/ATR-dependent DNA damage response through accumulation of reactive oxygen species (12915485).

RAD9 checkpoint clamp component A (RAD9) is a cell cycle checkpoint protein required for cell cycle arrest and DNA damage repair that possesses 3´ to 5´ exonuclease activity, which may contribute to its role in sensing and repairing DNA damage. RAD9 forms a checkpoint protein complex with RAD1 and HUS1, the 9-1-1 complex (19446481; 26021743; 20505337; 26088138). RAD9 is recruited by checkpoint protein RAD17 to the sites of DNA damage (21659603; 25091155) which is phosphorylated by ATR (11799063; 19020305).

Specific physical association between the BRCA1 RING domain and ATF1 stimulates the activity of ATF1 (10945975), which is involved in the stress response. ATF1 acts as a positive transcriptional regulator of PCNA (9883884; 9114015; 7479004; 7910946). CK2 (casein kinase II) contributes to cell survival by cooperating with other survival-promoting pathways, and has been shown to phosphorylate ATF1 and activate ATF1 transcription (9685505; 8600455). Aβ has been shown to enhance the phosphorylation of ATF1 (26053510) which has been posited to cause cell damage and cell death. p53 binds to CK2 and reduces the activity of CK2 (11180407; 10214938; 9180277) which regulates the apoptosis pathway, conversely CK2 phosphorylates and activates p53-binding to damaged DNA (10214938; 14640983; 9501176). CK2 also phosphorylates BRCA1 (10403822); LIG-1 (11331287; 10523317) and XRCC1 (21840775; 20471329). The ratio of phosphorylated to non-phosphorylated BRCA1 has been posited to modulate the DNA damage response in HD (22580959). Moreover, CK2 has been observed to be increased in the hippocampus and temporal cortex of AD patients compared to non-demented controls and interacts with Aβ (26732432; 8287280) and has been shown to be up-regulated in HD models (17971125).

The proto-oncoprotein, c-Abl, is a member of the non-receptor tyrosine kinases and has a pro-apoptotic role and is posited to act in the decision as to whether to activate the proapoptotic pathway when the DNA damage is too severe to be repaired. Alzheimer's and Parkinson's diseases patients have shown c-Abl activation, and overexpression in adult mouse neurons results in neurodegeneration and neuroinflammation (15474370; 21728062). The growth suppressor c-Abl increases the DNA binding activity of p53 (17339230; 15661746; 18490454; 15865930) and c-Abl interacts with p53 in response to DNA damage (10629029). c-Abl also causes the phosphorylation of PCNA (22238610; 23542172) and

facilitates nuclear foci formation of PCNA in cells stressed by DNA damage, while PCNA negatively regulates the stability of c-Abl and inhibits the apoptosis response to DNA damage (19156526). c-Abl phosphorylates ATR and causes its positive activation (20798688; 21151157), as well as the phosphorylation of EGFR (18721752; 16943190) promoting an increase in cell-surface expression of the EGFR. c-Abl interacts with JNK1(MAPK8-10) which may play an anti-apoptotic role (10022809; 19052872; 15696159). GADD45α has been associated with growth suppression, and GADD45α binds to and inhibits PCNA related negative growth control and inhibits apoptotic cell death (10828065; 7973727; 7784094). Exposure of neurons to Aβ has been shown to induce the up-regulation of GADD54α (11470486; 10448433) indicating DNA damage early in Aβ cytotoxicity. p53 acts as a transcriptional regulator for a large subset of proteins involved in NER, including: physical interaction and inhibition of BRCA1 transcription (14710355; 9482880; 25650659), actively binds and down-regulates HMGB1 (11748221; 15170359; 12534345; 11748232), activates DNA Ligase 1 transcription (10722849; 21332394), activates PCNA transcription (8570655; 8524244; 9651310; 15777783; 12947108), increases the secretion of EGFR and stimulates the EGFR signaling pathway (20190820; 26799187; 18359760; 9285564) and positively regulates GADD45α (8226988; 10749144; 9566896). The epidermal growth factor receptor (EGFR) directly binds to ATM and induces its phosphorylation and regulation (25601159; 26825989; 11466608), while depletion of endogenous EGFR impairs ATM-mediated DNA repair. ATM has been posited to bind to and phosphorylate EXO1 and regulate its activity (20019063; 18756267; 22326273). EGFR has been suggested to be one of the most significant AD risk genes, as Aβ oligomers can induce the activation of the EGFR (23019586) and over-expression of EGFR may trigger oxidative neuronal injury (10854274). PolyQ peptides have also been shown to cause the constitutive activation of EGFR and potently inhibit EGFR-mediated ERK activation in fly glial cells, with disruption of EGFR signaling and ensuing glial cell dysfunction posited to play a direct role in the pathogenesis of HD and other polyQ diseases in humans (15677486).

Cell Survival

Brain-derived neurotrophic factor (BDNF) promotes cell survival against oxidative DNA damage-induced death by enhancing DNA repair. BDNF has been shown to be reduced in Huntington's disease models (10825501; 22179319; 17959817) and its rescue results in decreased levels of neurodegeneration (18086127; 21048129). BDNF has also been shown to be significantly reduced in Alzheimer's disease (15935057) where a reduction of BDNF occurs early in the course of AD and correlates with loss of cognitive function, suggesting that BDNF play a role in synaptic loss and cellular dysfunction underlying cognitive impairment in AD. BDNF enhances BER through transcriptional activation by cyclic AMP response element-binding protein (CREB1). CBP acts as a co-activator of CREB1 through binding (7913207; 8413673; 9413984) to increases its activity. The ability of BDNF to activate CREB expression is abolished by shRNA of TrkB as well as inhibitors of TrkB, PI3 kinase or Akt kinase (24114393). Elevated levels of CREB1 have been shown in Huntington's disease models. CREB functionality is lost during the early stages of cell stress which contribute to the pathogenic process (14749423; 19632326). SIRT1 is a co-transcriptional regulator of CBP through direct binding (22826441; 22826441). SIRT1 has been shown to be up-regulated in AD and Amyloid Lateral Sclerosis models (17581637), and this is posited to promote neuronal survival mechanisms and has been shown to be impaired in Huntington's disease models (26815359; 24436303; 22227661; 22179319; 22179316). SIRT1 co-transcriptionally activates BDNF (22179316; 22179319) further enhancing BER. The neuroprotective effect of SIRT1 requires the presence of CREB-regulated transcription co-activator 1 (TORC1), a brain-specific modulator of CREB. The activity of SIRT1 in the deacetylatation and activation of TORC1 promotes TORC1 interaction with CREB. Moreover, TORC1 and SIRT1 act as co-transcriptional regulators of BDNF (22179316). PARP-1 binds to DNA through nonionic interactions (14567702; 23333033; 24588584), which conformationally activates PARP-1, this facilitates survival of proliferating cells under conditions

of DNA damage probably through its contribution to DNA base excision repair. PARP-1 is acetylated by CBP/p300 (16204234; 15607978). PARP-1 levels have been shown to be elevated in Alzheimer's disease models (27234294; 25274115; 27034851), and posited that the over-activation of PARP-1 can result in energy depletion and necrosis in response to high levels of DNA damage, but PARP-1 has been shown to be down-regulated in AD mice models (24121118).

MISMATCH REPAIR

DNA Mismatch Repair (MMR) is one of several DNA excision repair pathways that also include Base Excision Repair (BER) and Nucleotide Excision Repair (NER). The MMR system removes base–base mismatches and insertion/deletion mismatches, which may arise from a number of exogenous and endogenous factors such as polymerase errors, recombination errors, and chemical or physical damage. The MMR pathway and the key proteins, MutS and MutL, were originally identified in prokaryotes and are highly conserved in both prokaryotes and eukaryotes. Dysregulation of the MMR system has been implicated in the emergence of several cancers and in neurodegenerative diseases including Huntington's disease. CAG repeat diseases show dramatic instability in the striatum, with larger expansions noted with advancing age, while only modest instability occurs in the cerebellum. However there is a complex correlation between tissues showing varying amounts of repeat instability and MMR expression levels and there are marked variations in disease age-of-onset, progression and severity relating to MMR proteins (26774442). The mechanism of action relating repeat instability and MMR proteins is not understood at present, but one potential explanation was recently put forward whereby naturally occurring polymorphic variants of DNA repair genes may dramatically affect the levels of repeat instability (26774442). The central steps involved in MMR of DNA damage are: 1) Recognition and Initiation of mismatch/damage by MutS and MutL proteins; 2) Excision of the mismatch nucleotides by endonucleases; 3) the re-synthesis of the DNA strand by polymerases and ligases.

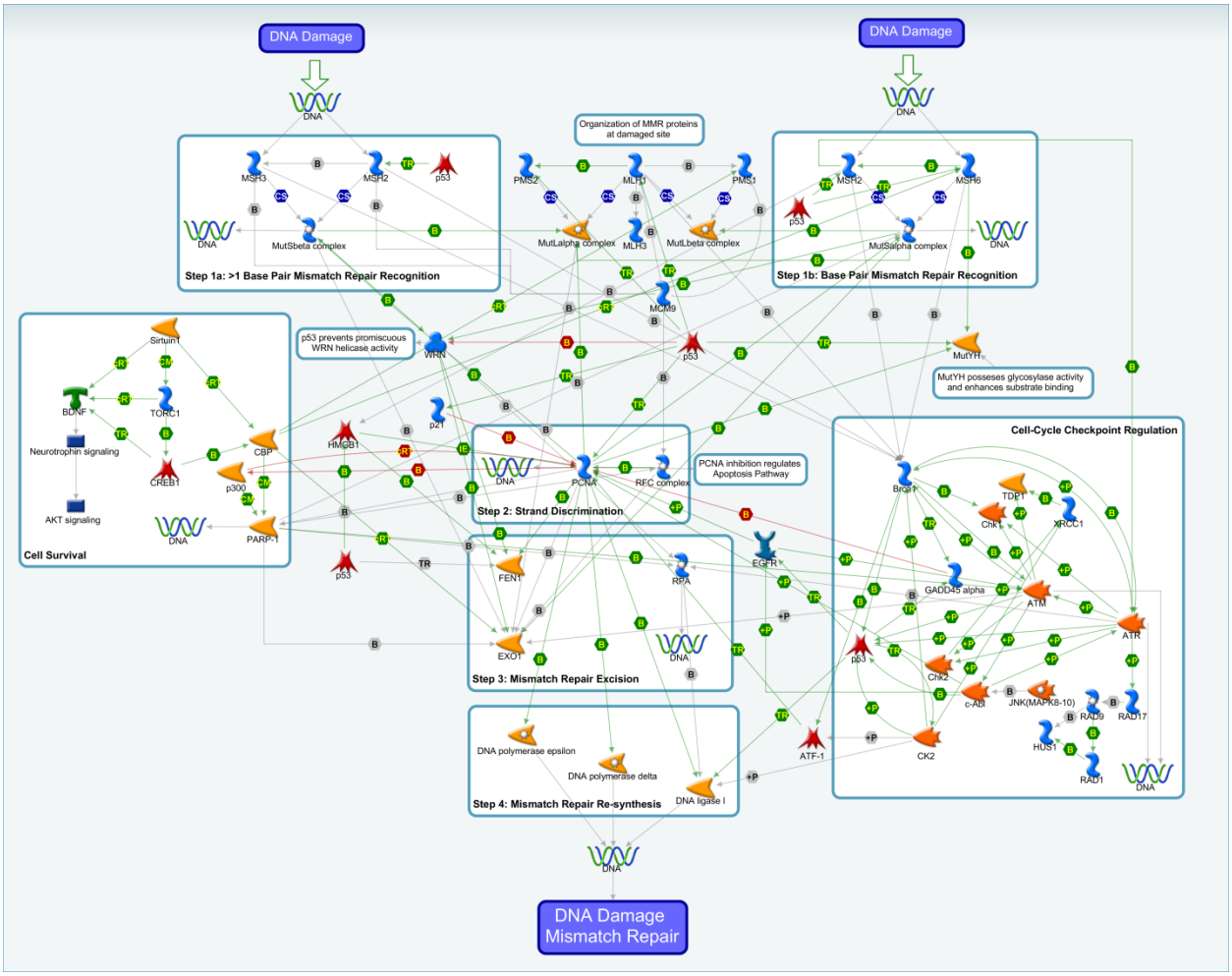

Figure 4: DNA Mismatch Repair

Step 1: Initiation

The recognition of a mismatched nucleotide in a DNA strand and initiation of mismatch repair is performed by MutS, which searches for mismatches by bending the DNA to locate the lesion. The MutS repair system is the result of two MutS complexes, MutSα and MutSβ. MutSα and MutSβ recognize and initiate separate MMR systems where MutSα recognizes base mismatches of 1 to 2 unpaired nucleotides, and MutSβ recognizes longer insertion-deletion loops (IDLs) that may comprise 1 to 15 unpaired nucleotides (24896128).

Step 1a: >1 Base Pair Mismatch Repair Initiation

The MutSβ complex is comprised of MSH2 and MSH3 (22179786; 19377479; 9774676; 8942985) which bind directly to DNA at three phosphates in an insertion-deletion loop (22179786; 24896128). The tumor suppressor protein p53 acts as a transcriptional activator of MSH2 (10984493; 11931386; 11350971; 11350971; 15064730). Knockdown of MSH2 and MSH3 suppressed large repeat expansions (26047474; 19436705). MSH2-MSH3 promotes CTG and CAG repeat expansions *in vivo* in *Saccharomyces cerevisiae.* MSH2-MSH3 directly interferes with normal Okazaki fragment processing by flap endonuclease1 (yeast homolog Rad27) and DNA ligase I (yeast homolog Cdc9) in the presence of trinucleotide repeat (TNR) sequences, thereby producing small expansion events (22938864). Deletion

of MSH2 was sufficient to eliminate the vast majority of striatal HTT CAG expansions in HD model mice (22970194) and act as a genetic enhancer both of somatic HTT CAG expansions and of HTT CAG-dependent phenotypes in mice.

Step 1b: Single Base Pair Mismatch Repair Initiation

MutSα complex is formed by MSH2 and MSH6 (17531815; 11048710; 9774676; 8942985), and this complex binds directly to homoduplex DNA through the binding with the base of a mismatched nucleotide (10480869; 11048711). The tumor suppressor protein p53 acts as a transcriptional activator of MSH2 (10984493; 11931386; 11350971; 11350971; 15064730) and MSH6 (16413492; 15064730). MSH2 has been shown to have a high affinity for TNR DNA structures, and this affinity increases with the length of the repeat sequence with binding preferentially to looped-out CAG repeat sequences suggesting strand asymmetry plays a role in MSH2 recognition (9215683). MSH6 expression levels have been reported to be increased in the cerebellum and this was posited to act as a mechanism to reduce somatic instability in this brain region (24191263). Moreover, knockdown of MSH6 promotes large expansions by compensatory increases in MSH3 and the MutSβ complex (26047474). Therefore, increased expression of MSH6 in regions of the brain where decreased somatic instability is observed provides a positive effect against CAG repeats (18930147; 26047474).

The organization of the MMR proteins on the DNA lesion is posited to be performed by the MutL complexes. The MutLα complex is formed from the interaction between PMS2 and MLH1 (18205192; 20978114; 21952876). PMS2 has been shown to decrease TNR expansion, as the absence of PMS2 results in increased accumulation of large expansions in the nervous system (cerebellum, cerebrum, and dorsal root ganglia) but not in non-neuronal tissues (23071719; 15198993). A recent genome-wide association study (GWAS) in HD found a significant association between the age at HD onset and PMS2 (27044000), implicating the protein in the disease and its progression. MLH1 also interacts with PMS1 to form the MutLβ complex (8066446; 10748159; 11292842) which binds to DNA lesions (22659005). PMS1 has been shown to be down-regulated in the motor cortex of ALS patients (17127558). MutLα complex binds with both MutSα (11948175; 11809883; 11441019; 16403449) and MutSβ (11809883; 20154325; 25825764). MutSα also binds and activates MutLβ (12799449; 11809883) with this binding regulated by MLH1 (12799449). MLH1 levels have been reported to be up-regulated in HD mice models. MLH1 has been shown to increase somatic instability and to promote TNR expansion in model systems (24204323) while down-regulation of MLH1 protein leads to a switch from repeat expansion to contraction (24971578). MLH1 gene levels have also been reported to be elevated in aged and demented brains (21846794). p53 was also shown to bind and transcriptionally activate PMS2 (15781865; 17677004) and MLH1(15781865; 17677004; 16413492). MLH1 interacts with MLH3 (18505871; 10570173) to form the MutLγ complex, and MLH3 has been implicated in DNA mismatch repair, meiotic recombination and has been implicated in mammalian microsatellite instability (18505871; 16702432). MLH3 has also been implicated in promoting TNR expansion (24204323). The MutLγ possesses endonuclease activity and binds DNA with a high affinity and shows a marked preference for Holliday junctions (24443562; 24403070; 18505871). MutLγ is recruited and persists at UVA-induced DNA lesions but DNA repair specific functions could not be detected in living cells (23696135). Functional studies have confirmed that MutLγ is a less efficient MMR complex than MutLα (18521850).

The Minichromosome maintenance 9 homologous recombination repair factor (MCM9) is a helicase and is involved in replication and homologous recombination, forming a complex with MMR initiation proteins (MSH2, MSH3, MLH1, PMS1, and the clamp loader RFC) (26300262) and is essential for MMR. Mcm9 null cells display microsatellite instability and MMR deficiency and MCM9 has been implicated in MMR-

proficient cancer (26806154). Another helicase, WRN, also directly binds to MutSα and MutSβ (17715146), specifically on forked DNA structures with a 3´ single-stranded arm. WRN is bound by p53 (10506209; 10364153) which inhibits WRN to prevent the promiscuous activities of WRN helicase (12080066; 15735006; 11427532; 18982914). Overexpression of WRN leads to augmented p53-dependent transcriptional activity and induction of p21 protein expression (10506209; 11280729), which can regulate cell-cycle pathways. PCNA and p21 form a complex (8101826), which regulates both cell-cycle arrest/apoptosis and DNA repair pathways (18782865; 16551699).

The transcription regulator p53 assists in the decision as to the fate of the MMR pathway between cell-cycle arrest and apoptosis or repair of DNA damage. p53 has been shown to be up-regulated in Alzheimer's disease (17399897; 9125193; 15548589) which has been posited to result in increased levels of neuronal apoptosis. It has also been reported that p53 interacts with HTT (huntingtin) *in vitro*, and it was posited that expanded repeat HTT causes aberrant transcriptional regulation through its interaction with p53, which may result in neuronal dysfunction and cell death in HD (10823891; 16278683; 21465263). ATR is a cell cycle checkpoint gene required for cell cycle arrest and DNA damage repair that has been shown to bind to MSH2; and MSH2 also activates ATR (21285353; 14657349). MSH2 (15886699; 24966277; 11498787; 10783165) and MSH3 (11498787; 14578343) are capable of forming a complex with BRCA1 and regulate the DNA damage response. Moreover BRCA1 also forms a complex with MSH6 (10783165), MLH1 (21240188; 10783165; 17148452) and ATM (10783165). BRCA1 forms a functional interaction with MSH6 and acts as a downstream effector of the adenosine nucleotide-activated MSH2-MSH6 signaling complex (11498787; 15975711). MutYH DNA glycosylase removes adenines from DNA mismatches and suppresses tumorigenesis by inducing cell death. MutYH physically associates with MSH2/MSH6 at the MSH6 subunit. This binding enhances MutYH glycosylase activity (11801590; 17114250; 15673720) and this is transcriptionally regulated by p53 (25310643). The expression of MutYH has been shown to be up-regulated in Parkinson's disease nigrostriatal dopaminergic neurons (17279544; 16773329).

The MutSα and MutSβ complexes are both able to bind to EXO1 (11427529; 11809771; 12629043; 16143102; 14636568) which plays a regulatory/structural role in assembly of the 3´ excision complex. EXO1 also possesses a cryptic 3´ to 5´ DNA hydrolytic activity. MutSα and MutSβ complexes bind PCNA (11005803; 11274057; 24981171; 9469823; 8858149; 11237611), which is believed to guide mismatch repair proteins to free termini in the newly replicated DNA strands. PARP-1 acts as a scaffold protein through the successive addition of ADP-ribose units to form long and branched chains of poly (ADP-ribose) and these polymers form a scaffold to recruit other proteins that are critical in MMR. PARP-1 directly interacts with MutSα (21945626), which may be involved in coupled checkpoint events. MutSα also directly interacts with the high mobility group box 1 protein (HMGB1) which facilitates protein-protein interactions and recognizes DNA damage. HMGB1 physically interacts with and stimulates MutSα (through MSH2) and is required at a step prior to the excision of mispaired nucleotide (15014079; 15808410; 16143102).

Step 2: Strand Discrimination

PCNA has been posited to act in strand discrimination in MMR. PCNA directly binds to and activates MutYH glycosylase (26377631; 11092888; 16879101) and MutLα (26283381; 9469823; 15225546). RFC complex acts as a clamp loader, while PCNA acts as a clamp to load MMR proteins (DNA polymerases) to the DNA lesion. RFC specifically binds to PCNA (25450506; 14530260; 11121020; 10051561) and loads PCNA onto the DNA (16980295; 9822671). PCNA expression levels have been shown to be elevated in Huntington's disease (24191263). HMGB1 directly influences the expression of PCNA, and it

has been shown that increased expression of HMGB1 correlates with increased levels of PCNA (25986235; 24481712; 22275109). Expression levels of HMGB1 were also shown to be different in HD model mice (19997493). EGFR phosphorylates PCNA (25825764; 25907674; 17115032; 22692198; 17115032), inhibits mismatch repair and promotes base mis-incorporation during DNA synthesis. This causes an alteration in PCNA interactions with mismatch-recognition proteins MutSα and MutSβ and interferes with PCNA-dependent activation of MutLα endonuclease, thereby inhibiting MMR at the initiation step. PNCA binds to, and is required for, the activation of MutLα (15225546; 16873062; 9469823; 21050827). RCF complex interacts with MCM9 helicase (22771115; 20532250), and this recruitment to the damage site has been posited to lead to MCM9 activation of MutL may participate in the excision of the mismatch through its helicase activity. c-Abl also phosphoryles PCNA (22238610; 23542172) and facilitates nuclear foci formation of PCNA in cells stressed by DNA damage, while PCNA negatively regulates the stability of c-Abl and inhibits the apoptosis response to DNA damage (19156526).

Step 3: Mismatch Repair Excision

The flap structure-specific endonuclease 1 (FEN1) removes 5´ overhanging flaps during DNA repair. The high mobility group box 1 (HMGB1) specifically interacts with and stimulates FEN-1 activities, acting as a cofactor for DNA damage repair (17803946). The HMGB1 activation of FEN-1 has been implicated in modulating CAG repeat expansion (19674974; 19997493). FEN1 interacts with PCNA (22556262; 15576034) resulting in the enhancement of FEN1 activity (24234453; 10744741; 14718165; 9778254). WRN has been shown to directly bind to FEN-1 and stimulate FEN-1 induced cleavage (12356323; 11598021; 15282207; 14657243). Together these proteins function to process DNA structures associated with the replication fork. FEN-1 expression levels have been shown to be elevated in Huntington's disease (24191263) and in HD mice models (19997493). The exonuclease 1 (EXO1) possesses hydrolytic activity and interacts with other components of the MMR system, including PCNA (15225546; 14676842). This interaction is believed to increase PCNA processivity of EXO1 in resection of DNA damage (23939618). EXO1 has been shown to be up-regulated in HD (22748968). EXO1 binds strongly to MLH1, and forms a complex with MLH1/PMS2 (MutLα) (11427529; 11429708). The primary role of RFC is the loading of PCNA onto the DNA lesion, which then leads to the excision of the mismatched bases by EXO1. It has been shown that RFC and PCNA are required for 3´ to 5´ excision of mismatches by EXO1 but with limited resection in the 5' to 3' direction (15225546). The activity of EXO1 has been posited to be regulated in part by WRN binding, as loss of the WRN helicase reduced exonucleolytic processing at nascent strands and led to severe genome instability (26275776; 12704184). The replication protein A2 (RPA) acts as a ssDNA-sensing ubiquitin ligase during the DNA damage response driven by ATR (24332808; 20616048), and defective RPA mutants inhibit the activation of ATR kinase in DNA damage response demonstrating that RPA acts as an activator of ATR kinase. The RPA-WRN binding has also been mapped in yeast and *C.elegans* (15965237; 22257160; 18558712), suggesting that RPA plays a role in the stimulation of helicase-catalyzed DNA unwinding. RPA expression levels have been shown to be elevated in Huntington's disease (24191263).

Step 4: Mismatch Repair Re-synthesis and Ligation

PCNA recruits DNA polymerase δ and ε to initiate the re-synthesis of the strands, whereas the DNA Ligase I (LIG-1) is recruited to the site of the DNA damage to seal the nick of the DNA strand after completing the mismatch repair process. LIG-1 has been shown to be highly expressed in the cerebellum of HD patients and mice models compared to the striatum, which the investigators posited was a reason why the cerebellum may not be as affected in the disease (24191263). LIG-1 has also been shown to play a role in CTG repeat maintenance in the maternal germline, mediating CTG expansions and avoiding maternal CTG contractions (21378394; 19628465), as defective LIG-1 was shown to reduce the

frequency of CTG expansions and increase CTG contraction frequencies on female transmissions. PCNA directly binds to and activates LIG-1 (22918593; 10959839; 9371766; 11331287; 15502161; 9649448), DNA pol δ (25813050; 23610416; 12403614) which is responsible for synthesis of the lagging strand, and DNA pol ε (20227374; 9878404; 11741962) which is responsible for synthesis of the leading strand. Increased repeat instability was linked to LIG-1 overexpression and expression of a mutant LIG-1 incapable of interacting with PCNA (19628465), suggesting that disruption of the LIG-1 PCNA interaction influences NTR instability.

Cell-Cycle Checkpoint Regulation

Tyrosyl-DNA phosphodiesterase 1 (TDP1) is activated downstream of the cell cycle (BRCA1-ATM-ATR-CHK1-CHK2) arrest pathway involved in DNA damage repair. TDP1 has been implicated in the removal of glycolate from single-stranded DNA containing 3´ phosphoglycolate by hydrolyzing the phosphodiester bond at a DNA 3´ end linked to a tyrosyl moiety suggesting a role in repair of free-radical mediated DNA double-strand breaks. TDP1 is post-translationally phosphorylated by ATM (19851285; 19851285) and this phosphorylation promotes cell survival and DNA repair. TDP1 has been shown to immunoprecipitate with XRCC1 (13679147) as a covalent complex. XRCC1 acts as a scaffolding protein that interacts with multiple repair enzymes, which allows these repair enzymes to then carry out their enzymatic steps in repairing DNA. CHK2 can form a complex with XRCC1 and phosphorylated XRCC1 (18971944), and this modification in turn affects XRCC1 interactions with other downstream MMR proteins including PCNA, LIG1, and LIG3, thereby stabilizing the XRCC1 to act as a scaffold protein and modulating DNA repair capacity (18971944; 23868975; 20471329) PolyQ repeats have been shown to induce ATM/ATR-dependent DNA damage response through accumulation of reactive oxygen species (12915485), and ATM may modulate the cytotoxicity of Aβ in Alzheimer's disease (14980204), as Aβ-induced apoptosis was significantly reduced in ATM knockout cell cultures. Moreover, ATR is induced during oxidative stress, and phosphorylated ATR accumulates in HD patients (12915485; 23602907)

The pathological accumulation of the Amyloid-β (Aβ) depletes neuronal BRCA1 which may contribute to cognitive deficits in AD (26615780); moreover, BRCA1 has been shown to be localized with Tau neurofibrillary tangles, which is a pathological hallmark of AD (17505559). *In vitro* and *in vivo* analysis demonstrates that BRCA1 and p53 bind each other (14978302; 9926942) which promotes p53 activation. BRCA1 promotes cell survival by directly regulating the DNA damage tolerance pathway through interaction and activation of CHK1 (23901102; 11836499) to regulate the G2/M checkpoint and DNA stability (11836499; 11836499). CHK2 activates BRCA1 via phosphorylation (15131084; 16675955; 18797466) in turn inducing ATR-CHK2 signaling through p53 activation of DNA damage response (18162465; 10673500) through ATR-dependent phosphorylation of ATM and subsequent ATM phosphorylation of CHK2 (17124492). BRCA1 positively regulates the transcription of GADD45α (10367887; 10962562; 11777930). This phosphorylation is also dependent upon CHK1 and CHK2 (12427729). BRCA1 is phosphorylated by the checkpoint kinases ATR (11114888) and ATM (10866324; 12024016; 11016625; 10550055; 23939379; 10866324).

The ATR serine/threonine kinase (ATR) participates in the kinase cascade that is activated in response to replication blocks and participates in cell-cycle arrest. ATR phosphorylates and interacts with ATM (17124492; 15322239; 18583959) and knockdown of ATM and ATR enhances cell death (23960094). ATR also actively phosphorylates CHK1 (21680713; 23667469; 23960094; 15110787) and CHK2 (23960094; 15367709; 12773400). This phosphorylation causes cell-cycle arrest, preventing the cells from entering the cell cycle to allow time for DNA damage to be repaired.

The ATM serine/threonine kinase (ATM) is a cell cycle checkpoint kinase that phosphorylates and functions as a regulator of a wide variety of downstream proteins including CHK1 (15322239; 19285939; 19530246; 18508566), CHK2 (19285939; 15322239; 15269203; 19530246; 18508566) and c-Abl (20798688; 9168117; 9168116). Both ATR (11358490; 22713662; 25336189; 22556425) and ATM (11358490; 9733514; 15159397; 10608806) phosphorylate p53, thus activating p53. The tumor suppressor protein p53 is partly responsible for transcriptional activation and p53 induces cell cycle arrest, apoptosis, senescence, DNA repair and changes in metabolism. PolyQ repeats have been shown to induce ATM/ATR-dependent DNA damage response through accumulation of reactive oxygen species (12915485).

RAD9 checkpoint clamp component A (RAD9) is a cell cycle checkpoint protein required for cell cycle arrest and DNA damage repair that possesses 3´ to 5´ exonuclease activity, which may contribute to its role in sensing and repairing DNA damage. RAD9 forms a checkpoint protein complex with RAD1 and HUS1, the 9-1-1 complex (19446481; 26021743; 20505337; 26088138). RAD9 is recruited by checkpoint protein RAD17 to the sites of DNA damage (21659603; 25091155) which is phosphorylated by ATR (11799063; 19020305).

Specific physical association between the BRCA1 RING domain and ATF1, and the expression of BRCA1 stimulates the activity of ATF1 (10945975), which is involved in the stress response. ATF1 acts as a positive transcriptional regulator of PCNA (9883884; 9114015; 7479004; 7910946), CK2 (casein kinase II) contributes to cell survival by cooperating with other survival-promoting pathways, and has been shown to phosphorylate ATF1 and activate ATF1 transcription (9685505; 8600455). Amyloid-β (Aβ) has been shown to enhance the phosphorylation of ATF1 (26053510), that has been posited to cause cell damage and cell death. p53 binds to CK2 and results in the down-regulation of CK2 (11180407; 10214938; 9180277) which regulates cell apoptosis pathway, whereas CK2 phosphorylates and activates p53-binding to damaged DNA (10214938; 14640983; 9501176). CK2 also phosphorylates BRCA1 (10403822); DNA Ligase I (11331287; 10523317), and XRCC1 (21840775; 20471329). The imbalance in non-phosphorylated and phosphorylated BRCA1 has been posited to modulate the DNA damage response in HD (22580959). Moreover, CK2 has been observed to be increased in the hippocampus and temporal cortex of AD patients compared to non-demented controls and interacts with Aβ (26732432; 8287280), and has been shown to be up-regulated in HD models (17971125).

The proto-oncoprotein, c-Abl, is a member of the non-receptor tyrosine kinases and has a pro-apoptotic role and is posited to act in the decision as to whether to activate the proapoptotic pathway when the DNA damage is too severe to be repaired. Alzheimer's and Parkinson's diseases patients have shown c-Abl activation and overexpression in adult mouse neurons results in neurodegeneration and neuroinflammation (15474370; 21728062). The growth suppressor c-Abl increases the DNA binding activity of p53 (17339230; 15661746; 18490454; 15865930) and c-Abl interacts with p53 in response to DNA damage (10629029). c-Abl phosphorylates ATR and causes its positive activation (20798688; 21151157), as well as the phosphorylation of EGFR (18721752; 16943190) promoting an increase in cell-surface expression of the EGFR. c-Abl interacts with JNK1(MAPK8-10), and JNK1 may play an anti-apoptotic role (10022809; 19052872; 15696159). GADD45 has been associated with growth suppression, and GADD45α binds to and inhibits PCNA related negative growth control and inhibits apoptotic cell death (10828065; 7973727; 7784094). Exposure of neurons to Aβ has been shown to induce the up-regulation of GADD54 (11470486; 10448433) indicating DNA damage during early in Aβ cytotoxicity. p53 acts as a transcriptional regulator for a large subset of proteins involved in MMR, including: physical

interaction and inhibition of BRCA1 transcription (14710355; 9482880; 25650659), actively binds and down-regulates HMGB1 (11748221; 15170359; 12534345; 11748232), induces FEN-1 transcription (16103874), activates DNA Ligase 1 transcription (10722849; 21332394), activates PCNA transcription (8570655; 8524244; 9651310; 15777783; 12947108), increasing the secretion of EGFR and stimulates the EGFR signaling pathway (20190820; 26799187; 18359760; 9285564), and positively regulates GADD45α (8226988; 10749144; 9566896). The epidermal growth factor receptor (EGFR) directly binds to ATM and causes its phosphorylation and regulation (25601159; 26825989; 11466608), and depletion of endogenous EGFR impairs ATM-mediated DNA repair. ATM has been posited to bind to and phosphorylate EXO1 and regulate its activity (20019063; 18756267; 22326273). EGFR has been suggested to be one of the most significant AD risk genes, as Aβ oligomers can induce the activation of the EGFR (23019586), and over-expression of EGFR may trigger oxidative neuronal injury (10854274). PolyQ peptides have also been shown to cause the constitutive activation of EGFR and potently inhibit EGFR-mediated ERK activation in fly glial cells, and posited that disruption of EGFR signaling and ensuing glial cell dysfunction could play a direct role in the pathogenesis of HD and other polyQ diseases in humans (15677486).

Cell Survival

Brain-derived neurotrophic factor (BDNF) promotes cell survival against oxidative DNA damage-induced death by enhancing DNA repair. BDNF has been shown to be reduced in Huntington's disease models (10825501; 22179319; 17959817) and its rescue results in decreased levels of neurodegeneration (18086127; 21048129). BDNF enhances MMR through transcriptional activation by cyclic AMP response element-binding protein (CREB1). CBP acts as a co-activator of CREB1 through binding (7913207; 8413673; 9413984) to increases its activity. CBP also co-activates EXO1 (26004186), and MSH6 (26004186; 16051665). Cognitive deficits are associated with reduced hippocampal expression of CBP in HD mice models (22116937). CBP expression is not significantly reduced in HD model animals but CREB1 phosphorylation is significantly increased, which in turn has been shown to increase CREB1 mediated transcription (14749423; 11532992). Elevated levels of CREB1 have been shown in Huntington's disease models. CREB functionality is lost during the early stages of cell stress which contribute to the pathogenic process (14749423; 19632326 SIRT1 is a co-transcriptional regulator of CBP through direct binding (22826441; 22826441). SIRT1 has been shown to be up-regulated in AD and Amyloid Lateral Sclerosis models (17581637), and this is posited to promote neuronal survival mechanisms and has been shown to be impaired in Huntington's disease models (26815359; 24436303; 22227661; 22179319; 22179316). SIRT1 co-transcriptionally activates BDNF (22179316; 22179319) further enhancing MMR. The neuroprotective effect of SIRT1 requires the presence of CREB-regulated transcription co-activator 1 (TORC1), a brain-specific modulator of CREB. The activity of SIRT1 in the deacetylatation and activation of TORC1 promotes TORC1 interaction with CREB. Moreover, TORC1 and SIRT1 act as co-transcriptional regulators of BDNF (22179316). PARP-1 binds to DNA through nonionic interactions (14567702; 23333033; 24588584), which conformationally activates PARP-1, this facilitates survival of proliferating cells under conditions of DNA damage probably through its contribution to DNA mismatch repair. PARP-1 is acetylated by CBP/p300 (16204234; 15607978). PARP-1 levels have been shown to be elevated in Alzheimer's disease models (27234294; 25274115; 27034851), and posited that the over-activation of PARP-1 can result in energy depletion and necrosis in response to high levels of DNA damage, but PARP-1 has been shown to be down-regulated in AD mice models (24121118). PARP-1 interacts with and directly activates EXO1 (21945626; 26519824; 26400172), interacts with RPA (21945626; 26456830), interacts with RFC (21945626; 21734457; 18093328), functionally and physically interacts with ATM (17428792; 19917246; 22226932; 17459151), and regulates the function of PCNA through direct binding (12930846; 9649317; 18093328; 20303835).

INTER-STRAND CROSSLINK REPAIR

Interstrand DNA crosslinks (ICLs) are covalently linked lesions that form between opposing strands of double-stranded DNA, thereby preventing transcription and replication by inhibition of DNA strand separation. ICLs form in the presence of bifunctional alkylating agents and are extremely cytotoxic, as even a single ICL in the genome can cause severe defects in a variety of vital DNA metabolic processes. Defective ICL repair causes chromosome instability syndromes such as Fanconi anemia. The major ICL repair pathway is coupled to DNA replication, being triggered by replication fork collision with an ICL. When this happens repair is initiated through the excision of the crosslink from one parental strand, releasing one daughter duplex from the ICL forming a double-stranded DNA break that is subsequently repaired. ICL repair is thus a rare instance in which stalled replication forks undergo programmed collapse.

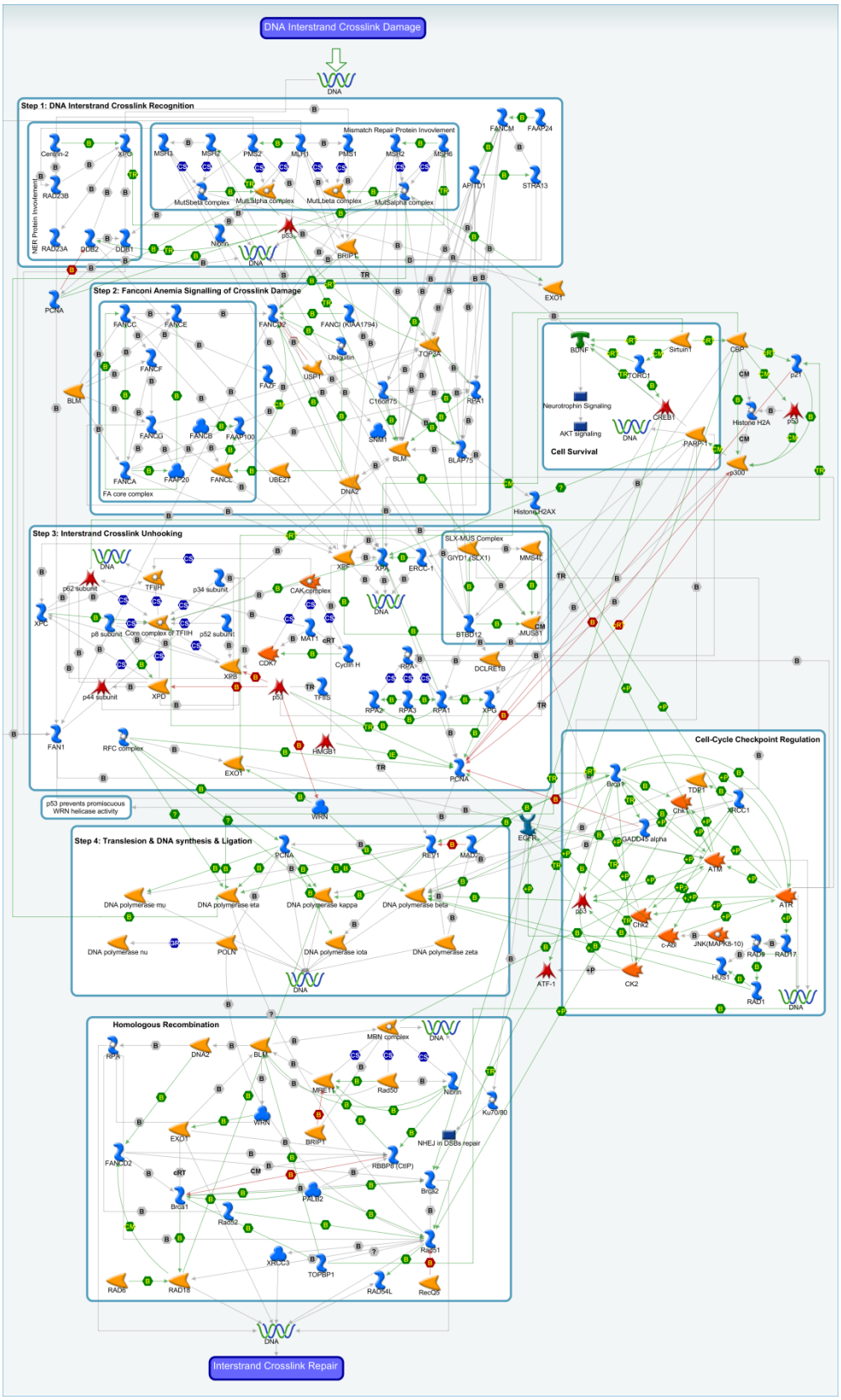

Figure 5: Interstrand Crosslink Repair

The first step of ICL repair involves recognition of the damage and incision of the DNA near the crosslink. ICL repair proteins recognize a crosslink as distortions in the local structure of the duplex due to destabilization of base stacking. Then ICL repair proteins incise one strand on either side of the ICL, producing an "unhooked" substrate whereby the excised fragment is still attached to the non-incised strand. Endonclease activity in the region adjacent to the 3' side of the crosslink results in the formation of a gap, and the crosslinked incision product is flipped out of the restored duplex. The incision leaves a double strand break (DSB) that is subject to homologous recombination (HR) or non-homologous end joining (NHEJ) repair synthesis to fill the gap left by the unhooking of the ICL. This process converts the crosslink to a monoadduct, after which the second cycle of repair can occur by conventional Nucleotide Excision Repair (NER). While NER and Homologous Recombinatino (HR) pathways are implicated in ICL repair, other proteins are also implicated in the pathway. The Fanconi Anemia (FA) proteins play prominent roles in multiple steps in ICL repair and acts in maintenance of genome stability after ICL damage.

Step 1: DNA Interstrand Crosslink Recognition

Mismatch Repair proteins

One of the mechanisms with which ICL are recognized is through the mismatch repair protein MutSβ, which recognizes insertion-deletion loops that comprise 1 to 15 unpaired nucleotides (24896128). Cells deficient in MSH2 are sensitive to crosslinking agents (10699935; 15891767), and MutSβ binds to a cisplatin ICL (19364127). Additionally, the recruitment of MSH3 to ICL has been shown to be dependent on the NER proteins DDB2 and XPC (19684342). The MutSβ complex is formed from the interaction between MSH2 and MSH3 (22179786; 19377479; 9774676; 8942985), and this complex interacts directly with DNA through binding three phosphates in an insertion-deletion loop (22179786; 24896128). Knockdown of MSH2 and MSH3, which form the MutSβ heterodimer, suppressed large repeat expansions (26047474; 19436705). Deletion of MSH2 was sufficient to eliminate the vast majority of striatal *Htt* CAG expansions in HD model mice (22970194), and activity of MSH2 acts as an enhancer both of somatic huntingtin (*Htt*) CAG expansions and of *Htt* CAG-dependent phenotypes in mice. The tumor suppressor protein p53 acts as a transcriptional activator of MSH2 (10984493; 11931386; 11350971; 11350971; 15064730). It has also been reported that p53 interacts with *HTT in vitro*, and it was posited that that expanded repeat *HTT* causes aberrant transcriptional regulation through its interaction with p53 which may result in neuronal dysfunction and cell death in HD (10823891; 16278683; 21465263). It is also interesting to note p53 has been shown to be up-regulated in Alzheimer's disease (17399897; 9125193; 15548589) which has been posited to result in increased levels of neuronal apoptosis.

The deletion of MSH6 or MSH2, which form the MutSα complex (17531815; 11048710; 9774676; 8942985), result in a lesion-specific increase in ICL sensitivity, elevation of ICL-induced chromosomal rearrangements and persistence of ICL-associated DNA double-strand breaks (22912599). MutSα complex interacts directly with DNA through binding the base of a mismatched nucleotide (10480869; 11048711). Additionally, it has been shown that MSH2 preferentially binds a cisplatin ICL DNA lesion that contains a mismatch compared with a cisplatin ICL substrate without a mismatch (23761438).The tumor suppressor protein p53 acts as a transcriptional activator of MSH2 (10984493; 11931386; 11350971; 11350971; 15064730) and MSH6 (16413492; 15064730). MSH6 expression levels have been reported to be increased in the cerebellum, which was posited to act as a mechanism to reduce somatic instability in this brain region (24191263). Knockdown of MSH6 promotes large expansions by compensatory increases in MSH3 and the MutSβ complex (26047474). Therefore, increased expression

of MSH6 in regions of the brain where decreased somatic instability is observed provides a positive effect against CAG repeats (18930147; 26047474). The activity of MutSα does not promote repeat expansion and it appears MSH6 can compete with MSH3 for MSH2 interactions; as mentioned above, both MSH3 and MSH2 are involved in CAG repeat expansion (26047474; 19436705; 22970194) .

MutLα complex binds with both MutSα (11948175; 11809883; 11441019; 16403449) and MutSβ (11809883; 20154325; 25825764). MutSα and MutSβ complexes bind PCNA (11005803; 11274057; 24981171; 9469823; 8858149; 11237611) which is believed to guide the repair proteins to free termini in the newly replicated DNA strands. The MutSα and MutSβ complexes are both able to bind to EXO1 (11427529; 11809771; 12629043; 16143102; 14636568) and this interaction results in a regulatory-structural role in assembly of the 3′ excision complex. It has also been posited that EXO1 possesses a cryptic 3′ to 5′ hydrolytic activity, but this is under dispute (16873062; 15225546; 11809771). PARP-1 acts as a scaffold protein through the successive addition of ADP-ribose units to form long and branched chains of poly (ADP-ribose), and these polymers form a scaffold to recruit other proteins. PARP-1 directly interacts with MutSα (21945626) which may be involved in coupled checkpoint events. MutSα also binds and activates MutLβ (12799449; 11809883), which is regulated by MLH1 (12799449). The MutL complexes have been shown to play a role in the recognition of ICL repair pathway through interactions with the DNA helicase FANCJ (BRIP1). As demonstrated by co-immunoprecipitation assays, BRIP1 directly interacts with MLH1 (23585563; 17581638; 17148452; 20173781; 17148452). It has been posited that the interaction between BRIP1 and MLH1 affects DNA damage signaling responses, as BRIP1-deficient cells exhibit delayed signaling and apoptosis that generate resistance to agents that induce DNA damage lesions (20978114). Furthermore, BRIP1-null cells accumulate DNA ICLs (17581638). BRIP1 also immunoprecipitates with the MutLα and MutLβ complex proteins PMS1 (17148452) and PMS2 (17148452; 17581638; 20978114), and these interactions were posited to implicate MMR proteins in recombination. PMS1 has been shown to be down-regulated in the motor cortex of ALS patients (17127558). PMS2 has been shown to decrease TNR expansion, as the absence of PMS2 results in increased accumulation of large expansions in the nervous system (cerebellum, cerebrum, and dorsal root ganglia) but not in non-neuronal tissues (23071719; 15198993). A recent genome-wide association study (GWAS) in HD found a significant association between the age of HD onset and PMS2 (27044000) implicating the protein in the disease and its progression. Nibrin has been posited to play a role in DNA double-strand break repair and DNA damage-induced checkpoint activation. MLH1 has been shown to bind Nibrin (17121922), the interaction of which participates in checkpoint regulation and cytotoxicity. MLH1 levels have been reported up-regulated in HD mice models where it is involved in somatic instability through promotion of TNR expansion in model systems (24204323), while down-regulation of MLH1 protein leads to a switch from expansion to contraction of TNR repeats (24971578). MLH1 gene levels have also been elevated in aged and demented brains (21846794). MLH1 (17148452; 20671156; 20603073; 20603015), PMS1 (20603015; 20671156; 20603073), and PMS2 (17148452; 20603015; 20671156), have all been shown to interact with FAN1, suggesting the interplay between the FA pathway and MMR.

Nucleotide Excision Repair proteins

MLH1 interacts with the RAD23B protein that is part of the global genome NER (GG-NER) system (20706999). In GG-NER, a helix distorting lesion or structure is recognized by XPC complexed with RAD23B and centrin 2 (CEN2). The DNA lesion is recognized by by the XPC-RAD23B complex (10873465; 16460043; 8692695; 8756644; 9372923; 15885096; 9164480) or XPC-RAD23A (9372924; 9164480), as both RAD23A and RAD23B stimulate the activity of XPC. Both RAD23A and RAD23B have been shown to accumulate in neuronal inclusions in brain material from HD and PD patients (16860562).

CEN2 has been posited to have a fundamental role in the structure and function of the microtubule-organizing center, possibly required for the proper duplication and segregation of the centrosome. CEN2 stimulates XPC-RAD23B binding and increases the yield of DNA adducts during recognition of bulky damages in DNA (22809153). Inhibition of XPC expression correlates with a decreased amount of CEN2 transcript and protein, indicating that XPC is required for the fine tuning of CEN2 gene expression (21676658). CEN2 directly binds and activates XPC through both electrostatic and apolar inter-molecular interactions by the burial of apolar bulky side-chains into the hydrophobic pocket of CEN2 (17897675; 16533048; 12890685). The interaction of XPC-CEN2 results in the stabilization of XPC, stimulating its activity (12890685; 15964821).

Damaged DNA binding protein 2 (DDB2) directly binds to damaged DNA (16951172) when certain DNA lesions do not significantly destabilize the DNA duplex. In complex with damaged DNA binding protein 1 (DDB1), DDB2 creates a kink that is recognized by XPC. DDB1 (16964240; 25675097) and DDB2 co-localize with PCNA and p21 at local UV-induced DNA-damage sites. DDB1 has been shown to interact with the Fanconi Anemia associated Nuclease 1 (FAN1) protein (20671156). DDB2-PCNA association is required for DDB2 proteolytic degradation (24200966), and this interaction influences cell cycle progression (26697842). DDB1 interacts with PMS2 (17148452) further supporting the interaction between the MMR and NER systems in DNA damage repair. p53 transcriptionally activates DDB2 (11971958; 14560002; 16140933; 16260627; 18922183) in humans but not in mice, and also transcriptionally activates and promotes expression of XPC (16413492; 9892649; 26029824). DDB2 has been posited to be up-regulated in Amyloid Precursor Protein (APP) expressing cells as a result of Aβ-dependent activation of these DDB2 following H_2O_2 treatment (26263968). Moreover, this demonstrates that Aβ secretion and oxidative stress leads to the over-expression of DDB2.

FANCM complex

An ensemble of Fanconi Anemia proteins have been shown to play an integral role in the recognition of ICL lesions. FANCM and FAAP24 are posited to recognize blocked replications forks and, via fork reversal, enable access to the subsequent downstream proteins that repair the damage. FANCM causes a concerted displacement and annealing of the nascent and parental DNA strands (18843105; 18206976) as it contains a highly conserved helicase domain and an associated ATP-dependent DNA translocase activity. FAAP24 directly binds to and activates FANCM (18285517; 20064461; 17289582), and the helix-hairpin-helix domains of FAAP24 and FANCM bind to ICL-like single/double-strand junction-containing DNA substrates (23661679; 23932590). FANCM and the two MHF proteins (APITD1 and STRA13) form a complex (24699063; 20347429; 23886707) through which suppression of APITD1 expression results in the destabilization of FANCM and STRA13. APITD1 binds and activates STRA13 (24390579; 23886707; 20347429; 22510687) to facilitate DNA binding while the MHF-FANCM complex acts in the recognition of DNA ICL's (22510687).

Step 2: Fanconi Anemia Signalling of Crosslink Damage

Fanconi Anemia core complex

The Fanconi Anemia core complex comprises nine proteins: FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FAAP100, FAAP20 and FANCL. The core proteins provide an essential E3 ligase function and activate the FA pathway. Three sub-complexes are believed to form the FA core complex:

1) FANCA, FANCG, and FAAP20 where the UBZ domain of FAAP20 is suggested to bind to ubiquitinated histone; while FANCG is posited as a possible scaffold for the sub-complex,
2) The FANCB-FANCL-FAAP100 sub-complex contains the E3 ligase, FANCL and
3) FANCC, FANCE, and FANCF sub-complex, in which FANCF has been shown to interact with FANCM and act as an adaptor protein

The FANCA-FANCG-FAAP20 sub-complex forms between FANCA and FANCG (20347428; 12239156; 21975120), FANCA and FAAP20 (22343915; 22705371), and between FANCG and FAAP20 (22705371; 22343915). FANCG has been shown to be up-regulated in AD patients (23752274). FAAP20 promotes the functional integrity and stabilization of the FA core complex via its direct interaction with FANCA. FAAP20 modulates the ubiquitin ligase activity of the FA core complex, which in turn regulates the FANCI/FANCD2 monoubiquitination reaction following DNA damage (22396592; 22343915). The FANCB-FANCL-FAAP100 sub-complex functions as the monoubiquitination module. FAAP100 is essential for the stability of the core complex and directly interacts with FANCB and FANCL to form a stable subcomplex (17396147; 24905007). FANCB directly binds to and activates FAAP100 (17396147) and binds to FANCL (16720839; 17396147). The FANCC-FANCE-FANCF sub-complex is an important step in the assembly of the larger FA core complex (16127171) and coimmunoprecipitates with FANCA, FANCC, and FANCG (12239156). FANCE mediates the interaction between the FANCC and FANCF proteins (16127171) and is required for FANCD2 monoubiquitination. FANCC and FANCE (24451376; 24469828; 12093742; 11157805), FANCE and FANCF (17082180; 18550849; 16720839; 24469828), and FANCC and FANCF (17082180; 18550849; 24469828) interact with each other. FANCC binds to FAZF (10572087; 14499622) that is posited to act as a transcriptional repressor (11986317; 10572087). A methylation-based EWAS study showed significant differences in methylation between PD cases and controls (25304910). The sub-complexes also interact to form the full FA complex, whereby FANCA physically interacts with FANCF (12724401; 15082718; 24469828), and FAAP20 physically interacts with FANCB (22343915; 22705371).

FA auxiliary proteins

The FANCL protein serves as an E2-conjugating enzyme recruitment module and interacts with UBE2T (26149689; 21775430; 16916645), which catalyzes the covalent attachment of ubiquitin to protein substrates (17550899; 17203973; 12938929). FANCL monoubiquitinates FANCD2 (19129235; 18277096; 15661754; 16784902; 16493006), which acts as a tumor suppressor and in the maintenance of genome integrity (18277096; 20937699). The interaction between FANCD2 and the MMR proteins MSH2 (21865299; 23993743), MSH6 (23993743) and MLH1 (21865299; 19934329) has been posited to regulate ICL resistance to damage repair, as it was shown that MSH2 depletion suppresses ICL sensitivity in FANCD2 deficient cells (24966277; 21975120; 21865299).

BRIP1 (FANCJ) binds to and affects the activity of FANCD2 (26336824; 25070891; 25659033). Transient depletion of BRIP1 adversely affects the stability of FANCD2 and its co-regulator FANCI. Moreover, BRIP1 has been posited to promote the assembly of FANCD2 nuclear foci (20676667).

FANCI binds to and activates FANCD2 (20671156; 20603015; 19561358). Posttranslational modifications of FANCI serves as a molecular switch in the activation of the FA pathway, as phosphorylation of FANCI protects the cells against chromosome breakage by DNA interstrand cross-linking agents (18931676; 22855611).

USP1 regulates the FA pathway by binding to and causing the deubiquitination of FANCD2 and recycling of the protein (15694335; 21389083; 18687060). Moreover, it has been posited that the inhibition of USP1 leads to hyper-accumulation of monoubiquitinated FANCD2 (15694335; 19075014).

DNA2 is a helicase/nuclease involved in the maintenance of mitochondrial and nuclear DNA stability. DNA2 binds to and activates FANCD2 (22987153) and it has been shown that depletion of DNA2 in FANCD2-deficient cells rescues the sensitivity of FANCD2 null cells to bifunctional alkylating agents (24626199). DNA2 has been posited to have a deleterious role in ICL repair as the activity of the protein may cause over-activity and unregulated resection of DNA (24626199).

FANCD2 binds to histone H2AX (17304220) which forms nucleosomes and is a fundamental repeating unit of chromatin. It has been posited that this interaction aids nucleosome assembly, acts as a histone-chaperone crucial for the histone dynamics and DNA crosslink repair in cells (22828868). Histone H2AX is positively regulated by the phosphorylation of the protein by both ATR (15286739; 19805520) and ATM (15286739; 18813293; 17391916). Histone modifications are altered in HD, and mono-ubiquitination of histone H2A may not function at the level of the individual gene but may rather influence transcription through global chromatin structure (25062675). H2A histone family members are specifically overexpressed in the blood and frontal cortex of patients with HD compared with controls (21969577).

SNM1 is a regulator of the mitotic cell cycle checkpoint and acts as a structure-specific DNA hairpin opening endonuclease during ICL repair by processing DNA intermediates including DNA hairpins or hairpin-like structures (22102580). The activity of SNM1 has been posited to result from the interaction with FANCD2 (18180189).

BLM complex

The BLM complex promotes dissolution of Holliday Junction-mobile DNA crossover structures that arise during homologous recombination-mediated repair of DNA double strand breaks. This functions to resolve linked DNA intermediates without exchange of genetic material, which is critical in somatic cells (23543275; 25131815; 24984776). The BLM complex is comprised of BLM, TOPIIIα, RPA-1, C16orf75 and BLAP75. The Bloom syndrome RecQ like helicase (BLM) protein has a central role in DNA damage signaling, repair, replication and telomere maintenance. BLM activity must be intricately controlled to prevent illegitimate recombination events that could have detrimental effects on genome integrity (25150915). BLAP75 mediates the interaction with BLM and TOP3A (18390547). The BLM complex interacts with the FA core complex through the interaction of the BLM protein with FANCA (22705371; 12724401), FANCC (12724401; 15616572), FANCE (12724401), FANCF (12724401) and FANCG (12724401). The BLM protein immunoprecipitates with the FANCM complex proteins FANCM (20347428; 16116422; 20064461) and APITD1 (20347429; 20347428; 24699063) which suggests cooperation between the ICL repair recognition and signaling pathways through BLM interaction with BRIP1 (21240188). Moreover, BLM interacts with the MMR proteins MSH2 (10783165; 20936779; 15064730), MSH6 (15064730; 20936779; 10783165) and MLH1 (12724401; 10783165; 19015241; 11325959). It has been posited that BLM and MLH1 function together in replication, recombination and DNA repair events independent of single base mismatch repair. The BLM protein is transcriptionally regulated by p53 (11399766; 15364958; 15220520) and mutations in BLM result in a dominant-negative effect of BLM helicase activity. DNA2 cooperates with BLM to mediate long-range DNA end resection (25122754; 25200081; 21325134). BLM has been posited to contribute to the neuronal cell cycle re-entry observed in brains affected by Alzheimer's disease (23572515).

Replication protein A (RPA) rapidly assembles on the single-stranded DNA region of the stalled fork and has been shown to be a requirement for DNA re-synthesis induced by ICL (10684938; 25922199) and can open forked DNA (27016742). RPA1 forms part of the BML complex and RPA stimulates BLM helicase activity, whereby it has been shown to co-immunoprecipitate with C16orf75 (18923082). RPA1 also physically interacts with BLAP75 (15775963; 19793864; 23543748). RPA directly binds to and activates the helicase activity of BLM (10825162; 18926708; 12724401; 10318934) to unwind DNA duplexes during replication, recombination, and repair. RPA also physically interacts with the FA core complex proteins FANCA (12973351; 22705371; 12724401) and FANCG (12724401), further supporting the concept that the BLM and FA complexes work together in the ICL repair pathways. The FA pathway acts upstream of RPA-ATR-CHK1 to generate the ICL signal (19748363). RPA1 binds to the proteins that form the FANCM complex, including FANCM (20347428; 18843105), APITD1 (20347428; 20347429; 22510687) and binds to activate BRIP1(20159562; 17596542; 22792074). RPA1 also co-localizes in nuclear foci after DNA damage, and is likely to be important for the role of the helicase to more efficiently unwind DNA repair intermediates to maintain genomic stability. FANCM/FAAP24-dependent RPA foci formation is required for efficient ATR-mediated checkpoint activation in response to ICL (20670894).

RMI2 (C16orf75) is also part of the BLM complex and stabilizes the complex (18923082), has been shown to activate BLM by binding (20826341; 20711169; 18923083) and is required for chromatin targeting of BLM in response to replication blocks. C16orf75 also binds to and activates BLAP75 (18923082; 20064461; 18923083). The FANCM complex interacts with C16orf75 in the BLM complex through interactions between FANCM-C16orf75 (20064461; 22392978) and FANCM-APITD1 (20347429).

Topoisomerase (DNA) III alpha (TOP3A) is an enzyme that catalyzes the transient breaking and rejoining of single stranded DNA, thus reducing the number of supercoils and altering the topology of DNA required for Holliday junction resolution by BLM. TOP3A co-immunoprecipitates with the BLM core protein, C16orf75, that stabilizes the complex (18923083; 18923082; 19245838). RPA1 is essential for both DNA unwinding by BLM and enforcing 5′ → 3′ resection polarity by DNA2 (12724401; 19793864; 21325134) and BLM (11406610; 15775963) which is posited to participate in the regulation of recombination in somatic cells. BLAP75 has been shown to directly bind and activate TOP3A (20360068; 16595695; 20360068), this interaction has been posited to promote TOP3A binding to double Holliday junction structures (16537486; 20445207). TOP3A co-immunoprecipitates with FANCA (22705371; 12724401; 12973351) and FANCG (12724401) which has been posited to link the BLM complex to the FA complex. TOP3A is co-transcriptionally regulated by p53 (11571296; 15220520) and, in turn, TOP3A actively regulates p53 transcription and its tumor suppression activity (24526736). TOP3A associates with the FANCM complex through its association with both FANCM (20347428; 20064461; 24699063) and APITD1 (20347428; 20347429; 24699063).

BLAP75 has been shown to partly maintain genome integrity and normal embryonic development (23900276). BLAP75 co-immunoprecipitates with FANCM (20064461; 17289582; 20347428) and has been posited to aid coordination of the dissolvasome and FA core complex activities. Moreover, BLAP75 binds to APITD1 (20347429; 20347428).

Step 3: Interstrand Crosslink Unhooking & Incision

A key event in crosslink repair is the unhooking step. An ICL prevents full strand separation but it is possible that unwinding can take place on both sides of an ICL. Dual incisions flanking the open structure can still flank the ICL and it has been posited that ERCC1-XPF is able to cleave several bases 5′ of an unpaired junction and, depending on the placement of an ICL in a duplex with respect to two single-stranded arms, is able to cut on either side of an ICL. Similarly, XPG may cut a few bases 3′ to a junction

between duplex and single-stranded DNA. Proteins necessary to form a productive, open pre-incision complex are TFIIH, XPA, RPA, and XPG (9351836; 9372913).

TFIIH complex

Transcription factor II H (TFIIH) is a basal transcription complex involved in transcription regulation. TFIIH is a multiprotein complex containing the core complex proteins (TFIIH p62, TFIIH p44, TFIIH p52, TFIIH p8, TFIIH p34, XPD and XPB) and the CAK (cyclin-activating kinase) complex (MAT1, CDK7 and Cyclin H). TFIIH is the only general transcription factor with known enzymatic activities, including DNA-dependent ATPase, ATP-dependent DNA helicase, and CTD (carboxy-terminal repeat domain of the largest RNA polymerase II subunit) kinase activities. TFIIH also regulates the transition from transcription initiation to elongation, functions as an essential component in nucleotide excision repair and has been implicated in mammalian cell cycle progression. The main function of TFIIH in ICL repair is to open the DNA around the crosslink and thereby allow the excision of the crosslinked lesion and its replacement by a new DNA fragment. Mutations in these subunits are associated with three genetic disorders: xeroderma pigmentosum (XP), Cockayne syndrome (CS) and trichothiodystrophy (TTD) (10064601; 26149386; 11062469; 10660593).

The TFIIH is recruited to the DNA ICL lesion through the binding of the TFIIH p62 subunit to XPC (26278177; 26909369; 26029824). p53 binds to THIIF p62 subunit (26029824) and it has been shown that diphosphorylation of p53 leads to a significant enhancement in p53 binding to THIIF p62 subunit (16793543; 18160537). XPC recruits TFIIH, and XPC associates with XPB to stimulate the XPB ATPase activity to initiate damaged DNA opening (18809580; 10734143). Patients carrying mutations in the XPB helicase subunit display the combined cancer and developmental-progeroid disorder xeroderma pigmentosum/Cockayne syndrome (19114557). XPB catalyzes the 3′ to 5′ DNA helicase activity. The THIIF p52 subunit interacts with XPB and stimulates its ATPase activity (17466626) by stabilizing XPB. Drosophila models show that point mutations introduced in human THIIF p52 subunit destabilize the interaction between THIIF p52 subunit and XPB which facilitate the assembly of the complex (17339330). The XPD helicase associates with XPC (18277385). XPD catalyzes the 5′ to 3′ DNA helicase activity. p53 has been shown to inhibit both helicases XPB and XPD, but does not inhibit their ATPase activities or transcriptional activities (7663514). Additionally, p53 was posited to act as a mechanism for damage recognition and a failsafe mechanism to prevent promiscuous DNA unwinding. The TFIIH p62 subunit has been shown to co-precipitate with XPB and XPD *in vitro* and *in vivo* (8652557; 15220921; 11259578; 8152490). The TFIIH p34 subunit has been suggested to play a role in splicing mechanisms in the first step of mRNA splicing (23147676) and has been shown to interact with the TFIIH p44 transcription factor and XPD in the TFIIH complex (25013903; 8652557). The p44 transcription factor interacts with XPD and stimulates its ATPase and 5′ to 3′ helicase activities (25268380; 25681444; 9771713; 10924514). The TFB5 (TFIIH p8) subunit interacts with and stimulates XPB ATPase activity to trigger DNA opening during DNA repair, and is implicated in regulating cellular levels of TFIIH (16669699; 19172752; 24127601). It was posited that p8 has a stabilizing function and protects TFIIH from degradation (15220921). TFIIH p8 also binds with and increases the activity of XPD (16427011), and its overexpression in trichothiodystrophy cells counteracts the detrimental effect of XPD mutations by restoring the cellular TFIIH concentration (16427011). TFIIH p8 is thought to act in conjunction with XPB as an ATP-driven motor that supplies the energy that is required to reorganize the intermediate DNA repair complex and thereby supports the repositioning of XPC–RAD23B and the unwinding of DNA by XPD (14981083).

The cyclin-dependent kinase (CDK)-activating kinase (CAK) subcomplex of TFIIH has been implicated in cell cycle control and is responsible for activating phosphorylation of several kinases, and is thought to

serve as a direct link between the regulation of transcription and the cell cycle. Cyclin H binds to and increases the activity of CDK7 (11319144; 8521393). CDK7 catalyzes the carboxyl-terminal domain (CTD) kinase activity. CDK7 also interacts with MAT1 (7588631; 8521393; 8521818) to form the CAK complex. CDK7 is significantly elevated in susceptible hippocampal neurons of Alzheimer disease patients in comparison with age-matched controls (11124424), and has been reported to be implicated in CAG instability (25026993). MAT1 acts as a co-transcriptional regulator of cyclin H (23765726; 20231280). The CAK component, MAT1, is rapidly recruited to UV-induced DNA damage sites and co-localizes with the TFIIH p62 subunit (23083890). The release of the CAK complex from TFIIH has been posited to initiate the incision/excision of the damaged oligonucleotide in NER (18614043; 20543986) and thereby regulating the helicase activities of the TFIIH complex (23083890).

Xeroderma pigmentosum proteins

XPF is a DNA endonuclease that is essential for unhooking of ICL to initiate the repair process. To secure successful ICL unhooking, XPF interacts with SLX4 (BTBD12) scaffolding protein to coordinate with other endonucleases (26453996; 21240275; 22902628; 19596236) and BTBD12 stimulates the activity of the XPF-ERCC1 nuclease (24726326). ERCC1 and XPF-defective cells show the most pronounced sensitivities to ICL-inducing agents. ERCC1-XPF nicks DNA specifically at junctions between double-stranded and single-stranded DNA when the single-strand is oriented 5′ to 3′ away from a junction. Nuclease activity resides entirely on the XPF module (11953324), while the ERCC1 is responsible for binding both the single strand-double strand DNA Y junction and XPA (16338413; 16076955; 17948053). XPF binds to ERCC1 (16076955; 15932882; 16338413) and stabilizes the endonuclease subunits. XPF-ERCC1 acts in unhooking DNA ICL in cooperation with FANCD2 and BTBD12 (24726325). SLX1 (GIYD1) acts as the catalytic subunit of the GIYD1-BTB D12 structure-specific endonuclease and binds to XPF (19596236; 19596235; 19595721). XPF-ERCC1 also physically interacts with the mismatch repair protein MSH2 (14706347) and co-localizes with the XPC after DNA damage (19279666). The XPF endonuclease interacts with the FA core complex proteins FANCA (14499622; 11401546; 12571280), FANCC (14499622) and FANCG (20518486). The interactions between XPF and FA proteins has been posited to act by influencing this incision process by interacting either with proteins involved in the unhooking step or with damaged DNA and acting as a damage sensor (18020456).

Xeroderma pigmentosum complementation group A (XPA) has been shown to directly bind to damaged DNA (8218288; 10373492; 9592168) and has been posited to act as the last recognition step of damage during the repair mechanism. It is hypothesized that XPA scaffolds the interaction between damaged DNA and several NER proteins (25056193). It has been posited that XPA acts to verify the presence of a lesion (9734359; 8538652) and recruits the structure specific endonuclease ERCC1-XPF heterodimer (19940136; 7876263) to incise the damaged strand of DNA 5′ to a lesion. The proliferating cell nuclear antigen (PCNA) has also been shown to be an essential interaction partner with XPA (23152873; 8814338). XPA directly binds to the ERCC1-XPF complex (8197174; 7598728; 7891694; 8972858; 15358100; 17948053) and plays a role in the localization or loading of an incision complex composed of ERCC1-XPF (22547097). ERCC1 is transcriptionally regulated by p53 (15220520; 11571296). HMGB1 interacts with XPA to facilitate the processing of ICL in human cells (26578599) as HMGB1 recognizes TFO-ICLs, and its depletion increases ICL-induced mutagenesis (19446504). HMGB1 has been posited to modulate the propensity of a given tissue for somatic CAG and other TNR expansion (19997493; 8913860; 8972860). Mutations in the human XPA gene cause UV-sensitivity (10699759; 12396616) and have a reduced capacity to repair UV-induced DNA damage. In *XPA* mutant *C. elegans,* the animals have shortened lifespan, UV sensitivity, damaged tissues and internal vacuoles (22091407; 18203746;

19879883). Moreover, XPA deficiency in human cultured cell do not substantially affect TNR instability in germline, kidney and/or liver but it does dramatically reduce CAG repeat instability in neuronal tissues such as striatum, hippocampus and cerebral cortex (21926083). ERCC1 deficient mouse models have been shown to possess impaired NER and develop age-dependent motor neuron abnormalities (20602234). Furthermore, ERCC1 deficiencies impact the dopaminergic system and are associated with human PD pathology (27210754). ERCC1-deficient mice exhibit an age-dependent decrease in neuronal plasticity and progressive neuronal pathology (21880916). DDB2 complex-mediated ubiquitination plays an important role in recruiting XPA to damaged sites (20368362; 19056823; 11278856), and has also been posited to assist in the recognition of damage through the efficient recruitment of XPA (11278856). Moreover, the binding between DDB2 and XPA has also been hypothesized to be important in clinical cases whereby mutations in XPA affect the binding in *in silico* studies (24063568). It has been shown that the MMR and NER proteins co-operate in the recognition of ICL, as XPA binds to both MSH2 and MSH3 (19468048; 21398635). XPA specifically binds to TFIIH and is essential for affecting the excision reaction (9287294; 9698541; 7876263; 15358100). The XPA DNA damage verification is in part regulated by sirtuin 1 (SIRT1), which is part of the cell survival pathway. SIRT1 binds to and deacetylates XPA, and optimizes NER pathway activity as it has been shown that SIRT1 expression is increased in preconditioned UV-irradiated cells (26317794). This causes a decrease in acetylated XPA and inhibition of SIRT1 abrogated XPA binding to DNA lesions (20670893; 26317794). It has been posited that ATR is required for the UV-induced nuclear focus formation of XPA and that the ATR checkpoint pathway may modulate NER activity through the regulation of XPA redistribution in human cells upon UV irradiation (16862173; 16540648; 19586908; 22174788; 23178497). Moreover, the ATR checkpoint pathway can modulate the cellular activity of NER through phosphorylation of XPA (16540648; 19586908). Replication protein A (RPA), in particular the RPA1 subunit, has been shown to bind and increase the activity of XPA (7876167; 7665601; 8972858; 7565690; 9535929; 10828957; 15358100) and was posited to influence the stabilization of the XPA-DNA interaction as mutant RPA defective in XPA interaction fails to stabilize an XPA - DNA complex (10828957). The proliferating cell nuclear antigen (PCNA) has also been shown to be an essential interacting partner to XPA (23152873; 8814338). PARP1 activation promotes its association with XPA (23038248; 24953096; 26880244) and inhibition of PARP1 activity decreases XPA. Finally, chromatin association and mutational analysis shows that mutant PARP1 lowers DNA binding affinity of XPA.

SLX-MUS Complex

The SLX–MUS complex (BTBD12-MUS81-MMS4L-GIYD1) forms a resolvase complex which has been posited to cleave Holliday Junctions by a nick and counter-nick mechanism (24831703; 26370409; 25131815). SLX4 (BTBD12) acts as a scaffold protein for the SLX-MUS complex (25224045), binding and activating GIYD1 (19596235; 22902628; 24080495; 21240276; 19595721); and SLX4 has been posited to promote symmetrical cleavage of static and migrating Holliday junctions. Moreover, BTBD12 binds to and activates EME1 (MMS4L) and MUS81 (24412650; 24076219; 21240275; 23093618; 19596236) thereby increasing their endonuclease activities. GIYD1 interacts with MUS81 (19596236; 27084631; 25224045; 19596235) and MMS4L (19596235; 19595721) in the SLX-MUS complex. GIYD1 binds to ERCC-1 (19595721; 19596235), which has been posited to act as a scaffold for the XPF-ERCC1 nuclease. MMS4L binds to and activates MUS81 (12686547; 12721304; 16189514; 19596236); this interaction is believed to be a structure-specific nuclease that is capable of resolving fork structures (12686547). MMS4L-MUS81 has been shown to prevent trinucleotide repeat expansion (24423876). p53 has been posited to transcriptionally regulate MMS4L (16980608). DCLRE1B binds to MUS81 (18469862) and has been shown to be influential in ICL repair as depletion of DCLRE1B by RNA interference rendered cells hypersensitive to ICL-inducing agents (15467758).

BTBD12 co-immunoprecipitates with DCLRE1B that is associated with telomeres (16606622; 22907656) and generates 3' single-stranded overhangs (20551906). There is an association between BTBD12 and MMR proteins as it has been shown that BTBD12 interacts with MSH2 (22902628; 19596235), MSH3 (19596235) and MSH6 (22912599; 22902628). It has been shown that DCLRE1B co-immunoprecipitates with BTBD12 (22907656) and they function epistatically. BTBD12 has been posited to affect cell survival by binding to PARP-1 (19596235), and PARylation of BTBD12 may have a novel role in the recruitment of BTBD12 to sites of DNA damage (25722289). The SLX–MUS complex interacts with the cell-cycle checkpoint regulation pathway through the interaction of GIYD1 with XRCC1 (19596235). The SLX–MUS complex is influenced by the FA pathway through the interaction between BLM and MUS81 (15805243), resulting in the increased activation of MUS81 endonuclease activity on the nicked Holliday junctions and 3' flap. Moreover, this interaction is believed to result in the restart of stalled replication forks and HR repair (24858046). CHK2 is a checkpoint kinase that physically interacts with MUS81 (11741546; 11073977) and is transcriptionally regulated by CREB1 (15753290).

RPA acts as a ssDNA-sensing ubiquitin ligase during the DNA damage response driven by ATR (24332808; 20616048). Defective RPA mutants inhibit the activation of ATR kinase in DNA damage response demonstrating that RPA acts as an activator of ATR kinase. ATR-mediated suppression of dormant origins shields active forks against irreversible breakage by preventing exhaustion of nuclear RPA (24267891). BTBD12 binds with RPA (25533185), which has been posited to direct the repair factors to the ICL. The endonuclease activity of XPG has been posited to be in part mediated by RPA, as a strong DNA interaction domain of RPA is positioned at the 5′ side of its binding region and a weak DNA-binding domain resides at the 3′ side (9716411). Polarity appears crucial for positioning of the excision repair nucleases XPG and ERCC1-XPF on the DNA. Upon binding of XPG and RPA the endonuclease activity of XPG was enhanced (9716411; 8626644; 7700386). RPA has been shown to interact with PCNA (12171929; 11254741; 20227374) and PARP1 (21945626; 26456830; 22246237). RPA expression levels have been shown to be elevated in Huntington's disease (24191263).

XPG is a latent endonuclease with a structural and catalytic role. XPG is recruited to the crosslinked region to complete the formation of a stable pre-incision complex. This fulfills a structural role in stabilizing the pre-incision complex to generate an open-stable structure. Dual incisions of the lesion at the 5′ and 3′ ends are performed by XPF and XPG, respectively (9351836; 10026181). Defects in XPG can cause either the cancer-prone condition xeroderma pigmentosum (XP) alone, XP combined with the severe neurodevelopmental disorder Cockayne Syndrome (CS) or the infantile lethal cerebro-oculo-facio-skeletal (COFS) syndrome which is characterized by dramatic growth failure, progressive neurodevelopmental abnormalities and greatly reduced life expectancy (25299392). The recruitment of XPG to the DNA lesion has been posited to be mediated by TFIIH, as XPG physically interacts with the core TFIIH protein complex (15572672;17000769; 11259578; 8652557; 17466625; 11141066). Moreover, it has been shown that XPG acts to stabilize the TFIIH complex as mutations in XPG found in XP-G/CS patient cells that prevent the association with TFIIH also resulted in the dissociation of CAK and XPD from the core TFIIH (17466625). In the absence of ATP TFIIH has been reported to inhibit the nuclease activity of both XPG and ERCC1-XPF (11141066), suggesting that TFIIH regulates the endonuclease activities of these proteins. XPG binds to XPC and it was shown that XPG/RAD2 displaces XPC/RAD4 from the repair complex in yeast models, which may be mediated by TFIIH (18277385; 23295669; 11259578). The acetylation of XPG has been shown to be in part mediated by PCNA (22954786), and binding of XPG to PCNA (16189514; 24674623; 9305916) protects PCNA from p21 induced inhibition (18079701). The association of XPG and PCNA may also regulate cell cycle arrest pathways (24674623). The interaction between p300 and XPG is regulated by p21, with human fibroblasts lacking p21 having abnormal accumulation of XPG at DNA damage sites. Therefore p300/CBP may facilitate repair by

acetylating not only histones but XPG as well (22954786) as p300/CBP has been shown to bind and covalently modify XPG. This acetylation of XPG has been shown to be in part mediated by PCNA (22954786). Binding of XPG and PCNA (16189514; 24674623; 9305916) protects PCNA from p21 induced inhibition (18079701) and may also regulate cell cycle arrest pathways (24674623). Mutations in p21 have been implicated in modulating Alzheimer's and Parkinson's diseases (25625488; 23306186). The ratio of p21/p21(thr145) in peripheral blood lymphocytes have been posited as potential biomarkers in Alzheimer's disease (22503900). An N-terminal fragment of HTT has been shown to repress the transcription of p21 (10823891), and p21 binds to HTT *in vivo* and *in vitro* and p21 overexpression enhances the aggregation of mutant HTT (18065495). p53 has been posited to act as a transcription factor for XPG with a putative binding site between the two having been identified (20018659; 20233728; 22848513).

HMGN1 is a PCNA interacting protein that enhances the binding of PCNA to chromatin (22393258). p53 acts as a transcriptional inducer of PCNA (16980608; 26677001) which activates Gadd45α through binding (9566896; 8226988; 1423616) and by transcriptional regulation. Gadd45α directly binds with and inhibits PCNA with this interaction being posited to serve to impede negative growth control (10828065; 7973727) demonstrated as decreased PCNA expression may result in apoptosis (11682006; 21253613). PARP-1 regulates the function of PCNA through direct binding (12930846; 9649317; 18093328; 20303835). RFC complex acts as a clamp loader, while PCNA acts as a clamp to load DNA polymerases to the DNA lesion and RFC specifically binds to PCNA (25450506; 14530260; 11121020; 10051561). PCNA expression levels have been shown to be elevated in Huntington's disease (24191263). The exonuclease 1 (EXO1) possesses a hydrolytic activity and also interacts with other components of the MMR system, including interactions with PCNA (15225546; 14676842). This interaction is believed to result in PCNA processivity to EXO1 in resection of the DNA damage (23939618). EXO1 has been shown to be up-regulated in HD (22748968). It has been shown the RFC and PCNA are critical for 3′ to 5′ excision of mismatches by EXO1 but limited resection in the 5′ to 3′ direction (15225546). The activity of EXO1 has been posited to be in part regulated by binding of WRN, as loss of the WRN helicase reduced exonucleolytic processing at nascent strands and led to severe genome instability (26275776; 12704184).

MTMR15 (FAN1) encodes a protein with 5′ flap endonuclease and 5′ to 3′ exonuclease activity. RPA rapidly assembles on the single-stranded DNA region of the stalled fork and FAN1 efficiently promoted DNA incision at the proper site of RPA-coated 5′-flapped DNA (25922199; 20935496). FAN1 is recruited to the stalled replication folk by binding to monoubiquitinated FANCD2 (22611161; 25135477; 26797144; 20671156) in conjunction with BLM (25135477). FAN1 was shown to precipitate with PCNA (20603015) and in complex with PARP-1 (20671156) and regulates FAN1 foci induction activity (23966292). Significant association of the rs3512 polymorph has been found against FAN1 in Huntington's disease and spinocerebellar ataxias (27044000).

Step 4: Translesion & DNA synthesis & Ligation

REV1 is a dCMP transferase and plays a non-catalytic function in translesion synthesis. It has been posited to assist in the first step in the bypass of abasic lesions by the insertion of a nucleotide opposite the lesion and REV1 promotes replication through the DNA crosslink. The REV1 polymerase plays a central role in mutagenic translesion DNA synthesis and has been shown to bind to PCNA (21690293; 16982685; 16857592). This interaction with PCNA has been posited to have an important non-catalytic role in coordinating translesion synthesis (15741181). REV1 binds to the p53 and affects p53-dependent cell death (25614517; 20018659). Moreover, loss of p53 increased the steady-state level of REV1 mRNA suggesting that p53 acts to regulate REV1 (16731756) and HR. BRCA1 has been shown to promote cell survival by directly regulating the DNA damage tolerance pathway in response to agents that create

crosslinks in DNA. BRCA1 directly recruits the REV1 translesion polymerase to the lesions through protein-protein interaction (23901102; 26187992). Moreover, REV1 has been shown to interact with MAD2b (14657033; 21926160; 20164194) with this interaction posited to play a role in translesion synthesis (25799990). MAD2b regulates polyubiquitination and overexpression of MAD2b augments REV1 degradation (23287467). MAD2b is a small subunit of DNA polymerase ζ (20088965) which has been shown to be essential for primordial germ cell maintenance through prevention of apoptotic cell death (23463509). When a replicative DNA polymerase is stalled by damaged DNA, a "polymerase switch" recruits specialized translesion synthesis DNA polymerases to sites of damage. REV1 reduces rates of CAG·CTG repeat expansion and contraction in yeast models (16979389). REV1 interacts with DNA polymerase μ (15189446; 15741181), DNA polymerase ι (14657033; 18242152; 19170759) and DNA polymerase ζ (22303021; 20164194; 15189446; 21926160; 22859295). REV1 and POLζ cooperate in lesion bypass and are considered the main players participating in an error-prone pathway of post-replication repair. Moreover, REV1 and Polζ perform an essential step that allows ICL-associated DSBs to be channeled into the homologous recombination (HR) repair pathway (20658647, 23065650). REV1 interacts with and activates DNA polymerase κ (14657033; 19170759; 18242152; 22859295) and DNA polymerase η (14657033; 18242152; 19170759; 22691049). DNA polymerase β (12063248; 20936779; 25184665), DNA polymerase ν (16611994; 12968183; 19995904), DNA polymerase μ (11724965), DNA polymerase ι (11724965; 16357261; 15342632; 18931444; 16763556; 26547069), DNA polymerase κ (11784855; 18162470; 20227374; 24848457), DNA polymerase η (16763556; 26170230; 16189514; 11585903) and POLA1 (18277385) interact with and are activated by PCNA. DNA polymerase β has been shown to be down-regulated in Alzheimer's disease models and patients (24121118; 17065437). In yeast models, both DNA polymerase η and ζ have been posited to most likely have a small role in trinucleotide repeat mutagenesis (12509280).

PCNA interacts with DNA pol β to form a complex (12063248; 21245343). The complex is loaded into DNA replication forks and mediates DNA replication in postmitotic neurons (25184665). The physical interaction between WRN and DNA pol β stimulates DNA pol β strand displacement DNA synthesis (12665521; 17173071) while WRN exonuclease activity can act cooperatively with DNA pol β (16449207). BRCA1 and DNA pol β were found to interact in co-immunoprecipitation assays (23826138) with DNA pol β expression being necessary for BRCA1 recruitment, suggesting a partnership between these repair factors in DNA damage repair. p53 activates DNA pol β through binding (11850801; 11179235; 12189182). TDP1 has been shown to associate with DNA pol β (23042675; 24183900) and also immunopercipitates with XRCC1 (13679147) forming a covalent complex. A positive association between AD risk and the presence of G/A genotype variant of the XRCC1 rs25487 polymorphism has been reported (25998844), but other polymorphisms in XRCC1 have not been associated with an increased risk (20553853). No differences in the frequency of XRCC1 gene polymorphisms between ALS patients and controls free of any neurological diseases have been measured (20719408). XRCC1 interacts with DNA pol β *in vivo*, *in vitro* and in human cells (8948628). This interaction induces suppression of strand displacement by DNA pol β, allowing for more efficient ligation after filling of a single base patch (8978692). DNA polymerase κ is recruited to repair sites by ubiquitinated PCNA, XRCC1 and DNA polymerase δ is recruited by the replication factor RFC (20227374). PCNA has been shown to improve the accuracy of nucleotide incorporation by DNA polymerase κ (POLκ) in the presence of RPA and RFC by reducing the apparent K_m (11784855; 18162470; 20227374). POLκ binds with mono-ubiquitinated PCNA, and has been posited to help POLκ to form nuclear foci after UV radiation (11784855; 18162470; 20227374). Moreover, both RPA (20227374; 11784855) and RFC (11784855) have been implicated in the recruitment of POLκ to the DNA site for re-synthesis. RFC and PCNA stimulate the DNA synthetic activity of DNA polymerase η. It has been posited from steady-state kinetics that this stimulation results

from an increase in the efficiency of nucleotide insertion resulting from a reduction in the apparent K_m of the incoming nucleotide (11585903; 17545166; 17608453). BRCA1 has been shown to directly bind to and recruit translesion DNA polymerase η (23901102). The catalytic activity of DNA polymerase η is increased through its interaction with the mismatch repair complex MutSα (15710654; 17190840; 21855803).

Homologous Recombination

Cells utilize the Homologous Recombination (HR) pathway in response to double strand breaks (DSBs) using the undamaged sister chromatid as a template for repair. This is an error-free pathway that plays a pivotal role during meiosis and during S and G2 phases of the cell cycle. Non-homologous end joining (NHEJ) is an error-prone repair system that is a non-templated DNA repair process in which DNA termini are directly ligated. NHEJ is an error-prone process that occurs throughout the cell cycle and is shown to be important in mitotic cells.

HR requires three major steps: end resection, strand invasion and resolution. End resection involves MRE11-RAD50-NBS1 (MRN) complex and EXO1, BLM and DNA2 to resect nucleotides and extend 3′ single strand DNA overhangs. The MRN complex detects double strand breaks, tethers the ends of the broken chromosomes, activates DNA damage response pathways and nucleolytically processes the DNA ends (25576492; 16163361; 15653682). NBS1 (Nibrin) binds to and increases the activity of MRE11 (19270065; 15758953; 9590181; 12447395). In turn, RAD50 also binds and increases the activity of MRE11 (7533895; 9013858), which has been posited to result in an ATP hydrolysis-driven conformational change in both DNA and the MRE11-RAD50 complex to coordinate its melting and endonuclease activity (26717941). RBBP8 (CtIP) binds to and increases the activities of MRE11 (17965729; 21052091) and nibrin (17965729), and this has been posited to facilitate controlling DSB resection, checkpoint signaling and HR. The inactivation of the NBS1 gene has been shown to lead to neurodegeneration (19345213). Defects in any component of the MRE11-RAD50-NBS1 complex are detrimental to cells and the MRE11 complex proteins (MRE11, RAD50, and NBS1) have all been found to be substantially reduced in the neurons of AD cortex (15337312). BLM has been shown to bind to the MRN complex (10783165) and has been posited to be required for the correct re-localization of the MRN complex after replication fork arrest (11916980). BLM also acts as a helicase (21325134). BRIP1 has been shown to bind to the MRN complex and specifically inhibit MRE11 nuclease, which has been posited to facilitate DSB processing and appropriate end resection (23530059). Both MRE11 and RAD50 have been shown to bind to BLM (20936779; 10783165) while BLM binds to and activates Nibrin (20719863; 10783165; 15026416). It has been posited that BLM is essential for timely Nibrin function (14517203). DNA2 (25200081; 21325134; 25122754) and WRN (25122754; 11919194; 22989712) have been shown to cooperate with the BLM helicase to catalyze the resection of DNA ends in cooperation with RPA (25122754; 24332808; 22257160) during homologous recombination (25122754). DNA2 binds to and activates FANCD2 (22987153) and has been posited to act as a mediator between homology-directed repair and the FA pathway (24626199). FANCD2 monoubiquitination is regulated by RAD18, thereby regulating its activity (21478670; 21355096).

RBBP8 (CtIP) binds to FANCD2 and stabilizes the protein (24556218). It has been suggested that CtIP cooperates with FANCD2 to promote fork restart and the suppression of new origin firing (24794434; 24556218; 24794430). EXO1 possesses 5′ to 3′ exonuclease activity and has been shown to be co-transcriptionally regulated by BRCA1 (24746700; 24705021). Moreover, WRN binds to and activates the nucleolytic functions of EXO1 (12704184; 15610765) while EXO1 binds to BLM (24705021) and RBBP8 (CtIP) which causes the localization of EXO1 to double-strand breaks (21052091; 22326273; 24705021). RBBP8 (CtIP) also binds with BRCA1 (20029420; 20351172; 11090615) which covalently modifies CtIP

and simulateneously reduces BRCA1 activity (11739404; 17525340; 14578343). PALB2 acts as a localizer of BRCA2 and binds directly to BRCA1 serving as a molecular scaffold in the formation of the BRCA1-PALB2-BRCA2 complex (19369211; 19584259; 17664283). PALB2 has also been shown to interact with RBBP8 (CtIP). It is suggested that interactions between CtIP and the BRCA complex may coordinate and fine-tune DNA repair through its ability to monitor processes including DNA resection (19369211). RAD51 has been proposed to be part of the BRCA complex as it has been shown to physically and functionally interact with PALB2 (20332121; 16793542; 22193777; 20871616), which in turn causes an enhancement of RAD51 recombinase activity (20871616). BRCA2 is involved in the maintenance of genome stability in HR, with FANCD2 being shown to interact with both BRCA1 (21414716; 14499622; 11239454) and BRCA2 (16127665; 15199141; 21414716). These interactions have been posited to be important in RAD51-mediated recombination in response to DNA damage. It has also been suggested that BRCA2 recruits RAD51 to sites of DNA damage (21670257; 25053826; 25833843), and this interaction promotes HR in protozoan DNA repair models (22505581). BRCA2 is transcriptionally regulated by ATF1 and CREB-1 (14680822).

RAD51 plays a role in homologous pairing and strand transfer of DNA. It is also found to interact with BRCA1 and BRCA2 which may be important for the cellular response to DNA damage. RAD51 binds to RPA (20936779; 19338310; 22797063) with RPA SUMOylation facilitating recruitment of RAD51 to the DNA damage foci to initiate DNA repair through HR (20705237). TOPBP1 regulates RAD51 phosphorylation and chromatin loading (26811421; 26811424; 16840526). During HR, mediators such as FANCD1/BRCA2 and RAD51, overcome this inhibition and stimulate DNA strand exchange by recruiting RAD51 to replace RPA from ssDNA (12077133). RAD51 phosphorylation mediated by CHK1 is required for RAD51 recruitment to damage sites (15665856; 20965415; 26317153) and further demonstrates that the CHK1 signaling pathway is part of protecting cells against lethal DNA lesions through regulation of HR. RAD51 foci formation has been shown to be facilitated by RAD18 (25417706; 26871286). In combination with XRCC3, RAD51 resolves the Holliday junctions to restore genetic information disrupted at double strand breaks (17110331; 14716019; 16395335; 9660962). A positive association of XRCC3 polymorphism and PD risk has been demonstrated (22224629). Moreover, CBP functions in DSB repair by transcriptionally activating the RAD51 (23285190). The depletion of CBP has been linked to HTT-induced neurotoxicity both in cellular models and transgenic mouse models (16766198; 15994095) and it has been proposed that CBP is sequestered in the protein aggregates of mutant HTT observed in the brain tissue of patients and in most experimental models of the disease (11264541; 10823891; 22116937; 16525063). CBP has also been shown to be dysregulated in AD, whereby activation of amyloid precursor protein-dependent signaling reduces CBP levels in primary neuronal cultures (14657026) and Aβ impairs CBP activity (11278679; 25888034). The polyglutamine-containing domain of HTT inhibits the enzymatic activity of both p300 and CBP (11607033). Polymerases POLN (19995904) and DNA polymerase η (16337601) have both been shown to have a functional role in HR, whereby POLN stimulates RAD51 to perform DNA synthesis after strand invasion and RAD51 enhances the D-loop extension activity of DNA polymerase η. RECQ5 encodes a protein that prevents aberrant HR by displacing RAD51 from ssDNA (24319145; 20348101; 18926708; 18003859). RAD52 binds single-stranded DNA ends and mediates the DNA-DNA interactions necessary for the annealing of complementary DNA strands. RAD52 interacts with RAD51 (15766559; 17118963; 9826763), which promotes ssDNA annealing (10438626). RAD52 binding to RPA has been shown to increase its affinity to ssDNA (12139939; 8702565; 11081631) and causes RPA displacement (12077133). RAD18 is an E3 ligase and has been posited to be an integral component in translating the damage response signal to orchestrate HR repair; its activity is increased upon binding to BRCA1 (23901102; 22036607). RAD18 interaction with RAD6 (21549715; 17720710) has been posited to mediate lesion bypass mechanisms (10908344; 22547805). RAD6 knockout models result in decreased expansion rates of Friedreich's ataxia GAA repeats in yeast (19595718). RAD18 has

been shown to recruit DNA polymerase κ to the site of repair (16611994; 20227374), as recruitment was significantly reduced in RAD18-depleted cells (23522793). RAD18 has been shown to be down regulated in Aβ treated cells *in vitro* (17970741). Binding of RAD54L to double-strand DNA induces a DNA topological change, which is thought to facilitate homologous DNA paring and stimulate DNA recombination (10209103; 16862129; 11459989). Binding between RAD51 and RAD54L causes increased activity of RAD54L (12205100). TOPBP1 regulates genome stability by binding to BLM, which is stabilized by TOPBP1 in S phase cells in order to suppress sister chromatid exchange (24239288; 25794620; 25794614). Moreover, the interactions between TOPBP1, BRCA1 (12697828) and RAD9 (25091155; 22925454) has been shown to rescue stalled forks and regulate checkpoint functions (11395493).

In non-homologous end joining (NHEJ), the double strand break is detected by Ku70/80 heterodimer (10854421) and tethering of the DNA ends occurs through Ku70/80 and DNA-dependent protein kinase (DNA-PKcs) complex (16488883; 14576316; 17241822). Secondly, the damaged DNA termini are processed by polynucleotide kinase phosphatase (PNKP) through its phosphorylation by DNA-PKcs (21824916) as well as gap-filling DNA polymerase μ (12077346), which binds to the Ku70/80 complex. Ku70/80 -DNA binding is reduced in extracts of post-mortem AD mid-frontal cortex that may be linked to reduced levels of Ku70/80 and DNA-PKcs proteins (15908050). Moreover, Ku70 has been shown to directly bind to mutant HTT . This interaction impairs Ku70 function and causes acceleration in lifespan shortening and locomotion disability in heterozygous null mutants or siRNA-mediated knock down mouse HD models (22096569; 20439996). Lastly, rejoining of the DSB ends is performed by the XRCC4-DNA ligase IV complex (17241822; 24582502; 26201248). It has been shown that there is reduced NHEJ activity in extracts of the cortices of AD brains compared to the normal subjects and significantly lower levels of DNA-PKcs are present in AD brain extracts (12928056).

Cell-Cycle Checkpoint Regulation

Downstream of the regulation of the cell cycle (BRCA1-ATM-ATR-CHK1-CHK2) arrest pathway involved in DNA damage repair is the activation of tyrosyl-DNA phosphodiesterase 1 (TDP1). TDP1 has been implicated in the removal of glycolate from single-stranded DNA containing 3′ phosphoglycolate by hydrolyzing the phosphodiester bond at the DNA 3′ end linked to a tyrosyl moiety, suggesting a role in repair of free-radical mediated DNA double-strand breaks. TDP1 is phosphorylated by ATM (19851285; 19851285) with this phosphorylation promoting cell survival and DNA repair. TDP1 has been shown to immunopercipitate with XRCC1 (13679147) forming a covalent complex. Mutations in TDP-1 are associated with Spinocerebellar ataxia with axonal neuropathy (16775218; 19211312), but wild-type TDP-1 has been implicated in trinucleotide repeat transcription-induced repeat instability (21628532). XRCC1 acts as a scaffolding protein that interacts with multiple repair enzymes, which allows these repair enzymes to then carry out their enzymatic steps in repairing DNA. CHK2 formed a complex with XRCC1and phosphorylated XRCC1 (18971944). PolyQ repeats have been shown to induce ATM/ATR-dependent DNA damage response through accumulation of reactive oxygen species (12915485). ATM may modulate the cytotoxicity of Aβ in Alzheimer's disease (14980204) as Aβ-induced apoptosis was significantly reduced in ATM knockout cell cultures. Moreover, ATR is induced during oxidative stress and phosphorylated ATR accumulates in HD patients (12915485; 23602907)

The pathological accumulation of the Amyloid-β (Aβ) depletes neuronal BRCA1 which may contribute to cognitive deficits in AD (26615780). Additonally BRCA1 has been shown to be localized with Tau neurofibrillary tangles, which is a pathological hallmark of AD (17505559). *In vitro* and *in vivo* analysis demonstrates that BRCA1 and p53 bind to each other (14978302; 9926942) to promote p53 activation. BRCA1 promotes cell survival by directly regulating the DNA damage tolerance pathway. BRCA1 also

interacts with and activates CHK1 (23901102; 11836499) to regulate the G2/M checkpoint (11836499; 11836499) involved in DNA stability. CHK2 activates BRCA1 via phosphorylation (15131084; 16675955; 18797466) which in turn increases ATR-CHK2 signaling and p53 activation of DNA damage response (18162465; 10673500). ATR-dependent phosphorylation of ATM activates ATM phosphorylation of CHK2 (17124492). BRCA1 positively regulates the transcription of GADD45α (10367887; 10962562; 11777930). BRCA1 phosphorylation is also dependent upon CHK1 and CHK2 (12427729). BRCA1 is phosphorylated by the checkpoint kinases ATR (11114888) and ATM (10866324; 12024016) through physical interaction. BRCA1 directly binds to and is phosphorylated by ATM (11016625; 10550055; 23939379; 10866324). The ATR serine/threonine kinase (ATR) participates in the kinase cascade that is activated in response to replication blocks ending in cell-cycle arrest. ATR interacts with and phosphorylates ATM (17124492; 15322239; 18583959) with knockdown of ATM and ATR enhancing cell death (23960094). ATR also phosphorylates CHK1 (21680713; 23667469; 23960094; 15110787) and CHK2 (23960094; 15367709; 12773400). This phosphorylation causes cell-cycle arrest, preventing the cells from entering the cell cycle until DNA damage can be repaired. The ATM serine/threonine kinase (ATM) is a cell cycle checkpoint kinase that phosphorylates and functions as a regulator of a wide variety of downstream proteins including CHK1 (15322239; 19285939; 19530246; 18508566), CHK2 (19285939; 15322239; 15269203; 19530246; 18508566), and c-Abl (20798688; 9168117; 9168116). The tumor suppressor protein p53 is partly responsible for transcriptional activation, with p53 inducing cell cycle arrest, apoptosis, senescence, DNA repair and changes in metabolism. Both ATR (11358490; 22713662; 25336189; 22556425) and ATM (11358490; 9733514; 15159397; 10608806) activate p53 through phosphorylation. PolyQ repeats have been shown to induce ATM/ATR-dependent DNA damage response through accumulation of reactive oxygen species (12915485).

RAD9 checkpoint clamp component A (RAD9) is a cell cycle checkpoint protein required for cell cycle arrest and DNA damage repair with 3′ to 5′ exonuclease activity. RAD9 may employ this enzymatic activity in sensing and repairing DNA damage. RAD9 forms a checkpoint protein complex with RAD1 and HUS1 and is recruited by checkpoint protein RAD17 to the sites of DNA damage (21659603; 25091155), which is phosphorylated by ATR (11799063; 19020305). The RAD9-RAD1-HUS1 interactions form the 9-1-1 complex (19446481; 26021743; 20505337; 26088138). The 9-1-1 complex interacts with DNA pol β and has a stimulatory effect on DNA pol β activity (15314187; 17426133).

Specific physical association between the BRCA1 RING domain and ATF1 stimulates ATF1 activity (10945975) in the stress response. ATF1 acts as a positive transcriptional regulator of PCNA (9883884; 9114015; 7479004; 7910946). CK2 (casein kinase II) contributes to cell survival by cooperating with other survival-promoting pathways and has been shown to phosphorylate ATF1 protein and activate ATF1 transcription (9685505; 8600455). Amyloid-β (Aβ) has been shown to enhance the phosphorylation of ATF1 (26053510) which has been posited to cause cell damage and cell death. p53 binds to CK2 and results in the down-regulation of CK2 (11180407; 10214938; 9180277) which regulates cell apoptosis pathway, whereas CK2 phosphorylates and activates p53-binding to damaged DNA (10214938; 14640983; 9501176). CK2 also phosphorylates BRCA1 (10403822); DNA Ligase I (11331287; 10523317) and XRCC1 (21840775; 20471329). The imbalance in non-phosphorylated and phosphorylated BRCA1 has been posited to modulate the DNA damage response in HD (22580959). CK2 observed upregulated in hippocampus and temporal cortex of AD patients compared to non-demented controls and interacts with Aβ (26732432; 8287280) as well as being upreguted in HD models (17971125).

The proto-oncoprotein, c-Abl, is a member of the non-receptor tyrosine kinases and has a pro-apoptotic role and is posited to act in the decision between activating the proapoptotic pathway when DNA damage is too severe to be repaired. Alzheimer's and Parkinson's diseases patients have shown c-Abl activation;

and overexpression in adult mouse neurons results in neurodegeneration and neuroinflammation (15474370; 21728062). The growth suppressor c-Abl increases the DNA binding activity of p53 (17339230; 15661746; 18490454; 15865930) and c-Abl interacts with p53 in response to DNA damage (10629029). c-Abl phosphorylates ATR and causes its positive activation (20798688; 21151157) as well as phosphorylating EGFR (18721752; 16943190) promoting an increase in cell-surface expression. c-Abl interacts with JNK1(MAPK8-10), which may have an anti-apoptotic role (10022809; 19052872; 15696159). GADD45 has been associated with growth suppression; while GADD45α binds to and inhibits PCNA related negative growth control and inhibits apoptotic cell death (10828065; 7973727; 7784094). Exposure of neurons to Aβ has been shown to induce the up-regulation of GADD45 (11470486; 10448433) indicating DNA damage during early in Aβ cytotoxicity. p53 acts as a transcriptional regulator for a large subset of proteins involved in ICL, including: physical interaction and inhibition of BRCA1 transcription (14710355; 9482880; 25650659), binding and down-regulation of HMGB1 (11748221; 15170359; 12534345; 11748232), induction of FEN-1 transcription (16103874), activation of DNA Ligase 1 transcription (10722849; 21332394), activation of PCNA transcription (8570655; 8524244; 9651310; 15777783; 12947108), increasing the secretion and signaling of EGFR pathway (20190820; 26799187; 18359760; 9285564), and positive regulation of GADD45α (8226988; 10749144; 9566896). The epidermal growth factor receptor (EGFR) directly binds to, phosphorylates and regulates ATM activity (25601159; 26825989; 11466608) while depletion of endogenous EGFR impairs ATM-mediated DNA repair. ATM has been posited to bind and phosphorylate EXO1, regulating its activity (20019063; 18756267; 22326273). EGFR has been suggested to be one of the most significant AD risk genes, as Aβ oligomers can induce the activation of the EGFR (23019586) and over-expression of EGFR may trigger oxidative neuronal injury (10854274). PolyQ peptides have also been shown to cause the constitutive activation of EGFR and potently inhibit EGFR-mediated ERK activation in fly glial cells. It has been posited that disruption of EGFR signaling and ensuing glial cell dysfunction could play a direct role in the pathogenesis of HD and other polyQ diseases in humans (15677486).

Cell Survival

Brain-derived neurotrophic factor (BDNF) promotes cell survival against oxidative DNA damage-induced death by enhancing DNA repair. BDNF has been shown to be reduced in Huntington's disease models (10825501; 22179319; 17959817) with its rescue resulting in decreased levels of neurodegeneration (18086127; 21048129). BDNF has also been shown to be significantly reduced in Alzheimer's disease (15935057) with a reduction of BDNF occuring early in the course of AD correlating with loss of cognitive function. These findings suggest that BDNF plays a role in synaptic loss and cellular dysfunction underlying cognitive impairment in AD. BDNF enhances repair through transcriptional activation by cyclic AMP response element-binding protein (CREB1). CBP acts as a co-activator of CREB1 through binding (7913207; 8413673; 9413984) and increases its activity. CBP also co-activates EXO1 (26004186). The ability of BDNF to activate CREB expression is abolished by shRNA of TrkB as well as inhibitors of TrkB, PI3 kinase, and Akt kinase (24114393). Elevated levels of CREB1 have been shown in Huntington's disease models, and CREB functionality is lost during the early stages of cell stress that contribute to the pathogenic process (14749423; 19632326). SIRT1 is a co-transcriptional regulator of CBP through direct binding (22826441; 22826441). SIRT1 has been shown to be up-regulated in AD and Amyloid Lateral Sclerosis models (17581637) where it is posited to promote neuronal survival mechanisms. SIRT1 has also been shown to be impaired in Huntington's disease models (26815359; 24436303; 22227661; 22179319; 22179316). SIRT1 co-transcriptionally activates BDNF (22179316; 22179319) further enhancing repair. The neuroprotective effect of SIRT1 requires the presence of CREB-regulated transcription co-activator 1 (TORC1), a brain-specific modulator of CREB. The activity of SIRT1 in the deacetylatation and activation of TORC1 promotes TORC1 interaction with CREB. Furthermore, TORC1 and SIRT1 act as co-transcriptional regulators of BDNF (22179316). PARP-1 binds to DNA through

nonionic interactions (14567702; 23333033; 24588584) which conformationally activates PARP-1; this facilitates survival of proliferating cells under conditions of DNA damage likely through its contribution to DNA base excision repair. PARP-1 is acetylated by CBP/p300 (16204234; 15607978). PARP-1 levels have been shown to be high in Alzheimer's disease models (27234294; 25274115; 27034851) and it has been posited that the over-activation of PARP-1 can result in energy depletion and necrosis in response to high levels of DNA damage, but PARP-1 has been shown to be down-regulated in AD mouse models (24121118).

DISCUSSION

DNA damage is a frequent event in the lifetime of replicating and non-replicating cells being caused by oxidation, radiation, chemical assault and even normal biological processes such as transcription and meiosis. The vast array of molecular monitors, repair mechanisms and regulatory pathways involved in detecting and repairing damage to the genome reinforce the critical role of genetic information in a high fidelity state. In this work we have explored the state of the scientific literature to identify and articulate the transcription factors, scaffolding proteins, enzymes and interactions involved in DNA Damage Response and Repair with a special emphasis on the effect in Huntington's, other trinucleotide repeat diseases and neurodegeneration in general.

The results of this search are detailed in the several pathway maps and extensively referenced discussions herein. The pathway maps are additionally available as interactive online forms through Clarivate Analytics Metacore application, and as tabular text files for viewable in network visualization software such as Cytoscape.

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