

# Chapter 1

## Krüppel-like Factors: Ingenious Three Fingers Directing Biology and Pathobiology

Ryozo Nagai, Ichiro Manabe, and Toru Suzuki

**Abstract** Krüppel-like transcription factors (KLFs) participate in diverse physiological and pathological processes, such as cell growth, cell differentiation, tumorigenicity, metabolism, inflammation, and tissue remodeling in response to diverse external stress. The importance of KLFs has recently been appreciated as detailed mechanisms of their molecular functions have been rapidly unraveled. However, many questions remain to be addressed: for instance, (1) how is gene expression of KLFs regulated—in a developmental stage-specific manner or in terms of cell–cell interaction; (2) how do KLFs interplay with other cofactors amid the transcriptional network; and (3) need to explore the tertiary structure of KLFs. Given the importance of KLFs in disease biology, extensive investigations on KLFs are expected to lead to identification of therapeutic targets for many diseases.

### Introduction

Mammalian Krüppel-like transcription factors (KLF) have recently gained recognition as critical regulators in cell proliferation and differentiation during normal development as well as in many disease states. KLFs constitute the KLF-half of the Sp1/KLF family of transcription factors, characteristically containing three consecutive and conserved cysteine and histidine (C2H2)-type zinc fingers in the DNA-binding domain located at the carboxyl terminal end. The paired cysteine and histidine in the DNA-binding domain are critical in spatially coordinating and anchoring the zinc atom. KLFs consist of at least 17 members that recognize GC- and GT-rich sequences and can either transactivate or repress target genes; and individual factors can act as both an activator and a repressor in a context-dependent manner.

Discovery of KLFs with DNA-binding properties similar to Sp1 have led us to reconsider the oversimplified notion that GC-boxes and CACCC-boxes (GT-boxes)

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R. Nagai (✉), I. Manabe, and T. Suzuki  
Department of Cardiovascular Medicine, Graduate School of Medicine,  
The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

are unique binding sites for Sp1, one of the first transcription factors to be cloned (Kadonaga et al. 1987). From early studies on Sp1, it was widely held that basal transcription levels of “housekeeping genes” are maintained by Sp1, which regulates GC-boxes or GT-boxes in promoter regions. However, KLF members are involved in a variety of cellular functions and even compete with Sp1 for their DNA-binding sites. Many gene promoters with GC- or GT-boxes are not necessarily constitutively active but are finely tuned by KLFs in cells during development and differentiation or in response to physical or metabolic stress. KLF members participate in regulation of gene transcription within a network of Sp/KLF factors through interaction with intracellular signal molecules and transcriptional cofactors, including nuclear receptors, histone chaperones, oncogenes, and tumor suppressors. Physiological and pathophysiological functions of KLF members have been studied extensively, and many important findings are rapidly accumulating. We present an overview here on the current understanding of the KLF family in biology and disease.

## KLFs in Development, Morphogenesis, and Differentiation

The ancestral gene of KLF is Krüppel in *Drosophila*, which is a factor activated in the center of the embryoid body during early development and involved in segmentation of the thorax and abdomen. In mammals, many KLF members participate in embryogenesis and fetal development. Homozygous KLF5 knockout mice (KLF5<sup>-/-</sup>) die at early embryonal stage before E8.5 (Shindo et al. 2002). KLF2<sup>-/-</sup> embryos die between E12.5 and E14.5, and KLF4<sup>-/-</sup> mice die at birth (Kuo et al. 1997; Segre et al. 1999).

The importance of KLFs in embryonic stem (ES) cell biology has recently been shown by Yamanaka et al., who found that pluripotent ES-like cells can be generated from murine or human fibroblasts by the introduction of four genes (i.e., KLF4, Oct3/4, Sox2, and c-Myc (Takahashi et al. 2007; Takahashi and Yamanaka 2006)). Yamanaka's group further demonstrated that KLF2 and KLF5 can replace KLF4 in combination with the three other genes to convert fibroblasts into ES-like cells. Jian et al. also recently reported that KLF2, 4, and 5 constitute a core circuitry in self-renewal of ES cells in which simultaneous depletion of KLF2, 4, and 5 results in ES cell differentiation (Jiang et al. 2008). These three KLFs cooperatively activate self-renewal genes and suppress differentiation. Parisi et al., on the other hand, indicated that depletion of KLF5 alone resulted in differentiation of mouse ES cells, which did not occur by depletion of KLF2 or KLF4, suggesting a central role of KLF5 to maintain ES cells in the undifferentiated state (Parisi et al. 2008). KLF5 is reported to play a role in maintenance of pluripotency of ES cells by promoting phosphorylation of Akt1, and early embryonic lethality of KLF5<sup>-/-</sup> mice is due to an implantation defect (Ema et al. 2008).

KLF members regulate differentiation of various cell types during development and normal growth. KLF1, the founder factor of the KLF family and also known

as EKLF, plays a critical role in erythropoiesis (Miller and Bieker 1993). KLF1 is not required for erythroid commitment but promotes maturation of erythrocytes by inducing the  $\beta$ -globin gene or genes essential for integrity of the erythrocyte membrane, cytoskeleton, and heme synthesis (Hodge et al. 2006; Perkins et al. 1995). KLF2 regulates the embryonic globin gene, whereas KLF13 is involved in the maturation of erythrocytes but its target genes remain elusive (Basu et al. 2005; Gordon et al. 2008). KLF6 also regulates hematopoiesis in the yolk sac (Matsumoto et al. 2006).

Adipocyte differentiation is another system intensively investigated in the context of transcriptional regulation by KLFs. KLF2, 3, 4, 5, 6, and 15 reportedly regulate adipogenesis (Birsoy et al. 2008; Li et al. 2005; Mori et al. 2005; Oishi et al. 2005; Sue et al. 2008; Wu et al. 2005); however, the mechanisms as to how these KLF members regulate adipogenesis are diverse. KLF2 and KLF3 are abundantly expressed in 3T3-L1 preadipocytes and are downregulated during differentiation. KLF2 directly inhibits PPAR $\gamma$ , and KLF3 represses C/EBP $\alpha$  together with co-repressor CtBP. KLF5 and KLF15 promote adipogenesis through transactivating the PPAR $\gamma$  gene; however, KLF5 expression is elevated during early differentiation and reduced in mature adipocytes, whereas KLF15 is upregulated later during adipocyte differentiation. KLF4 together with Krox20 is an early regulator of adipogenesis because they cooperatively activate C/EBP $\beta$ . Expression of KLF6 is increased during preadipocyte differentiation and in mature adipocytes. KLF6 together with HDAC3 inhibits Dlk1, which is a transmembrane protein containing an epidermal growth factor repeat domain and promotes preadipocyte proliferation. These studies clearly indicate that KLFs are involved in adipocyte differentiation, but how actions of these factors are orchestrated and whether they cooperatively interact is not known.

KLFs have also been implicated in epithelial cell differentiation (McConnell et al. 2007). In the intestinal epithelium, KLF4 and KLF5 show opposing roles in terms of proliferation and differentiation. KLF4 is expressed in terminally differentiated epithelial cells in the villi and upper crypt. KLF5, on the other hand, is localized to the proliferating epithelial cells at the base of the crypt. KLF4 is induced upon activation of the adenomatous polyposis coli (APC) gene and interacts with  $\beta$ -catenin, thus inhibiting its signaling (Dang et al. 2001). KLF4 also activates genes encoding negative regulators of the cell cycle, such as p27(Kip1), and suppresses cyclin D1 and cyclin B, which promote the cell cycle (Wei et al. 2008). KLF4 regulates the Sprr gene clusters and the keratin families contributing to epidermal barrier integrity (Turksen and Troy 2002). KLF5, to the contrary, is a positive regulator of cell proliferation with transformation activities. KLF5 regulates growth factor genes, such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF), in addition to the transforming growth factor–bone morphogenetic protein (TGF-BMP) signaling pathway (Wan et al. 2008).

Gene targeting of KLFs in mice has demonstrated their critical roles in the differentiation of many other cells: KLF2 in thymocytes, monocytes (Carlson et al. 2006), and vascular smooth muscle cells (SMCs) (Kuo et al. 1997); KLF4 in

monocytes (Alder et al. 2008), testicular Sertoli cells (Godmann et al. 2008), corneal epithelial and epidermal cells (Swamynathan et al. 2007); KLF5 in SMCs and respiratory epithelial cells (Shindo et al. 2002; Wan et al. 2008); KLF7 in olfactory sensory neurons (Kajimura et al. 2007); and KLF9 in intestinal epithelial cells (Simmen et al. 2007). KLF13 plays important roles in cardiac morphogenesis, as knockdown of the gene in the *Xenopus* embryo results in atrial septal defect and hypotrabeulation (Lavalley et al. 2006). Physical and functional interaction of KLF13 with GATA4, a transcriptional effector of cardiac development, may in part be responsible for this phenotype. However, the significance of KLF13 in the mammalian heart has not been reported to date.

## KLFs in Cell Proliferation, Apoptosis, and Oncogenesis

KLF4, KLF5, and KLF6 have been extensively studied in cell growth. KLF4 and KLF5 exhibit contrasting effects on cell growth in epithelial cells (Ghaleb et al. 2005). KLF5 is markedly induced in proliferating SMCs and fibroblasts, and it promotes proliferation of those cells, whereas KLF4 inhibits proliferation. Induction of KLF5 has been shown to be mediated by Erk-1 and the Wnt-1 signaling pathway (Kawai-Kowase et al. 1999; Ziemer et al. 2001). Introduction of KLF5 into NIH 3T3 and intestinal epithelial cells promotes growth and induces a transformed phenotype as indicated by anchorage-independent growth (Nandan et al. 2004). KLF5 positively regulates not only growth factor genes but also cell cycle promoting genes such as cyclin D1, cyclin B1, and *dk1/Cdc2* (Nandan et al. 2005).

In KLF5<sup>+/-</sup> mice, proliferation of vascular SMCs and cardiac fibroblasts, which occurred in response to mechanical injury and angiotensin II treatment, was markedly diminished (Shindo et al. 2002). Furthermore, infection of KLF5<sup>+/-</sup> mice with *Citrobacter rodentium*, a gram-negative bacterium, attenuated a hyperproliferative response of epithelium in the colon (McConnell et al. 2008).

Tumorigenicity of KLF5 is still controversial. KLF5 has been implicated in progression of colorectal cancer (Ghaleb and Yang 2008), although there has been a report showing that expression of KLF5 is reduced in intestinal tumors (Bateman et al. 2004). However, at least in colorectal cancer with mutated (activated) KRAS, KLF5 has been implicated in tumor progression because inhibition of KLF5 expression by mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK) inhibitors or KLF5-specific small interfering RNA led to reduced proliferation and transformation (Nandan et al. 2008).

Antiapoptotic activity of KLF5 is mediated in part by interaction with a 24-kDa proteolytic fragment of poly(ADP-ribose) polymerase-1 (PARP-1), a nuclear enzyme important in apoptosis. KLF5, particularly when acetylated, binds a 24-kDa proteolytic fragment of PARP-1 with high affinity and suppresses its apoptotic activities (Suzuki et al. 2007).

KLF4 and KLF6, on the other hand, are generally considered tumor suppressors. KLF4 inhibits the transition between the G<sub>1</sub> and S phases of the cell cycle,

which has been shown to occur by coordinated regulation of expression of numerous cell cycle regulatory genes including induction of p21/WAF1 and G1/S checkpoint genes (Zhang et al. 2000). The significance of KLF4 as a tumor suppressor has been extensively investigated *in vitro* and *in vivo*. KLF4 is activated by the tumor suppressor APC; and when KLF4<sup>+/-</sup> mice were crossbred with APC<sup>min/+</sup> mice, which develop adenomas in the intestine, they developed more intestinal adenomas than the APC<sup>min/+</sup> mice (Ghaleb et al. 2007). These reports indicate a role for KLF4 as a tumor suppressor in the gut. Conversely, experiments in cutaneous squamous epithelial cells showed KLF4 to be growth promoting because conditional overexpression of KLF4 in the basal layer of mouse skin led to hyperplasia, dysplasia, and squamous cell carcinoma (Foster et al. 2005). KLF4 levels are increased in mammary carcinoma and oropharyngeal squamous cell carcinoma (Foster et al. 2000), and KLF4 can act as a context-dependent oncogene through suppression of p53 (Rowland et al. 2005).

KLF6 is ubiquitously expressed and inducible by various stimuli. KLF6 is also considered a tumor suppressor. KLF6 promotes G<sub>1</sub> cell cycle arrest by inhibiting cell cycle-related gene expression; KLF6 upregulates expression of the p21/WAF1 gene and represses the cyclin D1 gene, thus disrupting cyclin D1-cyclin-dependent kinase (cdk) 4 complexes (Benzeno et al. 2004; Shie et al. 2000). KLF6 has been studied in detail in human prostate cancer in which the KLF6 locus is frequently deleted or mutated (Narla et al. 2001). Loss of heterozygosity (LOH) of the KLF6 gene is found in multiple cancers, including colorectal, hepatocellular, lung, and ovarian carcinomas (Narla et al. 2007; Watanabe et al. 2008). Furthermore, glioblastoma tumorigenicity could be suppressed by expression of KLF6 both *in vitro* and *in vivo* (Kimmelman et al. 2004). However, a role for KLF6 as a tumor suppressor is still controversial because silencing KLF6 leads to inhibition of cell proliferation and sensitization to apoptosis (Sirach et al. 2007). One explanation for this contradiction is the generation of splice variants of the KLF6 gene (KLF6-SV1-3) (Yea et al. 2008). KLF6-SV1 and SV2 are localized in the cytoplasm and antagonize the transcriptional activities of KLF6. Targeting KLF6-SV1 has been shown to induce spontaneous apoptosis in prostate cancer cells; and high levels of KLF6-SV1 expression in prostate tumor are associated with a low survival rate (Narla et al. 2008).

KLF2, KLF8, KLF10, and KLF11 have also been implicated in tumor growth. KLF2 inhibits cell growth by upregulating cell cycle checkpoint genes such as p21/WAF1 and WEE1. In ovarian cancer, KLF2 has been demonstrated to repress WEE1, which facilitates tumor cells to undergo apoptosis (Wang et al. 2005). KLF8 is known to be expressed in several types of human cancer. KLF8 upregulates cyclin D1 and is required for v-Src-induced transformation in NIH3T3 cells (Wang and Zhao 2007). KLF10 and KLF11 are TGF- $\beta$ -responsive genes (Ribeiro et al. 1999). KLF11 has been implicated in inhibition of cell growth *in vitro* and *in vivo* and in neoplastic transformation. The oxidative stress scavengers SOD2 and Catalase1 are target genes of KLF11 and are downregulated in transgenic mice for KLF11. A complex of KLF11 and co-repressor mSin3a suppresses cell growth by repressing TGF- $\beta$ -induced transcription from the Smad7 promoter (Fernandez-Zapico et al. 2003).

Phosphorylation of KLF11 by Erk-MAPK inactivates this pathway in pancreatic cancer cells with oncogenic Ras mutations (Ellenrieder et al. 2004). Despite these effects of KLF11, however, deletion of the KLF11 gene did not show any phenotypes in development and growth (Song et al. 2005).

## KLFs in Inflammation

KLF2, KLF4, KLF5, and KLF13 are known to be involved in modulating inflammation and/or differentiation of immune cells. KLF2 is expressed in lymphocytes, monocytes, and endothelial cells, playing a key role in thymocyte and T-cell migration by regulating sphingosine-1-phosphate receptor S1P1 (Carlson et al. 2006). KLF2 in monocytes and endothelial cells inhibits proinflammatory activation of these cells. Inhibition of KLF2 expression in monocytes by short interfering RNA increases inflammatory gene expression, whereas overexpression of KLF2 inhibits lipopolysaccharide (LPS)-mediated induction of proinflammatory cytokines and reduces phagocytosis, which are mediated by inhibiting the transcriptional activity of NF- $\kappa$ B (Das et al. 2006). KLF2 in endothelial cells also inhibits endothelial activation in response to proinflammatory stimuli (SenBanerjee et al. 2004). KLF2 is thought to be atheroprotective, as KLF2<sup>+/-</sup> /ApoE<sup>-/-</sup> mice show increased diet-induced atherosclerosis (Atkins et al. 2008).

KLF4 functions as a regulator of monocyte differentiation. Cre-excision of KLF4 in hematopoietic stem cells (HSCs) resulted in a reduced number of monocytes and an increase in granulocyte formation, whereas overexpression of KLF4 in HSCs resulted in differentiation to monocytes (Feinberg et al. 2007). In KLF4<sup>-/-</sup> chimeras, which were generated by transplanting KLF4<sup>-/-</sup> fetal liver cells into irradiated wild-type mice, showed a lack of circulating inflammatory (CD115<sup>+</sup>Gr1<sup>+</sup>) monocytes and reduced numbers of resident (CD115<sup>+</sup>Gr1<sup>-</sup>) monocytes (Alder et al. 2008). In macrophages, KLF4 expression was induced by proinflammatory cytokines such as interferon- $\gamma$  (IFN- $\gamma$ ), LPS, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and overexpression of KLF4 activated macrophage as shown by iNOS induction (Feinberg et al. 2005).

KLF5 regulates various genes involved in cell growth and angiogenesis, whereas proinflammatory cytokines, growth factors, angiotensin II, and S1P induce KLF5 expression (Shindo et al. 2002; Usui et al. 2004). KLF5<sup>+/-</sup> mice showed reduced chronic inflammation when angiotensin II was infused or a foreign body was placed around the artery (Shindo et al. 2002). KLF5 binds p50 subunit of NF- $\kappa$ B and promotes PDGF-A gene activation by KLF5 alone (Aizawa et al. 2004). Furthermore, overexpression of KLF5 in fibroblasts induced S100A, a potent proinflammatory protein (unpublished data). In intestinal epithelial cells, KLF5 mediates a LPS-induced proinflammatory response (Chanchevalap et al. 2006).

KLF13 is involved in the development of both B and T cells at multiple stages. In KLF13<sup>-/-</sup> mice, peripheral T cell activation was impaired. B-cell maturation in bone marrow was also impaired as revealed by partial arrest of B cells at the transition from CD43<sup>+</sup> to CD43<sup>-</sup> pre-B cells (Outram et al. 2008).



## KLFs in Phenotypic Modulation of Cell and Tissue Remodeling

Phenotypic modulation of cells underlies various diseases. In the cardiovascular system, for example, external stress such as pressure overload or angiotensin II loading induces changes in gene expression of cells, leading to hyperplasia of vascular SMCs, hypertrophy of cardiac myocytes, and tissue fibrosis. Structural rearrangement in organs is referred to as *tissue remodeling*.

The role of KLFs in tissue remodeling has been most extensively studied in the cardiovascular system. KLF5 is involved in phenotypic modulation of vascular SMCs and fibroblasts. KLF5 regulates genes of cell growth-related genes such as PDGF-A and PDGF-B, TGF- $\beta$ , and SMemb/NMHC-B, a molecular marker of dedifferentiated SMCs (Fujiu et al. 2005; Shindo et al. 2002; Usui et al. 2004; Wan et al. 2008; Watanabe et al. 1999). Introduction of short interference RNA into SMCs inhibits phenotypic modulation of the cell and increases expression of SMC differentiation markers (Liu et al. 2005). In KLF5<sup>-/-</sup> mice under various pathological conditions, hypertrophy and fibrosis in the heart, neointimal hyperplasia in the artery, fibrosis in the kidney, and angiogenesis in implanted cancers were mitigated compared to that in wild-type mice (Fujiu et al. 2005; Shindo et al. 2002). In the cardiovascular system, a complex of RAR $\alpha$ -RXR in SMCs and fibroblasts binds KLF5 as a coactivator and mediates KLF5-induced phenotypic modulation of cells and tissue remodeling (Shindo et al. 2002).

KLF4 has been thought to be a repressor of SMC differentiation markers in cultured SMCs such as smooth muscle  $\alpha$ -actin, smooth muscle myosin heavy chain, and SM22 $\alpha$  (Liu et al. 2005). KLF4 promotes phenotypic modulation of SMCs in vitro when stimulated with PDGF-BB. However, KLF4 can be a growth-promoting transcription factor in a context-dependent manner. Interestingly, conditional targeting of the KLF4 gene in vivo suppressed phenotypic modulation of SMCs but accelerated SMC proliferation in response to vascular injury (Yoshida et al. 2008). KLF15 has also been implicated in SMC proliferation following vascular injury because KLF15<sup>-/-</sup> mice developed exaggerated neointimal formation (Wang et al. 2008a).

Null mutants of KLF2 show apparently normal angiogenesis and vasculogenesis, but null embryos die in utero because of hemorrhaging and abnormal blood vessel structure (Kuo et al. 1997). They show endothelial cell necrosis, thin tunica media, and a reduction in pericytes and differentiated SMCs. KLF2 in endothelial cells is inducible by laminar flow and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins). Statins upregulate heme oxygenase-1 (HO-1), endothelial nitric oxide synthase (eNOS), and thrombomodulin, each of which has an antiinflammatory and an antiatherosclerotic effect on the vasculature (Ali et al. 2007; Halder et al. 2007; SenBanerjee et al. 2004; van Thienen et al. 2006).

In liver fibrosis, activation and proliferation of satellite cells are thought to play a critical role. KLF6 is induced in activated hepatic satellite cells and has been suggested to be involved in hepatic fibrosis (Friedman 2006; Ratzu et al. 1998). Another mechanism of organ fibrosis is epithelial-mesenchymal transition (EMT), which is mediated by TGF- $\beta$ . In renal fibrosis due to high glucose levels, elevated

KLF6 in renal proximal tubules has been suggested to induce EMT (Holian et al. 2008). KLF8, a downstream effector of focal adhesion kinase (FAK), has been implicated to play a crucial role in EMT in mammary gland epithelial cell lines. Aberrant expression of KLF8 in invasive human cancer is correlated with a decrease in E-cadherin expression; suppression of KLF8 in cancer cells restored E-cadherin expression and inhibited invasiveness of the cells (Wang et al. 2007).

In the presence of cardiac hypertrophy, gene expression in the myocardium is altered; that is, there is reduced expression of differentiation markers and induction of fetal genes. KLF10 is involved in the TGF- $\beta$ -Smad pathway, and knockout of the gene results in hypertrophic cardiomyopathy (Subramaniam et al. 2007). KLF15 is also known to play a role in cardiac phenotypes, as knockout of the gene results in cardiac hypertrophy (Fisch et al. 2007).

## KLFs in Metabolism

Roles of KLFs in adipocyte differentiation were described earlier in the chapter. In *Caenorhabditis elegans*, suppression of KLF1 gene expression by RNA interference results in increased fat accumulation in the intestine (Hashmi et al. 2008). In KLF5<sup>-/-</sup> mice did not develop obesity with a high-calorie diet as much as did wild-type mice, although they fed more (Oishi et al. 2005). This is due in part to enhanced lipid oxidation and energy uncoupling in skeletal muscles. SUMOylated KLF5 is associated with co-repressors containing PPAR $\delta$ ; it represses CPT1b, UCP2, and UCP3 in skeletal muscles, thereby suppressing lipid metabolism (Oishi et al. 2008).

KLF15<sup>-/-</sup> mice developed severe hypoglycemia after an overnight fast, which is caused by defective amino acid catabolism in liver and skeletal muscles. KLF15 regulates gene expression of alanine aminotransferase (ALT), which converts alanine to pyruvate (Gray et al. 2007). KLF15 also regulates insulin-sensitive glucose transporter GLUT4 in both adipocytes and muscle tissues, which seems to be due to synergistic activation of the GLUT4 gene by KLF15 and MEF2A (Gray et al. 2002).

3,5,3'-Triiodothyronine (T<sub>3</sub>) is a biologically active thyroid hormone that regulates the basal metabolic rate in vertebrates. T<sub>3</sub> is generated from prohormone thyroxine (T<sub>4</sub>) by deiodination enzymes Dio1 and Dio2. Dio1 is known to catalyze both activation and inactivation of T<sub>4</sub>. Expression of the Dio1 gene is strongly upregulated by KLF9, which is more enhanced in the presence of HNF4 $\alpha$  and GATA4 (Ohguchi et al. 2008).

## Transcriptional Regulatory Mechanisms of KLFs and Pharmacological Control

KLFs and Sp1 have common binding sites (i.e., GC-rich sites or CACCC-boxes) and activate promoter reporter constructs containing these sites in *in vitro* promoter assays. However, these binding sites *in vivo* are selectively discriminated



by individual factors. The mechanisms whereby Sp/KLF family members exhibit their specific biological functions through these similar DNA-binding sequences remain to be elucidated. To our current knowledge, formation of a transcription complex that consists of transcription factors with co-activators/co-repressors and chromatin-associated factors is a key regulatory mechanism for gene expression in a context-dependent manner. Protein modification of transcription factors is another mechanism of gene expression that may affect binding activities of transcription factors with cofactors. KLF5 has been thoroughly investigated in several laboratories, including ours, and provides an example of transcriptional regulation in which a member of KLF is incorporated. Therefore, we focus our discussion here on KLF5. In SMCs and fibroblasts, KLF5 forms a complex with RAR $\alpha$ -RXR and activates the PDGF-A gene. KLF5 associates with the p50 subunit of NF- $\kappa$ B in phorbol ester-induced pathological conditions in SMCs, which promotes KLF5-induced PDGF-A gene activation and possibly underlies the transition from acute to chronic inflammation (Aizawa et al. 2004). RAR $\alpha$ -RXR-induced activation of KLF5 is repressed by the RAR $\alpha$  agonist Am80 but further activates RAR $\alpha$  antagonist LE135 (Shindo et al. 2002). All-trans retinoic acid (ATRA), Am80, and acyclic retinoid NIK-333 induce SMC differentiation and inhibit dedifferentiation in which KLF5 and possibly KLF4 are involved (Fujii et al. 2005; Kada et al. 2007, 2008; Wang et al. 2008b).

During adipocyte differentiation KLF5 binds C/EBP $\alpha$  and C/EBP $\beta$  and upregulates PPAR $\gamma_2$ , which is essential for adipocyte differentiation (Oishi et al. 2005). In skeletal muscles, a complex of KLF5 and PPAR $\delta$  represses the UCP2 gene. C/EBP $\alpha$  activates UCP2 promoter in both adipocytes and skeletal muscle cells. C/EBP $\alpha$ -induced activation of UCP2 promoter is further activated by KLF5 in adipocytes but inhibited in skeletal muscle cells (Oishi et al. 2008).

In skeletal muscles, SUMOylated KLF5 forms a transcriptionally repressive complex with C/EBP $\beta$ , unliganded PPAR $\delta$  and corepressors, NCoR and SMRT, resulting in repression of the UCP2, UCP3, and CPT1b gene expression under basal conditions. The presence of a PPAR $\delta$  ligand, GW501516, changes the composition of this complex by recruiting desumoylation enzyme SENP1 and acetylation enzyme CBP, which then induces chromatin remodeling and activation of UCP2, UCP3, and CPT1b genes (Oishi et al. 2008). SUMOylation of KLF5 is also indicated to enhance nuclear localization and promote anchorage-independent growth of HCT116 colon cancer cells (Du et al. 2008). SUMOylation of KLFs leads to enhancement of their transrepression activities as known for KLF1 (Perdomo et al. 2005; Siatecka et al. 2007) and KLF8 (Wei et al. 2006).

Acetylation of KLFs enhances transcriptional activity. KLF5 is acetylated at a lysine residue proximal to the DNA binding domain by p300/CBP, and HeLa cells transfected with nonacetylatable KLF5 are sensitive to TNF $\alpha$ -induced apoptosis (Miyamoto et al. 2003). Histone deacetylase1 (HDAC1), on the other hand, negatively regulates the transcriptional activity of KLF5 (Matsumura et al. 2005). KLF5 activity is also inhibited by binding to SET/TAF-1 $\beta$ , an oncogenic protein that participates in chromatin assembly (Miyamoto et al. 2003). KLF5 interacts with a histone chaperone, acidic nuclear phosphoprotein 32B (ANP32B),

leading to transcriptional repression of a KLF5-downstream gene. Recruitment of ANP32B onto the promoter region requires KLF5 and results in promoter region-specific histone incorporation and inhibition of histone acetylation by ANP32B (Munemasa et al. 2008).

## Perspective

In recent years, many investigations on KLFs have been performed in laboratories worldwide. However, how KLFs show regulatory functions through binding to similar GC-rich sites or CACC-boxes remains an enigma. Multiple KLFs expressed in individual cells participate in differentiation of various cell types, some of which exert opposite functions. Furthermore, individual CACC-boxes existing in the same promoter region may respond to different types of stimuli as shown in the p21/Waf1 promoter (Gartel and Tyner 1999; Lu et al. 2000; Wang et al. 2000). It is important to note that DNA-binding characteristics likely differ in the *in vivo* context of chromatin DNA *in vivo* in contrast to the naked DNA state often used for biochemical experiments. One important example using transgenic mice showed that EKLF/KLF1 preferentially binds the  $\beta$ -globin locus site *in vivo* that had been shown to bind both EKLF and Sp1 in biochemical studies (Gilleman et al. 1998). Therefore, the KLF family apparently constitutes a complex network through which biological diversity and stress responses are regulated. However, whether individual KLF genes are independently regulated or gene expression of KLFs is coordinated through cross-talk among family members, as shown for KLF1, KLF3, and KLF8, requires more extensive examination (Eaton et al. 2008).

The mechanisms of action as a transcriptional factor are of primary importance in KLF research. Splice-variants of KLF6 transcripts have been reported to inhibit the native form of KLF6 (Narla et al. 2008). Whether similar splice-variants exist in other KLFs is not known and needs to be clarified. KLFs are subjected to posttranslational modifications as well—such as acetylation, phosphorylation, ubiquitination, and sumoylation—which at least in part are thought to affect their binding with transcriptional cofactors, thus mediating the differential biological effects of KLFs. Furthermore, KLFs interact with chromatin-associated factors including histone chaperones, acetylases/deacetylases, and nucleosome-remodeling enzymes, which allow the KLF transcription factor complex to access specific genes in packaged chromatin. KLFs may also posttranscriptionally modify other transcription factors and cofactors (Oishi et al. 2008). To understand the functions of KLFs, it is necessary to continue comprehensive analyses on each KLF member from these aspects. At the same time, molecular structures of KLFs need to be determined. In this volume, Chapter 2, contributed by Shigeyuki Yokoyama and colleagues, is devoted to the structures of KLFs. They reveal structures of DNA-binding domains and the protein-binding domains of KLFs. However, the structures of the N-terminal regions as a complex with co-activators or co-repressors have not been clarified. In analogy, Sp1 has been subjected to structural analysis of its cofactor complex

that acts through the regulatory region, and similar analyses are anxiously awaited for KLFs to understand their similarities and differences (Taatzes et al. 2002). It is important to determine the structures of the KLF–cofactor complex and examine how they recognize specific GC-boxes or CACC-boxes in the DNA sequences.

Many members of the KLF family are ubiquitously expressed, and their expression is closely associated with cell growth or growth inhibition, phenotypic modulation of cells, and tumorigenesis in which interactions between parenchymal cells and stromal cells (including inflammatory/immune cells and/or vascular cells) are implicated as playing critical roles. KLFs are involved in disease processes, including cardiovascular disease, certain types of cancer, and metabolic syndrome, in which the proinflammatory response to tissue stress or energy excess is underlying. It is therefore worthwhile to delete KLF genes in a cell type-specific manner and analyzing responses to external stress. Such experiments will provide new molecular and cellular insight into tumor growth and tissue remodeling.

Identification of low-molecular-weight compounds that can modulate the KLF–cofactor complex is another important research target given that this will lead to the development of new therapies. We discuss on this subject in detail in Chapter 18.

Research on the KLF family is still wide open and comprehensive understanding of family members will certainly help expand into new fields of molecular genetics, developmental cell biology and disease biology.

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