Published in final edited form as:

J Cell Biochem. 2017 May ; 118(5): 953–958. doi:10.1002/jcb.25723.

Precocious Phenotypic Transcription-Factor Expression during Early Development

Jennifer J. VanOudenhove^{1,2}, Ricardo Medina^{2,‡}, Prachi N. Ghule¹, Jane B. Lian¹, Janet L. Stein¹, Sayyed K. Zaidi¹, and Gary Stein^{1,*}

¹Department of Biochemistry and University of Vermont Cancer Center, University of Vermont College of Medicine, 89 Beaumont Avenue, Burlington, VT 05405, USA

²Department of Cell and Developmental Biology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, USA

Abstract

A novel role for phenotypic transcription factors in very early differentiation was recently observed and merits further study to elucidate what role this precocious expression may have in development. The RUNX1 transcription factor exhibits selective and transient upregulation during early mesenchymal differentiation. In contrast to phenotype-associated transcriptional control of gene expression to establish and sustain hematopoietic/myeloid lineage identity, precocious expression of RUNX1 is functionally linked to control of an epithelial to mesenchymal transition that is obligatory for development. This early RUNX1 expression spike provides a paradigm for precocious expression of a phenotypic transcription factor that invites detailed mechanistic study to fully understand its biological importance.

Keywords

Phenotypic transcription factor; Early development

Introduction

A fundamental, yet poorly understood dimension of developmental control is engagement of the phenotypic transcription factors in regulatory cascades that mediate gene expression to establish cell and tissue identity. When are phenotypic genes initially expressed during development? Is their control and are their regulatory activities modified to accommodate requirements for the initiation and progression of differentiation? Here we review recent evidence that selected phenotypic transcription factors are expressed during early differentiation—in advance of previously understood requirements for biological activity. Implications for how this precocious expression of phenotypic transcription factors contributes to mechanisms that mediate developmental processes associated with

*Corresponding author: Gary S. Stein, Department of Biochemistry and University of Vermont Cancer Center, University of Vermont College of Medicine, 89 Beaumont Avenue, Burlington, VT 05405, USA, Phone: 802-656-4874, Fax: 802-656-2140, gary.stein@uvm.edu.

‡Present address: Blueprint Medicines, 38 Sidney Street, #200 Cambridge, MA 02139

establishing cell and tissue specificity are explored. We focus on precocious, tissue-specific, expression of the RUNX1 transcription factor as a paradigm for this novel parameter of cellular control that is operative at the onset of differentiation.

The RUNX Transcription Factor Family

While the maintenance of pluripotency has been well studied [Boward et al., 2016; Boyer et al., 2005; Chambers et al., 2003; Huang et al., 2015; Kapinas et al., 2013], it is not well understood how differentiation signals regulate the transition from pluripotency to phenotypic establishment. Studies in human embryonic stem cells (hESCs) have shown that many genes responsible for early developmental events carry bivalent epigenetic modifications that poise them for rapid response to either activating or repressive transcription cues [Bernstein et al., 2006; Grandy et al., 2015; Szutorisz and Dillon, 2005]. Once a differentiation signal has been introduced, early factors are expressed that prime cells' gene expression program for lineage determination [Zaret and Carroll, 2011]. Transcription factors are primary regulators of gene expression, with clear potential to influence cell fate determination and differentiation of hESCs; the RUNX (Runt-related transcription factor) transcription factors are of particular interest because they have well-documented, but distinct, roles in development [Chuang et al., 2013].

The RUNX gene family is also known as the acute myeloid leukemia (AML), core-binding factor α (CBF α) or polyoma enhancer-binding protein-2 α (PEBP2 α) family. It includes three members: RUNX1, RUNX2, and RUNX3 [Ito, 2004]. Each of the RUNX-family transcription factors have unique developmental roles: RUNX1 is necessary for definitive hematopoiesis [Okuda et al., 1996], RUNX2 for bone formation [Otto et al., 1997], and RUNX3 for gastrointestinal and nervous system development [Inoue et al., 2002; Levanon et al., 2002; Li et al., 2002]. All three RUNX-family members are implicated in malignancies as either tumor suppressors or oncogenes in a context-specific manner [Blyth et al., 2005; Ito, 2004].

RUNX genes are transcribed from two promoters, the distal P1 and the proximal P2 [Bangsow et al., 2001; Drissi et al., 2000; Fujiwara et al., 1999; Ghoezi et al., 1996], and contain similar structural domains. There is a highly conserved 128 amino acid region in the N-terminal portion of the proteins known as the runt homology domain (RHD) due to its homology with the *Drosophila* Runt protein [Kagoshima et al., 1993; Ogawa et al., 1993b]. This RHD mediates both heterodimerization with the core binding factor β (CBF β) protein, which stabilizes the protein complex, and binding target DNA at the RUNX family consensus sequence PyGPyGGTPy (Py represents either pyrimidine base, cytosine or thymine) [Melnikova et al., 1993; Ogawa et al., 1993a]. CBF β alone does not bind DNA [Ogawa et al., 1993a]. A nuclear targeting signal (NLS) is on the C-terminal end of the RHD; it is required for the nuclear localization of RUNX proteins and consequently allows access to their DNA targets [Kanno et al., 1998].

The RUNX family members also have conserved regions on their C-termini. Each RUNX transcription factor has a nuclear matrix targeting signal (NMTS) in addition to the NLS. The NMTS is an ~31–38 amino acid sequence that is responsible for the subnuclear

localization of RUNX proteins to distinct nuclear sites to facilitate gene regulation [Stein et al., 2003; Zeng et al., 1998; Zeng et al., 1997]. The C-terminus of RUNX proteins also include additional domains that mediate gene regulation, such as an activation and inhibitory domain, the PPxY (or PY) motif, and the VWRPY motif. The PPxY motif is a proline-rich peptide sequence that interacts with proteins, such as Yes-associated protein (YAP), that contain a WW domain, whereas the VWRPY motif interacts with proteins, such as Groucho/TLE transcription corepressors, that contain tryptophan-aspartic acid repeats [Aronson et al., 1997; Chuang et al., 2013; Ito, 2004; Javed et al., 2000].

RUNX1 in Development

Developmental hematopoiesis begins with primitive hematopoiesis, in which a limited number of blood lineages (mostly large erythroblasts) that sustain early embryonic development are produced primarily from the yolk sac [Chen et al., 2014]. A second wave of blood development, termed definitive hematopoiesis, occurs intra-embryonically in the aorta-gonad-mesonephros. During this stage of development, hematopoietic stem cells (HSCs) are formed. HSCs have the ability to produce any of the hematopoietic lineages and, importantly, have long-term repopulation capacity [Chen et al., 2014]. RUNX1 is required for definitive hematopoiesis, as no HSCs are formed in the absence of RUNX1. When RUNX1 was genetically deleted in mice, embryonic lethality resulted due to major defects in the formation of the fetal liver and hemorrhaging in the central nervous system [Okuda et al., 1996; Wang et al., 1996]. RUNX1 appears to be mostly dispensable once HSCs are formed, however, loss of RUNX1 has some effects on differentiation toward specific hematopoietic lineages [Growney et al., 2005; Ichikawa et al., 2004; Ichikawa et al., 2008]. It is hypothesized that RUNX1 alters chromatin, through unfolding and modification of the epigenetic landscape, to allow early hematopoiesis [Hoogenkamp et al., 2009; Lichtinger et al., 2012].

The RUNX1 transcript is expressed as three major isoforms, one from the distal P1 promoter (isoform c) and two from a proximal P2 promoter (isoforms a and b) [Ghozi et al., 1996; Ran et al., 2013; Sroczynska et al., 2009]. A study of Runx1 isoform expression in early mouse hematopoietic development found that primitive erythrocytes produced prior to definitive hematopoiesis express mainly the proximal P2 promoter-derived isoforms [Bee et al., 2009]. However, in HSCs formed during definitive hematopoiesis, transcription occurs from both the P1 and P2 promoters, with isoforms from each promoter exhibiting non-redundant functions [Bee et al., 2010]. Once cells have migrated to the fetal liver to establish adult hematopoiesis, RUNX1c, transcribed from the P1 promoter, gradually becomes the main RUNX1 isoform in hematopoietic cells [Bee et al., 2009].

In human ESCs, studies of hematopoietic differentiation reveal an increase in RUNX1c mRNA levels at 8–12 days of differentiation from embryoid bodies that correlates with the emergence of the definitive hematopoietic lineage marker, CD34. Low RUNX1b mRNA levels have been observed throughout hematopoietic differentiation [Challen and Goodell, 2010; Zambidis et al., 2005]. Of note, in undifferentiated hESCs, the RUNX1 P2 promoter, but not the P1 promoter, is bivalently marked with repressive H3K27me3 and activating H3K4me3 epigenetic modifications, indicating that it is poised for rapid activation [Grandy

et al., 2015; Mikkelsen et al., 2007; Pope et al., 2014; Thurman et al., 2012]. This could indicate that RUNX1 transcription from the P2 promoter is more permissive early in development and may have a role that is not linked to emergence of HSCs. The role of RUNX1 in differentiation at very early time points in either directed or undirected differentiation of hESCs has been only minimally explored.

Emerging evidence indicates that RUNX1 has roles in non-hematopoietic cell lineages as well [Osorio et al., 2008; Scheitz and Tumber, 2013; Stifani et al., 2008]. Multiple reports have detailed a role for RUNX1 in epithelial biology [Scheitz and Tumber, 2013]. RUNX1 has been shown to modulate developmental activation and proliferation of hair follicle stem cells and inner olfactory nerve layer olfactory ensheathing cells [Hoi et al., 2010; Lee et al., 2014; Murthy et al., 2014; Osorio et al., 2008; Osorio et al., 2011]. RUNX1 is a key regulator of the differentiation of mammary epithelium stem cells from a state of ductal and lobular bipotency [Sokol et al., 2015]. Additional roles have been shown in other mesendodermal derivatives such as mesenchymal stem cells, myofibroblasts and skeletal progenitors [Kim et al., 2014; Lian et al., 2003].

Transient, Preemptive Expression of a Phenotypic Transcription Factor during Early Mesendodermal Differentiation

The recent report that, prior to their established functions in specifying lineage and cell type-specific identity, phenotype-associated transcription factors play a role in initial stages of differentiation is counterintuitive but significant [VanOudenhove et al., In Press, 2016]. This “preemptive” expression of RUNX1 as early as eight hours after initiation of differentiation, during mesenchymal differentiation, illustrates the contribution of a phenotypic transcription factor in initial parameters of developmental control (see overview in Fig. 1). The consequences are functional as well as potentially unique contributions of transcription factors to developmental stage specific gene expression.

The mechanistic role of RUNX1 in hematopoietic/myeloid differentiation has been extensively studied. Its role in genetic and epigenetic control has been established and validated through multiple lines of molecular, cellular, biochemical and *in vivo* genetic evidence. In contrast, a mechanistic understanding for the regulatory consequences of RUNX1 expression at the onset of mesendodermal differentiation is just beginning to emerge. The precocious burst of RUNX1 expression at the onset of mesendodermal differentiation is both selective and specific; other phenotypic transcription factors are not expressed during this developmental window. While RUNX1 expression from the distal P1 promoter is linked with the emergence of definitive hematopoietic stem cells [Chen et al., 2009; Lacaud et al., 2002; Okuda et al., 1996], there is limited understanding for the role of transcripts from the more ubiquitous proximal P2 promoter [Challen and Goodell, 2010; Fujita et al., 2001; Sroczynska et al., 2009]. The RUNX1 transcript that is synthesized during early mesendodermal differentiation is the RUNX1b isoform, transcribed from the P2 promoter. Interestingly, this P2 promoter is poised for expression, by virtue of bivalent H3K27me3 and H3K4me3 epigenetic modifications in undifferentiated hESCs, while the P1 promoter is not [Grandy et al., 2015; Mikkelsen et al., 2007; Pope et al., 2014; Thurman et

al., 2012]. Additionally, the transcripts from the two promoters are translated through different primary mechanisms, with the P1-derived transcript translated through a cap-mediated mechanism and the P2 transcripts translated through an internal ribosome entry site (IRES)-mediated mechanism [Pozner et al., 2000], which is often invoked to accommodate cellular stress, facilitate mitotic progression, and support competency for differentiation [Komar and Hatzoglou, 2011]. This further supports the idea that the RUNX1 expression from the P2 promoter has evolved specifically to be expressed early under differentiating conditions, indicating it could have an important role in early development.

A rapid, substantial increase in RUNX1 expression early in differentiation to mesendoderm suggests a potential role for RUNX1 that is unrelated to hematopoiesis. A role for RUNX1 in early mesendodermal differentiation of hESCs through regulation of cell migration and adhesion is indicated by impairment of these processes upon RUNX1 depletion. These findings are consistent with the emerging role of RUNX1 in controlling cell motility and migration in other biological systems. RUNX1 depletion in breast cancer cells results in a decreased migration and invasion phenotype [Browne et al., 2015]; similar results were found in ovarian cancer cells [Keita et al., 2013]. Prior to release of mouse hematopoietic stem cells from hemogenic endothelium, RUNX1b is responsible for inducing a cell adhesion and migration program [Lie-A-Ling et al., 2014]. RUNX1 depletion results in decreased cell motility and de-repression of epithelial markers, which suggests that there is a requirement for RUNX1 for fidelity of epithelial-mesenchymal transition (EMT).

RUNX1 regulates transforming growth factor β (TGF β) signaling during early mesendodermal differentiation in addition to its known roles in maintenance of hESC pluripotency [James et al., 2005; Wei et al., 2005], differentiation [Itoh et al., 2014; Watabe and Miyazono, 2009] and EMT [Xu et al., 2009]. The inhibition of motility and de-repression of epithelial genes observed upon RUNX1 depletion indicates that RUNX1 is upstream of the TGF β pathway [VanOudenhove et al., In Press, 2016]. Moreover, RUNX1 specifically occupies the TGF β 2 promoter and regulates the expression of the TGF β 2 gene, which encodes one of the three TGF β ligands. Though the TGF β ligands share >70% homology [Kingsley, 1994], TGF β -knockout mice show non-overlapping phenotypes, indicating that each ligand has specific functions in development [Sanford et al., 1997]. TGF β 2 knockout mice have cardiac, lung, craniofacial, limb, spinal column, eye, inner ear, and urogenital defects [Sanford et al., 1997], whereas the TGF β 1 knockout mice exhibit an autoimmune-like inflammatory disease or embryonic lethality due to defective yolk sac hematopoiesis and vasculogenesis, depending on the genetic background [Dickson et al., 1995; Kulkarni et al., 1993; Shull et al., 1992]. Consistent with the results from these mouse models, there is a specific requirement for TGF β 2, but not TGF β 1, to rescue the phenotype of RUNX1 depletion in hESCs [VanOudenhove et al., In Press, 2016].

In addition to a decrease in TGF β 2 ligand expression upon RUNX1 depletion, there is a decrease in phosphorylation of the downstream effector SMAD Family Member 2 (SMAD2) [VanOudenhove et al., In Press, 2016]. SMAD2 is required for competency to initiate gastrulation, as well as primitive streak/mesendoderm and mesoderm formation, with SMAD2 knockout being embryonic lethal before E8.5 [Nomura and Li, 1998; Weinstein et

al., 1998]. This is consistent with a role for RUNX1 in mesendodermal regulation upstream of TGF β 2-SMAD2 signaling.

Conclusion and Perspectives

Transient expression of the phenotypic transcription factor RUNX1 during early mesendodermal differentiation of hESCs suggests that RUNX1 contributes to differentiation in addition to its established role in hematopoietic lineage identity. There is compelling evidence that RUNX1 has a defined role in the epithelial to mesenchymal transition, and the associated competency for cell mobility and motility required for development of the mesendodermal germ layer. The current knowledgebase indicates that RUNX1 regulates cell motility and gene expression during mesendodermal differentiation specifically through TGF β 2 signaling, supporting a novel, evidence-based role for RUNX1 in early development. While these findings provide confidence in the importance of RUNX1 expression and bioavailability of the RUNX1 protein during a developmental window in which mesendodermal differentiation is initiated, they also present several important unanswered questions that include: what upstream regulatory mechanisms activate the transient, precocious expression of RUNX1?; Are epigenetic regulatory mechanisms associated with precocious RUNX1 expression?; How does RUNX1 contribute to epigenetic regulation of target genes for which the RUNX protein provides a scaffold on which histone-modifying and signaling proteins at target gene promoters during early development are strategically localized; and, How is this precocious RUNX1 expression downregulated during mesenchymal progression? The exquisitely selective expression of the RUNX1 transcription factor during mesenchymal differentiation is impressive. While genome-wide expression analysis reinforces the uniqueness of RUNX1 expression at the onset of mesendodermal differentiation it will be important to investigate the extent to which transcription factors precociously exhibit transient expression throughout early development, within the context of establishing the three germ layers.

From a broad biological perspective, RUNX1-mediated regulation of TGF β 2 signaling provides mechanistic insights into early mesendodermal differentiation. Precociously expressed RUNX1 is a selective and specific regulator of cell motility and EMT-associated gene expression.

Acknowledgments

The authors declare no conflicts of interest.

Contract grant sponsor: National Cancer Institute; **Contract grant number:** P01 CA082834 (GSS, JLS)

References

- Aronson BD, Fisher AL, Blechman K, Caudy M, Gergen JP. Groucho-dependent and -independent repression activities of Runt domain proteins. *Mol Cell Biol.* 1997; 17:5581–5587. [PubMed: 9271433]
- Bangsow C, Rubins N, Glusman G, Bernstein Y, Negreanu V, Goldenberg D, Lotem J, Ben-Asher E, Lancet D, Levanon D, Groner Y. The RUNX3 gene—sequence, structure and regulated expression. *Gene.* 2001; 279:221–232. [PubMed: 11733147]

- Bee T, Liddiard K, Swiers G, Bickley SR, Vink CS, Jarratt A, Hughes JR, Medvinsky A, de Bruijn MF. Alternative Runx1 promoter usage in mouse developmental hematopoiesis. *Blood Cells Mol Dis.* 2009; 43:35–42. [PubMed: 19464215]
- Bee T, Swiers G, Muroi S, Pozner A, Nottingham W, Santos AC, Li PS, Taniuchi I, de Bruijn MF. Nonredundant roles for Runx1 alternative promoters reflect their activity at discrete stages of developmental hematopoiesis. *Blood.* 2010; 115:3042–3050. [PubMed: 20139099]
- Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell.* 2006; 125:315–326. [PubMed: 16630819]
- Blyth K, Cameron ER, Neil JC. The RUNX genes: gain or loss of function in cancer. *Nat Rev Cancer.* 2005; 5:376–387. [PubMed: 15864279]
- Boward B, Wu T, Dalton S. Concise Review: Control of Cell Fate Through Cell Cycle and Pluripotency Networks. *Stem Cells.* 2016; 34:1427–1436. [PubMed: 26889666]
- Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, Guenther MG, Kumar RM, Murray HL, Jenner RG, Gifford DK, Melton DA, Jaenisch R, Young RA. Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell.* 2005; 122:947–956. [PubMed: 16153702]
- Browne G, Taipaleenmaki H, Bishop NM, Madasu SC, Shaw LM, van Wijnen AJ, Stein JL, Stein GS, Lian JB. Runx1 is associated with breast cancer progression in MMTV-PyMT transgenic mice and its depletion in vitro inhibits migration and invasion. *J Cell Physiol.* 2015; 230:2522–2532. [PubMed: 25802202]
- Challen GA, Goodell MA. Runx1 isoforms show differential expression patterns during hematopoietic development but have similar functional effects in adult hematopoietic stem cells. *Exp Hematol.* 2010; 38:403–416. [PubMed: 20206228]
- Chambers I, Colby D, Robertson M, Nichols J, Lee S, Tweedie S, Smith A. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell.* 2003; 113:643–655. [PubMed: 12787505]
- Chen, B., Mao, B., Huang, S., Zhou, Y., Tsuji, K., Ma, F. Human embryonic stem cell-derived primitive and definitive hematopoiesis. In: Atwood, CS., Meethal, SV., editors. *Pluripotent Stem Cell Biology - Advances in Mechanisms, Methods and Models.* Rijeka, Croatia: InTech; 2014. p. 87–113.
- Chen MJ, Yokomizo T, Zeigler BM, Dzierzak E, Speck NA. Runx1 is required for the endothelial to haematopoietic cell transition but not thereafter. *Nature.* 2009; 457:887–891. [PubMed: 19129762]
- Chuang LS, Ito K, Ito Y. RUNX family: Regulation and diversification of roles through interacting proteins. *Int J Cancer.* 2013; 132:1260–1271. [PubMed: 23180629]
- Dickson MC, Martin JS, Cousins FM, Kulkarni AB, Karlsson S, Akhurst RJ. Defective haematopoiesis and vasculogenesis in transforming growth factor-beta 1 knock out mice. *Development.* 1995; 121:1845–1854. [PubMed: 7600998]
- Drissi H, Luc Q, Shakoori R, Chuva De Sousa Lopes S, Choi JY, Terry A, Hu M, Jones S, Neil JC, Lian JB, Stein JL, Van Wijnen AJ, Stein GS. Transcriptional autoregulation of the bone related CBFA1/RUNX2 gene. *J Cell Physiol.* 2000; 184:341–350. [PubMed: 10911365]
- Fujita Y, Nishimura M, Taniwaki M, Abe T, Okuda T. Identification of an alternatively spliced form of the mouse AML1/RUNX1 gene transcript AML1c and its expression in early hematopoietic development. *Biochem Biophys Res Commun.* 2001; 281:1248–1255. [PubMed: 11243869]
- Fujiwara M, Tagashira S, Harada H, Ogawa S, Katsumata T, Nakatsuka M, Komori T, Takada H. Isolation and characterization of the distal promoter region of mouse Cbfa1. *Biochim Biophys Acta.* 1999; 1446:265–272. [PubMed: 10524201]
- Ghozi MC, Bernstein Y, Negreanu V, Levanon D, Groner Y. Expression of the human acute myeloid leukemia gene AML1 is regulated by two promoter regions. *Proc Natl Acad Sci U S A.* 1996; 93:1935–1940. [PubMed: 8700862]
- Grandy RA, Whitfield TW, Wu H, Fitzgerald MP, VanOudenhove JJ, Zaidi SK, Montecino MA, Lian JB, van Wijnen AJ, Stein JL, Stein GS. Genome-wide studies reveal that H3K4me3 modification in bivalent genes is dynamically regulated during the pluripotent cell cycle and stabilized upon differentiation. *Mol Cell Biol.* 2015; 36:615–627. [PubMed: 26644406]

- Growney JD, Shigematsu H, Li Z, Lee BH, Adelsperger J, Rowan R, Curley DP, Kutok JL, Akashi K, Williams IR, Speck NA, Gilliland DG. Loss of Runx1 perturbs adult hematopoiesis and is associated with a myeloproliferative phenotype. *Blood*. 2005; 106:494–504. [PubMed: 15784726]
- Hoi CS, Lee SE, Lu SY, McDermitt DJ, Osorio KM, Piskun CM, Peters RM, Paus R, Tumber T. Runx1 directly promotes proliferation of hair follicle stem cells and epithelial tumor formation in mouse skin. *Mol Cell Biol*. 2010; 30:2518–2536. [PubMed: 20308320]
- Hoogenkamp M, Lichtinger M, Krysinska H, Lancrin C, Clarke D, Williamson A, Mazzarella L, Ingram R, Jorgensen H, Fisher A, Tenen DG, Kouskoff V, Lacaud G, Bonifer C. Early chromatin unfolding by RUNX1: a molecular explanation for differential requirements during specification versus maintenance of the hematopoietic gene expression program. *Blood*. 2009; 114:299–309. [PubMed: 19339695]
- Huang G, Ye S, Zhou X, Liu D, Ying QL. Molecular basis of embryonic stem cell self-renewal: from signaling pathways to pluripotency network. *Cell Mol Life Sci*. 2015; 72:1741–1757. [PubMed: 25595304]
- Ichikawa M, Asai T, Saito T, Seo S, Yamazaki I, Yamagata T, Mitani K, Chiba S, Ogawa S, Kurokawa M, Hirai H. AML-1 is required for megakaryocytic maturation and lymphocytic differentiation, but not for maintenance of hematopoietic stem cells in adult hematopoiesis. *Nat Med*. 2004; 10:299–304. [PubMed: 14966519]
- Ichikawa M, Goyama S, Asai T, Kawazu M, Nakagawa M, Takeshita M, Chiba S, Ogawa S, Kurokawa M. AML1/Runx1 negatively regulates quiescent hematopoietic stem cells in adult hematopoiesis. *J Immunol*. 2008; 180:4402–4408. [PubMed: 18354160]
- Inoue K, Ozaki S, Shiga T, Ito K, Masuda T, Okado N, Iseda T, Kawaguchi S, Ogawa M, Bae SC, Yamashita N, Itohara S, Kudo N, Ito Y. Runx3 controls the axonal projection of proprioceptive dorsal root ganglion neurons. *Nat Neurosci*. 2002; 5:946–954. [PubMed: 12352981]
- Ito Y. Oncogenic potential of the RUNX gene family: ‘overview’. *Oncogene*. 2004; 23:4198–4208. [PubMed: 15156173]
- Itoh F, Watabe T, Miyazono K. Roles of TGF-beta family signals in the fate determination of pluripotent stem cells. *Semin Cell Dev Biol*. 2014; 32:98–106. [PubMed: 24910449]
- James D, Levine AJ, Besser D, Hemmati-Brivanlou A. TGFbeta/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells. *Development*. 2005; 132:1273–1282. [PubMed: 15703277]
- Javed A, Guo B, Hiebert S, Choi JY, Green J, Zhao SC, Osborne MA, Stifani S, Stein JL, Lian JB, van Wijnen AJ, Stein GS. Groucho/TLE/R-esp proteins associate with the nuclear matrix and repress RUNX (CBF(alpha)/AML/PEBP2(alpha)) dependent activation of tissue-specific gene transcription. *J Cell Sci*. 2000; 113(Pt 12):2221–2231. [PubMed: 10825294]
- Kagoshima H, Shigesada K, Satake M, Ito Y, Miyoshi H, Ohki M, Pepling M, Gergen P. The Runt domain identifies a new family of heteromeric transcriptional regulators. *Trends Genet*. 1993; 9:338–341. [PubMed: 8273148]
- Kanno T, Kanno Y, Chen LF, Ogawa E, Kim WY, Ito Y. Intrinsic transcriptional activation-inhibition domains of the polyomavirus enhancer binding protein 2/core binding factor alpha subunit revealed in the presence of the beta subunit. *Mol Cell Biol*. 1998; 18:2444–2454. [PubMed: 9566865]
- Kapinas K, Grandy R, Ghule P, Medina R, Becker K, Pardee A, Zaidi SK, Lian J, Stein J, van Wijnen A, Stein G. The abbreviated pluripotent cell cycle. *J Cell Physiol*. 2013; 228:9–20. [PubMed: 22552993]
- Keita M, Bachvarova M, Morin C, Plante M, Gregoire J, Renaud MC, Sebastianelli A, Trinh XB, Bachvarov D. The RUNX1 transcription factor is expressed in serous epithelial ovarian carcinoma and contributes to cell proliferation, migration and invasion. *Cell Cycle*. 2013; 12:972–986. [PubMed: 23442798]
- Kim W, Barron DA, San Martin R, Chan KS, Tran LL, Yang F, Ressler SJ, Rowley DR. RUNX1 is essential for mesenchymal stem cell proliferation and myofibroblast differentiation. *Proc Natl Acad Sci U S A*. 2014; 111:16389–16394. [PubMed: 25313057]
- Kingsley DM. The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms. *Genes Dev*. 1994; 8:133–146. [PubMed: 8299934]

- Komar AA, Hatzoglou M. Cellular IRES-mediated translation: the war of ITAFs in pathophysiological states. *Cell Cycle*. 2011; 10:229–240. [PubMed: 21220943]
- Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci U S A*. 1993; 90:770–774. [PubMed: 8421714]
- Lacaud G, Gore L, Kennedy M, Kouskoff V, Kingsley P, Hogan C, Carlsson L, Speck N, Palis J, Keller G. Runx1 is essential for hematopoietic commitment at the hemangioblast stage of development in vitro. *Blood*. 2002; 100:458–466. [PubMed: 12091336]
- Lee SE, Sada A, Zhang M, McDermitt DJ, Lu SY, Kempfues KJ, Tumber T. High Runx1 levels promote a reversible, more-differentiated cell state in hair-follicle stem cells during quiescence. *Cell Rep*. 2014; 6:499–513. [PubMed: 24462289]
- Levanon D, Bettoun D, Harris-Cerruti C, Woolf E, Negreanu V, Eilam R, Bernstein Y, Goldenberg D, Xiao C, Fliegau M, Kremer E, Otto F, Brenner O, Lev-Tov A, Groner Y. The Runx3 transcription factor regulates development and survival of TrkC dorsal root ganglia neurons. *EMBO J*. 2002; 21:3454–3463. [PubMed: 12093746]
- Li QL, Ito K, Sakakura C, Fukamachi H, Inoue K, Chi XZ, Lee KY, Nomura S, Lee CW, Han SB, Kim HM, Kim WJ, Yamamoto H, Yamashita N, Yano T, Ikeda T, Itohara S, Inazawa J, Abe T, Hagiwara A, Yamagishi H, Ooe A, Kaneda A, Sugimura T, Ushijima T, Bae SC, Ito Y. Causal relationship between the loss of RUNX3 expression and gastric cancer. *Cell*. 2002; 109:113–124. [PubMed: 11955451]
- Lian JB, Balint E, Javed A, Drissi H, Vitti R, Quinlan EJ, Zhang L, Van Wijnen AJ, Stein JL, Speck N, Stein GS. Runx1/AML1 hematopoietic transcription factor contributes to skeletal development in vivo. *J Cell Physiol*. 2003; 196:301–311. [PubMed: 12811823]
- Lichtinger M, Ingram R, Hannah R, Muller D, Clarke D, Assi SA, Lie ALM, Noailles L, Vijayabaskar MS, Wu M, Tenen DG, Westhead DR, Kouskoff V, Lacaud G, Gottgens B, Bonifer C. RUNX1 reshapes the epigenetic landscape at the onset of haematopoiesis. *EMBO J*. 2012; 31:4318–4333. [PubMed: 23064151]
- Lie-A-Ling M, Marinopoulou E, Li Y, Patel R, Stefanska M, Bonifer C, Miller C, Kouskoff V, Lacaud G. RUNX1 positively regulates a cell adhesion and migration program in murine hemogenic endothelium prior to blood emergence. *Blood*. 2014; 124:e11–20. [PubMed: 25082880]
- Melnikova IN, Crute BE, Wang S, Speck NA. Sequence specificity of the core-binding factor. *J Virol*. 1993; 67:2408–2411. [PubMed: 8445737]
- Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP, Lee W, Mendenhall E, O'Donovan A, Presser A, Russ C, Xie X, Meissner A, Wernig M, Jaenisch R, Nusbaum C, Lander ES, Bernstein BE. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature*. 2007; 448:553–560. [PubMed: 17603471]
- Murthy M, Bocking S, Verginelli F, Stifani S. Transcription factor Runx1 inhibits proliferation and promotes developmental maturation in a selected population of inner olfactory nerve layer olfactory ensheathing cells. *Gene*. 2014; 540:191–200. [PubMed: 24582971]
- Nomura M, Li E. Smad2 role in mesoderm formation, left-right patterning and craniofacial development. *Nature*. 1998; 393:786–790. [PubMed: 9655392]
- Ogawa E, Inuzuka M, Maruyama M, Satake M, Naito-Fujimoto M, Ito Y, Shigesada K. Molecular cloning and characterization of PEBP2 beta, the heterodimeric partner of a novel Drosophila runt-related DNA binding protein PEBP2 alpha. *Virology*. 1993a; 194:314–331. [PubMed: 8386878]
- Ogawa E, Maruyama M, Kagoshima H, Inuzuka M, Lu J, Satake M, Shigesada K, Ito Y. PEBP2/PEA2 represents a family of transcription factors homologous to the products of the Drosophila runt gene and the human AML1 gene. *Proc Natl Acad Sci U S A*. 1993b; 90:6859–6863. [PubMed: 8341710]
- Okuda T, van Deursen J, Hiebert SW, Grosveld G, Downing JR. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. *Cell*. 1996; 84:321–330. [PubMed: 8565077]

- Osorio KM, Lee SE, McDermitt DJ, Waghmare SK, Zhang YV, Woo HN, Tumbar T. Runx1 modulates developmental, but not injury-driven, hair follicle stem cell activation. *Development*. 2008; 135:1059–1068. [PubMed: 18256199]
- Osorio KM, Lilja KC, Tumbar T. Runx1 modulates adult hair follicle stem cell emergence and maintenance from distinct embryonic skin compartments. *J Cell Biol*. 2011; 193:235–250. [PubMed: 21464233]
- Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, Stamp GW, Beddington RS, Mundlos S, Olsen BR, Selby PB, Owen MJ. Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. *Cell*. 1997; 89:765–771. [PubMed: 9182764]
- Pope BD, Ryba T, Dileep V, Yue F, Wu W, Denas O, Vera DL, Wang Y, Hansen RS, Canfield TK, Thurman RE, Cheng Y, Gulsoy G, Dennis JH, Snyder MP, Stamatoyannopoulos JA, Taylor J, Hardison RC, Kahveci T, Ren B, Gilbert DM. Topologically associating domains are stable units of replication-timing regulation. *Nature*. 2014; 515:402–405. [PubMed: 25409831]
- Pozner A, Goldenberg D, Negreanu V, Le SY, Elroy-Stein O, Levanon D, Groner Y. Transcription-coupled translation control of AML1/RUNX1 is mediated by cap- and internal ribosome entry site-dependent mechanisms. *Mol Cell Biol*. 2000; 20:2297–2307. [PubMed: 10713153]
- Ran D, Shia WJ, Lo MC, Fan JB, Knorr DA, Ferrell PI, Ye Z, Yan M, Cheng L, Kaufman DS, Zhang DE. RUNX1a enhances hematopoietic lineage commitment from human embryonic stem cells and inducible pluripotent stem cells. *Blood*. 2013; 121:2882–2890. [PubMed: 23372166]
- Sanford LP, Ormsby I, Gittenberger-de Groot AC, Sariola H, Friedman R, Boivin GP, Cardell EL, Doetschman T. TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGFbeta knockout phenotypes. *Development*. 1997; 124:2659–2670. [PubMed: 9217007]
- Scheitz CJ, Tumbar T. New insights into the role of Runx1 in epithelial stem cell biology and pathology. *J Cell Biochem*. 2013; 114:985–993. [PubMed: 23150456]
- Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, Annunziata N, Doetschman T. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature*. 1992; 359:693–699. [PubMed: 1436033]
- Sokol ES, Sanduja S, Jin DX, Miller DH, Mathis RA, Gupta PB. Perturbation-expression analysis identifies RUNX1 as a regulator of human mammary stem cell differentiation. *PLoS Comput Biol*. 2015; 11:e1004161. [PubMed: 25894653]
- Sroczynska P, Lancrin C, Kouskoff V, Lacaud G. The differential activities of Runx1 promoters define milestones during embryonic hematopoiesis. *Blood*. 2009; 114:5279–5289. [PubMed: 19858498]
- Stein GS, Lian JB, Stein JL, van Wijnen AJ, Choi JY, Pratap J, Zaidi SK. Temporal and spatial parameters of skeletal gene expression: targeting RUNX factors and their coregulatory proteins to subnuclear domains. *Connect Tissue Res*. 2003; 44(Suppl 1):149–153. [PubMed: 12952189]
- Stifani N, Freitas AR, Liakhovitskaia A, Medvinsky A, Kania A, Stifani S. Suppression of interneuron programs and maintenance of selected spinal motor neuron fates by the transcription factor AML1/Runx1. *Proc Natl Acad Sci U S A*. 2008; 105:6451–6456. [PubMed: 18427115]
- Szutorisz H, Dillon N. The epigenetic basis for embryonic stem cell pluripotency. *Bioessays*. 2005; 27:1286–1293. [PubMed: 16299767]
- Thurman RE, Rynes E, Humbert R, Vierstra J, Maurano MT, Haugen E, Sheffield NC, Stergachis AB, Wang H, Vernot B, Garg K, John S, Sandstrom R, Bates D, Boatman L, Canfield TK, Diegel M, Dunn D, Ebersol AK, Frum T, Giste E, Johnson AK, Johnson EM, Kuttyavin T, Lajoie B, Lee BK, Lee K, London D, Lotakis D, Neph S, Neri F, Nguyen ED, Qu H, Reynolds AP, Roach V, Safi A, Sanchez ME, Sanyal A, Shafer A, Simon JM, Song L, Vong S, Weaver M, Yan Y, Zhang Z, Zhang Z, Lenhard B, Tewari M, Dorschner MO, Hansen RS, Navas PA, Stamatoyannopoulos G, Iyer VR, Lieb JD, Sunyaev SR, Akey JM, Sabo PJ, Kaul R, Furey TS, Dekker J, Crawford GE, Stamatoyannopoulos JA. The accessible chromatin landscape of the human genome. *Nature*. 2012; 489:75–82. [PubMed: 22955617]
- VanOudenhove JJ, Medina R, Ghule PN, Lian JB, Stein JL, Zaidi SK, Stein GS. Transient RUNX1 expression during early mesendodermal differentiation of hESCs promotes epithelial to mesenchymal transition through TGFβ2 signaling. *Stem Cell Rep*. 2016 (In Press).

- Wang Q, Stacy T, Binder M, Marin-Padilla M, Sharpe AH, Speck NA. Disruption of the Cbfa2 gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis. *Proc Natl Acad Sci U S A*. 1996; 93:3444–3449. [PubMed: 8622955]
- Watabe T, Miyazono K. Roles of TGF-beta family signaling in stem cell renewal and differentiation. *Cell Res*. 2009; 19:103–115. [PubMed: 19114993]
- Wei CL, Miura T, Robson P, Lim SK, Xu XQ, Lee MY, Gupta S, Stanton L, Luo Y, Schmitt J, Thies S, Wang W, Khrebtukova I, Zhou D, Liu ET, Ruan YJ, Rao M, Lim B. Transcriptome profiling of human and murine ESCs identifies divergent paths required to maintain the stem cell state. *Stem Cells*. 2005; 23:166–185. [PubMed: 15671141]
- Weinstein M, Yang X, Li C, Xu X, Gotay J, Deng CX. Failure of egg cylinder elongation and mesoderm induction in mouse embryos lacking the tumor suppressor smad2. *Proc Natl Acad Sci U S A*. 1998; 95:9378–9383. [PubMed: 9689088]
- Xu J, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. *Cell Res*. 2009; 19:156–172. [PubMed: 19153598]
- Zambidis ET, Peault B, Park TS, Bunz F, Civin CI. Hematopoietic differentiation of human embryonic stem cells progresses through sequential hematoendothelial, primitive, and definitive stages resembling human yolk sac development. *Blood*. 2005; 106:860–870. [PubMed: 15831705]
- Zaret KS, Carroll JS. Pioneer transcription factors: establishing competence for gene expression. *Genes Dev*. 2011; 25:2227–2241. [PubMed: 22056668]
- Zeng C, McNeil S, Pockwinse S, Nickerson J, Shopland L, Lawrence JB, Penman S, Hiebert S, Lian JB, van Wijnen AJ, Stein JL, Stein GS. Intracellular targeting of AML/CBFalpha regulatory factors to nuclear matrix-associated transcriptional domains. *Proc Natl Acad Sci U S A*. 1998; 95:1585–1589. [PubMed: 9465059]
- Zeng C, van Wijnen AJ, Stein JL, Meyers S, Sun W, Shopland L, Lawrence JB, Penman S, Lian JB, Stein GS, Hiebert SW. Identification of a nuclear matrix targeting signal in the leukemia and bone-related AML/CBF-alpha transcription factors. *Proc Natl Acad Sci U S A*. 1997; 94:6746–6751. [PubMed: 9192636]

Fig. 1.
Overview of the role of transient RUNX1 upregulation in early mesendodermal development.