The results of Yang *et al.* suggest that PML is a substrate of Chk2 in irradiated cells. Chk2 phosphorylates PML on Ser 117 *in vitro* and the same site is phosphorylated *in vivo* in response to radiation. The *in vivo* phosphorylation is compromised after transfection of a kinase-dead Chk2 mutant and is also defective in HCT-15 cells (which lack active Chk2 kinase), and in A-T cells (in which Chk2 activation after radiation is defective). Consistent with PML as a substrate of Chk2, Chk2 was shown to localize to PML-NB in untreated and irradiated cells. This result contradicts the diffuse nuclear staining previously reported for Chk2 (Ref. 16), but is consistent with co-immunoprecipitation results showing association of PML with Chk2 in non-irradiated cells.

Interestingly, the interaction between PML and Chk2 was weakened substantially in response to radiation; the dissociation between the two proteins was dependent on Chk2 kinase activity and required phosphorylation of PML on Ser 117. Having established PML as a substrate of Chk2, Yang *et al.* then addressed the consequence of PML phosphorylation on Ser 117. In two *p53*-null cell lines, overexpression of wild-type PML, but not an Ala 117 mutant, increased apoptosis after irradiation, providing evidence that PML-induced apoptosis is regulated by phosphorylation of Chk2.

These new findings by Yang *et al.* clearly link PML to Chk2 and p53-independent apoptosis and provide a framework for further studies aiming to understand how PML regulates apoptosis. Although it is somewhat premature to speculate on the molecular mechanisms involved, two distinct possibilities can be considered at this time. One possibility is that PML itself is a pro-apoptotic protein when phosphorylated on Ser 117. According to this hypothesis, PML that is phosphorylated on Ser 117 by Chk2 would participate directly in an apoptotic pathway by associating with proteins such as DAXX or caspases. Perhaps a more interesting model, consistent with the presence of multiple proteins in PML-NB and the radiation-induced dissociation of Chk2 from PML, is that PML itself has no direct link to apoptosis, but in the context of PML-NB functions to regulate the intracellular availability of pro-apoptotic PML-associated proteins.

More studies are needed to clarify what mechanism is correct. Nonetheless, the second model has a certain appeal. According to this model, before DNA damage, most of the Chk2 protein in the cell is sequestered in the PML-NB. After irradiation, ATM phosphorylates a sub-population of Chk2, which can then phosphorylate PML and PML-bound Chk2, decreasing the binding affinity between these two proteins and resulting in the release of Chk2 into the nucleoplasm, where it can participate in various apoptotic pathways (Fig. 1). Variations of this speculative model that allow the cell great flexibility in responding to stress can easily be considered. PML that is phosphorylated on Ser 117 by Chk2 may release not only Chk2, but also other proteins involved in the DNA damage response. In addition, other types of stress may induce distinct post-translational modifications of PML, allowing for the regulated release of different subsets of proteins, each suited for a particular type of stress. Evidence for this type of specificity is provided by Yang *et al.* through the observation that a PML Ala 117 mutant compromizes the apoptotic response to radiation, but not to other apoptotic agents, such as TNF, Fas and interferon. □

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1. Melnick, A. & Licht, J. D. *Blood* **93**, 3167–3215 (1999).
2. Guo, A. *et al. Nature Cell Biol.* **2**, 730–736 (2000).
3. Fogal, V. *et al. EMBO J.* **19**, 6185–6195 (2000).
4. Pearson, M. *et al. Nature* **406**, 207–210 (2000).
5. Alcalay, M. *et al. Mol. Cell Biol.* **18**, 1084–1093 (1998).
6. Torii, S., Egan, D. A., Evans, R. A. & Reed, J. C. *EMBO J.* **18**, 6037–6049 (1999).
7. Ishov, A. M. *et al. J. Cell Biol.* **147**, 221–234 (1999).
8. Salomoni, P. & Pandolfi, P. P. *Cell* **108**, 165–170 (2002).
9. Negorev, D. & Maul, G. G. *Oncogene* **20**, 7234–7242 (2001).
10. Wang, Z. G. *et al. Nature Genet.* **20**, 266–272 (1998).
11. Wang, Z. G. *et al. Science* **279**, 1547–1551 (1998).
12. Rego, E. M. *et al. J. Exp. Med.* **193**, 521–529 (2001).
13. Yang, *et al. Nature Cell Biol.* **4**, 865–870 (2002).
14. Bartek, J., Falck, J. & Lukas, J. *Nature Rev. Mol. Cell Biol.* **2**, 877–886 (2001).
15. Hirao, A. *et al. Mol. Cell. Biol.* **22**, 6521–6532 (2002).
16. Ward, I. M., Wu, X. & Chen, J. *J Biol Chem.* **276**, 47755–47758 (2001).

Polar exploration

The generation of anteroposterior and dorsoventral polarity is essential during oocyte development. In *Drosophila melanogaster*, the molecular components involved in establishing these axes have been studied extensively. Formation of each axis is also dependent on the successful repair of DNA double-strand breaks that occur during meiotic recombination. In a recent study (*Development* **129**, 5053–5064 (2002)), Steven Beckendorf and colleagues found that CSN5/Jab1, a component of the COP9 signalosome, interacts with a meiotic checkpoint. Unexpectedly, they found that this interaction influences the formation of both axes.

Translation of *gurken*, a TGF-α homologue, during two stages of oocyte development is required for the establishment of dorsoventral and anteroposterior axes in *Drosophila*. Translation of *gurken* at both stages depends on successful meiotic recombination and the repair of any DNA double-strand breaks generated during this process. The effects of double-strand breaks on Gurken seem to be controlled by a DNA damage checkpoint mediated by Mei-41, a member of the ataxia telangectasia mutated (ATM)/ataxia telangectasia and Rad3-related (ATR) family.

Mutations in *CSN5* prevent the normal accumulation of Gurken (see figure) and result in severe defects in polarity. Mutations in *CSN5* also affect the modification of Vasa, a protein known to be involved in the translation of Gurken. As the polarity phenotype caused by loss of zygotic *CSN5* and the mis-modification of Vasa can be suppressed by mutations in *mei-41* (see figure), it seems that in the absence of CSN5, a Mei-41 DNA-damage checkpoint is activated.

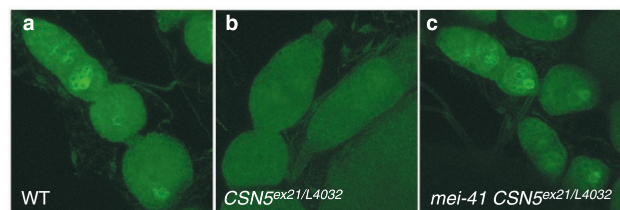

Figure 1 Effect of *CSN5* mutations on accumulation of Gurken. a, Wild-type germanium showing accumulation of Gurken. b, CSN^{ex21}/CSN^{L4032} germanium with loss of Gurken. c, Loss of *mei-41* restores *Gurken* expression. Image reproduced with permission from the Company of Biologists.

As the Mei-41 DNA damage checkpoint has previously been linked to the correct localization of Gurken and Vasa, this seems to be the manner by which CSN5 affects polarity. CSN is known to regulate protein stability through deneddylation of cullin, a subunit of the SCF (Skp1/cullin-1/F-box) ubiquitin ligase. Therefore, CSN5 could function to control the stability of proteins involved in the Mei-41 meiotic checkpoint. Although the precise targets of the CSN are unclear, future work will surely identify the precise function of CSN5 in polarity.

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