Repair orders

A large and varied group of proteins accumulates at the site of double strand DNA breaks (DSBs). But just what role each plays in the assembly process and in what order they arrive has been unclear. Bekker-Jensen et al. clear up one controversy on page 201, demonstrating that Mdc1/NFBD1 precedes 53BP1’s arrival and is required for 53BP1’s stable association with the chromatin surrounding DSBs.

Mdc1 and 53BP1 are two of the earliest proteins to accumulate at DSBs, but recent studies reported conflicting data as to how the proteins influence each other’s binding at damaged chromosomes. To find out, Bekker-Jensen et al. followed the assembly process in real time. They used a micro-laser to generate DSBs in tissue culture cells and started time-lapse imaging immediately.

GFP-tagged 53BP1 and Mdc1 began to accumulate at the sites of double strand breaks within minutes of damage. As expected, 53BP1 protein that lacked the Tudor domain, which is required for interaction with dimethylated histone H3 (H3-dmK79), did not aggregate at the breaks. Simultaneous imaging of a mixed culture of cells expressing labeled 53BP1 or Mdc1 showed that Mdc1 arrived at DNA breaks before 53BP1. In cells depleted of Mdc1 by siRNA treatment, much less 53BP1 bound to the DNA breaks. The 53BP1 protein that did bind drifted away more rapidly than in cells with Mdc1, likely due to an absence of phosphorylation of histone H2AX (γ-H2AX), which was previously shown to be important for 53BP1 binding. In the reciprocal experiment, Mdc1 binding was unaffected by 53BP1 siRNA depletion.

Bekker-Jensen et al. propose that after the initial sensor, the Mre11-Nbs1-Rad50 complex, detects a double strand break, ATM launches a phosphorylation cascade, including phosphorylation of histone H2AX. Mdc1 binds to γ-H2AX, altering the chromatin structure and unmasking H3-dmK79, a constitutive histone modification. 53BP1 forms a stable interaction with the exposed H3-dmK79 and settles in at the damage. The stepwise building of the Mdc1-53BP1 platform includes changes in local chromatin architecture around the DSBs and is probably important for increasing the local concentration of repair proteins. JCB

Viruses catch a wave

Scientists know from looking at fixed cells that viruses frequently associate with filopodia and microvilli. Using video microscopy, Lehmann et al. (page 317) find that after a virus attaches to the filopodial membrane, it surfs down the plasma membrane toward the cell body. Entry into the cell occurs only after the virus has reached the base of the filopodium.

Retroviruses labeled with YFP initially contacted the filopodia of cells grown in culture. After a brief period of moving randomly on the filopodial surface, the viruses moved steadily in a retrograde fashion. Once the virus reached the cell body, the viral and host cell membranes fused, as detected by the diffusion of the fluorescently labeled envelope protein of the virus.

Vesicular stomatitis viruses, pH-dependent viruses that require endocytosis before membrane fusion, also surfed to the base of the filopodium, where they colocalized with clathrin.

Viral surfing did not occur in cells lacking the cognate viral receptor or those treated with cytochalasin D, but it was unaffected by nocodazole. Similarly, inhibition of myosin II with blebbistatin slowed viral movement and reduced infection by about fivefold.

The researchers hypothesize that viral entry only occurs at hot spots for endocytosis, which are concentrated at the base of the filopodium. And, they note, a similar observation was seen when quantum dots were used to label a ligand that binds to cell surface receptors (Lidke et al. 2004. Nat. Biotechnol. 22:198). Although the role of actin and myosin was not explored in that study, Lehmann et al. think that retrograde transport may be a common requirement for endocytosis of molecules or particles that initially bind on the filopodial surface. Moreover, the endocytic hot spots coincide with sites of actin remodeling, which may be necessary to allow signals to move rapidly through the otherwise highly structured ring of cortical actin. JCB