enhancer and promoter decreased by around twofold. Conversely, when the distance between the enhancer and promoter increased, transcription stopped. These findings suggest that sustained physical proximity of regulatory elements is required for transcription.

Further characterizing the dynamics of long-range gene regulation, the authors found that a model with three states fit the distribution of distances between regulatory elements. These states represented three functional conformations, concerning physical distance (distal versus proximal) and transcriptional state (on versus off): distal off, proximal off and proximal on. Deletion of individual components in the reporter construct supported this interpretation. Tracking the populations of each state over time highlighted the striking observation that sustained proximity is required to transition into the on state.

The future of live-imaging transcription

Chen et al.[1] demonstrated the power of using in vivo imaging to confirm that enhancer–promoter proximity is required for sustained transcription. These results highlight the relationship between DNA topological association and physical proximity. That is, elements that strongly influence the conformation of chromosomes directly alter gene regulation. The *homie* insulator element chosen by the authors is a particularly strong example of this kind of element[9], as it was able to hijack transcription from the native *eve* promoter to the distal reporter construct, leading to competition between promoters resulting in developmental defects. Furthermore, while many regions in the genome are bound by similar insulator or architectural proteins (i.e., CTCF and BEAF32)[10], it is not clear how many of these regions are functional or how many elements have 'homing' activity. Future work could address the generalities of such insulator function and its dynamics, leading to a deeper understanding of transcriptional regulation.

These findings are consistent with the suggestion that chromosomal boundaries are rather stable, based on the similarity of boundaries across cell types from genomic data[11]. However, the dynamics of the loop structures that form such insulated neighborhoods[10] and the enhancer–promoter interactions within these regions are not yet fully understood. Furthermore, in the *Drosophila* genome, there is evidence for stable, long-range interactions between loci[12], consistent with the findings of Chen et al.[1]. In fact, the majority of enhancer interactions show no evidence of dynamic changes across development[12]. The experimental approaches used by Chen et al.[1] could be applied to study the dynamics of such neighborhoods of gene expression and enhancer–promoter interactions.

Intriguingly, the finding that stable physical proximity of regulatory elements is required for transcription seems to be at odds with recent work demonstrating that active transcription sites are highly dynamic[13–15] and can diffuse more freely[16]. These differences may be the result of the insulator elements inducing a very stable chromatin conformation, working with timing on the order of tens of minutes. In contrast, recent studies focused on transcriptional dynamics occurring in time frames from milliseconds to minutes. Resolving the apparent inconsistencies between the stable, long-range chromatin topologies found by Chen et al.[1] and the dynamic local transcriptional environments may be the key to understanding the mechanisms of transcriptional regulation during development. It is intriguing that many of these results are converging on similarly sized transcriptional 'hubs', which may form transiently on the stable chromatin scaffolds formed by insulator proteins. Ultimately, continued work to connect genomics to these in vivo measurements promises to lead to a better understanding of how the molecular interactions within the nucleus lead to a fully functional organism. ❐

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References
1. Chen, H. et al. *Nat. Genet.* https://doi.org/10.1038/s41588-018-0175-z (2018).
2. Lim, B., Heist, T., Levine, M. & Fukaya, T. *Mol. Cell* **70**, 287–296 (2018).
3. ENCODE Project Consortium. *Nature* **489**, 57–74 (2012).
4. Lettice, L. A. et al. *Hum. Mol. Genet.* **12**, 1725–1735 (2003).
5. Bertrand, E. et al. *Mol. Cell* **2**, 437–445 (1998).
6. Garcia, H. G. G., Tikhonov, M., Lin, A. & Gregor, T. *Curr. Biol.* **23**, 2140–2145 (2013).
7. Saad, H. et al. *PLoS Genet.* **10**, e1004187 (2014).
8. Dubarry, N., Pasta, F. & Lane, D. *J. Bacteriol.* **188**, 1489–1496 (2006).
9. Fujioka, M., Wu, X. & Jaynes, J. B. *Development* **136**, 3077–3087 (2009).
10. Hnisz, D., Day, D. S. & Young, R. A. *Cell* **167**, 1188–1200 (2016).
11. Dixon, J. R. et al. *Nature* **518**, 331–336 (2015).
12. Ghavi-Helm, Y. et al. *Nature* **512**, 96–100 (2014).
13. Mir, M. et al. *Genes Dev.* **31**, 1784–1794 (2017).
14. Cisse, I. I. et al. *Science* **341**, 664–667 (2013).
15. Tsai, A. et al. *eLife* **6**, e28975 (2017).
16. Gu, B. et al. *Science* **359**, 1050–1055 (2018).

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PLANT EPIGENETICS

Plant H3K27me3 has finally found its readers

Two new studies show that a plant-specific complex composed of EBS, or its homolog SHL, and EMF1 acts as a chromatin reader within the Polycomb pathway and effects gene repression. Two domains of EBS and SHL bind distinct chromatin modifications that are associated with active and repressed chromatin.

Kristin Krause and Franziska Turck

Higher eukaryotes share an epigenetic mechanism that allows them to tightly control the expression of genes involved in cell type specification. Within this pathway, organisms ranging from plants to humans share a protein complex, called Polycomb repressive complex 2 (PRC2), which adds an epigenetic mark to nucleosomes in the form of a trimethylated lysine at position 27 of the amino acid chain of histone H3 (H3K27me3)[1,2]. It is assumed that this epigenetic modification is

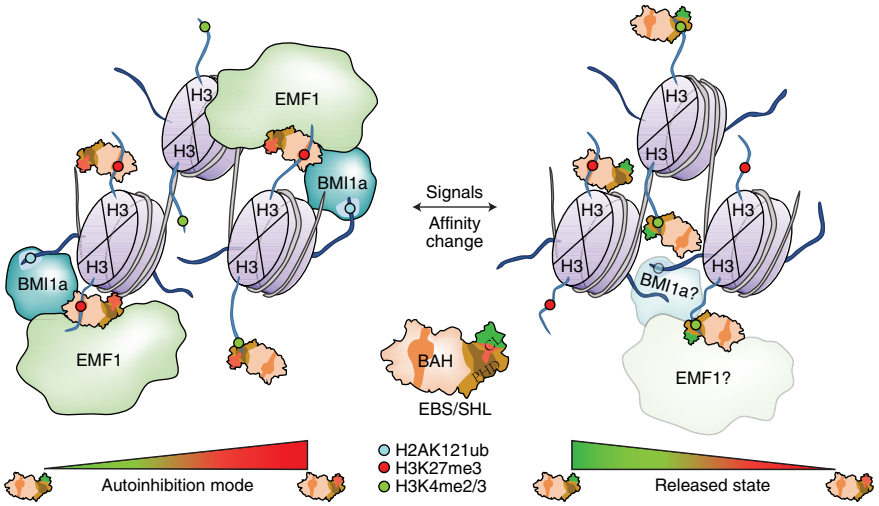

Fig. 1 | EBS and SHL effect Polycomb-mediated gene silencing by binding to H3K27me3 and H3K4me2 or H3K4me3. In the autoinhibition mode, the C-terminal loop (CL; red) folds into the canonical aromatic cage (dark brown) of the PHD domain (ochre), thereby reducing H3K4me2 or H3K4me3 binding and promoting H3K27me3 binding to the aromatic cage (dark orange) of the BAH domain (light orange). EBS and SHL are able to interact with BMI1a and EMF1, which establish H2AK121ub and chromatin compaction. In the released state, the C-terminal loop (green) does not block the PHD domain's binding site, allowing EBS and SHL to preferentially interact with H3K4me2 and H3K4me3. It is unclear whether BMI1a and EMF1 are able to associate in the released state and which signals cause a switch between the states.

interpreted by a chromatin reader complex that contains both components that bind to H3K27me3 and components that effect local chromatin compaction, which is required for stable gene repression. In animals, proteins containing a chromodomain with affinity for the H3K27me3 mark are crucial in establishing a link between the modification and the reader complex. Plants possess a similar chromodomain protein, called LIKE HETEROCHROMATIN PROTEIN 1 (LHP1), that binds to chromatin regions carrying the epigenetic modification; however, loss of LHP1 function only affects a small subset of genes controlled by the Polycomb pathway[1,3].

Neither the animal chromodomain proteins nor LHP1 carry domains that directly affect chromatin state. In animals, chromodomain proteins interact directly with a group of bifunctional proteins carrying domains mediating ubiquitination and chromatin compaction, the BMI1 proteins[2]. In plants, these functions appear to have split into two separate protein groups, one carrying only the ubiquitination function (BMI1-related) and the other, called EMBRYONIC FLOWER 1 (EMF1), carrying an unstructured C-terminal tail that mediates chromatin compaction[4,5].

In this issue, two related reports provide data indicating that, in addition to LHP1, EARLY BOLTING IN SHORT DAYS (EBS) and its homolog SHORT LIFE (SHL) are readers of the H3K27me3 mark in plants[6,7]. Yang et al.[6] provide protein structural data demonstrating that binding of the epigenetic mark occurs through a BAH (bromo-adjacent homology) domain present in these proteins. Li et al.[7] show that gene repression is effected by a complex formed by EBS or SHL, EMF1 and BMI1-related proteins.

EBS and SHL link the Polycomb mark to active chromatin modifications

EBS and SHL are multidomain proteins in which the N-terminal BAH domain is followed by a PHD domain with a C-terminal extension[6]. The PHD domain was previously described to bind to di- or trimethylated Lys4 residues of histone H3 (H3K4me2 and H3K4me3), which represent epigenetic marks associated with actively transcribed genes. It was proposed that EBS recruitment by H3K4me2 or H3K4me3 leads to gene repression via an associated histone deacetylase[8]. By combined structural and biochemical analysis, Yang et al.[6] showed that binding of histone H3 peptides modified at Lys4 or Lys27 is mutually exclusive. EBS protein binds H3K27me3 peptides with higher affinity than H3K4me3 peptides; however, a truncated EBS protein that lacked the C-terminal extension showed increased affinity for H3K4me3. Structural data suggested that this switch in affinity is explained through competitive binding of the C-terminal extension of EBS to the binding pocket for H3K4me2 and H3K4ne3 within the PHD domain. According to Yang et al.[6], a truncated version of EBS poorly complements the early flowering phenotype observed in *ebs* mutants. However, Li et al.[7] showed that natural splice variants of *EBS* and *SHL*, which encode C-terminally truncated proteins, were able to complement the strong phenotype observed in *ebs shl* double mutants.

Interestingly, despite preferred binding of the H3K27me3 modification by full-length EBS in vitro, genome-wide binding data presented by both groups show that the majority of EBS target genes are associated with H3K4me3-marked chromatin. Indeed, a significant overlap with H3K27me3 was only detected for SHL, but not for EBS[7]. These data suggest that, by default, the C-terminal loop is kept out of the H3K4me2 and H3K4me3 binding pocket. Alternatively, other factors may contribute to targeting to specific chromatin regions. In both scenarios, an interacting protein, such as EMF1 or BMI1, could be involved, as protein interactions may affect the conformation of EBS or SHL or determine chromatin targeting.

Despite the sporadic binding to H3K27me3-marked target genes, the functional genetic analysis of morphological and molecular phenotypes performed by Li et al.[7] confirms earlier studies suggesting that EBS and SHL are relevant for repression of PRC2 target genes and that their function is largely redundant[8]. Such redundancy is consistent with the observation that EBS and SHL largely target the same genes. Nevertheless, there is an as-yet-unexplained preference of EBS to enrich at 5′ genic regions and of SHL to enrich at 3′ genic regions, suggestive of subfunctionalization.

EBS, SHL and LHP1 represent potential readers of the H3K27me3 modification. A combination of loss-of-function alleles for all three genes caused enhanced developmental abnormalities in mutant plantlets, resulting in a phenotype similar to that of strong *emf1* mutants. Such abnormalities included the formation of a typical 'Polycomb callus' after prolonged growth of plantlets in vitro. Formation of such a callus is typical for strong PRC2 mutants[1,3]. It is therefore not altogether surprising that the *ebs shl lhp1* triple mutants showed a significant global reduction in H3K27me3 levels[7]. Accordingly, transcriptional reprogramming in *ebs shl lhp1* triple mutants was very similar to that observed in *emf1* mutant seedlings. Importantly, genes that were upregulated in the mutants were often direct targets of EBS,

LHP1 and EMF1, which was not the case for downregulated genes, confirming a predominantly repressive role for EBS.

The BAH–EMF1 complex may not be so plant specific after all

Taken together, plant complexes reading H3K27me3 contain two distinct classes of proteins with affinity for H3K27me3, LHP1 and EBS/SHL, as well as EMF1 and a protein related to BMI1 (Fig. 1). Although these four proteins are specific to plants, their functional domains are old friends and are also encountered in animal H3K27me3 readers such as the canonical PRC1, which contains domains present in LHP1, EMF1 and BMI1[2]. Furthermore, recent reports have shown that animal proteins with a BAH domain, such as human BAHD1, can bind to H3K27me3 and are able to promote chromatin compaction[9], indicating that BAH domains within the Polycomb pathway represent another example of convergent evolution. ❒

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References
1. Mozgova, I. & Hennig, L. *Annu. Rev. Plant Biol.* **66**, 269–296 (2015).
2. Chittock, E. C., Latwiel, S., Miller, T. C. R. & Müller, C. W. *Biochem. Soc. Trans.* **45**, 193–205 (2017).
3. Förderer, A., Zhou, Y. & Turck, F. *Curr. Opin. Plant Biol.* **29**, 169–178 (2016).
4. Beh, L. Y., Colwell, L. J. & Francis, N. J. *Proc. Natl Acad. Sci. USA* **109**, E1063–E1071 (2012).
5. Calonje, M., Sanchez, R., Chen, L. & Sung, Z. R. *Plant Cell* **20**, 277–291 (2008).
6. Yang, Z. et al. *Nat. Genet.* https://doi.org/10.1038/s41588-018-0187-8 (2018).
7. Li, Z., Fu, X., Wang, Y., Liu, R. & He, Y. *Nat. Genet.* https://doi.org/10.1038/s41588-018-0190-0 (2018).
8. López-González, L. et al. *Plant Cell* **26**, 3922–3938 (2014).
9. Bierne, H. et al. *Proc. Natl Acad. Sci. USA* **106**, 13826–13831 (2009).

Competing interests
The authors declare no competing interests.

CANCER GENOMICS

Copy number signatures in ovarian cancer

A new study uncovers novel copy number signatures in ovarian cancer genomes. This work sheds light on mutational processes driving ovarian cancer, reveals the distribution of copy number features across the patient population and identifies new genomic properties related to treatment response.

Sohrab P. Shah

Taming the complexity of ovarian cancer genomes

High-grade serous ovarian cancer (HGSOC) genomes are replete with complex somatic structural alterations[1]. The etiologic nature of these alterations is poorly understood, and their biological and clinical consequences are only minimally defined. Brenton and colleagues[2] have contributed work representing a significant step forward in inferring the mutational processes giving rise to different classes of structural alterations, leading to statistical association with mutated pathways and prognostic association with clinical outcome. Their work provides a new and different viewpoint on the landscape of mutational processes in HGSOC through the lens of copy number signatures (Fig. 1).

Structural alterations typically accrue as a result of endogenous DNA repair deficiency—a defining property of HGSOC. As such, distinct DNA repair deficiencies in ovarian cancers elicit specific patterns of somatic mutation in the corresponding genomes, giving rise to a key concept: the pattern, and not necessarily the gene content, of alteration is a critical property of interest. In ovarian cancer, the genomic consequences of DNA repair deficiency have been shown at nucleotide-level scales in the form of point mutation[3–5] and rearrangement breakpoint[5] signatures through whole-genome sequencing and computational analysis. The most well-known and well-studied example is the scenario in which *BRCA1* and/or *BRCA2* loss leads to a particular form of DNA repair defect known as homologous recombination deficiency (HRD). However, *BRCA1* and *BRCA2* loss only explain some cancers with HRD. Point mutation signatures can reflect HRD[6] more comprehensively and may represent a theoretically meaningful marker of response to new therapeutic agents called PARP inhibitors. The contribution of Brenton and colleagues[2] takes this concept further by focusing on copy number alterations. Copy number alterations represent a subset of structural variations and dominate the landscape of HGSOC genomes[1,3]. Their computational detection from genome sequencing is enabled through the compilation of read abundance over segmental regions of the genome, without a need for the nucleotide-level scales required for identification of rearrangement breakpoints or point mutations. This makes their detection feasible in low-coverage (and therefore low-cost) whole-genome sequencing data. Brenton and colleagues[2] astutely asked whether low-coverage whole-genome sequencing coupled with a new statistical method for computing copy features from these genomes could be used as a route to identifying mutational processes in HGSOC.

The authors' findings contribute both methodological and conceptual advances to the cancer genomics field by introducing copy number signatures, derived from novel 'features' of copy number alterations. Through computation of measurable copy number features informed by hallmarks of known mutational processes, the authors reduce each genome into a features vector that can be decomposed using the popular non-negative matrix factorization (NMF) approach, commonly used in point mutation signature analysis[7]. They contribute two additional novel components: application to cancer genomes from the BriTROC-1 clinical trial led by the investigators and the technique of inferring copy number features from low-pass (0.1× coverage) whole-genome sequencing data. The first point ensures high-quality, uniformly collected clinical outcomes data, while the latter point provides proof of principle that