DNA REPAIR

The big and the small picture

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URLs

ATM

<http://ca.expasy.org/uniprot/Q13315>

ATR

<http://ca.expasy.org/uniprot/Q13535>

BRCA1

<http://ca.expasy.org/uniprot/P38398>

53BP1

http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=71586&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

TOPBP1

http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=11073&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

RAP80

<http://ca.expasy.org/uniprot/Q96RL1>

BACH1

http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=5716&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

CTIP

http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=5932&itool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

Systems biology approaches can reveal the broad range of players that are involved in a cellular process and, at the same time, open avenues of investigation around specific players, as is nicely illustrated by four reports in *Science*.

Matsuoka *et al.* carried out a large-scale proteomics analysis of proteins that were phosphorylated in response to DNA damage on consensus sites recognized by **ATM** and **ATR** kinases. They identified >900 DNA-damage-inducible phosphorylation sites on >700 proteins. The extent of phosphorylation of known ATM and ATR substrates was much higher than anticipated; for example, among the mediator class of proteins (including **BRCA1**, **53BP1** and **TOPBP1**), 33 regulated sites were found, of which only six had been previously identified.

The phosphorylation of a small subset of candidate substrates was validated by immunoprecipitation and western blot. In addition, the authors chose a random selection of substrates that had not previously been implicated in the DNA damage response and tested cells that were depleted for these substrates in functional assays. Out of 37 proteins, 35 scored in at least one of four assays and more than half of the proteins scored in two or more assays — suggesting that the pool of candidate substrates is enriched with proteins that function in the DNA damage response.

Using gene-ontology analysis, 421 out of >700 candidate substrates were annotated with a biological process. Among these, 202 substrates were assigned to the nucleic-acid metabolism category, of which 46 have a function in DNA replication, repair or recombination and 101 have a function in mRNA transcription. The post-transcriptional modification category was surprisingly large. Together, these analyses imply the existence of a broad transcriptional, post-transcriptional and chromatin response to DNA damage. Many of the substrates cluster into protein

networks that are involved in DNA replication, DNA repair, the cell cycle and the spindle checkpoint. Several signalling pathways were also implicated in the DNA damage response. Elucidation of these many newly found connections will take time, but this study demonstrates the extraordinarily broad cellular response to DNA damage.

This DNA-damage phosphorylation information has already proved useful in identifying two ATM/ATR substrates as binding partners of the tumour suppressor **BRCA1**. The BRCT repeats of **BRCA1** constitute a phosphopeptide recognition domain, which was used by Wang *et al.* to affinity-purify phosphopeptides and identify the novel protein **Abraxas** and the ubiquitin-interacting motif (UIM)-containing protein **RAP80**. It is thought that **RAP80** is recruited to **BRCA1** through binding to **Abraxas**. Functional analyses showed that both proteins are required for DNA damage resistance, control of the G2-M checkpoint and DNA repair.

Two other groups, led by Bijan Sobhian and Hongtae Kim, respectively, also identified **RAP80** as a **BRCA1** interaction partner. Mutational analysis revealed that

the UIM domains are required for colocalization of **RAP80** to double-strand breaks (DSBs) and for

polyubiquitin binding. All three groups showed that **RAP80** is responsible for recruiting **BRCA1** to DNA damage-induced foci. Together, these findings suggest that **RAP80** targets the **BRCA1** complex to polyubiquitylated structures at DSBs, although

the nature of these polyubiquitylated substrates remains unclear.

Given that **BRCA1** has additional interaction partners, including **BACH1** and **CTIP**, it is proposed to exist in distinct, mutually exclusive complexes. It will be interesting to dissect the role of each of these complexes in the DNA damage response and in tumorigenesis.

Arianne Heinrichs,

Chief Editor,

Nature Reviews Molecular Cell Biology

ORIGINAL RESEARCH PAPERS Matsuoka, S. *et al.* ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* **316**, 1160–1166 (2007) | Wang, B. *et al.* **Abraxas** and **RAP80** form a **BRCA1** protein complex required for the DNA damage response. *Science* **316**, 1194–1198 (2007) | Sobhian, B. *et al.* **RAP80** targets **BRCA1** to specific ubiquitin structures at DNA damage sites. *Science* **316**, 1198–1202 (2007) | Kim, H. *et al.* Ubiquitin-binding protein **RAP80** mediates **BRCA1**-dependent DNA damage response. *Science* **316**, 1202–1205 (2007)