REVIEW

A double take on bivalent promoters

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Histone modifications and chromatin-associated protein complexes are crucially involved in the control of gene expression, supervising cell fate decisions and differentiation. Many promoters in embryonic stem (ES) cells harbor a distinctive histone modification signature that combines the activating histone H3 Lys 4 trimethylation (H3K4me3) mark and the repressive H3K27me3 mark. These bivalent domains are considered to poise expression of developmental genes, allowing timely activation while maintaining repression in the absence of differentiation signals. Recent advances shed light on the establishment and function of bivalent domains; however, their role in development remains controversial, not least because suitable genetic models to probe their function in developing organisms are missing. Here, we explore avenues to and from bivalency and propose that bivalent domains and associated chromatin-modifying complexes safeguard proper and robust differentiation.

His one pro eins and heir pos - ransla ional modifica- ions ha e emerged as impor an pla ers in he reg la- ion of gene e pression and o her chroma in-associa ed processes. The fo r core his ones H2A, H2B, H3, and H4 are s bjec o a hos of co alen modifica ions, in- cl ding me h la ion, ace la ion, phosphor la ion, and biq i ina ion, among o hers (Vaq ero e al. 2003; Campos and Reinberg 2009; Bannis er and Ko arides 2011). These marks are ho gh o e er heir f nc ion hro gh direc mod la ion of chroma in s r c re and hro gh effec or pro eins ha fea re modifica ion-specific binding domains (Ta erna e al. 2007; Voig and Reinberg 2011). Moreo er, se eral his one modifica ions ha e been implica ed as carriers of epigene ic informa ion ha can be ransmi ed hro gh cell di ision, ins r c ing gene e pression pa erns in he da gh er cells (Probs e al. 2009; Marg eron and Reinberg 2010).

Genome- ide mapping s dies of chroma in modifica- ions in ES cells ha e re ealed he presence of dis inc his one marks a cer ain genomic domains, s ch as H3K4me1 and ace la ion of H3K27 (H3K27ac) i hin ac i e enhancers as ell as H3K4me3 and H3K27me3

appear o be e cep ions in o her organisms. Using gas- r la s age *Xenopus* embr os ndergoing he midblas la ransi ion, Akkers e al. (2009) de ec ed er fe bi- alen domains. Moreo er, genes i h signals for H3K4me3 and H3K27me3 origina ed largel from dis- inc areas of he embr o, of en being e pressed in par s of he embr o, and onl a minori of hem corresponding o bi alen genes in mo se ES cells (Akkers e al. 2009). Al ho gh he se of la e s age *Xenopus* embr os ha ha e alread ndergone s bs an ial lineage specifica ion ma par iall e plain his discrepanc i h he ebrafish s dies, i ne er heless seems pla sible ha modes of gene reg la ion differ be een *Xenopus*, ebrafish, and higher er ebra es.

This no ion is s ppor ed b he compara i el la e appearance of repressi e his one marks d ring lineage specifica ion in *Xenopus* de elopmen (Schneider e al. 2011), s gges ing ha bi alen domains migh be re- s ric ed o cer ain organisms. Indeed, hile *Drosophila* fea res a reper oire of PcG and r G comple es similar o ha in mammals, bi alen domains appear o be absen . Anal sis of *Drosophila* embr os and es is-de- ri ed s em cells did no ield e idence for significan coe is ence of bo h marks (Sch e engr ber e al. 2009; Gan e al. 2010). Inheren differences in gene reg la ion be een ar hropods, lo er er ebra es, and mammals ma acco n for his apparen discrepanc . For e ample, CpG island promo ers, he si es of bi alen domains, are o er helmingl more common in mammals. Ins ead, reg la ion of RNA pol merase II (Pol II) pa sing ma cons i e an al erna i e means o coordina e he e pres- sion of earl de elopmen al genes in *Drosophila* (M se

single-cell approaches i h genome- ide anal ses. Firs ,

H3K4me3-con aining n cleosomes (Voig e al. 2012), in line i h MEFs e hibi ing fe er bi alen genes.

I has been arg ed ha H3K4me3 and H3K27me3 canno coe is on n cleosomes beca se PRC2 is inhibi ed b he ac i e marks H3K4me3 and H3K36me3 (Schmi ges e al. 2011). Moreo er, MS-based s dies fo nd ha H3K4me3 and H3K27me3 do no coe is on indi id al his ones in HeLa cells (Yo ng e al. 2009). Gi en he recen obser a ion ha sis er his ones i hin a n cleosome are of en neq all modified (Voig e al. 2012), bi alen domains co ld fea re as mme ricall modified n cleosomes. Indeed, n cleosomes i h onl one H3K4me3 mark co ld s ill be me h la ed b PRC2, pres mabl on he nmodified H3 ail, hereas inhibi- ion of PRC2 req ired he presence of H3K4me3 on bo h copies of H3 (Voig e al. 2012). MS anal sis of ES cell-deri ed his ones confirmed he presence of hese marks on dis inc copies of H3 in i o. In concl sion, hese da a s gges ha bi alen domains fea re n cleosomes ha carr H3K4me3 and H3K27me3 on opposi e H3 copies. Of no e, his obser ed as mme r in H3K27me3 and H3K4me3 is compa ible i h he repor ed red c ion in H3K27me3 signals a some H3K4me3-marked n cleosomes rela i e o heir neighbors ha do no carr H3K4me3 (Pan e al. 2007; Marks e al. 2012).

Generation of bivalent domains

Con rolling heir access o genomic loci is ho gh o be a major a of reg la ing he ac i i of r G and PcG pro eins, he cen ral pla ers in se ing p and main ain- ing bi alenc . Se eral recr i men mechanisms ha e been proposed, incl ding specific DNA seq ence elemen s, DNA me h la ion s a s, par ic lar his one modifica ions, TFs, and noncoding RNAs (ncRNAs), among o hers. No s rprisingl , man of hese elemen s ha e been implica ed in he genera ion of bi alen domains as ell. One of he ke cl es as o ho bi alen domains migh be genera ed came from anal ses of heir nderl ing DNA seq ences. I as nco ered earl on ha bi alen domains s rongl correla e i h CpG islands in ES cells (Berns ein e al. 2006). CpG islands are a prominen fea- re of promo ers in er ebra e genomes and are presen a ~70% of all promo ers (Sa ono e al. 2006; Dea on and Bird 2011). Vir all all CpG-rich promo ers in ES cells are de oid of DNA me h la ion (Weber e al. 2007; Fo se e al. 2008; Meissner e al. 2008; Mohn e al. 2008) hile being rime h la ed a H3K4 (G en her e al. 2007; Mikkelsen e al. 2007). Con ersel , essen iall all H3K4me3 si es map o CpG islands (Mikkelsen e al. 2007; Pan e al. 2007), hich conseq en l

comple ha is associa ed i h elonga ing RNA Pol II, media ing recr i men of SET1 o ranscribed loci d ring earl elonga ion (Krogan e al. 2003). A similar mechanism migh recr i SET1 and MLL comple es in mammals, leading o addi ional deposi ion of H3K4me3 d ring ranscrip ion. Ac i e ranscrip ion migh herefore reinforce H3K4me3 deposi ion a ac i el ranscribed genes and, o a lesser e en , a minimall ranscribed bi alen loci.

CpG islands and PRCs

CpG islands like ise pla an impor an role in es ablishing and main aining H3K27me3 a bi alen domains (Fig. 4B). In con ras o H3K4me3, ho e er, no all CpG islands are marked i h H3K27me3. Moreo er, hereas H3K4me3 is highl locali ed a promo ers and h s marks onl a min e frac ion of n cleosomes, he dis- rib ion pa erns of H3K27me3 are more comple . H3K27me3 marks ~10%–15% of all H3 his ones in ES cells as assessed b q an i a i e MS (Pe ers e al. 2003; Voig e al. 2012). If considering H3K27me2 as ell, ~50% of all n cleosomes in ES cells are modified b PRC2 (Voig e al. 2012). Man ChIP-seq s dies re ealed "la ns" of H3K27me3 mos l spanning in ergenic regions and inac i e genes (e.g., see Pa ler e al. 2009; Yo ng e al. 2011; Marks e al. 2012). H3K27me3 is also enriched in s b elomeric regions (Rosenfeld e al. 2009) and a long erminal repea re ro ransposons (Leeb e al. 2010). These regions likel acco n for he b lk of H3K27me2/3 presen in he ES cell genome. In addi ion, a rela i el smaller amo n of H3K27me3 also e hibi s more locali ed pa erns aro nd he TSS, some imes e ending in o he promo er (e.g., see Mikkelsen e al. 2007; Yo ng e al. 2011). In ES cells, hese TSSs are almos e cl si el bi alen (Mikkelsen e al. 2007; K e al. 2008). In eres ingl , hen anal ing he genomic locali- a ion of componen s of he PRC2 comple , defined peaks are predominan l fo nd aro nd gene promo ers (Bo er e al. 2006; Bracken e al. 2006; K e al. 2008), indica ing more efficien recr i men or re en ion a promo ers. PRC2 appears o be more spread o o er

i h a sligh bias o ard GC-rich seq ences (Fig. 4B; Li e al. 2010). Recr i men of Jarid2 and he PRC2 core componen E h2 appear o be codependen , b he e ac role of Jarid2 in recr i ing PRC2 remains nclear. Similarl , AEBP2, a inc finger pro ein ha binds DNA i h lo specifici , in erac s and colocali es i h PRC2 a some promo ers (Kim e al. 2009). PHF1 (PCL1), MTF2 (PCL2), and PHF19 (PCL3), or hologs of *Drosophila* Pol - comb-like (PCL), also in erac i h PRC2 and ha e been implica ed in i s recr i men (Marg eron and Reinberg 2011; Simon and Kings on 2013). These and o her pro- eins sho n o ransien l in erac i h PRC2 ma media e i s recr i men o specific loci, b i remains nclear he her an of hese pro eins can comple el acco n for i s preference for CpG islands in ES cells.

Targe ing of PRC2 comple es o specific genomic si es in mammals likel occ rs hro gh m l iple means. Gi en he pa ci of seq ence-specific fac ors iden ified o da e, o her modes of in erac ion ma e plain PRC2 recr i men o CpG islands. PRC2 forms m l iple con- ac s i h n cleosomes ha genera e affini for chroma in in a seq ence-independen fashion (Fig. 4B; see also Marg eron and Reinberg 2011). Al ho gh each s ch in erac ion is of lo affini , he combina ion of hese in erac ions ma allo for a consolida ed and spa iall acc ra e recr i men of PRC2 based on local chroma in fea res, akin o coincidence de ec ion (Marg eron and Reinberg 2011; Voig and Reinberg 2011). Specificall , Jarid2 and AEBP2 each in erac i h DNA and i h PRC2, and he PRC2 core componen s RbAp46/48 and Eed bind o his ones H3 and H4. Whereas Eed also binds o H3K27me3 and migh f nc ion in perpe a ing he mark (Marg eron e al. 2009), H3K4me3 abroga es RbAp46/48 recogni ion of H3 and inhibi s PRC2 ac i i (Schmi ges e al. 2011). Similarl , H3K36me3 inhibi s PRC2 ac i i (Schmi ges e al. 2011; Y an e al. 2011)

asn78.82and i scompo03.9(sl)0( )(co5(781 ha 03.83PRC2))0(R)14B;83.42in ef s5661inhib eea16.73forei781 ha 0s0-1.2203TD[((Schi)2a

ligase and a member of he PCGF pro ein famil , bridging RING1B and i s in erac ion par ners. PRC1 forms se eral s bcomple es i h niq e s b ni composi ion (Gao e al. 2012; Ta ares e al. 2012; Simon and Kings on 2013). RING1B occ pies ~40%–50% of all bi alen domains in ES cells (K e al. 2008; Brookes e al. 2012). RING1B-bo nd bi alen genes are highl enriched for de elopmen al fac ors and are ell conser ed be een mice and h mans. Moreo er, he e hibi larger regions of H3K27me3 and are more likel o remain repressed pon differen ia ion (K e al. 2008). PRC1 comple es ha con ain CBX pro eins ma be recr i ed, a leas in par , b binding o H3K27me3. In mo se ES cells, CBX7 is likel he predominan CBX pro ein ha helps recr i PRC1 o H3K27me3-con aining si es (More e al. 2012, 2013). Ho e er, o her H3K27me3-independen de erminan s con rol PRC1 arge ing and depend on he s b ni composi ion of each par ic lar PRC1 comple (Fig. 4B). Candida es incl de TFs s ch as E2F6, YY1, and REST as ell as ncRNAs (Simon and Kings on 2013).

No abl , Fb l10/KDM2B as recen l sho n o recr i some PRC1 comple es o nme h la ed CpG islands ia i s CXXC domain, rendering i an in rig ing candida e for arge ing some PRC1 comple es o bi alen promo ers (Farcas e al. 2012; He e al. 2013; W e al. 2013). KDM2B is presen a lo le els a ir all all nme h la ed CpG islands in ES cells b is e cl ded from si es of DNA me h la ion. Ho e er, s bs an ial KDM2B-PRC1 binding is obser ed onl a a frac ion of all nme h la ed CpG islands. This s gges s a "sampling" mechanism hereb KDM2B-PRC1 comple es con in all probe nme h- la ed CpG loci for heir s scep ibili o repression, and s able recr i men ma f r her depend on pre-e is - ing repressi e de erminan s (Farcas e al. 2012). Like ise, fac ors in ol ed in ranscrip ion ma pre en acc m la- ion of high le els of hese PRC1 comple es a ac i e loci.

Se eral s dies ha e connec ed PRCs o H2A.Z. As men ioned abo e, H2A.Z is enriched a bo h ac i e and bi alen promo ers b no a loci marked e cl si el b H3K27me3 (K e al. 2012; H e al. 2013). Loss of H2A.Z red ces PRC2 occ panc a bo h bi alen promo ers and enhancers (Cre gh on e al. 2008; H e al. 2013). Ini ial repor s s gges ed ha arge ing of H2A.Z o bi alen promo ers ma depend on PRC1/2 comple es and ice ersa (Cre gh on e al. 2008). Ho e er, recen s dies indica e ha H2A.Z deposi ion is independen of he PRCs (Illing or h e al. 2012). Ne er heless, nders and- ing H2A.Z recr i men ma shed ligh on ho PRCs are arge ed o specific loci. This q es ion is of special in eres gi en ha OCT4 arge ing is dependen on H2A.Z in ES cells (H e al. 2013). In addi ion o in erac ing i h he MLL comple es, OCT4 has also been sho n o in erac i h PRC1 s bcomple es as ell as i h he his one deace lase-con aining N RD comple (Pardo e al. 2010; an den Berg e al. 2010). The N RD comple facili a es PRC2 recr i men hro gh i s deace la ion of H3K27 (Re nolds e al. 2012). No abl , deple ion of N RD leads o dereg la ion of se eral bi alen genes and is accompanied b increased H3K27ac and red ced H3K27me3 (Re nolds e al. 2012). Taken oge her, hese s dies nderscore he impor ance of H2A.Z as a cen ral pla er orches ra ing he deposi ion of pl ripo enc fac- ors and epigene ic reg la ors a bi alen loci.

A model for the generation and maintenance of bivalent domains

SET1A/B/MLL comple es is media ed a leas in par b CXXC domain-con aining pro eins or hro gh he ac ion of TET en mes, OGT, and his one arian s. In he presence of ac i a ing signals and TFs s ch as OCT4, H3K4me3 a hese promo ers is reinforced and s s ained b co ranscrip ional deposi ion. The ac of prod c i e ranscrip ion as ell as he ensemble of TFs and coac- i a ors ma s ffice o e cl de PcG pro eins from ac i e genes hro gh compe i ion for binding o he nderl ing GC-rich DNA seq ences or hro gh rep lsion of PRC2 b n cleosomes s mme ricall modified i h H3K4me3 or H3K36me3. Sp rio s H3K27me3 ma be remo ed from

anal sis of PcG and r G gene knocko s. Al ho gh loss of H3K4me3 or H3K27me3 is no limi ed o bi alen loci in hese models, al able informa ion can be ob ained especiall for PcG pro eins, as he as majori of heir genic arge s in ES cells correspond o bi alen genes. None heless, he absence of H3K27me3 and PRCs a repe i i e elemen s and o her arge s m s be considered hen in erpre ing PcG m an pheno pes. Differen gro ps ha e obser ed a general propensi of PcG m an ES cells o p-reg la e de elopmen al genes, s ppor ing a cr cial role for PcG pro eins and h s bi alen domains in de elopmen . For ins ance, se eral bi alen genes are prema rel e pressed in *Eed*$^{-/-}$ ES cells (A ara e al. 2006; Bo er e al. 2006). Like ise, *Suz12*$^{-/-}$ ES cells sho higher e pression of lineage-specific genes (Pasini e al. 2007). Ho e er, despi e he mise pression of lineage genes, cell iabili and self-rene al are no compromised in PRC2-deficien ES cells (Pasini e al. 2007; Chamberlain e al. 2008; Shen e al. 2008; Leeb e al. 2010). The o erall mild defec s of PRC2-deficien ES cells in self-rene al ma be par iall e plained b PRC1-media ed compensa or effec s and he absence of TFs ha co ld rob s l ac i a e he affec ed genes in he ndifferen ia ed s a e. Indeed, sim l aneo s deple ion of RING1B and EED in ES cells pro okes an e en s ronger inclina ion o ard differen ia ion, al ho gh self-rene al can s ill be preser ed nder caref l c l re condi ions (Leeb e al. 2010). In con ras o he rela i el mild effec s on self-rene al, all PRC2-deficien ES cells e hibi aberran differen ia ion po en ial (Pasini e al. 2007; Chamberlain e al. 2008; Shen e al. 2008; Leeb e al. 2010), hich parallels he pos -implan a ion le hali pheno pes obser ed in PRC2 knocko mo se models (Fa s e al. 1995; O'Carroll e al. 2001; Pasini e al. 2004). Taken oge her, hese knocko models demons ra e ha PRCs pres mabl o a large degree hro gh con rol of bi alen arge genes encoding de elopmen al fac ors are i al for proper differen ia ion.

No abl , he recen disco er ha FBXL10/KDM2B is ke in arge ing a s bse of PRC1 comple es o CpG-rich promo ers ma allo for he specific mod la ion of PRCs a bi alen loci (Farcas e al. 2012; He e al. 2013; W e al. 2013). I s deple ion in ES cells ca ses derepression of PcG arge genes comparable i h RING1B knocko cells and leads o prema re and defec i e differen ia ion (He e al. 2013; W e al. 2013), nderscoring he impor ance of PcG repression as a safeg ard mechanism a bi alen loci for proper de elopmen , especiall in he con e of lineage specifica ion.

Histone modifications, binding proteins, and PRCs in reversible silencing at bivalent promoters

Bo h he his one modifica ions and he pro ein comple es presen a bi alen promo ers likel media e he impac of bi alenc on ranscrip ion. Man pro eins ha bind H3K4me3 and f nc ion as effec ors ha e been described, mos of hich are associa ed i h ac i e ranscrip ion (Fig. 6). The PHD finger of he TAF3 s b ni of TFIID recogni es H3K4me3 (Verme len e al. 2007), hereas he TAF1 s b ni binds o ace la ed l sines on his ones H3 and H4 ia i s bromodomains (Jacobson e al. 2000). These in erac ions likel con rib e o re-

absence of DNA me h la ion a hose si es (W and Zhang 2011; Williams e al. 2012). Moreo er, H2A.Z a CpG-rich promo ers ma f r her an agoni e DNA me h- la ion (Zilberman e al. 2008). H3K4me3, possibl along i h o her fac ors s ch as H2A.Z, ma h s f nc ion o a large degree b keeping genes in a s a e permissi e for ac i a ion b precl ding irre ersible repression hro gh DNA me h la ion (Fig. 6). A oidance of DNA me h la- ion is essen ial for bi alen genes as ell, as he are req ired o re ain plas ici for s bseq en ac i a ion or repression.

Ho e er, an ine i able conseq ence of s ch a permis- si e chroma in s a e ma be a res l an lo le el of ranscrip ion emana ing from hese bi alen promo ers. Indeed, as pre io sl men ioned, mos promo ers of pro- ein-coding genes (G en her e al. 2007) and essen iall all bi alen promo ers (Brookes e al. 2012) harbor he ini ia ing (S5P) form of RNA Pol II in ES cells, indica i e of ranscrip ional compe ence. F r hermore, se eral gro ps ha e ascer ained he presence of lo b appreciable le els of reg la ed ranscrip ion arising from PRC-bo nd bi alen loci (Kanhere e al. 2010; Walker e al. 2010; Min e al. 2011; Brookes e al. 2012). In eres ingl , he e en of RNA Pol II engroeen e3(e)65.4(el.7(gr)o13.1(rn65.4(g.1(.1(p h la 6-8(c)-1f)-26.9(nd)]TJ0-1.220305.0114mod l19.7(a10.9e

Se eral s dies ha e sho n ha PcG pro eins are remo ed from specific loci follo ing de elopmen al signals hro gh he ac ion of TFs, his one deme h lases, and enhancers and hro gh he in rod c ion of modifica- ions ha co n erac binding of PcG pro eins (Deles e al. 2012). Bo h UTX and JMJD3 are capable of deme h- la ing H3K27me3, and bo h pro eins are req ired for proper differen ia ion (Agger e al. 2007; Lee e al. 2007). UTX is par of he MLL3/4 comple es (Lee e al. 2007), hereas JMJD3 in erac s i h pro eins in ol ed in ran- scrip ional elonga ion, s ch as he FACT s b ni SPT16, and i h KIAA1718, a deme h lase for H3K9me2, H3K27me2, and H4K20me1 (Chen e al. 2012). In rig - ingl , KIAA1718 recogni es H3K4me3 ia i s PHD finger (Hor on e al. 2010), rendering i an ideal candida e for remo ing H3K27 me h la ion in he con e of bi alenc . Bo h UTX and JMJD3 rarel locali e o bi alen promo ers

bi alen genes ma likel be incl ded in hose regions as ell, being s bjec ed o addi ional means o s abili e heir silencing.

Conclusion

In he shor his or since heir disco er , bi alen domains ha e garnered grea a en ion as a means o poise gene e pression in ES cells and be ond. C rren e idence s gges s ha bi alen domains f nc ion in he fine-ning of gene e pression d ring de elopmen . The sim l-aneo s presence of ac i e and repressi e modifica ions and associa ed comple es helps o main ain bi alen loci in a s a e ha is bo h responsi e o de elopmen al c es and a he same ime refrac or o s b hreshold noise. Despi e remendo s progress o ard nders anding he es ablishmen of bi alenc as ell as he ac ion of marks and comple es in poising ranscrip ion, f re ork is clearl req ired o direc l probe he impor ance of bi a-lenc in de eloping organisms and f r her o r kno ledge of e ac l ho PcG pro eins reg la e ranscrip ion. The bi alenc field is s ill in de elopmen .

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