Cellular function of the Fanconi anemia pathway

To the editor—Fanconi anemia (FA) cells have a well-known sensitivity to oxygen[1–3], although the molecular basis of this sensitivity remains unknown. The article by Cumming *et al.*[4] in the July 2001 issue of *Nature Medicine* proposes that one of the six cloned FA proteins, FA group C protein (FANCC), regulates glutathione S-transferase P1-1 (GSTP1), a cytoplasmic enzyme involved in detoxifying reactive oxygen species (ROS) and by-products of oxidative stress. The authors show the following: 1) a GST–FANCC fusion protein interacts with GSTP1; 2) ectopic overexpression of human FANCC and human GSTP1 in a murine IL-3–dependent cell line (expressing endogenous murine FANCC and GSTP1) protects these transfected cells from apoptosis during cytokine withdrawal; and 3) overexpressed FANCC increases GSTP1 activity. Although no genetic evidence is provided to support this interaction, the study suggests a regulatory role of FANCC in the detoxification of ROS and electrophilic metabolites in the cytoplasm.

Although this model is attractive, its validity depends on the localization of endogenous FANCC to the cytoplasm. There is considerable disagreement on this issue. Some studies[5,6], using overexpression of FANCC in transfected cells, suggest a cytoplasmic localization, whereas other studies[7–9], examining endogenous FANCC, localize the protein to the nucleus. Moreover, the functional form of FANCC appears to be nuclear[10].

I wish to propose an alternative model for the ROS sensitivity of FA cells, based on the localization of FANCC to the nucleus. Many studies have demonstrated that FANCC is a subunit of a nuclear complex containing several other cloned FA proteins (A, E, F and G)[7–9,11]. In response to DNA damage, this FA protein complex becomes 'activated', leading to the monoubiquitination of the downstream FANCD2 (the Fanconi anemia subtype D2 protein) and its targeting to DNA repair foci, including the BRCA1 (the breast cancer susceptibility protein)[12,13]. In FA-C (Fanconi anemia subtype C cells), which lack functional FANCC, the nuclear FA complex does not assemble properly[8] and the FANCD2 protein is not activated, leading to a defect in DNA repair[13]. Moreover, ROS generated by hydrogen peroxide can activate the monoubiquitination of FANCD2 in normal cells, but does not activate the FA pathway in cells from FA complementation groups A, C, F or G (unpublished observation). Hence, FA cells are sensitive to ROS, not because of a failure to detoxify ROS, but because of a failure to respond to ROS-mediated DNA damage.

Additional studies will be required to distinguish between these two models. For instance, the measurement of intracellular ROS levels and oxidative DNA damage in FA cells versus functionally complemented FA cells may determine whether the FA defect occurs before or after DNA repair.

Finally, FANCC may have two discrete functions: one as a cytoplasmic regulator of GSTP1 and one as a subunit of a nuclear complex regulating DNA repair. However, this seems unlikely based on genetic arguments. Disruption of the *Fancc* gene in mice[14,15] generates a cellular and organismal phenotype which is indistinguishable from genetic disruption of the *Fanca* (ref. 16) or *Fancg* (ref. 17) gene. Accordingly, FANCC appears to function primarily as a critical subunit of the nuclear FA protein complex (A,C,E,F,G complex) and is less likely to have additional cellular functions outside of this pathway.

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Cumming and Buchwald reply—D'Andrea raises some concerns over our recent report characterizing the role of the FANCC protein as a redox regulator of GSTP1 (ref. 4). His first concern relates to the use of ectopic overexpression of FANCC and GSTP1 in addition to the lack of genetic evidence to support a FANCC–GSTP1 interaction. In our report we clearly showed by co-immunoprecipitation analysis an interaction between endogenous FANCC and GSTP1 in a normal human lymphoblast cell line. Moreover, this interaction was not detected in a lymphoblast cell line derived from an FA-C patient, which contains mutations in *FANCC* and as a result does not express a full-length FANCC polypeptide. However, the FANCC–GSTP1 interaction was restored in the FA-C cell line following transduction with a retroviral vector that allowed expression of wild-type FANCC at a level comparable to that found in the normal cell line.

Secondly, D'Andrea challenges the validity of our model based on his proposal that FANCC localizes and functions exclusively in the nucleus. Several studies have clearly demonstrated that FANCC interacts with other FA proteins, including FANCA, FANCE, FANCF and FANCG, in the nucleus to form the FA protein complex[7,8,18]. The formation of this nuclear complex seems to be necessary for the downstream monoubiquitination of FANCD2 and for the correction of the DNA cross-linker hypersensitivity phenotype of FA cells[13]. However, multiple studies have shown that abundant levels of endogenous FANCC are found both in the cytoplasm and in the nucleus[7,8,19,20]. Moreover, several studies besides ours have shown that FANCC physically and functionally interacts with a number of cytoplasmic proteins, including NADPH cytochrome P-450 reductase, signal transducer and activator of transcription 1 and Hsp70 (refs. 4,21,22). Moreover, a structure-function study in which three highly conserved motifs of FANCC were mutated revealed that the mutants complemented the DNA cross-linker hypersensitivity phenotype and corrected the aberrant posttranslational activation of FANCD2, but did not correct STAT1 activation in FA-C cells[23]. These findings indicate that FANCC is multifunctional and that separate structural domains exist for the nuclear damage function and the cytoplasmic anti-apoptotic functions.

Finally, D'Andrea states that our dual function model of FANCC as a redox regulator of cytoplasmic proteins and a component of a complex for DNA-repair signaling is unlikely based on genetic evidence from mice with targeted disruptions of *Fanca*, *Fancc* or *Fancg*. Although all of these mice demonstrate DNA cross-linker hypersensitivity, they did not exhibit spontaneous hematological defects, a key feature of the human disease. However, mice with combined disruptions of the cytoplas-