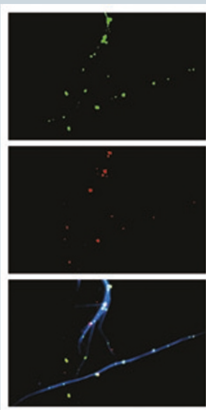
Inhibiting signals



Chromosome segregation is a highly regulated process in eukaryotes. At the center of this regulatory mechanism is the spindle checkpoint, which monitors chromosome attachment to the mitotic spindle. It generates signals that inhibit the onset of anaphase when a chromosome is not successfully attached to the spindle microtubule. One crucial event in the spindle checkpoint is the binding of Mad2 to unattached kinetochores, the multiprotein complexes at centromeres. In contrast, the way in which checkpoint signals are silenced upon chromosome-microtubule attachment is not well characterized. Previous studies showed that BubR1, a kinase essential for spindle checkpoint signaling in several organisms, is activated by direct interaction with CENP-E, a centromere-associated motor. CENP-E is involved in the capture and stabilization of spindle microtubules by the kinetochores. Cleveland and colleagues have now tested whether the capture of microtubule by CENP-E silences the checkpoint signals. They showed that, although other components of the kinetochore can mediate microtubule capture, a motorless CENP-E variant at the centromere produces a persistent checkpoint signal independent of microtubule capture by the kinetochore. Furthermore, this CENP-E variant cannot block recruitment of Mad2 to attached kinetochores. Purified CENP-E, BubR1 and assembled microtubules form a ternary complex *in vitro*, and this association inhibits the BubR1 kinase activity essential for the spindle checkpoint. The *in vitro* formation of the ternary complex and the inhibition of the BubR1 kinase activity require the CENP-E motor domain. Taken together, these results indicate that CENP-E directly links microtubule capture to the inhibition of the BubR1 kinase, thereby shutting off spindle checkpoint signaling. (*J. Cell Biol.* published online 6 September 2005, doi:10.1083/jcb.200505040) *HPF*

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Polymerase swap

DNA replication requires many proteins. At the replication fork, a protein complex called clamp loader positions a ring-shaped DNA-sliding clamp onto the leading and lagging DNA strands. This clamp acts as a tether for many proteins required for replication. For example, in *Escherichia coli*, two copies of the high-fidelity polymerase III (Pol III) core load onto the clamp on each strand and replicate the DNA. If Pol III encounters a damaged base on the leading strand, a low-fidelity polymerase such as Pol IV or Pol V replaces Pol III to bypass the lesion, allowing replication to resume. The mechanism by which the polymerases trade places on the clamp and DNA is not known. O'Donnell and colleagues now show that Pol III and Pol IV form a ternary complex with the clamp and pivot on its surface. During uninterrupted replication, Pol III retains control of the primed template DNA. However, if Pol III stalls, Pol IV gains controls of the DNA while both enzymes remain tethered to the clamp. Once stalling is relieved, Pol III quickly regains control of the DNA. This switching of enzymes ensures that the low-fidelity polymerase interacts with the DNA only when it is needed. The data do not explain how the swap between the two enzymes is triggered. The authors propose that the stalled enzyme may generate a conformational change in the clamp that results in the displacement of Pol III from the DNA, thereby providing access to the low-fidelity polymerase. Alternatively, Pol III may bind less tightly to the DNA when it is stalled and this may allow Pol IV to displace it from the DNA. Further studies are needed to elucidate the conformational changes that regulate competition between the two enzymes for the DNA. (*Mol. Cell* **19**, 805–815, 2005) *EJ*

Making sense of antisense

During transcription, the two strands of the duplex DNA are separated, and the sense strand is used as a template for transcription of messenger RNA (mRNA). Transcription of the opposite (antisense) strand can also occur, generating antisense RNA transcripts that can hybridize with DNA or with the corresponding sense RNA transcripts. The pairings with sense RNA might regulate gene expression by affecting transcription or mRNA degradation by RNA-mediated interference (RNAi). In a recent issue of *Science*, researchers from the RIKEN genomics groups and the FANTOM Consortium report on their analysis of the complete collection of coding and noncoding transcribed elements of the mouse genome (the 'transcriptome'). By computational analysis, they identified complementary RNA transcripts—potential sense-antisense (S-AS) pairs—and found that the percentage of S-AS pairs was much higher than previously thought: 30–40% of all transcripts may participate in these pairings. The group then monitored the effect of disturbing the expression of each member in a pair and found that, in general, expression of the two members of a pair was positively correlated. They also discovered that disrupting one member of an S-AS pair using RNAi could alter the transcription of the other partner, although not always in a negative way. This work provides new insight on S-AS pairs and lays the groundwork for future experiments to more fully explore the mechanistic details of the modulation of gene expression by these pairs. (*Science* **309**, 1564–1566, 2005) *DM*

Equalizing the sexes

In species where females have two copies of the X chromosome and males have only one, there is the potential for females to produce twice as much of X-linked gene products. However, an essential process called dosage compensation ensures that males and females express equal amounts of gene products regardless of the number of X chromosomes. In *Drosophila melanogaster*, two different models have been proposed for how dosage compensation is achieved. In the activation model, a male-specific-lethal (MSL) complex upregulates the transcription of X-linked genes two-fold in males to match the amounts generated in females. In contrast, the inverse dosage model proposes that MSL does not affect the X-linked genes but instead represses male autosomal gene expression to balance overall gene expression from all chromosomes. Now the Becker and Kuroda laboratories independently provide *in vivo* evidence in favor of the activation model for dosage compensation in flies. Both groups used RNAi to examine the effect of decreased MSL expression. They found that depletion of the MSL complex resulted in diminished expression from most X-linked genes but did not affect expression levels of autosomal genes. These findings indicate that in flies, MSL activates expression of X-linked genes in males. The question of how the MSL complex accomplishes this feat will require additional investigation. (*Genes Dev.* published online 1 October 2005, doi:10.1101/gad.1343105 and doi:10.1101/gad.1343705) *EJ*

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