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TALE Factors Poise Promoters for Activation by Hox Proteins

Seong-Kyu Choe,[1,2,3] Franck Ladam,[1,2] and Charles. G. Sagerström[1,*]
[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605, USA
[2]These authors contributed equally to this work
[3]Present address: Department of Microbiology and Center for Metabolic Function Regulation, School of Medicine, Wonkwang University, 460 Iksan-daero, Iksan, Jeonbuk 570-749, South Korea
*Correspondence: charles.sagerstrom@umassmed.edu
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SUMMARY

Hox proteins form complexes with TALE cofactors from the Pbx and Prep/Meis families to control transcription, but it remains unclear how Hox:TALE complexes function. Examining a Hoxb1b:TALE complex that regulates zebrafish *hoxb1a* transcription, we find maternally deposited TALE proteins at the *hoxb1a* promoter already during blastula stages. These TALE factors recruit histone-modifying enzymes to promote an active chromatin profile at the *hoxb1a* promoter and also recruit RNA polymerase II (RNAPII) and P-TEFb. However, in the presence of TALE factors, RNAPII remains phosphorylated on serine 5 and *hoxb1a* transcription is inefficient. By gastrula stages, Hoxb1b binds together with TALE factors to the *hoxb1a* promoter. This triggers P-TEFb-mediated transitioning of RNAPII to the serine 2-phosphorylated form and efficient *hoxb1a* transcription. We conclude that TALE factors access promoters during early embryogenesis to poise them for activation but that Hox proteins are required to trigger efficient transcription.

INTRODUCTION

Hox proteins are homeodomain-containing transcription factors that regulate genes required for specification of cell fates. Hox proteins form complexes with cofactors from the TALE (three amino acid loop extension) family of homeodomain transcription factors (reviewed in Mann et al., 2009). The TALE family consists of four closely related Pbx proteins that are ubiquitously expressed, four Meis proteins with restricted tissue distribution, and two ubiquitously expressed Prep proteins (reviewed in Moens and Selleri, 2006). Hox proteins form dimers with either a Pbx protein or a Meis/Prep protein, as well as trimers with one Pbx and one Meis/Prep protein (reviewed in Mann et al., 2009). Complex formation is required in part to improve the affinity and sequence selectivity of Hox proteins (Slattery et al., 2011), but it remains largely unclear how Hox:TALE complexes otherwise function to initiate transcription of target genes.

HoxA1 (the earliest expressed Hox protein in mouse; Hoxb1b in zebrafish) acts in a trimeric complex with one Pbx and one Meis/Prep factor to drive transcription of other *hox* genes (e.g., *HoxB1* and *HoxB2* in mammals; *hoxb1a* and *hoxb2a* in zebrafish; Figure 1A; Ferretti et al., 2000, 2005; Jacobs et al., 1999; Vlachakis et al., 2001) and represents a useful model to study the function of Hox:TALE complexes. Disruption of *HoxA1/hoxb1b* leads to abnormal hindbrain development (Barrow and Capecchi, 1996; Carpenter et al., 1993; Dollé et al., 1993; Goddard et al., 1996; Mark et al., 1993; McClintock et al., 2002; Studer et al., 1996) and causes the Bosley-Salih-Alorainy syndrome, which is characterized by facial weakness, mental retardation, and autism spectrum disorder in humans (Tischfield et al., 2005). Hox:TALE-mediated activation of *hox* transcription involves several events. For instance, chromatin is remodeled so that the *hox* clusters decondense (Chambeyron and Bickmore, 2004; Chambeyron et al., 2005) and make novel interchromosomal contacts (Würtele and Chartrand, 2006). Accordingly, expressed *hox* promoters possess distinct nucleosome organization (Weicksel et al., 2013) and nucleosomes are covalently modified to a state supportive of active transcription (Soshnikova and Duboule, 2009). Additionally, RNA polymerase II (RNAPII) and the general transcription machinery must be recruited prior to transcription. Hence, Hox:TALE complexes may regulate transcription by controlling one or more of these events.

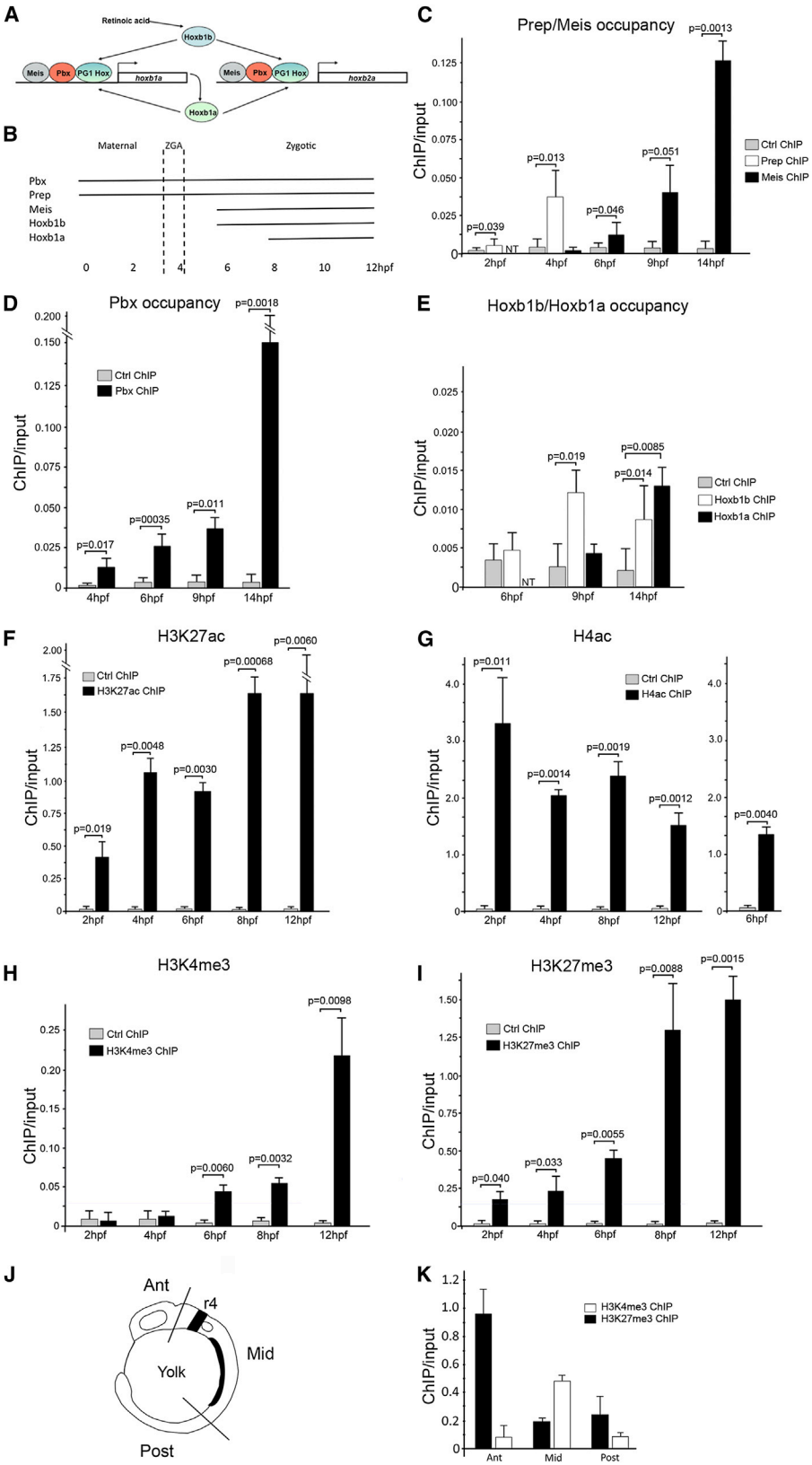
We have examined the function of a Hoxb1b:TALE complex in the regulation of transcription during zebrafish embryogenesis. We find that TALE factors occupy the *hoxb1a* promoter during early blastula stages and recruit both chromatin-modifying enzymes and RNAPII. However, *hoxb1a* transcription is inefficient until gastrula stages when Hoxb1b binds the promoter and triggers P-TEFb-mediated phosphorylation of RNAPII, thereby driving efficient *hoxb1a* transcription.

RESULTS

TALE Factors Occupy the *hoxb1a* Promoter at Blastula Stages

hoxb1a and *hoxb2a* expression in rhombomere 4 (r4) of the zebrafish hindbrain is initiated by a trimeric complex consisting of Hoxb1b together with one Prep/Meis protein and one Pbx protein (Choe et al., 2002, 2009; Vlachakis et al., 2001; Waskiewicz et al., 2001, 2002). The Hoxb1b protein is present only transiently, but once Hoxb1a is expressed, it acts in place of Hoxb1b to maintain *hoxb1a* and *hoxb2a* expression (Figure 1A).

In zebrafish, Prep and Pbx proteins are maternally provided, whereas *hoxb1b* (the earliest *hox* gene) and several *meis* genes



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become expressed at early gastrula stages (6 hr postfertilization [hpf]) and *hoxb1a* transcripts are first detected at ~8 hpf (Figure 1B). Hence, TALE cofactors may have early roles at the *hoxb1a* promoter independently of Hoxb1b. Using chromatin immunoprecipitation (ChIP) analysis, we detect Prep and Pbx at the *hoxb1a* promoter by 4 hpf (Figures 1C and 1D), although they may be present as early as 2 hpf (Prep protein in Figure 1C). Meis proteins are detected at the *hoxb1a* promoter by 6 hpf (Figure 1C) and Hoxb1b by 9 hpf (Figure 1E), in agreement with these proteins not being maternally contributed. Notably, Hoxb1b occupancy is coincident with the stage when *hoxb1a* expression is first detected. Lastly, Hoxb1a is observed at its own promoter by 14 hpf (Figure 1E). Furthermore, disruption of Prep/Meis function (using a dominant negative construct) leads to loss of Pbx from the *hoxb1a* promoter (Figures S1E and S1F available online), consistent with Pbx proteins binding DNA as heterodimers with Prep proteins. We conclude that a Pbx:Prep complex is present at the *hoxb1a* promoter several hours prior to binding of Hoxb1b at the promoter and well before the onset of *hoxb1a* transcription.

Active and Repressive Histone Marks Are Detected at *hox* Promoters at Blastula Stages

Zebrafish *hox* genes are broadly marked by histone modifications already during blastula stages (Lindeman et al., 2011; Vastenhouw et al., 2010; Wu et al., 2011). Accordingly, we find acetylation of histone H3 on lysine 27 (H3K27ac) and broad acetylation of histone H4 (H4ac)—two modifications associated with actively transcribed genes or genes poised for expression—at the *hoxb1a* promoter already at 2 hpf (Figures 1F and 1G). Trimethylation of histone H3 on lysine 4 (H3K4me3)—another mark associated with transcribed or poised promoters—is observed at 6 hpf (Figure 1H), a few hours before detectable *hoxb1a* transcription. Trimethylation of histone H3 on lysine 27 (H3K27me3)—a repressive mark associated with nonexpressed genes—is also detectable at the *hoxb1a* promoter by 2 hpf (Figure 1I). The coincidence of H3K4me3 and H3K27me3 modifications at the *hoxb1a* promoter at 6 hpf (prior to its expression) likely represents a bivalent mark as reported previously for zebrafish embryos (Vastenhouw et al., 2010). In contrast, the presence of H3K27me3 at *hoxb1a* promoters at 8–12 hpf (when *hoxb1a* is expressed) likely reflects the fact that not all cells in the embryo express *hoxb1a* at these stages. Indeed, dissection of 12–14 hpf zebrafish embryos (Figure 1J) revealed that anterior tissue (that never expresses *hoxb1a*) displays high levels of H3K27me3 and low levels of H3K4me3 at the *hoxb1a* promoter, while middle tissue (that contains *hoxb1a*-expressing and nonexpressing cells) is enriched for H3K4me3 and posterior tissue (that transiently expressed *hoxb1a* earlier during development) displays low levels of both marks (Figure 1K). We conclude that the *hoxb1a* promoter is marked by histone modifications already at blastula stages, well before the *hoxb1a* gene becomes transcribed and note that a similar pattern is observed at the *hoxb2a* promoter (Figures S1A–S1D).

TALE Factors Promote Deposition of Active Chromatin Marks in the Early Embryo

The presence of Prep and Pbx at the *hoxb1a* locus as chromatin modifications appear during blastula stages suggested that TALE factors might modulate chromatin marks prior to the onset of *hoxb1a* transcription. We therefore examined the effect of TALE cofactors on histone modifications at 4 hpf. This time point is several hours prior to the onset of *hox* expression and represents a stage when embryonic cells remain multipotent and undifferentiated (Ho and Kimmel, 1993). We find that H3K27ac is increased dramatically at the *hoxb1a* promoter when Pbx4+Meis3, or Pbx4+Prep1, expression is increased by mRNA injection into the fertilized egg (Figure 2A). Similar effects are observed at the *hoxb2a* and *ppp1r14ab* promoters that are also regulated by Hoxb1b:TALE complexes (Figures S2A and S2B), but not at the *otx1* and *pax2* promoters that are not controlled by Hoxb1b:TALE complexes (Figure 2H). Additionally, H3K27me3 is markedly decreased at the *hoxb1a* promoter following injection of Pbx4+Prep1 mRNA (Figure 2B) while H3K4me3 is increased (Figure 2C), but neither chromatin mark is affected at promoters that are not controlled by Hoxb1b:TALE complexes (Figures 2I and 2J). Similar changes in histone modification levels are observed when cofactors are expressed individually (Figures S2C and S2D). Because Hox proteins are not yet expressed at 4 hpf, TALE factors must act independently of Hox proteins to affect histone modifications at this stage. Indeed, even when Pbx and Prep are overexpressed, we do not observe endogenous Hoxb1b at the *hoxb1a* promoter by 4 hpf (Figure S2E). We also note that Hoxb1b+Pbx4+Prep1 injection has the same effect as Pbx4+Prep1 injection on H3K27Ac (Figure 2A) and H3K27me3 (Figure 2B), but increases the level of H3K4me3 approximately 2-fold (Figure 2C). Hence, TALE cofactors appear to affect histone modifications largely independent of Hox proteins.

We next examined the effect of reducing TALE factor levels by coinjecting antisense morpholino oligonucleotides (MOs) that disrupt translation of Pbx2 and Pbx4 (the predominant Pbx proteins at this stage; Waskiewicz et al., 2002) together with mRNA encoding a dominant negative construct that blocks Meis and Prep function (Choe et al., 2002). We find that reducing cofactor levels in this manner leads to a reduction in H3K27ac (Figure 2D) and H3K4me3 (Figure 2F), as well as an increase in H3K27me3 (Figure 2E), at the *hoxb1a* promoter. Similar effects are observed

Figure 1. TALE Factors Occupy the *hoxb1a* Promoter Coincident with the Appearance of Histone Modifications at Early Blastula Stages

(A) Diagram of *hoxb1a* and *hoxb2a* regulation by Hox, Pbx, and Prep/Meis factors. PG1, paralog group 1.

(B) Diagram summarizing temporal expression of Hox, Pbx, and Prep/Meis factors. ZGA, zygotic genome activation.

(C–E) Time course of Prep/Meis (C), Pbx (D) and Hoxb1a/Hoxb1b (E) occupancy at the *hoxb1a* promoter. NT, not tested.

(F–I) Time course of H3K27ac (F), H4ac (G), H3K4me3 (H), and H3K27me3 (I) modifications at the *hoxb1a* promoter.

(J) Diagram of zebrafish dissection into anterior (Ant), middle (Mid), and posterior (Post) pieces.

(K) Detection of histone modifications in anterior, middle, and posterior pieces.

Data are presented as the average of a minimum of three repeats with error bars indicating SD. Statistical significance was determined using Student's t test in Microsoft Excel; p values < 0.05 are indicated. See also Figure S1.

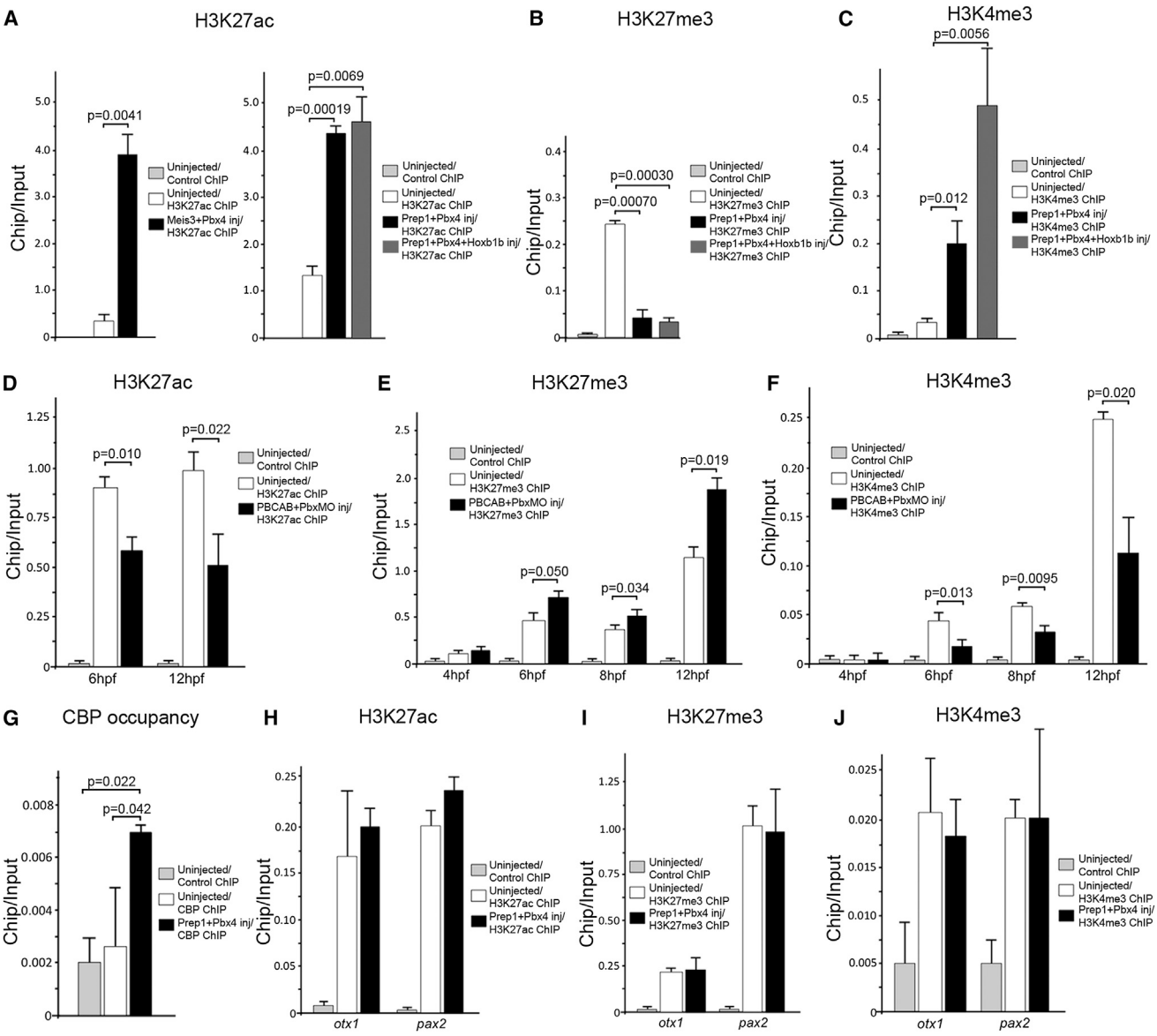


Figure 2. TALE Factors Promote Deposition of Active Chromatin Marks in the Early Embryo

(A–C) Detection of H3K27ac (A), H3K27me3 (B), and H3K4me3 (C) modifications in 4 hpf embryos overexpressing TALE factors, or TALE factors + Hoxb1b.

(D–F) Detection of H3K27ac (D), H3K27me3 (E), and H3K4me3 (F) modifications in 4–12 hpf embryos following disruption of Pbx (PbxMO) and Prep/Meis (PBCAB) function.

(G) Occupancy of CBP at the *hoxb1a* promoter in 4 hpf embryos overexpressing TALE factors.

(H–J) Detection of H3K27ac (H), H3K27me3 (I), and H3K4me3 (J) modifications at two promoters (*otx1* and *pax2*) not regulated by TALE factors.

Data are presented as the average of a minimum of three repeats with error bars indicating SD. Statistical significance was determined using Student's t test in Microsoft Excel; p values < 0.05 are indicated. See also Figure S2.

at the *hoxb2a* promoter (Figures S2G–S2I). These changes in histone modifications are detectable as early as 6 hpf and remain at least to 12 hpf. Because *hoxb1b* expression is being initiated around 6 hpf, these effects could conceivably be due to disruption of a TALE:Hox complex rather than a TALE dimer. To test this possibility, we used MOs to block Hoxb1a and Hoxb1b activity, as reported previously (McClintock et al., 2002). We find no effect of Hoxb1a/b MOs on histone modifications at 8 hpf (Figures S2J–S2M), but observe Hox-dependent changes by 12 hpf. This result demonstrates that TALE factors modulate histone modifications independently of Hox proteins at least up to 8 hpf—in agreement with the fact that Hox proteins are not detectable at the *hoxb1a* promoter until 9 hpf (Figure 1E).

The effect of TALE factors on histone modification levels could be explained if these factors recruit histone-modifying enzymes. In particular, acetylation of H3K27 is catalyzed by CBP (Tie et al., 2009), and it has been reported previously that Pbx binds CBP and HDACs (Choe et al., 2009; Saleh et al., 2000). Indeed, using

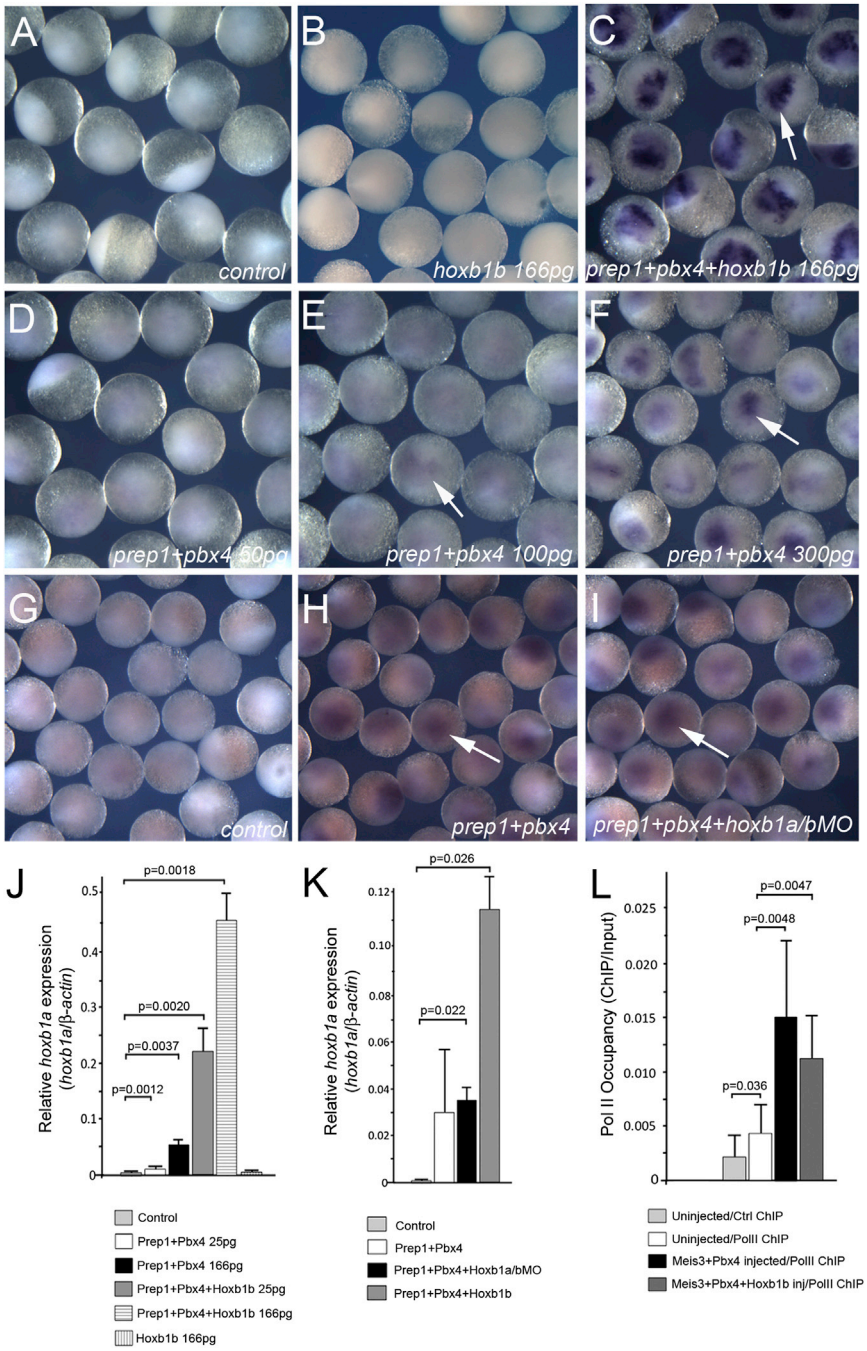


Figure 3. TALE Factors Recruit RNA Polymerase II but Drive Only Weak *hoxb1a* Transcription

(A–I) In situ hybridization to *hoxb1a* transcripts in 4 hpf zebrafish embryos injected with mRNA and MOs as indicated in lower right corner of each panel. Arrows indicate examples of positive signals.

(J and K) Quantitative PCR detection of *hoxb1a* transcripts in 4 hpf zebrafish embryos injected with the indicated mRNAs and MOs.

(L) Occupancy of serine-5 phosphorylated RNA polymerase II at the *hoxb1a* promoter of 4 hpf embryos overexpressing TALE factors or TALE factors + Hoxb1b.

Data are presented as the average of a minimum of three repeats (except in K, which was done in duplicate) with error bars indicating SD. Statistical significance was determined using Student's t test in Microsoft Excel; p values < 0.05 are indicated.

ChIP analysis we find that Pbx4+Prep1 recruits CBP to the *hoxb1a* promoter at 4 hpf (Figure 2G). We conclude that TALE factors recruit histone-modifying enzymes to promote deposition of active chromatin marks at early stages of embryogenesis.

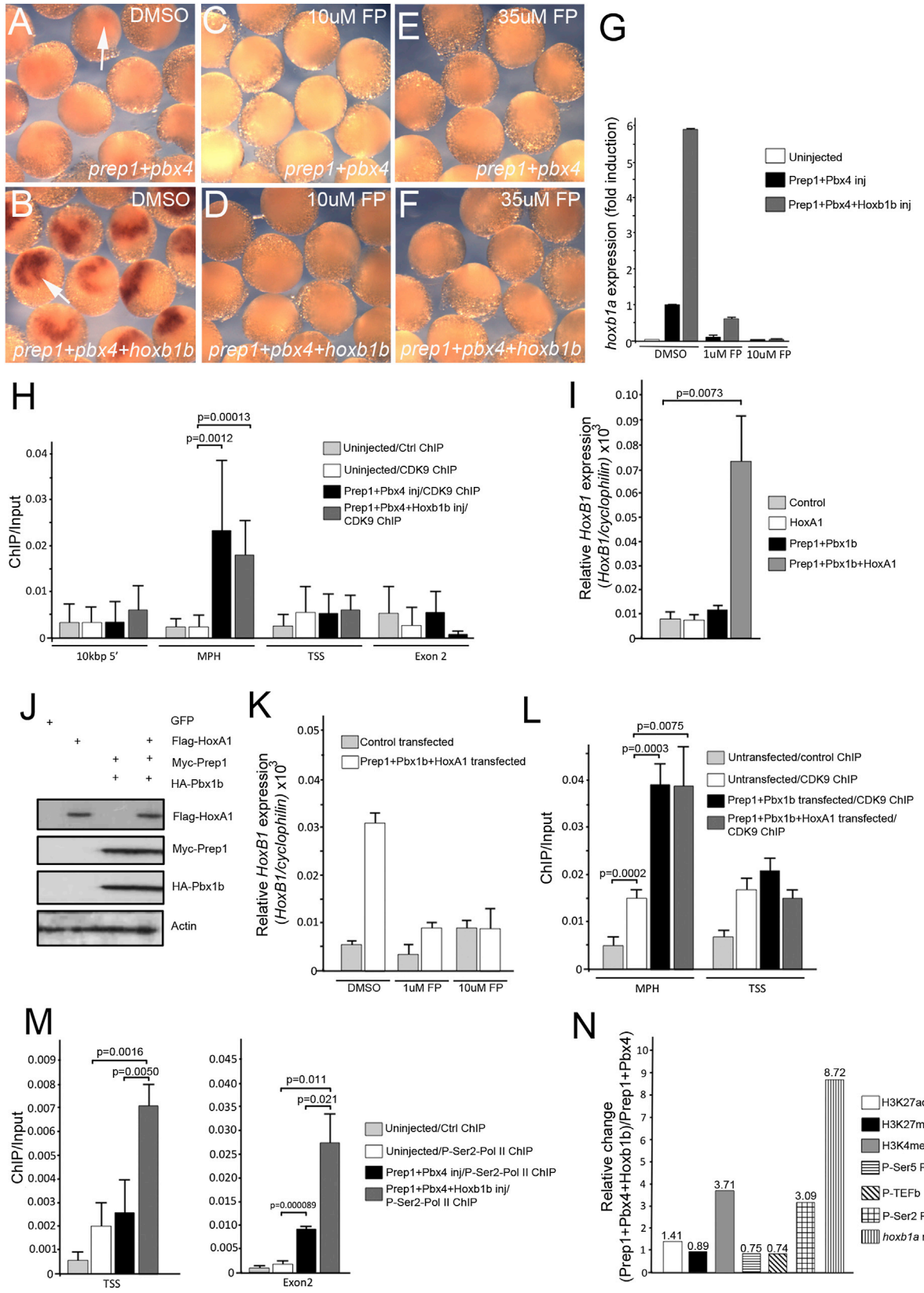
TALE Factors Recruit RNA Polymerase II but Drive Inefficient *hoxb1a* Transcription

Because TALE factors promote an active chromatin state, we next examined if they also support *hoxb1a* transcription. We find that Pbx4+Prep1 induces *hoxb1a* transcription in a dose-dependent manner at 4 hpf (Figures 3D–3F and 3J). Disruption of Hox function does not affect this transcription; Figures 3G–3I, and 3K), confirming that it is mediated by TALE proteins independent of Hox proteins. However, TALE-mediated *hoxb1a* transcription is inefficient, because inclusion of Hoxb1b increases transcription from 4- to 30-fold (Figures 3C, 3J, and 3K).

The increased transcription mediated by Hoxb1b could be explained if Hoxb1b recruits additional RNAPII to the *hoxb1a* transcription start site (TSS). We initially examined RNAPII phosphorylated on serine 5 (P-Ser5-RNAPII), which represents RNAPII that is bound to the TSS and has initiated transcription, but remains paused without engaging in active elongation of transcripts (reviewed in Adelman and Lis, 2012). ChIP analysis revealed that P-Ser5-RNAPII is barely detectable at the *hoxb1a* promoter in 4 hpf embryos, but occupancy is significantly increased upon introduction of TALE factors (Figure 3L). However, inclusion of Hoxb1b does not increase P-Ser5-Pol II levels further. Our results indicate that TALE proteins are sufficient to recruit P-Ser5-RNAPII to the *hoxb1a* promoter and drive weak transcription, but that Hoxb1b is required for efficient transcription of the *hoxb1a* gene.

Hoxb1b Triggers P-TEFb-Mediated Activation of RNA Polymerase II

Paused P-Ser5-RNAPII is converted to an actively elongating form by P-TEFb, which phosphorylates both serine-2 of RNAPII (P-Ser2-RNAPII) and the negative elongation factors DSIF and NELF. Together, these phosphorylation events convert paused RNAPII into an active form capable of proceeding into



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the gene and producing full-length transcripts. We find that *hoxb1a* transcription induced by Pbx4+Prep1 or Pbx4+Prep1+Hoxb1b is inhibited by flavopiridol, a small molecule inhibitor of P-TEFb (Chao and Price, 2001), in a dose-dependent manner (Figures 4A–4G), demonstrating that *hoxb1a* transcription is P-TEFb-dependent.

Transcription of some genes is activated when P-TEFb is recruited by sequence specific transcription factors (reviewed in Peterlin and Price, 2006), suggesting that Hoxb1b may act by recruiting P-TEFb. However, using ChIP analysis, we find that TALE factors are sufficient to recruit P-TEFb and that Hoxb1b does not increase P-TEFb levels further (Figure 4H). Notably, we make the same observation for regulation of the murine *HoxB1* gene in F9 cells (Figures 4I–4L). To test whether Hoxb1b is instead required for active phosphorylation of RNAPII by P-TEFb, we carried out ChIP experiments for P-Ser2-RNAPII. We find low levels of P-Ser2-RNAPII in the presence of TALE cofactors at 4 hpf (Figure 4M), consistent with the low level of transcription we observe under these conditions (Figure 3). Strikingly, inclusion of Hoxb1b leads to a robust increase in P-Ser2-RNAPII both at the TSS and over exon 2 of the *hoxb1a* gene (Figure 4M). We conclude that TALE cofactors recruit P-TEFb to the *hoxb1a* promoter and that Hoxb1b is required to initiate P-TEFb-mediated conversion of paused RNAPII into elongating RNAPII.

DISCUSSION

Previous studies have demonstrated a requirement for Hox:TALE complexes in driving transcription of Hox-target genes (reviewed in Mann et al., 2009). Our results define the specific contributions and sequential order of action for these factors. In particular, we demonstrate that the complex is 'split' temporally during embryogenesis such that TALE factors have early roles in poising the *hoxb1a* locus for activation, while Hoxb1b is required to trigger *hoxb1a* transcription. Our findings are consistent with previous work demonstrating that Pbx and Meis proteins recruit CBP to actively transcribed genes (Choe et al., 2009; Huang et al., 2005; Saleh et al., 2000; Wang et al., 2010), but here we show that TALE factors also recruit histone-modifying enzymes independent of Hox proteins and prior to gene activation.

We note that histones at the *hoxb1a* and *hoxb2a* promoters are highly acetylated already at the earliest stage assayed, although TALE factors are not yet detectable, suggesting that TALE factors increase acetylation beyond a basal level that is established independently of TALE factors. H3K27me3 is also detectable prior to TALE factor binding, but is reduced in the presence of TALE factors. Hence, TALE factors may act to protect the *hoxb1a* locus, which is targeted for early expression, from the repressive properties of the H3K27me3 mark. In contrast, the H3K4me3 mark is barely detectable at blastula stages, but appears in the presence of TALE factors, suggesting that the cofactors may promote de novo deposition of this mark. It is also striking that the extent of H3K4me3 modification correlates with transcription such that we observe low levels in the presence of TALE factors, when transcription is limited, and higher levels in the presence of TALE+Hoxb1b, when transcription is elevated. Lastly, we note that the H3K27ac and H3K4me3 histone marks are not completely abolished upon disruption of Pbx and Prep/Meis function. While this is most likely due to the cofactors not being completely eliminated in our experiments (e.g., the Pbx MOs target translation from maternal mRNA, but Pbx proteins are also maternally deposited), it remains possible that other pathways are also involved in controlling histone modifications at *hox*-regulated promoters.

We quantified the contribution of Hoxb1b to *hoxb1a* expression by deriving a ratio for the effect of Pbx4+Prep1+Hoxb1 relative to the effect of Pbx4+Prep1 (Figure 4N). The ratios for H3K27ac levels, H3K27me3 levels, P-Ser5-RNAPII recruitment, and P-TEFb recruitment are all close to 1.0, suggesting that Hoxb1b does not have an effect on these parameters beyond that contributed by the TALE factors. In contrast, the ratios for H3K4me3 levels, P-Ser2-RNAPII recruitment, and mRNA levels are significantly higher than 1.0, indicating that Hoxb1b affects these parameters. Because phosphorylation of serine 2 in RNAPII (a P-TEFb substrate) increases in the presence of Hoxb1b, while P-TEFb levels are unaffected, it appears that Hoxb1b stimulates P-TEFb activity, leading to increased transcription. Inactive P-TEFb is thought to be associated with the 7SK RNA complex (Nguyen et al., 2001; Yang et al., 2001) and recent work demonstrates that a sizeable pool of 7SK is associated with poised promoters (Ji et al., 2013). Hence, the 7SK complex may be recruited by TALE factors and Hoxb1b may act to

Figure 4. Hoxb1b triggers P-TEFb-Mediated Activation of RNA Polymerase II

(A–F) Embryos were injected with mRNA as indicated in lower right corner of each panel and incubated in DMSO (A and B), 10 μM flavopiridol (C and D), or 35 μM flavopiridol (E and F) until 4 hpf, followed by in situ hybridization to detect *hoxb1a* expression. Arrows point to examples of *hoxb1a* expression.

(G) Quantitative PCR detection of *hoxb1a* transcripts in 4 hpf zebrafish embryos injected with the indicated mRNAs and treated with the indicated concentrations of flavopiridol.

(H) CDK9 (the catalytic component of P-TEFb) occupancy at a region 10 kb upstream of the *hoxb1a* promoter, at the TALE:Hox binding site (MPH), at the transcription start site (TSS), or at Exon 2 in 4 hpf embryos overexpressing TALE factors or TALE factors + Hoxb1b.

(I) Quantitative PCR detection of *HoxB1* transcripts in F9 cells transfected with the indicated plasmids.

(J) Western blot detection of transcription factors following transfection of F9 cells.

(K) Quantitative PCR detection of *HoxB1* transcripts in F9 cells transfected with the indicated plasmids and treated with the indicated concentrations of flavopiridol.

(L) CDK9 occupancy at the TALE:Hox binding site (MPH) and the transcription start site (TSS) of the *HoxB1* gene in F9 cells transfected with the indicated plasmids.

(M) Occupancy of serine-2 phosphorylated RNA polymerase II at the *hoxb1a* transcription start site (left) or exon 2 (right) of 4 hpf embryos overexpressing TALE factors or TALE factors + Hoxb1b.

(N) Ratio of Prep1+Pbx4+Hoxb1b signal/Prep1+Pbx4 signal for various parameters related to *hoxb1a* expression.

Data are presented as the average of a minimum of three repeats with error bars indicating SD. Statistical significance was determined using Student's t test in Microsoft Excel; p values < 0.05 are indicated.

locally redistribute P-TEFb from 7SK to the *hoxb1a* locus, possibly acting together with Brd4, which is known to associate with active P-TEFb and help it localize to chromatin (Yang et al., 2005). Lastly, TALE factors were initially identified in mammals as proto-oncogenes and we hypothesize that the oncogenic potential of these factors stems, at least in part, from their ability to prepare promoters for transcriptional activation.

EXPERIMENTAL PROCEDURES

Plasmid Constructs, In Situ Hybridization, and Quantitative RT-PCR

meis3, *prep1*, *pbx4*, and *hoxb1b* constructs for mRNA synthesis, as well as the PBCAB dominant negative construct, have been reported previously (Choe et al., 2002). Antisense morpholinos to *pbx4* and *pbx2* (Erickson et al., 2007) and *hoxb1a/b* (McClintock et al., 2002) have been published. Embryo injections, in situ hybridizations and quantitative RT-PCR using gene specific primers (see Supplemental Experimental Procedures), were carried out as previously reported (Choe et al., 2009). Human HoxA1 (id105018) and Pbx1a (id109104) were obtained from Addgene. Human Prep1 was cloned from HEK293 cDNA. Human Prep1, HoxA1, and Pbx1b (amplified from Pbx1a) were each subcloned into the pCS2 expression vector. F9 cell transfections were carried out by mixing 4 μg of plasmid with 10 μg of Lipofectamine 2000 (Life Technologies) for each transfection. Antibodies for western blots were anti-HA (Roche; #1867423), anti-Myc (Roche; #11667149001), and anti-flag (Sigma; #f1804). All animal work was approved by the University of Massachusetts Medical School institutional animal care review board.

Antisera and ChIP

Antisera to Meis, Pbx, and Hoxb1b were reported previously (Choe et al., 2009). Rabbit antisera were raised to full-length Prep1 and Hoxb1a using standard protocols. All other antibodies are commercially available (see Supplemental Experimental Procedures). ChIPs were performed as reported previously (Choe et al., 2009), except ChIP for Ser2-RNAPII, which was done using the ChIP-IT kit from Active Motif. qPCR was performed using gene specific primers (see Supplemental Experimental Procedures) and normalized to 1% input sample. Control ChIPs were carried out with preimmune serum, Ig-matched antibodies, or beads alone. Data are presented as the average of a minimum of three repeats with error bars indicating SD. Statistical significance was determined using Student's t test in Microsoft Excel.

Flavopiridol Treatments

Flavopiridol (F3055, Sigma-Aldrich) was dissolved in DMSO and diluted in fish water immediately prior to treatment. Embryos were incubated in flavopiridol or the corresponding concentration of DMSO from 0 hpf until being harvested at 4 hpf.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and two figures and can be found with this article online at http://dx.doi.org/10.1016/j.devcel.2013.12.011.

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