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Review

# Fanconi anemia proteins and endogenous stresses

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ABSTRACT

Each of the thirteen identified Fanconi anemia (FA) genes is required for resistance to DNA interstrand crosslinking agents, such as mitomycin C, cisplatin, and melphalan. While these agents are excellent tools for understanding the function of FA proteins in DNA repair, it is uncertain whether a defect in the removal of DNA interstrand crosslinks (ICLs) is the basis for the pathophysiology of FA. For example, DNA interstrand crosslinking agents induce other types of DNA damage, in addition to ICLs. Further, other DNA-damaging agents, such as ionizing or ultraviolet radiation, activate the FA pathway, leading to monoubiquitination of FANCD2 and FANCI. Also, FA patients display congenital abnormalities, hematologic deficiencies, and a predisposition to cancer in the absence of an environmental source of ICLs that is external to cells. Here we consider potential sources of endogenous DNA damage, or endogenous stresses, to which FA proteins may respond. These include ICLs formed by products of lipid peroxidation, and other forms of oxidative DNA damage. FA proteins may also potentially respond to telomere shortening or replication stress. Defining these endogenous sources of DNA damage or stresses is critical for understanding the pathogenesis of deficiencies for FA proteins. We propose that FA proteins are centrally involved in the response to replication stress, including replication stress arising from oxidative DNA damage.


**Contents**

1. Introduction ..... 43
2. Reactive oxygen species and the generation of ICLs by products of lipid peroxidation ..... 44
   2.1. Intracellular reactive oxygen species, oxidative stress, and antioxidants ..... 44
   2.2. Endogenous ICLs can result from oxidative stress ..... 45
3. Fanconi anemia and oxidative DNA damage ..... 45
   3.1. Evidence suggesting a relationship between FA proteins and oxidative stress/oxidative DNA damage ..... 45
   3.2. The role of inflammatory ROS in FA leukemogenesis ..... 46
4. Telomeres and Fanconi anemia ..... 47
   4.1. Telomeres and activation of DNA damage responses ..... 47
   4.2. Telomeres, Fanconi anemia and FA proteins ..... 47
5. Replication stress and FA ..... 47
   5.1. Evidence for the potential relationship of FA proteins to DNA replication ..... 47
   5.2. ATR regulates FANCD2 monoubiquitination and the cellular response to replication stress ..... 47
   5.3. Potential functions of FA proteins at the replication fork ..... 48
6. Conclusion ..... 49
   Conflicts of interest ..... 49
   Acknowledgements ..... 50
   References ..... 50

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## 1. Introduction

At present, thirteen Fanconi anemia (FA) complementation groups and the corresponding genes have been identified (reviewed in [1,2]). Importantly, the shared clinical and cellular phenotypes of patients from each complementation group suggest that the encoded proteins may function in a biochemical pathway. This pathway is the FA–BRCA pathway, including the basic FA pathway that consists of the FA core complex (composed of FANC-A, B, C, E, F, G, L, and M) and its substrates for monoubiquitination, FANCD2 and FANCI [3–10]. FANCD2 monoubiquitination is induced by various types of DNA damage, including exposure to DNA interstrand crosslinking agents, ionizing radiation (IR), and ultraviolet radiation [3]. Replication stress, generated by treatment of cells with aphidicolin (APH) or hydroxyurea (HU), also induces FANCD2 monoubiquitination [11,12].

The FANCD1/BRCA2, FANCN/PALB2, and FANCJ/BRIP1 proteins are also components of the FA–BRCA pathway. Biallelic mutation of the corresponding genes leads to FA, while monoallelic mutation is associated with breast cancer susceptibility in the general population [13–16]. FANCD1/BRCA2, FANCN/PALB2, and FANCJ/BRIP1 are not required for FANCD2 monoubiquitination [17–19], and may therefore function downstream of the basic FA pathway.

Among the phenotypes that are characteristic of cells derived from FA patients, irrespective of the complementation group, is hypersensitivity to DNA interstrand crosslinks (ICLs) induced by agents such as mitomycin C (MMC), cisplatin, melphalan, and psoralen/UV-A [3,20,21]. ICLs block strand separation necessary for passage of the replication fork and for transcription, and are thereby highly deleterious to cells [22,23]. It should be noted, however, that DNA interstrand crosslinking agents also induce other types of DNA damage, including alkylation of bases and DNA intrastrand crosslinks [22,24] (Fig. 1). In fact, alkylation typically predominates over ICLs [25].

FA cells also display chromosome abnormalities, such as chromosome breakage and the formation of radial chromosomes, in the presence of diepoxybutane or MMC [26]. In fact, such chromosome instability is used in the diagnosis of Fanconi anemia ([27] and "Fanconi Anemia and its Diagnosis" Auerbach, this issue). Additionally, FA cells also accumulate in G2-M of the cell cycle in response to treatment with DNA interstrand crosslinking agents ([21,28,29] and "Genotype-Phenotype Correlations in Fanconi Anemia" Neveling et al., this issue). Together, these cellular phenotypes suggest that FA proteins may function in cellular responses to DNA damage.

While comparative studies have suggested that FA cells display a greater sensitivity to DNA interstrand crosslinking agents [20,21], there is evidence that FA cells may also be sensitive to other stresses. For example, there is conflicting evidence which suggests that FA cells may be hypersensitive to agents that induce oxidative DNA damage [21,30,31]. Further, deficiency of FA proteins is associated with sensitivity to formaldehyde, which crosslinks protein to DNA [32]. And it has been reported that some FA cells may show sensitivity to DNA monoalkylating agents [20]. Together, these results call into question whether FA proteins respond exclusively to ICLs.

Some human diseases, such as Xeroderma pigmentosum (XP), are caused, in part, by defective repair of lesions that clearly originate from environmental sources of DNA damage. XP patients display abnormal skin pigmentation and a greatly increased incidence of skin cancer that is associated with exposure to solar radiation [33,34]. In contrast, it is not clear that the phenotypes of Fanconi anemia patients derive from exposure to exogenous agents that induce ICLs. For example, a striking array of tissues and organs are potentially affected by congenital abnormalities ([35] and "Fanconi Anemia and its Diagnosis" Auerbach, this issue). Further, FA patients display an increased incidence of a wide variety of cancers, including leukemia and tumors of the head/neck, urogenital tract, digestive tract, lung, and brain [36]. An environmental agent that would affect this diverse spectrum of organs is not readily apparent. Thus, FA proteins may instead have a role in responding to sources of DNA damage or replication stress that are intrinsic to the cell. Identifying the roles of FA proteins in responding to endogenous stresses is critical as a foundation for understanding the pathophysiology of FA.

In this review, we will consider endogenous stresses which may activate the FA pathway and for which FA proteins may mediate a cellular response that results in increased cell survival or proliferation. Among these stresses, lipid peroxidation generates compounds that can generate ICLs (reviewed in [37,38]). Since FA cells are hypersensitive to ICLs, endogenous DNA interstrand crosslinking agents are obviously a relevant source of DNA damage. Oxidation of bases and sugar moieties is a major source of endogenous DNA damage [39] and is another stress to which FA cells may be sensitive. We will consider evidence for a function of FA proteins in responding to oxidative stress and to oxidative DNA damage. Additionally, telomeres shorten with proliferation and critically short telomeres are recognized as DNA double-strand breaks [40,41]. We will review evidence for increased telomere shortening in FA. Finally, FANCD2 is recruited to stalled replica-

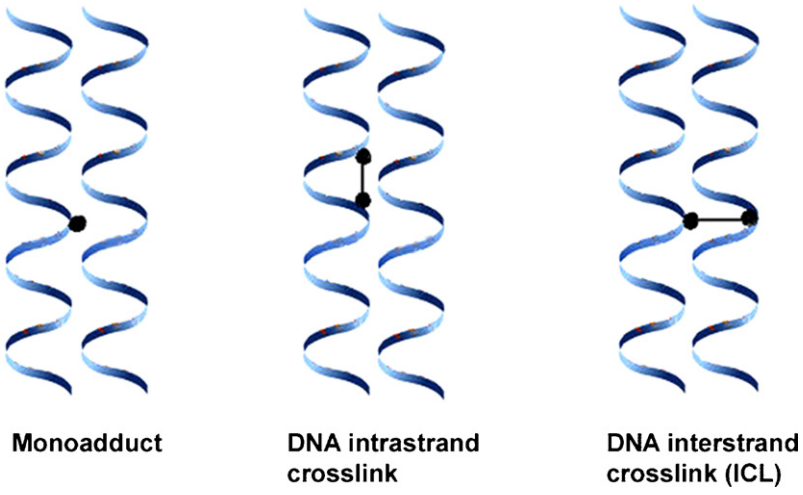


**Fig. 1.** DNA adducts formed by interstrand crosslinking agents. Bifunctional compounds can form monoadducts that affect a single nucleotide. Or these compounds can form adducts that affect two nucleotides in the same strand or two paired strands to generate DNA intrastrand and interstrand crosslinks, respectively. This figure was adapted from [187] with permission from Bentham Science Publishers.