more sensitive than columnar primitive cells to the noxious effects resulting from the gastroesophageal reflux and chronic inflammatory condition, which likely trigger Barrett's esophagus (Herfs et al., 2009)?

If correct, the model presented by Wang and colleagues would change the view of metaplasia, shifting the focus of attention from "after" to "before the fact." For further "conjectures and refutations," we refer to the authors' interesting discussion of the "competitive or opportunistic cell replacement mechanism," which may be at the basis of Barrett's esophagus and other metaplastic conditions with increased cancer risk.

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Stress Signaling Etches Heritable Marks on Chromatin

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Transgenerational inheritance of epigenetic states allows organisms to pass on adaptive responses to the environment to their offspring. Seong et al. (2011) now reveal how stress-induced signaling through dATF-2 disrupts heterochromatin and leaves heritable marks that influence patterns of gene expression in subsequent generations.

The packing of DNA into condensed and inaccessible heterochromatin exerts a powerful influence on the genome, not only by suppressing transcription but also by governing proper segregation of chromosomes during cell division, silencing transposons and suppressing illegitimate recombination (Grewal and Elgin, 2007; Moazed, 2009). Heterochromatin nucleation involves the parallel actions of both the RNA interference (RNAi) machinery and sequence-specific DNA-binding proteins, and changes in the activity of either of these pathways provide a window of opportunity for chromatin states to change without corresponding alterations in DNA sequence. Such epigenetic changes, if passed to the next generation, may add an almost intangible layer of phenotypic variation at an organismal level. Although much attention has focused on how environmental stresses initiate "a highly programmed sequence of events within the cell that serve to cushion the effects of the shock" (McClintock, 1984), we are only now beginning to learn about how stress modifies chromatin structure, and whether such stress-induced chromatin modification is heritable. In this issue, Seong et al. (2011) show that stress-induced phosphorylation of the transcription factor dATF-2 triggers the loss of heterochromatin structures at several regions of the genome in *Drosophila*, and that the disrupted heterochromatin state is transmitted to the next generation in a non-Mendelian fashion.

Previous work in the fission yeast *Schizosaccharomyces pombe* revealed that the RNAi pathway targets histone H3 lysine 9 (H3K9) methylation and the Swi6 protein (a homolog of animal HP1 proteins) to repetitive DNA sequences for heterochromatin assembly at the centromeres (Volpe et al., 2002). However, in RNAi mutants, heterochromatin assembly can still occur at low efficiency. The transcription factors Atf1 and Pcr1, which belong to the activating transcription factor/cAMP response element-binding protein (ATF/CREB) family of proteins, bind to their recognition sequences and cooperate with chromatin-modifying complexes such as SHREC (SNF2- and histone deacetylase-containing repressor complex) to nucleate and spread heterochromatin assembly independently at the centromeres (Jia et al., 2004). Notably, Atf1 activity is regulated by stress-activated

protein kinases (SAPKs), and it has been long known that stress induces defects in heterochromatin assembly (Jia et al., 2004 and references therein).

The *Drosophila* homolog (dATF-2) of the fission yeast Atf1 binds to the cyclic AMP response element (CRE) and is phosphorylated by SAPKs such as p38. Seong and colleagues (2011) now take advantage of position effect variegation (PEV) of *white* gene silencing as a read-out to determine whether dATF-2 contributes to heterochromatin formation in fly. When a gene is juxtaposed close to heterochromatin, the inactivating influence of the heterochromatin can spread to affect the gene in some, but not all, cells of the same cell type. In the *white* PEV system, variable spread of pericentric heterochromatin gives rise to mottled patterns of eye pigmentation in which some cells express the *white* gene and are therefore red, whereas others do not and are therefore white. The authors found that loss of function of *dATF2* induces derepression of the *white* gene in the PEV assay system. Indeed, dATF-2 colocalizes with HP1 on heterochromatin and binds to CREs in heterochromatin. Together, these data show that CRE-dependent association of dATF-2 with chromosomes correlates with heterochromatin formation.

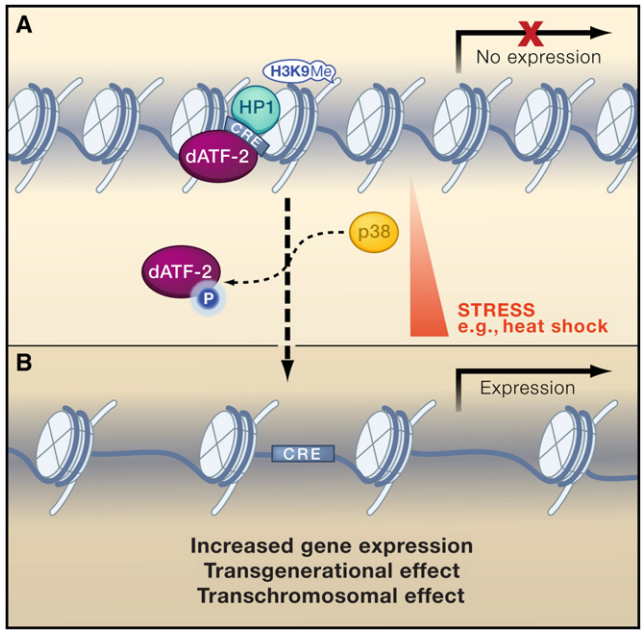

Figure 1. Stress-Induced Regulation of Heterochromatin Formation by dATF-2

(A) In low stress conditions, dATF-2 specifically binds to its recognition sequence CRE (cyclic AMP response element) and likely cooperates with HP1 and chromatin-modifying complexes containing a histone methyltransferase to nucleate and spread heterochromatin assembly.

(B) Upon heat shock or osmotic stress, dATF-2 is phosphorylated by stress-activated protein kinases including p38, which in turn leads to release of phosphorylated dATF-2 from the heterochromatin region. This results in the disruption of heterochromatin and then increases the expression of genes located in the region. This epigenetic change is heritable and occurs by transcommunication between chromosomes.

To determine whether environmental stresses such as heat shock or osmotic stress could alter the binding of dATF-2 to CREs, and whether such alterations lead to changes in chromatin state, the authors examined the phosphorylation of dATF-2 by SAPKs. Heat shock and osmotic stress induce the phosphorylation of dATF-2, which correlates with a reduction in both the binding of dATF-2 to CREs located in the pericentromeric heterochromatin and levels of H3K9 dimethylation at these heterochromatic sites (Figure 1). Chromatin immunoprecipitation assays in cultured cells reveal that osmotic stress reduces the levels of H3K9me2 at heterochromatic sites bound to dATF-2 but not those bound to a mutant dATF-2, in which the p38 phosphorylation sites (two threonines) were replaced by alanines, suggesting that disruption of heterochromatin by stress is mediated by dATF-2 phosphorylation. The authors also show that heat shock during early development (0–3 hr after egg laying), when heterochromatin is established, disrupts heterochromatin formation, which correlates with the release of phosphorylated dATF-2 from heterochromatic CREs. This disruption is independent of RNAi-based mechanisms. Based on these data, Seong and colleagues (2011) propose a new model for how stress induces disruption of heterochromatin: namely, dATF-2 is required for heterochromatin formation, as in the case for its fission yeast homolog Atf1, and p38 and its upstream kinase Mekk1 negatively regulate dATF-2-dependent heterochromatin formation.

The authors found that the effects of stress on heterochromatic reporter genes can be transmitted through the germline. However, the phenotypes examined (eye color and wing notches) persist to a lesser extent in successive generations and disappear unless the stress is repeated. Intriguingly, transgenerational derepression of the *white* gene occurs even when unstressed animals harboring the *white* gene are mated with stressed animals, suggesting that transmission of stress-induced disruption of heterochromatin occurs by transcommunication between chromosomes. This is reminiscent of paramutation, the temperature-sensitive process by which homologous DNA sequences communicate in *trans* to establish meiotically heritable expression states (Chandler, 2010). Genetic screens in plants for mutants unable to undergo paramutation identified several genes with homology to genes that mediate RNA-directed transcriptional silencing, suggesting that the underlying mechanisms regulating paramutation may be shared with those of RNAi.

Finally, to examine "natural" target genes of heterochromatin that are heritably affected by stress, the authors performed a microarray analysis and found that a number of genes involved in various biological functions were upregulated in successive generations of stress-treated flies. We now know that forced changes to the transcriptome dramatically alter cell fate (for example, in induced pluripotent stem cells; Takahashi and Yamanaka, 2006). It will be interesting to discover what happens to the inheritance of the new epigenetic state after multiple stress treatments in successive generations. The work by Seong and colleagues (2011) opens the door to studies of how the environment could influence germ cells in such a way

that epigenetic states and thereby transcriptomes are altered in a directed and heritable way. The work presents an interesting example of the Lamarckian paradigm (Martienssen, 2008; Koonin and Wolf, 2009), in which the environment directs evolution, and induced changes in the epigenetic state may in turn create variations in gene expression to be explored, selected for, and coopted through the generations.

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Stuck in the Middle: Drugging the Ubiquitin System at the E2 Step

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The discovery of a small-molecule allosteric inhibitor of the CDC34 ubiquitin-conjugating enzyme (E2) by Ceccarelli et al. raises the possibility that it will be generally feasible to selectively inhibit ubiquitin transfer at this central step in the ubiquitin pathway.

There is significant interest in targeting the ubiquitin proteasome system (UPS) with small molecules for treatment of diseases such as cancer and neurodegeneration (Bedford et al., 2011). In this pathway, ubiquitin is transferred to substrates through cycles of an E1-activating enzyme-E2-conjugating enzyme-E3 ubiquitin ligase cascade, leading to production of a polyubiquitin chain covalently attached to the substrate that targets it for rapid degradation by the proteasome (Figure 1A). Though molecules targeting the E1 class of enzymes as well as the proteasome are already in the clinic or in trials, the development of inhibitors of components in the middle of the pathway have lagged behind. This is despite the fact that E2s and E3s control the substrate specificity and the type of ubiquitin linkage formed and therefore potentially represent more specific targets for small-molecule-based therapy. In this issue of *Cell*, Ceccarelli et al. (2011) report the identification of the first inhibitor of an E2 enzyme, in this case the human E2 CDC34 (also called UBE2R1), which plays a critical role in the elimination of cell-cycle regulatory proteins by the proteasome (Skaar and Pagano, 2009). Unexpectedly, the inhibitor acts allosterically by binding to a pocket distant from the active site. This study indicates that it is possible to develop a highly selective inhibitor of an E2 enzyme and raises the possibility of targeting other E2s in a similar manner.

The success of proteasome inhibitors clinically has fueled the interest in developing inhibitors that block specific E1-E2-E3 pathways (Bedford et al., 2011). By targeting one or a small number of E3-dependent processes, the effects on overall protein homeostasis may be restricted to the precise pathway intended. CDC34 is one of approximately two dozen ubiquitin E2s in mammals but is unique in that it functions solely with one specific class of E3s: the cullin-RING ligase (CRL). Cullins employ a RING finger protein (RBX1 or RBX2), together with a conserved pocket in cullins themselves, to recruit ubiquitin-charged CDC34 (Deshaies and Joazeiro, 2009; Kleiger et al., 2009). CRL activity also requires modification of cullin by the ubiquitin-like NEDD8 protein, a process that is inhibited by a small-molecule inhibitor of the neddylation E1 enzyme (Figure 1B) (Bedford et al., 2011). As CRLs are responsible for controlling the turnover of cancer-related genes, including the cell-cycle inhibitor p27 (Skaar and Pagano, 2009), this class of E3s has received particular attention in cancer drug discovery.

To identify small-molecule inhibitors of p27 ubiquitination, Ceccarelli et al. employed a fully reconstituted p27 ubiquitination reaction involving E1, CDC34, the E3 $CRL1^{SKP2\text{-}CKS1}$, and p27 in a high-throughput screen, identifying CC0651 as a prime candidate for an inhibitor of this pathway. Given the number of components and the complexity of the process being examined in this assay, CC0651 could block one of numerous steps. Using a variety of related E3 CRL complexes and