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Lesson #2: glutamate receptors mediate a diversity of brain functions under physiological conditions; thus, pharmacological blockade disrupts these normal functions, resulting in a diversity of unwanted effects including impaired learning and memory, psychosis, and others. This raises the important question as to what normal brain functions might be mediated by ASIC receptors. Interestingly, earlier work from the Welsh laboratory revealed defects in synaptic plasticity, learning and memory, and fear conditioning in *ASIC1a*$^{-/-}$ mice (Wemmie et al., 2002, 2003). This raises the possibility that neuroprotective drugs targeting ASIC1a must somehow selectively block this receptor under pathological conditions in order to be tolerated in humans.

While sobering, these practical concerns for developing neuroprotective therapies should not diminish from the impressive achievement of the authors: identification of a molecular mechanism by which acidosis contributes to neuronal destruction in ischemic brain tissue. Indeed their work moves "acidotoxicity" to the center stage in analyses of molecular mechanisms of ischemia.

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Watching the DNA Repair Ensemble Dance

Repair of damaged DNA is a dynamic process that requires careful orchestration of a multitude of enzymes, adaptor proteins, and chromatin constituents. In this issue of *Cell*, Lisby et al. (2004) provide a visual glimpse into how the diverse signaling and repair machines are organized in space and time around the deadliest genetic lesions—the DNA double-strand breaks.

All eukaryotic cells face a challenge of maintaining the integrity of their genome despite continuous exposure to environmental and metabolic insults that damage DNA. Some of these chemical and structural DNA modifications are particularly deadly. For instance, DNA double-strand breaks (DSBs) can destabilize the genome by promoting mutations and chromosomal rearrangements. This undermines cell viability and, on the organismal level, may lead to fatal diseases such as premature aging or cancer.

Given the danger of DSBs, it is not surprising that a large number of proteins, integrated into complex functional networks, cooperate to rapidly detect and eliminate them (Shiloh, 2003). But how is the multifaceted DSB response "choreographed" so that each molecular "dancer" involved knows when to arrive on the stage, how long and with whom to perform, and when to give way to those that are scheduled to follow? Amazingly, nature has provided cells with a score for a fascinating play called "DNA repair." Although we have known some of the "dancers" for quite awhile, only now are we actually beginning to see the performance unfold in front of our eyes.

In this issue of *Cell*, Michael Lisby, Rodney Rothstein, and their colleagues approach DSB repair from a truly challenging angle (Lisby et al., 2004). They study this complicated process in its physiological environment—the nucleus of a living cell. Indeed, the cell nucleus may represent the "ultimate test tube" to validate other analytical approaches and create workable models of how DSB response actually works. The intact nucleus supplies all determinants for the physiological pace of molecular interactions involved in DSB metabolism, and it allows the natural interplay of the focal DSB repair and signaling with other, "pan-nuclear" processes such as those delaying cell cycle progression or regulating gene expression. It is difficult to imagine that even a sophisticated in vitro approach could satisfy all these requirements.

Luckily, the utilization of the green fluorescent protein (GFP) has allowed unprecedented tracking of proteins in real time and in their natural locations. And indeed, the results of live-cell imaging have been gratifying, providing new mechanistic insights into complex biological processes. To sort out the order of events at DSBs in vivo, Lisby et al. combined the GFP technology with powerful genetic manipulations in the budding yeast *Saccharomyces cerevisiae*. They exchanged numerous genes involved in DSB response by their replicas fused to spectral variants of GFP, thereby preserving the endogenous expression control, and studied redistribution of now "visible" DSB regulators in live yeast cells exposed to ionizing radiation (IR, a known DSB inducer). One could argue that similar strategies were applied before (see, e.g., Melo et al., 2001), but what is so special about the new study is the *scale* on which Lisby and colleagues approached this task. The numerous yeast strains generated during this monumental project allowed imaging of the DSB response in an unprecedented breadth, from DSB recognition to homologous recombination (HR), the key mechanism to repair DSBs in yeast. And all this with an option to directly follow redistribution of the DSB regulators around the so-called "repair centers," the cytologically detectable nuclear

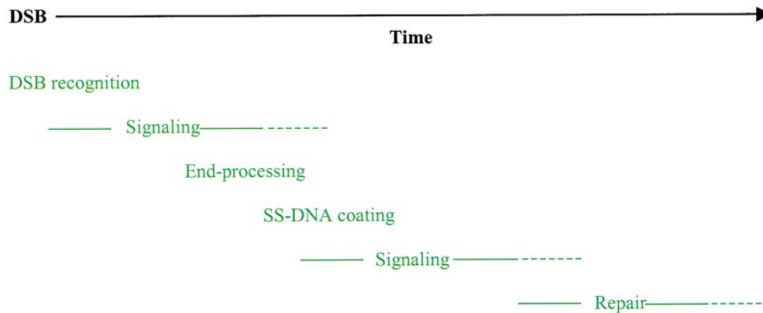

Figure 1. Temporal Order of Protein Assembly in DSB Repair Centers

The budding yeast proteins (in blue), their mammalian homologs (in red), and the main function of a protein or complex (in italics) are indicated. The corresponding order of the key biochemical processes elicited by DSBs is indicated below the arbitrary time axis (see Lisby et al., 2004 for the real time values for protein recruitment in *S. cerevisiae*). Asterisk indicates the switch between engagement of the Mre11/Rad50/Xrs2 and Rad52 group of HR proteins in the DSB repair centers. Whether interaction of the mammalian MRE11/RAD50/NBS1 complex and RAD52 group of proteins is also mutually exclusive and whether factors similar to the yeast Sae2 contribute to this process remain to be established. Question mark reflects the fact that mammalian cells likely evolved more DSB mediators compared to yeast; the indicated 53BP1 and MDC1 proteins are among the candidates to perform functions analogous to Rad9.

foci that integrate and repair DSBs (Lisby et al., 2003). The result is a fascinating insight into the "choreography" of the crucial life-saving, evolutionarily conserved process (Figure 1).

The first issue that this work helps clarify is the DSB recognition. By combined timelapse and genetic analyses the authors show that the first activity to arrive to DSBs is the MRX complex, a heterotrimeric assembly of Mre11, Rad50, and Xrs2 proteins. Although there is a hierarchy within the complex itself (Mre11 recruitment depends on Rad50), the recognition of DSBs by the assembled MRX does not require any other DSB regulator. Instead, MRX is required for recruitment of Tel1 (the proximal protein kinase activated by DSBs) and likely contributes to the initial nucleolytic resection of DSBs to generate single-stranded DNA (SS-DNA) critical for the subsequent protein assemblies. Thus, as predicted (Petrini and Stracker, 2003), MRX emerges as a true DSB sensor determining the timing and magnitude of all downstream processes. This seems to be a conserved feature of the DSB response, as mammalian NBS1 (Xrs2 homolog) is needed for proper activity of ATM (Tel1 homolog) (Bakkenist and Kastan, 2004), and NBS1 also recruits to DSBs astonishingly rapidly (Lukas et al., 2004).

The next step is marked by, and critically dependent on, the multicomponent RP-A complex that recognizes SS-DNA and thus arrives to the sites of DNA lesions several minutes after MRX. The coating of the SS-DNA by RP-A protects these unstable DNA intermediates and directs recruitment of the Mec1/Ddc2 kinase complex. The Mec1/Ddc2 activity is then required to recruit Rad9 mediator together with Rad53 kinase that further propagates the DSB signaling to diverse molecular effectors. Among other factors whose RP-A-dependent recruitment coincides with (but is independent of) Mec1/Ddc2 are Rad24 and the Ddc1/Mec3/Rad17 complex. All the above proteins persist in the repair foci for several hours, and although many features of these assemblies have been described before, the present study is valuable in placing them on a temporal scale relative to the upstream and downstream events (Figure 1).

Strikingly, the genuine DSB repair proteins are the latest to arrive. The assembly of the HR factors around DSBs is initiated by Rad52, which in turn determines recruitment of other HR components like Rad51, Rad55/Rad57, Rad54, Rdh54, and Rad59. Interestingly (and this is one of the novel findings of this study), the recruitment of HR machinery temporally coincides with the disassembly of the MRX foci (Figure 1). It even appears

that Rad52 recruitment terminates the "window of opportunity" for sensors such as MRX to interact (at least in a cytologically discernible manner) with the DSB lesions. Furthermore, the authors found that Sae2, a protein originally identified in processing DSBs in meiosis (Keeney and Kleckner, 1995), facilitates the Rad52-mediated displacement of MRX from DSBs. So, there seems to be a major switch in the DSB metabolism in terms of the accessibility of its sensor and repair components. Although the purpose of such a switch (and the role of Sae2) remains elusive, its identification paves the way to elucidate this key transition in DSB repair.

While Lisby et al. provide so far the most complete temporal framework of the DSB-associated events, their work (inevitably) also creates new challenges. For instance, it is shown that while MRX, RP-A, and several other factors could interact with the DSB repair centers throughout the cell cycle, the recruitment of the HR machinery is restricted to late S and G2 phase. This makes sense as HR requires sister chromatid templates for replacement of the DSBs. But what limits the access of Rad52 to the RP-A-coated SS-DNA during G1 and early S? Is there an active mechanism that prevents illegitimate (and therefore potentially harmful) recombination events before the sister chromatids become available? Or does DNA damage in late S/G2 generate hitherto unidentified DNA intermediates that cooperate with RP-A to productively recruit recombination and repair proteins?

Another unresolved issue is why cells tend to avoid concomitant presence of MRX and HR proteins in the repair centers. This study does not determine the dynamics of protein interaction with such centers, and in mammalian cells both RAD52 (Essers et al., 2002) and NBS1 (Lukas et al., 2003) undergo a dynamic exchange between the DSB sites and the neighboring nucleoplasm. If such phenomenon exists also in yeast, then why can MRX and the HR proteins not coexist at repair centers in a competitive fashion, as proposed in current models? Does this mean that the engagement of the HR machinery eliminates the structure(s) that the MRX complex recognizes in the first place? Regardless of the answer, the results in the present study caution against interpreting the MRX disappearance from the DSBs as a sign of a completed repair program.

Finally, recent studies of DSB-induced checkpoint events in live mammalian cells revealed that although proteins like Chk2 are firmly integrated in the DSB signaling, its residence time at the actual DSBs is so short that it never manifests itself as a cytologically detectable accumulation around DSBs (in other words, it does not form "foci") (Lukas et al., 2003). This observation sets a precedent that the microscopic appearance in the DSB repair centers is not the only criterion for an intimate involvement of a protein in DSB signaling and repair. It would be interesting to know whether such transient interaction modes exist also in yeast, and if so, what is their biological significance. On the other hand, other studies in mammalian cells showed that the sustained concentration of numerous DSB regulators requires phosphorylation of histone H2AX in vast regions surrounding the actual DSBs (Celeste et al., 2003). It would be illuminating to determine whether and how the choreography of the DSB repair described by Lisby et al. (2004) is modulated by epigenetic changes in the chromatin.

Live-cell imaging provides a new dimension to the knowledge generated by biochemistry and genetics and, as shown by Lisby and colleagues, "watching" proteins in their physiological habitat can also open new and surprising questions and identify new phenomena that would otherwise remain hidden in more conventional "test tubes."

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Wnt Signaling Went *derailed* Again: a New Track via the LIN-18 Receptor?

In this issue of *Cell*, Inoue et al. (2004) reports that LIN-18, an atypical receptor tyrosine kinase related to mammalian Ryk and *Drosophila* Derailed, mediates Wnt signaling in parallel to LIN-17/Frizzled (Fz) during worm vulval development. LIN-18/Ryk and LIN-17/Fz appear to exhibit distinct Wnt specificity, and surprisingly, the LIN-18 intracellular domain may be dispensable.

Wnt proteins are secreted signaling molecules critical for animal development. While Fz serpentine receptors have been established as Wnt receptors, other transmembrane receptors are beginning to share the lime-