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Milestones in Fanconi Anemia Research

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Abstract

The recessive disease Fanconi anemia (FA) is a prototype chromosome instability syndrome which shows a high level of spontaneous and induced chromosomal aberrations in combination with a significantly increased cancer risk. Thus, the underlying defect must be directly or indirectly involved in a fundamental cellular task of long-lived mammalian cells, the maintenance of genomic integrity. In addition to the delineation of the FA clinical and cellular phenotypes, prominent milestones in FA research include proof of extensive genetic heterogeneity, identification of (so far) 12 disease genes, and elucidation of the FA pathway involved in the repair of crosslinks at arrested replication forks. What is referred to as the FA/BRCA pathway represents only part of a complex network of protein-protein interactions which is far from being understood. The ultimate milestone in FA research would be the achievement of an individualized and curative therapy. Currently, hematopoietic stem cell transplantation is a promising but still high-risk therapy, and gene therapy is still at the experimental stage. Nonetheless, in a significant proportion of patients, a kind of ‘natural gene therapy’ can be observed which results from intragenic recombination or from compensating second site mutations. The elucidation of these somatic events and their underlying mechanisms can be considered a milestone in genetic research. The remarkable progress in FA research during the last 10–15 years was fostered by the foundation of FA patient support groups in many countries. These support groups are gratefully acknowledged as important motivational milestones in FA research.

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‘Many phenomena that basic research tries to explain would simply be unknown had they not been uncovered by the study of diseases. Phenomena such as spontaneously enhanced chromosome instability in Fanconi anemia or Bloom syndrome with all their consequences for somatic mutation and cancer formation were discovered accidentally in the process of examining certain patients for diagnostic reasons’. This statement in the introductory chapter of Vogel and Motulsky’s famous textbook, ‘Human Genetics – Problems and Approaches’ [1]

is still valid today. Currently, following the identification of 12 different genes leading to the Fanconi anemia (FA) phenotype, the statement can be extended to include the understanding of basic biological principles related to the maintenance of genome stability, the elucidation of relevant pathways and their interconnection in genetic networks. Here, a brief and necessarily subjective review is given of what most researchers in the field might consider milestones in FA research. More details are presented in subsequent articles of this volume and in a number of excellent reviews [2–9].

The Foundations of FA Research: Clinical Observations

In 1927, the Swiss pediatrician Guido Fanconi (1892–1979) first described 3 brothers with a specific combination of bone marrow failure (pancytopenia) and various physical anomalies, such as short stature, hypogonadism and hyperpigmentation, pointing to a new autosomal recessive disorder [10]. Generally, the substantiation of a new genetic disease is more likely to be based on particularly severely affected individuals, as these individuals receive preferential medical attention. As a consequence, the disease in FA index cases is regularly more severe than in their affected sibs [11]. Only around 70–75% of FA patients display the ‘classical’ FA phenotype which consists of short stature in combination with a typical spectrum of congenital defects, including radial ray defects, pigmentary changes, urogenital malformations, atresia of the external ear canal, and many others [12]. Endocrine abnormalities reminiscent of premature aging occur in nearly all FA patients with developmental defects. They include hyperinsulinemia, growth hormone insufficiency and hypothyroidism [13]. Reproduction is severely impaired due to failure of spermatogenesis in males and a low pregnancy rate in females [14].

Following the initial and follow-up descriptions by Fanconi himself [10, 15], a landmark paper in FA research was the publication by Gmyrek and Syllm-Rappoport in 1964 who reviewed phenotypic features and clinical course of 129 patients [16]. Much of what we know today about the spectrum of congenital malformations and course of the disease is already mentioned in this paper. From a European perspective, another landmark publication was the 1976 paper by Traute Schroeder and colleagues, entitled ‘Formal Genetics of Fanconi’s Anemia’ [17]. Based upon an extensive collection of pedigrees, the authors provided unequivocal proof of autosomal recessive inheritance despite the surprising fact that none of the families examined showed evidence for consanguinity. There was a slight preponderance of affected males which retrospectively would be compatible with a small proportion of X-linked recessive cases which we now know are due to mutations in the *FANCB* gene, the only FA

gene located on the X-chromosome. Because the authors noted a high intrafamilial correlation for age of onset and for the severity of malformations, they correctly predicted genetic heterogeneity and stated that ‘apart from the standard type, an especially mild type with late onset, few malformations, and a relatively benign course seems to exist’ [17]. They could not have been more correct, since today we are well aware that ‘mild’ mutations exist in some of the FA genes, and that the phenomenon of revertant mosaicism may mitigate the clinical course. Around 1982, Arleen Auerbach started the Fanconi Anemia International Registry in the United States which has made a number of landmark contributions to our understanding of the clinical and genetic heterogeneity of FA so far [18–22].

A definitive clinical milestone in FA research was the realization that patients exhibit a high risk for myelodysplastic syndrome (MDS), and for developing overt malignancies [23]. Leukemia is found in about 10% of younger patients (especially acute myeloblastic leukemia, AML), and solid tumors, particularly squamous cell carcinomas of the oral cavity and genital area have been reported in almost 10% of older patients. This, in combination with bone marrow failure, explains the significantly reduced life expectancy of FA patients. The cumulative incidence of solid tumors reaches 30% by the age of 45 years and remains the major threat to older FA patients [24, 25]. Since malignant growth is one of the possible endpoints of somatic mutations, the high cancer risk reflects the genetic defect in FA, which is related to the defective maintenance of genomic integrity. The discovery of spontaneous chromosomal instability as cytogenetic expression of the (at that time still elusive) genetic defect marks the first of the many experimental milestones of FA research.

Chromosomal Instability and Sensitivity to DNA Crosslinking Agents

On the time scale shown in figure 1, the first milestone following Fanconi’s original description of three patients was the discovery of chromosomal instability. In 1964, Traute Schroeder and colleagues, then working at the University of Heidelberg, reported increased spontaneous chromosomal instability in blood lymphocytes of two affected brothers [26]. Less than a year later, this key observation was confirmed by the Zurich group of Werner Schmid, with Guido Fanconi himself as senior author of the paper [27]. The chromosomal aberrations described by the Schroeder and Schmid groups were mainly of the chromatid type and manifested at metaphase as chromatid breaks or chromatid interchanges. Obviously, these originate during the S-phase of the cell

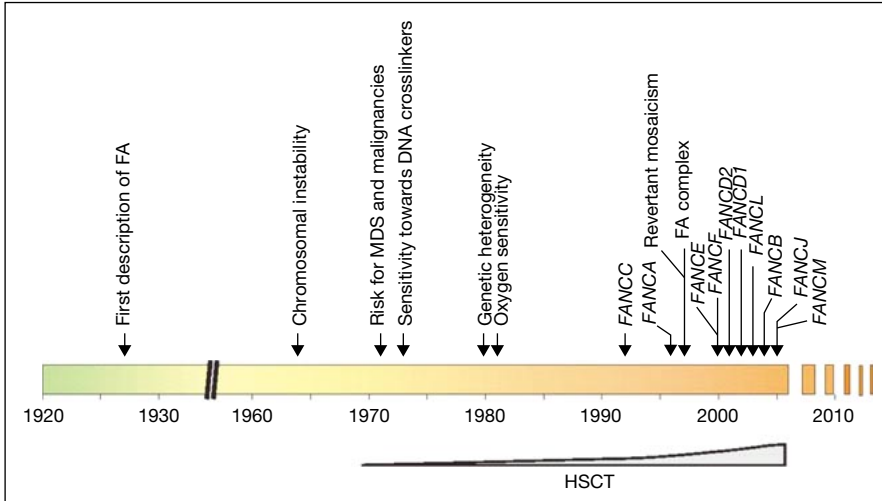


Fig. 1. Milestones in FA-research. The figure does not yet include *FANCN* as the most recently identified FA gene whose product proved identical to PALB2, a protein that functions downstream of FANCD2 as ‘partner and localizer of BRCA2’ [26, 77].

cycle and involve newly replicated DNA. Since each chromatid break is the visible manifestation of a DNA double-strand break, the characteristic chromosomal instability of FA indicates that the underlying defect in this genetic disease is directly or indirectly involved in a fundamental cellular process, the repair of DNA double-strand breaks. Almost at the same time, spontaneous chromosomal instability was also observed by James German and colleagues in Bloom syndrome [28]. While in FA cells the breakpoints of the interchanges are almost randomly distributed, in Bloom syndrome they preferably involve homologous regions, indicating the impairment of different DNA repair pathways [29]. Looking back at these early data from today’s vantage point it is quite remarkable how careful observations at the cytogenetic level were able to predict molecular differences.

About 10 years after the discovery of spontaneous chromosomal instability as a cytogenetic hallmark of FA, Sasaki and Tonomura showed that the spontaneous chromosome instability in FA is associated with a high rate of induced chromosomal aberrations after treatment with DNA crosslinking agents [30]. The determination of cellular sensitivity towards crosslinking agents such as diepoxybutane, mitomycin C, cisplatinum or nitrogen mustard still serves as gold standard for the confirmation of the clinical diagnosis of FA [31]. Because of the highly variable clinical phenotype, each putative patient requires confirmation via the demonstration of increased in vitro sensitivity towards crosslinking

agents. As an alternative to chromosome breakage studies, crosslink sensitivity can also be assessed via cell cycle analysis [32].

The term chromosome instability syndrome now covers an increasing number of recessive disorders which share a high level of spontaneous and induced chromosomal aberrations. However, the type of chromosome anomalies and the specificity of the clastogens vary considerably among the different disorders. For instance, in Ataxia telangiectasia and Nijmegen breakage syndrome, the break points of the spontaneous translocations and inversions in lymphocytes preferentially involve the T-cell receptor and immunoglobulin gene loci. Other than FA, these patients are highly sensitive to ionizing radiation, but they share a common characteristic of all chromosome instability syndromes which is a sharply increased risk of malignancy [8].

Analysis of Genetic Heterogeneity

In most organisms, the existence of (intergenic) heterogeneity in recessive traits can easily be studied by crossing pairs of mutants and analyzing their offspring. In the case of heterogeneity the progeny of such crossings has a normal phenotype due to complementation of the parental defects. In our species, family studies have only rarely provided evidence for the presence of distinct genes. The arrival of somatic cell genetics allowed researchers to fuse different human cell lines and thus perform experimental complementation studies *in vitro*. In FA, normalization of the characteristic hypersensitivity to crosslinkers in proliferating cell hybrids should indicate complementation, and thus prove the existence of genetic heterogeneity. This was first demonstrated by Zakrzewski and Sperling in 1980 after fusion of an SV40 transformed FA cell line with diploid fibroblasts derived from another patient. In contrast to the parental cells, the resulting cell hybrids did not show hypersensitivity to DNA cross-linkers proving mutual complementation of distinctive gene defects [33]. However, due to the inherent chromosomal instability and frequent chromosome loss caused by the SV40 genome, using SV40 transformed cell lines has severe shortcomings. These problems were overcome by using Epstein-Barr virus immortalized B lymphoblasts from different FA patients. EBV transformed lymphoid cell lines remain mostly diploid and can be sustained with different selectable markers for the isolation of cell hybrids [34]. Such cell fusion studies using lymphoid cell lines were first performed in the laboratory of Manuel Buchwald in Toronto and later on, most extensively, in the laboratory of Hans Joenje in Amsterdam. These tedious cell fusion studies were of fundamental importance for the ensuing progress of FA research, and the Amsterdam laboratory of Hans Joenje deserves credit for having provided evidence for at least 12 and possibly even

more FA complementation groups [9]. Reference cells lines for each newly described complementation group were generously provided by the Amsterdam group to laboratories all over the world, boosting FA research.

The genes underlying 12 complementation groups have been identified so far. Their cDNAs are extremely useful for assigning unclassified patients to a specific complementation group. For this, the relevant cDNAs are inserted into retroviral or episomal expression vectors, which are then transferred into the patient's cell line and tested for complementation [35]. This was first exemplified in 1997 by using a recombinant retroviral vector which stably integrates into the host genome [36]. In the following years, vector design and transfection efficiencies were substantially improved by the laboratories of David Williams and Helmut Hanenberg such that assignment of FA patients to the respective complementation groups has become part of diagnostic routine [37, 38]. Interestingly, a number of patient cell lines are not complemented by any of the known FA cDNAs rendering the actual figure of complementation groups higher than 12. Clearly, experimental demonstration and molecular proof of extensive genetic heterogeneity has been another major milestone in FA research.

Identification of FA Genes

As illustrated on the time scale of figure 1, the early nineties of the 20th century marked the beginning of a new, molecular area in FA research. The first FA gene identified in 1992 belonged to complementation group C and was termed *FACC* (FA complementation group C complementing). Since then, the nomenclature has changed and today is based on a truncation of the name Fanconi and the letter of the respective complementation group. Therefore the 12 FA genes are: *FANCA*, *FANCB* (syn. *FAAP95*), *FANCC*, *FANCD1* (syn. *BRCA2*), *FANCD2*, *FANCE*, *FANCF*, *FANCG* (syn. *XRCC9*), *FANCI* (syn. *BRIP1*), *FANCL* (syn. *FAAP43*, *PHF9*), *FANCM* (syn. *FAAP250*, *KIAA1596*) and *FANCN* (syn. *PALB2*). Figure 2 shows the human chromosome map with the location of the 12 known FA genes. With the exception of *FANCC* and *FANCG*, which are both located on chromosome 9, and *FANCA* and *FANCN*, which are both located on chromosome 16, the other FA genes are spread over different chromosomes, including *FANCB* on the X.

The advent of the Human Genome project paved the way for the identification of human disease genes by the technique of positional cloning, reported for the first time in 1986 [39]. Positional cloning is based on the knowledge of a gene's location in the genome, the isolation of candidate genes from the critical region and the identification of mutations in a candidate gene in patient DNA. A prerequisite for the success of this approach is the availability of many

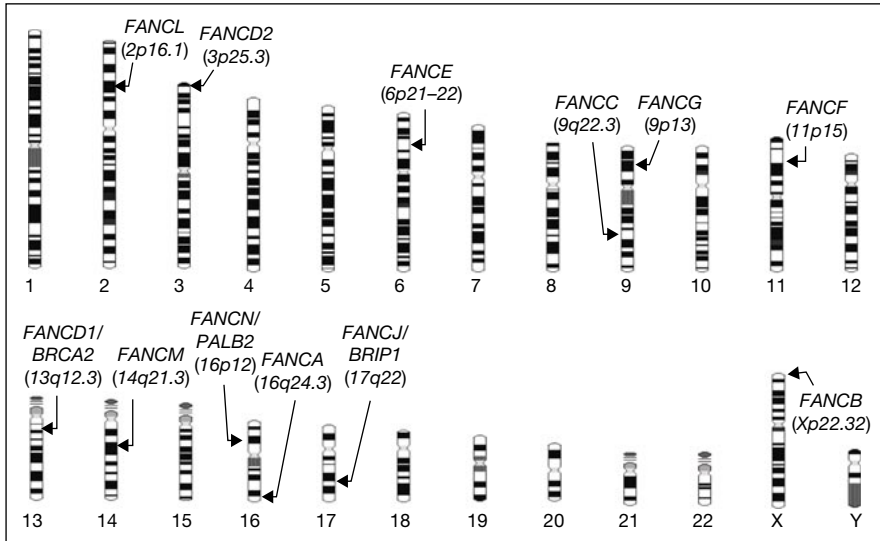


Fig. 2. Human karyotype map with locations of the 12 known Fanconi anemia genes. Figure taken from reference [75], with permission.

affected individuals, usually from different families, but belonging to the same complementation group. Thus, due to the extensive genetic heterogeneity, this approach has obvious limitations in FA. In contrast, the older technique of ‘functional cloning’ of a disease gene depends on fundamental information about the basic biochemical defect and – due to our ignorance – is rarely applicable. In the pre-genomic era, many different biochemical lesions have been attributed to FA cells, including alterations affecting DNA ligase activity, the intracellular distribution of topoisomerase, UV excision repair or cellular NAD^+ levels. Today we know that these were only secondary phenomena.

Expression cloning is a form of functional cloning which does not require knowledge of the primary defect. In the case of FA it is based on the transfection of cells with a normal cDNA library or, alternatively, fusing patient cells with so called mini cells containing different human chromosomes/chromosomal regions. Successfully complemented cells are no longer sensitive to cross-linkers since they now contain a functional FA cDNA. The next step is the identification of the complementing cDNA/genomic DNA. This approach led to the cloning of the first gene underlying Fanconi anemia (*FANCC*) in 1992 by Manuel Buchwald’s group in Toronto, opening up the molecular avenues in FA research [40]. Most of the other FA genes were identified by the expression cloning approach (*FANCA*, *FANCE*, *FANCF*, *FANCG*, and *FANCD2*). *FANCA* was also independently identified by positional cloning, as was *FANCI*.

Based on the observation that many FA proteins form a complex together with other unidentified proteins, FAAPs (Fanconi Anemia Associated Proteins) that could be isolated by immunoprecipitation, three additional FA genes were identified. Using this biochemical approach, Weidong Wang collaborating with the Joenje group showed that FAAP43, a protein with a molecular weight of 43 kDa, was absent in the only patient belonging to complementation group L, thereby defining FAAP43 as the elusive *FANCL* gene [41]. Subsequently, the FA-associated proteins FAAP95 and FAAP250 were shown to be defective in patients belonging to complementation groups B (X-linked) and M [9].

Altogether, these studies confirmed that each complementation group corresponds to a distinct FA gene – with one exception. The exception is group D, which comprises the *FANCD1* and the *FANCD2* genes. This heterogeneity is not well understood, but might be due to the fact that *FANCD1*, in contrast to almost all other FA genes, acts downstream of *FANCD2* which might have affected the results of the initial complementation studies. Most importantly, *FANCD1* was subsequently shown to be identical to *BRCA2* by systematic screenings of FA patients for mutations in *BRCA2* [42]. It came as a great surprise, of course, that *FANCD1* corresponds to *BRCA2*, a prominent cancer gene known to be involved in homology directed DNA repair via its association with the RAD51 recombinase. A single patient initially assigned to complementation group H was later shown to carry biallelic mutations in *FANCA* such that the reference cell line had to be reassigned to group FA-A [43].

In many of the FA genes no functional domains are apparent in the protein sequences and no strong homologies exist in nonvertebrate species. Consequently, their biochemical function has remained obscure. One exception was the *FANCG* gene which was shown to be identical to *XRCC9*, a gene known to be involved in DNA repair [44]. The situation changed completely when in 2001 the groups of Markus Grompe and Alan D'Andrea discovered the *FANCD2* gene which is highly conserved in plants (*A. thaliana*), nematodes (*C. elegans*), and insects (*Drosophila*), indicating the possible conservation of a 'basic' FA pathway in lower organisms [45]. In addition, two of the most recently identified FA genes, *FANCI* and *FANCF*, are identical to conserved genes with known DNA maintenance functions. *FANCI* is identical to the BRIP1/BACH1, a DNA helicase, which interacts with BRCA1. *FANCF* has homology with both helicases and endonucleases, including the archaeal Hef protein. Another conserved FA gene, *FANCL*, has a ring finger motif that is typical for E3 ubiquitin ligases [9].

Most FA patients belong to the complementation groups A, C and G. However, there are also ethnic differences, which are mainly due to founder mutations. Thus, most FA patients in the Afrikaans-speaking population in South Africa belong to group A [46], whereas in the Ashkenazi-Jewish population,

group C is most frequent [47]. Interestingly, there is no strong correlation between a given complementation group and a given clinical phenotype, simply because different mutations in the same FA gene can lead to strikingly different phenotypic consequences. As a rule, null mutations lead to more severe and earlier disease manifestation than mutations that result in a partially functional gene product [5]. There is no doubt that identification of each single FA gene represents a milestone in FA research, both with respect to the practice of medical genetics and the understanding of FA gene and protein function.

Elucidation of FA Pathways and Networks

Elucidation of the FA pathway should link the clinical and cellular FA phenotype to the mutations in the affected genes. However, recent insights into the molecular pathophysiology of FA have indicated an enormous degree of complexity in which individual pathways are only parts of a network of protein-protein interactions. Nonetheless, the most important advances in medical research are often obtained by reducing complexity to basic principles. In the case of FA, one major unanswered question is how chromosome instability is linked to the susceptibility of FA cells to DNA crosslinking agents.

Some important milestones in this research were:

- The proof in 1997 that the FA proteins, A and C, form a nuclear complex and the subsequent realization that the FA proteins B, E, F, G, L, and M are also part of this core complex [48, 49].
- The discovery that the FANCD2 protein takes a central position in the FA pathway. The FANCD2 protein is activated by the addition of a ubiquitin molecule in response to DNA damage [50].
- The finding that the FANCD2 protein directly interacts with known DNA repair proteins such as BRCA1 and BRCA2/FANCD1, linking DNA double-strand-break (DSB) repair with DNA cross-link repair [50, 51].

It is now well established that each of the proteins that are assembled to the FA core complex is needed for monoubiquitination of FANCD2. The catalytic function is obviously provided by the FANCL protein with its E3 ubiquitin ligase domain. Thus, all these 8 proteins act upstream of the evolutionarily conserved FANCD2 protein. The posttranslational modification of FANCD2 appears to be crucial for its activity. Proficient monoubiquitination is easily recognized by the appearance of a larger FANCD2 isoform (FANCD2-L). Western blotting thus permits convenient subtyping of FA cell lines, since patients with defects in any of the core complex genes fail to monoubiquitinate FANCD2 and display only the small (FANCD2-S) isoform [52]. Activated, intact FANCD2 protein is targeted to discrete nuclear foci. The combination of proteins

comprising these foci varies with the cell cycle and the type of DNA damage. FANCD2 co-localizes in these foci with FANCD1/BRCA2 and RAD51 (proteins involved in error-free repair) or BRCA1 and RAD50 (proteins involved in error prone repair). FA foci are found in S-phase cells and after treatment with crosslinkers and ionizing radiation. Thus, the association with proteins involved in DNA repair indicates that the FA pathway is directly involved in the DNA-damage-response during S-phase [5, 9].

Following DNA damage, the FA core complex directly binds to chromatin, whereby the FANCM protein with its helicase motif seems to be essential for recognizing crosslinked DNA [53]. It has been speculated that the activated core complex stabilizes arrested replication forks blocked by crosslinks, thereby promoting repair via DNA double-strand-break intermediates, either by translesion synthesis or by homologous recombination. FANCD1/BRCA2, FANCI, and FANCN are involved in these repair processes and act downstream of FANCD2 in the FA pathway(s). Clearly, we are only just beginning to understand the complexity of FA mediated crosslink repair, and confirmation of theoretical models by biochemical studies is still very limited.

Moreover, it is still unknown how the high sensitivity of FA cells to chromosomal breakage by atmospheric oxygen is linked to the FA pathway [54]. Reducing oxygen tension in tissue culture incubators from 20% to 5% normalizes the cellular FA phenotype with respect to chromosomal breakage and G2-phase delay [55]. The molecular explanation of the obvious oxygen sensitivity of FA cells will represent another milestone in FA research. It also needs to be clarified whether, in the patients themselves, oxidative stress is a primary or secondary phenomenon, and to which extent oxidative stress contributes to the features of the FA clinical phenotype [56, 57].

What is the reason for the excessive apoptosis of bone marrow stem cells in FA patients resulting in hematopoietic failure? What is the functional relevance of the interaction of FANCC with STAT1, hsp70, NADPH cytochrome p450 reductase, FAZF, GRP94 and cdc2 [7], and in how many additional pathways are the other FA proteins involved? Clearly, the complexity of protein-protein interactions is bewildering and, in addition, depends on the specific stage of development and the individual tissue. The combination of reductionistic approaches with systems biology might lead to insight into these networks. It is safe to predict that the way to this goal will be paved with future milestones in FA research.

The Difficult but Promising Road to Therapy

Deeper insight into the molecular pathogenesis of FA will hopefully result in a better understanding of its pathophysiology, which is the prerequisite for a

rational, individualized therapy. From the patient's point of view, this would clearly be the ultimate milestone in FA research. Currently, there is no causal therapy for the genetic instability underlying the clinical manifestations of FA. In this situation, prenatal diagnosis is requested by couples at risk [58]. Both functional studies, e.g. determination of sensitivity towards DNA crosslinking agents, and direct FA gene mutation analysis can be performed with fetal cells. Although prenatal diagnosis is an important by-product of FA research, it merely represents a makeshift solution rather than a genuine therapeutic milestone.

A number of conventional treatments are available to combat bone marrow failure, including steroid and cytokine medications, but the first line of therapy is hematopoietic stem cell transplantation (HSCT), pioneered in FA among others by Elaine Gluckman and her coworkers [59]. As indicated graphically in figure 1, both the number of patients transplanted and the success of HSCT have greatly increased during recent years, mostly due to improvements of conditioning regimens and graft T-cell depletion [60]. Even with suitable donors HSCT still carries a relatively high risk. In this context, a key observation made in the laboratory of Heidemarie Neitzel has great impact on the difficult decision whether and when to subject a patient to transplantation. Neitzel and colleagues found that specific clonal chromosomal aberrations, most significantly gains of 3q, precede the onset of myelodysplasia and/or acute myelocytic leukemia [61]. Knowing whether a potentially devastating conversion to malignant cell growth is in the offing is an important step forward in the medical management of FA patients. In combination with the establishment of a rapid and sensitive FISH-assay [62], this has important consequences for early intervention and treatment of FA patients. Given a low a priori chance of 1 in 4, and given the small sizes of present day families, matching sibling donors are available in only a minority of affected families. In such a precarious situation, preimplantation genetic diagnosis (PGD) for the selection of an HLA identical embryo as potential stem cell donor has been proposed and performed [63, 64]. The first case involving an FA family made headlines worldwide, both positive and negative ('designer baby'). Clearly, PGD is neither a desirable milestone in FA research nor a breakthrough for patient care but rather an expression of the desperate situation of parents faced with limited therapeutical possibilities. Both the economical and psychological burden of PGD are tremendous and the success rate is, for biological reasons, very low such that PGD is unlikely to have a major impact on the medical care of FA families.

Despite considerable efforts, gene therapy is still at the experimental stage [65]. Nonetheless, in a significant proportion of FA patients a kind of 'natural gene therapy' can be observed. This is based on the observation that some patients have two sets of peripheral blood cells: MMC sensitive and

MMC insensitive [66, 67]. In some cases, the wildtype cells completely replace the defective ones. Skin fibroblasts, however, remain MMC sensitive. This self-correction, which ideally should take place in a hematopoietic stem cell [68] may more often than not lead to improvement of the hematological status [67, 69, 70]. In recessive diseases, such as FA, different molecular mechanisms have been shown to be instrumental in somatic reversion, including intragenic recombination, gene conversion, or back-mutation. In addition, partial restoration of protein function can be due to compensating mutations *in cis* of one of the affected alleles [66, 71]. The elucidation of these mechanisms can surely be considered a milestone in genetic research. A dogma of classical genetics was that recombination occurred only between genes but not within a gene. In the fifties of the last century, this dogma was rejected for prokaryotes and fungi, in the sixties, for *Drosophila*, and, in the nineties, for our species. Intragenic recombination is highly increased in compound heterozygotes with the chromosomal instability syndrome Bloom syndrome [72]. In FA the occurrence of reverted cells in peripheral blood indicates a likely proliferative advantage of reversed cells within the FA bone marrow. In addition, analysis of the clonal expansion of these stem or progenitor cells is expected to give insight into their proliferating potential, which is of relevance for somatic gene therapy in general. Unfortunately, in the murine FA knockout mouse models, hematopoiesis is almost unaffected and tumor incidence is not increased [73, 74]. Thus, with respect to the development of somatic gene therapy and the understanding of FA pathophysiology, these models are only of limited value.

The transition from the cellular to the (molecular) genetic approach about 15 years ago opened completely new perspectives for FA research and gave exciting new insights into basic problems in cell biology. We now know that the FA family of genes plays an important role in the maintenance of genomic stability, and thereby in the prevention of cancer and premature aging. This progress was accompanied and promoted by the foundation of FA patient support groups in many countries. Their goal is to catalyze and support scientific research on Fanconi anemia. In the US, under the guidance of Lynn and Dave Frohnmayer, the Fanconi Anemia Research Fund has raised considerable sums for FA research. In addition, the FARE, as it is known, has edited several brochures for FA patients and their physicians, provides newsletters and information on research projects, and organizes annual meetings both for scientists and FA families. Parallel to this development in the USA, Ralf Dietrich founded a patient support group in Germany with aims and activities similar to those of FARE. The enormous contribution of the Deutsche Fanconi-Anämie-Hilfe e.V. to progress in the understanding of FA can hardly be overstated. As scientists we greatly appreciate the support and motivation provided by the family

support groups. As such, each of these groups clearly represents a most welcome milestone in the common quest to improve the life expectancy and quality of life of FA patients.

Note Added in Proof

The elusive gene underlying complementation group FA-I (Levitus et al., 2004) has been identified as a paralog and binding partner of FANCD2, bringing the total number of identified FA genes to 13. FANCI was detected as an ATM/ATR kinase target protein required for resistance to mitomycin C. Like FANCD2, the newly detected FANCI protein is monoubiquitinated at a highly conserved lysine residue. In the chain of events leading to DNA crosslink repair, FANCI and FANCD2 appear to form an interdependent complex ('ID-complex') required for mutual ubiquitination, activation and association with chromatin (Smogorzewska et al., 2007).

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