Fanconi anaemia (FA) is a genetic, life-threatening disorder featuring progressive bone marrow failure, birth defects, leukaemia, increased incidence of solid tumours, spontaneous chromosomal instability and hypersensitivity to cross-linking reagents. Because of FA’s relationship to DNA damage hypersensitivity and its association with susceptibility to neoplastic transformation, the FA research field has gained much attention in recent decades, especially after the discovery of the connection between FA and the well-known breast cancer (BRCA) genes.

Clinical Features
FA is a very heterogeneous condition clinically, and about 70% of patients display a wide variety of abnormalities, classic among which are missing or misshapen thumbs and radii, kidney, gastrointestinal and neurocognitive and developmental delay. The most important clinical feature of FA is marrow failure in the form of aplastic anaemia, myelodysplastic syndrome or acute myeloid leukaemia (AML). The number of FA children surviving childhood has greatly increased with the advent of improved outcomes after bone marrow transplantation for bone marrow failure and AML, but those patients surviving until adulthood have to face a greatly increased risk of solid tumours, typically squamous cell carcinomas such as head and neck and gynaecological tumours.

Because FANCD1/BRC2 conveys an inherited risk of breast, ovarian and pancreatic cancer for individuals carrying a single mutated allele, the FA pathway is thought to be involved in cancer development. Many studies support the notion that somatic inactivation of the FA pathway may contribute to the pathogenesis of sporadic cancers, including AML. For pancreatic cancer, although screens for germline mutations in FANCC, FANCA and FANCG failed to detect any pathogenic alleles in familial tumours, truncations of FANCC were found in sporadic pancreatic cancers of early-onset cases. Expression of FANCF is decreased in most ovarian cancers compared with those in normal ovarian tissue, in part because of methylation of the FANCF promoter, suggesting a role for epigenetic modifications in FA tumorigenesis. Restoration of this pathway is associated with demethylation of FANCF, leading to acquired cisplatin resistance.

Fanconi Anaemia Genes
At the cellular level, the distinguishing and diagnostic features of FA are spontaneous genomic instability and hypersensitivity to DNA interstrand cross-linkers such as mitomycin C (MMC) and diepoxybutane (DEB). FA complementation groups were established by the pair-wise fusion of patient-derived cell lines and the subsequent resistance to genotoxic reagents. The genes were subsequently cloned via expression, purification, positional and candidate approaches. A summary of identified FA genes is shown in Table 1.

Protein Biochemistry of Fanconi Anaemia

Core Complex Formation
Eight FA genes (FANCA, FANCC, FANCB, FANCG, FANCF, FANCE, FANCL and FANCM) encode for members of a multi-subunit protein complex termed the FA core complex. Although the stoichiometry is unclear, most subunits are required for general complex stability and proper nuclear localisation, which is essential for mono-ubiquitlation of the downstream effector FANCD2. FANCA and FANCG readily associate and stabilise each other. Other known interacting pairs within the FA core complex include FANCF/FANCC and FANCB/FANCL. FANCF functions as a molecular adaptor, bridging between the subcomplexes A:G and C:E, whereas FANCM may link the subcomplex B:L to the subcomplex A:G. The FA core complex is also reported to weakly associate with other DNA repair proteins, including Bloom’s helicase, topoisomerase II& and replication protein A. Although the functional significance of these inter-complex associations is uncertain, they nevertheless imply a role of FA in DNA damage response. The amount of the chromatin-bound fraction of FA complex increases in the S phase when the FA pathway is activated, and is facilitated by FANCM. Similar results were observed in a newly developed cell-free Xenopus system. The loading and unloading of the xFA core complex is strictly dependent on entry and exit during the S phase, supporting the role of FA in DNA replication.

Mono-ubiquitillation of FANCD2
FANCD2 is mono-ubiquitinated on lysine 561 in response to DNA damage as well as S-phase progression, which is dependent on the FA core complex. This is a pivotal event to activate the FA pathway. FA-D2 cell lines expressing mutant FANCD2 (K561R) are as sensitive to MMC as parental cell lines. The whole FA core complex is hypothesised to be the ubiquitin E3 ligase for FANCD2 with FANCL as the catalytic subunit. FANCE may serve as a link between FANCD2 and the core complex, as it is found to be bound to both. In addition, knockdown of the E2 ligase UBE-2T, a binding partner of FANCL, leads to a drastic reduction of the level of mono-ubiquitinated FANCD2 (FANCD2-L isoform). However,
dependent on the FA pathway. Following incision, ICLs are processed for the first step of interstrand cross-link (ICL) incision and is not in mammalian cells, the nucleotide excision repair pathway is responsible for the maintenance of mono-ubiquitilation of either one is required for the expression of mono-ubiquitilated D2. Interestingly, the newly identified FANCI is a paralogue of FANCD2 and is mono-ubiquitilated in a similar manner,59 and is also reported to associate with the IkappaB kinase (IKK) complex during mitosis,62 presumably to regulate the exit of FA complexes from the nucleosome. The FANCE subunit of the core complex is phosphorylated on two conserved sites (threonine 346 and serine 383) by CHK1.63 Interestingly, the non-phosphorylatable FANCE mutant (FANCE-T346A/S374A) supports aberrations and hypersensitivity to MMC, as with FA mutant cells.54

Phosphorylation of Fanconi Anaemia Core Complex Proteins

A number of FA member proteins are phosphorylated in response to DNA damage or cell-cycle progression. FANCM contains multiple predicted ATR phosphorylation sites and is hyper-phosphorylated following MMC treatment.24 FANCA is phosphorylated in an FA-complex-dependent manner,55 and is also reported to associate with the IkappaB kinase (IKK) signalling via interaction with IKK2, a kinase required for rapid, stress-dependent phosphorylation of the FA core complex.64 FANCG is phosphorylated on serine-7, a modification required for full correction of MMC resistance in FA-G cells,61 as well as at serine 383 and serine 387 during mitosis,62 presumably to regulate the exit of FA complexes from the nucleus. The FANC subunit of the core complex is phosphorylated on two conserved sites (threonine 346 and serine 374) by CHK1.63 Interestingly, the non-phosphorylatable FANC mutant (FANC-E346A/S374A) supports FANCD2 mono-ubiquitilation, FANCD2 foci assembly and normal S-phase progression, but fails to complement the transfected FA-E mutant cells, suggesting yet another role for CHK1.63

Non-nuclear Roles of the Fanconi Anaemia Pathway

Tumour Necrosis Factor-α, Interferon-γ, and Pro-apoptotic State

Tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) are well-known negative modulators of haematopoiesis by triggering excessive apoptosis. In vivo, TNF-α and IFN-γ are overexpressed in stimulated bone marrow lymphocytes from FA-C patients.65 In vitro, both the number and size of erythroid colony-forming units and erythroid burst-forming units grown from FA patients are subject to inhibition by TNF-α.65 Murine models further support the theory that FA proteins are involved in cytokine signalling pathways and that cytokine-mediated apoptosis has a major role in the pathogenesis of bone marrow failure observed in FA patients.65

<table>
<thead>
<tr>
<th>Complementation Groups</th>
<th>FA Gene</th>
<th>Chromosomal Location</th>
<th>Protein Domain/Motif</th>
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<tbody>
<tr>
<td>A</td>
<td>FANCA</td>
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<tr>
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<td>FANCB</td>
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<td>FANCC</td>
<td>9p22.3</td>
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<tr>
<td>K</td>
<td>FANCL</td>
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* Loss of conservation of key functional residues.
Increased apoptosis may be mediated via TNF-related apoptosis-inducing ligand in FA-A cells, although FA-C cells are resistant to TRAIL. Protein kinase regulated by RNA (PKR) plays a critical role in cell growth and apoptosis and is implicated in the FA pathway. In primary human bone marrow cells, mutation in FANCA, FANCC or FANCG markedly increases the association between PKR and FANCC, leading to hyperactivation of PKR and hypersensitivity to cytokine-mediated cytotoxicity. FANCC binds to the signal transducer and activator of transcription 1 (STAT1) following IFN-γ treatment and is required for proper docking of STAT1 at the IFN-γ receptor-ε-chain for survival cues. Together, augmented activity of PKR and failed activation of STAT1 by IFN-γ may thus tip the balance from survival to death in FA-C haematopoietic progenitor cells.

**Fanconi Anaemia Oxygen Sensitivity**

There is an extensive body of evidence indicating that the FA pathway has a role in oxidative stress response. FANCA associates with proteins with redox activities such as NADPH cytochrome P450 reductase and glutathione S-transferase. FANCG protein interacts with cytochrome P450 reductase 72 and is implicated in the FA pathway. In primary human bone marrow cells, mutation in FANCA, FANCC or FANCG markedly increases the association between PKR and FANCC, leading to hyperactivation of PKR and hypersensitivity to cytokine-mediated cytotoxicity. FANCC binds to the signal transducer and activator of transcription 1 (STAT1) following IFN-γ treatment and is required for proper docking of STAT1 at the IFN-γ receptor-ε-chain for survival cues. Together, augmented activity of PKR and failed activation of STAT1 by IFN-γ may thus tip the balance from survival to death in FA-C haematopoietic progenitor cells.

**Figure 1: Model of the Fanconi Anaemia Pathway**

- **DNA damage/replication fork stalling**
- **DNA repair**
- **Recombination and repair**
- **Fanconi A complex**
- **Fanconi C complex**
- **Fanconi D2-L complex**
- **Fanconi D2-S complex**
- **FA effector complex?**