mat region. The spreading reaction is mediated in part by Swi6 (ref. 5), and Noma *et al.*⁸ now show that it involves RITS as well.

Their interpretations are based on several observations. First, they found that a mutation in the chromodomain of Chp1 abolishes RITS binding to chromatin. They also found a loss of RITS binding in *clr4*⁻ mutants, which lack methylated H3-Lys9 binding sites for RITS. In addition, they found that RITS spreads in a Swi6-dependent manner. These data support the view that Swi6 mediates the recruitment of Clr4, which provides the necessary H3-Lys9 binding sites for RITS. The authors note that siRNAs and Dcr1 are not required for spreading or subsequent maintenance of RITS at heterochromatic loci. Instead, the Chp1 chromodomain is primarily responsible for the affinity of RITS for silent chromatin. The sequence-independence of this spreading reaction presumably allows RITS to exert control over sequences incapable of initiating the RNAi response.

Enforcing the silence

Drawing on the analogy of RNAi as a genetic immune response⁹, several authors have hypothesized that RNAi might guide chromatin-silencing complexes back to the gene that produced the dsRNA^{3–5,7}. Further, if components of the RNAi machinery remain bound to transcriptionally silenced loci, rare transcripts that escape silencing could be rec-

ognized and destroyed before transcription is completed. In a key set of experiments, Noma *et al.* show that the free, chromatin-unbound form of RITS does not associated with siRNAs (with the caveat that small quantities of siRNAs might have been missed). When bound to chromatin through H3-Lys9, however, RITS seems to become especially receptive to siRNAs. RITS may acquire its siRNAs from post-transcriptional (*trans*-acting) RNAi activity or more locally from aberrant RNAs processed at the source of transcription. Once bound to chromatin and primed with siRNAs, RITS probably becomes the center of localized RNA destruction, ensuring that stray transcripts from silent chromatin are cleaved in *cis* (presumably by Ago1)⁶. Thus, although siRNAs are not necessarily required to target RITS to specific chromatin sites, siRNAs and RITS both seem to be involved in the predicted *cis*-acting RNAi function (Fig. 1).

Initiating events

The authors also discuss briefly the issue of what factors might be involved in the earliest events of Dcr1-dependent, sequence-specific heterochromatin formation. It is important to incorporate Dcr1 and siRNAs into any model for the initiation of silencing, because the genetic data for this at pericentromeres and other loci and quite good^{4,10}. Although silencing at *mat* is genetically more complex

than at pericentromeres, there are good indications that the two silent domains are regulated in a similar manner. Aside from the facts that pericentromeres and *mat* contain nearly identical sequences and bind the same set of proteins^{4,5}, a *dcr1*⁻ *swi6*⁻ double mutant fails to initiate and maintain *mat* silencing⁸. Given that *swi6*⁻ alone does not have this effect⁵, siRNAs are probably required in some form to designate *mat* as a silent domain.

The discovery that RITS binds efficiently to chromatin without siRNAs⁸ puts a damper on early predictions that RITS might serve as the primary initiator of chromatin silencing. An alternate possibility is that a larger silencing complex of some sort⁸ interacts with Clr4 (ref. 10). However this question is ultimately resolved, it's a good bet that the answer will come from *S. pombe*, where the combination of genetics and biochemistry has been very productive.

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A new gene on the X involved in Fanconi anemia

Nazneen Rahman & Alan Ashworth

A new study shows that Fanconi anemia complementation group B is caused by mutations in a previously uncharacterized gene located on the X chromosome. Its unique chromosomal localization identifies *FANCB* as a potential weak link in a key DNA-repair and tumor-suppressor pathway.

There are few better examples of the proposition that studying rare genetic disorders can illuminate important biological pathways than research into Fanconi anemia, which has identified several genes important for DNA repair. Fanconi anemia is characterized by congenital

defects, progressive bone marrow failure and cancer susceptibility¹. Its distinctive cellular phenotype involves chromosomal instability and hypersensitivity to DNA cross-linking agents. Eleven distinct complementation groups for Fanconi anemia have been defined using somatic cell fusion techniques, and the genes underlying eight of these complementation groups have been identified. Now, Meetei *et al.*² on page 1219 describe an X-linked gene defective in individuals with Fanconi anemia complementation group B (FA-B). Because of its unique chromosomal location, the gene encoding this newly discovered component of the Fanconi anemia complex may represent a

particularly vulnerable component of the DNA repair pathway responsible for maintaining genomic integrity.

Six of the proteins involved in Fanconi anemia (FANCA, FANCC, FANCE, FANCF, FANCG and FANCL) are part of a protein complex that functions as a ubiquitin ligase, of which FANCL is probably the catalytic subunit³. In response to DNA damage or in S phase of the cell cycle, this complex catalyzes the monoubiquitination of FANCD2 (Fig. 1). A defect in any one of these core proteins results in failure to monoubiquitinate FANCD2, emphasizing the importance of this modification. But exactly how this complex

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functions in DNA repair is not known. An eighth gene involved in Fanconi anemia, corresponding to complementation group D1, has been identified as the breast cancer susceptibility gene *BRCA2* (ref. 4). In *BRCA2*-defective cells, the core Fanconi anemia complex is intact and *FANCD2* is appropriately ubiquitinated, suggesting that *BRCA2* acts downstream of the other proteins involved in Fanconi anemia (Fig. 1).

From B to X

Meetei *et al.*² analyzed proteins associated with the core Fanconi anemia complex⁵ and identified a new component, FAAP95, encoded by a gene of unknown function. They then examined this protein more closely to see whether it is an important functional component of the complex. They found that knockdown of FAAP95 by short interfering RNA in cultured cells resulted in reduced ubiquitination of *FANCD2* and produced the sensitivity to DNA cross-linkers that is characteristic of Fanconi anemia. They also found that FAAP95, like other core proteins, localized to the nucleus under normal conditions but became cytoplasmic in cells defective in *FANCA*, another core component of the complex. Taken together, these data provided strong evidence that FAAP95 functions in the Fanconi anemia core complex and further suggested that mutations of FAAP95 might underlie some cases of the disease.

Because the gene encoding FAAP95 is located on the X chromosome, the authors suspected that mutations in this gene might cause FA-B, which has only been observed in males. Notably, FA-B cell lines have defective *FANCD2* ubiquitination, which is also seen in response to FAAP95 depletion². The authors confirmed this suspicion by sequencing the gene from two FA-B cell lines and finding that both cell lines carried inactivating mutations in the gene. They then found that two other Fanconi anemia cell lines previously unassigned to complementation groups also carry mutations in this gene. They showed that a cDNA expressing FAAP95 was able to complement the sensitivity of FA-B cell lines to DNA cross-linkers, providing strong evidence that mutations in this gene cause the FA-B phenotype.

Before this study, Fanconi anemia was thought to be inherited exclusively in an autosomal recessive fashion. Thus, the location of *FANCB* on the X chromosome generates new issues in genetic counseling for the disease. Males who inherit a mutation in *FANCB* will be severely affected because they carry only a single copy of the gene. The situation is more complicated in females, as X-chromosome inactivation⁶ would be expected to result in

Figure 1 Schematic depiction of the Fanconi anemia DNA repair pathway. DNA damage activates the complex of Fanconi anemia-associated proteins *FANCA*, *FANCB*, *FANCD1*, *FANCD2*, *FANCG* and *FANCL*⁵. *FANCL*⁵ is a ubiquitin ligase that probably causes the ubiquitination of *FANCD2*. This protein, together with another Fanconi anemia-associated protein, *BRCA2* and *RAD51*, is involved in the repair of DNA damage.

half of the somatic cells of heterozygous individuals who carry the *FANCB* mutation expressing only the wild-type protein and the other half only the mutant form. Meetei *et al.* show, however, that blood cells and skin fibroblasts from female carriers express only the wild-type allele, presumably because of selection against cells lacking the wild-type protein². Nevertheless, some cells selectively expressing the mutant form might remain in particular tissues. Because these cells are likely to manifest genomic instability and be prone to transformation, this should be taken into account in the clinical management of females carrying the mutation in *FANCB*. Finally, it has been suggested that inactivation of genes involved in Fanconi anemia might have a role in sporadic cancer^{7–9}. Because *FANCB* is expected to be present as a single functional copy in most cells, it could potentially be inactivated by a single mutational event, distinct from the two-hit paradigm typical of most tumor-suppressor genes.

Case closed?

A previous study describing a truncating mutation in *BRCA2* in an FA-B cell line led to the suggestion that *FANCB* and *BRCA2* were the same gene⁴. This *BRCA2* mutation, 3033delAAAC, is predicted to result in loss of more than half the protein, including functionally important domains. Moreover, this same mutation confers breast cancer susceptibility in heterozygotes and is predicted to cause Fanconi anemia in biallelic carriers. This raises the puzzling question of why two potentially causative mutations should be present in a single individ-

ual. The first possibility is that it is due to chance. But apart from certain founder populations, the frequency of *BRCA2* mutations is only ~1 in 800 (ref. 10), requiring a somewhat extraordinary coincidence. Perhaps we need to consider the possibility, then, that the *BRCA2* mutation contributes to the phenotype in this FA-B case. Is there any evidence to support this? No two-generation FA-B family has ever been reported, though admittedly, only four families have been identified with *FANCB* mutations, and, therefore, this subtype may be very rare. Nevertheless, an affected uncle and nephew pair or affected male cousins would almost certainly be reported, and it may be that such families occur very rarely. One possibility for their absence is that mutations in additional genes are required for expression of the phenotype (e.g., *BRCA2* in the case of the reference FA-B cell line). Digenic predisposition has been described in other conditions¹¹ and is a plausible cause of some cases of Fanconi anemia. Thus, it remains possible that the case for *BRCA2* involvement in FA-B is not yet closed.

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