Mutations are the basis for genetic variation and a prerequisite for evolution, a process enabling living organisms to adapt to changing environments. A certain degree of mutagenesis is therefore needed to sustain viable populations. However, accumulation of deleterious mutations will inevitably reduce the fitness of a population and be the source of both inherited and somatic disease. Thus, a fine balance exists between the need for creating variability and the preservation of the genetic code. DNA damage is the source of mutations, and several thousand lesions are induced spontaneously in every human cell every day due to the chemical instability of DNA. The bases are susceptible to hydrolytic deamination reactions, of which a major product is the mutagenic deamination of cytosine to uracil. DNA is also oxidised as a result of aerobic metabolism resulting in strand breaks and a large number of modified DNA bases. Base excision repair (BER) is the predominant repair pathway for most classes of spontaneous DNA-base damage. BER is initiated by a set of damage specific DNA glycosylases that excise the altered bases leaving an abasic (AP) site as a common intermediate for further processing.

Uracil-DNA glycosylase (UNG) recognises uracil in DNA resulting from deamination of cytosine or misincorporation of dUTP. Mutants of Escherichia coli and Saccharomyces cerevisiae lacking this glycosylase have an increased frequency of GC to AT transition mutations due to deamination of cytosine. In collaboration with Dr. Hans E. Krokan’s laboratory at the Norwegian University of Science and Technology, we have successfully generated UNG-deficient mice (Nilsen et al. (2000) Mol. Cell (5), 1059-1065). Contrasting the phenotype observed in E.coli and S.cerevisiae, the mice show only a modest increase in spontaneous mutation frequency (less than 1.5-fold). However, isolated nuclei from UNG-deficient embryonic fibroblasts (MEF) accumulate misincorporated dUMP residues during replication. This results in an elevated steady-state level of dUMP residues in the genome. Thus we conclude that UNG, which is localised to replication foci through interaction with proliferating cell nuclear antigen (PCNA), serves its main purpose in the rapid removal of misincorporated uracil residues during replication. The presence of a significant complementary uracil-excising activity became apparent from the biochemical analysis of UNG-deficient mice. We have recently identified this major activity to be the SMUG1 uracil-DNA glycosylase (Nilsen et al., (2001) EMBO J. (20), 4278-86). Thus, the lack of a mutator phenotype in UNG-deficient mice suggests that SMUG1 might be the main anti-mutator protecting cells from the deleterious effect of cytosine deamination.

The misincorporation of dUMP has up until now been regarded as relatively innocuous as the resulting U: A base pair is not mutagenic, and bacteria and yeast can survive with up to 20% of genomic TMP substituted with dUMP without any obvious deleterious consequences. Nevertheless, the UNG-family of uracil-DNA glycosylases is highly conserved from bacteria to
mammals. To elucidate the biological effects of dUMP misincorporation in mammalian cells we are currently monitoring life span and cause of death in UNG-deficient mice and in their wild type counterparts (approx. 100 animals in each group). The UNG-deficient mice appear to have an increased mortality compared to wild type mice. The overall cancer rate is not elevated, but the UNG-deficient mice develop leukaemia whereas the wild type mice develop solid tumours. Moreover, there is higher incidence of multiple tumours in the mutant mice. The elucidation of the phenotypes induced by UNG-deficiency is currently our primary research interest, and we are focusing on obtaining detailed pathology of ageing mice. We are also analysing whether increased dUMP content in the genome has any effect on the proliferative capacity of cells of the immune system.

The human base excision repair pathway has previously been reconstituted using naked oligonucleotides as substrates. To introduce an added level of complexity, we are now conducting studies to elucidate the effect chromatin has on repair of uracil-containing DNA. We are using a reconstituted mononucleosome substrate containing a fixed U: A base pair to study the kinetics of uracil removal by the human Uracil-DNA glycosylase (UNG) and reconstituting the base excision repair pathway using purified human proteins, AP-endonuclease 1, DNA polymerase β, XRCC1 and DNA ligase III.