DNA repair : two pieces of the puzzle

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DNA metabolizing processes are, in general, so fundamental that viable mammalian mutants or human patients affected in one of these systems are expected to be extremely rare. Indeed, well-documented cases of natural mutations in genes controlling DNA replication, transcription, recombination or chromosome segregation have not been described. The exception is a class of disorders that are collectively designated DNA repair syndromes. This heterogeneous group of inherited diseases is caused by impairments in DNA repair systems that operate in normal cells to minimize the noxious effects of DNA damage induced by ubiquitously occurring genotoxic agents[1]. The general clinical features of syndromes such as xeroderma pigmentosum (XP), Fanconi's anaemia (FA), Bloom's syndrome (BS) and ataxia telangiectasia (AT) include hypersensitivity to at least one type of DNA damaging agent, genetic instability and predisposition to cancer (see Table). Each of these rare, autosomal recessive disorders is thought to reflect a defect in a different repair pathway or in a complex cellular response system to a challenge posed by inflicting DNA injury. Most XP patients carry a deficiency in the nucleotide excision repair (NER) process; in FA, a system dealing with certain types of cross links is impaired, BS is thought to result partially from an abnormally functioning DNA ligase I and AT displays a defective response to damage induced by ionizing radiation. Only XP was known to possess a primary defect in genes directy involved in repair of DNA damage.

The main human DNA repair disorders

Disease[a]	Tumour predisposition	Primary sensitivity	Defective process	Number compl. gps	Number genes cloned
XP	skin cancer	UV	NER/PRR	8	4[b]
CS	-	UV	NER[c]	2	1[b]
FA	leukaemia	cross-links	cross-link repair?	4	1
AT	lymphoma	X-ray	X-ray response	6[d]	0
BS	wide variety	many agents	DNA ligase?	1	0
'46 BR'	lymphoma?	many agents	DNA ligase I	1	1

[a] For abbreviations see text.
[b] (Unpublished data)
[c] Defect limited to the NER subpathway of preferential repair of the transcribed strand of active genes.
[d] Including a related radiosensitive disorder: Nijmegen breakage syndrome.

However, two new reports have characterized the genes involved in two other putative DNA repair syndromes: Strathdee and coworkers have isolated a gene responsible for one form of Fanconi's anaemia[2] and Barnes *et al.* identified deleterious mutations in the essential DNA ligase I gene of a unique patient with a not yet categorized syndrome, displaying all the hallmarks of a DNA repair disease[3].

The notion that most DNA repair processes involve the concerted action of multiple proteins explains the extensive genetic heterogeneity underlying many genetic instability syndromes. A clear example is presented by XP[4], which can be caused by mutations in at least seven distinct NER genes. An eighth form of the disease — called XP-variant — displays anomalies in the poorly understood post replication repair (PRR) system. In addition, patients suffering from a related but distinct disorder Cockayne Syndrome (CS), harbour defects in a specific NER subpathway focussing on the preferential repair of the transcribed strand of active genes. Finally, a curious overlap of seemingly unrelated syndromes is found in a fraction of Trichothiodystrophy patients that suffer from a defect in sulphur metabolism, but simultaneously exhibit an XP-NER deficiency[5].

Following years of slow progress, there have recently been several breakthroughs mainly in XP and CS, where three genes have been cloned and the cloning of at least two more NER genes will be reported soon. The isolation of these genes proved unexpectedly difficult. The most obvious strategy involves transfection of genomic DNA into cells of patients and 'rescue' of the sequences responsible for correction of the repair defect. However, in contrast to some of the rodent lines human cells appeared particulary unsuitable for this approach because of their inability to stably integrate intact exogenous DNA sequences in sufficiently large quantities[6]. In fact two of the XP-correcting genes cloned recently, *XPBC*[7] and *XPDC*[8], were originally isolated using NER-deficient rodent mutants for genomic DNA transfections. Following their isolation, these genes turned out also to be involved in XP. The only XP gene successfully isolated after largescale genomic DNA transfections into human cells is the XPA correcting (*XPAC*) gene[9]. However, the construction of high quality cDNA libraries in extrachromosomally replicating (EBNA-based) mammalian expression vectors as done by Strathdee *et al.* in the course of the cloning of the *FACC* gene[2] circumvents the limitations inherent to the use of human cells. EBNA vectors can be easily transfected, maintained and recovered from a variety of human cell lines and should prove particularly valuable for the

isolation of additional human (repair) genes.

The recently cloned *FACC* gene[2] is the first from the FA series. The disorder is characterized by progressive pancytopaenia, con-genital malformations, and pre-disposition to leukaemia. Cells derived from FA patients display hypersensitivity to DNA cross-linking agents and an increased level of spontaneous and induced chromo-somal aberrations. The biochemical defect in FA is not pinpointed unequivocally but probably affects one of the cellular pathways dealing with the elimination of interstrand cross-links. In accompanying work Strathdee, Duncan and Buchwald have extended the number of known FA complementation groups from 2 to 4, by cell fusion using FA cell lines with different dominant selectable marker genes[10]. The fact that all three FA strains tested in this study seemed to fall into different complementation groups promises an even greater genetic heterogeneity than revealed already and points to a considerable molecular complexity underlying the affected cellular process.

The *FACC* cDNA confers wild–type resistance to DNA cross-linking agents upon transfection into cells of FA complementation group C. In addition, a leucine to proline substitution (L553P) is found at the fifth amino acid before the C-terminus in one of the alleles of the FA-C line used. Another sequence change was observed in 2 unrelated and unclassified FA patients. However, definite proof that these mutations inactivate the gene is lacking. The predicted 557 amino acid sequence of the FACC protein does not shed light on the potential function of the gene product. No overall homology is found to known proteins, and no obvious functional domains stand out from the primary sequence. Hence, the function of the protein and the process in which it is involved remain for the time being enigmatic. One speculative idea is that FA is defective in a recombinogenic repair pathway essential for the removal of interstrand cross links.

In contrast, the function of the protein that appeared to be affected in patient 46BR(ref. 3) was known *a priori*: DNA ligase I. This enzyme was first suspected to carry the primary mutation causing Bloom's syndrome[11,12]. The unique 46BR patient shares some clinical and cellular features with BS, including severe immunodeficiency, retarded growth, cancer predisposition and cellular sensitivity to a variety of DNA damaging agents. However, the two disorders are not identical. The strongly increased frequency of spontaneous sister chromatid exchange (SCE) typical of BS cells is much lower in the cells derived from the 46BR individual. Furthermore, BS cells exhibit delayed rejoining of large DNA replication intermediates, whereas 46BR cells display a retarded rate of rejoining of short Okazaki fragments. These findings point to a (different) defect in DNA ligation in both disorders, and suggest that 46BR is the sole representant of a novel, extremely rare repair disorder.

Previously, evidence was obtained for altered biochemical properties of partially purified DNA ligase I in several BS lines[11,12]. However, no coding mutations have been encountered in the ligase I gene of a number of BS patients[2,13]. Instead both ligase I alleles of 46BR were found to contain a clear mutation[3]. A highly conserved glutamic acid in the active site of the enzyme is replaced by lysine (E566K) in one allele. This change was shown to cause almost complete impairment of enzyme activity *in vitro*. The mutation in the second allele affected a conserved arginine that was substituted with a tryptophan (R77W)[3]. This alteration probably produces a partially functional enzyme, as it is difficult to envisage that a cell can survive without the predominant DNA ligase. The crucial function of this enzyme for various vital cellular processes provides a plausible explanation for the extreme rarity of the "46BR-syndrome", as only a very limited subset of alterations will be compatible with cellular viability. In the meantime, the BS-ligase connection remains unresolved. As suggested by Barnes *et al.*[3] one of the most likely alternatives is that a process modulating ligase I activity is disturbed in BS or that another ligase (ligase III) partly copurifying with ligase I, is affected.

Without doubt, the isolation of these genes will provide the key to understanding the cellular systems impaired in both disorders. Furthermore, the cloned sequences will permit generation of mouse models that mimick mutations in patients, using targeted gene inactivation by homologous recombination in totipotent embryonal stem cells. The main task ahead will be to understand how the molecular defect translates into the clinical phenotype, most importantly the predisposition to cancer. □

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