Chapter 3
Krüppel-like Factor Proteins and Chromatin Dynamics

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Abstract Krüppel-like factors (KLFs) are transcription regulatory proteins. Members of this protein family are characterized by a highly conserved C-terminus that has three zinc finger domains that bind to GC-rich sequences in DNA. The N-terminal domains of these proteins contain regulatory regions that can activate or repress transcription in a context-specific manner. KLFs interact with a wide range of co-activators or co-repressors to accomplish their transcription regulatory function. These interactions provide a complex stage for the chromatin dynamics to unfold and regulate diverse biological functions. This chapter focuses on expanding our understanding of molecular mechanisms of transcription regulation by KLFs and their impact on chromatin dynamics.

Introduction

Krüppel-like factors (KLFs) constitute a family of diverse transcription regulatory proteins characterized by an N-terminal domain that contains transcriptional regulatory motifs (Bieker 2001; Black et al. 2001; Cook et al. 1999; Cook and Urrutia 2000; Turner and Crossley 1998) and a highly conserved C-terminus that has three Cys2His2 zinc finger domains to bind to DNA. The members of this family bind to similar, yet distinct GC-rich target sequences, and they function either as activators or repressors (Bieker 2001; Lomberk and Urrutia 2005; Turner and Crossley 1998). KLF activator proteins, such as KLF1 and KLF4, function by interacting with histone acetylases, requiring interaction with the co-activator CBP/p300 and p300/CBP-associated factor (PCAF) (Geiman et al. 2000; Zhang et al. 2001a). On the other hand, KLF repressor proteins form a repressor complex with CtBP through use of a canonical PVDLS/T motif, a CtBP-interacting domain

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(KLF3 and KLF8) (Turner and Crossley 1998; van den Ent et al. 1993), or they utilize the SIN3A-HDAC complex, which binds to a SIN3A-interacting domain, or SID (KLF10, KLF-11, KLF-13, KLF-16). Such complex formation has profound effects on chromatin dynamics, which affect virtually all known biological processes governing normal and abnormal mammalian development, differentiation, survival, and aging. Among others, histones play a central role in the chromatin dynamics as their N-terminal tails are subject to covalent modifications by the opposing actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs), as well as other enzymatic activities. This reversible acetylation along with other histone modifications—collectively known as the Histone Code—alter either focal or global chromatin domains and thereby influence the activation or repression of gene transcription. In this chapter, we discuss the molecular mechanisms that have emerged during last two decades that have shed light on the transcriptional regulation by KLF family members and their effect on chromatin dynamics.

Classification of KLF Proteins and Their Co-repressor/ Co-activator Interactions

The discovery of Sp1 as a transcriptional regulator led to an extensive search, during last two decades, for proteins that are structurally and functionally related to Sp1. During the early 1990s, our laboratory characterized two novel, transforming growth factor-β (TGF-β)-inducible Sp1-like proteins—transcription factors TIEG1 and TIEG2—which are now known as KLF10 and KLF11, respectively (Cook et al. 1998; Tachibana et al. 1997). Since then, database comparisons and library screening in addition to extensive work performed by many dedicated laboratories worldwide have revealed the existence of 24 different proteins that share a remarkable similarity with Sp1 within their zinc finger domains and also bind to GC-rich sequences to regulate gene expression, thus belonging to the KLF/Sp1-like family of proteins (Buttar et al. 2006; Kaczynski et al. 2003; Lomberk and Urrutia 2005). For instance, we and several others have shown that both KLF10 and KLF11 proteins bind to and regulate the function of promoters that contain Sp1-like sequences (Cao et al. 2005; Fernandez-Zapico et al. 2003; Lomberk et al. 2008; Neve et al. 2005; Subramaniam et al. 2007; Wang et al. 2007; Zhang et al. 2007). The existence of a family of Sp1-like proteins posed several important biological questions regarding their function. For example, do Sp1-like proteins: (1) have redundant or distinct roles in mammalian cell physiology; (2) form homodimers and/or heterodimers; (3) work in a cell type-specific manner; (4) participate in a hierarchical cascade of gene expression; and/or (5) antagonize each other's functions to fine-tune specific cellular processes?

Several seminal publications with promising results by our group and several others over last two decades have helped move this field forward in addressing these questions. The ubiquitous expression of Sp1 in murine cells suggests that most, if not all, mammalian cells require Sp1 for proper function. The validity of this

hypothesis was supported by finding that the knockout of this gene leads to gross morphological defects in a large number of tissues (Liu et al. 1996; Marin et al. 1997). In contrast, several other members of the Sp1-like family (e.g., KLF1, KLF2, Sp4) are expressed in a tissue-enriched manner (Abdelrahim et al. 2004; Carlson et al. 2006; Lin et al. 2005; Nielsen et al. 1998; Safe and Abdelrahim 2005; Watanabe et al. 1998). This selective pattern of expression raises the possibility that these proteins have a cell-specific function. This is particularly true in the case of KLF1 knockouts, where there are selective defects in erythropoiesis (Drissen et al. 2005; Eaton et al. 2008; Funnell et al. 2007). Because several Sp1-like proteins that recognize identical DNA sequences can be co-expressed in a single mammalian cell, it raised the question of redundancy or distinct transcriptional regulatory activity. By utilizing the biochemical comparison paradigm, we identified that whereas Sp1 acts as a potent transcriptional activator on reporter plasmids carrying GC boxes, KLF10 and KLF11 proteins act as transcriptional repressors (Cao et al. 2005; Fernandez-Zapico et al. 2003; Kaczynski et al. 2002; Neve et al. 2005). Moreover, there is a competition between Sp1-like proteins that function as "on" or "off" switches for similar promoters (Kaczynski et al. 2001; Sogawa et al. 1993; Turner and Crossley 1999). Another interesting finding is that certain members of the Sp/KLF family can activate transcription if the promoter contains multiple GC boxes but behave as repressors on promoters containing a single copy of this sequence (Imataka et al. 1992). This supports the notion that the regulation of gene expression by Sp1-like proteins may depend on promoter context. Overall, the discovery of Sp1-like transcriptional repressors, in addition to those members that activate transcription, has represented a significant step in the transcriptional field, and it challenged the early paradigm of "Sp1 activates all GC-rich sites." As modeled by the current, more accurate paradigm, GC-rich sites are not necessarily the target of Sp1 in isolation; rather, these sites may be activated or repressed depending on the family member by which it is recognized. Collective studies in this field have emphasized the complex nature of the biological effects generated by the existence of various KLF proteins, which in large part are dictated by the co-activators and co-repressors that facilitate the chromatin dynamics occurring on a given promoter.

To first understand the function ascribed to these transcription factors and subsequently their effect on chromatin dynamics, it is important briefly to revisit a few basic structural properties of these proteins. At least three domains are required for any family member of these Sp/KLF transcription factors: the DNA-binding zinc finger domain, a nuclear localization signal (NLS) domain, and a transcriptional regulatory domain. Within the DNA-binding domain, comprised of three Cys_2His_2 zinc finger motifs each of 25–30 amino acid residues, the sequence identity among the family members is higher than 65%, again emphasizing a role in the regulation of similar promoters (Kaczynski et al. 2003). Some zinc finger proteins recognize DNA sequences slightly different from the one that is predicted from its amino acid sequence, which is likely to be due to a mechanism of cooperative binding when the interaction of one finger with DNA modifies the selectivity of another finger. Similarly, DNA recognition by these zinc fingers may reflect a "wobbler effect" similar to the one that operates during peptide synthesis. Regardless of the exact

mechanism, currently Sp1-like proteins have been divided into two groups based on the selectivity between two highly similar GC-rich sites, either a CGCCC or CACCC core sequence (Crossley et al. 1996; Hagen et al. 1995; Kingsley and Winoto 1992; Matsumoto et al. 1998; Shields and Yang 1998; Thiesen 1990). Returning to the basic questions of KLF biology that remain to be answered, whether proteins compete against each other for recognition of these two different sites or acquire different binding selectivity by posttranslational modifications or combinations of homo-/hetero dimerization must be determined. Because there are several thousands of these sites genome-wide, this information would be useful to advance a large number of studies that are focused on the mechanism of expression of distinct genes while utilizing caution against adopting an Sp1-centric assumption.

In contrast to the high conservation of the zinc finger domain that defines the members of this family, the structure and function of the transcriptional regulatory domain in the N-terminal portion of the proteins, as well as the location of their NLS, are variable. The location of the NLS can categorize these proteins into two major groups: one containing the signal within the zinc finger domain and the second with the NLS directly upstream of this region (Pandya and Townes 2002; Shields and Yang 1997). Along with the structural variability in the N-terminus, the ability of distinct family members to regulate transcription and subsequently affect cellular processes are divergent as well. For instance, KLF11 behaves as a potent transcriptional repressor, distinguishing it from the powerful transcriptional activation of Sp1 (Cook et al. 1998). The functional distinctions between the members of this family are embedded within the high level of variability in the N-terminal portion of the protein, which contains specific activation and repression domains. These domains, in turn, interact with distinct co-activators and co-repressors, thus regulating the chromatin dynamics and consequently transcription of a promoter in its own unique manner. In summary, although the presence of the similar zinc finger domain classifies these proteins in the KLF family, it is the N-terminal region thatprovides the functional identity to each member.

Structural and Epigenetic Aspects of KLF–Co-activator Interactions

Several members of the Sp/KLF family of transcription factors have been shown to interact with co-activators. Interestingly, the interaction of the Sp/KLF proteins and their co-activators appears to be selective and may contribute to transcriptional specificity. Beyond physical interaction, however, functionally most important is the chromatin remodeling capacity that KLF recruitment of these co-activators to a promoter facilitates. Some Sp/KLF members are able to promote transcription through glutamine-rich regions within their N-terminal domain, such as Sp1 and Sp3, which interact with components of the general transcription factor TAFII130 to recruit the RNA polymerase II complex (Gill et al. 1994). However, because the DNA of promoter regions is not in isolation but, rather, is

within the context of the chromatin landscape, generally speaking transcriptionally activating KLF proteins require the assistance of co-activators, which can remodel the chromatin at the target site via complexes containing histone acetyltransferase (HAT) activity, such as p300/CBP and PCAF. CBP (CREB-binding protein) and p300 (EP300, E1A binding protein) are transcriptional co-activators that are structurally and functionally closely related.(Vo and Goodman 2001). Together with an acetylase (p300/CBP associated factor, or PCAF) they have been shown to bind numerous transcription factors. Transcription factor binding by these co-activators generally results in increased target gene expression.

All of these transcriptional co-activators contain intrinsic histone/protein acetyltransferase domains (HAT/PAT). Functionally, histone acetylation at target gene promoters provides a binding site for bromodomain-containing proteins, including the HATs themselves and the SWI/SNF family of chromatin remodelers, allowing structural relaxation of chromatin, thereby facilitating access to transcriptional machinery (Yang 2004). In addition to histone acetylation, p300/CBP and PCAF have been shown to acetylate several transcription factors. The functional significance of transcription factor acetylation is still under investigation, although it has been speculated that it affects their stability, DNA binding, and interaction with other proteins (Chen et al. 1999; Gu et al. 2001).

One KLF family member found to utilize the p300/CBP co-activator complex through direct transcription factor acetylation is KLF1 (erythroid KLF, or EKLF). KLF1 is necessary for the establishment of optimized chromatin structure and high-level expression of the β-globin gene that is characteristic of the erythroid cell lineage (Zhang et al. 2001b). CBP/p300 acetylates KLF1 at residues Lys288 and Lys302. Interestingly, KLF1 acetylation is necessary for transactivation of β-globin expression. Acetylation of KLF1 also facilitates interaction with the SWI/SNF chromatin remodeling complex, which further facilitates target gene expression. In contrast to KLF1, however, the acetyltransferase activity of CBP/p300 is not necessary for KLF13 (FKLF2)-mediated transactivation of human γ-globin expression (Song et al. 2002). KLF13 instead depends on the PCAF acetyltransferase to upregulate γ-globin. Interestingly, although CBP/p300 cooperatively facilitates KLF13 DNA binding and hence γ-globin transactivation along with PCAF, only PCAF (not CBP/p300) acetyltransferase activity is necessary for this response. In addition, CBP and PCAF acetylate KLF13 in its zinc finger domain, causing differential effects (Song et al. 2003). Acetylation by CBP disrupts the DNA binding of KLF13; and the regulation of DNA binding by KLF13 via PCAF and CBP can be synergistic or antagonistic, depending on acetylation status. This is exemplified by the observation that PCAF blocks CBP acetylation and thus prevents CBP disruption of KLF13 binding to DNA, whereas CBP-mediated acetylation of KLF13 prevents PCAF stimulation of KLF13 DNA binding. Selective recruitment of specific co-activator domains in addition to selective utilization of co-activators may be one mechanism conferring target gene specificity of the diverse but structurally related Sp/KLF family members.

A different mechanism underlies regulation of the KLF4-mediated inflammatory response in macrophages (Feinberg et al. 2005). KLF4 acts downstream of pro-inflammatory cytokines—e.g., interferon-γ (IFN-γ) and tumor necrosis factor-α

(TNF-α)—and activates inducible nitric oxide synthase expression (iNOS). In doing so, KLF4 antagonizes the antiinflammatory signal mediated by TGF-β1 and Smad3 via plasminogen activator inhibitor 1 (PAI1). Rather than binding the PAI1 promoter directly, KLF4 competes with Smad3 for CBP/p300 binding. Thus, KLF4 is an indirect trans-repressor of PAI1 by competitive antagonism via co-activator binding.

All of these mechanisms are operative to some extent in the regulation of KLF5 (Matsumura et al. 2005). HDAC1 deacetylase competes with p300 for a common binding site on the first zinc finger of KLF5. HDAC1 has been shown to bind KLF5 directly and diminish its DNA-binding affinity. Conversely, p300 acetylates and activates KLF5-mediated transcription. The HDAC1-mediated reduction in DNA binding causes a decrease in expression of platelet derived growth factor A (PDGF-A), a KLF5 target gene. HDAC1 therefore inhibits KLF5 directly by decreasing DNA binding as well as indirectly by competitively antagonizing binding of acetylase p300. Studies continue to emerge with more KLF family members utilizing CBP/p300 as a co-activator, such as KLF2 (SenBanerjee et al. 2004) and more recently KLF11 (R. Urrutia and R. Stein, unpublished results). These direct interactions with CBP/p300 recruit HAT activity to the site of the promoter and thus presumably facilitate an active chromatin state for transcription (Fig. 1). Further investigations will likely reveal additional KLF members that not only interact with CBP/p300 but with PCAF as well to allow more dynamic control of the chromatin landscape surrounding the Sp/KLF site.

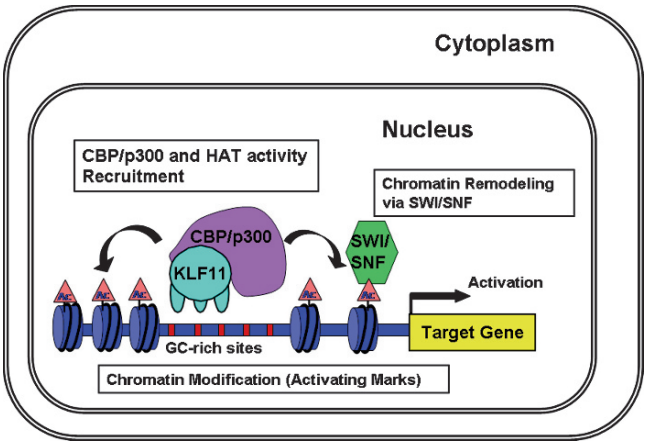

Fig. 1 Chromatin dynamics of KLF-mediated activation. Using KLF11 as an example, this cartoon depicts a model for KLF-mediated activation that involves the recruitment of CBP/p300 to a target gene promoter. The recruitment of CBP/p300 to the promoter also provides histone acetyltransferase (*HAT*) activity, which facilitates modification of surrounding histones to create "active" chromatin with acetylated histones. Addition of acetylated marks to histones signals activation of transcription through recruitment of other bromodomain-containing proteins, such as the SWI/SNF family of chromatin remodelers, allowing structural relaxation of chromatin and, thus, access to transcriptional machinery. A similar mechanism is proposed for the KLF family members that utilize p300/CBP-associated factor

The Sp/KLF family is comprised of distinct but structurally related transcription factors. As our understanding of this family has evolved, it has become clear that no single classification scheme can accurately characterize the function of any member distinctly. Individual KLF transcription factors function as activators or repressors in a context-dependent manner. In addition to co-activator binding, recruitment of selective cofactors in specific complexes further determines the specificity of KLF-directed gene regulation. As mentioned for KLF1 and KLF5, the KLF transcription factors themselves are subject to post-translational modifications, such as acetylation, further augmenting or regulating their specific biological capability. The development of novel inhibitors of specific enzymatic activity of PCAF, CBP, and p300 acetyltransferases will not only the elucidate the role of these cofactor–KLF complexes but also contribute to novel therapeutic possibilities.

Structural and Epigenetic Aspects of KLF-Co-repressor Interactions

In 1998, the primary functional subfamilies of KLF transcriptional repressors were identified and characterized almost concurrently. A subset of KLF family members were found to utilize the C-terminal-binding protein (CtBP) co-repressors (Turner and Crossley 1998), and our laboratory discovered the KLF/TIEG TGF-β-inducible early gene subfamily of transcriptional repressors that function via the Sin3-HDAC system (Cook et al. 1998, 1999; Zhang et al. 2001a). Subsequently, an extended subfamily of Sin3-mediated repressors was described, known as the KLF/BTEBs [BTE (basic transcription element)-binding proteins] (Kaczynski et al. 2001, 2002). Initially, these KLF subfamilies were classified based entirely on structural features; however, because KLF/TIEGs and KLF/BTEBs utilize the same co-repressor system (i.e., Sin3-HDAC), these two groups may actually represent the same functional subfamily. Therefore, to focus on the relation of KLF proteins to chromatin dynamics, we discuss the KLF proteins according to their mechanisms of action—i.e., CtBP- and Sin3-dependent KLF repressors.

Ctbp-Dependent KLF Repressors

The CtBP-dependent KLF repressors include KLF3, KLF8, and KLF12, which have a five-amino-acid motif, PXDLS (Pro-Xaa-Asp-Leu-Ser), that interacts with CtBP (Schuierer et al. 2001; Turner and Crossley 1998; van Vliet et al. 2000). It is noteworthy that outside of this small CtBP-recognition motif, no additional significant similarity occurs in the N-terminal region of these three KLF proteins. Originally characterized as the binding protein of the C-terminal portion of the adenovirus E1A protein, CtBPs are highly evolutionarily conserved and share significant amino acid similarity to NAD-dependent 2-hydroxy acid dehydrogenases

(Boyd et al. 1993; Schaeper et al. 1995). Although the function of CtBPs as transcriptional co-repressors has been well established, their mechanism of action is still emerging (Cook et al. 1998; Nibu et al. 1998a, 1998b; Poortinga et al. 1998; Postigo and Dean 1999). One mechanism for gene silencing via CtBP proteins is through the recruitment of HDACs (Koipally and Georgopoulos 2000; Sundqvist et al. 1998). However, evidence exists for the involvement of additional co-repressors as CtBP transcriptional repression also occurs independently of HDACs (Koipally and Georgopoulos 2000; Meloni et al. 1999; Phippen et al. 2000). Other transcriptional repressor families have been reported to interact with CtBP, including Ikaros and members of polycomb (Koipally and Georgopoulos 2000; Sewalt et al. 1999). Therefore, gene silencing via CtBP appears to occur also through the physical rearrangement of nucleosomes, as several of these interacting proteins are fundamental parts of chromatin-remodeling complexes. During KLF-CtBP-mediated repression, it remains unclear as to which co-repressor CtBP recruits and whether the choice of co-repressor is KLF- or promoter-dependent.

Even though KLF3 was initially assumed to function only as an activator, as shown in studies on a minimal promoter, the achieved activation was still significantly less than other KLF proteins, and this effect required an excess of KLF3 protein (Crossley et al. 1996). Subsequently, KLF3 was found to be a potent repressor that mapped to a domain located within a 74-amino-acid sequence in the N-terminus (Turner and Crossley 1998). Using yeast two-hybrid screening, CtBP2 was identified as the co-repressor for this KLF family member. KLF3 appears to have additional co-repressors as disruption of CtBP interaction does not completely abolish its transcriptional repression. Additional two-hybrid screening revealed an interaction between KLF3 and FHL3, a member of the FHL (four and a half LIM domain) family (Turner et al. 2003). FHL proteins have been implicated in cytoskeletal organization and more recently observed within the nucleus, associated with co-regulation of transcription (Du et al. 2002; McLoughlin et al. 2002; Muller et al. 1991). Thus, similar to the KLF proteins that interact with various co-activators, KLF3 interacts with distinct multiprotein complexes to achieve transcriptional repression of GC-rich promoters.

Identified “in silico” owing to its similarity to KLF3, KLF8 also associates with CtBP through a PVDLS recognition motif (van Vliet et al. 2000). Similar to KLF3, the N-terminus, specifically the CtBP-binding site, of KLF8 is responsible for its transcriptional repression activity but not complete. Again, loss of CtBP binding does not abolish the repression capacity of KLF8, suggesting the existence of additional co-repressors for this KLF protein (van Vliet et al. 2000). Whether a FHL protein also interacts with KLF8, as with KLF3, or other, yet unidentified co-repressors are involved in its full repression activity remains to be determined.

Originally identified from studies of its target gene, KLF12 is a repressor of the activator protein-2α (AP-2α) gene, which also encodes a mammalian transcription factor (Imhof et al. 1999). This repression occurs through a PVDLS sequence located within the N-terminus of KLF12, which facilitates a direct interaction with CtBP1 (Schuierer et al. 2001). Although much of KLF12 repression is associated with its binding to CtBP, the C-terminal portion of KLF12 containing the three zinc

fingers is also capable of partial repression of the same promoter, AP-2α, suggesting that the zinc finger domain may provide a site for an additional co-repressor interaction or sterically interfere with activator binding to nearby sequences (Roth et al. 2000). Interestingly, a mechanism of trans-regulation exists between KLF12 and its gene target, as induction of *KLF12* expression leads to subsequent downregulation of AP-2α expression and vice versa with AP-2α acting as a negative regulator of *KLF12* expression (Roth et al. 2000). The biological relevance of this reciprocation remains unknown; however, it is clear that although KLF12 is a CtBP-mediated transcriptional repressor it shows distinct structural and functional differences from KLF3 and KLF8.

Sin3-Dependent KLF Repressors

As mentioned, the KLF/TIEGs and KLF/BTEBs, which include KLF9, KLF10, KLF11, KLF13, and KLF16, utilize the HDAC system to facilitate transcriptional repression through direct interaction with the scaffold co-repressor protein Sin3A (Lomberk and Urrutia 2005). Mammalian Sin3 (mSin3) proteins, part of large, multiprotein complexes capable of local chromatin modification, are orthologues of the Sin3p transcriptional repressor in *Saccharomyces cerevisiae* (Kadosh and Struhl 1998; Kasten et al. 1997; Vidal et al. 1991; Wang et al. 1990). The mSin3-HDAC complexes are composed of many subunits, including mSin3A/mSin3B, HDAC1, HDAC2, RBAP46 [Rb [retinoblastoma protein (Rb)-associated protein 46], RBAP48 (Rb-associated protein 48), SAP18 (Sin3-associated polypeptide 18), and SAP30 (Sin3-associated polypeptide 30) (Hassig et al. 1997; Laherty et al. 1992; Zhang et al. 1997). The Sin3 protein has multiple protein interaction domains, which allows it to function as a central scaffold for assembly of the entire complex. HDAC activity is essential for mediating the repression capacity of the complex, evidenced by significant disruption of repression activity on either mutation of the HDAC binding site in Sin3 or treatment with HDAC inhibitors (Kadosh and Struhl 1998; Sommer et al. 1997). The structure of Sin3 itself is comprised of four evolutionarily conserved imperfect repeats of ~100 residues, each predicted to form a four-helix-bundle fold, known as a paired amphipathic helix (PAH) region (Ayer et al. 1995; Halleck et al. 1995; Wang et al. 1990). To facilitate recruitment to a specific target sequence on a promoter, these PAH domains mediate binding with various transcription factors, such as the KLF proteins.

Initial biochemical characterization of these proteins demonstrated that the N-terminal domains of both KLF/TIEGs (KLF10 and KLF11) contain three distinct transcriptional repressor domains (R1, R2, R3) (Cook et al. 1999). Within KLF11, the repression of reporter gene activity achieved a minimum of 75% in the R1 (amino acids 24–41), R2 (151–162), and R3 (273–351) domains (Cook et al. 1999). Based on low-resolution secondary structure prediction algorithms, the R1 domain had been predicted to adopt an α-helical conformation, which was later confirmed by circular dichroism analysis (Zhang et al. 2001a). Congruent with this

idea, proline mutations in the central core of the R1 domain (amino acids 30–39; AVEALVCMSS) disrupted its repression activity (Cook et al. 1999). Subsequent studies using either the wild-type R1 domain or its mutant led to the purification of a 160-KDa R1-binding protein. This high-affinity binding protein was later identified as Sin3a (Zhang et al. 2001a), and the core R1 domain was characterized as the α-helical repression motif SID (Sin3-interacting domain), which is further discussed below. Upon detailed biochemical and functional analyses, the KLF11 SID was found to interact specifically with the PAH2 domain of Sin3a to repress transcription. Subsequent analysis of the N-terminal domains of three other repressor proteins discovered in our laboratory—KLF13, KLF14, KLF16—clearly showed related R1 domains that function as Sin3-interacting-domains (SIDs), analogous to the corresponding domain in KLF10 and KLF11 (Kaczynski et al. 2001, 2002; Zhang et al. 2001a). KLF9, which is the first identified and cloned of this subfamily of KLF proteins that bind to BTE sites (Ratziu et al. 1998), also shares a SID in its N-terminus.(Zhang et al. 2001a). Thus, because the SID is a defining structural and necessary functional feature of these KLF repressors, this subset of KLF members are intricately linked to HDAC-mediated chromatin modification and transcription repression via mSin3A binding (Fig. 2) (Hassig et al. 1997). For instance, we have found that KLF14 represses the TGFβRII promoter via a co-repressor complex containing mSin3A and HDAC2 (Truty et al. 2008). Furthermore, TGF-β pathway

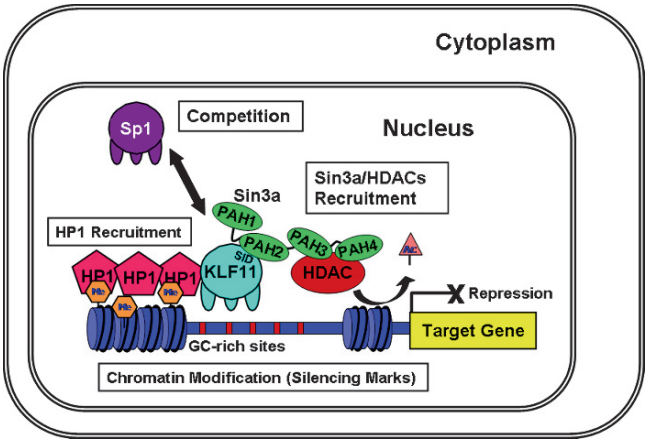

Fig. 2 Chromatin dynamics of KLF-mediated repression. Using KLF11 as an example, this cartoon depicts a model for KLF11-mediated repression that involves the recruitment of histone deacetylases (*Sin3a-HDACs*) to a target gene promoter (through the SID of KLF11 and PAH2 domain of Sin3a) and inhibition of Sp1 binding through competition. The recruitment of Sin3a-HDAC to the promoter facilitates remodeling of surrounding chromatin with silencing marks, namely the deacetylation of histones. (This process would be similar for the CtBP-dependent KLF repressors because at least one mechanism also involves the recruitment of HDACs.) Removal of acetylation signals short-term repression of a target gene and, in addition, primes the histone for receiving additional long-term silencing marks, such as methylation of K9 on histone H3, through the interaction between KLF11 and HP1

activation leads to the recruitment of a KLF14-mSin3A-HDAC2 repressor complex to the TGFβRII promoter, as well as remodeling of chromatin to decrease histone marks that associate with transcriptional activation (e.g., histone acetylation) and increase marks associated with transcriptional silencing (e.g., methylated K20 of histone H4) (Truty et al. 2008). Interestingly, at the time that this domain was discovered in the KLF family, a similar SID was thought to be a unique domain described only for the tumor suppressor and MYC oncogene inhibitors Mad1, Ume6, and Pf1 (Brubaker et al. 2000; Washburn and Esposito 2001; Yochum and Ayer 2001). This discovery marked a high point in the study of tumor-suppressor proteins by demonstrating that the presence of the SID domain is not exclusive to Mad1 but, rather, a more widespread molecular mechanism used by tumor suppressors in epithelial cells.

As mentioned, the SID within KLF proteins has some structural and functional resemblance to the better characterized SID of Mad1, the basic helix–loop–helix protein that dimerizes with Max to antagonize the function of the c-Myc oncoprotein (Zhang et al. 2001a). Additional members of the Mad family, including Mad1, Mad3, Mad4, and Mxi-SR, also have an N-terminal SID, which interacts with Sin3a through its PAH2 domain (Ayer et al. 1995). First found through circular dichroism (CD) and mutational analyses and then confirmed via nuclear magnetic resonance (NMR) structural analysis, the Mad1 SID was found to adopt an amphipathic α-helical conformation (Brubaker et al. 2000; Eilers et al. 1999). These findings also supported the concept hat this α-helical structure binds to the PAH2 domain by docking into a hydrophobic pocket in the base of this four-helix-bundle structure. As the SID interactions of KLF11 and Mad1 are both with the PAH2 domain, we sought to evaluate whether there are structural similarities (Pang et al. 2003; Truty et al. 2008). A comprehensive investigation into SIDs of KLFs and Mad1 repressor proteins suggested that SIDs of both KLFs and Mad1 have the AA/VXXL core consensus and a similar propensity for helix formation, but the two SIDs can be classified into two subtypes on the basis of their sequence—in particular, the residues outside the AA/VXXL core sequence (Zhang et al. 2001a). Even with structural similarities, the affinity of the KLF SID is lower than that of the Mad1 SID for the Sin3a PAH2 domain. This difference in affinities was evaluated with molecular modeling experiments combined with molecular dynamics simulation of the Mad1 SID–PAH2 complex, as compared with the KLF11 SID–PAH2 complex, to substantiate that this is a result of distinct binding mechanisms (Pang et al. 2003). These structural differences between the binding of KLF SID and that of the Mad1 SID offered new insight into transcriptional regulation via KLF repressor proteins.

The discovery of the SID in both the Mad1 and KLF repressor proteins also raised the question as whether these domains function in a constitutive or a regulated manner. Interestingly, we showed that the pro-proliferative EGF-ras-MEK1-ERK1 pathway phosphorylates residues near the SID in KLF11, leading to its dissociation from Sin3a and consequent inactivation of the Sin3-dependent KLF11 repressor function (Ellenrieder et al. 2002). Thus, these data demonstrated that the SID functions in a manner that can be influenced by cell signaling. Because KLF11 is induced by TGF-β to mediate an antiproliferative pathway, its inactivation by

EGF signaling may contribute to its inactivation in cells where this mechanism is hyperactive, such as pancreatic cancer.

Future of KLF Proteins in Chromatin Dynamics

Recently, in *Drosophila melanogaster*, Sp/KLF proteins have been found to bind to a site necessary for the activity of a Polycomb-group response element (PRE) from the *engrailed* gene (Brown et al. 2005). These PREs are DNA elements recognized by the Polycomb-group (PcG) of transcriptional repressors through chromatin modification, suggesting further the complexity of chromatin cofactors involved in KLF-mediated transcriptional activation and/or repression.

In addition, in efforts to determine the interacting partners of the other repressor domains (R2, R3) of KLF11, our laboratory has discovered that KLF11 binds to the Gβ subunit of the heterotrimeric G-protein (Mathison et al. 2008; Zhang et al. 2007). This interaction occurs through the R3 domain of KLF11; and more specifically, mutation of alanine 347 to a serine significantly disrupts the binding between these two proteins. Interestingly, this mutation is naturally occurring in a French family with maturity-onset diabetes of the young (MODY) (Neve et al. 2005). These findings are significant, suggesting that activation of GPCRs can mediate short-term responses via their Gα-subunit and Gβγ complex, and long-term transcriptional effects can be triggered via Gβ translocation into the nucleus and functional cooperation with transcription factors such as KLF11. Concurrently, we also found that KLF11 interacts with the chromatin protein HP1α through the extreme C-terminal tail after the zinc finger domains (Lomberk et al. 2008a, 2008b). HP1 proteins play a role as "gatekeepers" of long-term epigenetic gene silencing that is mediated by histone H3 lysine-9 methylation via recruitment of the G9a or SUV39H1 histone methylases (Lomberk et al. 2006). Therefore, this finding offers a new model for the function of KLF11, which may work not only in transient repression via HDAC but also in long-term repression via histone methylation/HP1 (Fig. 2).

Much remains to be discovered in regard to the role of KLF proteins in chromatin dynamics. Ongoing studies continue to implicate additional nonhistone chromatin proteins in mediating KLF function. We now understand that a gene promoter is not simply a DNA sequence to be recognized by a KLF protein. The promoter is occupied by a complex array of chromatin proteins associated with either an active or a repressed state. If this chromatin state opposes the necessary biological function at a given moment, the chromatin landscape requires modification and remodeling to switch a promoter "on" or "off," which can be accomplished onlyby recruitment of the appropriate co-activators or co-repressors. These transitions between "active" and "inactive" chromatin states are catalyzed by the specific targeting of these megadalton multiprotein co-activators/co-repressors to DNA, which can be directed to a particular sequence only via sequence-specific DNA-binding proteins, such as the KLF proteins.

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