sequent training (which cannot occur at the shorter spacing intervals). This proposal underscores the importance of the generation of discrete waves of MAPK activity with each trial.

In summary, the authors have demonstrated the importance of activation and inactivation of MAPK in the first 2 (out of 10) training trials leading to long-term memory formation in flies. To explore this model further, it will now be important to test whether a similar on-off switch for MAPK activity occurs across trials 3–10 in normal flies, and in gain-of-function *corkscrew* mutant flies trained using the 40 min interval protocol. If these predictions are confirmed, this significantly advances our understanding of the spacing effect, as it indicates that it is not only the activation kinetics of MAPK signaling that determine the optimal spacing of training sessions, but also the generation of discrete waves of MAPK that is critical. Ultimately, an understanding of such activation profiles in patients suffering from disorders of the Ras/MAPK signaling pathway such as Noonan's syndrome could, in principle, lead to the development of optimal learning strategies that would allow the encoding of lasting memories.

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DNA Double-Strand Breaks Come into Focus

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DOI 10.1016/j.cell.2009.09.017

The Mre11-Rad50-Nbs1 (MRN) complex senses DNA double-strand breaks and recruits different repair pathway and checkpoint proteins to break foci. Two new studies (Williams et al., 2009; Lloyd et al., 2009) identify Nbs1 as a key factor in this process and reveal how an N-terminal protein recruitment module in Nbs1 binds to different response factors through shared phosphopeptide motifs.

Of the various types of DNA damage, double-strand breaks (DSBs) may be the most cytotoxic because of their potential to cause gross chromosomal aberrations, often linked to cell death or cancer. Cells therefore go to great lengths to repair DSBs, mounting a highly complex multistep response that includes modifications to large chromatin domains ("repair foci") through, e.g., ubiquitination, phosphorylation, and binding of numerous repair factors, scaffolding mediators, and posttranslational modifiers (Harper and Elledge, 2007). A keystone in the response to DSBs in eukaryotic cells is the Nbs1 protein, one of the earliest repair factors to bind to DSBs. However, Nbs1 also acts later in the repair process to regulate the DNA damage checkpoint and to recruit other repair factors to DSBs. Two papers in this issue of *Cell* (Williams et al., 2009; Lloyd et al., 2009) now provide structural and molecular insight into the mechanism by which Nbs1 performs these later repair functions.

DSBs are repaired by two major pathways, homologous recombination (HR) and nonhomologous end joining (NHEJ). Homologous recombination uses the sister chromatid as a template for new DNA synthesis and is highly accurate but limited to the S and G2 phases of the cell cycle. In NHEJ, in contrast, DNA ends are directly ligated without the need for sister chromatids, but this repair pathway is potentially mutagenic and can lead to chromosome aberrations. Repair pathway selection appears to be controlled in part by phosphorylation of repair factors, but the underlying molecular mechanisms are unclear. Nbs1, which plays a key role in both DSB repair pathways, interacts with different response proteins in a phosphorylation-dependent manner.

Nbs1 is mutated in Nijmegen breakage syndrome (Carney et al., 1998; Varon et al., 1998), which is characterized by chromosomal instability, microcephaly, immunodeficiency, and a susceptibility to cancer. Nbs1 together with the endo/exonuclease Mre11 and the ATP binding protein Rad50 form

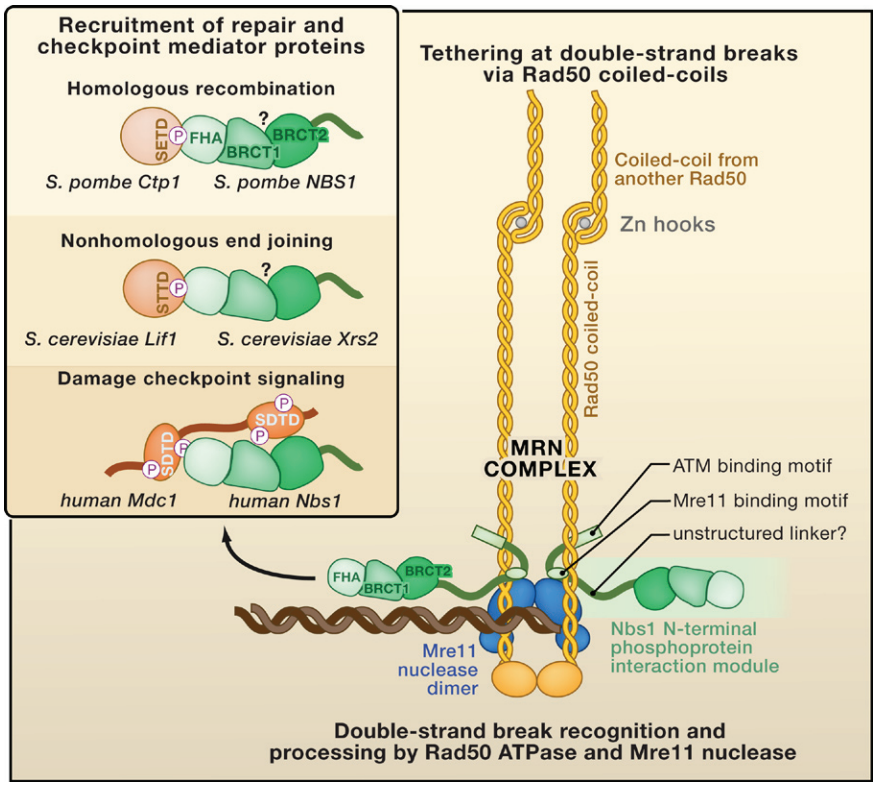

Figure 1. Nbs1 and DNA Double-Strand Break Repair

The MRN complex (Mre11, blue; Rad50, yellow; Nbs1, green) is a key sensor of DNA double-strand breaks (DSBs). DSBs trigger the activation of the DNA damage checkpoint and can be repaired by one of two different pathways: homologous recombination or nonhomologous end joining. All of these responses are mediated at some level by Nbs1. (Inset) The FHA domain of Nbs1 binds to a phosphothreonine-containing motif in Ctp1 and Lif1, proteins involved in homologous recombination and non-homologous end joining, respectively. In addition, the BRCT domains of human Nbs1 bind to the same motif when it contains phosphoserine, leading to an interaction with the human checkpoint factor Mdc1.

the Mre11-Rad50-Nbs1 (MRN) complex (Figure 1). Binding of the MRN complex to DSBs is one of the first steps in the DNA damage response pathway. MRN has multiple functions: it tethers DSBs via Rad50 coiled-coils, participates in the nucleolytic processing of DNA ends, and triggers DSB signaling by recruiting and activating the kinase ATM (Williams et al., 2007). ATM then orchestrates the DNA damage response by phosphorylating a wide variety of response factors, including the tumor suppressor proteins Chk2, Brca1, and p53.

However, Nbs1 also acts downstream of ATM. Nbs1 interacts with the scaffolding adaptor protein Mdc1 to maintain the DNA damage checkpoint and is also important in the direct recruitment of repair factors to DSBs such as CtIP/Sae2/Ctp1 and Lif1 (Palmbos et al., 2008). The CtIP/Sae2/Ctp1 complex, together with MRN, primes DSBs for DNA end resection during homologous recombination (Mimitou and Symington, 2008; Takeda et al., 2007), whereas Lif1 is part of the DNA ligase IV complex involved in nonhomologous end joining.

The promiscuous downstream role of Nbs1 in various DSB signaling and repair pathways is not well understood. The two new studies by Williams et al. (2009) and Lloyd et al. (2009) now combine high-resolution X-ray crystallography of the N-terminal phosphoprotein interaction module of fission yeast Nbs1 with genetic and biochemical data on yeast and human Nbs1 to reveal Nbs1's molecular adaptor role. The structural studies show that a surprisingly tight compact module at the N-terminus of Nbs1 is formed by a forkhead associated (FHA) domain and two Brca1 C terminus (BRCT) domains. This protein interaction module appears to be connected to the C-terminal Mre11 binding motif via a long unstructured region, suggesting that the N-terminal module recruits response factors to DSBs in a spatially flexible way that is constrained by distance (Williams et al., 2009).

The FHA domain binds to a Ser-Asp-Thr-Asp-like motif when phosphorylated on threonine. These motifs are found in fission yeast Ctp1, budding yeast Lif1, and as multiple copies in human Mdc1. Williams and colleagues reveal further details of the phosphopeptide interaction in a cocrystal structure of a Ctp1 peptide (containing a phosphorylated threonine) bound to the FHA domain. They explain that the specificity for a phosphorylated threonine over a phosphorylated serine or tyrosine is due to a tailored binding pocket. The conservation of the Ser-Asp-Thr-Asp motif (or more generally Ser-X-Thr motif) in Mdc1, Ctp1 and Lif1 suggests not only a conserved mode of interaction, but also that cells can easily control molecular events at DSBs via casein kinase 2 (CK2). CK2 is a multifunctional kinase that phosphorylates Ser/Thr-Asp motifs and is implicated in DNA repair, cell-cycle control, and circadian rhythms.

What about the two BRCT domains? Lloyd et al. find that the two BRCT domains of human (but not fission yeast) Nbs1 also bind to Ser-X-Thr motifs, but only when the serine residue is phosphorylated. As a consequence, double-phosphorylated motifs, which occur in human Mdc1 for instance, have the potential to bind to both FHA and BRCT domains. The multiple Ser-X-Thr sites may allow for fine-tuning of the interaction between single Mdc1 and Nbs1 molecules (Lloyd et al., 2009) or crosslinking of multiple Nbs1 molecules during formation of repair foci, but also could interact with additional partners. In any case, the intriguing role of phosphopeptide-dependent interaction networks and the role of multiple kinases including CK2 and cyclin-dependent kinases (Huertas et al., 2008) suggest a complex mode of cell cycle-dependent pathway choice and timing of the damage response that needs to be addressed in future studies.

The crystal structures also provide a molecular framework for understanding disease-associated Nbs1 mutations. Several missense mutations map to the core of BRCT1, presumably perturbing its fold

or directly interfering with phosphopeptide interactions. However, the most frequent mutation in patients with Nijmegen breakage syndrome (~90% of patients) is a five base-pair deletion in exon 6 (657 del5), which maps to the linker region between the two BRCT domains. This mutation results in expression of two artificially truncated polypeptides, p26 and p70. The split occurs between the two BRCT repeats and has two consequences. It disrupts the BRCT repeat, likely abolishing its direct role in phosphopeptide interactions. In addition, the FHA domain in p26 is not physically attached to the Mre11 binding motif in p70, thus abolishing Nbs1's ability to recruit repair factors to DSBs, which are bound by Mre11 and Rad50.

The new work provides a mechanistic look at how Nbs1 recruits repair and checkpoint mediators to DSBs and reveals the molecular basis for Nijmegen breakage syndrome. Yet there is still much to be learned. For instance, the role of the BRCT domains needs further investigation. What are the ligands for the BRCT domains in yeast and what is the role of the ATM-dependent phosphorylation at serine 278 in this region? Likewise, we need to understand how the MRN complex activates the ATM kinase in the first place. This would take us from the "head" of Nbs1 to its "tail." So, one end is just the beginning of the next installment of this intriguing story.

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