

DNA repair

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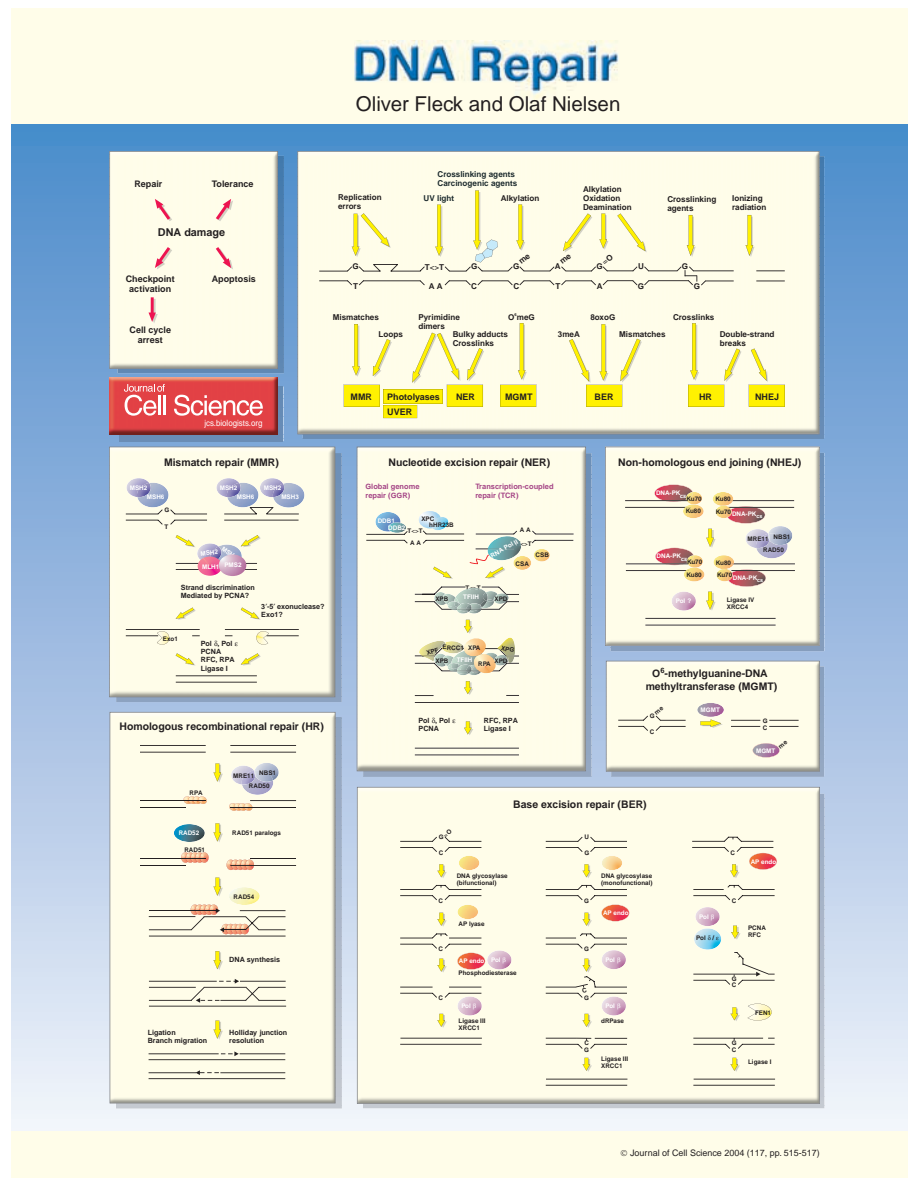
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Organisms are permanently exposed to endogenous and exogenous agents that damage DNA. If not repaired, such damage can result in mutations, diseases and cell death. The cellular responses to DNA damage include processes that deal

with its consequences (e.g. tolerance and apoptosis) as well as direct correction of the damage by DNA repair mechanisms, which may require activation of checkpoint pathways. There are various forms of DNA damage, such as base modifications, strand breaks, crosslinks and mismatches. There are also numerous DNA repair pathways. Each repair pathway is directed to specific types of damage, and a given type of damage can be targeted by several pathways. Major DNA repair pathways are mismatch repair (MMR), nucleotide excision repair (NER), base excision repair (BER), homologous recombination repair (HR), and non-homologous end joining (NHEJ). These

pathways each require a number of proteins. By contrast, O-alkylated bases, such as O⁶-methylguanine can be repaired by the action of a single protein, O⁶-methylguanine-DNA methyltransferase (MGMT). MGMT removes the alkyl group in a suicide reaction by transfer to one of its cysteine residues. Photolyases are able to split covalent bonds of pyrimidine dimers produced by UV radiation. They bind to a UV lesion in a light-independent process, but require light (350-450 nm) as an energy source for repair. Another NER-independent pathway that can remove UV-induced damage, UVER, is present in only a few organisms, such as the yeast *Schizosaccharomyces pombe*. A key factor in UVER is the endonuclease Uve1/UVDE, which cuts 5' of various types of damage. Recent work has uncovered novel pathways, such as transcription-coupled BER, break-induced replication, and nucleotide incision repair as well as interconnections between known pathways. For simplicity, we do not consider these here. Although most repair proteins are usually homologous between organisms, their designations are often different. Here we generally use the names of human proteins.



Mismatch repair

The main task of MMR is to remove base mismatches and small insertion/deletion loops (IDLs) introduced during replication. In *Escherichia coli*, the main players in MMR are MutS, MutL and MutH. MutH nicks the non-methylated strand and thereby enables discrimination between the newly synthesized strand and the template. MMR is bidirectional, i.e. nicking and degradation can occur from either the 5' or 3' side of the mismatch. In eukaryotes, several MutS and MutL homologues are involved in MMR; MutH homologues appear to be absent. Inactivation of human MMR causes hereditary nonpolyposis colorectal cancer (HNPCC) and some types of sporadic tumor. In the course of human MMR, base mismatches are bound by the MutS-homologous heterodimer MSH2-MSH6, while small IDLs can be bound by MSH2-MSH6 and MSH2-MSH3. Subsequently, the MutL-homologous heterodimer MLH1-PMS2 is recruited.

(See poster insert)

In some eukaryotes additional MutL homologues exist. These form heterodimers with MLH1 and may play a minor role in MMR. It is not yet understood how eukaryotes distinguish between the new and the old strand. Strand discrimination may be either mediated by the replication accessory factor PCNA or could be simply achieved by recognition of nicks, gaps or free 3' ends that are present in the nascent strand during replication. In a downstream step, the newly synthesized strand is degraded, which removes the mismatch. MMR patches are ~100 to >1000 nucleotides in length. EXO1 is involved in 5' to 3' excision. It is not yet clear which factors participate in 3' to 5' excision, but DNA Pol δ and ϵ and EXO1 may be involved. MMR is completed after DNA synthesis by the replication machinery and ligation of the remaining nick.

Nucleotide excision repair

NER removes a variety of forms of DNA damage, including photoproducts induced by UV and other bulky lesions. NER consists of two subpathways: global genome repair (GGR), which removes damage in the genome overall and transcription-coupled repair (TCR), which specifically repairs the transcribed strand of active genes. The main difference between GGR and TCR is the requirement for different factors during the initial recognition steps. UV-DDB, consisting of DDB1 and DDB2, and XPC-hHR23B are involved in the recognition step of GGR, while TCR is thought to be initiated by RNA polymerase II stalled at a lesion. Additional factors required for TCR are CSA and CSB. The proteins acting further downstream in GGR and TCR are likely to be identical. First, transcription factor IIIH (TFIIH), a complex consisting of nine subunits, is recruited to the damaged site. At this step the initial recognition factors are probably released from the damaged DNA. Two subunits of TFIIH, XPD and XPA, exhibit helicase activity of opposite polarity, and unwind the DNA around the lesion. The next factors that bind to the damaged site are XPG and XPA-RPA. XPA-RPA verifies whether the NER complex is correctly assembled and ensures proper incision of the

damaged strand. After binding of XPF-ERCC1, dual incision occurs by XPG and XPF-ERCC1, which cut 3' and 5' to the damage, respectively. In this way, the damage is released in a 24-32 nucleotide long oligonucleotide. Repair is completed by DNA synthesis and ligation. The typical disorder caused by a defect in NER is xeroderma pigmentosum (XP), while Cockayne syndrome (CS) and trichothiodystrophy (TTD) are due to impaired TCR and in the latter case eventually also to affected transcription.

Base excision repair

BER mainly repairs non-bulky lesions produced by alkylation, oxidation or deamination of bases. Cells contain several DNA glycosylases, each of them exhibiting a specific substrate spectrum. After cleavage of the N-glycosylic bond by a DNA glycosylase, the damaged base is released and an apurinic/aprimidinic (AP site) is created. An AP site can also occur spontaneously and represents damage itself. Bifunctional glycosylases have an intrinsic AP lyase activity, which cleaves the sugar-phosphate backbone 3' to the AP site. The resulting fragmented sugar residue is removed by a phosphodiesterase activity, contributed by either an AP endonuclease or by DNA polymerase β . The one-nucleotide gap is filled by Pol β and ligated. Processing of AP sites produced by a monofunctional DNA glycosylase requires 5' incision by an AP endonuclease (the major human AP endonuclease is APE1). Pol β incorporates a nucleotide and its deoxyribosephosphodiesterase (dRPase) activity removes the 5' moiety. The remaining nick is sealed by ligation. During a minor, long-patch BER pathway, 2-8 nucleotides are removed together with the damaged nucleotide. Long-patch BER may be required in the presence of modified AP sites where the 5' moiety cannot be removed by a dRPase activity. After strand displacement by Pol β , and Pol δ or Pol ϵ , a flap structure is formed, which is cleaved by FEN1. No human disease is currently known to be associated with a defect in BER, which may be due to embryonic lethality or functional redundancy and/or because accumulation of damage, usually

processed by BER, has no biological consequence. In fact, knockout mice lacking factors acting downstream of DNA glycosylases exhibit an embryonic lethal phenotype, while a defect in a single DNA glycosylase does not cause any phenotypic abnormality.

Homologous recombination repair

Double-strand breaks (DSBs) can be repaired by either HR or NHEJ. HR uses a homologous DNA template and is highly accurate, whereas NHEJ rejoins the broken ends without using a template and is often accompanied by loss of some nucleotides. The relative contribution of each pathway depends on the cell-cycle stage, with NHEJ being more active in G1 and HR dominating during S and G2 phases. During HR DSBs are converted to 3' single-stranded DNA (ssDNA) tails, which are bound by RPA. Processing of DSBs probably requires MRE11-RAD50-NBS1. RAD52 interacts with RPA and promotes binding of RAD51 to the ssDNA, which may be stabilized by RAD51 paralogues (RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3 in human, RAD55 and RAD57 in yeast). Subsequently, the RAD51-bound ssDNA invades a homologous molecule in a reaction stimulated by RAD54. After DNA synthesis and ligation, two Holliday junctions are formed and branch migration can occur. The Holliday junctions are finally resolved by resolvases, which in eukaryotes are not yet identified.

HR also represents an error-free subpathway of damage tolerance, allowing replicational bypass of lesions through a template switch. Alternatively, damage tolerance can be achieved by error-free and error-prone translesion synthesis carried out by specialized DNA polymerases. HR-dependent lesion bypass may sometimes produce a 3' flap that can be cleaved by MUS81-EME1 or resolved by TOP3-RECQ.

Non-homologous end joining

NHEJ is initiated by binding of Ku70-Ku80 dimers to the DNA ends. In higher eukaryotes the DNA protein kinase catalytic subunit (DNA-PK_{cs}) is

subsequently recruited. DSBs that are not suitable for ligation may be processed by MRE11-RAD50-NBS1 and other nucleases, such as FEN1. In addition, a DNA polymerase may be required. Finally, the DNA ends are rejoined by XRCC4-DNA ligase IV.

Defective repair of DSBs can result in chromosomal instability, which is characterized by rearrangements and loss of chromosomes. A number of human syndromes, such as Ataxia telangiectasia (AT) and related disorders, Nijmegen breakage syndrome (NBS), as well as breast and ovarian cancer caused by mutation of BRCA1 or BRCA2, are associated with defects in DSB repair. However, these syndromes are a consequence of defects in regulation of DSB repair (e.g. in checkpoint activation) rather than due to a direct inactivation of HR or NHEJ.

Further reading

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