Hit-and-Run leaves its mark: Catalyst transcription factors and chromatin modification

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Understanding how transcription factor (TF) binding is related to gene regulation is a moving target. We recently uncovered genome-wide evidence for a Hit-and-Run model of transcription. In this model, a master TF hits a target promoter to initiate a rapid response to a signal. As the hit is transient, the model invokes recruitment of partner TFs to sustain transcription over time. Following the run, the master TF hits other targets to propagate the response genome-wide. As such, a TF may act as a catalyst to mount a broad and acute response in cells that first sense the signal, while the recruited TF partners promote long-term adaptive behavior in the whole organism. This Hit-and-Run model likely has broad relevance, as TF perturbation studies across eukaryotes show small overlaps between TF-regulated and TF-bound genes, implicating transient TF-target binding. Here, we explore this Hit-and-Run model to suggest molecular mechanisms and its biological relevance.

Keywords:
dynamic regulation; gene regulatory networks; "Hit-and-Run"; TF binding; transcriptional model; transcriptional regulation

Introduction

Dynamic action of transcription factors (TFs) and associated changes in chromatin state has emerged as a very active field of research [1–6]. Studies of individual TFs in yeast [3], Drosophila, human [5] and Arabidopsis [7] have identified similarities in mode of action, wherein a TF can affect changes in gene expression long after its own attachment and dissociation from the target gene. With advances in transcriptome sequencing and high-resolution imaging technologies of single TF molecules, many of these studies have focused on the binding activity of individual TFs [2, 5, 8–10], and the resultant alteration of target gene expression [7, 11]. We recently reported that dynamic binding of bZIP1—a TF implicated in nutrient signaling in plants [12, 13]—triggers rapid and transient genome-wide responses to a nitrogen nutrient signal [7]. Our study which was performed by TF perturbation in isolated root cells where the nitrogen signal is first perceived, allowed the identification of direct bZIP1 targets based either on genome-wide expression or TF-binding profiles, assayed simultaneously. We captured transient TF-target binding, and showed that bZIP1 was bound to the promoter of a set of transient targets within 1 and 5 minutes of nuclear entry, but not at later time-points (30 and 60 minutes) [7]. Surprisingly, these targets regulated by the transient binding of bZIP1 (within 1–5 min) were actively transcribed 5 hours later when the TF was no longer bound. Furthermore, these transient TF targets had an over-representation of non-bZIP1 cis-elements in their promoters [7]. These cis-elements constitute potential binding sites for partner TFs that might take over the transcriptional control after bZIP1 has left its binding site.

Our genome-wide findings for the transient targets of bZIP1 invoked a classic, but largely forgotten "Hit- and-Run" model of transcription, proposed by Walter Schaffner in the 1980's [14]. This model posits that a TF can act as a trigger to assemble a stable transcriptional complex that includes RNA Polymerase II and possibly other TFs, enabling transcription to continue even when the founding TF is no longer bound to the DNA. Since that initial hypothesis [14], the "Hit-and-Run" mode of transcription has been invoked to explain observations of sustained transcriptional activity at the unbound promoter of individual TF target genes [8, 15]. Our study [7] provides the first genome-wide evidence for this "Hit-and-Run" model, showing that a

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Abbreviations:
TF, transcription factor; **TSS,** transcription start site.

single master TF (bZIP1) can initiate changes in the expression of hundreds of genes, whose regulation persists after the initiating TF dissociates from the target promoters.

The purpose of this essay is to propose an explanation for the mechanistic basis for this "Hit-and-Run" model, and to infer how this rapid transcriptional activation in root cells—where the N-signal is first perceived—can contribute to long-term biological responses in a whole plant. Explicitly, we propose that this mode of transcriptional regulation [7, 14] may involve alteration of the chromatin status of the promoters in the target genes of a master TF. Further, we propose that these altered promoters have an increased competence to bind secondary TFs that can then modulate their expression level [5]. Moreover, as TF perturbation studies across eukaryotes show a large discrepancy between the TF-regulated and TF-bound genes [16–18], this transient mode of TF binding is likely to have broad relevance beyond plants. We posit that this "Hit-and-Run" mode of TF action enables a TF to act catalytically to mediate rapid, yet sustained responses to external or internal stimuli.

"Hit-and-Run" transcriptional regulation

Molecular evidence for the "Hit-and-Run" model of transcriptional regulation was uncovered at the single target gene level in animals [15], and more recently at a genome-wide scale for the TF bZIP1, a central integrator of metabolic signaling in plants and other eukaryotes [7]. Para et al. studied the role of bZIP1 in the context of a nitrogen (N) signal and surprisingly found that the transiently bound bZIP1 targets were most relevant to N signal transduction [7]. Paradoxically, the stably bound and regulated targets are the focus of most TF studies. However, a focus on the stably bound targets would have missed the role of bZIP1 in N signaling. An additional paradox was that the majority of the regulated bZIP1 targets were found to be still transcriptionally activated or repressed after the bZIP1 TF was no longer bound to their promoters [7]. Here, we explore the mechanisms by which this prolonged transcription can occur following transient bZIP1 binding.

Recent studies on the nature of transcriptional activation/repression suggest that the DNA binding activity of TFs is highly dynamic [19], and can be best described as quantitative continua [20]. Under this emerging paradigm, TFs bind to and are released from their target sites on a time-scale of seconds [20]. As the number of TF molecules increases, the effective "bound" time increases, and thus results in higher levels of activation of the target genes [20]. This quantitative model relies on the increase in available TF protein levels to cause significant target activation. By contrast, the "Hit-and-Run" model we present in Para et al. [7], relates to the rapid and robust activation of target genes, without requiring substantial increases in the TF protein level. The immediate advantage of such a "Hit-and-Run" mechanism for a master TF is that the signal transduced through the TF is not delayed by the kinetics of de novo protein synthesis required to generate enough TF molecules (e.g. one dedicated to each gene). The second advantage of this mechanism is that the relatively small number of master TF molecules allows a rapid shutdown through a competing or attenuating signal. We note that the "Hit-and-Run" model does not preclude the effect of post-translational modifications on the gene network. In fact, the TF partners that take over the regulation of the target genes might themselves be activated by interacting with bZIP1 or through other post-transcriptional modification pathways originating from the external signal.

As demonstrated for bZIP1, a TF can also cause activation of a target resulting from a hit within 1–5 minutes [7], and the active transcription can occur hours after it dissociates from the target (i.e. after it has "run"). Therefore, the question we address in this essay is: how can a TF generate long-term changes in the expression of its target genes, despite only interacting with their promoters in the initial 1–5 minutes after nuclear entry. Below, we review several molecular explanations for continued transcription of TF targets after the master TF has "run" to its next target, and the underlying evidence.

Is continued regulation of "Hit-and-Run" targets mediated through proximal binding sites of partner TFs?

Another surprising discovery reported in Para et al., was that the group of transient targets under "Hit-and-Run" control by bZIP1 is larger (781 genes) than the stably bound targets (120 genes), the latter typically considered the "gold standard" targets (TF-bound and TF-regulated at the same time-point) [7]. This finding implies that the majority of N-responses mediated by bZIP1 involve: transient TF-target associations but prolonged transcriptional responses. How might this occur? As the promoters of these transient bZIP1 targets are enriched in cis-elements for other TFs [7], this provides support that these secondary TFs are potentially recruited by bZIP1, as postulated in the original "Hit-and-Run" model [14]. One potential mechanism for continuous activation of target genes is that the transient bZIP1 "hit" to a promoter alters DNA accessibility to favor the binding of additional TF regulators. Within this model, the effect of the master TF is binary (i.e. "on/off switch"), while the secondary TFs would potentially act in a more traditional dosage-dependent manner (i.e. "dimmer switch") as described by Biggin [20]. A possible mechanism for the bZIP1 induced recruitment or activation of these TF partners is depicted in Fig. 1 and discussed below.

A "hit" by a "catalyst TF" might mediate changes in the histone acetylation state of promoters

How can a transient "hit" by a "catalyst TF" potentiate transcription that continues after it has "run" to its next target? One potential mechanism which we posit in this essay is that the "hit" of the "catalyst TF" initiates epigenetic changes in the promoters of its transient target genes. Support for this hypothesis comes from the interplay of TFs and chromatin modification complexes which has been discovered repeatedly

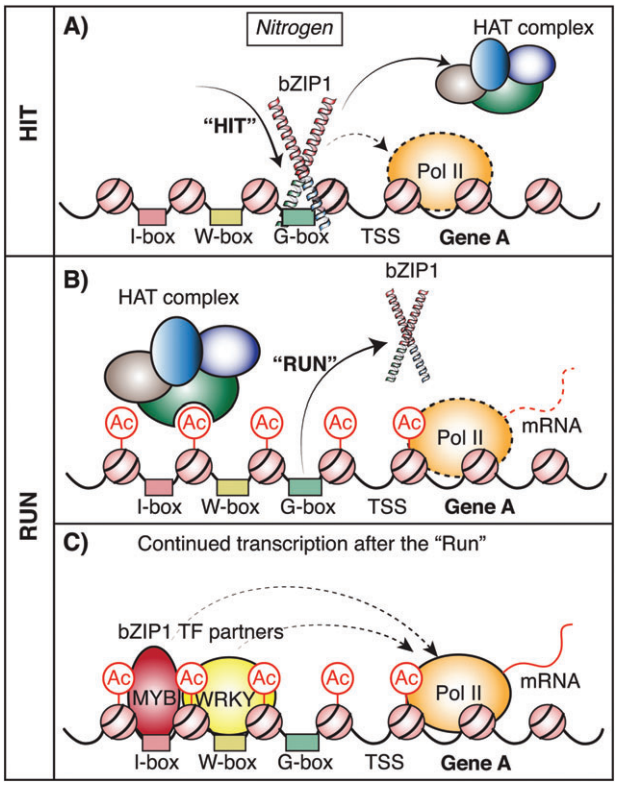

Figure 1. "Hit-and-Run" transcription: A proposed mechanism to explain how transcription initiated by the bZIP1 transient "hit", can continue after the "catalyst TF" "runs" off to its next target [7]. Our hypothesis in this essay proposes that when bZIP1 transiently "hits" a target, it recruits a chromatin modifier, Histone Acetyl Transferase (HAT complex) (Panel A). Next, the HAT complex acetylates histones associated with the promoter of bZIP1 transient targets (Panel B). This histone acetylation at the transcription start site (TSS) of the promoter region can provide accessibility for partner TFs, which would enable transcription of the transient bZIP1 targets to continue after the "catalyst TF" moves on to its next target (the "run") (Panel C). In the specific example of bZIP1 [7], along with the known bZIP binding site (G-box [12]), other cis-elements including W-Box and I-Box motifs are over-represented in the promoters of hit-and-run targets. These additional cis-motifs were previously shown to bind WRKY [41] and MYB [42] TFs respectively, implicating bZIP1 "partners" involved in continued transcription after bZIP1 has "run" to its next target in the genome.

across unicellular (yeast [3]) and multicellular eukaryotes (Drosophila [4], mammals [5], and Arabidopsis [7, 21]). TFs have been shown to recruit histone methylation, acetylation, and deacetylation complexes to the promoters of their target genes and establish an altered state of transcriptional competence for these genes [1].

According to this hypothesis, a specific "hit" by the bZIP family of TFs may alter the histone acetylation status immediately upstream of the transcription start site (TSS) of the target genes (Fig. 1). Evidence of such a mode of action was recently uncovered for bZIP11, another S1 bZIP family member that can dimerize with bZIP1 [22]. bZIP11 has been shown to recruit the histone acetylation machinery (HAT) to activate the promoters of known target genes [23]. Similarly, the human bZIP proteins, JDP2, and ATF3, were shown to recruit the histone deacetylase machinery (HDAC) to repress the expression of their targets [24]. These two studies demonstrate the ability of the bZIP family of TFs to recruit either the HAT machinery associated with transcriptional activation, or the HDAC machinery associated with repressing the expression of target genes. The idea of TFs altering the histone code is well supported by parallel studies in the mammalian TFs Sox2 [25] and FoxA1 [26]. Both Sox2 [25] and FoxA1 [26] TFs recruit the histone methylation apparatus to deposit a methylation mark on H3K4 that primes their target genes for activation.

Changes in the histone acetylation status of a promoter can alter its accessibility for TF regulators [27] by altering the chromatin state of the promoters. The increased availability of the promoters could represent an obvious mechanism for a "catalytic TF" (e.g. bZIP1) to recruit additional partner TFs through epigenetic modifications. In effect, this acetylation state would enable bZIP1 to indirectly leave its "mark" on its target genes, and ensure that their expression could persist long after its dissociation from the promoter (Fig. 1B). Support for the model of bZIP-mediated changes in histone acetylation is provided by the observation that the G-box recognition site for bZIP1 [12] lies in closer proximity to the TSS of its target genes than the cis-elements of the recruited partner TFs (Table 1) [7]. This proximal position of bZIP1 binding to the TSS would enable the "catalyst TF" to alter acetylation of histones directly upstream of the G-Box in the promoter and downstream into the start of the gene. Indeed, histone acetylation is known to generally peak around the TSS for active genes [28, 29]. Therefore, a bi-directional increase in histone acetylation that is centered at the bZIP1 binding site proximal to the TSS would simultaneously increase the accessibility of the secondary TF recognition sites and the TSS. As a result, a promoter organization favoring the placement of bZIP binding sites closer to the TSS and the secondary TF sites more distally (Table 1), could have been selected during plant evolution. Such changes in histone acetylation status of the target promoter, would alter the local chromatin structure, resulting in repressive or permissive chromatin configurations for the binding of the other recruited TF partners [30] and the resulting transcription machinery (Fig. 1C). Again, the concept of TFs acting cooperatively on a related set of promoters was recently reported in mammalian adipogenesis [31], suggesting that the basic mechanisms of transcriptional regulation are broadly conserved across eukaryotes.

Once the chromatin status of the promoter of the transient TF target gene is altered by the TF "hit" (Fig. 1A), the "catalyst TF" is then free to dissociate

Table 1. The bZIP1 "catalyst TF" binds most proximal to the TSS, compared to its potential recruited partner TFs

Cis-regulatory motifs over-represented in bZIP1 transient targets	Median distance to TSS (bp)
bZIP1	
G/C-Box (bZIP binding)	160
Partner TFs	
I-Box (MYB binding)	370
SORLIP1 (unknown)	268
W-Box (WRKY binding)	312

Proximity of TF binding sites in bZIP1 transient targets [7] relative to the canonical transcription start site (TSS) is shown. The known bZIP1 binding site G/C Box [12] lies closer to the TSS, compared to the secondary TF binding sites that are specifically enriched in the promoters of the transient targets of bZIP1 [7], as also depicted in Fig. 1.

from the promoter (Fig. 1B). The assembly of the transcriptional machinery (RNA Pol II complex) may be initiated by the master TF (Fig. 1A) and/or by the secondary TFs after the master TF "runs" (Fig. 1C). In both cases, the master TF is responsible for recruitment of the HAT complex. The released "catalyst TF" molecule is then available to interact with the promoters of other target genes, to initiate transient binding, or to become part of a stable transcription initiation complex. Within the current paradigm of transcription dynamics as quantitative association continua [19, 20], the "Hit-and-Run" mechanism can be viewed as a means by which transient association of low-levels of a TF could support large expression changes. As discussed in Para et al. [7], this mechanism is distinct from TF "tread-milling" where transient binding results in low level expression [11]. The "Hit-and-Run" mechanism would also eliminate the dilution effect inherent to the association of a few TF molecules with a very large number of recognition sites in the genome [19].

The "run": Does nucleosome positioning affect transcription factor dissociation?

Single molecule studies have demonstrated that TF binding to DNA is a dynamic process with rapid association and dissociation events [11, 19]. The "run" portion of the "Hit-and-Run" model is distinct in that once bZIP1 dissociates from the promoter the TF does not bind again to the promoter [7], suggesting that re-establishment of binding is inhibited in some fashion. A recent study on the effect of nucleosome positioning relative to TF binding site [2], offers insights into a possible mechanism to explain the hypothesis that bZIP1 re-binding to its transient target is hindered. This study showed that when nucleosomes are positioned at the TF binding site the TF binding affinity is reduced 500-fold, and TF dissociation rate is increased 1,000-fold. Also, nucleosome positioning is heavily influenced by histone acetylation [32] and therefore a TF that recruits the Histone Acetyltransferase (HAT) machinery would affect nucleosome positioning. A recent study [23] on the recruitment of the HAT machinery in Arabidopsis showed that multiple members of the bZIP S1 subfamily bind the ADA2b protein and recruit the HAT complex. The ADA2b protein is a known transcriptional coactivator that physically interacts with the GCN5 protein in the HAT complex and plays a role in H3 and H4 acetylation in Arabidopsis [33, 34]. Although this study [23] found no evidence for bZIP1 binding to ADA2b, it found strong evidence that a closely related family member, bZIP11, binds ADA2b and recruits the HAT complex. The bZIP TF family has been reported to act through extensive homo- and hetero-dimerization networks in humans [35] and plants [22]. The plant study in particular showed that bZIP1 does form dimers with bZIP11 [22]. Therefore, bZIP1 may, through dimerization with bZIP11 and/or bZIP44 [22], recruit the HAT complex, and alter the histone code of the transient promoters to which it binds. The acetylation of such promoters would lead to two effects: (i) Activate the promoters to allow continued binding by secondary TFs and (ii) alter the histone mark on its own binding site leading to heightened dissociation of bZIP1 from these promoters.

Early and transient "Hit-and-Run" targets of bZIP1 detected in isolated cells, mediate long-term downstream responses in planta

Finally, what are the in planta biological implications of the "Hit-and-Run" transcription mechanism? These rapid and transient bZIP1 targets captured specifically in root cells (where the N-signal is first sensed), are the intermediates that control long-term nitrogen responses in plants, as described in Para et al. [7] and expanded on below. Acting as a "catalyst TF", bZIP1 transiently binds to the promoters of other TFs (e.g. LBDs [36]) to initiate a transcriptional cascade in response to a nitrogen nutrient signal [7]. These early and transient TF targets of bZIP1 are in turn relevant to the downstream nitrogen responses mediated by bZIP1 in planta (including N-induced changes in development), as evidenced by transgenic and mutant studies [12]. In one example, the transient bZIP1 TF targets (LBD38 & 39) detected in isolated root cells [7], have themselves been shown to mediate changes in nitrogen uptake and assimilation and root development in planta [36]. In another example, WRKY70 is regulated by bZIP1 in a "Hit-and-Run" manner [7]. This activation of WRKY70 by a bZIP1 transient "hit" will lead to the downstream, long-term effect on plant growth as described in a functional analysis of WRKY70 [37]. Thus, the rapid and transient targets of bZIP1 captured in isolated root cells [7] include important TFs that are regulators of the downstream processes in planta, to mediate changes in growth

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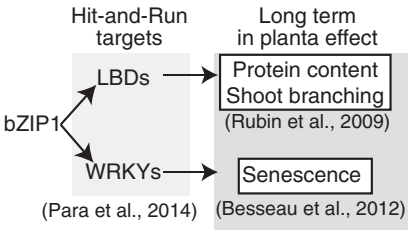

Figure 2. Transient "Hit-and-Run" targets of bZIP1 detected specifically in root cells are early responders that mediate bZIP1 downstream responses in planta. Rapid and transient "Hit-and-Run" targets of bZIP1 detected in isolated root cells [7] include TFs such as specific members of LBD and WRKY families [7]. These LBDs and WRKYs have been shown to mediate life-time traits of plants such as total protein content, shoot branching patterns [36] and leaf senescence [37].

and development in response to the changing nitrogen environment (Fig. 2) [12].

"Hit-and-Run" enables a TF to act as a "catalyst TF"

Another biological implication of the "Hit-and-Run" model is that it can enable a small number of TF molecules to act catalytically to rapidly initiate a large genome-wide response. The bZIP family of TFs, especially the S-group of bZIPs that includes bZIP1, is post-translationally modified in response to nutrient sensing [13]. This post-translational mechanism can therefore allow existing TF molecules to be activated and rapidly initiate broad responses to the nutrient signal. The combined advantage of a post-transcriptional activation mechanism coupled with a "Hit-and-Run" activation mechanism, allows an organism to rapidly alter its genetic program with a small number of TF molecules and without the delay of a multi-step transcriptional cascade.

Experimental approaches to determine the histone code during "Hit-and-Run" regulation

The hypothesis put forward herein, that the "Hit-and-Run" mode of transcriptional regulation involves changes in the histone code, is readily testable. The primary prediction of this hypothesis is that the acetylation status of histones associated with the bZIP1 transiently bound promoters (e.g. Class III transient bZIP1 targets in Para et al.) [7] is altered shortly after activation by the TF "hit" (Fig. 1). In Para et al., [7] the moniker "Class III targets" refers to the set of genes whose promoters are transiently bound by the catalyst TF but continue to be differentially regulated after the catalyst TF is no longer bound to the promoter. Specifically, the histones in close proximity to the known bZIP1 binding sites [7] would be hypothesized to show an increased acetylation signal after bZIP1 binding. Therefore, a time-course assay of the genome-wide histone acetylation status following induced TF nuclear localization in the cell-based TARGET system [7, 38], would reveal temporal changes in histone modifications specifically associated with the promoters of bZIP1 transient targets. The highly transient nature of such TF interactions would be difficult, if not impossible, to capture through biochemical techniques such as immunoprecipitation, since the time required to fix intact tissues would exceed the interaction time of the TF and its target. Nonetheless, the acetylation footprint of the TF should be detectable after the TF is released.

In the model proposed herein, the "catalyst TF" recruits specific HATs to the activated set of targets, and specific HDACs to the repressed set of targets. Therefore, the role of the histone acetylation in the "Hit-and-Run" mechanism can be validated through the global study of bZIP1 targets in HAT and HDAC mutant backgrounds.

Catalyst versus pioneer modes of TF action

Finally, some parallels in the mode-of-action can be drawn between bZIP1, a "catalyst TF" that responds to environmental cues in plants [7], and "pioneer TFs" such as Zelda in Drosophila [4] and FoxA1 in mammals [26, 39] that respond to developmental cues. Both models invoke a master TF that affects a large set of genes by altering promoter accessibility through changes in chromatin status. Recently, it has been shown that Zelda binds to thousands of genes and modifies their chromatin accessibility, leading to early and robust activation of these genes by the TF Dorsal during early embryo development [4]. Similarly, a sequential and simultaneous binding pattern of multiple TFs leads to adipocyte differentiation in mammals [31]. A recent, study of pioneer TF binding to closed chromatin regions details the ability of a set of pioneer TFs (FoxA, Oct4, Sox2, Klf4, and c-Myc) to bind partial sites accesible on nucleosomes [40]. Nonetheless, crucial differences remain between the "Hit-and-Run" regulation by the bZIP1 master TF and the pioneer TFs such as Zelda and FoxA1. The most glaring difference is that the pioneer TFs are stably bound to their targets through the relevant developmental stage, while bZIP1 transiently binds to its targets. Also, while pioneer TFs are typically developmental regulators of early embryogenesis or cell differentiation, bZIP1 is probably one of many master TFs that exhibit this "catalyst TF" mode-of-action in response to environmental cues.

Conclusions and outlook

In summary, we recently reported genome-wide evidence for a "Hit-and-Run" model of transcriptional control. In this model a "catalyst TF" initiates transcription of a target by the "hit", recruits partners and then "runs", leaving its partner TFs to continue the work it started. Here, we further explored this proposed "Hit-and-Run" transcription model and discussed its possible molecular mechanism and biological relevance. The hypothesis put forward in this essay is that the "Hit-and-Run" mode of transcription involves chromatin modifications that make a transient target gene more accessible to partner TFs. These partner TFs continue to regulate the transient target, enabling the catalyst TF to "run" off to another target. The implication of change in the chromatin state of transient targets is readily testable by a time-course ChIP-Seq assay for histone modifications in the cell-based system for TF perturbation [7, 38]. Finally, commonalities in the mode of action of bZIP1 as a "catalyst TF" that responds to an environmental cue in

plants [7], and "pioneer" TFs regulating development and differentiation in animals [4, 40], suggests that variations on the "Hit-and-Run" theme could be relevant to transcriptional regulation of gene networks across eukaryotes.

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The authors declare that there is no conflict of interest.

References

1. **Voss TC**, **Hager GL.** 2014. Dynamic regulation of transcriptional states by chromatin and transcription factors. *Nat Rev Genet* **15**: 69–81.
2. **Luo Y**, **North JA**, **Rose SD**, **Poirier MG.** 2014. Nucleosomes accelerate transcription factor dissociation. *Nucleic Acids Res* **42**: 3017–3027.
3. **Knight B**, **Kubik S**, **Ghosh B**, **Bruzzone MJ**, et al**.** 2014. Two distinct promoter architectures centered on dynamic nucleosomes control ribosomal protein gene transcription. *Genes Dev* **28**: 1695–1709.
4. **Foo SM**, **Sun Y**, **Lim B**, **Ziukaite R**, et al**.** 2014. Zelda potentiates morphogen activity by increasing chromatin accessibility. *Curr Biol* **24**: 1341–1346.
5. **Zaret KS**, **Carroll JS.** 2011. Pioneer transcription factors: establishing competence for gene expression. *Genes Dev* **25**: 2227–2241.
6. **Magnani L**, **Eeckhoute J**, **Lupien M.** 2011. Pioneer factors: directing transcriptional regulators within the chromatin environment. *Trends Genet* **27**: 465–474.
7. **Para A**, **Li Y**, **Marshall-Colon A**, **Varala K**, et al**.** 2014. Hit-and-run transcriptional control by bZIP1 mediates rapid nutrient signaling in Arabidopsis. *Proc Natl Acad Sci USA* **111**: 10371–10376.
8. **Spivakov M.** 2014. Spurious transcription factor binding: non-functional or genetically redundant?. *BioEssays* **36**: 798–806.
9. **Morisaki T**, **Muller WG**, **Golob N**, **Mazza D**, et al**.** 2014. Single-molecule analysis of transcription factor binding at transcription sites in live cells. *Nat Commun* **5**: 4456.
10. **Coulon A**, **Chow CC**, **Singer RH**, **Larson DR.** 2013. Eukaryotic transcriptional dynamics: from single molecules to cell populations. *Nat Rev Genet* **14**: 572–584.
11. **Lickwar CR**, **Mueller F**, **Hanlon SE**, **McNally JG**, et al**.** 2012. Genome-wide protein-DNA binding dynamics suggest a molecular clutch for transcription factor function. *Nature* **484**: 251–255.
12. **Kang SG**, **Price J**, **Lin PC**, **Hong JC**, et al**.** 2010. The arabidopsis bZIP1 transcription factor is involved in sugar signaling, protein networking, and DNA binding. *Mol Plant* **3**: 361–373.
13. **Baena-Gonzalez E**, **Rolland F**, **Thevelein JM**, **Sheen J.** 2007. A central integrator of transcription networks in plant stress and energy signalling. *Nature* **448**: 938–942.
14. **Schaffner W.** 1988. Gene regulation. A hit-and-run mechanism for transcriptional activation?. *Nature* **336**: 427–428.
15. **Eadara JK**, **Hadlock KG**, **Lutter LC.** 1996. Chromatin structure and factor site occupancies in an in vivo-assembled transcription elongation complex. *Nucleic Acids Res* **24**: 3887–3895.
16. **Gorski JJ**, **Savage KI**, **Mulligan JM**, **McDade SS**, et al**.** 2011. Profiling of the BRCA1 transcriptome through microarray and ChIP-chip analysis. *Nucleic Acids Res* **39**: 9536–9548.
17. **Monke G**, **Seifert M**, **Keilwagen J**, **Mohr M**, et al**.** 2012. Toward the identification and regulation of the *Arabidopsis thaliana* ABI3 regulon. *Nucleic Acids Res* **40**: 8240–8254.
18. **Hughes TR**, **de Boer CG.** 2013. Mapping yeast transcriptional networks. *Genetics* **195**: 9–36.
19. **Becker M**, **Baumann C**, **John S**, **Walker DA**, et al**.** 2002. Dynamic behavior of transcription factors on a natural promoter in living cells. *EMBO Rep* **3**: 1188–1194.
20. **Biggin MD.** 2011. Animal transcription networks as highly connected, quantitative continua. *Dev Cell* **21**: 611–626.
21. **Wang L**, **Kim J**, **Somers DE.** 2013. Transcriptional corepressor TOPLESS complexes with pseudoresponse regulator proteins and histone deacetylases to regulate circadian transcription. *Proc Natl Acad Sci USA* **110**: 761–766.
22. **Ehlert A**, **Weltmeier F**, **Wang X**, **Mayer CS**, et al**.** 2006. Two-hybrid protein-protein interaction analysis in Arabidopsis protoplasts: establishment of a heterodimerization map of group C and group S bZIP transcription factors. *Plant J* **46**: 890–900.
23. **Weiste C**, **Droge-Laser W.** 2014. The Arabidopsis transcription factor bZIP11 activates auxin-mediated transcription by recruiting the histone acetylation machinery. *Nat Commun* **5**: 3883.
24. **Darlyuk-Saadon I**, **Weidenfeld-Baranboim K**, **Yokoyama KK**, **Hai T**, et al**.** 2012. The bZIP repressor proteins, c-Jun dimerization protein 2 and activating transcription factor 3, recruit multiple HDAC members to the ATF3 promoter. *Biochim Biophys Acta* **1819**: 1142–1153.
25. **Liber D**, **Domaschenz R**, **Holmqvist PH**, **Mazzarella L**, et al**.** 2010. Epigenetic priming of a pre-B cell-specific enhancer through binding of Sox2 and Foxd3 at the ESC stage. *Cell Stem Cell* **7**: 114–126.
26. **Lupien M**, **Eeckhoute J**, **Meyer CA**, **Wang Q**, et al**.** 2008. FoxA1 translates epigenetic signatures into enhancer-driven lineage-specific transcription. *Cell* **132**: 958–970.
27. **Verdin E**, **Ott M.** 2015. 50 years of protein acetylation: from gene regulation to epigenetics, metabolism and beyond. *Nat Rev Mol Cell Biol* **16**: 258–264.
28. **Roudier F**, **Ahmed I**, **Berard C**, **Sarazin A**, et al**.** 2011. Integrative epigenomic mapping defines four main chromatin states in Arabidopsis. *EMBO J* **30**: 1928–1938.
29. **Charron JB**, **He H**, **Elling AA**, **Deng XW.** 2009. Dynamic landscapes of four histone modifications during deetiolation in Arabidopsis. *Plant Cell* **21**: 3732–3748.
30. **Eberharter A**, **Becker PB.** 2002. Histone acetylation: a switch between repressive and permissive chromatin. Second in review series on chromatin dynamics. *EMBO Rep* **3**: 224–229.
31. **Siersbaek R**, **Rabiee A**, **Nielsen R**, **Sidoli S**, et al**.** 2014. Transcription factor cooperativity in early adipogenic hotspots and super-enhancers. *Cell Rep* **7**: 1443–1455.
32. **Gansen A**, **Toth K**, **Schwarz N**, **Langowski J.** 2015. Opposing roles of H3- and H4-acetylation in the regulation of nucleosome structure-a FRET study. *Nucleic Acids Res* **43**: 1433–1443.
33. **Vlachonasios KE**, **Kaldis A**, **Nikoloudi A**, **Tsementzi D.** 2011. The role of transcriptional coactivator ADA2b in Arabidopsis abiotic stress responses. *Plant Signal Behav* **6**: 1475–1478.
34. **Mao Y**, **Pavangadkar KA**, **Thomashow MF**, **Triezenberg SJ.** 2006. Physical and functional interactions of Arabidopsis ADA2 transcriptional coactivator proteins with the acetyltransferase GCN5 and with the cold-induced transcription factor C BF1. *Biochim Biophys Acta* **1759**: 69–79.
35. **Newman JR**, **Keating AE.** 2003. Comprehensive identification of human bZIP interactions with coiled-coil arrays. *Science* **300**: 2097–2101.
36. **Rubin G**, **Tohge T**, **Matsuda F**, **Saito K**, et al**.** 2009. Members of the LBD family of transcription factors repress anthocyanin synthesis and affect additional nitrogen responses in Arabidopsis. *Plant Cell* **21**: 3567–3584.
37. **Besseau S**, **Li J**, **Palva ET.** 2012. WRKY54 and WRKY70 co-operate as negative regulators of leaf senescence in *Arabidopsis thaliana*. *J Exp botany* **63**: 2667–2679.
38. **Bargmann BO**, **Marshall-Colon A**, **Efroni I**, **Ruffel S**, et al**.** 2013. TARGET: a transient transformation system for genome-wide transcription factor target discovery. *Mol Plant* **6**: 978–980.
39. **Jin HJ**, **Zhao JC**, **Wu L**, **Kim J**, et al**.** 2014. Cooperativity and equilibrium with FOXA1 define the androgen receptor transcriptional program. *Nat Commun* **5**: 3972.
40. **Soufi A**, **Garcia MF**, **Jaroszewicz A**, **Osman N**, et al**.** 2015. Pioneer transcription factors target partial DNA motifs on nucleosomes to initiate reprogramming. *Cell* **161**: 555–568.
41. **Yu D**, **Chen C**, **Chen Z.** 2001. Evidence for an important role of WRKY DNA binding proteins in the regulation of NPR1 gene expression. *Plant Cell* **13**: 1527–1540.
42. **Giuliano G**, **Pichersky E**, **Malik VS**, **Timko MP**, et al**.** 1988. An evolutionarily conserved protein binding sequence upstream of a plant light-regulated gene. *Proc Natl Acad Sci USA* **85**: 7089–7093.