What are the consequences of living in multilevel societies? Primates living in multilevel societies are characterized by pronounced sexual size dimorphism. Being large may increase a male's mating success when competition over access to females is frequent and intense. Primate males in multilevel societies also boast extravagant secondary sexual traits, such as the elongated nose of proboscis monkeys. In a crowded and anonymous social environment wherein traditional means of getting to know each other may not work, selection must have placed a premium on such amplified signals of individual identity, rank or attractiveness. The cognitive consequences of living in multilevel societies are largely unexplored. One would expect individuals to possess significant cognitive skill to navigate such a complex social landscape. One could also hypothesize that complex social organization translates into the need for more intricate social knowledge.

Where can I find out more?

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Quick guide

Fanconi anemia pathway

Alfredo Rodríguez[1,2] and Alan D'Andrea[1,*]

What is the Fanconi anemia (FA) pathway? The FA pathway is a biochemical network that assists in DNA repair, DNA replication and other cellular processes. During fork progression, nucleotide incorporation and movement of the replication fork can be impeded by several obstacles, including damaged bases within the DNA, DNA–protein complexes, DNA–RNA hybrids (R-loops) and certain DNA structures, such as fragile sites and G quadraplexes. The principal function ascribed to the FA pathway is the removal of a critical barrier, the DNA interstrand crosslink (ICL), which is known to interfere with DNA replication and genetic transcription. ICLs can have an exogenous origin, resulting from psoralen and cisplatin used in cancer chemotherapy, or from endogenous products, such as aldehydes and nitrous acid.

How is the FA pathway activated? The FA pathway is activated by ICLs during S phase (Figure 1A). ICLs are detected by the UHRF1 protein and the FANCM–MHF1–MHF2 complex, which respectively recruit the FANCD2-I heterodimer and the FA core complex to chromatin. The FA core complex is a ubiquitin E3 ligase composed of ten proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, FAAP100, FAAP20 and FAAP24) that, in conjunction with the UBE2T/FANCT E2 conjugating enzyme, monoubiquitylate FANCD2-I. The FA core complex is assembled from three subcomplexes: the BL100 subcomplex (FANCB, FANCL and FAAP100), which guides the assembly of the whole complex; the CEF subcomplex (FANCC, FANCE and FANCF), which forms a bridge for the interaction between the FA core complex and its target FANCD2-I; and the AG20 subcomplex (FANCA, FANCG and FAAP20), which is not critical for the assembly and ubiquitin

ligase activity of the FA complex but is required for its localization to the nucleus. In the presence of ICLs, the RAD18 ubiquitin ligase ubiquitylates PCNA, the DNA polymerase loading clamp, and facilitates the recruitment of FANCD2-I to the chromatin for ubiquitylation.

What happens after ICL detection? After their detection, ICLs are repaired by the FA pathway. Ubiquitylated FANCD2-I recruits SLX4/FANCP, a scaffolding protein for the DNA endonucleases MUS81, SLX1 and XPF/ERCC4/FANCQ. These endonucleases cleave the DNA strand contiguous to the ICL and generate a DNA adduct and an ICL-derived double-strand break. The DNA adduct is bypassed by the REV1, REV7/FANCV and REV3 translesion synthesis complex, whereas the ICL-derived double-strand break is repaired by homologous recombination. Although deubiquitylation of the FANCD2-I complex by USP1–UAF1 is required for the correct functioning of the FA pathway, the exact timing of this deubiquitylation is currently unknown.

The FA pathway ensures fidelity in the repair of the ICL-derived double-strand break by blocking the error-prone non-homologous end-joining pathway and funneling the ICL-derived double-strand break to FA-pathway-dependent homologous recombination repair. In this high fidelity pathway, the double-strand break is resected by the DNA exonucleases CtIP, MRN, and EXO1, thereby generating a 3' single-stranded DNA overhang that is initially coated by replication protein A (RPA). Once the RAD51/FANCR recombinase recognizes the RPA-covered single-stranded DNA, it promotes the eviction of RPA from the 3' overhang and stimulates the formation of a recombination filament, aided by the BRCA2/FANCD1, FANCN/PALB2, RAD51C/FANCO, BRIP1/BACH1/FANCJ, and XRCC1/FANCU proteins. The recombination filament performs homology search for the final repair process. Importantly, detection of ICLs leads not only to this DNA repair process but also to activation of the ATR–CHK1 cell-cycle checkpoint, thereby slowing DNA replication and allowing repair to occur.

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How about other roles for the FA pathway? The FA pathway is also involved in replication fork stability. DNA damage can arise during DNA synthesis due to replication fork slowing/stalling, which exposes nascent DNA strands to degradation by DNA exonucleases, such as MRE11. However, these nascent DNA strands can be protected by several components of the FA pathway, including the FA core complex, monoubiquitinated FANCD2-I, FANCO/RAD51, FANCS/BRCA1 and FANCD1/BRCA2. FANCD2, for example, prevents the accumulation of single-stranded DNA and pathological replication intermediaries, binds nascent DNA and restrains the replication fork. FANCI, on the other hand, has been shown to activate dormant origin firing in response to low levels of replication stress, thereby guaranteeing that DNA replication is completed in a timely manner. However, FANCI cannot promote dormant origin firing if the replication stress is severe, in which case the full FA pathway is activated (Figure 1B,D).

Another source of endogenous fork stalling is R-loops (Figure 1C). In the presence of these DNA–RNA hybrids, the FA pathway stabilizes the replication fork and, in association with RNAse H1, provides enzymatic activity to resolve the hybrid. Interestingly, an increase in the number of R-loops has been observed after cellular treatment with formaldehyde or mitomycin C. In this context, the FA pathway has an important role in coordinating replication and transcription.

The FA pathway has also been linked with telomere length maintenance. Telomeres are nucleoprotein structures that cap the ends of linear chromosomes and consist of the tandem repeat 5'-TTAGGG-3' together with associated proteins. Telomeric sequences shorten after every cell division. This shortening can be compensated for by human telomerase. Another mechanism, known as alternative lengthening of telomeres (ALT), maintains telomere length through homologous recombination in ~15% of human tumors, and this ALT mechanism appears to require FA proteins.
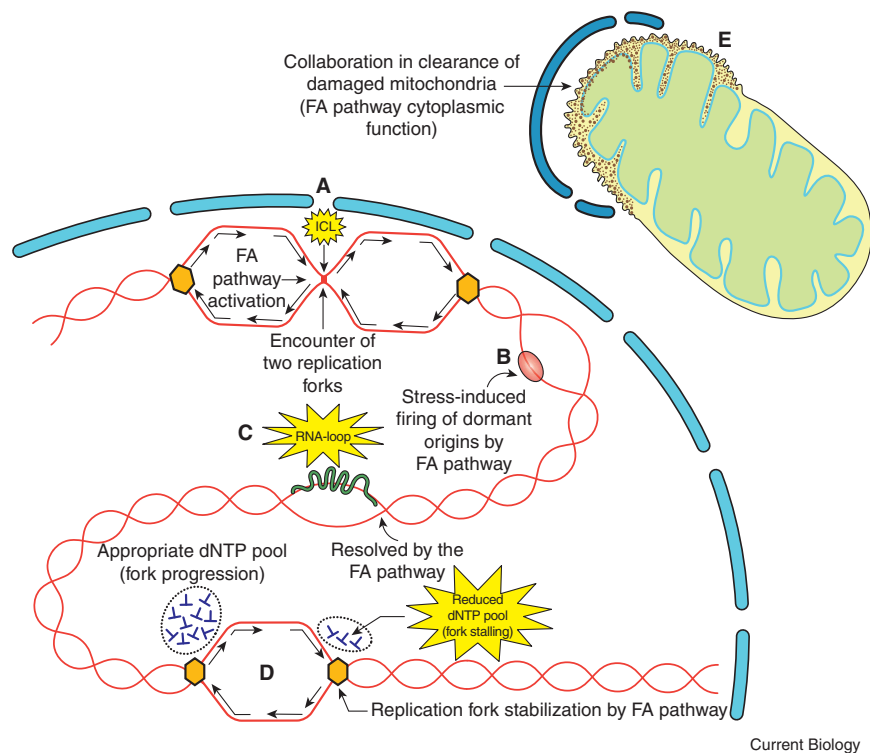

Figure 1. The Fanconi anemia pathway executes several activities related to the maintenance of DNA integrity.
(A) The initial activity ascribed to the FA pathway has been the detection and repair of the DNA ICLs that hold together both DNA strands and impede DNA replication. (B) In response to replicative stress, some FA proteins collaborate in the activation and firing of dormant origins of replication to counteract the replicative slow-down. (C) FA proteins collaborate in the elimination of RNA–DNA hybrids known as R-loops: these structures stall DNA replication forks and, if wrongly repaired, increase genomic instability. (D) Fork stalling can occur after dNTP pool depletion: in such a scenario, FA proteins stabilize the replication fork and impede its degradation by DNA exonucleases. (E) In addition to their nuclear activity, FA proteins might collaborate in the clearance of damaged mitochondria (mitophagy): in this manner, FA proteins might prevent the accumulation of reactive oxygen species and their potential induction of DNA damage.

In addition, the FA pathway is involved in cytokinesis. Under certain conditions, ultra-fine DNA bridges can be observed during cytokinesis as delicate strings that link condensed chromosomes in both emerging nuclei. These bridges appear when DNA replication occurs under exposure to hydroxyurea, mitomycin C or aphidicolin, and reflect the presence of under-replicated DNA in early mitotic cells. Since the FA pathway copes with replication stress, it prevents the appearance of these DNA bridges and becomes necessary for successful cytokinesis. Inhibiting the formation of these bridges is essential for genomic stability, given that they can lead to chromosome breakage and to the formation of binucleated cells as a result of interfering with cell division (i.e. cytokinesis failure).

A role for the FA pathway has recently been described in specific cases of autophagy, such as selective mitophagy (Figure 1E). FANCA, FANCF, FANCC, FANCL, FANCD2, FANCS/BRCA1 and FANCD1/BRCA2 proteins appear to contribute to the clearance of damaged mitochondria in a role that is totally independent of their function in nuclear DNA damage repair. Although the effects on mitophagy may be indirect, they suggest that the FA pathway might have a cytoplasmic role. The accumulation of damaged mitochondria could cause the accumulation of reactive oxygen species and the production of inflammatory cytokines, such as

tumor necrosis factor α (TNFα) and interleukin 1β, both of which are elevated in FA.

What happens when the pathway is lost? Biallelic germline mutations in the *FANCA*, *FANCB*, *FANCC*, *BRCA2/FANCD1, FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *BRIP1/FANCJ*, *FANCL*, *PALB2/FANCN*, *SLX4/FANCP*, *XPF/FANCQ* and *UBE2T/FANCT* genes cause bona fide FA, the most frequently inherited bone marrow failure syndrome. FA is characterized by hypersensitivity to ICL-inducing agents, predisposition to myelodysplastic syndrome/acute myeloid leukemia, solid tumors and congenital malformations, including absent radii, and cardiac and renal malformations (~30% of FA individuals might have subtle or absent physical anomalies). Mutations in *FANCA*, *FANCC* and *FANCG* account for approximately 90% of all reported FA cases. Mutations in *BRCA1/FANCS, RAD51/FANCR, RAD51C/FANCO and XRCC2/FANCU* genes cause a FA-like syndrome, without bone marrow failure or leukemia predisposition. In addition, an increased risk to breast and ovarian familial cancer has been observed in the carriers of monoallelic mutations in the *FANCD1/BRCA2, FANCS/BRCA1, FANCJ/BRIP1, FANCM, FANCN/PALB2* and *FANCO/RAD51C* genes. Finally, a sizeable fraction of high-grade serous ovarian cancer, triple-negative breast cancer, and metastatic prostate cancer have been shown to have alterations in the FA pathway.

There is a consensus that the bone marrow failure in FA patients is caused by attrition of the hematopoietic stem and progenitor cell compartment, which has been mainly attributed to DNA damage accumulation and activation of apoptosis. However, a pro-inflammatory bone marrow environment is also detected in FA, which could be due to defects in mitophagy mediated by the FA pathway. In this scenario, pro-inflammatory TNFα may contribute to apoptosis of FA hematopoietic stem cells through activation of the extrinsic apoptotic pathway.

What are the current treatments for FA? Currently, androgen therapy and transplantation of hematopoietic stem cells from allogeneic-matched donors remain the best options for treatment of the FA-associated hematologic manifestations. Unfortunately, androgen therapy is not effective in the long term and has been associated with liver malignancies. Promising recent work is focusing on *ex vivo* hematopoietic stem cell gene therapy and re-introduction of the corrected cells into the FA patient. Pharmacological interventions also seem feasible, such as the use of metformin or inhibitors of the TGF-β pathway. TGF-β inhibition has been shown to restore DNA damage repair and facilitate improved survival of FA cells.

Where can I find out more?
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Primer

Spermatogenesis

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Most organisms consist of two cell lineages — somatic cells and germ cells. The former are required for the current generation, and the latter create offspring. Male and female germ cells are usually produced during spermatogenesis and oogenesis, which take place in the testis and the ovary, respectively.

Spermatogenesis involves the differentiation of spermatogonial stem cells into spermatocytes via mitotic cell division and the production of haploid spermatids from the tetraploid primary spermatocytes via meiotic cell division. Spermatids subsequently give rise to spermatozoa in the final phase of spermatogenesis, called spermiogenesis. These fundamental steps, where mitotic proliferation precedes meiosis during spermatogenesis, are observed in a wide variety of organisms. However, developing a comprehensive understanding of the cell biology and genetics of spermatogenesis is difficult for most species because it occurs within a complex testicular environment characterized by the intimate association of developing sperm with accessory cells. In this Primer, we summarize the processes of spermatogenesis occurring in two pivotal model animals — mouse and *Caenorhabditis elegans* — and compare them to consider which important features might be evolutionarily conserved.

Mouse spermatogenesis
In the mouse testis, germ cells differentiate into mature spermatozoa within seminiferous tubules, which are highly organized, complicated structures. As shown in Figure 1, seminiferous tubules contain germ cells at many different developmental stages and in intimate association with somatic Sertoli cells. A basement membrane and myoid cells surround the seminiferous tubules, and Leydig cells and blood vessels are found in the interstitium.