

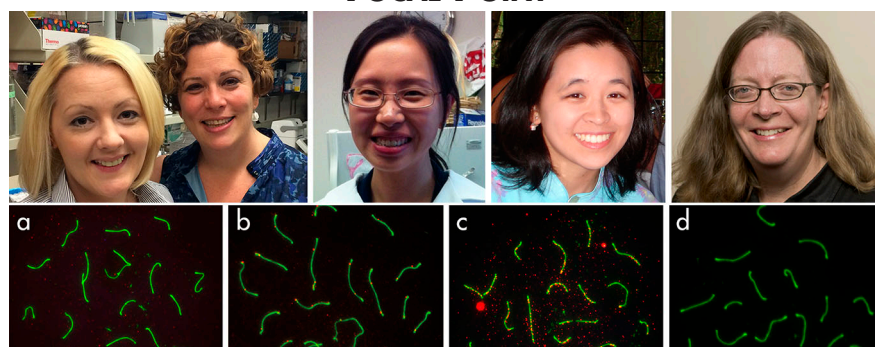
CNTD1's crossover act

The cyclin-related protein selects the sites of homologous recombination in meiotic cells.

Before meiotic divisions, chromosomes form crossovers, which represent the reciprocal exchanges between the DNA molecules of the homologous partner chromosomes. These crossovers aren't just important for reassorting genetic traits; they're essential for the correct alignment and segregation of meiotic chromosomes. To ensure that every homologous chromosome pair forms at least one crossover—and, perhaps, to aid homologues finding each other in the first place—meiotic cells generate a vast excess of DNA double-strand breaks (DSBs), most of which are then repaired by “noncrossover” recombination pathways that restore intact DNA molecules without resulting in a crossover. Holloway et al. reveal that a cyclin-related protein called CNTD1 helps determine which DSB sites mature into crossovers and which don't (1).

Mouse spermatocytes initially form around 200–300 DSBs, which are subsequently pared down to just 20–30 crossover sites. “Crossovers are placed in a specific manner,” explains Paula Cohen, from Cornell University in Ithaca, New York. “You have to have at least one on every chromosome pair, and, if there are two, they can't be too close together. So how does the cell orchestrate repair of all these DSBs at high fidelity, while ensuring that the breaks that become crossovers are distributed appropriately?”

Several proteins have been identified that help to specify which DSBs mature into crossovers. The MutS γ complex (consisting of the subunits MSH4 and MSH5) binds to around half of the initial break sites, and a subset of these subsequently recruit the SUMO ligase RNF212 (2, 3). Finally, the MutL γ complex (consisting of MLH1 and MLH3) binds to the 20–30 sites where mature crossovers form, and the excess MutS γ sites are eliminated (4). In 2012, Rayka Yokoo and Anne Villeneuve at Stanford University identified a cyclin-related protein in *C. elegans* named COSA-1 that is required to convert a subset of DSBs into mature crossovers and functions in conjunction with



(Left to right) Kim Holloway, Paula Cohen, Xianfei Sun, Rayka Yokoo, and Anne Villeneuve describe how the cyclin-related protein CNTD1 selects the sites of crossover formation during meiosis. Meiotic cells generate a large pool of potential crossover sites by introducing an excess of DNA double-strand breaks, only a few of which mature into crossover sites conjoining the DNA of homologous chromosomes. Chromosome spreads from late pachytene spermatocytes show that, in wild-type cells (panels a and b), precrossover sites marked by MSH4 (red, a) are largely absent and have been replaced by mature crossover sites containing MLH1 (red, b). In CNTD1-deficient spermatocytes (panels c and d), precrossover sites persist (red, c), and mature crossovers (red, d) are absent.

MutS γ (5). “Whereas the MutS γ complex is present in plants, animals, and fungi, COSA-1 orthologues are only found in animals. Thus, we felt that it was important to look at this protein in mice,” says Villeneuve. Yokoo and Villeneuve therefore collaborated with Cohen and her colleagues Kim Holloway and Xianfei Sun to examine the function of COSA-1's mouse orthologue, CNTD1 (1).

CNTD1-deficient mice were infertile; male animals, for example, were unable to produce spermatozoa. Meiotic spermatocytes lacking CNTD1 generated DSBs and paired up their homologous chromosomes as usual. But the cells failed to form mature crossover sites containing the MutL γ complex, indicating that, like its *C. elegans* orthologue, CNTD1 is required for crossover maturation. The absence of CNTD1 caused immature, precrossover sites containing MutS γ and RNF212 to persist throughout meiotic prophase I. But this wasn't simply due to the failure of crossover maturation: MutS γ foci were gradually eliminated in MutL γ -deficient spermatocytes unable to form mature crossovers. This suggests that mouse CNTD1 has an additional role in removing excess precrossover sites. “It promotes the maturation of some potential crossover sites and decommmissions others,” Villeneuve explains.

“CNTD1 dictates which sites become crossovers,” agrees Cohen. A recent study proposed a similar function for the E3 ubiquitin ligase HEI10 (6), and Holloway et al. think that CNTD1 works in conjunction with this enzyme. In late meiotic prophase, HEI10 specifically concentrates at designated crossover sites, but this localization is lost in the absence of CNTD1. Because CNTD1 contains a cyclin-like domain, the protein may also cooperate with a cyclin-dependent kinase such as CDK2, which also localizes to mature crossover sites in wild-type cells but not in CNTD1-deficient spermatocytes.

“The temporal order will be difficult to establish,” says Cohen. “But these decisions [surrounding crossover site selection] are probably driven by posttranslational modifications such as ubiquitination, SUMOylation, and phosphorylation.” The researchers now want to identify the targets of these posttranslational modifications and to investigate how cells sense that they have formed enough crossovers to proceed through meiosis and eliminate the excess pool of precrossover sites.

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