An early warning system for DNA damage

On page 245, Stokes and Michael show that when DNA damage is present, not only is the replication checkpoint system activated, but an additional system directly notifies replication forks of the problem. Previous work had identified two mechanisms of damage-induced replication arrest, the checkpoint system and a general slowing of fork progression, but the new work uncovers a third mechanism.

After observing that alkylation-damaged DNA induces a checkpoint-independent block to undamaged DNA replication in *Xenopus laevis* extracts, the authors found that the damaged DNA activates a diffusible inhibitor that stops new replication forks from progressing. To stop fork progression, the inhibitor must be present during a short window of time early in the assembly of prereplication complexes. Paradoxically, its effect is to prevent the binding of the processivity factor PCNA, a much later event in fork assembly. The inhibitor may work by blocking the binding of an unknown prereplication complex factor that is required for later PCNA binding.

Besides ensuring redundancy in the critical process of damage-induced replication arrest, the new system may also provide an earlier warning of trouble than the checkpoint, which responds to stalled replication forks in S-phase. By shutting down fork assembly even on undamaged sequences, the inhibitor would generate multiple checkpoint signals.

Laminin-2 on the brain

Myelination involves a vast spreading of cell membrane, but the mechanism underlying this is largely unknown. On page 397, Chun et al. show that laminin-2 induces cell spreading in oligodendrocytes, the myelinating cells of the CNS, by signaling through β1 integrins and integrin-linked kinase (ILK). They also resolve an apparent difference between mice and humans. In previous studies, humans with a defect in the laminin-2 gene have shown abnormalities in both the peripheral and central nervous systems, whereas laminin-2 mutant mice appeared to have only peripheral myelination defects. In the new study the authors show that, contrary to previous reports, myelination also fails in the central nervous system (CNS) of the mutant mice. Although the CNS of the mutant mice appears normal by conventional microscopy, electron microscopy clearly reveals myelination defects in several large clusters of small-diameter axons. Laminin-2 induces a cascade of events in differentiated oligodendrocytes in culture, acting through β1 integrins and PI3-kinase to stimulate integrin-linked kinase and promote the dramatic cell spreading characteristic of myelinating oligodendrocytes. Chun et al. also found high levels of integrin-linked kinase expression in CNS oligodendrocytes in vivo.

Since laminin-2 localizes to the surfaces of axons, its absence may prevent oligodendrocytes from spreading around certain CNS axons. In the areas of the laminin-2 mutant mouse CNS that are properly myelinated, other laminins may be able to substitute for laminin-2.

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A close look at cerebellar learning

The cerebellum handles many motor coordination and reflexive behaviors, and learned adaptations of these behaviors are thought to require synaptic long-term depression (LTD). On page 295, Feil et al. identify a signaling pathway essential for LTD in cerebellar Purkinje cells but, surprisingly, this pathway is required only for a subset of motor reflex adaptations.

Using a conditional gene knock-out approach, the authors disrupted the expression of cGMP-dependent protein kinase type I (cGKI) in Purkinje cells, which normally express high levels of the kinase. The disruption virtually abolishes cerebellar LTD in the mutant mice, but the animals perform normally in several tests of motor coordination. However, adaptation of the vestibulo-ocular reflex of the mutant mice, which keeps images stable on the retina during head movements, is defective.

Feil et al. propose that cGKI links nitric oxide and cGMP signaling to phosphatase inhibition and AMPA receptor endocytosis, leading to synaptic LTD and motor learning. The behavioral results imply that LTD is only required for specific forms of motor learning, though, and not for general motor coordination. The authors are now trying to test their molecular model by conditionally deleting other components of the proposed signaling pathway.

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Gross cerebellar structure is fine without LTD.

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Damaged DNA stalls new replication (right).

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ILK and Laminin-2 help oligodendrocytes to spread.