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PRIMER

RUNX transcription factors: orchestrators of development

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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 specification 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 direct 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 'PyGPyGGTPy' (Kamachi et al., 1990; Wang and Speck, 1992), (2) protein-protein interactions (Lilly et al., 2016; 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 transactivation 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)

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Development (2019) 146, dev148296. doi:10.1242/dev.148296

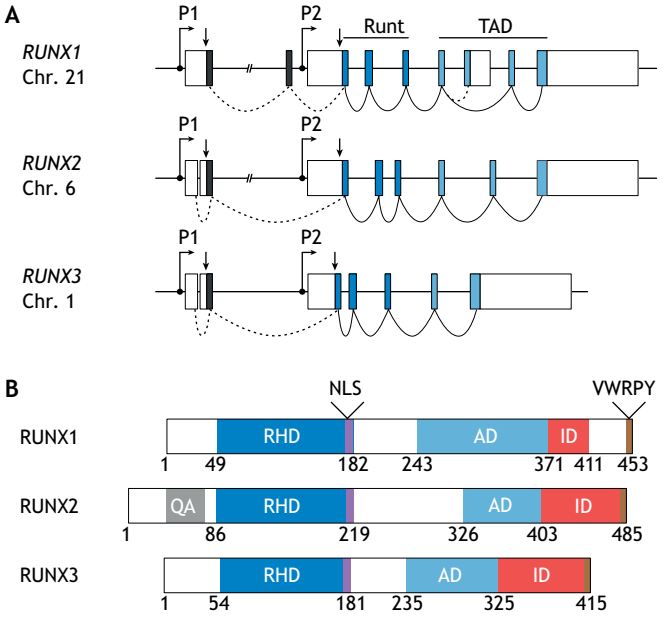

Fig. 1. RUNX1, RUNX2 and RUNX3 genes and proteins. (A) Conserved genomic structure of human *RUNX1* (Entrez Gene ID: 861), *RUNX2* (Entrez Gene ID: 860) and *RUNX3* (Entrez Gene ID: 864). The initiator ATG codons of P1- and P2-derived transcripts are indicated by downward pointing arrows. Exons coding for Runt and transactivation domains (TAD) are shown in dark and light blue, respectively, and UTRs are in white. Dominant splicing is indicated by a continuous line, and some alternative splicing is shown by a dashed line. (B) Schematic of human RUNX1 (NP_001001890), RUNX2 (NP_001265407) and RUNX3 (NP_004341) protein structures and domains, including the conserved Runt homology domain (RHD), nuclear localization signal (NLS), transactivation (AD) and inhibitory (ID) domains, C-terminal Groucho/TLE binding site (VWRPY), and the RUNX2-specific glutamine/alanine-rich (QA) sequence.

(Kamachi et al., 1990; Ogawa et al., 1993; Wang et al., 1993). CBFβ acts as a non-DNA-binding regulatory element, which allosterically increases the DNA-binding affinity and stability of the complex by interacting with RUNX through the RHD (Bravo et al., 2001; Huang et al., 2001; Tang et al., 2000; Yan et al., 2004). Of note, CBFβ-independent functions of RUNX have also been suggested in the literature (Bresciani et al., 2014), including a non-transcriptional role for RUNX1 and RUNX3 in the Fanconi anemia DNA-repair pathway (Tay et al., 2018; Wang et al., 2014b). Usually regarded as weak transcription factors by themselves, RUNX proteins might have the potential to act as 'pioneer' transcription factors, which are able to engage condensed chromatin to facilitate its opening, and promote the recruitment of other transcriptional regulators (Zaret and Carroll, 2011). Indeed, this seems to be the case for RUNX1 in hematopoiesis (Hoogenkamp et al., 2009; Lichtinger et al., 2012) and RUNX3 in cell cycle progression (Lee et al., 2019); however, the extent to which these observations could apply to other settings and other RUNX proteins remains to be determined. Importantly, RUNX factors can function as both transcriptional activators and repressors. For instance, RUNX1 is able to drive PU.1 (Spi1) expression in myeloid and B cells, whereas it represses the same gene in T cells and megakaryocytes (Huang et al., 2008). This gene- and context-specific ambivalence stems from the myriad of RUNX/CBFβ interactors.

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; Kitabayashi et al., 2001; 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). Methylations 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; Vu 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., 2008). 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

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; Telfer 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 proplatelets, 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 (Tsuzuki and Seto, 2012; Tsuzuki et al., 2007).

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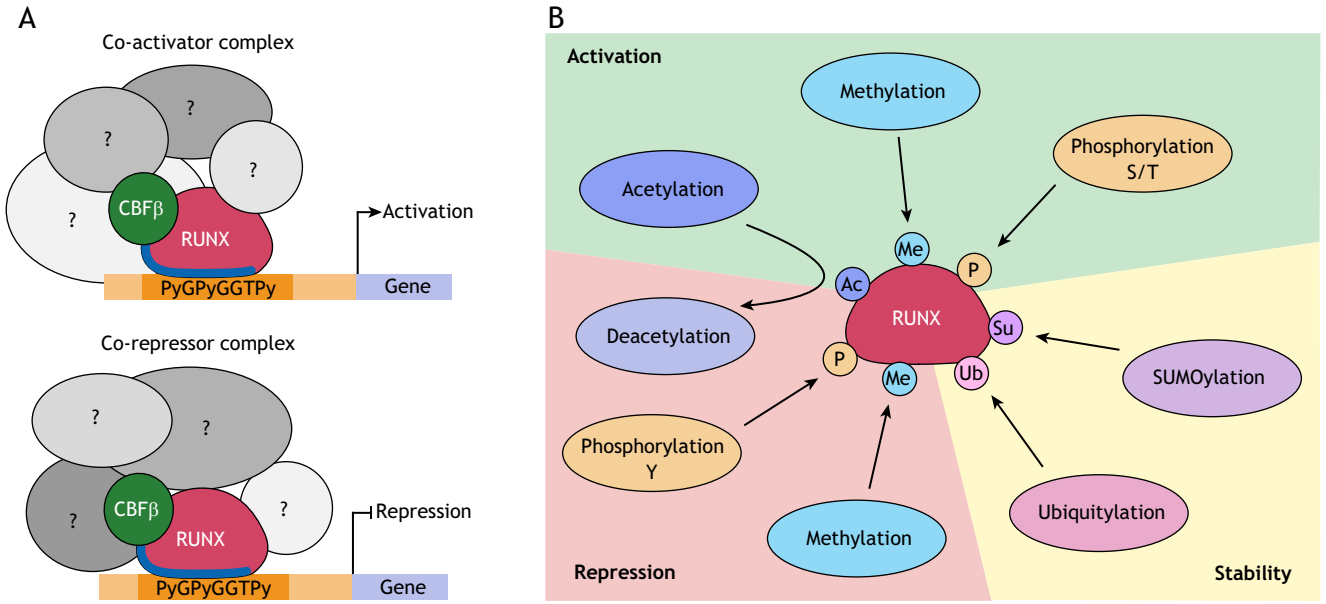

Fig. 2. RUNX activity and stability. (A) RUNX factors can act as transcriptional activators or repressors depending on binding partners and post-translational modifications. CBFβ dimerizes with the Runt binding domain to enhance DNA-binding to the RUNX consensus motif. The Runt domain is indicated in blue. (B) Summary of the main post-translational modifications affecting RUNX protein activity. Ac, acetylation; Me, methylation; P, phosphorylation; S, serine; Su, SUMOylation; T, threonine; Ub, ubiquitylation; Y, tyrosine.

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 originally 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 Groner, 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

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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; Woolf 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 IgE, 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_H to DJ_H recombination.	(Seo et al., 2012a)
Runx1	cKO	*Wnt1*-Cre	Nervous system	Defects in pain perception (nociception) resulting from impaired differentiation of $TrkA^+$ 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 $CGRP^+$ nociceptive neurons, changes in pain responses.	(Kramer et al., 2006)
*Runx1*a	iOE	*Sox10*-rtTA	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.	(Hoi et al., 2010; Lee et al., 2013; Osorio et al., 2011)
Runx1	iOE	*Krt14*-rtTA	Hair follicles	Promotion of hair germ early progenitor fate in bulge cells, hair degeneration during anagen.	(Lee et al., 2014)

Continued

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Table 1. Continued

Gene	Alteration	Strategy	Tissue	Main phenotypes	References
Runx1	cKO	*β-actin*-CreER	Hair follicles	Impaired HFSC proliferation and cell cycle progression, delayed anagen onset.	(Hoi et al., 2010; Osorio et al., 2008)
Runx1	cKO	*MMTV*-Cre	Mammary gland	Reduction of mature luminal cells (ER^+).	(van Bragt et al., 2014)
Runx1	cKO	*Mck*-Cre	Muscle	Atrophy of denervated myofibers, disorganized myofibrils, excessive autophagy.	(Wang et al., 2005b)
Runx1	cKO	*Myf5*-Cre	Muscle	Combined deletion with *Mdx* impairs muscle regeneration, age-dependent muscle waste, decreased motor capabilities and shorter lifespan.	(Umansky et al., 2015)
Runx1	cKO	*αMHC*-Cre	Cardiac system	Protection against adverse cardiac remodeling following myocardial infarction, maintenance of the ventricular wall thickness and contractile function.	(McCarroll et al., 2018)
Runx1	gKO+rescue	*Tie2*-Cre	NA	Neonatal lethality, rescue of definitive hematopoiesis, sternal development defects.	(Liakhovitskaia et al., 2009, 2010)
Runx2	cOE	*Col1a1*-Cre	Skeletal development	Reduced bone formation and osteopenia.	(Geoffroy et al., 2002; Kanatani et al., 2006; Liu et al., 2001)
Runx2	cOE	*Col2a1*-Cre	Skeletal development	Acceleration of endochondral ossification due to precocious chondrocyte maturation, absence of permanent cartilage.	(Ueta et al., 2001)
Runx2	cKO	*Col1a1*-Cre	Skeletal development	Perinatal lethality, altered bone formation.	(Adhami et al., 2015)
Runx2	cKO	*Col1a1*-Cre	Skeletal development	Mild defects of bone formation.	(Takarada et al., 2013)
Runx2	cKO	*Col2a1*-Cre	Skeletal development	Defects of endochondral ossification.	(Chen et al., 2011; Takarada et al., 2013)
Runx2	cKO	MMTV-Cre	Mammary gland	Decreased mammary regeneration, lactation defects due to impaired alveolar differentiation.	(Ferrari et al., 2015)
Runx2	cOE	MMTV-Cre	Mammary gland	Abnormal branching of the mammary epithelial tree, epithelial hyperplasia, delayed alveolar differentiation, blocked milk production.	(McDonald et al., 2014; Owens et al., 2014)
Runx3	cKO	*Mx1*-Cre	Hematopoietic system	Myeloproliferative disorder, splenomegaly, increase in HSPCs.	(Wang et al., 2013)
Runx3	cKO	*Cd4*-Cre	Immune system	Reduction of CD8 single-positive thymocytes, alteration of Th1 differentiation.	(Egawa et al., 2007; Naoe et al., 2007)
Runx3	iKO	*Ert2*-Cre	Immune system	Reduction of tissue-resident memory $CD8^+$ T cells.	(Milner et al., 2017)
Runx3	cKO	*NKp46*-Cre and *Vav1*-Cre	Immune system	Defects of innate lymphoid cells differentiation.	(Ebihara et al., 2015)
Runx3	cKO	*Cd11c*-Cre *Cebpα*-Cre	Immune system	Impaired splenic $CD4^+$/CD11b $(ITGAM)^+$ dendritic cell compartment.	(Dicken et al., 2013)
Runx3	cKO	*Col1a1*-Cre	Skeletal development	Severe congenital osteopenia.	(Bauer et al., 2015)
Runx3	cKO	*Col1a2*-Cre	Skeletal development	Normal bone development.	(Bauer et al., 2015)
Runx3	cKO	TK-Cre	Nervous system	Impaired differentiation of proprioceptive neurons ($TrkC^+$).	(Kramer et al., 2006)
Runx3	cOE	*Isl1*-Cre and *Hb9*-Cre	Nervous system	Suppression of TrkB expression in DRG neurons and increase in $TrkC^+$ neurons.	(Kramer et al., 2006)
Runx2/3	Double gKO	NA	NA	Embryonic lethality, absence of chondrocyte differentiation.	(Yoshida, 2004)
Runx1/2	*Runx1* cKO *Runx2* gKO	*Prx1*-Cre	Skeletal system	Absence of sternal development.	(Kimura et al., 2010)
Runx1/3	Double cKO	*Mx1*-Cre	Hematopoietic system	Lethality induced by BMF, reduction of all mature hematopoietic lineage compartments, defective Fanconi anemia DNA-repair pathway.	(Wang et al., 2014b)

cOE, conditional overexpression; cKO, conditional knockout; DJ_H, Diversity and Joining segments of the immunoglobulin heavy chain; gKO, germline knockout; iKO, inducible knockout; iOE, inducible overexpression; NA, not applicable; OE, overexpression; V_H, Variable segments of the immunoglobulin heavy chain.

in hematopoiesis (de Bruijn and Dzierzak, 2017; Ebihara et al., 2017; Gao et al., 2018; Thambyrajah et al., 2016b; Tober et al., 2016; Voon et al., 2015).

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., 2008; McGrath et al., 2015; Palis et al., 1999; Yoshimoto 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

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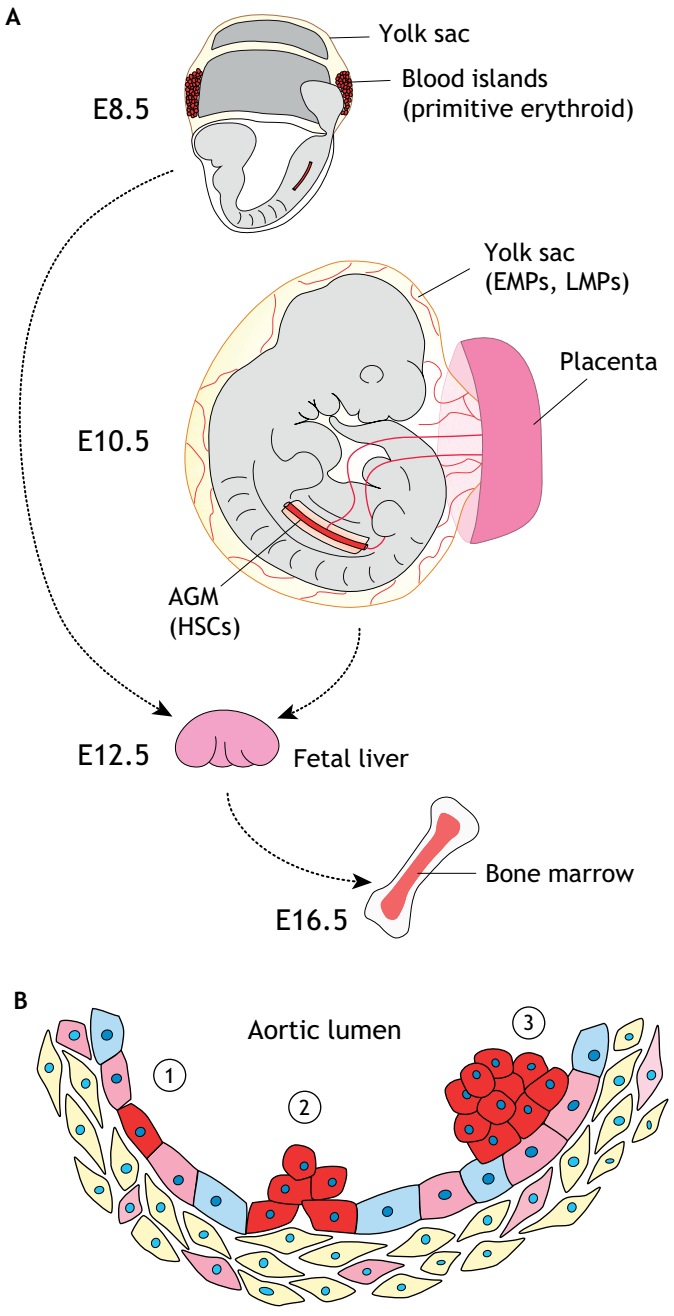

Fig. 3. RUNX1 and embryonic hematopoiesis. (A) Timeline of embryonic blood development. *Runx1* is expressed at all the different sites of hematopoietic development during the different hematopoietic waves. EMPs, erythro-myeloid progenitors; HSCs, hematopoietic stem cells; LMPs, lymphoid-myeloid progenitors. (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).

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; Kissa 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; Thambyrajah 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; Lacaud et al., 2004; Lie-A-Ling et al., 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; 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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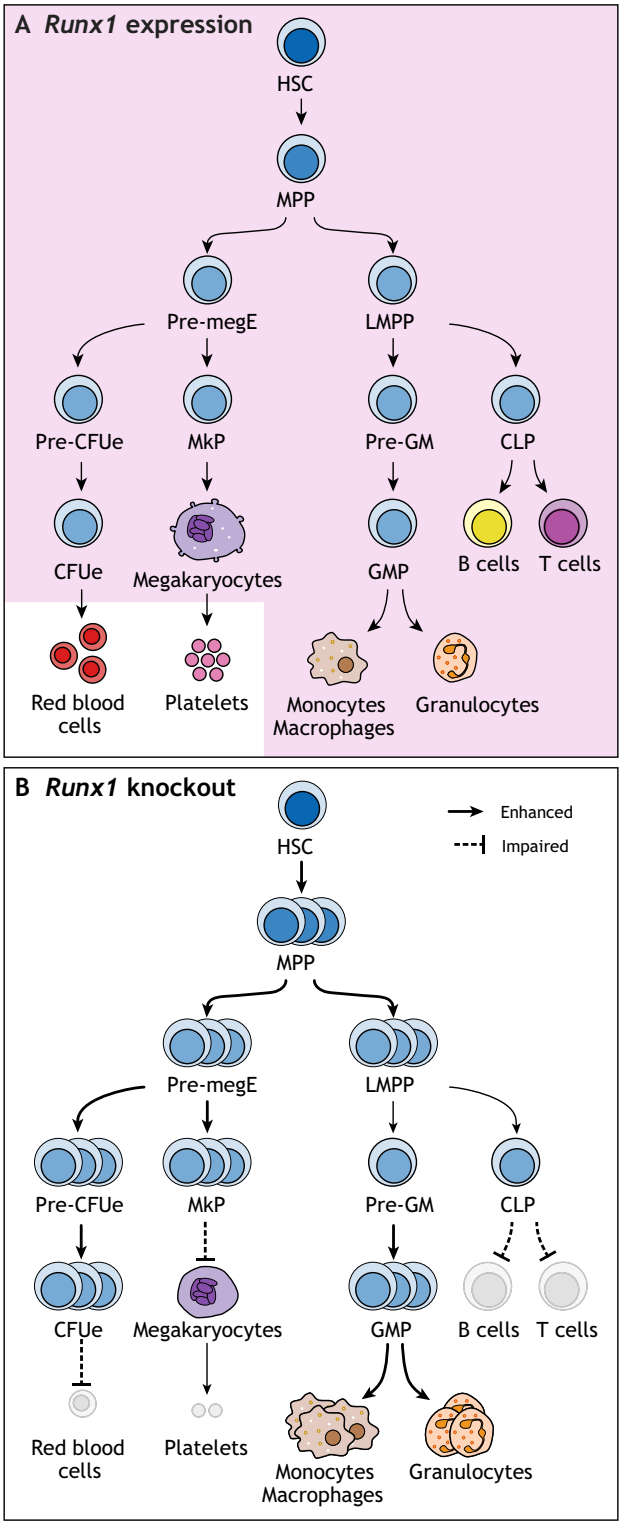

Fig. 4. *Runx1* expression and impact of *Runx1* knockout on adult hematopoiesis in mice. (A) Expression of *Runx1* (red background) in the different hematopoietic populations of the adult hematopoietic system. (B) Impact of hematopoietic *Runx1* knockout on the frequencies of these populations. Enhanced processes are indicated by bold arrows, impaired processes are indicated by bars. CFUe, erythroid colony-forming unit; CLP, common lymphoid progenitor; GMP, granulocyte/monocyte progenitor; 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.

et al., 2011, 2013). Enforced *RUNX1* expression in the K562 human cell line promotes megakaryopoiesis by directly repressing the erythroid lineage (Kuvardina et al., 2015). Surprisingly, the loss of *Runx1* also causes an increased commitment to the megakaryocytic lineage (Behrens et al., 2016), perhaps due to the properties of the different RUNX1 isoforms (Box 1).

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; Growney 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). Early CD4/CD8 double-negative (DN) T cell progenitors commit to

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 (Kamikubo, 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/CBFβ 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).

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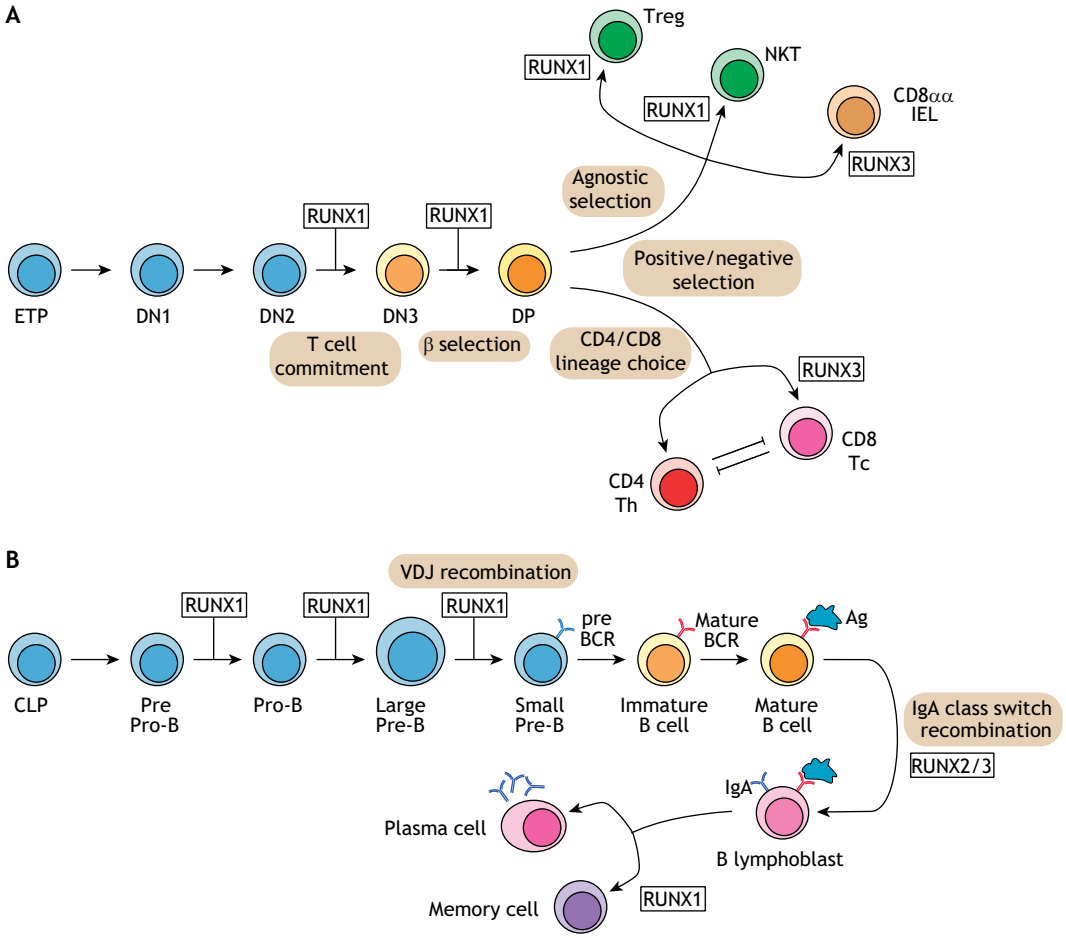

Fig. 5. RUNX-mediated regulation of B and T cell development. (A) RUNX1 and RUNX3 regulate T cell commitment and lineage fate decisions. RUNX1-directed transitions (T cell commitment, DN-to-DP transition, Treg and NKT cell selection) and RUNX3-directed transitions (CD8 Tc and CD8αα IEL lineage choice) are indicated. (B) RUNX proteins regulate B cell differentiation. RUNX1-directed transitions (early B cell differentiation, VDJ recombination, memory cell commitment) and RUNX2/3-directed transitions (IgA class switch recombination) are indicated. Ag, antigen; BCR, B cell receptor; CLP, common lymphoid progenitor; DN, CD4/CD8 double-negative cell; DP, CD4/CD8 double-positive T cell; ETP, early T-cell progenitor; IEL, intra-epithelial cell; NKT, natural killer T cell; Tc, cytotoxic T cell; Th, helper T cell; T reg, regulatory T cell.

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; Taniuchi 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; Seo et al., 2012a). 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

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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 mucosal immune response (reviewed by Eberl et al., 2015). *Runx1* is important for basophil development (Mukai et al., 2012), but also in lymphoid tissue inducer cells, which are fundamental for the development of secondary lymphoid tissues such as lymph nodes and lymphatic tissue of the small intestine, known as Peyer's patches (Tachibana et al., 2011).

Skeletal development

Alongside the crucial roles of RUNX proteins in hematopoiesis, RUNX2 is a master regulator of skeletal development, playing multiple roles in the specification and differentiation of both the osteoblast and the chondrocyte lineage (reviewed by Komori, 2018). The formation of bony skeleton occurs through two distinct processes (Fig. 6). *De novo* bone formation in soft tissue is achieved by the differentiation of mesenchymal stem cells (MSCs) into osteoblasts, which mature into osteocytes and eventually form bone, in a process called intramembranous ossification (Marks and Odgren, 2002). Alternatively, MSCs can differentiate into chondrocytes, which form cartilage. This cartilage can either be permanent, or vascular invasion permits the arrival of osteoclast and mesenchymal cells, instigating endochondral ossification. The resident chondrocytes terminally differentiate and apoptose, whereas the mesenchymal cells differentiate into osteoblasts and form bone on the cartilage structures (Komori, 2018).

The role of *Runx2* in bone formation was uncovered using knockout mouse models. *Runx2*-null mice die shortly after birth as a result of respiratory failure, and the analysis of their skeletons revealed them to be composed of cartilage and not bone. In these mice, osteoblasts are completely absent and there is virtually no bone matrix protein expression (Inada et al., 1999; Komori et al., 1997). 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; Maruyama et al., 2007; Otto 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*

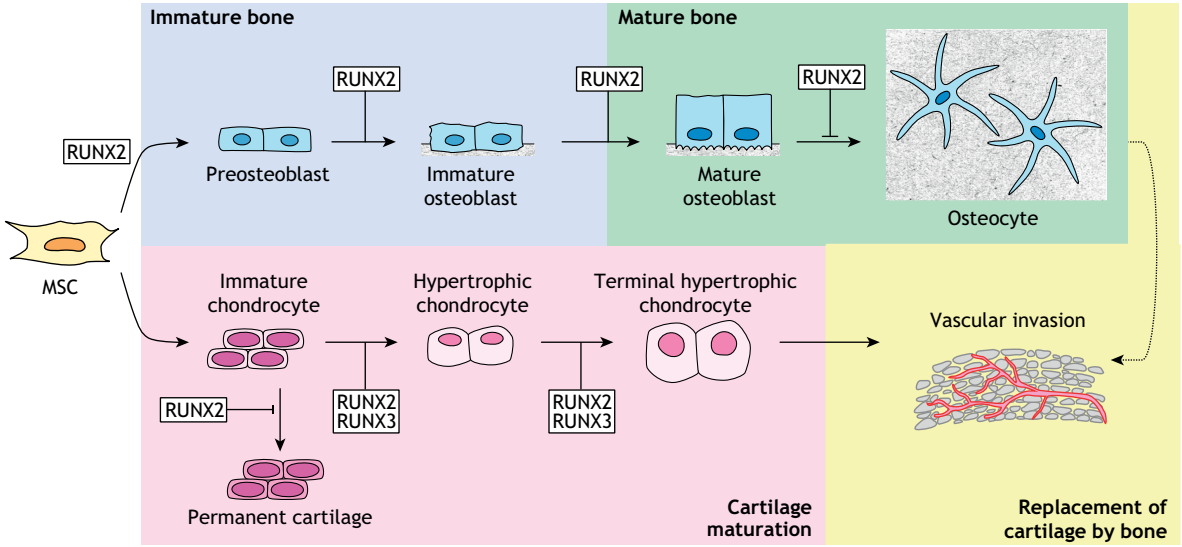

Fig. 6. Roles of the RUNX factors in osteoblast and chondrocyte differentiation. Bone is generated through the differentiation of mesenchymal stem cells (MSCs) towards either bone-forming osteoblasts or cartilage-forming chondrocytes. The osteoblast fate choice and the maturation pathway resulting in 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.

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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 (Lallemend 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.

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Whereas low *Runx1* expression in bulge stem cells enhances self-renewal (Hoi et al., 2010), higher RUNX1 levels promotes 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 (*Cdkn1a*)-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 (McCarroll 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.

Acknowledgements
We apologize for the many relevant studies that could not be discussed or cited owing to length restrictions. The authors thank the laboratories' members for critical reading of the manuscript.

Competing interests
The authors declare no competing or financial interests.

Funding
Research in the authors' laboratory is supported by the Medical Research Council (MR/P000673/1), the Biotechnology and Biological Sciences Research Council (BB/I001794/1; BB/R007209/1), the European Union's Horizon 2020 (GA6586250) and Cancer Research UK (C5759/A20971).

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