PROSPECT
Journal of Cellular Biochemistry 107:194–202 (2009)

Journal of Cellular Biochemistry

Is Runx a Linchpin for Developmental Signaling in Metazoans?

James A. Coffman*
Mount Desert Island Biological Laboratory, Salisbury Cove, Maine 04672

ABSTRACT

The Runt domain (Runx) is a 128 amino acid sequence motif that defines a metazoan family of sequence-specific DNA binding proteins, which appears to have originated in concert with the intercellular signaling systems that coordinate multicellular development in animals. In the model organisms where they have been studied (fruit fly, mouse, sea urchin, and nematode) Runx genes are essential for normal development, and in humans they are causally associated with a variety of cancers, manifesting both oncogenic and tumor suppressive attributes. During development Runx proteins support both cell proliferation and differentiation, and function in both transcriptional activation and repression. Runx function is thus context-dependent, with the context provided genetically by *cis*-regulatory sequence architecture and epigenetically by development. This context dependency makes it difficult to formulate reductionistic generalizations concerning Runx function in normal and carcinogenic development. However, a growing body of literature links Runx function to each of the major intercellular signaling systems in animals, suggesting that the general function of Runx transcription factors may be to potentiate and govern genomic responsiveness to developmental signaling. J. Cell. Biochem. 107: 194–202, 2009.

KEY WORDS: Runx; DEVELOPMENT; SIGNALING; SEA URCHIN EMBRYO; GENE REGULATORY NETWORK; TRANSCRIPTION FACTOR; EPIGENETIC

Heritable aspects of animal development are controlled by gene regulatory networks (GRNs) that formally specify when and where genes are expressed during ontogeny [Davidson, 2006]. The operation of GRNs depends on epigenetic systems of intercellular signaling that coordinate proliferation, patterning, and differentiation of cells within multicellular tissues. Metazoan development is governed by a small number of such systems; namely, the Wnt, Notch, TGFβ/BMP, receptor tyrosine kinase (RTK), JAK/STAT, Hedgehog, and nuclear receptor pathways, many of which appear to have originated in the earliest metazoans [Pires-daSilva and Sommer, 2003]. For each of these pathways, signals provided by extracellular ligands are carried to the cell nucleus via the intracellular modification and hence activation of transcriptional regulatory proteins, resulting in the activation or repression of genes that contain binding sites for those proteins within their *cis*-regulatory sequences.

The DNA binding sequences recognized by signal-transducing transcription factors are often short and degenerate, and hence commonplace in the genome, which raises the question of how the relatively miniscule subset of functional target sites is selectively engaged during development. The answer lies at least in part in the clustering of target sites within *cis*-regulatory modules, which facilitates cooperative protein–protein interactions. In addition to the sites for the factors bearing signals, these sites include targets for proteins that play architectural roles such as DNA bending and looping, subnuclear targeting, and recruitment of non-DNA binding chromatin-modifying enzymes that further increase site-selective specificity [Zaidi et al., 2005; Davidson, 2006]. Transduction of signals to *cis*-regulatory target sequences therefore occurs within the context of multi-protein complexes, the composition of which is determined by the sequence structure of the *cis*-regulatory module.

The Runt domain (Runx) is a highly conserved 128 amino acid sequence motif that defines a metazoan family of sequence-specific DNA binding proteins required for the ontogeny of each of the animal species in which it has been functionally studied, as well as for the regulation of somatic stem cells and development of the lineages to which they give rise [Coffman, 2003; Ito, 2008; Nimmo and Woollard, 2008]. Runx genes facilitate developmental coordination of cell proliferation and differentiation, in part by nucleating assembly of regulatory protein complexes within *cis*-regulatory systems that support the transduction of intercellular signals [Westendorf and Hiebert, 1999; Coffman, 2003]. Indeed, as reviewed below Runx proteins are functionally implicated in most of the major metazoan developmental signaling pathways, including those

Grant sponsor: NIH; Grant number: GM070840.
*Correspondence to: Dr. James A. Coffman, PO Box 35, Old Bar Harbor Road, Salisbury Cove, ME 04672.
E-mail: jcoffman@mdibl.org
Received 18 February 2009; Accepted 19 February 2009 • DOI 10.1002/jcb.22143 • 2009 Wiley-Liss, Inc.
Published online 27 March 2009 in Wiley InterScience (www.interscience.wiley.com).

mediated by TGFβ/BMP, RTKs, Wnt, and Notch. These functions often involve intramodular protein–protein interactions between Runx proteins and signal-transducing transcription factors in the Smad, Ets, Tcf/Lef, and nuclear receptor families. In addition to these interactions, Runx proteins heterodimerize with a non-DNA binding beta subunit (CBFβ), which allosterically enhances Runx binding to DNA [Yan et al., 2004]. Like many of the signaling pathways described above, both the Runt domain and CBFβ appear to be metazoan inventions: both are found encoded in the genomes of all metazoans examined to date, including the genomes of cnidarians and two sponges, whereas neither has been found encoded in any non-metazoan eukaryotic genome [Rennert et al., 2003; Sullivan et al., 2008].

As indicated above and discussed in more detail below, recent studies implicate Runx as a critical collaborator in each of the signaling systems involved in animal development. This remarkable fact offers a potential explanation for the paradoxical attributes of Runx, a transcription factor involved in both activation and repression that is required for both cell proliferation and differentiation during development, and which plays both oncogenic and tumor suppressive roles in cancer. It is hypothesized that Runx is generally required as a genetic/epigenetic gate that both potentiates and governs the specific effects of developmental signaling in proliferating progenitor cells.

Runx AS A TRANSCRIPTIONAL REGULATOR OF CELL FATE

The Runt domain derives its name from the *Drosophila* developmental regulatory gene *runt*, whose mutation produces arrested embryos with segmentation defects stemming from its essential function in the developmental GRN that controls patterning along the anterior-posterior axis of the embryo [Nusslein-Volhard and Wieschaus, 1980; Gergen and Butler, 1988]. Another *Drosophila* gene, *lozenge*, which has an even longer history of genetic studies associated with its essential role in eye development as well as development of antennae and limbs, also contains a Runt domain [Daga et al., 1996]. The sequencing of the *Drosophila* genome revealed that it encodes two additional unnamed Runx genes, the functions of which remain enigmatic. Interestingly, all four Runx genes lie on the X chromosome, a synteny that is conserved among insects and represents and an insect-specific gene expansion [Bao and Friedrich, 2008; Duncan et al., 2008].

Although the genetic studies of *Drosophila runt* and *lozenge* demonstrated that these genes control developmental specification of cell fate, the biochemical function of their products was not revealed until sequence-specific transcriptional regulatory proteins from mammalian cells, purified by oligonucleotide affinity chromatography using functionally characterized viral *cis*-regulatory elements, were shown to be *runt* homologues [Kagoshima et al., 1993; Ito, 2008]. The DNA binding domain of these proteins consisted of a 128 amino acid sequence motif that was shown to be present in *Drosophila runt* as well as in *AML1*, a human gene frequently mutated by chromosomal translocations that give rise acute myeloid leukemia [Meyers et al., 1993]. *AML1*, now referred to as *Runx1*, is one of three mammalian Runx genes.

Individual gene knockouts revealed that each mammalian Runx gene is critical for the development of a specific tissue or organ system: *Runx1* for hematopoiesis, *Runx2* for osteogenesis, and *Runx3* for neurogenesis in the dorsal root ganglia [reviewed in Coffman, 2003; Ito, 2008]. More refined studies have shown that *Runx1* and *Runx2* are each critical for specific cell fate decisions made respectively along hematopoietic and osteogenic developmental trajectories [see, e.g., Stein et al., 2004; Anderson, 2006 for reviews]. Thus, *Runx1* and *Runx2* are often referred to as "tissue-specific" regulatory genes or even "master control genes." Unfortunately, such terminology tends to promote reductionistic oversimplifications of the functional niche filled by Runx in the developmental physiology of cell fate specification; in fact, all three Runx genes play important developmental roles in multiple tissues, and it is possible (if not probable) that Runx is required globally for normal development, with the different paralogues manifesting (to an unknown extent) functional redundancy and cross-regulation. To date no triple Runx knockouts have been carried out in mice, and although it is commonly assumed that the knockout of the single *CBFbeta* gene (which produces a phenotype similar to that of the Runx1 knockout) is equivalent to complete loss of Runx function, this assumption is highly questionable, as discussed below. As with many gene families, Runx gene multiplicity in vertebrates (and independently in insects) may be more a reflection of developmental GRN complexity than of diversification of protein function.

Along with fruit flies, sea urchin embryos and nematodes are relatively simple developmental models that have provided important insights concerning Runx function in developmental specification of cell fate. The sea urchin Runt-1 protein was discovered biochemically by virtue of its sequence-specific interaction with (and function as a transcriptional activator of) a tissue-specific actin gene [Coffman et al., 1996]. Subsequent studies demonstrated that Runt-1 is globally expressed in early development, and required throughout the embryo for cell-type-, tissue-, and/or region specific gene expression [Coffman et al., 2004], as well as for cell survival and proliferation [Dickey-Sims et al., 2005; Robertson et al., 2008]. The single *Caenorhabditis elegans* Runx gene *rnt-1* is required for development of hypodermal seam cells that give rise to the rays of the male tail [Ji et al., 2004; Nimmo et al., 2005]. However, the latter function does not appear to involve developmental decisions related to cell fate specification per se; rather, it reflects a requirement for *rnt-1* in proliferation of the stem cell progenitors of seam cells [Nimmo et al., 2005; Nimmo and Woollard, 2008].

Runx INVOLVEMENT IN CELL CYCLE CONTROL, GROWTH, AND CANCER

As noted above, the first mammalian Runx gene to be identified as such was *AML1* (*Runx1*), a frequent target of leukemogenic translocations. Thus, a good deal of Runx-related research has focused on the role of this family in leukemias, and more recently, other forms of cancer involving each of the mammalian Runx genes

[Ito, 2004]. The facts that leukemogenic *Runx1* translocations produce proteins that have both lost normal functions and gained abnormal functions, and that *Runx3* loss-of-function is associated with gastrointestinal tumorogenesis, are consistent with the proposition that Runx is normally a "tumor-suppressor" [Ito, 2004, 2008]. Also consistent with this notion is the fact that both *Runx1* and *Runx2* promote the differentiation of specific cell types in blood and bone, respectively. Thus, it might be assumed that leukemogenesis associated with mutant forms of *Runx1* is simply attributable to the inability of pluripotent progenitors carrying these mutations to differentiate, which causes them to continue proliferating by "default." However, this simplified view is brought into question by the fact that Runx expression is actually required for proliferation in some contexts [Coffman, 2003; Nimmo and Woollard, 2008; Robertson et al., 2008]. Indeed, unaltered forms of all three mammalian Runx genes are both over-expressed and causally associated with proliferation in various kinds of cancer, consistent with the proposition that in these contexts they are "oncogenes" [Cameron and Neil, 2004].

Runx proteins engage mechanisms that control cell cycle progression at multiple stages, including the critical G1/S and G2/M transitions [Zhang et al., 2008b]. As might be expected, both positive (activating cell cycle progression) and negative (inhibiting cell cycle progression) interactions have been identified, in some cases involving the same Runx protein in different contexts. Moreover, these interactions involve both protein-DNA interactions between Runx and its target genes that encode cell cycle control proteins (e.g., cyclin D and p21 [Bernardin-Fried et al., 2004; Cha et al., 2008]), and direct protein–protein interactions between Runx factors and cell cycle control proteins (e.g., cyclin D3 [Peterson et al., 2005], pRb [Thomas et al., 2004], and E1A [Cha et al., 2008]). The interaction between Runx proteins and the cell cycle control machinery is bi-directional, as Runx protein abundance is regulated in a cell cycle dependent manner via cyclin-cdk-mediated phosphorylation [Biggs et al., 2006; Shen et al., 2006]. Finally, in addition to regulating progression through and exit from the cell cycle, Runx proteins control cell growth by regulating expression of genes required for protein synthesis, including rRNA genes transcribed by RNA polymerase I [e.g., Young et al., 2007a].

Beyond its roles in regulating cell growth and proliferation, Runx contributes to cancer by regulating both apoptosis and metastasis. Not surprisingly, these regulatory functions can be both positive and negative, depending on context. For example, both *Runx1* and *Runx3* promote apoptosis in different mammalian lineages, which contributes to their functionality as tumor suppressors [Fukamachi and Ito, 2004; Hug et al., 2004]; a similar pro-apoptotic role has been found for Drosophila *lozenge* [Wildonger and Mann, 2005]. On the other hand, in post-blastula stage sea urchin embryos Runt-1 is anti-apoptotic [Dickey-Sims et al., 2005], a function that has also been attributed to mammalian Runx2 in Myc-induced lymphomas [Blyth et al., 2006]. Finally, *Runx2* has been shown to promote metastasis of bone and breast cancers, in part by activating the expression of matrix metalloproteinases [Pratap et al., 2005], whereas *Runx3* expression inhibits metastasis of colon cancer cells [Peng et al., 2008]. Thus, the only broad generalization that can be made regarding Runx function in carcinogenesis is that Runx is causally associated with both the suppression and development of cancer.

Runx INVOLVEMENT IN INTERCELLULAR SIGNALING

In animal development, cell growth and proliferation are normally dependent upon the same intercellular signaling systems that control cell fate specification, a regulatory linkage that was undoubtedly a prerequisite for animal evolution. Moreover, this contingency manifests both as positive and negative effects on cell cycle progression that are dependent on both *cis*-regulatory/GRN architecture and developmental context, which as discussed in the foregoing is also something that can be said of Runx function. It is therefore perhaps not surprising that Runx has been found to be part of the circuitry that controls each of the major intercellular signaling pathways associated with animal development, each of which is known to be "short-circuited" in some forms of cancer.

Much of what is known concerning Runx and intercellular signaling has come from studies of vertebrate osteogenesis, which involves synergistic interactions between Runx2 and the BMP/TGFβ pathway [reviewed in Lian et al., 2006]. Runx2 facilitates TGFβ superfamily signaling both by acting as a cooperative co-factor with Smad transcription factors, with which they interact physically [Miyazono et al., 2004], and by activating the transcription of the TGFβ type I receptor [McCarthy et al., 2003]. Moreover, these systems are mutually linked, as BMP signaling induces expression of *Runx2* [Lian et al., 2006] and *Runx1* [Pimanda et al., 2007] during osteogenesis and hematopoiesis, respectively. Both branches of the TGFβ superfamily (i.e., BMP and TGFbeta/activin/nodal) utilize Runx as a cofactor, with context-specific effects. For example, the tumor suppressive functionality of Runx3 involves its requirement as an activator of Bim in TGFβ-induced apoptosis [Yano et al., 2006].

Runx proteins also play critical roles in Wnt signaling. *Runx2* expression is activated during osteogenesis in response to Wnt signaling [Lian et al., 2006], and Runx2 and canonical Wnt signaling collaborate to activate *fgf18* expression via physical interactions between Runx2 and TCF/Lef [Reinhold and Naski, 2007]. On the other hand, interactions between Runx2 and β-catenin/Lef1 inhibit Runx2-mediated activation of *osteocalcin* [Kahler and Westendorf, 2003], and Runx3 was recently shown to attenuate tumorogenic β-catenin signaling via direct physical interactions with both β-catenin and TCF4 [Ito et al., 2008]. In *C. elegans*, Rnt-1 collaborates with Wnt signaling to regulate asymmetric divisions in the T-blast stem-cell lineage [Kagoshima et al., 2005], while in sea urchin embryos, Runt-1 expression promotes blastula stage expression of several of the zygotically induced *wnt* genes, including *wnt-8*, a key component of the endomesoderm GRN [Robertson et al., 2008]. The latter involves intra-modular collaboration with Tcf/Lef-β-catenin and Blimp-1 transcription factors within a compact *cis*-regulatory module [Robertson et al., 2008]. Furthermore, GSK-3 activity, which is negatively regulated by canonical Wnt signaling, controls Runt-1 protein levels, suggesting that Wnt signaling may post-translationally regulate

Runt-1 expression [Robertson et al., 2008]. These findings, together with the well-known mitogenic effects of wnt signaling (which occurs in part via activation of cyclin D), led us to propose that mutual linkage of wnt signaling and Runx expression may constitute an ancient regulatory circuit for the control of cell proliferation in metazoan development (Fig. 1) [Robertson et al., 2008].

The Notch signaling pathway is often used for cell patterning via local interactions within pluripotent progenitor fields that are specified by Wnt signaling [Hayward et al., 2008]. In vertebrate hematopoiesis, Notch signaling activates *Runx1* expression [Burns et al., 2005], which in turn establishes competency for further specification of sublineages via Notch signaling [Rothenberg, 2007]. In sea urchin embryos, Notch signaling is required for specification of the non-skeletogenic mesoderm in response to expression of *delta*, which is activated via a putative Runx target site in a promoter-proximal *cis*-regulatory module. Thus, Notch signaling, like Wnt and TGFbeta signaling, appears to be mutually linked to Runx expression in some contexts.

Hedgehog signaling plays critical roles in the control of cell proliferation and differentiation, and is a frequent target for metastatic tumorogenesis. In mouse lower molar development, Runx2 regulates the expression of *sonic hedgehog* (*shh*) and its downstream effectors [Wang et al., 2005]. Likewise in chondrocytes and breast cancer cells, Runx2 activates expression of the *indian hedgehog* (*Ihh*) gene, and interacts physically with the Gli transcriptional effector of Hedgehog signaling, which promotes chondrocyte proliferation [Komori, 2005] as well as metastasis of breast cancer to bone [Pratap et al., 2008]. *Runx2* expression during chondrogenesis is in turn down-regulated in response to Ihh signaling via parathyroid hormone-like hormone (*Pthlh*) expression and PKA signaling [Komori, 2005].

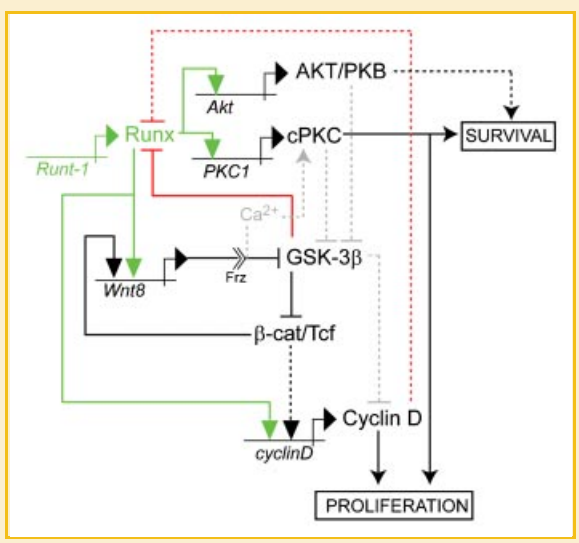

Fig. 1. A genetic/epigenetic circuit through which Runt-1 regulates cell proliferation and survival in the mid-to-late blastula stage sea urchin embryo. Genes are represented by standard symbols (horizontal lines terminating in bent arrows). Positive *cis*-regulatory inputs are shown as arrows terminating on genes; negative (inhibitory) protein–protein regulatory interactions are shown as bars terminating on the named proteins. Solid lines represent experimentally verified or well-established interactions/effects; dashed lines represent hypothetical interactions/effects gleaned from the literature. For supporting references see text, Dickey-Sims et al. [2005], and Robertson et al. [2008]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Nuclear receptors are transcription factors that bind and transduce signals provided by lipophilic ligands such as steroid hormones and metabolites. Runx proteins have been shown to interact physically and functionally with several members of the nuclear receptor superfamily, including the estrogen and vitamin D receptors [McCarthy et al., 2003; Sierra et al., 2003], and the orphan receptor RORgamma [Zhang et al., 2008a]. Moreover, *Runx2* expression is activated as part of the anabolic response to parathyroid hormone (PTH), which occurs via activation of CREB and in collaboration with FGF2 signaling [Sabbieti et al., 2009].

Finally, Runx proteins interact antagonistically with STAT transcription factors [Ogawa et al., 2008], and synergistically with downstream effectors of receptor tyrosine kinases (including FGFs, EGFs, and IGFs), in particular the Ets transcription factors. Interaction between mammalian Runx1 and Ets1, and similarly between *Drosophila* Lozenge and Pointed, are facilitated by the proximity of Runx and Ets target sequences within *cis*-regulatory modules, which promotes cooperative DNA binding via mutual alleviation of negative regulatory domains in each protein [Kim et al., 1999; Jackson Behan et al., 2005]. In mammalian cells Runx expression is required for Ras-induced cellular sencescence [Kilbey et al., 2008], and Runx protein activity is regulated by MAPK-mediated phosphorylation [Kwok et al., 2009]. Indeed, as discussed below Runx proteins appear to be substrates for diverse post-translational modifications that occur in response to a variety of extracellular signals and physiological states, and reciprocally, Runx factors regulate the expression of key kinases involved in intracellular signal transduction, including PKC [Hug et al., 2004; Dickey-Sims et al., 2005] and AKT [Fujita et al., 2004; A.J. Robertson and J.A. Coffman, unpublished data] (Fig. 1). Interestingly, the latter two kinases are both well known to play important roles in the control of cell death and survival.

Runx, NUCLEAR ARCHITECTURE, AND EPIGENETICS

Unlike many transcription factors, native Runx proteins (in cultured cells at least) are not part of the soluble nucleoplasm released with detergent extraction. Rather, they remain tightly associated with the detergent-insoluble nuclear "matrix," via a conserved sequence referred to as the "nuclear matrix targeting sequence" or NMTS [Harrington et al., 2002]. The NMTS localizes Runx to specific nuclear sub-domains, and this localization is required for signal transduction via Smads and nuclear receptors [Zaidi et al., 2002]. Moreover, the mammalian Runx proteins associate with nucleolar organizing regions and regulate ribosomal RNA synthesis [Ali et al., 2008]. Finally, mammalian Runx2 remains associated with specific promoters within chromosomes during mitosis, which has been suggested to provide an epigenetic mechanism that facilitates maintenance of transcriptional regulatory states in dividing cells [Young et al., 2007b].

Runx proteins participate in epigenetic regulation through their physical and functional interactions with chromatin modifying enzymes [Taniuchi and Littman, 2004]. For example, Runx recruits the Groucho/TLE corepressor (and through it histone deacetylases) to *cis*-regulatory systems via its linkage with a highly conserved C-terminal VWRPY sequence motif. Runx-mediated recruitment of Groucho is dependent on *cis*-regulatory sequence context, as the VWRPY motif (unlike the WRPW motif of the bHLH protein Hairy and its homologues) binds Groucho with a relatively weak affinity [Jennings et al., 2006]. Thus, Runx-mediated recruitment of Groucho is enabled by cooperative interactions with additional Groucho-interacting transcription factors that bind adjacent *cis*-regulatory sequences [Canon and Banerjee, 2003]. In other contexts Runx proteins recruit the histone deacetylases via their binding to the mSin3a co-repressor [Durst and Hiebert, 2004], and contribute to gene silencing by recruiting the histone methyltransferase SUV39H1 [Reed-Inderbitzin et al., 2006]. Conversely, Runx proteins contribute epigenetically to gene activation by recruiting histone acetylases such as p300, CBP, MOZ, and MORF [Yang, 2004].

Runx-mediated recruitment of chromatin modifying enzymes is regulated both genetically, by *cis*-regulatory context (as described above for Groucho), and epigenetically, by post-translational modification of the Runx proteins themselves. Runx proteins are subject to a variety of post-translational modifications, including phosphorylation, acetylation, and ubiquitination [Bae and Lee, 2006], which affect both Runx protein activity and stability and occur in response to intercellular signaling (e.g., via PKA, PKC, and MAPKs), as well as in response to cell cycle physiology (e.g., via cyclin D/cdk4 and cylcin B/cdk1 [Biggs et al., 2006; Shen et al., 2006]). Not surprisingly, such regulation is circuitous; as discussed above Runx proteins regulate the expression of numerous signal-responsive regulatory enzymes, including some that control the epigenetic state of chromatin (A.J. Robertson and J.A. Coffman, unpublished results).

Finally (and perhaps most importantly), the Runx participates in the regulation of cell and tissue architecture, by way of target genes that encode ECM components and remodeling enzymes (e.g., collagen and collagenases, respectively), cell adhesion molecules (e.g., cadherins, N-CAM, and integrins), and cytoskeletal components (e.g., actin and tubulin). Again, such regulation is circuitous; for example it has been shown that mammalian *Runx2* expression is activated in response to mechanical stress [Ziros et al., 2008] and three-dimensional tissue architecture [Stiehler et al., 2009].

Runx AND THE DEVELOPMENTAL PHYSIOLOGY OF GENE REGULATION

From the foregoing review we might pose the following generalizations about the role of Runx in animal development: (1) Runx proteins are critical for normal developmental specification of cell fate; (2) Runx proteins play critical roles in coupling cell cycle regulation to differentiation; (3) Runx proteins regulate developmental signaling via each of the major intercellular signal transduction pathways; and (4) Runx proteins are required for epigenetic regulation of gene expression via their association with the nuclear matrix and chromatin modification as well as through the role they play in the structural remodeling of cells and tissues. Of these four generalizations, only the first is likely to be provocative, as it tends to undermine the common reductionistic notion that Runx factors control fate as tissue-specific "master control genes"; a common perception is that Runx drives the development of some tissues at the expense of others. This notion obtains largely from cell culture studies, in which cell fate and differentiation can be driven down specific trajectories by overexpression of Runx factors (most notably, Runx2 in osteoblastogenesis), and from the fact that loss of function mutants or knockout of each Runx gene in fruit flies and mice produces local (region or tissue-specific) rather than global developmental defects. In contrast, knockdown of the single Runx gene expressed in sea urchin embryos produces global developmental defects, perturbing the expression of genes that mark each of the embryonic territories [Coffman et al., 2004].

How might these different views of Runx function (i.e., general vs. lineage-specific requirement) be reconciled? A potentially fruitful approach to answering this is to ask what functional niche Runx fulfills in the developmental physiology of gene regulation. Studies of early sea urchin embryogenesis have provided some intriguing clues that are relevant to answering this question. Although there are two Runx genes encoded in the genome of the purple sea urchin *Strongylocentrotus purpuratus*, only one of these (*Runt-1*) is expressed during embryogenesis. Runt-1 mRNA is absent from the egg, and begins to accumulate at late cleavage stage, reaching maximum per embryo steady state levels by late blastula stage [Coffman et al., 1996; Robertson et al., 2002] (Fig. 2). Morpholino antisense-mediated knockdown of Runt-1 produces global blastula stage block in cell proliferation (Fig. 2), which occurs just as cells in two of the embryonic territories (aboral ectoderm, skeletogenic

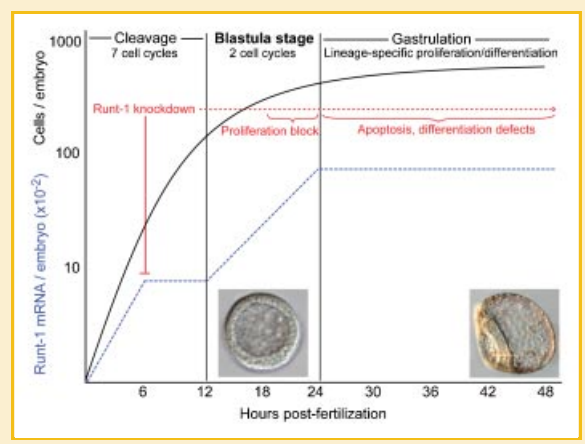

Fig. 2. The canonical temporal pattern of cell proliferation during sea urchin embryogenesis (black curve), related to the temporal pattern of *Runt-1* transcript accumulation (blue dashed line) and effects of Runt-1 knockdown (shown in red). Runt-1 transcripts accumulate 10-fold (per embryo) between 12 and 24 h post-fertilization (hpf), corresponding to blastula stage [Coffman et al., 1996]. During the second half of this period (18–24 hpf) Runt-1 morphants display a block in cell proliferation [Robertson et al., 2008], and subsequently extensive apoptosis and differentiation defects [Coffman et al., 2004; Dickey-Sims et al., 2005]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

mesoderm) are entering their penultimate rounds of division prior to terminal differentiation. The proliferation block is associated with deficits in the expression of several *wnt* genes [Robertson et al., 2008], cyclin D, conventional protein kinase C, and Akt/protein kinase B (see Fig. 1), and is followed by extensive apoptosis and differentiation defects (Fig. 2). The fact that Runt-1 appears to control multiple components of key signaling systems suggests that in its absence, developing progenitor cells do not receive (or cannot respond to) the lineage-specific signals that they need to proliferate, grow and survive; as a consequence, they "default" to apoptosis [Raff, 1992].

Interestingly, over-expression of Runt-1 has no affect at all on the early embryo (J.A. Coffman, unpublished observations), although it does rescue development in Runt-1 morphants [Coffman et al., 2004]. Thus, Runt-1 does not appear to be rate-limiting for early embryogenesis. This is in sharp contrast to the dominant effect of Runx2 overexpression in cultured mammalian cells, which drives osteogenic fate. There are two possible explanations for this difference. On the one hand it is possible is that Runt-1 is only required for relatively late development (blastula stage and beyond, which would not be susceptible to injected mRNA), and not for early (cleavage stage) embryogenesis. On the other hand, it is possible that Runt-1 is actually required globally for early embryogenesis (as suggested by the presence of maternal Runt-1 protein; J.A. Coffman, unpublished data), but is not rate-limiting and is in fact present in excess as maternal protein.

The configuration of chromatin, and the ratio of nucleus to cytoplasm, changes dramatically as development proceeds. During early development (cleavage stage embryos) chromatin is relatively open (which is also true in stem cells), and the nucleus to cytoplasm ratio is relatively low. Later in development (blastula stage and beyond) chromatin is more closed as cells begin to exit the cell cycle and differentiate, and the nucleus to cytoplasm ratio is relatively high. These changing conditions are likely to have profound effects on the physiology of gene regulation. During early development, the relative openness of chromatin will decrease the probability that any given transcription factor will bind to functionally relevant target sites, given the vast number of functionally irrelevant target sites that are available. Clustering of *cis*-regulatory target sites to facilitate cooperative interactions is probably an important mechanism for increasing this probability. This problem would be much less acute in later development, when large parts of the genome are subject to epigenetic silencing in a lineage-specific manner. Under such circumstances a different problem arises: genes that must be responsive to signals (but not constitutively active) need to remain in a relatively open configuration over multiple cell generations, which may require that they be epigenetically "marked."

As reviewed above, mammalian Runx proteins are known to function both cooperatively with a wide variety of other transcription factors, and as epigenetic marks that provide some memory of regulatory state over multiple cell generations within developmentally specified cell lineages [Young et al., 2007b]. Moreover, there is as yet little evidence that Runx proteins are required for very early embryogenesis. Indeed, the sea urchin gene Runt-1 is conspicuously absent from the GRN that controls endomesoderm specification, although it is globally expressed in the early embryo. Nevertheless, it remains possible that Runt-1 is globally necessary for normal GRN function, that is, that it functions in the background in a global support capacity by cooperatively facilitating interactions between low-specificity factors and their target sites. This cooperative "targeting" function may be further enhanced via interactions between Runt-1 and the nuclear matrix. This has been difficult to address owing to the fact that Runt-1 is present as maternal protein in the early embryo. However, studies of the sea urchin *wnt8 cis*-regulatory module C provides some intriguing clues that are relevant to this problem. A Runx target site in module C is necessary for module C enhancer activity at blastula stage, as base substitutions that abolish Runx binding also abolish module C-mediated activation of a GFP reporter [Robertson et al., 2008]. On the other hand, the reporter gene construct containing these same base substitutions is actually overexpressed in late cleavage stage embryos [Robertson et al., 2008]. One possible explanation for this is that module C might confer transcriptional repression throughout most of the embryo in early development, on account of its interaction with TCF and consequent recruitment of Groucho. In this case Runx binding might cooperate with TCF, both to facilitate binding of the latter, and to recruit Groucho via the C-terminal VWRPY sequence of Runt-1, thereby repressing global low-level "leaky" expression. Additional work is required to test this hypothesis, and to investigate the function (if any) of maternal Runt-1 in early development and operation of the developmental GRN.

Runx AND CBFβ: ARE THEY FUNCTIONALLY SYNONYMOUS?

In every animal model studied to date, Runx transcription factors heterodimerize with a beta subunit (CBFβ, a non-homologous protein that doesn't itself bind DNA, but which allosterically enhances the DNA binding affinity of Runx [Yan et al., 2004]. Because Runx proteins purified from nuclear extracts are invariably bound to CBFβ, and because knockout of *CBFβ* in mouse produces a phenotype that is very similar to that of the *Runx1* knockout (i.e., failure of definitive hematopoiesis), it is commonly assumed that CBFβ is constitutively required for Runx function. However, this assumption is undermined by recent functional studies of the sea urchin CBFβ homologue. Unlike Runt-1 morphants, CBFβ morphants do not display extensive apoptosis or loss of *PKC1* expression; furthermore, unlike other Runx targets, *PKC1* promoter sequences are not recovered in ChIP using anti-CBFβ antibodies, although they are recovered by ChIP using anti-Runt-1 antibodies [Robertson et al., 2006]. Finally, cell survival can be rescued in Runt-1 morphants by injection of mRNA encoding a Runt-1 point mutant that cannot dimerize with CBFβ, indicating that CBFβ is not required for the anti-apoptotic function of Runt-1 [Robertson et al., 2006]. That a similar situation may obtain in mammals is suggested by a recent study indicating that *Runx1* retains some functionality in CBFbeta-deficient mice [Yokomizo et al., 2008].

The possibility that CBFβ is not constitutively required for Runx function undermines the assumption that knockout of the single

CBFβ gene in mouse is equivalent to complete loss of Runx function. It raises the possibility that CBFβ functions as a regulatory subunit that comes into play in specific developmental contexts. Consistent with this possibility, the pattern of CBFβ expression overlaps with but is not identical to that of Runt-1; for example, unlike Runt-1, CBFβ is not present as maternal protein in sea urchin eggs or early zygotes [Robertson et al., 2006]. Thus, if maternal Runt-1 protein does function in early development, it does so without CBFβ. It is possible that a similar situation obtains in mammals. The extent to which the different Runx genes compensate for one another during development of single knockout mice remains largely unknown, although there is one study that addresses this problem in the context of tooth development in a *Runx2*/*Runx3* double knockout mouse [Wang et al., 2005]. A triple Runx knockout, which has yet to be produced, is the only way to investigate the possibility that Runx is required for early embryogenesis, which would also test the hypothesis that Runx transcription factors play a CBFβ-independent role in the early embryo.

Under what circumstances might Runx function independently of CBFβ? One possibility is CBFβ-mediated stabilization of Runx binding is not conducive to dynamically responsive or low-level gene activity that may be required in some contexts, for example in actively cycling cells. As noted above, mammalian Runx proteins are cell-cycle regulated, and it is possible that this regulation is modulated by CBFβ, which has been shown to protect Runx1 from ubiquitination and consequent proteolysis [Huang et al., 2001]. Clearly more work is needed to ascertain the extent to which Runx proteins function in the absence of CBFβ in different model systems, and the role of CBFβ in modulating Runx function in cell cycle control and development.

CONCLUSIONS AND FUTURE PROSPECTS

The central hypothesis posed here is that Runx transcription factors are generally required for pre-differentiation developmental signaling in animals, functioning to enhance target selectivity and activity of signal-responsive transcription factors in early development, and to epigenetically mark those genes that must remain responsive to developmental signaling through later stages of development. Like most transcription factors, Runx proteins undoubtedly play diverse roles; for example, it is clear that they participate in the activation of genes associated with cell differentiation, as in the case of the sea urchin *CyIIIa* actin gene. Moreover, it is likely that multiple mechanisms (and transcription factor families) have evolved to fulfill similar functional niches, as many developmental signaling genes appear not to utilize Runx in their regulation. Nevertheless, the fact that Runx genes have undergone limited and independent diversification (compared to other transcription factor families) during animal phylogenesis, and the fact that they originated in concert with and are mutually linked (upstream, downstream, and in parallel) with each of the major metazoan signaling systems, strongly suggests that they occupy an essential niche in the physiology of developmental signaling in animals.

Gene regulatory networks provide the inherited program of ontogeny [Davidson, 2006]. In the GRNs to which they have been functionally linked, Runx genes operate in specified embryonic domains or tissue progenitor fields. In most of these cases Runx function is fulfilled by one of multiple paralogues. Sea urchin embryos, which express a single Runx gene, provide a relatively simple and synchronous biochemical, molecular and cellular model of development with a well-characterized GRN [e.g., Davidson, 2006; Oliveri et al., 2008; Smith and Davidson, 2008; Sethi et al., 2009]. The virtual absence of Runx from the current model of the GRN that controls early sea urchin embryogenesis may indicate that Runx is specifically required for later stages of development (i.e., in specified progenitor cells prior to their terminal differentiation); alternatively it might simply reflect that Runx plays a globally supportive role in earlier development that is not rate limiting, and hence not readily apparent in the absence of appropriate perturbations. Distinguishing between these possibilities, and elucidating the functional niche filled by Runx in the developmental physiology of animals, remains an important task for future research.

ACKNOWLEDGMENTS

I apologize to colleagues in this field who have made seminal contributions that I did not cite due to space limitations. The work in my laboratory reviewed here is supported by a grant from the NIH (GM070840).

REFERENCES

Ali SA, Zaidi SK, Dacwag CS, Salma N, Young DW, Shakoori AR, Montecino MA, Lian JB, van Wijnen AJ, Imbalzano AN, Stein GS, Stein JL. 2008. Phenotypic transcription factors epigenetically mediate cell growth control. Proc Natl Acad Sci USA 105:6632–6637.

Anderson MK. 2006. At the crossroads: Diverse roles of early thymocyte transcriptional regulators. Immunol Rev 209:191–211.

Bae SC, Lee YH. 2006. Phosphorylation, acetylation and ubiquitination: The molecular basis of RUNX regulation. Gene 366:58–66.

Bao R, Friedrich M. 2008. Conserved cluster organization of insect Runx genes. Dev Genes Evol 218:567–574.

Bernardin-Fried F, Kummalue T, Leijen S, Collector MI, Ravid K, Friedman AD. 2004. AML1/RUNX1 increases during G1 to S cell cycle progression independent of cytokine-dependent phosphorylation and induces cyclin D3 gene expression. J Biol Chem 279:15678–15687.

Biggs JR, Peterson LF, Zhang Y, Kraft AS, Zhang DE. 2006. AML1/RUNX1 phosphorylation by cyclin-dependent kinases regulates the degradation of AML1/RUNX1 by the anaphase-promoting complex. Mol Cell Biol 26:7420–7429.

Blyth K, Vaillant F, Hanlon L, Mackay N, Bell M, Jenkins A, Neil JC, Cameron ER. 2006. Runx2 and MYC collaborate in lymphoma development by suppressing apoptotic and growth arrest pathways in vivo. Cancer Res 66: 2195–2201.

Burns CE, Traver D, Mayhall E, Shepard JL, Zon LI. 2005. Hematopoietic stem cell fate is established by the Notch-Runx pathway. Genes Dev 19:2331–2342.

Cameron ER, Neil JC. 2004. The Runx genes: Lineage-specific oncogenes and tumor suppressors. Oncogene 23:4308–4314.

Canon J, Banerjee U. 2003. In vivo analysis of a developmental circuit for direct transcriptional activation and repression in the same cell by a Runx protein. Genes Dev 17:838–843.

Cha EJ, Oh BC, Wee HJ, Chi XZ, Goh YM, Lee KS, Ito Y, Bae SC. 2008. E1A physically interacts with RUNX3 and inhibits its transactivation activity. J Cell Biochem 105:236–244.

Coffman JA. 2003. Runx transcription factors and the developmental balance between cell proliferation and differentiation. Cell Biol Int 27:315–324.

Coffman JA, Kirchhamer CV, Harrington MG, Davidson EH. 1996. SpRunt-1, a new member of the runt domain family of transcription factors, is a positive regulator of the aboral ectoderm-specific CyIIIA gene in sea urchin embryos. Dev Biol 174:43–54.

Coffman JA, Dickey-Sims C, Haug JS, McCarthy JJ, Robertson AJ. 2004. Evaluation of developmental phenotypes produced by morpholino antisense targeting of a sea urchin Runx gene. BMC Biol 2:6.

Daga A, Karlovich CA, Dumstrei K, Banerjee U. 1996. Patterning of cells in the Drosophila eye by Lozenge, which shares homologous domains with AML1. Genes Dev 10:1194–1205.

Davidson EH. 2006. The regulatory genome: Gene regulatory networks in development and evolution. San Diego: Academic Press/Elsevier.

Dickey-Sims C, Robertson AJ, Rupp DE, McCarthy JJ, Coffman JA. 2005. Runx-dependent expression of PKC is critical for cell survival in the sea urchin embryo. BMC Biol 3:18.

Duncan EJ, Wilson MJ, Smith JM, Dearden PK. 2008. Evolutionary origin and genomic organisation of runt-domain containing genes in arthropods. BMC Genomics 9:558.

Durst KL, Hiebert SW. 2004. Role of RUNX family members in transcriptional repression and gene silencing. Oncogene 23:4220–4224.

Fujita T, Azuma Y, Fukuyama R, Hattori Y, Yoshida C, Koida M, Ogita K, Komori T. 2004. Runx2 induces osteoblast and chondrocyte differentiation and enhances their migration by coupling with PI3K-Akt signaling. J Cell Biol 166:85–95.

Fukamachi H, Ito K. 2004. Growth regulation of gastric epithelial cells by Runx3. Oncogene 23:4330–4335.

Gergen JP, Butler BA. 1988. Isolation of the Drosophila segmentation gene runt and analysis of its expression during embryogenesis. Genes Dev 2:1179–1193.

Harrington KS, Javed A, Drissi H, McNeil S, Lian JB, Stein JL, Van Wijnen AJ, Wang YL, Stein GS. 2002. Transcription factors RUNX1/AML1 and RUNX2/Cbfa1 dynamically associate with stationary subnuclear domains. J Cell Sci 115:4167–4176.

Hayward P, Kalmar T, Arias AM. 2008. Wnt/Notch signalling and information processing during development. Development 135:411–424.

Huang G, Shigesada K, Ito K, Wee HJ, Yokomizo T, Ito Y. 2001. Dimerization with PEBP2beta protects RUNX1/AML1 from ubiquitin-proteasome-mediated degradation. EMBO J 20:723–733.

Hug BA, Ahmed N, Robbins JA, Lazar MA. 2004. A chromatin immunoprecipitation screen reveals protein kinase Cbeta as a direct RUNX1 target gene. J Biol Chem 279:825–830.

Ito Y. 2004. Oncogenic potential of the RUNX gene family: 'overview'. Oncogene 23:4198–4208.

Ito Y. 2008. RUNX genes in development and cancer: Regulation of viral gene expression and the discovery of RUNX family genes. Adv Cancer Res 99:33–76.

Ito K, Lim AC, Salto-Tellez M, Motoda L, Osato M, Chuang LS, Lee CW, Voon DC, Koo JK, Wang H, Fukamachi H, Ito Y. 2008. RUNX3 attenuates beta-catenin/T cell factors in intestinal tumorigenesis. Cancer Cell 14:226–237.

Jackson Behan K, Fair J, Singh S, Bogwitz M, Perry T, Grubor V, Cunningham F, Nichols CD, Cheung TL, Batterham P, Pollock JA. 2005. Alternative splicing removes an Ets interaction domain from Lozenge during Drosophila eye development. Dev Genes Evol 215:423–435.

Jennings BH, Pickles LM, Wainwright SM, Roe SM, Pearl LH, Ish-Horowicz D. 2006. Molecular recognition of transcriptional repressor motifs by the WD domain of the Groucho/TLE corepressor. Mol Cell 22:645–655.

Ji YJ, Nam S, Jin YH, Cha EJ, Lee KS, Choi KY, Song HO, Lee J, Bae SC, Ahnn J. 2004. RNT-1, the C. elegans homologue of mammalian RUNX transcription factors, regulates body size and male tail development. Dev Biol 274:402–412.

Kagoshima H, Shigesada K, Satake M, Ito Y, Miyoshi H, Ohki M, Pepling M, Gergen P. 1993. The Runt domain identifies a new family of heteromeric transcriptional regulators. Trends Genet 9:338–341.

Kagoshima H, Sawa H, Mitani S, Burglin TR, Shigesada K, Kohara Y. 2005. The C. elegans RUNX transcription factor RNT-1/MAB-2 is required for asymmetrical cell division of the T blast cell. Dev Biol 287:262–273.

Kahler RA, Westendorf JJ. 2003. Lymphoid enhancer factor-1 and beta-catenin inhibit Runx2-dependent transcriptional activation of the osteocalcin promoter. J Biol Chem 278:11937–11944.

Kilbey A, Terry A, Cameron ER, Neil JC. 2008. Oncogene-induced senescence: An essential role for Runx. Cell Cycle 7:2333–2340.

Kim WY, Sieweke M, Ogawa E, Wee HJ, Englmeier U, Graf T, Ito Y. 1999. Mutual activation of Ets-1 and AML1 DNA binding by direct interaction of their autoinhibitory domains. EMBO J 18:1609–1620.

Komori T. 2005. Regulation of skeletal development by the Runx family of transcription factors. J Cell Biochem 95:445–453.

Kwok S, Partridge NC, Srinivasan N, Nair SV, Selvamurugan N. 2009. Mitogen activated protein kinase-dependent inhibition of osteocalcin gene expression by transforming growth factor-beta1. J Cell Biochem 106:161–169.

Lian JB, Stein GS, Javed A, van Wijnen AJ, Stein JL, Montecino M, Hassan MQ, Gaur T, Lengner CJ, Young DW. 2006. Networks and hubs for the transcriptional control of osteoblastogenesis. Rev Endocr Metab Disord 7:1–16.

McCarthy TL, Chang WZ, Liu Y, Centrella M. 2003. Runx2 integrates estrogen activity in osteoblasts. J Biol Chem 278:43121–43129.

Meyers S, Downing JR, Hiebert SW. 1993. Identification of AML-1 and the (8;21) translocation protein (AML- 1/ETO) as sequence-specific DNA-binding proteins: The runt homology domain is required for DNA binding and protein-protein interactions. Mol Cell Biol 13:6336–6345.

Miyazono K, Maeda S, Imamura T. 2004. Coordinate regulation of cell growth and differentiation by TGF-beta superfamily and Runx proteins. Oncogene 23:4232–4237.

Nimmo R, Woollard A. 2008. Worming out the biology of Runx. Dev Biol 313:492–500.

Nimmo R, Antebi A, Woollard A. 2005. mab-2 encodes RNT-1, a C. elegans Runx homologue essential for controlling cell proliferation in a stem cell-like developmental lineage. Development 132:5043–5054.

Nusslein-Volhard C, Wieschaus E. 1980. Mutations affecting segment number and polarity in Drosophila. Nature 287:795–801.

Ogawa S, Satake M, Ikuta K. 2008. Physical and functional interactions between STAT5 and Runx transcription factors. J Biochem 143:695–709.

Oliveri P, Tu Q, Davidson EH. 2008. Global regulatory logic for specification of an embryonic cell lineage. Proc Natl Acad Sci USA 105:5955–5962.

Peng Z, Tang H, Wang X, Zhou C, Fan J, Wang L, Jia Z, Li Q, Le X, Wei D, Xie K. 2008. Inhibition of the growth and metastasis of human colon cancer by restoration of RUNX3 expression in cancer cells. Int J Oncol 33:979–984.

Peterson LF, Boyapati A, Ranganathan V, Iwama A, Tenen DG, Tsai S, Zhang DE. 2005. The hematopoietic transcription factor AML1 (RUNX1) is negatively regulated by the cell cycle protein cyclin D3. Mol Cell Biol 25:10205–10219.

Pimanda JE, Donaldson IJ, de Bruijn MF, Kinston S, Knezevic K, Huckle L, Piltz S, Landry JR, Green AR, Tannahill D, Gottgens B. 2007. The SCL

transcriptional network and BMP signaling pathway interact to regulate RUNX1 activity. Proc Natl Acad Sci USA 104:840–845.

Pires-daSilva A, Sommer RJ. 2003. The evolution of signalling pathways in animal development. Nat Rev Genet 4:39–49.

Pratap J, Javed A, Languino LR, van Wijnen AJ, Stein JL, Stein GS, Lian JB. 2005. The Runx2 osteogenic transcription factor regulates matrix metalloproteinase 9 in bone metastatic cancer cells and controls cell invasion. Mol Cell Biol 25:8581–8591.

Pratap J, Wixted JJ, Gaur T, Zaidi SK, Dobson J, Gokul KD, Hussain S, van Wijnen AJ, Stein JL, Stein GS, Lian JB. 2008. Runx2 transcriptional activation of Indian Hedgehog and a downstream bone metastatic pathway in breast cancer cells. Cancer Res 68:7795–7802.

Raff MC. 1992. Social controls on cell survival and cell death. Nature 356:397–400.

Reed-Inderbitzin E, Moreno-Miralles I, Vanden-Eynden SK, Xie J, Lutterbach B, Durst-Goodwin KL, Luce KS, Irvin BJ, Cleary ML, Brandt SJ, Hiebert SW. 2006. RUNX1 associates with histone deacetylases and SUV39H1 to repress transcription. Oncogene 25:5777–5786.

Reinhold MI, Naski MC. 2007. Direct interactions of Runx2 and canonical Wnt signaling induce FGF18. J Biol Chem 282:3653–3663.

Rennert J, Coffman JA, Mushegian AR, Robertson AJ. 2003. The evolution of Runx genes. I. A comparative study of sequences from phylogenetically diverse model organisms. BMC Evol Biol 3:4.

Robertson AJ, Dickey CE, McCarthy JJ, Coffman JA. 2002. The expression of SpRunt during sea urchin embryogenesis. Mech Dev 117:327–330.

Robertson AJ, Dickey-Sims C, Ransick A, Rupp DE, McCarthy JJ, Coffman JA. 2006. CBFbeta is a facultative Runx partner in the sea urchin embryo. BMC Biol 4:4.

Robertson AJ, Coluccio A, Knowlton P, Dickey-Sims C, Coffman JA. 2008. Runx expression is mitogenic and mutually linked to Wnt activity in blastula-stage sea urchin embryos. PLoS ONE 3:e3770.

Rothenberg EV. 2007. Regulatory factors for initial T lymphocyte lineage specification. Curr Opin Hematol 14:322–329.

Sabbieti MG, Agas D, Xiao L, Marchetti L, Coffin JD, Doetschman T, Hurley MM. 2009. Endogenous FGF-2 is critically important in PTH anabolic effects on bone. J Cell Physiol 219:143–151.

Sethi AJ, Angerer RC, Angerer LM. 2009. Gene regulatory network interactions in sea urchin endomesoderm induction. PLoS Biol 7:e29.

Shen R, Wang X, Drissi H, Liu F, O'Keefe RJ, Chen D. 2006. Cyclin D1-cdk4 induce runx2 ubiquitination and degradation. J Biol Chem 281:16347–16353.

Sierra J, Villagra A, Paredes R, Cruzat F, Gutierrez S, Javed A, Arriagada G, Olate J, Imschenetzky M, Van Wijnen AJ, Lian JB, Stein GS, Stein JL, Montecino M. 2003. Regulation of the bone-specific osteocalcin gene by p300 requires Runx2/Cbfa1 and the vitamin D3 receptor but not p300 intrinsic histone acetyltransferase activity. Mol Cell Biol 23:3339–3351.

Smith J, Davidson EH. 2008. From the cover: Gene networks in development and evolution special feature Sackler colloquium: Gene regulatory network subcircuit controlling a dynamic spatial pattern of signaling in the sea urchin embryo. Proc Natl Acad Sci USA 105:20089–20094.

Stein GS, Lian JB, van Wijnen AJ, Stein JL, Montecino M, Javed A, Zaidi SK, Young DW, Choi JY, Pockwinse SM. 2004. Runx2 control of organization, assembly and activity of the regulatory machinery for skeletal gene expression. Oncogene 23:4315–4329.

Stiehler M, Bunger C, Baatrup A, Lind M, Kassem M, Mygind T. 2009. Effect of dynamic 3-D culture on proliferation, distribution, and osteogenic differentiation of human mesenchymal stem cells. J Biomed Mater Res A 89:96–107.

Sullivan JC, Sher D, Eisenstein M, Shigesada K, Reitzel AM, Marlow H, Levanon D, Groner Y, Finnerty JR, Gat U. 2008. The evolutionary origin of the Runx/CBFbeta transcription factors—Studies of the most basal metazoans. BMC Evol Biol 8:228.

Taniuchi I, Littman DR. 2004. Epigenetic gene silencing by Runx proteins. Oncogene 23:4341–4345.

Thomas DM, Johnson SA, Sims NA, Trivett MK, Slavin JL, Rubin BP, Waring P, McArthur GA, Walkley CR, Holloway AJ, Diyagama D, Grim JE, Clurman BE, Bowtell DD, Lee JS, Gutierrez GM, Piscopo DM, Carty SA, Hinds PW. 2004. Terminal osteoblast differentiation, mediated by runx2 and p27KIP1, is disrupted in osteosarcoma. J Cell Biol 167:925–934.

Wang XP, Aberg T, James MJ, Levanon D, Groner Y, Thesleff I. 2005. Runx2 (Cbfa1) inhibits Shh signaling in the lower but not upper molars of mouse embryos and prevents the budding of putative successional teeth. J Dent Res 84:138–143.

Westendorf JJ, Hiebert SW. 1999. Mammalian runt-domain proteins and their roles in hematopoiesis, osteogenesis, and leukemia. J Cell Biochem 75(Suppl 32):51–58.

Wildonger J, Mann RS. 2005. The t(8;21) translocation converts AML1 into a constitutive transcriptional repressor. Development 132:2263–2272.

Yan J, Liu Y, Lukasik SM, Speck NA, Bushweller JH. 2004. CBFbeta allosterically regulates the Runx1 Runt domain via a dynamic conformational equilibrium. Nat Struct Mol Biol 11:901–906.

Yang XJ. 2004. The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. Nucleic Acids Res 32:959–976.

Yano T, Ito K, Fukamachi H, Chi XZ, Wee HJ, Inoue K, Ida H, Bouillet P, Strasser A, Bae SC, Ito Y. 2006. The RUNX3 tumor suppressor upregulates Bim in gastric epithelial cells undergoing transforming growth factor beta-induced apoptosis. Mol Cell Biol 26:4474–4488.

Yokomizo T, Yanagida M, Huang G, Osato M, Honda C, Ema M, Takahashi S, Yamamoto M, Ito Y. 2008. Genetic evidence of PEBP2beta-independent activation of Runx1 in the murine embryo. Int J Hematol 88:134–138.

Young DW, Hassan MQ, Pratap J, Galindo M, Zaidi SK, Lee SH, Yang X, Xie R, Javed A, Underwood JM, Furcinitti P, Imbalzano AN, Penman S, Nickerson JA, Montecino MA, Lian JB, Stein JL, van Wijnen AJ, Stein GS. 2007a. Mitotic occupancy and lineage-specific transcriptional control of rRNA genes by Runx2. Nature 445:442–446.

Young DW, Hassan MQ, Yang XQ, Galindo M, Javed A, Zaidi SK, Furcinitti P, Lapointe D, Montecino M, Lian JB, Stein JL, van Wijnen AJ, Stein GS. 2007b. Mitotic retention of gene expression patterns by the cell fate-determining transcription factor Runx2. Proc Natl Acad Sci USA 104:3189–3194.

Zaidi SK, Sullivan AJ, van Wijnen AJ, Stein JL, Stein GS, Lian JB. 2002. Integration of Runx and Smad regulatory signals at transcriptionally active subnuclear sites. Proc Natl Acad Sci USA 99:8048–8053.

Zaidi SK, Young DW, Choi JY, Pratap J, Javed A, Montecino M, Stein JL, van Wijnen AJ, Lian JB, Stein GS. 2005. The dynamic organization of gene-regulatory machinery in nuclear microenvironments. EMBO Rep 6:128–133.

Zhang F, Meng G, Strober W. 2008a. Interactions among the transcription factors Runx1, RORgammat and Foxp3 regulate the differentiation of interleukin 17-producing T cells. Nat Immunol 9:1297–1306.

Zhang L, Fried FB, Guo H, Friedman AD. 2008b. Cyclin-dependent kinase phosphorylation of RUNX1/AML1 on 3 sites increases transactivation potency and stimulates cell proliferation. Blood 111:1193–1200.

Ziros PG, Basdra EK, Papavassiliou AG. 2008. Runx2: Of bone and stretch. Int J Biochem Cell Biol 40:1659–1663.