The emergence of and transitions between distinct phenotypes in isogenic cells can be attributed to the intricate interplay of epigenetic marks, external signals, and regulatory elements. These elements include chromatin remodelers, histone modifiers, transcription factors, and regulatory RNAs. Mathematical models known as gene-regulatory networks (GRNs) are an increasingly important tool to unravel the workings of such complex networks. In such models, epigenetic factors are usually proposed to act on the chromatin regions directly involved in the expression of relevant genes. However, it has been well-established that these factors operate globally and compete with each other for targets genome-wide. Therefore, a perturbation of the activity of a regulator can redistribute epigenetic marks across the genome and modulate the levels of competing regulators. In this paper, we propose a conceptual and mathematical modeling framework that incorporates both local and global competition effects between antagonistic epigenetic regulators, in addition to local transcription factors, and show the counterintuitive consequences of such interactions. We apply our approach to recent experimental findings on the epithelial–mesenchymal transition (EMT). We show that it can explain the puzzling experimental data, as well as provide verifiable predictions.

**Significance**

Epigenetics refers to the role of structural modifications in the genome that encode the context governing gene-expression dynamics. Although there have been attempts at creating mathematical models of these structural changes, how these changes couple to the transcriptional circuitry has remained unclear. Here, we study the role of histone modifications—namely, the adding or removing of methyl groups from histone H3 tails—in interacting with regulatory factors governing the transitions between epithelial and mesenchymal phenotypes. Our results show the critical importance of taking into account off-target global effects due to the competition between different DNA segments for the same histone-modifying enzymes.
In the context of modeling EMT, most of the systems-biology modeling effort has focused exclusively on the transcriptional and translational dynamics (22). Conversely, mathematical modeling of the underlying epigenetic processes has largely been limited to coarse-grained phenomenological approaches (23, 24). Thus, recent high-resolution characterizations of the epigenetic and transcriptional changes during EMT and their response to epigenetic and transcriptional perturbations (4, 6, 25) provide both a need and an opportunity for the development of new models that can shed light on the principles governing the complex transcriptional–epigenetic interplay.

In summary, the aforementioned formulations, both for general GRNs and for EMT, do not consider genome-wide effects. Instead, they focus on the genetically local interactions of the regulatory factors with a single gene or a small set of genes. Although such an assumption might be justified in many cases, this ignores the fact that many regulatory factors, and especially epigenetic ones, act globally and can have hundreds or thousands of targets. Furthermore, such factors compete with each other, and perturbations to the activity or expression level of one of them can have considerable off-target effects, as will be reviewed next.

The Polycomb and Trithorax Groups of EFs

In this paper, we focus on the well-documented antagonism between the Polycomb (PcG) and Trithorax (TrX) protein groups. These protein families modulate histone tails that help maintain genes in silenced or active states and that act globally to regulate numerous cellular processes (26, 27). PcG and TrX act antagonistically, where the first is usually associated with silencing, while the latter is associated with activation (26, 27). For instance, PcG Repressive Complex 2 (PRC2), a PcG protein, is responsible for trimethylating Histone H3 lysine 27 (H3K27) to mark genes for silencing (28, 29). PRC2 has been reported to have more than 1,000 targets in a single human embryonic fibroblast cell (30), and it is estimated to target at least 10% of the genes in embryonic stem cells (ESCs) (31).

On the other hand, the COMPASS family of proteins (a subfamily of TrX proteins) is involved in depositing activating methylation marks. In particular, KMT2A/B (MLL1/2), KMT2C/D (MLL3/4), and SETD1A/B (KMT2F/G) deposit methylation marks at Histone H3 lysine 4 (H3K4) (32). These proteins differ in the genomic region targeted: SETD1A/B trimethylate H3K4 around transcription start sites (33), KMT2C/D monomethylate H3K4 around enhancer elements (34, 35), and KMT2A/B deposit H3K4me2 and H3K4me3 marks at developmental genes (36). It should be noted that these factors often have overlapping effects depending on the context. For instance, knockout of KMT2D causes genome-wide disappearance of H3K4me3 in mice B cells (37) and brain cells (38), and it has been shown to be essential for the maintenance of H3K4me2 marks in mice cardiomyocytes (39). In addition, KMT2D is required for acetylation H3K27 in conjunction with CREBBP and EP300 (40), where H3K27ac is an activating mark that is mutually exclusive with the silencing mark H3K27me3. Similar to PRC2, TrX group proteins act genome-wide. For instance, the transcription of 1,200 genes has been shown to be dependent on KMT2B in mouse ESCs (41), while KMT2D was shown to bind to 4,880 genes in mice cardiomyocytes (39). In summary, PcG and TrX proteins act genome-wide, deposit marks on similar histone sites, and have opposing functions.

EF Competition

As reviewed above, antagonistic EFs deposit functionally opposing histone marks. Do they compete for the same (or nearby) genomic sites? PcG and TrX proteins are recruited to genes by regulatory sequences known as PcG response elements (PREs) and TrX response elements (TREs), respectively (42). Existing evidence in Drosophila shows that PREs are also TREs and that PcG/TrX proteins compete for them (19, 42). In addition, activating methylation marks (e.g., H3K27ac, H3K4me3, and H3K36me3) inhibit PRC2’s ability to methylate H3K27 (43–45). On the other hand, PRC2’s activity reduces the ability of CREBBP/EP300/KMT2D to deposit H3K27ac activating marks (46, 47). While it might be possible for H3K4me3 and H3K27me3 to exist in the same vicinity [a phenomenon known as bivalency (48)], they are mutually exclusive on the same histone tail (49). Alternative mechanisms of competition include PRC2 acting indirectly on nearby nucleosomes by recruiting other factors to remove activating marks (50, 51). Therefore, it is usually assumed that PcG and TrX counteract each other genome-wide (19, 27).

In addition to direct competition with other EFs, PRC2 is antagonistic to active transcription. PRC2 activity leads to chromatin compaction (52), which makes it harder for activating TFs to access their target sites. On the other hand, PRC2 can read the epigenetic context to avoid acting on genes that are transcriptionally active (53–55). Possible mechanisms include PRC2 binding to nascent RNA (56), relative aversion to open chromatin (57), likely competition with TFs (55), or enhancer–PRE communication (58), among others (21).

PcG Dilution and Redistribution upon Suppression of Competitors

Since PcG and its competitors vie for similar genomic sites, knockout of one factor can have far-reaching off-target effects via redistribution or dilution of its competing factors. Therefore, new genes might get activated or silenced. Such knockout experiments have been conducted in the literature with a particular attention to PcG proteins and the corresponding histone mark (H3K27me3). Below, we review experiments that provide evidence supporting the sequestration and redistribution of PcG proteins.

The protein MES4 is an H3K36 methyltransferase that is antagonistic to PcG. In ref. 59, loss of MES4 in Caenorhabditis elegans caused a reduction of H3K27me3 levels (deposited by PcG) at its target sites. Meanwhile, genomic sites that lost the antagonistic mark H3K36me3 gained H3K27me3. Similarly, in mice (60), an H3K36M mutant inhibits the activity of H3K36 methyltransferases. The authors observed sequestration of PRC2, as evidenced by increased levels of chromatin-bound EZH2 and SUZ12 (subcomponents of PRC2). In addition, many genes lost H3K27me3, and their expression levels increased. This indicated that the loss of H3K36 methylation can provide new substrates for PRC2. Redistribution of H3K27me3 was also observed in H3K36M mutants in Drosophila (61). In a recent investigation, rapid depletion of the BAF complex (a chromatin remodeler that is antagonistic to PcG) redistributed PcG from highly occupied domains to new genomic sites in mouse ESCs (62). Additional pieces of evidence are included in Discussion.

Overall, PcG proteins are highly sensitive to perturbations to other EFs and epigenetic marks. Such interventions reshape the global epigenetic landscape leading to aberrant changes in transcription. This, in turn, has been identified as a contributing factor to various malignancies (63, 64).

Organization of the Paper

We first propose a general modeling framework and outline its underlying assumptions based on the experimental literature. We
then show how the epigenetic competition model can explain paradoxical knockout results in single- and multiple-knockout experiments in ref. 6. We then propose a combined model encompassing epigenetic competition and gene regulation to account for different patterns of gene activity in the observed EMTs. Then, we use our model to offer several predictions, some of which have already been verified. We conclude with a discussion that complements our literature review in the introduction and points to new directions. The mathematical details are included in SI Appendix.

### Results

**The Proposed Modeling Framework Accounts for Global Effects.** Consider a GRN consisting of a number of genes interacting with each other via TF binding and/or micro-RNA–mediated posttranscriptional regulation. The interactions can either be inhibitory or activating. A subset of genes in the GRN are also subject to the influence of global EFs, such as histone modifiers (e.g., PcG or TrX). Fig. 1 depicts the schematics of the network. We describe elements of the model next. The mathematical details are included in SI Appendix.

**EFs.** The EFs can generally be histone modifiers, chromatin remodelers, and/or DNAmethyltransferases. We focus here on PcG and TrX EFs and assume the presence of at least one repressing PcG protein and one activating TrX protein. Based on our review of the literature on the antagonism and redistribution of EFs and epigenetic marks, we make the following four assumptions:

1. **Competition:** The EFs compete for binding to similar genomic targets, which could involve in the regulation of the same gene. For example, if a PcG protein binds to a PRE/TRE, or if it deposits a repressive mark, then another TrX protein cannot bind to the same gene, and vice versa. This assumption is justified by our previous review of the literature documenting the antagonism between the two groups of proteins across the genome.

2. **Global targeting:** The EFs have targets genome-wide and are not limited to the local GRN. This is justified by the fact that common EFs are known to have hundreds or even thousands of genomic targets.

3. **Scarcity:** The levels of the EFs are limited. More formally, we assume that the total levels are constant in the time scale of interest. This is justified by our review of the knockout experiments that observe the dilution of the EFs at their original targets and their redistribution to new targets.

4. **Localization:** If an EF is bound to a target on a specific gene, then it cannot simultaneously bind to targets on other genes. This is justified by the observation that EFs (e.g., PRC2) are physically localized to their targets. In addition, the aforementioned dilution and redistribution effects imply that a specific EF complex cannot affect two genes simultaneously.

Thus, when an EF is knocked out, there are sufficient binding sites to sequester the available EFs of another type and make them localize to other genomic loci.

**Genes.** We use a coarse-grained model of the genes. The individual nucleosomes (that serve as substrates for the various histone-modifying enzymes) are not explicitly modeled. Instead, each gene is modeled as a collection of states that account for the possible histone marks, PRE/TRE occupancies, and TF binding sites. Hence, a given gene can be quantified as distribution of the aforementioned states. The rest of the genome is modeled as a single “mega-gene” with a very large copy number compared to the local GRN genes.

**Modeling the PREs/TREs.** The PRE/TRE component allows the EFs to affect the target gene. For a given gene, the PRE/TRE can be in one of the following states (shown in Fig. 1B):

1. **Neutral:** There is no PcG or TrX bound to it, and there are no histone marks.

2. **Active:** Either 1) an activating EF is bound to it (e.g., a TrX protein), or 2) it has an activating histone mark (e.g., H3K27ac or H3K4me3).

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![Fig. 1.](https://doi.org/10.1073/pnas.2210844119)
3. Repressed: Either 1) a repressive EF is bound to it (e.g., a PcG protein), or 2) it has a silencing histone mark (e.g., H3K27me3).

Note that the last two states of the PRE/TRE (Active and Repressed) can have the EF either bound or unbound. The localization effect described earlier arises only for the bound states 2a and 3a. We do not explicitly include a bivalent state, as it can be effectively modeled with a gene whose repressed and active states are both present with nonnegligible proportions.

**Interaction between TFs and PREs/TREs.** Our model also allows for interaction between the PRE/TRE state and TF-binding state, as shown in Fig. 1 B and C. This is motivated by the observation that PcG proteins cannot act on genomic loci under active transcription genes, as we have reviewed earlier. The interaction between PRE/TRE and TF-binding states can be modeled by disallowing a PcG protein from silencing a gene while an activating TF is bound to it. In addition, a TF cannot bind to a gene that has been silenced by PcG. Mathematical details concerning the implementation of this effect are provided in SI Appendix, section 2. Note that such an interaction can create regulatory feedback from the local GRN compartment to the EF compartment—active transcription at the target gene repels repressing EFs. Hence, the aggregate effect of the transcriptional activity of many target genes can alter the global level of an EF.

**The Genome-Wide Competition Context Reverses Expected Knockout Results.** In order to illustrate the model behavior, we consider first a toy example of a single gene that is only regulated by activating and repressing EFs. If the repressive EFs are dominant, then the gene is strongly repressed, while it is strongly active when the activating EFs are dominant. When none of the EFs are present at the gene, we assume that the gene is weakly active. In this scenario, knocking out an activating EF of a gene is expected to reduce expression, while knocking out a repressive EF is expected to increase expression. However, under a competition scenario, opposite effects might occur. We review several cases below.

**Two EFs.** We first consider one activating EF and one repressive EF, as illustrated in Fig. 2 A, B, D, and E. When the competition effects are minimal, knockout of a repressor will induce strong activation of the gene, as shown in Fig. 2A. Similarly, the knockout of an activator will result in strong repression, as shown in Fig. 2D. However, when the two EFs compete for targets across the genome, then knocking out one of them can create many new targets for the competing EF. Depending on the EF’s binding affinity to the gene under consideration, as compared to newly available targets, the competing EF can be diluted genome-wide and redistributed to new targets. This is illustrated in Fig. 2B, where knockout of the repressor does not produce strong activation due to the dilution of the activator. Similarly, knockout of the activator does not produce strong repression due to the dilution of the repressor, as shown in Fig. 2E.

The case with only two EFs is not sufficient to recapitulate all possible outcomes. For instance, it cannot capture a gene that is strongly repressed after the knockout of its only repressor. Such behavior can be recapitulated by a model with three EFs, as we show next.

**Three EFs.** We consider cases with two activators and one repressor and with two repressors and one activator. As reviewed before, there are multiple EFs that have overlapping functions. For instance, H3K4 can be trimethylated by multiple factors. Therefore, the function of a knocked-out EF can be “rescued” by an alternative activator. For example, Hanna et al. (65) showed that H3K4me3 levels are elevated at many genomic locations after the knockout of SETD1B (which is an H3K4me3 methyltransferase) due to compensation by MLL2 (KMT2B),...
an alternative methyltransferase. A similar pattern exists for repressors. For instance, it has been observed that the loss of DNA methylation and H3K9 trimethylation is rescued by silencing via H3K27 trimethylation (66).

In order to illustrate our modeling of the aforementioned behavior, Fig. 2F shows two activators, $T$, $T'$, and one repressor, $P$. Knockout of $T$ and the dilution of $P$ are not sufficient to explain the strong activation of the gene. Instead, the presence of an alternate activator, $T'$, is required for strong activation. To keep $T'$ undiluted, despite the knockout of $T$, the model requires asymmetry between $T'$ and $P$ in terms of their affinity to target sites across the genome. Finally, for completeness, we depict in Fig. 2F the case of two repressors and one activator, wherein the alternate repressor $P'$ rescues the repression of the gene, despite the knockout of the repressing EF $P$. This case corresponds to the experiment reported in ref. 66.

The Model Explains Paradoxical Knockout Results. Our modeling framework can explain multiple counterintuitive results from EF knockout experiments. To illustrate this, we consider the results of knocking out EED (a PRC2 component) and KMT2D (a component of the KMT2D–COMPASS complex) in the HMLE cell line (6). A total of 413 genes were identified as targets of PRC2 that had significant expression in the control or PRC2-KO cells. When examining the changes in the expression levels of these genes upon PRC2 and KMT2D knockout, we found multiple genes with paradoxical changes in expression levels. Below, we will use our modeling framework to interpret the observed behaviors. The balance between the global and local affinity parameters determines the model’s behavior. In order to model the experiments, we study a single constitutively expressed gene that is subject to the effect of three EFs: one repressing ($P$; e.g., PRC2) and two activating ($T$, $T'$; e.g., KMT2D and an alternative activator). Consider the three experimental setups: control, $P$ knockout, and $T$ knockout. Under such an experiment, we are interested in six possible behavioral phenotypes. Each phenotype is characterized by an unambiguous ordering of the expression levels between the three cases. Fig. 3 shows that all phenotypes are possible if the global context is considered. In particular, it is shown that the interplay between global and local affinities of the EFs determines the observed phenotype.

We illustrate our framework by studying specific examples from the results reported in ref. 6. The majority of the PRC2 target genes (67.71%) exhibited their highest expression when PRC2 was knocked out, which is expected if the local context is dominant. One such example is CDH2 (N-cadherin), shown in Fig. 4A. Nevertheless, we still see, unexpectedly, that CDH2 becomes partially activated (compared to the control case) when KMT2D is knocked out. This can be interpreted either as PRC2 not being fully diluted upon KMT2D knockout, due to the weak affinity of the alternate activators (e.g., KMT2C) to CDH2, or a combination of both effects. Next, we study more striking examples.

Activator knockout results in strong activation. In 61 of the PRC2 targets, a paradoxical behavior was reported. The highest expression level was observed when the activating EF KMT2D was knocked out. This includes multiple EMT-related genes, such as TWIST1, ZEB1, ZEB2, and PRRX1. Fig. 4B shows the case of TWIST1 as an example. In the control case, TWIST1 is strongly repressed. When PRC2 (the repressing EF) is knocked out, TWIST1’s expression is increased, but is not strongly activated (when compared to the third case), which is counterintuitive.

| Phenotype Parameters | Expression Level |
|----------------------|------------------|
|                      | Control | $P$-KO | $T$-KO |
| Repressor $P$ | Global Association Ratio | Medium-High | Low-Medium | High |
|                   | Local Association Ratio | Low-Medium | Medium-High | Low |
| Activator $T$ | Global Association Ratio | High | Medium | High |
|                   | Local Association Ratio | Medium | Low-Medium | Low |
| Alternative Activator $T'$ | Global Association Ratio | High | Medium-High | Low |
|                   | Local Association Ratio | Low | Low-Medium | Low |

Fig. 3. The balance between local and global affinities determines the knockout phenotype. The behavior of each EF across the genome is characterized by a mix of local and global parameters. In this table, we consider a single constitutively expressed gene. We consider two parameters for each EF: the local association ratio to the gene of interest and the global association ratio to the rest of the genome. We keep the remaining parameters constant. Each entry in the table indicates the average of the corresponding parameter conditioned on the phenotype under consideration. The three experimental scenarios are: Control (both PRC2 and KMT2D are present), $P$-KO (PRC2 is knocked out), and $T$-KO (KMT2D is knocked out). A more detailed version of this table is provided in Fig. 8.
Fig. 4. Our model explains the differing behavior of PRC2 targets under two knockout experiments, as presented in ref. 6. Under PRC2 and KMT2D knockouts, the panels show our model’s behavior for specific parameter sets that can explain the response of CDH2 (corresponds to the third column in Fig. 3) (A), TWIST1 (corresponds to the fifth column in Fig. 3) (B), and CNTN1 (corresponds to the first column in Fig. 3) (C). A very thick arrow denotes sequestration of the corresponding EF by other genes, a dotted arrow depicts dilution of the corresponding EF, and a lightly shaded arrow denotes an absent regulatory link due to knockout.

The small magnitude of the increase in TWIST1 expression upon PRC2 knockout can be explained by dilution of the activating EFs upon PRC2 knockout. Hence, our model’s interpretation is that TWIST1 is operating at its nominal level without the presence of EF regulators.

The third case is even more surprising, where the knockout of KMT2D (an activating EF) causes TWIST1 to be strongly activated to an expression level that is multiple times greater than its expression level when PRC2 is knocked out. As explained in the previous subsection, PRC2 dilution cannot, by itself, explain this paradoxical disparity. This is since PRC2’s dilution cannot be worse than a full PRC2 knockout. Mathematically, this implies the existence of an alternative activator, $T'$ (e.g., KMT2C), that rescues the expression of TWIST1 when PRC2 is diluted upon KMT2D knockout. This raises the following question: Why is the activating effect of $T'$ only observed when KMT2D is knocked out? One possibility within our modeling framework is that $T'$ binds weakly to targets across the genome compared to $P$. Therefore, when $T$ is knocked out, $P$ outcompetes $T'$ across the genome, and it gets diluted. This leaves $T'$ free to activate TWIST1.

Repressor knockout results in repression. Another paradoxical behavior can be noticed when examining PRC2 targets that have the highest expression level in the control case. Such genes number 46 out of the 413 PRC2 targets. This set includes CNTN1 (Contactin 1) (Fig. 4C), which is highly expressed in the control
case, despite being a target of PRC2. Using our competition paradigm, this can mean that the activating EFs are dominant. Surprisingly, when the repressor PRC2 is knocked out, the expression of CNTN1 is significantly decreased. We interpret this as the outcome of dilution of the activators of CNTN1, caused by the knockout of PRC2. The result is less surprising when KMT2D is knocked out. As seen in Fig. 4B, loss of PRC2’s competitor at CNTN1 allows for stronger repression. Another possible interpretation of the behavior under our modeling framework is that PRC2 is dominant locally, but its affinity to sites across the genome is much higher compared to the activator. Consequently, PRC2 cannot maintain repression of CNTN1 in the control case.

Overall, the power of our model stems from its versatility and its ability to account for local and global effects simultaneously. In the following sections, we will use our modeling framework to analyze the effect of PRC2 and KMT2D knockout on EMT in HMLER cells (6).

**Global EFs Modulate the Behavior of the Local GRN.** Zhang et al. (6) characterized the changes in the expression levels of multiple EMT markers in HMLER cells in response to the knockout of two histone methyltransferases, PRC2 and KMT2D. As shown in Fig. 5A, they reported an increase in the mesenchymal regulators ZEB1 and PRRX1 expression levels upon the knockout of both PRC2 and KMT2D, with a much higher fold change upon KMT2D knockout. Thus, both PRC2 and KMT2D knockout resulted in phenotypic change away from the epigenetic state, although to different extents. In contrast to changes in ZEB1 expression, SNAI1 expression, as compared to the control case, decreased upon KMT2D knockout and was the maximum under PRC2 knockout. This behavior is in disagreement with the traditional transcriptional picture, wherein SNAI1 is believed to activate ZEB1 expression, resulting in a positive correlation between their expression levels (67).

The change in ZEB1 and PRRX1 levels in response to PRC2 and KMT2D knockouts can be easily explained using the framework described in the previous section since both of these TFs are targets of PRC2, as reported by ref. 6. SNAI1, on the other hand, was not identified as a PRC2 target. To explain the unexpected changes in SNAI1 expression under epigenetic perturbations, we must integrate the transcriptional circuit involving ZEB1, PRRX1, and SNAI1 with our model of EF competition, as described below.

**Integration of EFs competition and local transcription regulation.** We consider a system of coupled toggle switches, one involving ZEB1 and miR-200 (67) and another involving SNAI1 and PRRX1 (68). As shown in Fig. 5E, the two switches are coupled via the activation of ZEB1 and repression of miR-200 by SNAI1.

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**Fig. 5.** The proposed modeling framework explains the EMT response under EF knockouts. (A) Experimental RNA-seq results from ref. 6. (B) The miR-200–ZEB1 circuit (67). (C) The PRRX1–SNAI1 circuit (68). (D) Model simulations recapitulate the experimental results in A. (E) Our proposed model combining ternary epigenetic competition with the miR-200–ZEB1 circuit (67, 69) and the PRRX1–SNAI1 circuit (70). P denotes PRC2, T denotes KMT2D, and T’ denotes an alternative epigenetic activator, such as KMT2C.
When PRC2 is knocked out, its targets (ZEB1 and PRRX1) are no longer strongly repressed, and they get modestly up-regulated. However, they do not get strongly activated due to the dilution of their activators (caused by PRC2 knockout). Consequently, the PRRX1–SNAI1 toggle switch will exhibit a high SNAI1 state. However, SNAI1 will be unable to activate ZEB1 in this scenario due to the dilution of ZEB1’s epigenetic activator upon PRC2 knockout. When KMT2D is knocked out, PRC2 is diluted at ZEB1 and PRRX1, and, hence, the alternative activator $T'$ (which can be KMT2C) can fully activate both PRRX1 and ZEB1. The activation of PRRX1 results in the PRRX1–SNAI1 circuit switching to a high-PRRX1, low-SNAI1 state, in agreement with the experimentally reported behavior.

The Local Transcriptional Context Determines the Effect of the EF Activity. In the previous subsection, we have emphasized the effect of EFs on the local GRN. On the other hand, the transcriptional response in a GRN can also be influenced by epigenetic perturbations, mediated by the antagonistic interactions between PcG proteins and active transcription, as shown in Fig. 1B (see SI Appendix for detailed models). To demonstrate this effect, we consider the case of a single self-activating gene, here, ZEB1, as described below.

**Effect of single EF knockouts.** ZEB1 is known to activate its own promoter (67). Therefore, we study the interaction between the self-activating feedback loop and the EF competition circuit. To that end, we consider change in the ZEB1 promoter activation level as a function of ZEB1 concentration under the knockout of individual EFs. The results are pictorially illustrated in Fig. 6A. In the control case (when both PRC2 and KMT2D are present), the activation level of the ZEB1 promoter increases very slowly with the ZEB1 protein concentration due to the inhibitory effect of PRC2. However, if PRC2 is knocked out, we see a sharp, Hill-function-like increase in ZEB1 promoter activation as a function of ZEB1 protein concentration. The activation level is low for low ZEB1 levels due to the dilution of KMT2D and other activators away from the ZEB1 promoter upon PRC2 knockout. However, when ZEB1 is abundantly available at its own promoter, the activation level increases due to the self-activatory loop. In the third case, if KMT2D is knocked out instead, the ZEB1 promoter remains highly active, even at low concentrations of ZEB1, and there is no substantial change in the activation level of the promoter with ZEB1 concentration. This is because KMT2D knockout is accompanied both by the dilution of repressive PRC2 away from the ZEB1 promoter and strong activity of the alternative activator (e.g., KMT2C) at ZEB1. Finally, the alternative activator knockout makes little difference on ZEB1 promoter activation, as compared to the control case. Thus, overall, Fig. 6A shows that epigenetic perturbations do not simply up-regulate or down-regulate their target genes: Such interventions can also change the response function of GRN, as shown here for the case of a GRN involving a single self-activating gene.

**Effect of double EF knockouts.** To further illustrate the complexity of the transcription–epigenetic interplay, we next consider the effect of knocking out two EFs in different orders. Fig. 6C shows that starting from a GRN state with low ZEB1 expression (phenotypically corresponding to an epithelial state), the GRN will switch to a state with only modestly higher gene-expression level (corresponding to a quasi-mesenchymal phenotype). If this is followed by KMT2D knockout, the ZEB1 expression will decrease only slightly. PRC2 knockout, followed by KMT2D knockout, will thus result in epithelial cells switching to a quasi-mesenchymal state. In contrast, if KMT2D is knocked out in epithelial cells, Fig. 6D shows that the cells will switch to a mesenchymal state, one with very high ZEB1 expression. Thereafter, PRC2 knockout will lower the ZEB1 expression only slightly. Thus, double PRC2–KMT2D knockout in epithelial cells will have distinct phenotypic consequences: While PRC2 knockout followed by KMT2D knockout will cause these cells to switch to a quasi-mesenchymal state, KMT2D knockout followed by PRC2 knockout will result in the cells switching to a highly mesenchymal state.

**The Modeling Framework Provides Verifiable Predictions.** In the previous sections, we have developed a model that integrates the local GRN and global EFs. Using our framework, we can provide several predictions regarding the system studied in ref. 6, as discussed below.

**H3K27me3 redistributes and PRC2 is absent at the promoter of ZEB1 when KMT2D is knocked out.** According to our model, an essential mechanism for the activation of ZEB1 and PRRX1 is PRC2’s dilution when KMT2D is knocked out. The model predicts a redistribution of PRC2 across the genome, resulting in the redistribution of H3K27me3 marks. In addition, our model predicts PRC2’s absence at the promoter of ZEB1 and PRRX upon KMT2D knockout. Indeed, it has been observed (6) that PRC2 is absent at the promoter of ZEB1 when KMT2D is knocked out and that H3K27me3 is redistributed to other genomic loci.

**EMT does not occur upon the knockout of the alternative epigenetic activator (e.g., KMT2C).** Our proposed model (Fig. 5E) requires the existence of an alternative activator $T'$. Biologically, $T'$ can correspond to an alternate lysine methyltransferase, KMT2C. Using the same parameters used in the simulations depicted in Fig. 5D, we performed an in silico experiment by knocking out the
investigated as therapy options in cancer (71–73). Fig. 7
inhibitors of EZH2 (a subcomponent of PRC2) are being
we next consider the case of gradual EF knockouts. Indeed,
Instead of the all-or-none knockout experiments analyzed before,
Gradual knockouts of PRC2 and KMT2D have different signatures.
Knocking out PRC2 followed by KMT2D will not convert epithe-
lial cells into a highly mesenchymal state.
Simultaneous knockout of KMT2D and PRC2 will not result in strong
activation of EMT genes. In silico experiments using the same
parameters used for generating Fig. 5D show that the result of
the simultaneous PRC2–KMT2D double knockout resembles
the case of PRC2 knockout far more than the case of KMT2D
knockout; i.e., ZEB1 and other EMT genes are not strongly
activated under simultaneous PRC2–KMT2D double knockout.
Knocking out PRC2 followed by KMT2D will not convert epite-
phelial cells into a highly mesenchymal state.

The activation curves shown in Fig. 6A provide us with a prediction regarding the
cellular response to the PRC2–KMT2D serial knockout exper-
iment: PRC2 knockout will convert epithelial cells to a quasi-
mesenchymal state. Thereafter, if PRC2 knockout is followed by
KMT2D knockout, Fig. 6C shows our model prediction that the
cells will stay in a quasi-mesenchymal state and will not switch to
a highly mesenchymal state.

Gradual knockouts of PRC2 and KMT2D have different signatures.
Instead of the all-or-none knockout experiments analyzed before,
we next consider the case of gradual EF knockouts. Indeed,
inhibitors of EZH2 (a subcomponent of PRC2) are being
investigated as therapy options in cancer (71–73). Fig. 7A shows
that ZEB1 is more active when PRC2 is partially knocked out,
compared to when it is fully knocked out. More precisely, it can be
seen that ZEB1 becomes rapidly activated when PRC2’s presence
fraction goes from 0.6 to 0.5. This rapid activation would indicate
the GRN behavior from a regime dominated by PcG to one
dominated by TrX. However, when PRC2 is fully depleted, the
levels of TrX get gradually diluted away from the ZEB1 promoter,
leaving the PRE/TRE of ZEB1 in an unmodified state and weakly
active.

Our model would suggest that this effect can be more pro-
nounced: Simulations with different model parameters, as shown
in Fig. 7B, would indicate the possibility of a situation where a
small dip in the level of PRC2 can cause a rapid collapse in its
activity at the ZEB1 promoter. This can be contrasted with the
mechanism of activation in the case of a gradual KMT2D
knockout. In that scenario, the activity of the ZEB1 PRE/TRE
builds up slowly as PRC2 gets increasingly diluted and as the
alternative epigenetic activator gets the full chance to activate
ZEB1, as shown in Fig. 7C. From these results, we can make the
counterintuitive prediction that that the partial knockout of an
epigenetic repressor—here, PRC2—can have a stronger repressive
effect on gene activity, as compared to a complete PRC2 knockout.

**Discussion**

In this work, we have described a modeling framework that
combines local transcriptional regulation with global epigenetic
control and showed that complex interplay between transcrip-
tional and epigenetic control can lead to rich gene-expression
dynamics. We have used our modeling framework to understand
the effects of various epigenetic perturbations on EMT, a crucial
cellular process involved in both health and disease. We note
that interplay between epigenetic competition, the EMT tran-
scriptional network, and the baseline transcriptional context can
result in counterintuitive experimental observations and generate
unique paths for cells to transition between epithelial and mes-
enchymal states. Furthermore, our results indicate that the rest of
the genome exerts an indirect effect on the behavior of the local
GRN by competing for the same EFs. So when one of the EFs is
knocked out, the new empty sites across the rest of the genome try
to sequester the other EFs present at the local GRN. As a result,
the local landscape is reshaped, leading to new steady states. A key
property of the model is the asymmetry between the behaviors of
different EFs, as shown in Fig. 3. In other words, the number of
global sites that are made available to a specific EF depends on the
identity of the eliminated and the competing EFs and the local
and global affinities of the considered EFs.

**Further Evidence of Redistribution and Dilution of PcG Proteins.** Our modeling framework relies upon the competition
between PcG and TrX proteins to modify histones at the same
or nearby genomic sites and the redistribution of PcG that
accompanies TrX knockout. Such an effect is not just restricted
to TrX and PcG proteins. Another line of research has investigated
the relationship between DNA methylation, H3K9me3, and
H3K27me3. Despite the fact that all three are silencing marks,
they do not usually mark the same genomic locations. DNA
methylation and H3K9me3 mark constitutive heterochromatin,
while H3K27me3 marks facultative heterochromatin (74, 75).
Induced DNA hypomethylation can cause H3K27me3 to
disappear from its normal locations and accumulate at new
genomic sites in Arabidopsis thaliana (74), mouse somatic
cells (76), and mouse ESCs (41). Hence, inhibition of DNA
demethylation can open up new locations for PRC2 recruitment,

![Fig. 7](image_url)
sequestering it from PcG-repressed genes (41). Similarly, elimination of H3K9me3 has been shown to cause H3K27me3 to disappear from its normal genomic locations and redistribute to new genomic loci in the fungus Neurospora (75, 77). It is worth noting that PRC2 sequestration and redistribution is not limited to competition scenarios. For instance, it has been observed that PRC2 redistributes across the genome upon modifying ATRX, a chromatin remodeler that assists PRC2’s binding (78).

**Disorder in PcG/TrX Proteins Causes Disease and Is a Target for Therapeutics.** Given the global activity of PcG and TrX proteins, it is expected that their knockout will have far-reaching and detrimental effects on cells. Indeed, the inactivation or aberrant activation of such proteins has been shown to play a key role in the emergence of cancer (79). PRC2 has been studied extensively in that context (80, 81), and its catalytic component EZH2 has been tested as a therapeutic target in multiple clinical trials (71–73). Similarly, disorders in COMPASS proteins are very common in cancers (70, 82) and have been proposed as key regulators and potential therapeutic targets (83–87). Our results imply that such therapeutic interventions must proceed with the utmost caution by accounting for the global context.

**Similar EFs Can Play Different Roles.** Our results show that EFs with the same enzymatic activity, such as KMT2C/D, both of which deposit the same methylation mark on histone tails, can exhibit very different biological and functional behaviors. This has been reported in multiple experimental contexts. For example, Zhang et al. (6) found that while KMT2D knockout could induce transition to a mesenchymal state in HMLER cells, KMT2C was not identified to be among the key EMT inducers. Similarly, in MCF10A cells, TGF-β–induced EMT is accompanied by the up-regulation of the H3K27me3 demethylase KDM6B, while the enzymatically similar KDM6A is down-regulated during the process (88).

**Competition Effects in Molecular Biology.** Competition effects have been studied earlier in the context of synthetic biological circuits, where circuits compete for RNA polymerases and ribosomes. It has been shown that such competition can cause nontrivial coupling between isolated components and affect protein-expression performance (89, 90). It has also been investigated in the context of the design of Boolean genetic circuits via CRISPRi, which uses dCas9 as a shared resource (91). More detailed models of messenger RNA’s competition for ribosomes during the transcription process have also been proposed (92, 93). Similarly, competition for the same micro-RNAs between transcripts that have the same or similar micro-RNA–binding sequence motifs has been shown to induce coupling between the expression levels of seemingly independent proteins (94, 95).
Experiments Involving Epigenetic Perturbations Must Be Analyzed with Care. One crucial takeaway from the analysis presented in this manuscript is the possibility of widespread cross-talk between the genomic targets of different EFs. Most experimental studies analyzing the effect of epigenetic perturbations follow a set procedure: Characterize the transcriptional profiles before and after knocking out an epigenetic modifier, identify the set of differently expressed genes (which often number in the hundreds), and carry out gene-set enrichment analysis (96) or Gene Ontology enrichment analysis (97) using the differently expressed genes. In light of the framework described here, it is unsurprising that the outcome of epigenetic perturbations is hundreds of differently expressed genes, a list that is then arbitrarily whittled down, depending on the biological interests of the researchers carrying out the analysis. This is usually followed by choosing a pathway or biological process of interest and analyzing how it is affected by the given epigenetic modifier. One would then conclude with identifying that epigenetic modifier as a key regulator of that biological process. Our analysis shows that any such conclusion could be highly unreliable outside the context of the specific experimental setup. For example, in the study by Zhang et al. (6), SNAI1 expression is up-regulated upon PRC2 knockout, even though it is not a direct target of PRC2. While a simple differential gene-expression analysis might lead one to identify PRC2 as a key regulator of SNAI1 expression, our analysis shows that the effect of PRC2 on SNAI1 expression can only be explained by the complex interplay between epigenetic control and the GRN involving SNAI1, ZEB1, and PRRX1. Thus, PRC2 knockout may have no effect on SNAI1 expression in cells wherein the SNAI1–ZEB1–PRRX1 GRN is inactive. Moreover, we show that the effect of epigenetic perturbations on gene expression will depend on the cell's transcriptional state at the time of the epigenetic perturbation. This would suggest that the same epigenetic perturbation could have very different effects on genotypically identical cells in different phenotypic states. For example, PRC2 knockout has been shown to trigger differentiation in primed mouse ESCs, but not in naive ESCs (98). Thus, careful analysis of the effects of epigenetic perturbations will also require that the effects be analyzed within the transcriptional context of the cells.

Materials and Methods

Datasets. The datasets used in this study include the full RNA-sequencing (RNA-seq) data sheet and the list of PRC2 targets for the experiments reported in ref. 6. The data have been provided by Y.Z., who is the lead author in the aforementioned study.

Mathematical Modeling. The details of the mathematical models and numerical simulations are provided in SI Appendix and are very briefly summarized here.

Constitutively expressed genes subject to EF competition. In SI Appendix, section 1, we describe the reaction network models for a general network of N EFs and n genes. We show that each interaction of an EF with a gene can be characterized by two parameters: the association ratio and the marking ratio. In particular, the results in Fig. 3 are generated by studying the effect of the parameters on the three experimental scenarios discussed in The Model Explains Paradoxical Knockout Results. A more detailed version of Fig. 3 is shown in Fig. 8.

Self-activating genes subject to EF competition. In SI Appendix, section 2, we provide our mathematical model for the interaction between the transcriptional and epigenetic components of regulation for a group of n self-activating genes and N EFs. We illustrate this numerically by showing the effect of EF perturbations on the activation function of a single self-activating gene.

A general local GRN subject to EF competition. In SI Appendix, section 3, we model a generic local GRN with TF and micro-RNA regulations subject to EF competition. We provide our concrete model for the network in Fig. 5E, along with the parameters utilized in the simulations.

Parameter selection. The feasible parameters are not unique. The simulation parameters have been chosen to reproduce the qualitative behavior of the experimental results. For the epigenetic competition circuit, the parameters have been chosen based on a screen similar to those shown in Fig. 8 and SI Appendix, Tables 1–3. The parameters of the local GRN have been chosen by refining an initial parameter set generated by the software package RACIPE (9). The parameters are listed in SI Appendix.

Software. Numerical simulations have been performed via MATLAB R2020a on the discovery high-performance computing cluster at Northeastern University.

Data, Materials, and Software Availability. Previously published data were used for this work (GSE158115) (99). The code used to generate the figures is posted at https://github.com/malirdwi/EpigeneticFactorCompetition.

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