observation raises several intriguing questions. First, how does Dicer-2 'know' which partner to choose when confronting exogenous and endogenous dsRNA. One possibility is that there is a subpopulation of Dicer-2, perhaps post-translationally modified, that specifically recruits Loquacious and is devoted to the esiRNA pathway. Further biochemical analysis may reveal whether this conjecture is correct.

A second mysterious question raised by the esiRNA pathway is, where in the cell does Dicer processing take place? In this regard, Dicers have been thought to be cytoplasmic enzymes. However, it seems highly unlikely

that the substrates for esiRNA production would be exported to the cytoplasm for processing. Surprisingly, none of the seven papers comments on this issue. In the absence of evidence to the contrary, it seems possible and perhaps likely that there may be a dedicated nuclear RNAi apparatus in animal cells. If this proves to be true, it raises the prospect that small RNAs could be involved in every level of gene expression in animals; perhaps we are not so different from plants after all.

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Damage control

Efficient repair of DNA damage is essential to all organisms. Cells must sense the damage and activate the appropriate signals to allow DNA to be repaired. If DNA damage is extensive, the cell might transiently delay the cell cycle to ensure the damage is repaired before the cell divides. In more extreme cases, cell cycle arrest may be permanent, resulting in senescence, or the cell may undergo cell death.

The DNA damage response (DDR) in mammalian cells comprises early damage sensors, such as the MRN complex (MRE11/Rad50/NBS1), that detects double-stranded breaks; mediator proteins, MDC1 and 53BP1, which are recruited by MRN; and activated kinases such as ATM that phosphorylate the histone variant H2AX. H2AX phosphorylation marks the chromatin at the site of DNA damage and serves as a signal to help recruit the DNA repair machinery in a complex cascade of events whose sequence and interdependencies have been difficult to define. Recently, two groups independently investigated the consequences of accumulating specific DDR proteins at a defined DNA site, and found that the DDR can be activated without DNA damage.

DDR proteins accumulate in distinct DNA repair foci at the sites of DNA lesions. To reconstruct DDR foci in the absence of DNA damage, both groups fused genes for DDR proteins to those encoding a fluorescent protein and the bacterial LacR repressor protein. The fusion proteins were then expressed in cells containing an array of repeats of the LacR target sequence, *lacO*, where they became immobilized to this DNA site, forming a focus that can be visualized by fluorescence microscopy. Adding IPTG causes the focus to disassemble, so one can tell the effects of overproduction of the protein from those due to its concentration on a chromatin region.

Soutoglou and Misteli (*Science*, published online 15 May 2008; doi:10.1126/science.1159051) found that tethering of NBS1 led to recruitment of MRE11 as well as MDC1 and 53BP1. Likewise, tethering of MRE11 led to accumulation of NBS1, MDC1 and 53BP1. Moreover, targeting of NBS1,

MRE11, MDC1 or ATM was sufficient to activate the DDR in mouse cells, as indicated by several phosphorylation marker events and cell cycle delay (left hand panel immobilization of a simple repair factor on chromatin (red), middle panel DDR activation is indicated by phosphorylation of H2AX (green), right hand panel colocalization). The fact that the DDR can be triggered without a DNA lesion indicates that although the lesion itself is important for triggering the response, it is not instrumental for subsequent steps of the DDR. The authors also

provided some insight into who recruits whom—as expected, they found that tethered upstream factors recruited downstream components, but they also noticed that downstream factors such as MDC1 could recruit upstream factors, pointing to a feedback loop that might be

important for amplification of the initial DDR signal. H2AX was required for the checkpoint response, indicating that chromatin modifications are important for full DDR activation.

Toczyski and colleagues (*Mol. Cell* **30**, 267–276) tethered a different set of DDR proteins to yeast chromatin, those recruited to single-stranded DNA overhangs formed during processing of double-stranded breaks: the damage-specific DNA clamp, called the 9-1-1 complex, and the complex Mec1–Ddc2. Mec1 is the yeast ortholog of the mammalian ATR. It phosphorylates the adaptor molecule Rad9 (an ortholog of 53BP1) at the site of DNA damage, leading to recruitment and activation of the checkpoint kinase Rad53 (a Chk2 ortholog). Tethering of both the 9-1-1 and the Mec1–Ddc2 complexes to a chromatin site could activate the checkpoint responses, with Rad53 phosphorylation and downstream events including cell cycle arrest occurring in the absence of DNA damage. Specific chromatin modifications were required for efficient checkpoint signaling.

Both these papers used an elegant and powerful technique to dissect the events involved in DNA damage signaling and checkpoint responses, and this approach may be useful in studying other protein complexes involved in DNA-dependent processes.

Inês Chen