COMMENTARY

Spotting new DNA damage-responsive chromatin-binding proteins

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In response to DNA damage, cells initiate multiple repair mechanisms that all contribute to the survival of both the cell and the organism. These responses are numerous and variable, and can include cell cycle arrest, transcriptional activation of DNA repair genes and relocalization of repair proteins to sites of DNA damage. If all else fails, in multicellular organisms the initiation of apoptosis is also a potential cellular response to DNA damage. Despite a wealth of information about these events, it is clear that we do not yet have a comprehensive picture of the cellular responses to DNA damage. In this issue of the *Biochemical Journal*, a proteomics approach was used by Lee et al. to identify proteins that bind to chromatin in a DNA damage-inducible manner. The proteins identified, nucleophosmin, hnRNP C1 (heterogeneous nuclear ribonucleoprotein C1) and hnRNP C2, were proteins that would not necessarily have been predicted to behave this way. These studies have the potential to be extended and contribute to our knowledge of the cellular response to DNA damage.

Key words: chromatin, DNA damage, heterogeneous nuclear ribonucleoprotein (hnRNP), nucleolus, nucleophosmin, proteomics.

DNA DSBs (double-strand breaks) are arguably the most deleterious lesion a cell can sustain, with potentially lethal consequences. Moreover, the inaccurate repair of DNA DSBs can lead to chromosomal translocations. Indeed, loss of proteins required for the signalling and repair of DNA DSBs results in increased incidence of tumorigenesis in multicellular eukaryotes. Not surprisingly, cells have rigorous and redundant mechanisms for detecting and repairing these lesions.

One of the most rapid responses to DNA breaks is the activation of a family of the PIKKs (phosphoinositol kinase-like kinases); in humans, these include ATM (ataxia telangiectasia mutated), ATR (ataxia telangiectasia mutated-related) and DNA-PK (DNA-dependent protein kinase). These kinases have been shown to phosphorylate a histone variant, H2AX, in the vicinity of the DNA DSBs. Ultimately, when the lesions are not immediately repaired, these kinases set off a signal transduction cascade that results, in part, in the accumulation of DNA damage signalling and repair proteins at the sites of the DNA DSBs into 'foci' that are visible by immunofluorescence [1].

Although a wealth of information has been uncovered about the proteins present in the vicinity of DNA DSBs after its occurrence, it is likely that not all of the players have been identified. Studies that attempt to identify novel proteins involved in DNA damage responses are therefore of great value. One such study, in this issue of the *Biochemical Journal* [2], falls into this category. The authors hypothesize that proteins involved in this response would bind to chromatin in a DNA damage-dependent manner. Moreover, this association would be relatively salt-resistant. These criteria could then be applied in a proteomic approach to purify chromatin under high-salt conditions both before and after DNA damage to identify proteins that are present in the high-salt fractions after damage. Importantly, the authors demonstrated that the DNA DSB repair protein Ku bound to the high-salt chromatin fractions in a DNA damage-dependent manner, demonstrating the validity of their approach.

Four candidate proteins were thus detected in two-dimensional SDS/PAGE gel analysis and, identified by mass spectrometry. Of the four proteins identified, three of them, nucleophosmin, hnRNP C1 (heterogeneous nuclear ribonucleoprotein C1) and hnRNP C2, were subsequently shown to bind to chromatin in a DNA damage-dependent manner using chromatin fractionation and Western blotting. Interestingly, however, these proteins do not associate with phosphorylated H2AX in DNA damage-induced foci. Nucleophosmin does relocalize from its normal location in the nucleolus, but is found diffused throughout the nucleus after DNA damage induction. The hnRNP C1 and C2 proteins are located throughout the nucleus before damage, and this does not detectably change after DNA damage. Although it is possible that the abundance of these proteins masks a physiologically relevant subset at DNA damage-induced foci, it is also possible that these proteins play a more global role in chromatin binding that is not specifically localized to the DNA lesions. What then, might that role be?

Nucleophosmin, which is primarily involved in ribosome biogenesis, has already been implicated in DNA damage responses. It can bind to the ARF (alternative reading frame) tumour suppressor protein and sequester it in the nucleolus [3]. After DNA damage, the nucleolus is disrupted [4], and this may release ARF from nucleophosmin. ARF would then be free to bind to Mdm2 (murine double minute 2), the ubiquitin ligase that targets the p53 tumour suppressor gene for degradation [5]. Thus, in response to DNA damage, nucleophosmin would contribute to the stability of p53, which exerts its protective functions, at least in part, through its activity as a transcription factor to up-regulate genes involved in cell cycle arrest and/or apoptosis. This function of nucleophosmin does not require either its ability to relocalize from the nucleolus or its ability to bind to chromatin. However, it has been reported that nucleophosmin can act as a histone chaperone and possesses chromatin remodelling activity [6]. Global changes in chromatin structure (not necessarily at the site of a DNA lesion) have been proposed to be the mechanism by which ATM is activated [7]. Therefore one attractive possibility is that the release of the abundant nucleophosmin from the nucleolus after DNA damage, and its global association with nuclear chromatin, results in structural changes that contribute to the activation of ATM.

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The hnRNPs are abundant nucleic acid-binding proteins that are involved in a variety of cellular processes, including ribosome activity. The two proteins isolated in this study, hnRNP C1 and C2, are found in the nucleus and have been reported to associate with telomerase [8]. Multiple links between DNA DSB repair and telomere biology have been elucidated in recent years. For example, the DNA DSB repair protein Ku has been shown to be required for DSB repair by the non-homologous end-joining pathway, and for the appropriate maintenance of telomere length and localization [9]. Ku is a heterodimeric protein that is, in mammalian cells, a component of the DNA-PK holoenzyme [9], one of the DNA damage-inducible PIKK family members mentioned above. It is notable, therefore, that hnRNP C1 and C2, in addition to being associated with telomerase, have been identified recently as substrates for DNA-PK [10]. Whether phosphorylation by DNA-PK is important for its association with chromatin is not known; nor is the physiological significance of chromatin association after DNA damage.

In addition to an association with telomeres and telomere-binding proteins, hnRNP C1 and C2 have been shown to act on mRNA species to influence translation. For example, XIAP (X-chromosome-linked inhibitor of apoptosis) is translationally controlled by an IRES (internal ribosome entry site), and this was recently shown to be bound by hnRNP C1/C2 [11]. Moreover, functional data to suggest that hnRNP C1/C2 enhance translation of XIAP were generated [11]. In another study, the IRES of the proto-oncogene c-*myc* transcript was also found to be bound by hnRNP C1/C2 [12]. The massive relocalization of hnRNP C1/C2 into the chromatin fraction after DNA damage might then be a reflection of its association with chromatin-bound mRNAs, and this association could then influence the translation of proteins such as XIAP and c-*myc* that may contribute to the cellular response to DNA damage.

The approach used in this study to identify proteins that bind to chromatin after DNA damage was successful in identifying three proteins that would not necessarily have been predicted to behave in this manner. Notably, the three proteins that were identified are very abundant, and, as the authors point out, this is undoubtedly a consequence of the limitations of using a proteomics-based approach, in which visualization and sequencing of target proteins requires a minimum level of protein. Many DNA damage detection and signalling proteins are not likely to be as abundant as nucleophosmin and hnRNPs, suggesting that many proteins that are re-localized to chromatin after DNA damage might not be identifiable using these techniques.

Does this mean that the proteins that can be identified in this manner have been exhausted? Not necessarily. It is worth noting that Ku, which the authors demonstrate is present in the high-salt chromatin fraction after DNA damage, was not identified in this study. However, the majority of Ku is still in the soluble fraction in the preparations used in these assays. It is possible, therefore, that by using a much higher dose of DNA damage in order to elicit a higher number of DNA lesions, a greater proportion of DNA repair and signalling proteins will be in the high-salt chromatin fractions being analysed, thus increasing the chances of identifying them by this approach. In addition, the appearance of DNA repair and signalling proteins in foci is variable: for example, 53BP1 is detected in foci before the appearance of Mre11 [13]. Therefore an analysis after different periods of time post-DNA damage will be likely to yield a different proteomic pattern. An additional variable that might be altered in subsequent studies is the protein gel parameters. The proteins are separated by charge in one dimension, and size in another, before the gel is stained and analysed for changes in the proteome before and after DNA damage. Obviously, analysing the same samples using a range of different electrophoresis parameters may yield new candidate proteins.

This study has demonstrated the utility of using a proteomics-based approach to identifying new players in the cellular response to DNA damage, and can certainly be extended and modified to continue to yield novel insights. In the meantime, we now know that three very abundant proteins associate with chromatin after the induction of DNA damage, and it will be very interesting to uncover the biological significance of this behaviour.

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