PRIMER

RUNX transcription factors: orchestrators of development

Renaud Mevel1, Julia E. Draper1, Michael Lie-a-Ling1, Valerie Kouskoff2,* and Georges Lacaud1,*

ABSTRACT

RUNX transcription factors orchestrate many different aspects of biology, including basic cellular and developmental processes, stem cell biology and tumorigenesis. In this Primer, we introduce the molecular hallmarks of the three mammalian RUNX genes, RUNX1, RUNX2 and RUNX3, and discuss the regulation of their activities and their mechanisms of action. We then review their crucial roles in the specific and maintenance of a wide array of tissues during embryonic development and adult homeostasis.

KEY WORDS: Bone, Development, Hematopoiesis, RUNX, T cell, Embryonic

Introduction

The RUNX family of transcription factors orchestrate various developmental and cellular processes, such as cell proliferation, differentiation and cell lineage specification. The RUNX genes were named after the discovery of the developmental regulatory gene runt, which was found to be essential for early embryonic segmentation after being identified in a mutagenesis screen for the development of Drosophila melanogaster (Gergen and Butler, 1988; Nüsslein-Volhard and Wieschaus, 1980). RUNX genes have been described in the majority of sequenced metazoan genomes, with single copies of a RUNX gene present in most bilaterians, and at least three genes in insects and vertebrates (Rennert et al., 2003). The mammalian RUNX transcription factors consist of RUNX1, RUNX2 and RUNX3.

In this Primer, we introduce the hallmarks of the RUNX family of transcription factors and discuss their diverse means of regulation and mechanisms of action. We then review the main developmental roles of the RUNX factors, focusing principally on mammals, to highlight their importance from embryogenesis to adult homeostasis in a wide range of tissues.

The RUNX family of transcription factors

Conservation of RUNX loci and transcriptional regulation

The genomic architecture of the three mammalian RUNX genes is very similar (Fig. 1A), and highly conserved across metazoans. In vertebrates, all RUNX genes contain two alternative promoters: a distal P1 promoter and a proximal P2 promoter, which is thought to represent the primordial promoter (Levanon and Groner, 2004; Rennert et al., 2003). The main protein isoforms encoded by these transcripts differ in their N-terminal amino acid sequences; distal isoforms are usually longer and always begin with the MAS(D/N)S amino acid sequence, whereas proximal isoforms begin with the MRIPV motif (Bangsow et al., 2001; Miyoshi et al., 1995; Xiao et al., 1998). The 500 million years of conservation of this dual promoter structure for the three RUNX genes in vertebrates suggests specific – but currently unclear – functions for each promoter or for their respective transcripts (Levanon and Groner, 2004; Rennert et al., 2003). Indeed, differential activity of the two promoters has been reported across a wide range of developmental stages and tissues, suggesting divergent context-specific requirements (Bangsow et al., 2001; Bee et al., 2010; Chung et al., 2007; Draper et al., 2016; Harada et al., 1999; Liu et al., 2011; Rini and Calabi, 2001; Sroczynska et al., 2009).

Alternative splicing events add further diversity to RUNX transcripts originating from the P1 and P2 promoters (see Box 1). Interestingly, all RUNX genes express a panel of isoforms with increased or reduced transactivation activities (Bae et al., 1994; Bangsow et al., 2001; Geoffroy et al., 1998; Jin et al., 2004; Puig-Kröger et al., 2010; Telfer and Rothenberg, 2001; Terry et al., 2004). Also, sequence variations occurring in the 5′ and 3′ untranslated regions (UTRs) of the multiple RUNX transcripts affect the stability and translation efficiency of the different RUNX mRNAs. P1-derived transcripts, generally bearing a shorter 5′ UTR, have been shown to directly cap-mediated translation more efficiently than the P2-derived 5′ UTR (Pozner et al., 2000). In addition, microRNAs can regulate RUNX transcripts by targeting isoform-specific 3′ UTRs (Ben-Ami et al., 2009; Zhang et al., 2011). Currently, the specific physiological role of all these different isoforms remains largely unknown.

Structural homologies between the RUNX proteins

The defining component of the RUNX proteins is the presence of the highly conserved Runt homology domain (RHD), a 128-amino-acid sequence located near the N terminus (Fig. 1B) (Rennert et al., 2003). The RHD is essential for (1) binding to the DNA at the consensus RUNX motif ‘PyGPyGGPy’ (Kamachi et al., 1990; Wang and Speck, 1992), (2) protein-protein interactions (Lilly et al., 2003; Nagata et al., 1999) and (3) the nuclear localization of the RUNX factors (Michaud et al., 2002; Telfer et al., 2004). The C terminus is less conserved and contains an activation domain, an inhibitory domain and a five amino acid C-terminal motif (VWRPY in most cases), known as the recruitment signal for the Groucho/TLE (transducin-like enhancer of split) family of co-repressors (Levanon et al., 1998; Seo et al., 2012b; Yarmus et al., 2006). Within the transcriptional domain, RUNX proteins also bear a conserved nuclear matrix-targeting signal sequence, which plays a role in the regulation of RUNX activity and nuclear localization (Zaidi et al., 2001; Zeng et al., 1998).

Mechanism of action

RUNX transcription factors are part of a heterodimeric complex formed by dimerization between the α subunit, RUNX, and its main partner, the core binding factor subunit β (CBFβ) (Fig. 2A)
Box 1. Spotlight on Runx1 isoforms in adult hematopoiesis

RUNX isoforms display well-defined structural differences and distinct developmental expression patterns. Now, isoform-specific functionalities are beginning to emerge. In adult hematopoiesis, the P1-derived RUNX1c is dominant, whereas P2-derived RUNX1b is confined to progenitor subsets of the granulocyte/macrophage, lymphoid lineages and megakaryocytes (Bee et al., 2009; Draper et al., 2016; Teffer and Rothenberg, 2001). Downregulation of Runx1b is a prerequisite for terminal differentiation of these lineages, except megakaryocytes. In myeloid cells, Runx1b expression correlates with increased proliferation and colony-forming unit-culture activity in the bipotential pre-megakaryocyte/erythroid (PreMegE) progenitor (Draper et al., 2016). In P1-null mice, inactivation of Runx1c results in lineage-specific defects, reminiscent of total Runx1 deficiency (Bee et al., 2010; Draper et al., 2016, 2017). In these mice, PreMegE progenitors produce more erythroid, and fewer megakaryocyte, progenitors (Draper et al., 2017). Unlike in complete Runx1 knockout models (Growney et al., 2005; Ichikawa et al., 2004), Runx1c-null megakaryocytes differentiate and produce platelets, suggesting that Runx1c specifies megakaryocytes, whereas Runx1b drives the maturation of committed megakaryocytes. In a human B lymphoblastoid cell line infected with Epstein–Barr virus, Runx1c – but not Runx1b – inhibits growth (Brady et al., 2013). Finally, a shorter isoform designated RUNX1a, transcribed from the P2 promoter and lacking the transactivation domain, has been identified in humans (Komeno et al., 2014). RUNX1a is proposed to act as a dominant negative (Levanon et al., 2001), that enhances hematopoietic commitment (Ran et al., 2013) and increases HSPC renewal (Tszuzuki and Seto, 2012; Tsuzuzuki et al., 2007).

Post-translational modifications

RUNX factors are actively regulated at the post-translational level to enable fine-tuning of their transcriptional potency, stability and localization (Fig. 2B) through several signaling pathways. Here, we briefly describe the main post-translational modifications that regulate RUNX protein activity; more in-depth reviews describing interactors and target genes have been published previously (Chuang et al., 2013; Goyama et al., 2015; Ito et al., 2015). RUNX transcriptional activity is generally associated with its acetylation or interaction with chromatin modifiers such as p300 (EP300)/CBP (CREBBP) or MOZ (KAT6A) (Jin et al., 2004; Pelletier et al., 2002; Wang et al., 2011a). Furthermore, serine and threonine phosphorylation by diverse signaling cascades can enhance RUNX activity in a variety of cellular contexts (Aikawa et al., 2006; Guo and Friedman, 2011; Imai et al., 2004; Kim et al., 2008; Wee et al., 2008; Zhang et al., 2008a). Methylation of RUNX factors play ambivalent roles, as they can increase DNA binding (Zhao et al., 2008) or favor transcriptional repression depending on the cellular context (Herglotz et al., 2013; Yu et al., 2013). Repressive activity of the RUNX proteins are further controlled by tyrosine phosphorylation (Goh et al., 2010; Huang et al., 2012), association with histone deacetylases (HDACs) (Jeon et al., 2006; Lutterbach et al., 2000), and interaction with transcriptional regulators, such as SIN3A and Groucho/TLE family members (Imai et al., 1998, 2004; Levanon et al., 1998; Zhao et al., 2008a). As both acetylation of non-histone proteins and ubiquitylation occurs at lysine residues, HDACs can reduce the transcriptional activity of RUNX by promoting their ubiquitylation (Jin et al., 2004). Indeed, the stability of the RUNX factors is tightly regulated by ubiquitin-mediated degradation and depends on a combination of other post-translational modifications. Additionally, the CDC20 subunit of the anaphase-promoting complex (APC) can regulate the level of RUNX proteins during cell cycle progression (Biggs et al., 2006). Finally, the stability of RUNX has also been reported to be decreased by SUMOylations (Kim et al., 2014). Although these post-translational modifications have mostly been studied in isolation, it is evident that RUNX activators and repressors have mostly been studied in isolation, it is evident that RUNX
proteins are regulated by complex crosstalk between these modifications, which represent a major level of regulation of RUNX transcriptional activity. Importantly, deregulations of these subtle regulatory mechanisms have been shown to play important roles in the context of disease (Ito et al., 2015).

**Auto-regulation, cross-regulation and functional redundancy**

Analyses of the promoters of each of the RUNX members have revealed the presence of consensus RUNX binding sites, raising the possibility of auto-regulatory loops (Ghozi et al., 1996), which was confirmed for Runx2 in the context of bone formation (Drissi et al., 2000; Ducy et al., 1999). Similarly, Runx1 has since been reported to regulate its own expression both in vitro (Pimanda et al., 2007) and in vivo (Martinez et al., 2016). Cases of cross-talk between RUNX factors have also been described, whereby RUNX3 regulates Runx1 expression level by repressing its transcription in human B lymphoid cell lines (Brady et al., 2009; Spender et al., 2005).

The extent to which RUNX factors play redundant roles remains largely unknown. A few studies have demonstrated that the RUNX proteins can substitute each other in specific instances. For example, it has been shown using an in vitro co-culture system of the murine embryonic para-aortic splanchnopleural region that ectopic expression of Runx2 or Runx3 is able to rescue the hematopoietic defects caused by Runx1 deficiency (Goyama et al., 2004).

Similarly, replacement of RUNX1 C terminus by the equivalent portions of RUNX2 or RUNX3 rescues the Runx1-null embryonic lethal phenotype (Fukushima-Nakase, 2005). Likewise, premature senescence was induced in murine embryonic fibroblasts by individually overexpressing one of the three RUNX genes (Kilbey et al., 2007). Studies in Runx1/Runx3 double knockout (DKO) mice have exposed functional redundancy between these genes in the Fanconi anemia DNA-repair pathway, independent of their transcriptional role (Tay et al., 2018; Wang et al., 2014b). Finally, Morita and colleagues have shown compensatory mechanisms between the three RUNX factors in the context of leukemia (Morita et al., 2017). The studies described above, as well as the strong structural homologies and potential auto- and cross-regulations, suggest that RUNX proteins could compensate for each other. New technologies such as single cell RNA transcriptomics are likely to reveal further co-expression in defined cellular compartments, and the specific spatiotemporal expression patterns of the RUNX genes is thought to explain, at least partially, their non-redundant functions and requirements in several developmental processes (Levanon and Gröner, 2004; Levanon et al., 2001).

However, even in the context of co-expression, such as in teeth development, comparison of the phenotypes observed in Runx2/ Runx3 DKO mice with those of single Runx2 knockouts showed no obvious compensations between the two factors (Wang et al., 2005a). In other contexts, partial, but not complete, redundancy has been reported. Only a certain degree of redundancy has been observed between Runx2 and Runx3 during chondrocyte development using single and double knockout mouse models (Yoshida, 2004), and partial redundancy has also been reported during lacrimal gland development (Voronov et al., 2013).

Taken together, although these studies reveal possible compensation between the RUNX genes in defined contexts, they also highlight their crucial and non-redundant functions that are partly, but not exclusively, associated with their intricate spatiotemporal regulation.

**Developmental functions of the RUNX family**

The functions of the RUNX family members have been uncovered using knockout mice. Deletion of each of the three RUNX proteins has severe consequences on survival; Runx1 knockout is embryonically lethal, Runx2 mice display neonatal lethality, and Runx3 knockouts have mixed survival rates depending on the model and strain used. These models initially revealed that Runx1 is essential for definitive hematopoiesis (Okuda et al., 1996; Wang et al., 1996), Runx2 is crucial for skeletal development (Ducy et al., 1999; Komori et al., 1997; Otto et al., 1997), and Runx3 plays an important role in neurogenesis (Inoue et al., 2002; Levanon et al., 2002). Later, studies using stage- and tissue-specific
conditional knockouts started to uncover a myriad of additional roles for RUNX proteins in other tissues, which were previously concealed by the severity of the developmental defects in the complete knockout models (Table 1). In the next section, we give an overview of the most thoroughly examined roles and requirements of the three mammalian RUNX proteins. Although these conclusions have mainly been drawn from mouse experiments, we do indicate when other models, such as human, have been used.

### RUNX in the hematopoietic system

Of the three RUNX proteins, RUNX1 and RUNX3 are the major players in the hematopoietic system. RUNX1 is generally considered as a master regulator of hematopoiesis owing to its crucial role in the ontogeny of the whole hematopoietic system whereas RUNX3 has important functions in the lymphocyte and myeloid lineages. We summarize below the main roles of RUNX in the hematopoietic system; for in-depth information, readers are directed to excellent recent reviews about the role of RUNX factors.

#### Table 1. Summary of RUNX mouse models, and their associated phenotypes in development

| Gene  | Alteration | Strategy      | Tissue                      | Main phenotypes                                                                                                                                                    | References |
|-------|------------|---------------|----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Runx1 | gKO        | NA            | NA                         | Embryonic lethality (E12.5). Hemorrhages in central nervous system, lack of HSC emergence, defective differentiation of neurons from the cholinergic lineage.       | (Okuda et al., 1996; Theriault et al., 2004; Wang et al., 1996) |
| Runx2 | gKO        | NA            | NA                         | Neonatal lethality associated with respiratory failure. Absence of bone ossification, impaired osteoblast differentiation, delayed hair follicle development.         | (Glotzer et al., 2008; Komori et al., 1997; Otto et al., 1997) |
| Runx3 | gKO        | NA            | NA                         | Embryonic, neonatal and postnatal lethality. Gastric epithelial hyperplasia, inflammatory bowel disease, defects in immune cells differentiation, impaired T cell development, defects in motor functions, delayed skeletal ossification, alveolar hyperplasia in the lungs. | (Bauer et al., 2015; Brenner et al., 2004; Fainaru et al., 2005; Inoue et al., 2002; Levanon et al., 2002; Li et al., 2002; Taniuchi et al., 2002; Wolff et al., 2003) |
| Runx1 | cKO        | Vav1-Cre      | Hematopoietic system       | Reduced HSPC growth, ribosome biogenesis, metabolism, increased stress resistance.                                                                              | (Cai et al., 2015) |
| Runx1 | cKO        | Vav1-Cre      | Hematopoietic system       | Reduced apoptosis and proliferation, and minimal impact on the frequency of long-term repopulation of HSCs.                                                      | (Cai et al., 2011) |
| Runx1 | cKO        | Mx1-Cre       | Hematopoietic system       | Mild HSPC expansion and myeloid proliferation in aged mice.                                                                                            | (Growney et al., 2005) |
| Runx1 | cKO        | Mx1-Cre       | Hematopoietic system       | HSC expansion and subsequent exhaustion.                                                                                                                         | (Jacob et al., 2010; Motoda et al., 2007) |
| Runx1 | cKO        | Mx1-Cre       | Hematopoietic system       | Impaired megakaryocyte maturation and platelet production, increased hematopoietic progenitor cells, development of mild myeloproliferative phenotype, defective T and B lymphocyte development, inhibition of common lymphocyte progenitor (CLP) production. | (Growney et al., 2005; Ichikawa et al., 2004) |
| Runx1 | cKO        | Lck-Cre       | Immune system              | Differentiation block during T cell development, CD4 derepression and thymic hypocellularity.                                                                     | (Taniuchi et al., 2002) |
| Runx1 | cKO        | Cd4-Cre       | Immune system              | Impaired T cell development, reduction of CD4 single-positive thymocytes.                                                                                         | (Egawa et al., 2007) |
| Runx1 | cKO        | Foxp3-Cre     | Immune system              | Lymphoproliferation, autoimmune disease, hyperproduction of IgG, reduced Foxp3 expression.                                                                       | (Kitoh et al., 2009) |
| Runx1 | cKO        | Mb1-Cre       | Immune system              | Developmental block during early B lymphopoiesis, resulting in the lack of IgM+ B cells and reduced V<sub>H</sub> to DJ<sub>H</sub> recombination.                      | (Seo et al., 2012a) |
| Runx1 | cKO        | Wnt1-Cre      | Nervous system             | Defects in pain perception (nociception) resulting from impaired differentiation of TrkA<sup>+</sup> DRG neurons.                                                        | (Chen et al., 2006; Liu et al., 2008) |
| Runx1 | gKO+rescue | Runx1::Tg     | Nervous system              | Alteration of neuronal differentiation and axonal projections in CNS and PNS.                                                                                    | (Yoshikawa et al., 2007) |
| Runx1 | gKO+rescue | Runx1::Tg     | Nervous system              | Reduced hypoglossal axon projections to the intrinsic vertical and transverse tongue muscles.                                                                   | (Yoshikawa et al., 2015) |
| Runx1 | cKO        | Vglut3-Cre    | Nervous system             | Altered development of VGLUT3 (SLC17A8)-persistent neurons, perception of mechanical pain unaffected.                                                          | (Lou et al., 2013) |
| Runx1 | cOE        | Lsl1-Cre      | Nervous system             | Neonatal lethality, increase in the number of CGRP<sup>+</sup> nociceptive neurons, changes in pain responses.                                               | (Kramer et al., 2006) |
| Runx1a | iOE        | Sox10::TTA    | Nervous system              | Retarded fetal growth, pigment defects, megacolon, and dystrophic DRG.                                                                                         | (Kanaykina et al., 2010) |
| Runx1 | cKO        | Krt5-Cre      | Epidermis                  | Defects in hair shape and structure.                                                                                                                                | (Raveh et al., 2006) |
| Runx1 | cKO        | Krt14-Cre     | Hair follicles             | Impaired HFSC renewal, telogen block, delayed entry into the hair cycle.                                                                                         | (Ho et al., 2010; Lee et al., 2013; Osorio et al., 2011) |
| Runx1 | iOE        | Krt14::TTA    | Hair follicles             | Promotion of hair germ early progenitor fate in bulge cells, hair degeneration during anagen.                                                                  | (Lee et al., 2014) |

Continued
Embryonic hematopoiesis

The vertebrate hematopoietic system is established through three main successive waves of blood cell generation. The first two waves take place in the extra-embryonic yolk sac and produce sequentially primitive erythrocytes, then erythro-myeloid and lymphoid progenitors (Costa et al., 2012; Frame et al., 2016; Lux et al., 2011, 2012). The third wave takes place in the intra-embryonic aorta-gonad-mesonephros (AGM) region, and generates the hematopoietic stem cells (HSCs) that will sustain the hematopoietic system during adulthood (Medvinsky and Dzierzak, 1996) (Fig. 3A). Both stem and progenitor hematopoietic cells (HSPCs) arise from hemogenic and progenitor hematopoietic cells (HSPCs) arise from hemogenic

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endothelial cells through an endothelial-to-hematopoietic transition (EHT) (Fig. 3B) (Bertrand et al., 2010; Boisset et al., 2010; Eilken et al., 2009; Jaffredo et al., 1998; Kiss and Herbomel, 2010; Lam et al., 2010; Lancrin et al., 2009; Zovein et al., 2008). HSPCs then move to the fetal liver, where they further expand and mature before seeding the bone marrow that serves as the main hematopoietic organ during adulthood (Mikkola and Orkin, 2006).

Runx1 is expressed in all these sites of de novo blood cell emergence, as well as in all the hematopoietic cells, with the exception of mature erythrocytes (Lacaud et al., 2002; Lorsbach et al., 2004; North et al., 1999, 2002; Sroczynska et al., 2009; Stefanska et al., 2017; Swiers et al., 2013; Zeigler et al., 2006). Disruption of Runx1 results in the complete absence of all hematopoietic cells other than primitive erythroid cells (Lacaud et al., 2002; Okuda et al., 1996; Wang et al., 1996). RUNX1 is essential for both initiation and completion of EHT by epigenetic silencing of the endothelial program (Lancrin et al., 2012; Liakhovitskaia et al., 2014; Lie-A-Ling et al., 2014; Thambyrarah et al., 2016a; Tober et al., 2013), and redistribution of hematopoietic transcription factor binding (Lichtinger et al., 2012), to form a stable epigenetic state, at which point RUNX1 is dispensable (Chen et al., 2009; Lancrin et al., 2009; North et al., 1999) (Fig. 3B). Precise modulation of RUNX1 levels and activity is essential for the efficiency and correct timing of hematopoietic progenitor emergence (Cai et al., 2000; Eliades et al., 2016; Faedda et al., 2004; Lancrin et al., 2009; Lie-A-Ling et al., 2014, 2018). This is in part sustained by the sequential activation of Runx1 promoters: proximal P2 transcripts are detected during EHT, whereas distal P1 transcripts are only found once hematopoietic commitment is completed (Bee et al., 2009; Challen and Goodell, 2010; Fujita et al., 2001; Lancrin et al., 2009; Sroczynska et al., 2009; Zambidis et al., 2005). Accordingly, the activity of the P2 proximal promoter is crucial for blood cell emergence whereas abrogation of P1 distal transcripts results in more subtle defects (Lam et al., 2009; Mukai et al., 2012; Pozner et al., 2007; Sroczynska et al., 2009).

Adult hematopoiesis

Runx1 is broadly expressed in most adult blood cells (Lorsbach et al., 2004; North et al., 2004) (Fig. 4A). However, despite this broad expression, Runx1 appears to be partially dispensable in adult hematopoiesis, as indicated by the absence of lethality when deleted either from the onset of HSC development at embryonic day (E) 11.5 using the Vav1-Cre mouse line (Chen et al., 2009) or in established adult hematopoiesis with the pan hematopoietic inducible Mx1-Cre system (Cai et al., 2011; Growney et al., 2005; Ichikawa et al., 2004; Jacob et al., 2010; Putz et al., 2006). Runx1 deletion in adult mice was shown to result either in expansion (Growney et al., 2005; Ichikawa et al., 2004) or exhaustion (Jacob et al., 2010) of phenotypic HSCs. A more recent study suggests that deregulation of expression of HSC cell surface markers in the absence of RUNX1 might explain some of these conflicting results, and that loss of Runx1 does not substantially alter the frequency of functional long-term repopulation of HSCs based on bone marrow chimerism (Cai et al., 2011) (Fig. 4B). Runx1 deletion causes significant expansion of the entire bone marrow hematopoietic stem and progenitor cell compartment (Cai et al., 2011; Growney et al., 2005; Ichikawa et al., 2004; Jacob et al., 2010) and decreased HSC apoptosis and ribosome biogenesis, which have been proposed to contribute to preleukemic states (Cai et al., 2015) (Box 2, Fig. 4B).

Hematopoietic deletion of Runx1 in adult mice also results in decreased B and T cell numbers, and lower platelet counts (Growney et al., 2005; Ichikawa et al., 2004). Although RUNX1 is downregulated in mature erythrocytes and platelets, in vivo and in vitro studies have demonstrated a key role for RUNX1 in balancing specification of platelet-producing megakaryocytic lineage commitment through multiple interactions with, for example, AP-1, p300, GATA and ETS transcription factors (Elagib, 2003; Pencovich

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**Fig. 3. RUNX1 and embryonic hematopoiesis.** (A) Timeline of embryonic blood development. Runx1 is expressed at all the different sites of hematopoietic development during the different hematopoietic waves. EMPs, erythro-myeloid progenitors; HSCs, hematopoietic stem cells; LMPs, lymphoid-myeloid progenitors. (B) RUNX1 is crucial for the endothelial-to-hematopoietic transition. Runx1 is expressed in endothelial cells of the ventral wall of the mouse dorsal aorta and in mesenchymal cells situated below (pink cells). A rare subset of endothelial cells, the hemogenic endothelial cells (red cells), are committed to forming blood cells (1). Runx1 is required for these cells to undergo morphological changes and bud from the endothelial lining into the lumen of the dorsal aorta (2). These precursors further proliferate and differentiate into mature blood cells in intra-aortic clusters (3).
**Impact of hematopoietic different hematopoietic populations of the adult hematopoietic system.**

Progenitor; Pre-megE, pre-megakaryocyte/erythroid progenitor.

pre-erythroid colony-forming unit; Pre-GM, pre-granulocyte/monocyte

MkP, megakaryocyte progenitor; MPP, multipotent progenitor; Pre-CFUe, HSC, hematopoietic stem cell; LMPP, lymphoid-primed multipotent progenitor; MkP, megakaryocyte progenitor; MPP, multipotent progenitor; Pre-CFUe, pre-erythroid colony-forming unit; Pre-GM, pre-granulocyte/monocyte progenitor; Pre-MegE, pre-megakaryocyte/erythroid progenitor.

Despite its high expression in HSPCs, the requirements for RUNX3 in adult hematopoiesis have only been uncovered recently. Conditional hematopoietic Runx3 deletion results in mild HSPC expansion and myeloid proliferation in aged mice (Wang et al., 2013), which is reminiscent of the Runx1 conditional knockout model (Cai et al., 2011; Gronwney et al., 2005). Investigations into compensatory mechanisms utilized by RUNX1 and RUNX3 in adult hematopoiesis led to the development of a Runx1/Runx3 DKO model (Wang et al., 2014b). These mice die within 25 weeks of induction of the DKO phenotype, as a result of either bone marrow failure (BMF) or myeloproliferative disorders. Such seemingly contradictory phenotypes are reminiscent of Fanconi anemia, an inherited BMF syndrome caused by defective DNA repair, in which Runx1/3 play crucial roles (Tay et al., 2018; Wang et al., 2014b).

The reduction of all mature hematopoietic lineage compartments observed in DKO mice appears to be responsible for the BMF phenotype, which occurs in spite of the HSPC expansion (Wang et al., 2014b). More recently, downregulation of RUNX3 in human and mouse HSCs was found to be associated with aging and reduced erythroid potential (Balogh et al., 2019).

Immune system

RUNX proteins play multiple crucial roles in various immune cell subsets, both innate and acquired. In particular, RUNX1 and RUNX3 are involved at multiple stages of the complex cell-fate decisions of T lymphocyte development in the thymus (Fig. 5A).

**Box 2. RUNX factors in cancer**

RUNX genes are associated with hallmarks of cancer development, including proliferation and epithelial-to-mesenchymal transition, and have oncogenic or tumor-suppressive functions. Mutations in all three RUNX genes, and CBFβ, are frequently identified in cancers and their roles are being actively investigated (Blyth et al., 2005; Chuang et al., 2013; Groner, 2017; Ito et al., 2015). Indeed, RUNX1 (formerly AML1, acute myeloid leukemia 1) was first discovered in the human t(8;21) translocation (Miyoshi et al., 1991). Since then, point mutations and translocations involving RUNX1 have not only been linked with forms of leukemogenic and hereditary diseases (Sood et al., 2017), but also to epithelial tumors such as skin and oral cancers (Scheitz et al., 2012). RUNX1 is also implicated in the tumorigenesis of hormone-related organs, including breast, ovarian, uterine and prostate cancers (Riggio and Blyth, 2017). Misexpression of RUNX2 is associated with osteosarcoma development (Martin et al., 2011), as well as breast and prostate cancer bone metastasis (Chuang et al., 2017). RUNX3 is linked to solid-tissue tumorigenesis in the gastrointestinal system, pancreas and lung, where it exerts context-dependent pro- and anti-tumorigenic effects. However, its role in tumorigenesis remains controversial (Chuang et al., 2017; Lotem et al., 2017). Compensation between the RUNX genes means that assessment of redundancy during tumorigenesis is necessary (Kimikubo, 2018). Although transcription factors are difficult to target with pharmaceuticals, pan-inhibitors of RUNX factors have shown promising anti-tumor effects in pre-clinical settings by small-molecule inhibition of RUNX/CBF complexes (Illendula et al., 2016), or via pyrrole-imidazole polyamides, which selectively target the consensus RUNX motif in chromatin to prevent transcription (Morita et al., 2017).
the T cell lineage in the thymus and differentiate from DN1 to DN3T cells in a RUNX1-dependent process (Ikawa et al., 2004; Kawamoto et al., 1999, 2000; Krueger and von Boehmer, 2007; Perry et al., 2004; Petrie, 2007). RUNX1 acts by inducing Bcl11b expression (Kueh et al., 2016), which in turn primes the T cell lineage-specifying genes, including Thpok (Zbtb7b) and Runx3 (Kojo et al., 2017; Liu et al., 2010). Conversely, deletion of Runx1 in T cells (Lck-Cre) results in a block at the DN3 stage, preventing their transition to robustly proliferating, pre-T cell receptor-expressing T cells (Egawa et al., 2007; Sato et al., 2003). In addition, RUNX proteins are crucial in determining the lineage choice between CD4 (T-helper)- and CD8 (cytotoxic)-positive T cells. A single transcriptional silencer is crucial for the repression of Cd4 transcription by RUNX1 in DN thymocytes, and by RUNX3 in the cytotoxic T-lineage (Collins et al., 2011; Sawada et al., 1994; Setoguchi et al., 2008; Siu et al., 1994; Steinke et al., 2014; Tanuchi et al., 2002; Zou et al., 2001). Additionally, RUNX3 positively regulates Cd8 expression to participate in the induction of the cytotoxic T cell fate (Hassan et al., 2011; Kohu et al., 2005).

Beyond regulating CD4/CD8 lineage choice, RUNX1 and RUNX3 are also essential in subtypes of effector T cells, including the specification of T-helper cell 17 cells (Wang et al., 2014a; Zhang et al., 2008b), mature CD8 cytotoxic T cells (Egawa and Littman, 2008; Woolf et al., 2003) and FOXP3+ regulatory T cells (Bruno et al., 2009; Kitoh et al., 2009; Ono et al., 2007; Rudra et al., 2009). RUNX1 is also crucial for the generation of natural killer T cells (Tachibana et al., 2011), whereas RUNX3 is implicated in T-helper type 1 cell-lineage specification (Djuretic et al., 2007), and tissue-resident memory CD8T cells (Milner et al., 2017). RUNX3 is also essential for memory cytotoxic T lymphocyte differentiation (Wang et al., 2018), and has also been suggested to play a role in the development of dendritic epidermal T cells (Woolf et al., 2007) and CD8αα intra-epithelial cells (Reis et al., 2013).

In the bone marrow, deletion of Runx1 (using Mx1-Cre) leads to defects in early B cell development (Growney et al., 2005; Ichikawa et al., 2004) that result in a loss of IgM+ B cells and altered VDJ recombination (Seo et al., 2012a) (Fig. 5B). Mechanistically, RUNX1 and CBFβ are thought to regulate and cooperate with the transcription factor EBF for progression to the pro-B cell stage (Maier et al., 2004) (Fig. 5B). RUNX2 and RUNX3 are involved later in mature effector B cells but their precise role remains to be dissected. In vitro, Runx3 knockout results in the absence of IgA production and appears to be required as a downstream target of transforming growth factor β (TGFβ) to
mediate IgA class switch recombination (Fainaru et al., 2004; Shi and Stavnezer, 1998). However, in vivo, loss of Runx3 leads to equal or elevated IgA levels (Brenner et al., 2004; Watanabe et al., 2010). Interestingly, upon transplantation into mice, Runx2/Runx3 DKO splenocytes are impaired in their ability to produce IgA, in contrast to observations in single knockouts (Watanabe et al., 2010).

The contribution of RUNX1 and RUNX3 extends to other compartments of the immune system. Runx3-deficient mice have altered myeloid dendritic cell maturation, and lack Langerhans cells, the dendritic cells of the skin (Fainaru et al., 2004 2005). Although these mice have normal development of natural killer (NK) cells, Runx3 has been shown to be important for the proliferation and maturation of IL15-induced NK cells (Levanon et al., 2014). Interestingly, Runx3 is crucial for the lineage commitment and function of innate lymphoid cells (Ebihara et al., 2015; Miyamoto et al., 2019; Yin et al., 2018), a recently described cell type of the function of innate lymphoid cells (Ebihara et al., 2015; Miyamoto et al., 1997). Interestingly, Runx3 has been shown to be important for the proliferation and maturation of IL15-induced NK cells (Levanon et al., 2014). Interestingly, increased extracellular glucose concentration is able to rescue the Runx2-null phenotype, but restoring Runx2 in cells lacking the glucose transporter Glut1 (Slc2a1) still leads to impaired bone formation (Wei et al., 2015).

Runx2 expression in MSCs leads to the acquisition of an osteoblast phenotype and is essential for osteoblast differentiation, particularly in preosteoblasts, through the regulation of osterix (Sp7) expression (Ducy et al., 1997; Inada et al., 1999; Komori et al., 1997). Runx2 expression peaks in preosteoblasts and immature osteoblasts, and decreases with osteoblast maturation (Maruyama et al., 2007). The function of Runx2 in committed osteoblasts, however, is more controversial; Runx2 overexpression in committed osteoblasts (Col1a1-Cre) results in reduced bone formation and osteopenia (Geoffroy et al., 2002; Kanatani et al., 2006; Liu et al., 2001), but so does conditional Runx2 deletion in a model that produces a C-terminally truncated RUNX2 protein (Adhami et al., 2015). Overall, these results suggest that high Runx2 expression halts osteoblast maturation and prevents their transition to osteocytes by maintaining a more immature state, and that Runx2 is crucial for de novo bone formation.

Although Runx2 knockout mice have entirely cartilaginous skeletons, severe inhibition of chondrocyte maturation is evident (Inada et al., 1999) and accompanied by defects in vascular invasion of the cartilage (Himeno et al., 2002; Zelzer et al., 2001). Runx2 is expressed in mature chondrocytes (Enomoto et al., 2000), and its depletion in vitro leads to a loss of the differentiated phenotype (Enomoto et al., 2004). In vivo overexpression of Runx2 in chondrocytes promotes chondrocyte maturation and endochondral ossification, whereas overexpression of a dominant-negative Runx2

![Fig. 6. Roles of the RUNX factors in osteoblast and chondrocyte differentiation.](image)

Bone is generated through the differentiation of mesenchymal stem cells (MSCs) towards either bone-forming osteoblasts or cartilage-forming chondrocytes. The osteoblast fate choice and the maturation pathway resulting in intramembranous ossification are largely controlled by RUNX2. Cartilage differentiation is regulated by both RUNX2 and RUNX3 (adapted from Komori, 2018). Inhibitory roles are indicated by bars.
or deletion of Runx2 has opposite effects (Chen et al., 2011; Takarada et al., 2013; Takeda et al., 2001; Ueta et al., 2001). In contrast, Runx2 is thought to inhibit chondrocyte proliferation and hypertrophy specifically in the perichondrium, a layer of fibrous tissue surrounding the cartilage (Hinoi et al., 2006). Taken together, RUNX2 is a key factor of chondrocyte maturation.

Besides RUNX2, the two other mammalian RUNX proteins have also been implicated in bone formation, albeit to a lesser extent. Runx3 is also expressed in the embryonic skeleton and partially redundant with Runx2; Runx3 knockout mice experience delayed endochondral ossification, but Runx2/3 DKO mice present a complete absence of chondrocyte maturation (Yoshida, 2004). In pre-committed osteoblasts, however, RUNX3 has a non-redundant role in regulating proliferation (Bauer et al., 2015). RUNX1 has also been implicated in chondrogenesis (Wang et al., 2005c), notably during sternal development (Kimura et al., 2010; Liakhovitskaia et al., 2010), and together with RUNX2 and RUNX3 in the development of the dental system (Chu et al., 2018; Wang et al., 2005a; Yamashiro et al., 2002).

The nervous system

The nervous system of bilateral animals is made of the central nervous system (CNS), which consists of the brain and the spinal cord, and the peripheral nervous system (PNS), which comprises the nerves and ganglia outside of the CNS. In the PNS, the dorsal root ganglia (DRG) sensory neurons transmit somatosensory stimuli to the spinal cord, including nociceptive (pain), mechanoreceptive (mechanical pressure) or proprioceptive (relative position) signals. The roles played by the RUNX factors in the mammalian neural system were first revealed with the generation of Runx3 knockout mice, which suffered from limb ataxia caused by defects in the TrkC (NTRK3)− subset of neurons of the DRG (Inoue et al., 2002; Levanon et al., 2002).

During DRG development, a transient population of neuronal progenitors emerges around E10.5 and is characterized by the double expression of neurotrophic tyrosine kinase receptor family members TrkB (NTRK2)+ and TrkC+; these later differentiate into TrkB+ mechanoreceptive neurons or TrkC+ proprioceptive neurons. Runx3 expression is restricted to the TrkC+ proprioceptive neuronal lineage (Inoue et al., 2002; Levanon et al., 2002). Loss of Runx3 in TrkC+ neurons leads to upregulation of TrkB followed by neuronal cell death (Kramer et al., 2006; Levanon et al., 2002). Conversely, ectopic Runx3 expression suppresses TrkB and promotes TrkC+ expression (Kramer et al., 2006). In addition, prior to their death, TrkC neurons of Runx3 knockout embryos exhibit atypical proprioceptive axonal projections to peripheral and central targets, preferably innervating dorsal positions over the ventral spinal cord (Chen et al., 2006; Inoue et al., 2002; Nakamura et al., 2008), and ectopic Runx3 expression is sufficient to promote axonal projections to the ventral spinal cord (Kramer et al., 2006). Furthermore, RUNX3 has been recently shown to transcriptionally control positional information during axon extension (Lallemand et al., 2012). Overall, these studies demonstrate that Runx3 is crucial for the emergence and specification of proprioceptive neuronal circuits.

Besides RUNX3, RUNX1 is crucial for the diversification of sensory neuron lineages, in particular for when neurons differentiate into peptidergic [TrkA (NTRK1)+] and non-peptidergic (TrkA−) subtypes. Runx1 is expressed at prenatal and perinatal stages, and remains expressed postnatally in non-peptidergic sensory neurons that innervate the skin epidermis and hair follicles, where it actively regulates further specification of cutaneous sensory neurons (Chen et al., 2006; Gascon et al., 2010; Kramer et al., 2006; Lou et al., 2015; Luo et al., 2007; Marmigère et al., 2006; Yang et al., 2013; Yoshikawa et al., 2007). At the transcriptional level, RUNX1 modulates the expression of nociceptive genes, by positively regulating genes involved in the non-peptidergic subtype, and repressing peptidergic genes (Chen et al., 2006; Kramer et al., 2006; Liu et al., 2008; Ugarte et al., 2013; Yoshikawa et al., 2007). Both in vivo and in vitro studies have shown that altering Runx1 expression is associated with defective axon growth and branching to defined target sites (Chen et al., 2006; Kramer et al., 2006; Yoshikawa et al., 2007).

In keeping with its role during PNS nociceptive neuron development, Runx1 is expressed in defined subtypes of terminally differentiated post-mitotic neurons. It has been proposed to be involved in the regulation of motor neuron diversity and circuit formation. Indeed, inactivation of Runx1 is associated with the apoptosis of cranial ganglion sensory neurons (Theriault et al., 2004). Runx1-expressing neurons project their axons to muscles mediating tongue protrusion, and disruption of Runx1 reduces the number of hypoglossal axon projections to the intrinsic muscles of the tongue (Yoshikawa et al., 2015). Runx1 expression has also been reported in other motor neuron subtypes, such as spinal motor neurons, suggesting a possible broader contribution to the control of posture and movement (Stifani et al., 2008).

Finally, Runx1 plays a unique role in the neuronal tissue of the olfactory system. Unlike most neural tissues, the olfactory system undergoes constant tissue regeneration. It connects the olfactory bulb and cortex in the brain to the olfactory epithelium in the nasal cavity. Runx1 expression is found in a specific type of undifferentiated mitotic olfactory sensory neurons where it participates in maintaining the pool of progenitor cells by regulating proliferation and delaying differentiation (Theriault et al., 2005). RUNX factors have also been implicated in the homeostasis of glial populations of the CNS and the PNS, including Schwann cells (Hung et al., 2015; Li et al., 2016) and astrocytes (Takarada et al., 2013). Interestingly, RUNX1 has been shown to suppress the proliferation of olfactory ensheathing cells, a specific glial population that ensheaths the non-myelinated axons of olfactory neurons (Murthy et al., 2014).

Hair follicles and epidermis

RUNX proteins, and in particular RUNX1, participate in hair follicle (HF) morphogenesis and maintenance. During development, Runx1 is expressed in both the HF epithelium and the surrounding mesenchyme (Levanon et al., 2001; Osorio et al., 2011; Raveh et al., 2006). In the forming epithelium, disruption of Runx1 expression delays HF development (Osorio et al., 2011). Loss of Runx1 in mesenchymal cells does not initially impact early HF development, but subsequently leads to the emergence of defective hair follicle stem cell (HFSC) precursors, which differentiate preferentially in enlarged sebaceous cysts instead of healthy hair bulbs (Osorio et al., 2011). After follicular morphogenesis, the hair growth cycle starts postnatally; Runx1 is expressed in specific HF compartments and absent in the surrounding mesenchyme (Osorio et al., 2011; Raveh et al., 2006). During the hair growth phase (anagen), Runx1 is broadly found in bulge cells. Loss of Runx1 impairs HFSCs self-renewal and delays entry into anagen (Hoi et al., 2010; Osorio et al., 2011). Conversely, ectopic expression of Runx1 during anagen initiates hair degeneration (Lee et al., 2014). During the resting phase (telogen), Runx1-null HFSCs are able to exit quiescence either with time (Hoi et al., 2010; Osorio et al., 2011) or following injury (Osorio et al., 2008), indicating that Runx1 is dispensable for this process. Like in other tissues, Runx1 dosage has an important role in the regulation of skin epithelial cell fate.
Whereas low Runx1 expression in bulge stem cells enhances self-renewal (Hoi et al., 2010), higher RUNX1 levels promote their transition towards early progenitor hair germ cells (Lee et al., 2014). Mechanistically, RUNX1 orchestrates HF specification and maturation by modulating Wnt signaling (Osorio et al., 2011), and regulates HFSCs proliferation in a P21 (Cldn1a)-dependent manner (Hoi et al., 2010; Lee et al., 2013). Additionally, RUNX1 has been implicated in the lipid metabolism of skin epithelial cells by regulating fatty acid production (Jain et al., 2018). Runx1 is also expressed in mouse keratinocytes, where it collaborates with p63 (Trp63) to regulate the balance between proliferation and differentiation (Masse et al., 2012; Qu et al., 2018). In addition to Runx1, Runx2 is expressed in the dermal papillae and the bulb epithelium (Glotzer et al., 2008), whereas Runx3 is expressed in the dermal layer in placode and hair germ stages, and in the dermal papillae throughout the hair cycle (Raveh et al., 2005). However, deletion of neither Runx2 nor Runx3 results in major defects related to HF development.

Mammary gland
Specific roles of RUNX proteins in the development and functions of the mammary gland are also starting to emerge. The mammary gland is generated from the embryonic mammary placode and develops mainly after birth into a branched network of collecting ducts and tubes. The mammary gland undergoes further dynamic changes, greatly affected by hormone levels during estrous cycles, pregnancy and lactation. The epithelium of the mammary gland is composed of two distinct cell types forming a bilayer structure, in which the alveolar luminal secretory cells, responsible for milk production, are surrounded by basal or myoepithelial cells (Inman et al., 2015).

All three RUNX genes are expressed at different levels within the mouse mammary epithelium (Blyth et al., 2010). Runx1 is more highly expressed than Runx2, whereas Runx3 expression is barely detectable (McDonald et al., 2014; Owens et al., 2014; van Bragt et al., 2014). RUNX1 and RUNX2 levels are higher in the basal than in the luminal compartment (Kendrick et al., 2008; McDonald et al., 2014; van Bragt et al., 2014), and RUNX1 is completely absent in the alveolar luminal cells (van Bragt et al., 2014). The expression of the RUNX genes appears to fluctuate extensively; Runx1 is highly expressed in the epithelium of virgin females and post-lactation, but it gradually decreases throughout pregnancy to reach its lowest levels in late pregnancy and lactation (Blyth et al., 2010; van Bragt et al., 2014). This has been linked to the extensive tissue remodeling that takes place during pregnancy, which results in the large expansion of alveolar luminal cells that do not express Runx1 (Inman et al., 2015; van Bragt et al., 2014). In particular, Runx1 regulates the fate of the estrogen receptor-positive luminal lineage in vivo, where it represses the alveolar transcription factor Elf5 and promotes expression of a more mature luminal transcriptional program. Indeed, deletion of Runx1 results in a reduction of mature luminal cells (van Bragt et al., 2014). In vitro, 3D morphogenesis studies utilizing the non-tumorigenic basal-like MCF10A cell line have shown that Runx1 is essential to promote the differentiation of acinar structures into ductal and lobular tissue (Sokol et al., 2015; Wang et al., 2011b). Together, these results suggest that RUNX1 is required for the differentiation of luminal cells. In contrast, overexpression of Runx2 in the same cell line creates defects in acini formation and promotes proliferation (Owens et al., 2014; Pratap et al., 2006). In the HC11 cell line, an in vitro model of murine mammary cell differentiation, ectopic Runx2 expression potentiates the formation of mammospheres (Ferrari et al., 2015), structures retrospectively reflecting the presence of stem-like cells, therefore suggesting that Runx2 expression maintains a more stem/progenitor state (Owens et al., 2014). Accordingly, disruption of Runx2 expression decreases primary mammosphere formation in vitro and mammary regeneration in vivo (Ferrari et al., 2015). Furthermore, mouse models of both conditional overexpression and deletion of Runx2 result in abnormal development of the murine mammary epithelium, associated with lactation defects due to altered alveolar differentiation (McDonald et al., 2014; Owens et al., 2014).

Other tissues: muscle, cardiomyocytes, lacrimal gland and gastrointestinal tract
RUNX factors have been implicated in the biology of several other tissues that are subject to frequent repair and regeneration processes. High Runx1 expression has been reported in denervated skeletal muscles (Zhu et al., 1994), and in vivo deletion of Runx1 in skeletal muscle (Mck-Cre) has revealed a role for RUNX1 in protecting denervated myofibers from excessive atrophy, autophagy and muscle wasting (Wang et al., 2005b). Interestingly, although Runx1 is not expressed in naïve developing or adult striated muscle, its expression peaks following myopathic damage and is proposed to regulate the balance of myoblast proliferation and differentiation during muscle regeneration (Umansky et al., 2015).

Both RUNX1 mRNA and protein levels increase in cardiomyocytes following myocardial infarction, where RUNX1 modulates calcium uptake and contractile functions. Interestingly, this study also indicated that injured Runx1-deficient mice are protected from the adverse effects of cardiac remodeling (McCarron et al., 2018).

In the lacrimal gland, which secretes the aqueous layer of the tear film, expression of both Runx1 and Runx3 is increased during tissue regeneration after inflammation-induced lacrimal gland damage. Furthermore, deletion of Runx1 is associated with impaired epithelial development of the gland (Voronov et al., 2013).

Runx3 deficiency in mice has been linked to the development of hyperplasia of the gastrointestinal tract, suggesting a role in regulating homeostasis of this tissue (Fukamachi et al., 2004; Ito et al., 2011; Li et al., 2002). Similarly, hyperplasia has been described in the lungs of Runx3-null mice, which suggests a role in regulating alveolar differentiation (Lee et al., 2011). However, it remains to be determined whether these observations are the result of cell-autonomous effects or broader consequences of Runx3 loss in other compartments, such as the immune system (Lotem et al., 2017).

Conclusions
In the last 30 years, vast progress has been made in understanding the function and regulation of RUNX transcription factors. They have been shown to play key roles in developmental and cellular processes, such as self-renewal, proliferation, cell lineage specification and differentiation in diverse tissues. However, our understanding of their functions and mechanisms of action remains limited to specific cell types and developmental stages, corresponding to loss-of-function phenotypes.

Moving forward, we need to expand our knowledge of the mechanisms behind the generation of their diverse isoform repertoire and their specific functions. We need to understand how RUNX activities are modulated by context-dependent post-transcriptional modifications, and co-factor interactions. We should also interrogate how RUNX factors positively or negatively regulate their own expression, while interacting with other transcriptional
regulators. One underexplored aspect is to define how RUNX proteins cooperate and potentially compensate for each other. Another challenge will be to characterize the integration of RUNX activities with signaling pathways at the molecular level. Finally, it will be essential to map the precise genome-wide targets of RUNX1, RUNX2 and RUNX3 in stage-specific cell populations or even at the single cell level. Indeed, recent technical advances in single cell technologies now provide us with exciting new tools to address these challenges. Because all of the RUNX proteins have been linked with cancer (Box 2) and other disorders, understanding their molecular activities could have profound value in the diagnosis and treatment of a wide array of diseases.

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