



Clare Hall

## Genetic Recombination

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Mammalian cells possess a large repertoire of repair processes that maintain the integrity of our genetic material. But some individuals have defects in DNA repair that can result in tumour formation: for example, defects in the repair of DNA double-strand breaks are responsible for some inheritable breast cancers. The research in our laboratory is focused on understanding how cells maintain chromosome stability to allow the faithful reproduction of the genome. In particular we want to understand how chromosomal breaks are repaired and how this process is important for tumour avoidance.

Our DNA is continually subjected to damage, either from endogenous sources such as reactive oxygen species that are produced as by-products of oxidative metabolism, from the breakdown of replication forks during normal cell growth, or by agents in the environment such as ionising radiation or carcinogenic chemicals. Fortunately, cells have evolved to cope with damage by employing elaborate and effective repair processes that are specialised to recognise certain lesions in DNA and to repair them.

DNA double-strand breaks represent one of the more dangerous forms of damage, as they can lead to aberrant gene translocations. The consequences of aberrant repair can

be catastrophic to the cell and lead to cancer. One major pathway of double-strand break repair involves the enzymes of genetic recombination. Although genetic recombination normally occurs in germ-line cells at meiosis, where it provides a mechanism for the exchange and reassortment of genetic information, it also plays a critically important role in somatic cells for the repair of damaged or broken chromosomes. Our interest in the contribution of genetic recombination to the repair of DNA double-strand breaks stems from observations indicating that cell lines derived from individuals predisposed to breast cancer through mutations in *BRCA2* exhibit a genome instability phenotype characteristic of a recombination/repair defect.

#### Recombinational repair and breast cancer

The importance of gaining a thorough understanding of homologous recombination is highlighted by observations indicating that individuals with mutations in *BRCA2* have an extremely high probability (70% during their lifetime) of developing breast or ovarian cancers. The process of homologous recombination (HR) requires a number of proteins including RAD51, RAD52, RAD54, the RAD51 'paralogs' (RAD51B, RAD51C, RAD51D, XRCC2, XRCC3), *BRCA2* and RP-A. Many of these proteins have been purified in this laboratory, and we use biochemical, cytological and molecular biological approaches to understand how they function within the cell to repair DNA breaks (McIlwraith *et al.*, *Molecular Cell* 2008; 32: 313).

Of particular importance for HR is RAD51, a protein that catalyses the key reactions required for DNA pairing and strand exchange. In response to DNA damage RAD51 localises to distinct sub-nuclear assemblies (foci) where the repair reactions take place. The localisation of RAD51 to repair foci is dependent upon the breast cancer-associated tumour suppressor *BRCA2*. It is now clear that *BRCA2* controls RAD51 activity throughout the cell cycle and in response to DNA damage. The two proteins interact directly,

mainly through the 8 BRC regions of BRCA2 that map within exon 11, but also at an unrelated site close to the C-terminus of BRCA2. The C-terminal interaction domain is thought to supply regulatory functions, through phosphorylation at S3291, whereas the BRC repeats represent a scaffold for multiple RAD51 binding and relocalisation.

Much of our understanding of the molecular actions of BRCA2 comes from the study of small fragments of the protein. However, we were recently successful in purifying a BRCA2-RAD51 complex from human cells grown in culture and this holds great promise for future biochemical studies.

We also achieved a long-term goal, in that we were successful in identifying the human Holliday junction resolvase as GEN1 (Ip *et al.*, Nature 2008; 456: 357). The resolvase was identified using a two pronged approach: GEN1 was identified by mass spectrometry following extensive fractionation of HeLa cell-free extracts, whereas its yeast counterpart (Yen1) was detected using a TAP-fusion library screen for nucleases capable of Holliday junction resolution. Although these resolvases catalyse Holliday junction cleavage by a mechanism analogous to that shown by the *E. coli* Holliday junction resolvase RuvC, they represent a new sub-class of the Rad2/XPG family of structure-specific nucleases. Preliminary indications are that, like BRCA2, GEN1 deletions/frameshift mutations may be linked to increased risk of breast cancer.

#### Crosslink repair and Fanconi anemia

Fanconi anemia (FA) is a rare autosomal disorder characterised by congenital abnormalities, bone marrow failure and increased incidence of cancer. Cells derived from individuals with FA exhibit a chromosome instability phenotype and are hypersensitive to agents that form DNA crosslinks. These cell lines can be classified into 12 complementation groups, and the genes responsible have now been identified. One of these genes is FANCM, which encodes a putative DNA helicase that is thought to be involved in the recognition of crosslinks as they impede the progress of the DNA replication apparatus. Our biochemical analysis of FANCM is continuing with particular emphasis on defining its interaction partners.

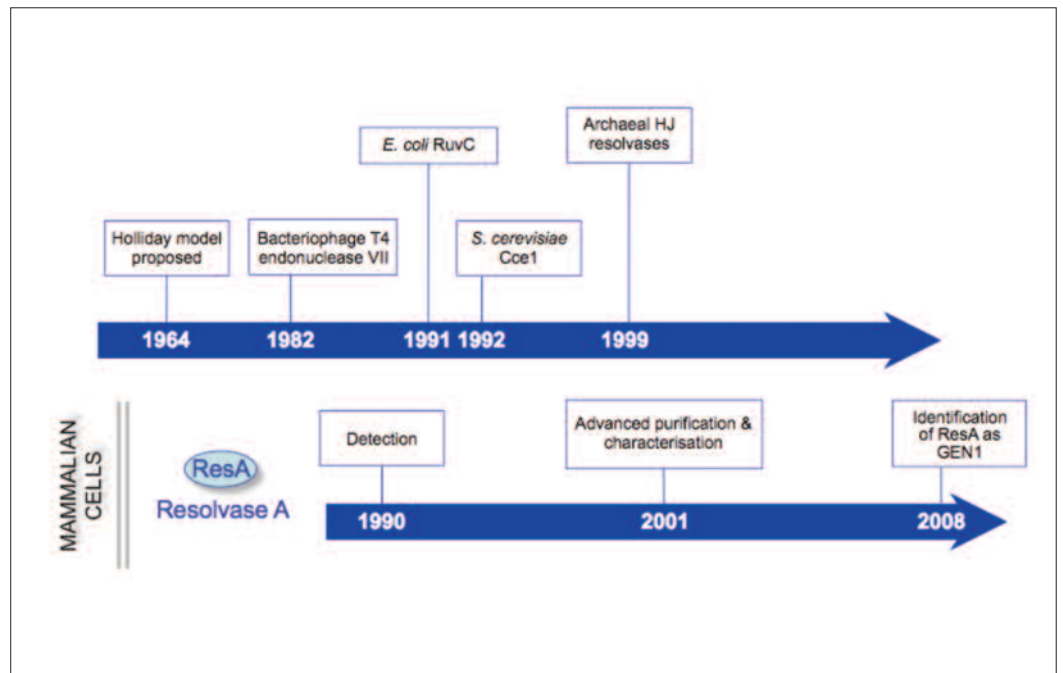


Figure 1. Timeline in the identification of Holliday junction resolvases. Following Robin Holliday's proposal describing the formation of a four-way DNA intermediate (now known as a Holliday junction) in homologous recombination and recombinational repair, enzymes that promote their resolution have been identified from a variety of organisms. The identification of GEN1 as the human HJ resolvase is the culmination of an 18 year-long study.

#### Defective DNA repair and neurological disorders

For some time we have been interested in how defects in some DNA repair processes are associated with neurological disorders (Rass *et al.*, Cell 2007; 130: 991). We have been successful in defining the molecular defect associated with a disease known as Ataxia with Oculomotor Apraxia I (AOA1), which is due to defects in a protein known as Aprataxin. The biochemical properties of Aprataxin suggest that it acts as a proofreader for DNA ligases (Ahel *et al.*, Nature 2006; 443: 713 and Rass *et al.*, J. Biol. Chem. 2008; 283: 33994), indicating that the neurological problems associated with AOA1 are caused by the progressive accumulation of persistent nicks that cannot be repaired when Aprataxin is inactive.

Our studies of Aprataxin led us to note that a repair protein known as APLF (Aprataxin-PNK-Like Factor), whose precise cellular function is unknown, contains a novel form of zinc finger that binds to poly(ADP-ribose). Remarkably, these zinc fingers are found in a number of proteins associated with the DNA damage response and checkpoint regulation (Ahel *et al.*, Nature 2008; 451: 81). These studies provided the first example of a zinc finger motif that interacts with poly(ADP-ribose), often considered to be the third form of nucleic acid.

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