instability is a common property within networks of low-affinity protein interactions (*22*). It would allow for stochastic abortion of sites that initiate but fail to cross a growth- or cargo-mediated checkpoint (*19*, *23*–*25*) before investing energy in membrane bending. During invagination, further exchange would allow clathrin reorganization and bending of the lattice into a defined cage that requires active disassembly.

REFERENCES AND NOTES

1. F. M. Brodsky, *Annu. Rev. Cell Dev. Biol.* **28**, 309–336 (2012).
2. H. T. McMahon, E. Boucrot, *Nat. Rev. Mol. Cell Biol.* **12**, 517–533 (2011).
3. S. M. Ferguson, P. De Camilli, *Nat. Rev. Mol. Cell Biol.* **13**, 75–88 (2012).
4. E. Cocucci, F. Aguet, S. Boulant, T. Kirchhausen, *Cell* **150**, 495–507 (2012).
5. E. Ungewickell, D. Branton, *Nature* **289**, 420–422 (1981).
6. J. Heuser, *J. Cell Biol.* **84**, 560–583 (1980).
7. T. Kirchhausen, *Curr. Opin. Struct. Biol.* **3**, 182–188 (1993).
8. A. Grassart *et al.*, *J. Cell Biol.* **205**, 721–735 (2014).
9. J. B. Doyon *et al.*, *Nat. Cell Biol.* **13**, 331–337 (2011).
10. F. Aguet, C. N. Antonescu, M. Mettlen, S. L. Schmid, G. Danuser, *Dev. Cell* **26**, 279–291 (2013).
11. W. Kukulski *et al.*, *J. Cell Biol.* **192**, 111–119 (2011).
12. W. Kukulski, M. Schorb, M. Kaksonen, J. A. G. Briggs, *Cell* **150**, 508–520 (2012).
13. Materials and methods are available as supplementary materials on *Science* Online.
14. S. Morlot, A. Roux, *Annu. Rev. Biophys.* **42**, 629–649 (2013).
15. T. Kirchhausen, D. Owen, S. C. Harrison, *Cold Spring Harb. Perspect. Biol.* **6**, a016725 (2014).
16. D. Loerke, M. Wienisch, O. Kochubey, J. Klingauf, *Traffic* **6**, 918–929 (2005).
17. X. Wu *et al.*, *J. Cell Biol.* **155**, 291–300 (2001).
18. L. von Kleist *et al.*, *Cell* **146**, 471–484 (2011).
19. D. Loerke *et al.*, *PLOS Biol.* **7**, e57 (2009).
20. J. Grove *et al.*, *Mol. Biol. Cell* **25**, 3581–3594 (2014).
21. S. Saffarian, E. Cocucci, T. Kirchhausen, *PLOS Biol.* **7**, e1000191 (2009).
22. E. M. Schmid, H. T. McMahon, *Nature* **448**, 883–888 (2007).
23. M. Mettlen, D. Loerke, D. Yarar, G. Danuser, S. L. Schmid, *J. Cell Biol.* **188**, 919–933 (2010).
24. A. G. Henry *et al.*, *Dev. Cell* **23**, 519–532 (2012).
25. M. Ehrlich *et al.*, *Cell* **118**, 591–605 (2004).

ACKNOWLEDGMENTS

We thank members of the Kaksonen and Briggs laboratories for helpful discussion; B. Podbilewicz, J. Ries, and P. Lénárt for critically reading the manuscript; the EMBL electron and advanced light microscopy facilities; and A. Politi and J. Ellenberg for single-site FRAP software. O.A was supported by the Marie Curie Actions COFUND (European Commission Cofunding of Regional, National, and International Programme) [grant no. 229597; EMBL Interdisciplinary Postdoc (EIPOD)]. Computer codes used are available upon request.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/348/6241/1369/suppl/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S2
Movies S1 to S7
References (*26*–*31*)

19 February 2015; accepted 13 May 2015
10.1126/science.aaa9555

TRANSCRIPTION

Recruitment of RNA polymerase II by the pioneer transcription factor PHA-4

H.-T. Hsu,[1] H.-M. Chen,[1] Z. Yang,[2] J. Wang,[3] N. K. Lee,[1] A. Burger,[4] K. Zaret,[2] T. Liu,[3,5] E. Levine,[4] S. E. Mango[1]*

Pioneer transcription factors initiate cell-fate changes by binding to silent target genes. They are among the first factors to bind key regulatory sites and facilitate chromatin opening. Here, we identify an additional role for pioneer factors. In early *Caenorhabditis elegans* foregut development, the pioneer factor PHA-4/FoxA binds promoters and recruits RNA polymerase II (Pol II), often in a poised configuration in which Pol II accumulates near transcription start sites. At a later developmental stage, PHA-4 promotes chromatin opening. We found many more genes with poised RNA polymerase than had been observed previously in unstaged embryos, revealing that early embryos accumulate poised Pol II and that poising is dynamic. Our results suggest that Pol II recruitment, in addition to chromatin opening, is an important feature of PHA-4 pioneer factor activity.

Embryonic development depends on precise patterns of gene expression that are orchestrated by key transcription factors such as pioneer transcription factors. Pioneer factors function at the earliest stage of transcriptional onset to facilitate chromatin opening at cis-regulatory sites, which enables additional factors to bind DNA (*1*). The founding pioneer factor is mammalian FoxA1, which associates with liver genes and promotes chromatin accessibility before transcriptional activation. In vitro, FoxA proteins bind nucleosomes and block chromatin compaction by H1 linker histones (*1*), and in vivo FoxA proteins open chromatin with the histone variant H2A.Z (*2*). It is unknown whether chromatin opening is the sole mechanism of transcriptional priming induced by pioneer transcription factors.

In *Caenorhabditis elegans*, *pha-4* encodes a selector gene that specifies foregut fate (*3*). *pha-4* is orthologous to FoxA proteins (*4*, *5*) and interacts with H2A.Z (*2*), raising the question of whether *pha-4* functions as a pioneer transcription factor in addition to its selector activities. We performed five tests that revealed that *pha-4* had pioneer activity. First, PHA-4 associated with target genes *M05B5.2*, *ceh-22*, and *myo-2* beginning at the 8E stage ("E" for endodermal cells), when PHA-4 was first detected (Fig. 1, A and B, and fig. S1A). We observed binding to promoters that are activated at early, mid-, or late embryogenesis and confirmed that the mid- (*ceh-22*) and late-stage (*myo-2*) genes were not expressed in our 8E sample (gastrulation stage). These data indicate that PHA-4 associates with endogenous foregut promoters hours before transcriptional onset, which is as expected for a pioneer factor. Second, we determined that PHA-4 bound nucleosomal DNA in vitro equivalently to its orthologs FoxA1 and FoxA2 (Fig. 1C). Third, chromatin sites bound by PHA-4 in vivo [measured with chromatin immunoprecipitation (ChIP)] (*6*) lacked stable nucleosomes [measured with formaldehyde-assisted isolation of regulatory elements (FAIRE)] (fig. S1C) (*7*), indicating PHA-4 association with open chromatin. Moreover, regions bound by PHA-4 were enriched for activating histone marks H3K4me2, H3K4me3, and acetylated H3K27 (fig. S1C) (*8*). Fourth, single-cell analysis with artificial chromosomes (Fig. 1D) revealed that chromatin was open in the foregut, where PHA-4 is expressed, but not in other cell types, which lack PHA-4, nor with a target promoter bearing a mutated PHA-4-binding site (Fig. 1E) (*9*). Fifth, we tracked PHA-4 association with chromatin during mitosis and observed that a portion of PHA-4 was retained on DNA in dividing foregut cells (fig. S2) (*10*). Together, the results reveal that PHA-4 fulfilled the criteria of a pioneer transcription factor (Fig. 1 and fig. S1B). It associated with binding sites early in development, bound DNA packaged in nucleosomes in vitro, and decompacted chromatin in vivo.

To examine the role of PHA-4 in transcription, we mapped Pol II occupancy by means of genome-wide ChIP-sequencing (ChIP-seq). Previous studies with *C. elegans* Pol II had focused on relatively late time points, after transcription was established for many genes (*6*, *11*). Our interest was earlier stages, before transcriptional onset. We analyzed early embryos after PHA-4 bound to target genes but before their transcription (~8E stage) and compared those embryos to mid-stage, transcriptionally active embryos (bean stage) (staging is provided in fig. S3). To localize Pol II, we mapped its position relative to the transcription start site (TSS) (*11*) and calculated three scores: promoter occupancy for Pol II spanning the TSS (Fig. 2A), Pol II within gene bodies (Fig. 2B), and the poising index as the ratio of the promoter to the gene body values (Fig. 2C). The poising index reflects the relative quantity of

[1]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA. [2]Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA, USA. [3]Department of Biochemistry, University at Buffalo, Buffalo, NY, USA. [4]Department of Physics and Center for Systems Biology, Harvard University, Cambridge, MA, USA. [5]Department of Biostatistics, University at Buffalo, Buffalo, NY 14214, USA.
*Corresponding author. E-mail: smango@mcb.harvard.edu

Pol II close to the site of transcriptional initiation (*12*). Poising has been detected in diverse organisms including, to a degree, *C. elegans* (*12*, *13*).

We began by surveying the whole genome. In early embryos, most genes showed little Pol II at either promoters or gene bodies (Fig. 2, A and B), suggesting that most of the genome was inactive. However, ~20% of genes had Pol II near the TSS and little Pol II within gene bodies, leading to a high poising index (≥2.5) (Fig. 2C). As development progressed, the Pol II signal for both promoters and gene bodies increased, resulting in a broad range of poising values (Fig. 2C and fig. S4C). This result suggested that the mid-stage poising scores reflected a surge in Pol II activity at the level of initiation and elongation, and that poising in *C. elegans* is temporally regulated, similar to other animals (*12*). Most genes had docked Pol II, in which Pol II bound just upstream of the TSS (*14*) (Fig. 2D) (*11*). Pol II "pausing" was also observed 3′ to the TSS, like other species (*12*, *14*), but we observed fewer cases of pausing as compared with docking. We suggest that poising in *C. elegans* is more prevalent than had been previously recognized. Earlier studies observed some poising in starved larvae and in samples bearing mixtures of stages (*6*, *13*, *14*). In our samples, poising was associated with both early and mid-stages, with index values typically higher in early embryos because occupancy of Pol II within gene bodies was low. Our analysis gives a picture of Pol II loading and transcriptional onset during embryogenesis.

We next examined Pol II at foregut-associated genes. We observed an enrichment of poised Pol II: 27% of foregut genes were poised early compared with 17% for the whole genome (Fig. 2C). At the bean stage, 36% of PHA-4–bound promoters had a poising index >2.5, compared with 29% for the whole genome. We confirmed the ChIP-seq result by means of ChIP–quantitative polymerase chain reaction (PCR) for four foregut genes exhibiting different Pol II poising scores (*11*). For example, Pol II was poised at the *ceh-22* promoter in early embryos before transcriptional onset, but it subsequently decreased at the TSS and increased in the gene body (Fig. 2E and fig. S4B). Quantitative reverse transcriptase–PCR (RT-PCR) analysis demonstrated that *ceh-22* mRNA was activated in mid-embryos, as expected (Fig. 3, B and C) (*11*, *15*). These data suggest that poised Pol II often reflects preparation for transcriptional activation (*12*). Consistent with this idea, genes with poised Pol II were associated with Gene Ontology (GO) terms "embryonic development" and "embryonic morphogenesis" (table S1). However, poising likely has additional roles because we also detected embryonic poised Pol II at a subset of genes not expressed in embryos (such as *mex-3*) or associated with GO terms such as "post-embryonic development."

Pol II poising at developmentally expressed genes has been observed in *Drosophila* embryos

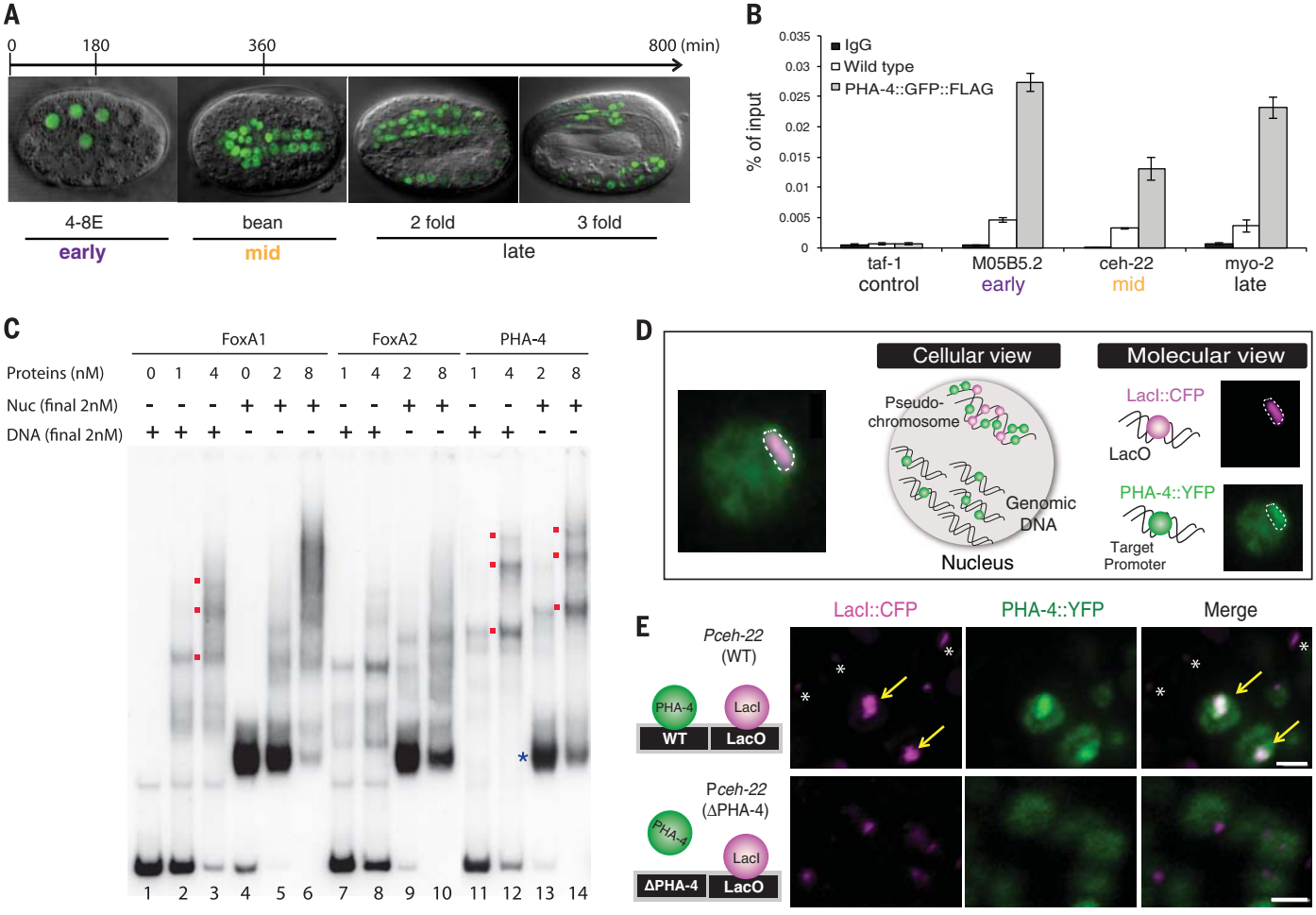

Fig. 1. PHA-4 is a pioneer factor. (**A**) PHA-4::green fluorescent protein (GFP) (green) during stages of embryogenesis. Early embryos are enriched for 8E stage, and mid-embryos are enriched for bean stage (*11*). (**B**) PHA-4::GFP::FLAG (*11*) binding endogenous targets *M05B5.2* (expressed early), *ceh-22* (mid), and *myo-2* (late), detected with ChIP–quantitative PCR. Wild-type embryos lack FLAG, a negative control. *taf-1* is not a PHA-4 target. $n = 3$ replicates, mean ± SEM. (**C**) PHA-4 binds nucleosomes. Shown is recombinant PHA-4 compared with FoxA proteins incubated with the albumin enhancer containing a FoxA1 binding site as free DNA (DNA) or nucleosomal (Nuc) DNA. Bound PHA-4 generated slow migrating bands (red). (**D**) Artificial chromosomes with PHA-4::yellow fluorescent protein (YFP) bound target promoters in single cells. (**E**) PHA-4::YFP (green) bound artificial chromosomes (purple, LacI) bearing the *ceh-22* promoter (arrows). PHA-4::YFP binding was abolished when *ceh-22* carried a mutated PHA-4 binding site (ΔPHA-4; bottom). Asterisks mark artificial chromosomes in nonforegut cells. Scale bar, 2 μm.

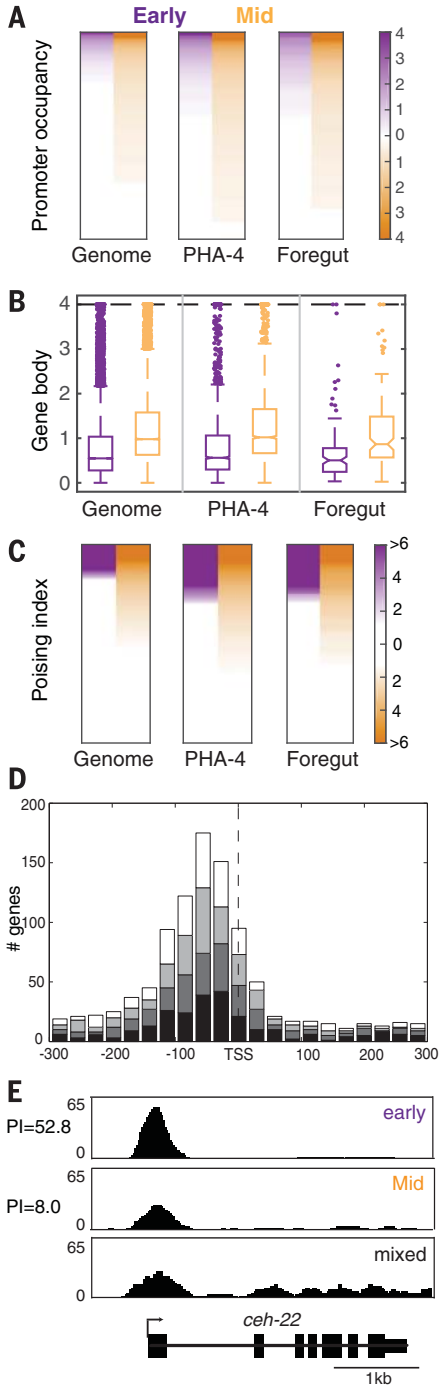

Fig. 2. Early embryos accumulate poised Pol II. (**A**) Pol II occupancy at promoters (enrichment over input). (**B**) Normalized gene activity scores for Pol II occupancy within gene bodies. (**C**) Poising index. For (A) to (C), numbers are provided in table S3; "Early" and "Mid" denote stages. (**D**) Pol II is enriched upstream of the TSS. Genes with Pol II peaks near defined TSSs (*11*) were divided into four quartiles according to gene body activity scores (from low, black, to high, white) and graphed for Pol II across the gene. (**E**) Pol II at the *ceh-22* locus in early, mid-, and late or mixed stage embryos. Mixed population sample is from (*6*).

(*16*), but poising in *C. elegans* had been associated predominantly with starvation (*6*, *13*). A likely explanation for the difference between the prior studies and ours is the embryonic stage (*11*). For example, we found that mixtures of embryos with a range of ages lacked poised Pol II for *ceh-22*, underscoring the importance of staging (fig. S4A).

To test whether *pha-4* affects Pol II occupancy, we performed Pol II ChIP with a *pha-4(ts)* temperature-sensitive strain that combines *pha-4(zu225nonsense)* with *smg-1(cc546ts)* (fig. S6A) (*17*). *smg-1(cc546)* alone served as a control. Growth of *pha-4(ts)* was complicated because *pha-4* is an essential gene, and therefore we relied on ChIP–quantitative PCR, which requires less material.

At restrictive temperature, *pha-4* mutants failed to accumulate poised Pol II at three tested loci (*ceh-22*, *T06D8.3*, and *K10D3.4*) or elongating Pol II at one (*M05B5.2*) (Fig. 3A and fig. S6B) (*11*). *mig-38*, which has PHA-4 bound but is expressed broadly, was not affected by *pha-4(ts)* (Fig. 3A). Recent studies found that Pol II poising was not tissue-specific for *Drosophila* muscle (*16*), but our analysis indicates that PHA-4 helps Pol II associate with its target foregut genes in worms.

Pioneer transcription factors promote chromatin opening at target genes to modulate gene expression. We therefore wondered whether chromatin opening by PHA-4 affected Pol II loading. We adapted FAIRE (*7*, *11*) to track regions of the *C. elegans* genome with absent or unstable nucleosomes. In older, wild-type embryos,

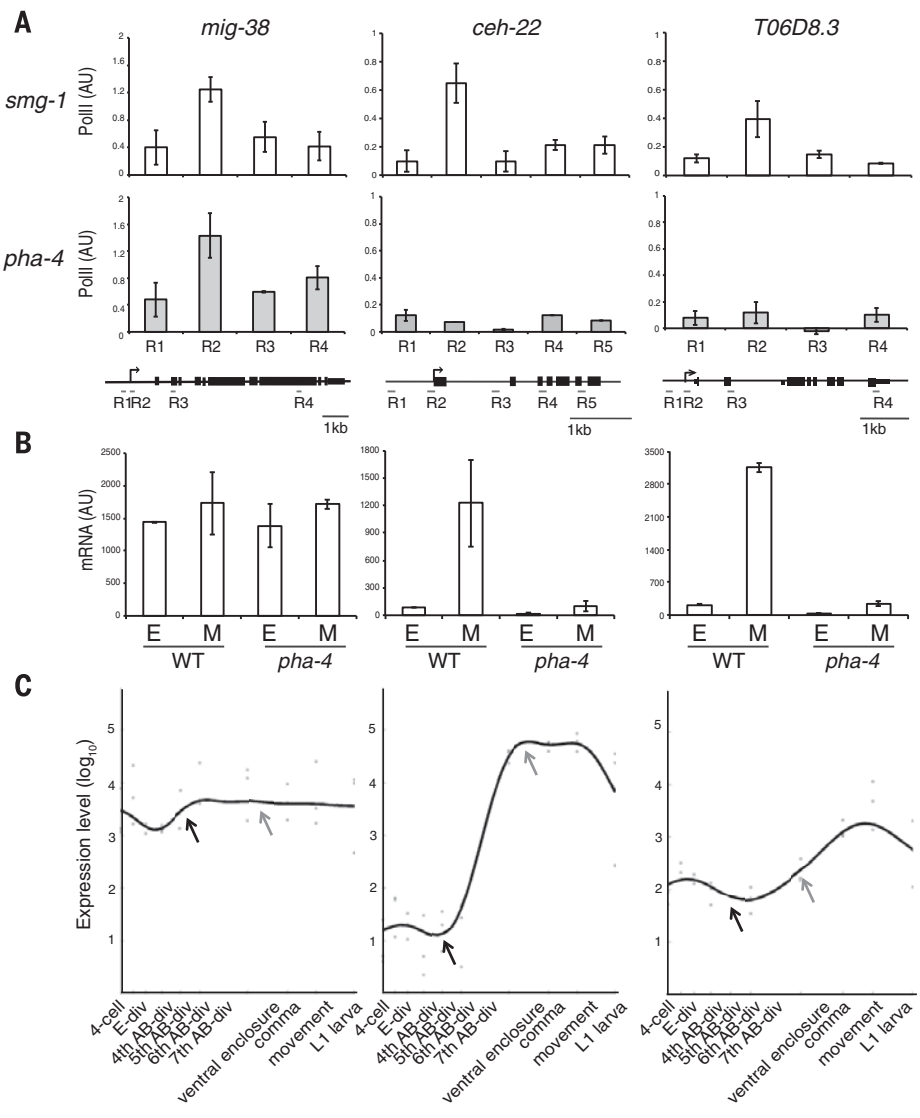

Fig. 3. PHA-4 is required for Pol II occupancy at foregut genes. (**A**) Pol II occupancy at *mig-38* (poised, ubiquitous) versus *ceh-22* and *T06D8.3* (poised, foregut) in *smg-1* control versus *pha-4(ts)* embryos, normalized to *eft-3* (set at 1) and *srw-99* (set at 0). Error bars indicate $n = 3$ replicates, mean ± SEM. (**B**) mRNA abundance (quantitative RT-PCR) for *mig-38*, *ceh-22*, and *T06D8.3* for wild-type or *pha-4(ts)* embryos early (E) or mid (M). $n = 3$ replicates, mean ± SEM. (**C**) Gene expression profiles from (*20*). Early stage (dark gray arrowhead) is at the fifth and sixth AB-div. Mid-stage (light gray arrowhead) is equivalent to ventral enclosure.

we found a strong correlation between PHA-4 binding and open chromatin, characterized by a high FAIRE signal, at both promoters (H2A.Z^{+}, H3K4me3^{+}, and H3K27ac^{+}) and enhancers (H3K4me1/2^{+}) (fig. S7). Conversely, Pol II occupancy was only weakly correlated with open chromatin (fig. S7A), which is similar to *Drosophila* (*18*). To determine the contribution of *pha-4* to chromatin opening, we surveyed three foregut genes by means of FAIRE–quantitative PCR at early and mid-stages after *pha-4* inactivation (Fig. 4A). At the 8E stage, *pha-4(ts)* embryos had a FAIRE signal equivalent to wild-type embryos for *ceh-22* and *K10D3.4*, suggesting that PHA-4 did not contribute to chromatin opening at these early stages. *T06D8.3*, however, showed a ~10% decrease in FAIRE, suggesting nucleosomes depended on *pha-4* for at least some opening. In mid-stage embryos, reduction of *pha-4* lead to a decrease in open FAIRE regions spanning the PHA-4 binding site and the Pol II binding site for *T06D8.3* and *K10D3.4*. The effect was less dramatic for *ceh-22*, with a small reduction at the PHA-4 binding region. Nevertheless, PHA-4 still promoted *ceh-22* opening. These data suggest that PHA-4 promotes chromatin opening at mid-stages, at least for the surveyed genes, but has less of an effect early.

We extended these results in three ways. First, we determined that *pha-4(ts)* had no impact on three nonforegut genes (*eft-3*, *mig-38*, and *srw-99*) (Fig. 4A). Second, we used artificial chromosomes bearing PHA-4 target promoters and fluorescently tagged PHA-4 to examine chromatin opening in single cells (*9*). We observed decompaction of artificial chromosomes in the foregut of mid-stage embryos but not early embryos (Fig. 4, B to D) (*9*). Artificial chromosomes in nonforegut cells failed to decompact at either stage (Fig. 4, B and C). Third, a comparison of wild-type 4E embryos (with little to no PHA-4) and 8E embryos (with detectable PHA-4) revealed a decrease in FAIRE values at the 8E for both foregut and nonforegut genes (fig. S7). Thus, PHA-4 binding did not induce detectable decompaction at the 8E stage compared with the 4E. The data suggest that PHA-4 induces decompaction predominantly at mid-stages, after Pol II binding.

This study reveals widespread poised Pol II during *C. elegans* development and shows that the pioneer transcription factor PHA-4 contributes to Pol II recruitment at poised and transcribed genes within the foregut. PHA-4 activity is critical during early embryonic stages when we observe Pol II recruitment, suggesting these early events are essential for proper organogenesis (*17*). *C. elegans* embryos develop in 13 hours, with rapid

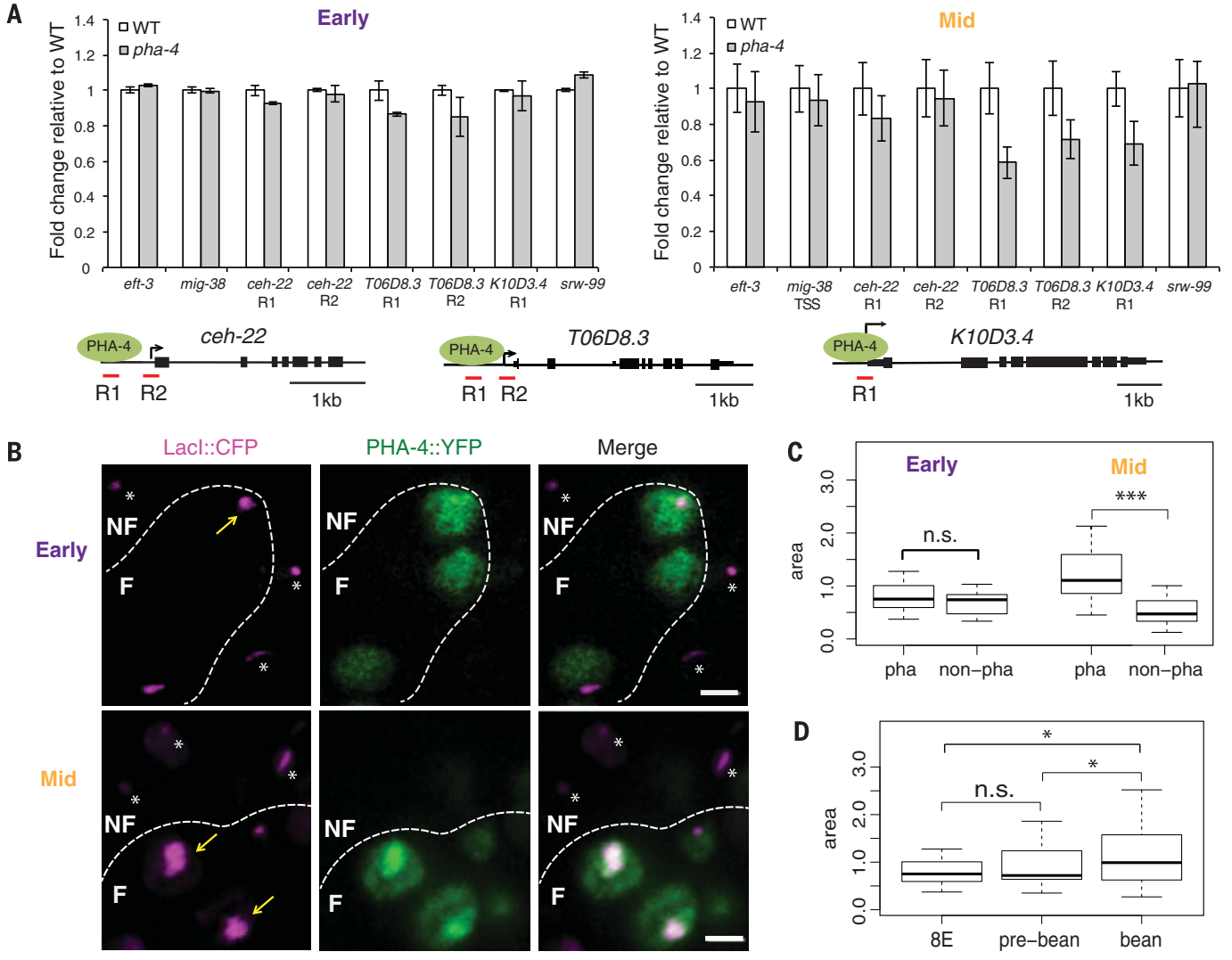

Fig. 4. PHA-4 promotes chromatin openness during mid-stage embryogenesis. (**A**) Chromatin opening tracked by FAIRE–quantitative PCR. Early (left), FAIRE signals for three poised foregut genes were similar between wild-type (white) and *pha-4(ts)* (gray). Mid- (right), FAIRE signals were reduced at poised foregut genes in *pha-4(ts)* embryos. The gene structures show positions for PHA-4 binding (R1) and TSS (R2) sites. n = 3 replicates, mean ± SEM. (**B**) Artificial chromosomes (CFP::LacI, purple) bearing the *ceh-22* promoter bound by PHA-4::YFP (green) in early and mid-stage embryos. Dotted lines distinguish foregut (F) from nonforegut (NF). Scale bar, 2 μm. (**C**) Areas of artificial chromosomes carrying the *ceh-22* promoter in foregut (pha) versus nonforegut (non-pha). (**D**) Areas of artificial chromosomes carrying the *ceh-22* promoter in the foregut at different stages. *P = 0.01 to 0.05; ***P < 0.001.

changes in gene expression. Pol II poising may accommodate these dynamics by promoting rapid and/or synchronous transcriptional onset (*12*, *19*). Recruitment of poised Pol II is followed by decompaction of chromatin. One appealing hypothesis is that deposition of Pol II at TSS regions may participate in chromatin opening, along with PHA-4 (*18*). This scenario predicts that Pol II binds to regions that would otherwise contain stable nucleosomes, a prediction that is borne out by our FAIRE analysis, and that Pol II interferes with the construction of nucleosomes. It will be of interest to see whether other pioneer factors or FoxA proteins also poise Pol II.

REFERENCES AND NOTES

1. M. Iwafuchi-Doi, K. S. Zaret, *Genes Dev.* **28**, 2679–2692 (2014).
2. D. L. D. L. Updike, S. E. S. E. Mango, *PLOS Genet.* **2**, e161 (2006).
3. S. E. Mango, *Annu. Rev. Cell Dev. Biol.* **25**, 597–628 (2009).
4. M. A. Horner *et al.*, *Genes Dev.* **12**, 1947–1952 (1998).
5. J. M. Kalb *et al.*, *Development* **125**, 2171–2180 (1998).
6. M. Zhong *et al.*, *PLOS Genet.* **6**, e1000848 (2010).
7. P. G. Giresi, J. Kim, R. M. McDaniell, V. R. Iyer, J. D. Lieb, *Genome Res.* **17**, 877–885 (2007).
8. J. W. K. Ho *et al.*, *Nature* **512**, 449–452 (2014).
9. T. H. I. Fakhouri, J. Stevenson, A. D. Chisholm, S. E. Mango, *PLOS Genet.* **6**, e1001060 (2010).
10. J. M. Caravaca *et al.*, *Genes Dev.* **27**, 251–260 (2013).
11. Materials and methods are available as supplementary materials on *Science* Online.
12. K. Adelman, J. T. Lis, *Nat. Rev. Genet.* **13**, 720–731 (2012).
13. L. R. Baugh, J. Demodena, P. W. Sternberg, *Science* **324**, 92–94 (2009).
14. C. S. C. S. Maxwell *et al.*, *Cell Reports* **6**, 455–466 (2014).
15. C. A. Kuchenthal, W. Chen, P. G. Okkema, *Genesis* **31**, 156–166 (2001).
16. B. Gaertner *et al.*, *Cell Reports* **2**, 1670–1683 (2012).
17. J. C. Kiefer, P. A. Smith, S. E. Mango, *Dev. Biol.* **303**, 611–624 (2007).
18. D. A. Gilchrist *et al.*, *Cell* **143**, 540–551 (2010).
19. A. N. Boettiger, M. Levine, *Science* **325**, 471–473 (2009).
20. M. Levin, T. Hashimshony, F. Wagner, I. Yanai, *Dev. Cell* **22**, 1101–1108 (2012).

ACKNOWLEDGMENTS

We thank J. Whetstine for ChIP advice, A. Schier and S. von Stetina for comments, and G. Marnellos and M. Clamp for informatics. Some strains were obtained from the Caenorhabditis Genetics Center (CGC), funded by NIHP40OD010440. S.E.M. received support from the MacArthur Foundation and grant NIHR37GM056264, E.L. received support from grant NSFMCB-1413134, and K.S.Z. received support from grant NIHR37GM36477. Sequencing data are accessible from the National Center for Biotechnology Information Gene Expression Omnibus: GSM1666978 R1.CE.8E.ChIP GSM1666979, R1.CE.8E.Input GSM1666980, R2.CE.8E.ChIP GSM1666981, R2.CE.8E.Input GSM1666982, R1.CE.BE.ChIP GSM1666983, and R1.CE.BE.Input.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/348/6241/1372/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S7
Tables S1 to S3
References

13 March 2015; accepted 12 May 2015
10.1126/science.aab1223

DRUG DEVELOPMENT

Phthalimide conjugation as a strategy for in vivo target protein degradation

Georg E. Winter,[1]* Dennis L. Buckley,[1]* Joshiawa Paulk,[1] Justin M. Roberts,[1] Amanda Souza,[1] Sirano Dhe-Paganon,[2] James E. Bradner[1,3]†

The development of effective pharmacological inhibitors of multidomain scaffold proteins, notably transcription factors, is a particularly challenging problem. In part, this is because many small-molecule antagonists disrupt the activity of only one domain in the target protein. We devised a chemical strategy that promotes ligand-dependent target protein degradation using as an example the transcriptional coactivator BRD4, a protein critical for cancer cell growth and survival. We appended a competitive antagonist of BET bromodomains to a phthalimide moiety to hijack the cereblon E3 ubiquitin ligase complex. The resultant compound, dBET1, induced highly selective cereblon-dependent BET protein degradation in vitro and in vivo and delayed leukemia progression in mice. A second series of probes resulted in selective degradation of the cytosolic protein FKBP12. This chemical strategy for controlling target protein stability may have implications for therapeutically targeting previously intractable proteins.

Phthalimide-based drugs emerged in the 1950s. Among the most notable was thalidomide, developed initially as a sedative but infamously withdrawn from human use owing to catastrophic teratogenicity (*1*). More recently, the phthalimides have been successfully repurposed for erythema nodosum leprosum, multiple myeloma (MM), and myelodyspasia. The efficacy of thalidomide, lenalidomide, and pomalidomide in MM (Fig. 1A) has prompted investigation into the mechanism of action of phthalimide immunomodulatory drugs (IMiDs). By ligand-affinity chromatography, cereblon (CRBN)—a component of a cullin-RING ubiquitin ligase (CRL) complex—was identified as the target of thalidomide (*2*). Recently, our group and others reported that phthalimides prompt CRBN-dependent proteasomal degradation of transcription factors (TFs) IKZF1 and IKZF3 (*3*, *4*). Crystallographic studies now establish that IMiDs bind CRBN to form a cryptic interface that promotes recruitment of IKZF1 and IKZF3 (*5*).

Ligand-induced target protein destabilization has proven to be an efficacious therapeutic strategy, in particular for cancer, as illustrated by arsenic trioxide–mediated degradation of the PML protein in acute promyelocytic leukemia (*6*) and estrogen receptor degradation by fulvestrant (*7*). Historically, target-degrading compounds have emerged serendipitously or through target-specific campaigns in medicinal chemistry. Chemical biologists have devised elegant solutions to modulate protein stability using engineered cellular systems, but these approaches have been limited to nonendogenous fusion proteins (*8*–*11*). Others have achieved the degradation of endogenous proteins through the recruitment of E3 ligases, but these approaches have been limited by the requirement of peptidic ligands (*12*–*14*), the use of nonspecific inhibitors (*15*), and by low cellular potency.

RING-domain E3 ubiquitin-protein ligases lack enzymatic activity and function as adaptors to E2 ubiquitin-conjugating enzymes. Inspired by the retrieval of CRBN using a tethered thalidomide (*2*), we hypothesized that rational design of bifunctional phthalimide-conjugated ligands could confer CRBN-dependent target protein degradation as chemical adapters. We selected BRD4 as an exemplary target. BRD4 is a transcriptional coactivator that binds to enhancer and promoter regions by recognition of acetylated lysines on histone proteins and TFs (*16*). Recently, we developed a direct-acting inhibitor of BET bromodomains (JQ1) (*17*) that displaces BRD4 from chromatin and leads to impaired signal transduction from TFs to RNA polymerase II (*18*–*20*). Silencing of BRD4 expression by RNA interference in murine and human models of MM and acute myeloid leukemia (AML) elicited rapid transcriptional down-regulation of the *MYC* oncogene and a potent antiproliferative response (*19*, *21*). These and other studies in cancer, inflammation (*22*), and heart disease (*23*, *24*) establish a desirable mechanistic and translational purpose to target BRD4 for selective degradation.

Having shown that the carboxyl group on JQ1 (*25*) and the aryl ring of thalidomide (*5*) can tolerate chemical substitution, we designed the bifunctional dBET1 to have preserved BRD4 affinity and an inactive epimeric dBET1(R) as a stereochemical control (Fig. 1, A and B). Selectivity profiling confirmed potent and BET-specific target engagement among 32 bromodomains (BromoScan) (Fig. 1C and tables S1 and S2). A high-resolution crystal structure (1.0 Å) of dBET1 bound to BRD4(1) confirmed the mode of molecular recognition, comparable to JQ1 (Fig. 1D, fig. S1, and

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02115, USA. [2]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA 02115, USA. [3]Department of Medicine, Harvard Medical School, Boston, MA 02115, USA.
*These authors contributed equally to this research. †Corresponding author: E-mail: james_bradner@dfci.harvard.edu

Recruitment of RNA polymerase II by the pioneer transcription factor PHA-4

H.–T. Hsu *et al.*

Science **348**, 1372 (2015);
DOI: 10.1126/science.aab1223