

Engineering a DNA damage response without DNA damage

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Abstract

Recent work has achieved the feat of activating the DNA damage checkpoint in the absence of DNA damage, revealing the importance of protein-chromatin associations for the activation, amplification and maintenance of the DNA damage response.

Detecting and repairing DNA damage is critical for the faithful transmission of genetic information. In response to DNA lesions, cells activate a complex cellular response, which at its core consists of a signal transduction cascade controlled by members of the PI(3) kinase-like kinase (PIKK) family [1,2]. This signaling cascade, often referred to as the DNA damage checkpoint, primarily aims to coordinate DNA repair with the arrest or slowing down of the cell cycle (the checkpoint). Failure to detect and repair DNA damage can lead to cell death or to genome aberrations that can promote the development of cancer.

Like other signaling systems, such as those that operate at the cell surface, DNA damage signaling is under tight spatial and temporal control. The spatial component of this pathway is particularly evident during the response to DNA double-strand breaks (DSBs), the most harmful type of DNA lesion. DSBs elicit the rapid accumulation of DNA damage signaling and repair proteins on the chromatin that surrounds lesions, forming distinctive subnuclear foci [1,2] that are easily detectable by fluorescence microscopy. What is extraordinary about this response is not that there is protein recruitment *per se*; rather, it is the scale of the phenomenon that is impressive. Indeed, protein recruitment after the induction of DSBs can extend for tens of kilobases on either side of the lesion [3,4]. By inference, DSBs promote the recruitment of dozens, if not hundreds, of molecules to the surrounding chromatin. As described below, protein recruitment at DNA lesions often occurs in a hierarchical manner and involves multiple post-translational

modifications. Why is DNA damage signaling organized this way? Recent work published by Evi Soutoglou and Tom Misteli in *Science* [5] and by David Toczyski and colleagues in *Molecular Cell* (Bonilla *et al.* [6]) address this critical question by reverse-engineering the DNA damage response, to spectacular effect.

Recruitment of checkpoint proteins to DNA lesions

Soutoglou and Misteli [5] and Bonilla *et al.* [6] investigate DNA damage signaling in mouse cells and in *Saccharomyces cerevisiae*, respectively. It is therefore useful to briefly compare and contrast the early response to DSBs in these species. In mammalian cells, DSBs are thought to be sensed by the MRE11/RAD50/NBS1 (MRN) complex, which recruits and activates ATM (ataxia telangiectasia mutated), a PIKK-family kinase that orchestrates the DNA damage response (for reviews see [1,2]). Activated ATM triggers the rapid phosphorylation of the histone 2A variant H2AX to form what is referred to as γ -H2AX. Strikingly, the size of the γ -H2AX chromatin domain can extend up to a megabase, which serves to demarcate the area on which DNA damage signaling and repair proteins accumulate. The phospho-epitope formed on γ -H2AX is specifically recognized by the proteins MCPH1 and MDC1. The consequences of MDC1 recruitment by γ -H2AX are the better understood. MDC1 amplifies ATM-dependent signaling by shielding γ -H2AX from the action of phosphatases and histone-exchange factors. In addition, it promotes the accumulation of additional MRN and activated ATM molecules at DNA

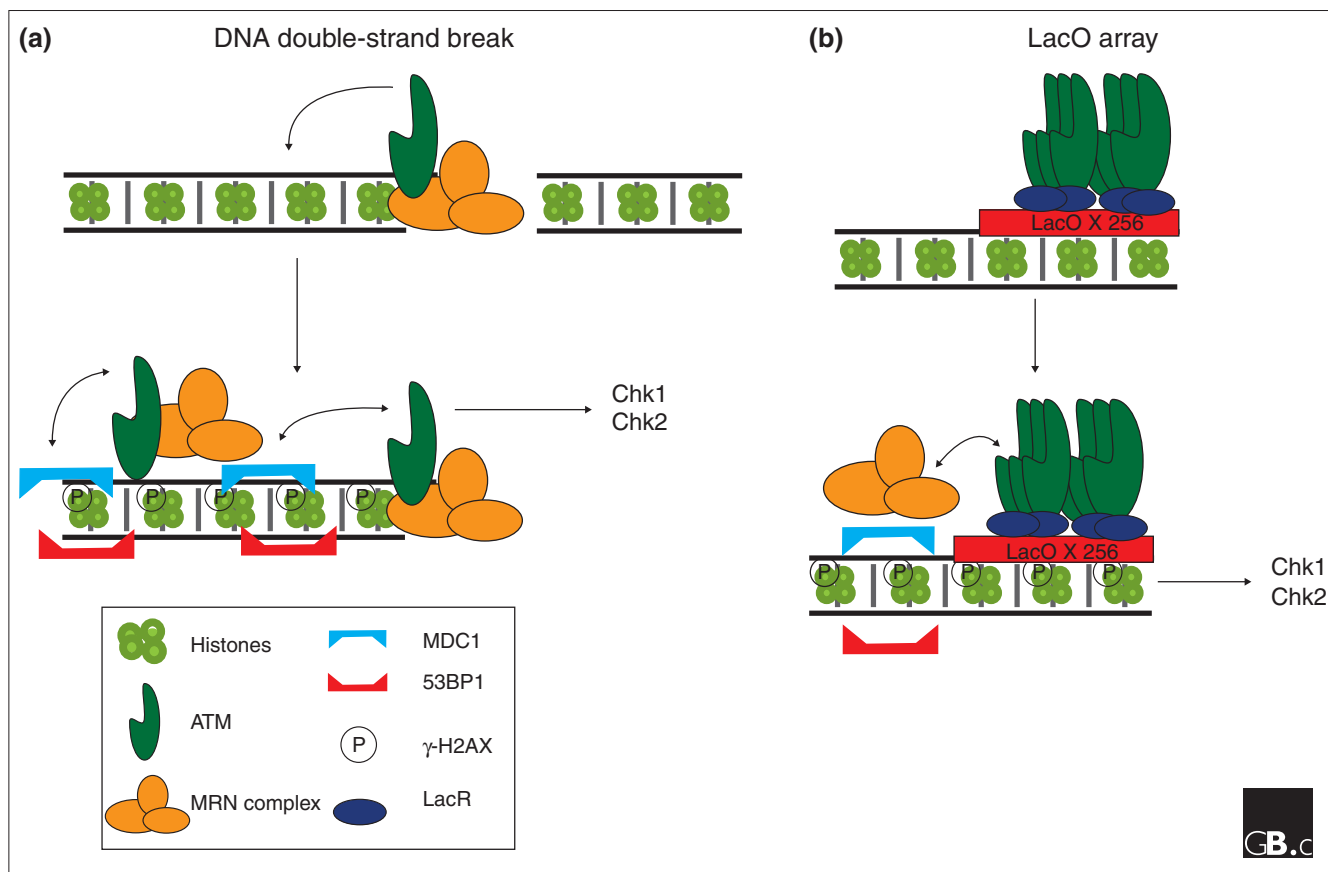


Figure 1

Engineering DNA damage signaling without DNA damage. **(a)** The response to DNA double-strand breaks (DSBs) in human cells. The MRN complex localizes to the DNA ends and promotes the recruitment of the protein kinase ATM. ATM phosphorylates histone H2AX to γ -H2AX, which promotes the assembly of MDC1, 53BP1 and RNF8 (not shown) at the site of the DSB. In turn, the recruitment of MDC1 promotes the amplification of the signaling cascade by recruiting additional ATM molecules over many kilobases. Chk1 and Chk2 are the checkpoint kinases that are activated by the DNA damage response and interact with cell-cycle components to cause the arrest of the cell cycle. **(b)** Tethering of ATM to an array of Lac operator sites (LacO) via the Lac repressor (LacR) promotes the formation of γ -H2AX and recruitment of MRN, MDC1 and 53BP1, followed by activation of Chk1 and Chk2.

lesions via specific phosphorylation-dependent interactions and also promotes the recruitment of other DSB signaling and repair proteins. This pathway is summarized in Figure 1a. DNA damage signaling ultimately leads to activation of the checkpoint protein kinases Chk1 and Chk2, which modify components of the cell-cycle machinery to cause cell-cycle arrest.

In *S. cerevisiae*, the recognition and response to DSBs does not primarily depend on ATM, but on a signaling pathway initiated by a complex of the protein kinase Mec1 and its regulatory protein Ddc2 [2]. Mec1 is the yeast homolog of the human protein kinase ATR (ataxia telangiectasia related), which in humans is primarily concerned with the checkpoint response to replication fork-blocking lesions. Mec1-Ddc2 defines one of two DNA damage signaling complexes in yeast that localize independently to DSBs, the other being the Rad17-Mec3-Ddc1 complex (referred to here as 9-1-1). The recruitment of Mec1-Ddc2 requires the 5'-3'

resection of the DSB to produce tracts of single-stranded DNA (ssDNA), which become coated with the ssDNA-binding protein replication protein A (RPA). RPA recruits the Mec1-Ddc2 complex via a direct interaction with Ddc2. The 9-1-1 complex is loaded onto sites of DNA damage at the junction between double-stranded DNA (dsDNA) and the RPA-bound ssDNA by a complex composed of Rad24 and the Rfc2-5 proteins. How the co-localization of these two complexes leads to activation of Mec1 remains unclear, although it is becoming increasingly evident that the 9-1-1 complex plays an important role [7,8].

The massive accumulation of signaling and repair proteins to sites of DNA damage prompted Soutoglou and Misteli [5] and Bonilla *et al.* [6] and to ask whether protein recruitment alone was sufficient to activate checkpoint signaling. Both groups performed the same simple yet elegant experiment: they integrated an array of Lac operators (LacO) repeats, the binding site for the Lac repressor (LacR), in the genomes of

mouse or *S. cerevisiae* cells. Figure 1b shows the experiment in human cells. The expression of proteins fused to the repressor then allowed their tethering to the chromatin of the LacO array. In essence, this strategy mimics the accumulation of repair and signaling proteins triggered by DNA lesions, with the important exception that there was no prior DNA damage.

Both groups made the startling observation that this experimental scheme resulted in a robust DNA damage response when the appropriate proteins were fused to LacR. The concordance of these results is particularly striking because, as outlined above, the DNA damage responses in yeast and in humans are initiated by different signaling pathways. Therefore, it seems that a universal feature of the initiation and amplification of DNA damage signaling systems in eukaryotes, be they ATM- or ATR/Mec1-based, is the concentration of key proteins on chromatin. In addition, beyond this exciting core finding, both studies revealed more about the inner workings of DNA damage signaling.

Plasticity of metazoan ATM-based DNA damage signaling

Soutoglou and Misteli [5] primarily assessed activation of DNA damage signaling by the formation of a γ -H2AX focus at the LacO array. Using this readout, they found that checkpoint signaling can be achieved by recruitment to the LacO array of any one of the proteins NBS1, MRE11, MDC1 or a fragment of ATM (ATM¹³⁰⁰⁻³⁰⁶⁰). Individual tethering of the effector kinases Chk1 or Chk2, the kinases whose actions lead to the cell-cycle arrest, does not prompt checkpoint signaling, however. These observations reveal a surprising plasticity in the initiation of the DNA damage response. Indeed, they implicitly suggest that initiation of DNA damage signaling may not be by a stereotypical cascade of protein recruitment at sites of DNA damage, but rather that an initial accumulation of any one of the MRN, ATM or MDC1 proteins might be sufficient to trigger ATM activation.

The DNA damage response elicited by tethering MRN or MDC1 to the LacO array was largely ATM-dependent and led to cell-cycle arrest at the G2 to M transition, thereby recapitulating many aspects of the cellular response initiated by DSBs [6]. A few important differences are apparent, however. One concerns the activation of the G2/M checkpoint. Recent work has suggested that the ATM-dependent G2/M checkpoint might not be sensitive enough to respond to a single DSB and that a threshold of around 20 DSBs might be necessary [9,10]. Why a checkpoint is observed when MDC1, MRN or ATM are tethered to the LacO array is unclear as it should mimic the response that occurs at a single DSB. It perhaps indicates that the physical tethering of these proteins overrides some control mechanism that establishes the threshold number

of DSBs or threshold ATM activity necessary to trigger the G2/M checkpoint.

A second discrepancy concerns the strange behavior of 53BP1 recruitment following the tethering of MDC1 and MRN. Indeed, 53BP1 accumulation at sites of DNA damage is almost entirely dependent on MDC1 [11], yet only the physical tethering of MRN, but not that of MDC1, leads to the formation of 53BP1 foci [5]. If the trivial possibility that the MDC1-LacR fusion is interfering with 53BP1 recruitment can be experimentally rejected, this would suggest that MDC1 recruitment to the LacO array might not be sufficient to trigger the ubiquitination cascade mediated by the E3 ubiquitin ligase RNF8 that is necessary for 53BP1 recruitment [12-14], whereas MRN somehow can. It will therefore be interesting to study how RNF8-dependent protein ubiquitination is normally initiated by the MRN complex at sites of DNA damage.

Yeast Mec1 signaling: 9-1-1 calls in

In contrast to the results in mouse cells, in yeast [6] the tethering of any one protein to the LacO array was unable to activate checkpoint signaling, as monitored by phosphorylation of the yeast Chk2 ortholog Rad53. Rather, Bonilla *et al.* [6] needed to simultaneously target LacR fusions of Ddc2 and components of the 9-1-1 complex to the LacO array in order to achieve Rad53 activation and a G2/M checkpoint. Importantly, the response required the presence of Rad9, the yeast homolog of 53BP1, demonstrating that the signaling triggered by the combined recruitment of Ddc2 and 9-1-1 recapitulated most aspects of a normal DNA damage response.

The power of *S. cerevisiae* as a genetic system allowed Bonilla *et al.* [6] to dissect the response triggered by the artificial recruitment of Ddc1 and Ddc2 to chromatin. Firstly, they found that the Rad24-based complex that loads 9-1-1 at ssDNA-dsDNA junctions became redundant, in terms of checkpoint activation, when 9-1-1 was tethered to the LacO array. Moreover, the integrity of the 9-1-1 complex itself was not essential to promote the activation of the DNA damage response. Indeed, when Ddc1 was immobilized to chromatin next to Mec1 via LacR fusions, checkpoint signaling could still be initiated in cells that had deletions in the genes coding for Mec3 and Rad17, the two partners of Ddc1 in 9-1-1. This latter result is particularly significant because it suggests that Rad24, Mec3 and Rad17 are primarily required to localize Ddc1, which is an activator of Mec1. Future work will address whether this activation is direct or via other proteins. Nevertheless, these experiments provide an elegant explanation for the previous observation that the Ddc2-Mec1 and 9-1-1 complexes are independently recruited to sites of DNA lesions, as they suggest that co-localization of two or more complexes to chromatin provides a fail-safe mechanism that prevents spurious activation of the DNA damage response.

Bonilla *et al.* [6] also took advantage of the LacR/LacO system to investigate the observation that Mec1 signaling in response to certain types of DSBs is regulated by a cell's position in the cell cycle. Indeed, cell-cycle position greatly influences the type of repair and signaling pathways (whether ATM or Mec1/ATR-based) elicited by DSBs [15-17]. This 'choice' is regulated by the status of the DNA lesion, namely whether it is resected to uncover the ssDNA tracts needed for ATR activation, or whether the DNA end is 'stable', which shunts the signaling pathway to ATM [18]. DNA-end resection is cell-cycle regulated, primarily by cyclin-dependent kinase (CDK) activity [15,16,18]. In G₁, when CDK activity is lowest, resection is less efficient, translating into a downregulated Mec1 response [16,19]. However, if CDK activity were solely required during Mec1-dependent signaling to promote DNA resection, one would predict that the tethering of Ddc1 (9-1-1) and Ddc2 to the LacO array should produce a cell-cycle-independent activation of Mec1. Surprisingly, when this experiment was carried out, Rad53 phosphorylation was not detected [6]. This result, along with a clever strategy that allowed Bonilla *et al.* to experimentally turn off CDK activity, revealed an unsuspected role for CDKs in maintaining an active checkpoint signal. The authors postulate that this occurs via the phosphorylation of Rad9, a key activator that promotes Rad53 phosphorylation by Mec1.

Taken together, both reports show that localization and accumulation of the appropriate proteins to chromatin is sufficient for the initiation and amplification of the DNA damage signal, whether DNA damage is present or not. These important observations not only provide a new experimental system for dissecting the DNA damage response but also put a new emphasis on the importance of the protein-chromatin associations. Finally, we note that just as the assembly of protein-chromatin complexes is essential for the initiation and amplification of the DNA damage response, their disassembly will assuredly be critical for termination of DNA damage signaling following repair.

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