breast tumors (Sjöblom et al., 2006). Therefore, EI24 and mEPG5 may specifically regulate autophagy in cancer cells. The identification of these new genes by Tian et al. (2010) highlights the importance of autophagy in human diseases and may lead to exciting new discoveries about the role of autophagy in cancer and other disorders.

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REFERENCES

Axe, E.L., Walker, S.A., Manifava, M., Chandra, P., Roderick, H.L., Habermann, A., Griffiths, G., and Ktistakis, N.T. (2008). J. Cell Biol. *182*, 685–701.

Dusetti, N.J., Jiang, Y., Vaccaro, M.I., Tomasini, R., Azizi Samir, A., Calvo, E.L., Ropolo, A., Fiedler, F., Mallo, G.V., Dagorn, J.C., et al. (2002). Biochem. Biophys. Res. Commun. *290*, 641–649.

Gu, Z., Flemington, C., Chittenden, T., and Zambetti, G.P. (2000). Mol. Cell. Biol. *20*, 233–241.

Mathew, R., Karantza-Wadsworth, V., and White, E. (2007). Nat. Rev. Cancer *7*, 961–967.

Meléndez, A., and Neufeld, T.P. (2008). Development *135*, 2347–2360.

Mizushima, N. (2007). Genes Dev. *21*, 2861–2873.

Mizushima, N., Levine, B., Cuervo, A.M., and Klionsky, D.J. (2008). Nature *451*, 1069–1075.

Sjöblom, T., Jones, S., Wood, L.D., Parsons, D.W., Lin, J., Barber, T.D., Mandelker, D., Leary, R.J., Ptak, J., Silliman, N., et al. (2006). Science *314*, 268–274.

Tian, Y., Li, Z., Hu, W., Ren, H., Tian, E., Zhao, Y., Lu, Q., Huang, X., Yang, P., Li, X., et al. (2010). Cell, this issue.

Zhang, Y., Yan, L., Zhou, Z., Yang, P., Tian, E., Zhang, K., Zhao, Y., Li, Z., Song, B., Han, J., et al. (2009). Cell *136*, 308–321.

ATM Creates a Veil of Transcriptional Silence

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The ATM kinase orchestrates diverse responses to DNA damage. By simultaneously monitoring transcription and DNA-damage responses in single cells, Shanbhag et al. (2010) now uncover a role of ATM in preventing transcription near DNA double-strand breaks.

The chromatin domains that flank DNA double-strand breaks (DSBs) harbor a plethora of posttranslational protein modifications. Although these modifications decorate megabase-size regions and are generally thought to promote DNA repair and cell survival, the functional roles of many remain to be determined, among them monoubiquitinated histone 2A (uH2A). Stemming from previous studies that implicate uH2A in transcriptional silencing (Weake and Workman, 2008), Greenberg and colleagues now examine whether uH2A may also exert similar gene silencing activities near sites of DNA damage (Shanbhag et al., 2010).

To do this, the authors borrow a previously described transcriptional reporter (Janicki et al., 2004) and re-engineer it so that a defined DSB can be generated at a stretch of sequence adjacent to the transcription unit. By employing fluorescence-based designs, the system makes it possible to simultaneously observe, both qualitatively and quantitatively, nascent transcription, protein production, as well as DNA-damage responses—all at the single-cell level.

Introduction of DSBs not only disrupts the physical integrity of interphase chromatin but is thought to interrupt numerous processes that take place at this dynamic structure. Whereas DSBs appear to inhibit DNA replication by preventing global origin firing and slowing the progression of local replication forks, it is not known whether and how these DNA lesions modulate local transcription. Now using this experimental setup, Shanbhag et al. (2010) address this question by measuring transcriptional activities adjacent to the engineered DSB site. They find that transcriptional activities at the chromosomally integrated reporter are largely repressed when a DSB is introduced. What's more interesting is that this DSB-associated gene silencing response is only effective on regions of chromatin proximal to the lesion and does not affect transcription at distal sites.

The authors call this phenomenon DNA *d*ouble-strand break-*i*nduced silencing in c*is* (DISC), and they uncover a strict requirement for the ataxia telangiectasia mutated (ATM) kinase in mediating DISC. Notably, DISC coincides with two hallmarks of transcriptional repression: stalling of RNA polymerase II (indicated by hypophosphorylation) and impaired chromatin decondensation, pointing to the notion that DISC affects through

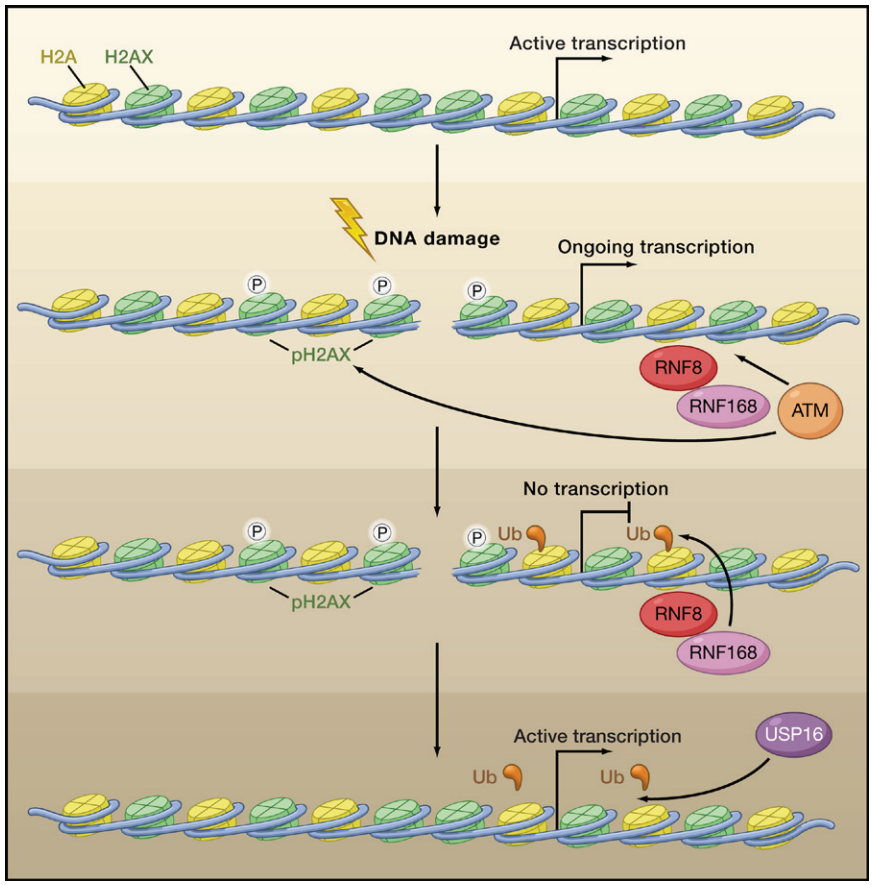

Figure 1. DNA Double-Strand Break-Induced Silencing In *cis* (DISC)
A model depicting how the kinase ATM (ataxia telangiectasia mutated) regulates gene silencing and the components involved in DNA double-strand break-induced silencing in *cis* (DISC). When a DNA double-strand break (DSB) is introduced at an actively transcribed region, it causes ATM activation and phosphorylation of histone variant H2AX. ATM promotes assembly of the H2A ubiquitin ligases RNF8 and RNF168 to the vicinity of the DSB, which triggers H2A ubiquitination (uH2A), leading to inhibition of transcription. Upon repair of the DSB or recovery from DNA-damage signaling, USP16 deubiquitinates uH2A, resulting in restored transcription. Red and yellow circles represent H2AX and H2A molecules, respectively.

canonical transcription regulatory mechanisms. Prompted by the implicated role of monoubiquitinated H2A (at lysine 119) in transcription inactivation, the authors asked whether uH2A molecules that contribute to the chromatin landscape surrounding a DSB may also impose effects on gene silencing. Indeed, the DISC effect is abrogated by removing this histone mark through the ectopic expression of a mutant H2A (K119/120R).

The authors further dissect the genetic bases for the DISC response. Based on previous work demonstrating that the E3 ubiquitin ligases RNF8 and RNF168 promote uH2A foci formation at DSBs (Huen and Chen, 2010), Greenberg and colleagues find that codepletion of the two ubiquitin ligases restores reporter activity. Conversely, silencing the deubiquitinase USP16, which resulted in sustained DSB-associated uH2A signal, prolongs the DISC effect. Together, these three lines of evidence strongly implicate a functional coupling between DNA-damage signaling, H2A ubiquitination, and local transcriptional regulation.

Perhaps the most intriguing part of the study is the revelation of a possible functional distinction between monoubiquitinated H2A, which is associated with DSBs, and K63-linked polyubiquitin chains, which are involved in DNA-damage responses. Accumulation of both classes of ubiquitin conjugates at DSBs depends on RNF8 and RNF168. In this model, the uH2A promotes transcriptional silencing, whereas K63-linked polyubiquitin chains recruit checkpoint and repair proteins, including BRCA1 and 53BP1. Although the data presented support the interesting notion that multiple ubiquitin species coexist at DSBs, with each devoted to a specific task, it remains enigmatic why reverting the DISC response requires simultaneous inactivation of both RNF8 and RNF168. Do these enzymes share similar substrates at the damage-modified chromatin? Do they independently target H2A for monoubiquitination?

In addition, although this study unveils a new role of ATM in regulating nascent transcription at sites flanking a DSB, it remains largely speculative how its kinase activity promotes DISC. Given the requirement for uH2A in gene silencing, it is likely that ATM promotes DISC at multiple levels along the DNA damage-signaling cascade (Figure 1), for instance by amplifying the γH2AX signal or by promoting DSB association of uH2A ubiquitinases (such as RNF8 and RNF168). This model predicts that the MRN complex (consisting of Mre11, Rad50, and Nbs1) and MDC1 (mediator of DNA-damage checkpoint protein 1) are required for DISC, seeing as these proteins are critical for ATM activation and accumulation of RNF8/RNF168 foci, respectively.

Moreover, the current dogma holds that chromatin becomes more accessible to DNA repair machineries shortly after DNA damage. In fact, ATM signaling seems to take a major part in ensuring proper repair of DSBs associated with heterochromatin structures as well as promoting global chromatin relaxation following DNA damage (Goodarzi et al., 2008; Noon et al., 2010; Ziv et al., 2006). In addition, signals arising from DSBs are also reported to attract a number of chromatin-remodeling complexes (Misteli and Soutoglou, 2009; Morrison and Shen, 2009). So, how does each of these activities integrate and complement the other to enforce optimal DNA repair and cell survival? How does the chromatin accommodate DISC while allowing access to DNA-damage repair factors? Answering these questions will likely require a combination of biological tools, but such efforts will undoubtedly reveal mechanistic insight into the interplay between DNA-damage repair and chromatin biology.

Finally, what is the biological relevance of DISC? Coupled with the fact that ATM also plays a role in DNA replication checkpoints (Bartek et al., 2004), perhaps this newly discovered strategy for inhibiting transcription at the vicinity of DSBs has evolved to prevent collisions between the cellular machineries for DNA repair, DNA replication, and transcription. What appears more certain is that ATM, with its ever-expanding repertoire of DNA-damage responses, is unlikely to step out of the spotlight anytime soon.

REFERENCES

Bartek, J., Lukas, C., and Lukas, J. (2004). Nat. Rev. Mol. Cell Biol. *5*, 792–804.

Goodarzi, A.A., Noon, A.T., Deckbar, D., Ziv, Y., Shiloh, Y., Lobrich, M., and Jeggo, P.A. (2008). Mol. Cell *31*, 167–177.

Huen, M.S., and Chen, J. (2010). Trends Biochem. Sci. *35*, 101–108.

Janicki, S.M., Tsukamoto, T., Salghetti, S.E., Tansey, W.P., Sachidanandam, R., Prasanth, K.V., Ried, T., Shav-Tal, Y., Bertrand, E., Singer, R.H., et al. (2004). Cell *116*, 683–698.

Misteli, T., and Soutoglou, E. (2009). Nat. Rev. Mol. Cell Biol. *10*, 243–254.

Morrison, A.J., and Shen, X.T. (2009). Nat. Rev. Mol. Cell Biol. *10*, 373–384.

Noon, A.T., Shibata, A., Rief, N., Lobrich, M., Stewart, G.S., Jeggo, P.A., and Goodarzi, A.A. (2010). Nat. Cell Biol. *12*, 177–184.

Shanbhag, N.M., Rafalska-Metcalf, I.U., Balane-Bolivar, C., Janicki, S.M., and Greenberg, R.A. (2010). Cell, this issue.

Weake, V.M., and Workman, J.L. (2008). Mol. Cell *29*, 653–663.

Ziv, Y., Bielopolski, D., Galanty, Y., Lukas, C., Taya, Y., Schultz, D.C., Lukas, J., Bekker-Jensen, S., Bartek, J., and Shiloh, Y. (2006). Nat. Cell Biol. *8*, 870–876.