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RUNX Genes Find a Niche in Stem Cell Biology

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ABSTRACT

The RUNX family of transcriptional regulators are well conserved throughout the animal kingdom, from the simple nematode worm *Caenorhabditis elegans* to vertebrates. Interest in the RUNX genes emerged principally as a result of the finding that chromosomal translocations disrupting RUNX protein function are observed in a large number of patients suffering with acute myeloid leukemia (AML). In the 20 years that RUNX genes have been under investigation, they have emerged as central players in the control of developmental decisions between proliferation and differentiation in a wide variety of biological situations. This review focuses on recent data highlighting the roles of RUNX genes in stem cells and illustrates the diversity of processes in which the RUNX proteins play a critical role. In particular, we focus on the role of RUNX1 in hematopoietic stem cells (HSCs) and hair follicle stem cells (HFSCs) and the importance of the solo *C. elegans* RUNX factor *rnt-1* in stem cell proliferation in the worm. Observations in a variety of stem cell systems have developed to the point where useful comparisons can be made, from which guiding principles may emerge. J. Cell. Biochem. 108: 14–21, 2009.

KEY WORDS: RUNX; STEM CELLS; HEMATOPOIESIS; HAIR FOLLICLE; *C. ELEGANS*

A central and recurrent theme in the development of multicellular organisms is the establishment of disparate cell and tissue types from a single fertilized zygote. Somatic stem cells provide the means by which from a limited pool of undifferentiated cells, multipotent daughters can arise, destined for a variety of tissue types. Stem cells are able to divide asymmetrically (self-renewal maintenance) to maintain overall stem cell number and provide more specialized progenitors, which may then embark on one or more specific developmental programmes before becoming terminally differentiated. Alternatively, stem cells may undergo symmetric (self-renewal expansion) division, to repopulate the stem cell niche. In tissue homeostasis, stem cells are pivotal since they are essential for supplying replacements for short-lived cells, as in the case of hematopoiesis, or in tissue repair. Stem cell developmental programmes therefore need to be tightly regulated; if developmental programmes of progenitors are not properly controlled they may fail to mature, or follow inappropriate paths to differentiation; if the homeostasis of the stem cell itself is disrupted, too few progenitors may be available. It is therefore apparent that at the heart of proper stem cell function are the mechanisms that regulate the developmental decision between cell division and differentiation.

The RUNX family of transcription factors have recently emerged as major players in the control of stem cell differentiation and proliferation in both cancer and developmental biology. With the advent of genome sequencing, RUNX genes have now been identified in a wide range of animal species, from worms to fish, sea urchins to mice. In mouse, Runx1 has recently been identified as a major regulator of both hematopoietic stem cell (HSC) emergence [Chen et al., 2009] and hair follicle stem cell (HFSC) activation [Osorio et al., 2008]. The simple metazoan worm, *Caenorhabditis elegans* possesses a single RUNX gene, *rnt-1* which is expressed in and functions to control the stem cell-like divisions of seam cells [Nimmo et al., 2005; Kagoshima et al., 2007], which form a specialized epidermal tissue type in the worm. In this review, we summarize recent findings relating to the precise role of RUNX genes in these stem cell systems and present *C. elegans* as an emerging, but exciting model organism with which to expand our understanding of this important family of proteins.

THE RUNX GENE FAMILY

The RUNX family of transcriptional regulators takes its name from *Drosophila* runt; a pair-rule gene involved in the establishment of embryonic segmentation [Gergen and Butler, 1988]. The literature contains several nomenclature systems for this family of genes, ostensibly reflecting the means by which the individual family

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members were identified. For example, the mouse PEBP2αA/RUNX2 (for polyoma enhancer binding protein) was discovered in a screen for proteins binding the enhancer of polyomavirus, a marker of embryonic cell differentiation [Kamachi et al., 1990]. The alternative notation, acute myeloid leukemia (AML), often used for human members of the RUNX family, reflects the discovery that *AML1/Runx1* is a common target of chromosomal translocations resulting in AML. The designation CBF (for enhancer core binding factor) is also sometimes referred to in the literature, denoting the ability of these gene products to bind the enhancer regions of murine leukemia viruses. A recent, standardized designation of RUNX has now been established for all mammalian members of this gene family [van Wijnen et al., 2004].

The proteins encoded by RUNX genes are principally defined by the presence of the Runt domain [Kagoshima et al., 1993], a highly conserved 128 amino acid sequence, which contains sites involved in DNA binding and heterodimerization of RUNX proteins with their conserved binding partner [Kamachi et al., 1990; Ogawa et al., 1993]. RUNX genes have been identified in a broad range of animal phyla, including mouse, *Drosophila* and sea urchin (*Strongylocentrotus purpuratus*), with more recent discoveries of RUNX family members in the puffer fish (*Takifugu rubripes*), sea squirt (*Cionia intestinalis*), and mosquito (*Anopheles gambiae*) [Rennert et al., 2003]. RUNX genes appear not to be represented in non-metazoan organisms and the possession of a single RUNX seems to be the primitive condition in bilaterians. Higher organisms typically possess multiple RUNX genes (four have been identified in *Drosophila*, three in mice, and three in humans); it is suggested that the specialization of RUNX genes into more region- or tissue-specific function has followed the more recent gene duplications in these organisms [Rennert et al., 2003].

RUNX family members are known to be able to function as transcriptional repressors or activators, depending on the context in which they bind to the RUNX motif. Consistent with their role in repression, most members of the family possess a conserved C-terminal VWRPY motif (with the exception of *C. elegans*, which possesses the similar C-terminal motif IWRPF, not yet demonstrated to be functional), required for interaction with Groucho/TLE1 to repress the expression of certain target genes. For example, in *Drosophila*, repression of *hairy* and *eve* by Runt requires interaction with Groucho via the VWRPY motif [Aronson et al., 1997]. The VWRPY domain is not required for repression of all target genes negatively regulated by RUNX, however; for example, Runt is required for repression of *otc* and *engrailed* in *Drosophila* independently of the presence of the Groucho-interacting motif; for review, see Wheeler et al. [2000].

Another important conserved characteristic of all members of the RUNX family is their association as a heterodimer with a binding partner CBFβ [Kamachi et al., 1990]. CBFβ is not able to bind DNA directly, but associates with the α-subunit via the Runt domain, to stabilize the α-subunit and increase its binding efficiency [Kagoshima et al., 1996]. In a mouse model, homozygous mutation of CBFβ results in embryonic lethality and failure of fetal liver hematopoiesis in a manner similar to that observed in *Runx1* mutants [Wang et al., 1996] illustrating the crucial role of CBFβ in RUNX gene function. Aside from increasing the binding specificity of RUNX1, evidence suggests that CBFβ also functions to stabilize human RUNX1 in murine cell cultures, by inhibiting its ubiquitination and subsequent proteolysis [Huang et al., 2001]. *C. elegans* RNT-1 protein has been shown to dimerize with BRO-1, a homolog of CBFβ [Kagoshima et al., 2007], demonstrating that the conserved nature of the RUNX/CBFβ heterodimer extends from worms to vertebrates.

RUNX GENES IN DEVELOPMENT AND DISEASE

The human Runx1 gene AML1 was originally identified as being at the breakpoint of t(8;21) chromosomal translocations in AML [Miyoshi et al., 1991]. This translocation is found in a particularly high proportion of AML-M2 leukemias and represents 18–20% of chromosomal anomalies in all leukemias [reviewed in Licht, 2001]. The fusion partner of this chimeric protein, ETO (or Eight-Twenty One oncoprotein) was previously unknown before its discovery as an oncoprotein [Erickson et al., 1992]. Fusion of AML1/RUNX1 with ETO retains the N-terminal region of the RUNX protein, but loses much of the C-terminus, including sites required for interaction with co-activators and with the sin3 and TLE co-repressors [Licht, 2001].

Several other chimeric proteins generated as a result of chromosome translocations are known to interfere with RUNX function in humans, including AML1/MTG16, AML1/EVI1, and TEL/AML1 [reviewed in Yamagata et al., 2005]. Chromosomal translocations involving RUNX1/AML1 are generally thought to act in a dominant negative fashion, probably either as a result of competing for RUNX binding sites with the wild-type protein and ultimately repressing RUNX function, or by sequestering CBFβ away from the native protein [Okuda et al., 1998]. CBFβ is also associated with chromosome translocations in AML, as an inversion of chromosome 16 (inv(16)(p13q22)) fuses the RUNX binding partner CBFβ with a smooth muscle myosin heavy chain gene [Liu et al., 1993]. Recently a ''gatekeeper'' function has been suggested for RUNX1 in acute leukemia [Niebuhr et al., 2008]. This designation follows observations that mutations or translocations involving *RUNX1* may not be sufficient in themselves to cause acute leukemia, but predispose to a future occurrence of the disease. *RUNX1* translocations are found in higher numbers in the population than the clinical incidence of leukemia, and individuals with such translocations have a preleukemic cell population with increased self-renewal capacity and impaired differentiation. Secondary mutations appear to be required for the establishment of overt leukemia.

Studies on mouse models have shown Runx1 to be absolutely required for definitive hematopoiesis; *Runx1*-deficient mice die between embryonic days 11.5 and 12.5 with no hematopoietic progenitors in the liver or yolk sac [Okuda et al., 1996; Wang et al., 1996]. Surprisingly, a conditional knockout of *Runx1* in mice has demonstrated that the gene product is not absolutely required for hematopoiesis in the adult, although lineage-specific hematopoietic abnormalities were observed in the knockout mice [Growney et al., 2005]. Further post-embryonic functions have been ascribed to Runx1, including the correct specification of nociceptive neurons

[Chen et al., 2006] and in the dermis and epidermis, where it is required for proper hair morphogenesis [Raveh et al., 2006].

In vertebrates, the second RUNX family member, RUNX2 has been shown to have a crucial role in bone formation. Heterozygous *Runx2* mutant mice appeared mostly normal for skeletal development, but had hypoplastic clavicles and nasal bones and some defects in cranial ossification. However, homozygous mutants died shortly after birth with short legs and a complete lack of ossification [Komori et al., 1997]. The phenotype associated with heterozygosity of the Runx2 mutant above, has a paralog in human disease; the disorder Cleidocranial Dysplasia (CCD). Patients suffering from CCD have hypoplastic clavicles and delayed closure of the fontanelles; the lesions in this disease have been attributed to missense mutations in the DNA binding domain of RUNX2 (also called OSF2 in this study) [Lee et al., 1997]. A further role for Runx2 has been shown in late stages of tooth formation [reviewed in Camilleri and McDonald, 2006].

The third vertebrate RUNX gene family member, RUNX3 has been implicated in gastric cancer; mouse knockout lines developed hyperplasia of the gastric mucosa and primary cell cultures derived from these mice proved insensitive to TGF-β1-mediated apoptosis [Li et al., 2002]. The authors noted in the same study that RUNX3 expression was undetectable in 47% of human gastric cancer cell lines examined, supporting their view that RUNX3 functions as a tumor suppressor gene in gastric cancer. A direct link between RUNX3 loss of function and stomach cancer has proved controversial [Levanon et al., 2003] although it is possible that the reason for the discrepancy between the findings published by various groups may lie in the choice of antibody used to demonstrate RUNX3 localization [Ito et al., 2009]. Roles for Runx3 have also been reported in establishing the correct specification of neutrophin receptors in dorsal root ganglion neurons [Inoue et al., 2007] and in axon guidance [Inoue et al., 2002].

RUNX GENES AND THE CONTROL OF CELL PROLIFERATION VERSUS DIFFERENTIATION

On the cellular level, RUNX genes have been shown to function both in the control of cell proliferation and differentiation. A good example of this duality of RUNX function can be illustrated by the role of the sea urchin RUNX family member, *SpRunt*. The product of this gene was identified initially as a transcriptional activator of the *CyIIIa* actin gene in embryogenesis [Coffman et al., 1996]. Interestingly, *SpRunt* is required in embryogenesis to support cell proliferation, yet its expression later on in development is necessary to promote terminal differentiation; a stage when proliferation is inhibited [reviewed in Coffman, 2003]. Further experimental observations supporting a role for RUNX genes in cell differentiation include transfection of the murine myeloid precursor cell line 32Dc13 with the chimeric AML1/ETO gene, which resulted in a failure of granulocytic differentiation [Ahn et al., 1998], and analysis of *Drosophila lozenge* mutants, which demonstrated that the RUNX gene *lozenge* is required for the correct differentiation of crystal cells during *Drosophila* hematopoiesis [Lebestky et al., 2000]. On the other hand, *C. elegans rnt-1*, like its sea urchin counterpart, has been shown to promote cell proliferation during development [Nimmo et al., 2005].

Likewise, conflicting roles for RUNX genes in regulating cell cycle progression have been reported. For example, RUNX1 levels increased threefold during the G1–S phase transition in hematopoietic cells [Bernardin-Fried et al., 2004], supporting a proliferative function, and overexpression of AML-1B in 32D myeloid progenitors resulted in a shortening of the G1 phase of the cell cycle, with increased numbers of cells in S phase [Strom et al., 2000]. Similarly, fusion of a KRAB repressor domain to an inducible AML1/RUNX1 DNA-binding domain resulted in decreased cell proliferation, with G1/S phase cell cycle arrest [Lou et al., 2000]. In contrast, RUNX2 is maximally expressed in G1/G0 in osteoblasts [Galindo et al., 2005], supporting an anti-proliferative function.

Far from simply being conflicting and difficult to interpret, however, these observations allow us to propose that cell context-specific regulation of RUNX proteins during development and during the cell cycle allows them to act as important switches between cell proliferation and differentiation [reviewed further in Nimmo and Woollard, 2008]. This is entirely consistent with the categorization of RUNX proteins as both ''oncogenes'' and ''tumor suppressors'' [Cameron and Neil, 2004].

RUNX GENES AND ''STEMNESS''

Being so closely associated with the cellular decision to proliferate or to differentiate, it is perhaps no surprise that RUNX genes are emerging as important players in stem cell biology. RUNX genes appear to function in several different processes in the ''career'' of a stem/progenitor cell. In some instances, for example, RUNX proteins have been implicated in the emergence and/or maintenance of stem cell populations, whereas in others a role has been identified in expansion/proliferation of stem cells. A good example of the first role is that of RUNX1 in vertebrate HSC emergence. Early experiments with mouse knockout strains showed that *Runx1* mutants died in embryogenesis with a complete failure of definitive hematopoiesis [Okuda et al., 1996; Wang et al., 1996]. HSCs are thought to emerge from vascular endothelial clusters; deletion of Runx1 from cells expressing the endothelial marker VEC resulted in 65% fetal lethality and various other defects characteristic of a failure in hematopoiesis. However, once the HSC-specific marker Vav1 was expressed, the requirement for Runx1 was lost [Chen et al., 2009]. It is thus clear that in vertebrate models, RUNX1 is absolutely required for emergence of the HSCs but that the principal requirement for RUNX1 protein expression is within a precisely defined timeframe.

Paradoxically, although a major role for establishment of HSCs has been ascribed to RUNX1 by many researchers, RUNX1 loss of function via the dominant-negative effects of AML1-ETO is associated with leukemia and cells expressing this fusion protein display an increased self-renewal capacity [Okuda et al., 1998]. Similarly, human CD34+ cells expressing the CBFβ–SMMHC fusion undergo enhanced proliferation with decreased differentiation potential of progenitors [Wunderlich et al., 2006]. These data are strongly suggestive of a multimodal role for the RUNX/CBFβ

heterodimer in hematopoiesis. Indeed, conditional knockout of *Runx1* in mice did not result in a complete loss of hematopoiesis, although the authors reported defects in some blood cell lineages; for example, an 80% reduction in platelets and decrease in megakaryocyte numbers [Growney et al., 2005]. A hypercellular phenotype was also described, with mild myeloid expansion in hematopoietic tissues. Levels of RUNX1 expression have been followed in various hematopoietic lineages [North et al., 2004]; expression of a reporter fusion was observed in the bone marrow in most myeloid blast cells and was seen in most cells during early T-lymphocyte maturation. RUNX1 appears not to be important in terminal maturation of erythrocytes, however, and Runx1 expression levels were observed to decrease with ongoing maturation of this lineage. Interestingly, the authors found that bone marrow from *Runx1* mutants was unable to repopulate irradiated mice, hinting at a role for Runx1 in maintenance of HSC viability. Runx1 also appears to act in bone marrow homeostasis, with a recent report demonstrating increased numbers of quiescent HSCs in the bone marrow of *Runx1* knockout mice and an increased number of myeloid progenitors [Ichikawa et al., 2008].

A large variety of experimental approaches and model systems are current utilized in the study of RUNX gene function in hematopoiesis; it is therefore inevitable that differences in the proposed precise function and importance of RUNX1 exist. However, it seems that this gene may be important at many different levels in the life of HSCs and their more committed progeny.

Runx1 is also specifically involved in the activation of quiescent stem cells—an example of this being in the hair follicle (HF) [Osorio et al., 2008] (Fig. 1). The development of a HF can, like hematopoiesis, be categorized into primitive and definitive waves, known as morphogenesis, and adult hair cycling, respectively. At the end of morphogenesis, adult HFSCs enter quiescence (telogen). Lying at the base of the HF, the dermal papilla acts as a signaling center, thought to activate HFSC proliferation at the appropriate time, leading to hair growth (anagen). HFSCs reside in the bulge (Fig. 1) and express Runx1 prior to their proliferation [Osorio et al., 2008]. In *Runx1* conditional knockout mice, HF morphogenesis was largely unperturbed, but adult hair regeneration did not occur after post-natal day 21. *Runx1* mutant mice were unable to re-grow hair within 2 weeks of gentle removal, with no specific hair lineage markers being detectable [Osorio et al., 2008]. HFSCs are certainly present in these mutants, however, as demonstrated by the presence of stem cell-specific markers. Runx1 disruption in the HF was found to prolong the hair cycle quiescent phase and impair HFSC colony formation, suggesting that Runx1 acts in HFs at the stem cell level, promoting passage of HFSCs from quiescence into proliferation, presumably in response to normal growth activation signals [Osorio et al., 2008]. Fascinatingly, the HFSCs retained their ability to proliferate and produce differentiated hair lineages in response to injury, suggesting that Runx1 functions downstream of the signaling pathway specifying normal adult hair cycling, but does not regulate the injury-driven activation of stem cells.

The *C. elegans* genome contains a single gene with homology to the RUNX family of transcription factors as well as a single CBFβ homologue (*rnt-1* and *bro-1*, respectively). *rnt-1* was originally identified by its sequence homology to other known RUNX family members [Lee et al., 2004] and a reverse genetics approach was utilized to generate targeted deletions in this gene [Kagoshima et al., 2005]. Hermaphrodites homozygous for a deletion in *rnt-1* appear grossly phenotypically normal, although male worms exhibited several defects including loss of rays from the tail. *rnt-1* was also

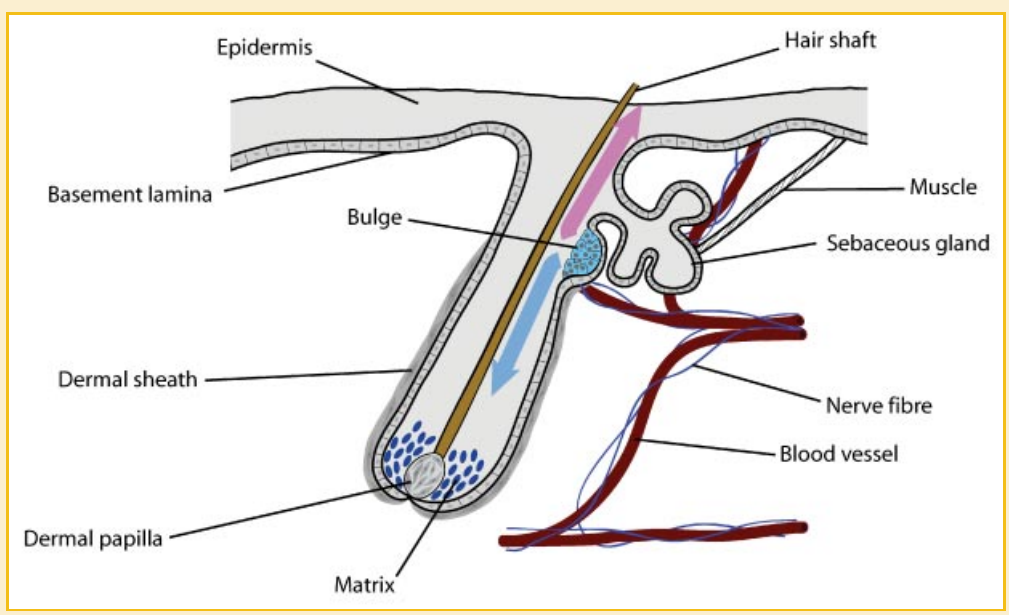

Fig. 1. Role of Runx1 in HFSC activation. Runx1 is required for developmental activation of hair follicle stem cells. Diagrammatic representation of hair follicle showing major structural components. Hair follicle stem cells (HFSCs) reside in the bulge and lie quiescent until stimulated to re-enter the cell cycle. Cues from the dermal papilla, nerves, and blood vessels stimulate quiescent HFSCs to leave the niche and generate transit amplifying matrix cells in a Runx1-dependent manner (blue arrow). Activation of quiescent HFSCs in response to wounding (pink arrow) does not require Runx1 activity. [After Fuchs et al., 2004].

identified at the same time in a forward genetic analysis of the male abnormal (mab) mutant *mab-2*, of interest due to its defects in the stem-like divisions of the V and T seam cell lineages [Hodgkin, 1983]. *mab-2* and *rnt-1* were subsequently shown to be allelic and to act to promote the proliferation of seam stem cells [Nimmo et al., 2005].

The seam cells of *C. elegans* comprise two rows of specialized epidermis with stem cell-like properties; at hatching worms have 10 seam cells per side which divide with a characteristic pattern of asymmetrical (self-renewal maintenance) and symmetrical (proliferative) divisions [Sulston and Horvitz, 1977]. Anterior daughters of asymmetric seam cell divisions mostly differentiate into hypodermal nuclei, which contribute to the body-wide hyp-7 syncytium, although some asymmetric divisions also give rise to neuronal cell types. Posterior daughters of asymmetric division retain the stem fate of further proliferation, eventually giving rise to 16 nuclei per side in hermaphrodites (18 nuclei in the male) at the fourth larval stage (L4) [Sulston and Horvitz, 1977] (Fig. 2). At adulthood, seam cells on each side of the worm terminally differentiate and fuse to form the seam syncytium, incapable of further division. In males, seam cells give rise to additional cell types which form the specialized sensory mating structures (rays) missing in *rnt-1* mutants, and which are derived from the daughters of the posterior-most seam cell lineages, V5, V6, and T. The ability of the seam to self-renew, proliferate and give rise to multipotent daughter cells illustrates the stem cell-like nature of these cells in the worm.

In *rnt-1* mutants, the number of seam stem cells is markedly reduced. Lineage analysis of *rnt-1* mutants revealed various failures of division from L2 onwards, suggesting a role for *rnt-1* in promoting the proliferation of seam cells rather than specification of seam cell identity per se (Fig. 2). Timely exit from the cell cycle is, at least in part, regulated by the KIP/CIP homologue *cki-1*. Worms lacking CKI-1 show hyperplasia of many tissues and expression of a *cki-1* reporter is concomitant with exit from the cell cycle [Fukuyama et al., 2003]. *rnt-1* mutants subjected to *cki-1* RNAi have an increase in seam cell number to near wild-type levels, whereas

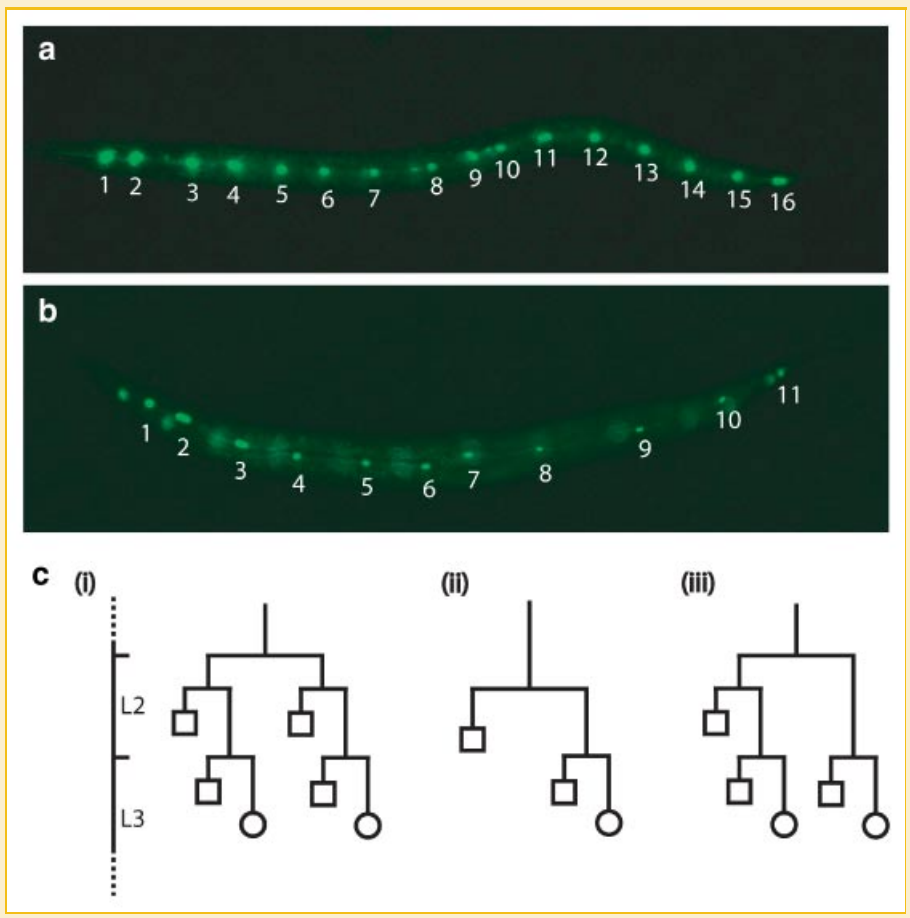

Fig. 2. Reduced stem cell proliferation in *C. elegans rnt-1* mutants. *rnt-1* mutants have failures in seam stem cell divisions, resulting in a decrease in numbers of seam and hypodermal nuclei at adulthood. At the end of the division programme, wild-type (WT) hermaphrodite animals have 16 seam nuclei per side (a). *rnt-1* mutant with decreased number of seam cell nuclei (b). c: Partial lineage traces illustrating division failures commonly observed in *rnt-1* mutants. (i) WT lineage including a symmetrical (proliferative) division at the beginning of the L2 stage, followed by asymmetric divisions at L3 to yield hypodermal (squares), and seam cells (circles). (ii) Failure of the L2 proliferative division in a *rnt-1* mutant leading to a decrease in final seam cell number. (iii) Failure of asymmetric division in the posterior branch of the lineage in *rnt-1* animals, leading to loss of one hypodermal nucleus. Numbered nuclei represent the syncytium on one side of the worm; indistinct green patches represent nuclei in the opposite seam, visible through the worm.

CKI-1::GFP expression is derepressed in a *rnt-1* mutant [Nimmo et al., 2005]. These results suggest that *cki-1* acts to limit seam cell division and that *rnt-1* either directly or indirectly acts to repress the expression of *cki-1* prior to the G1–S phase transition in seam cells that are destined to divide further. Consistent with this, RNT-1 is expressed in the posterior daughter of each asymmetric division, that is, the one that retains the "stem" fate of further proliferation, but not in the anterior daughter whose fate is to terminally differentiate [Sulston and Horvitz, 1977].

A role for *rnt-1* has also been defined in regulating the polarity of division of the T blast cell [Kagoshima et al., 2005]. In wild-type hermaphrodites, this cell divides to give an anterior daughter T.a, which undergoes further divisions yielding mainly hypodermal descendants and one sensory neuron. The descendants of the posterior daughter, T.p comprise three neurons, two structural "socket cells," and a single daughter destined to be lost by programmed cell death. In contrast to the previously described *rnt-1* study by Nimmo et al., the authors argue that RNT-1 is required for the stem cell fate of T cell daughters, in a similar manner to that seen in Wnt signaling defects [Kagoshima et al., 2005]. Thus, in *rnt-1* mutants, posterior T cell daughters appear to sometimes adopt the anterior, hypodermal fate. It could be argued that the failure of T cell daughters to proliferate prevents the acquisition of neuronal identity by seam cell descendants. However, whatever the mechanism by which seam cell numbers are reduced in *rnt-1* mutants, it is certainly apparent that RNT-1 lies at the heart of the decision by seam stem cells to proliferate or differentiate.

A potential CBFβ homologue in *C. elegans* was originally identified by Lee et al. [2004] and found to be 18% identical and 34% similar to mammalian CBFβ, although critical residues required for heterodimerization are well conserved. Deletion alleles for this gene (named *bro-1* after the *Drosophila* homologues brother and big brother) generate a ray-loss phenotype in V- and T-derived rays caused by reduced seam cell proliferation that is strikingly similar to that observed in *rnt-1* mutants [Kagoshima et al., 2007]. An important observation from *C. elegans* studies of *rnt-1* and *bro-1* is that overexpression of either gene leads to seam cell hyperplasia, caused by the symmetrization of various seam cell divisions [Kagoshima et al., 2007]. Most strikingly, overexpression of both RNT-1 and BRO-1 together results in massive hyperplasia, with more than three times the wild-type number of seam cells being produced in a "tumorous" phenotype (Fig. 3). Thus, RNT-1 and BRO-1 act to promote the self-renewal capacity of seam stem cells, at the expense of differentiation.

There is a striking parallel between the function of *rnt-1* in *C. elegans* seam cells and the role of *Runx1* in HFSCs, which goes beyond the simple observation that both stem cell systems are ectodermal in origin and comprise specialized epidermal cells. Like HFSCs, seam stem cell divisions usually occur between long quiescent phases. In the case of the worm, seam cells divide once at the beginning of each larval stage, with an extra symmetrical "amplifying" division at the beginning of larval stage 2 (L2) (in males, a further symmetrical division at the beginning of L3 in the posterior-most seam lineages produces extra progenitor cells in preparation for building sensory structures associated with the male tail). Between divisions, seam cells remain quiescent until the molt

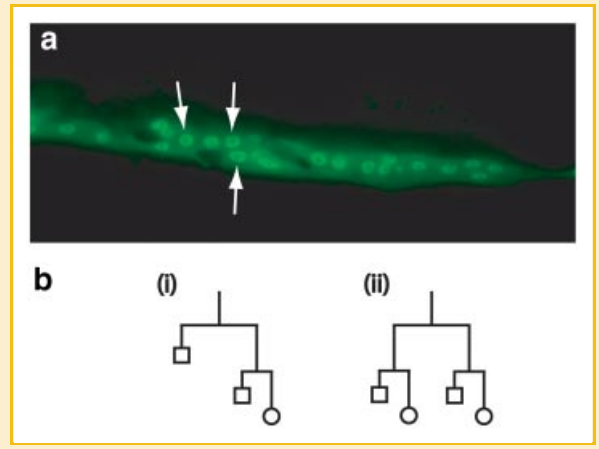

Fig. 3. RNT-1/BRO-1 overexpression-induced seam stem cell hyperplasia. Overexpression of RNT-1 and BRO-1 leads to seam cell overproliferation. a: Worms overexpressing RNT-1::GFP and BRO-1::GFP reporters display hyperplasia of the seam with clusters of multiple seam nuclei (arrows). b: Seam hyperplasia results from extra proliferative (symmetrical) divisions; (i) Illustrative fragment of a WT seam lineage with a seam cell dividing asymmetrically twice to yield two hypodermal nuclei (squares) and a single seam daughter (circle). (ii) Aberrant seam lineage in animal overexpressing RNT-1 and BRO-1 with an extra symmetrical proliferative division yielding an extra seam cell. Multiple proliferative divisions would lead to a huge expansion of seam cell nuclei, as shown in (a).

preceding each larval transition. In *rnt-1* (and *bro-1*) mutants, the transition from stem cell quiescence to proliferation does not occur properly, mirroring the situation in the HF of Runx1 mutant mice. We can even take this analogy a stage further; there is no obvious embryonic defect in either the HFs of Runx1 mutant mice or the seam cells of *rnt-1* mutant worms. *Runx1* mutant mice develop normal hair shafts a few days after birth (although there are mild structural defects of the hair coat consistent with Runx1 expression in the hair cortex [Raveh et al., 2006]) with normally developed HFs. Likewise, *rnt-1* mutant worms are born with the correct number of seam cells in the correct positions [Nimmo et al., 2005]. It is only during the post-embryonic "definitive wave" of development that defects emerge in both systems, with stem cells being unable to proceed properly from quiescence into proliferation. It is suggested that *Runx1* mutant HFSCs fail to respond properly to normal growth signals [Osorio et al., 2008]. Perhaps this is also the case in *C. elegans*, where the growth signal could be provided by the molt, or some other intrinsic timing mechanism. It is intriguing in this respect that the *rnt-1* mutant phenotype of failed seam cell proliferation is enhanced by starvation, particularly at the earliest larval stage [Nimmo et al., 2005]. Perhaps RNT-1 is part of the machinery that transduces nutritional information during development, in order that growth and development can be properly coordinated.

CONCLUSIONS

An emerging body of data thus suggests important roles for RUNX genes in regulating stem cells. Defects in *Runx1* impair stem cell

emergence in blood versus stem cell activation in hair. The solo *C. elegans* RUNX homologue, *rnt-1*, is also required for stem cell activation/proliferation. As with other aspects of RUNX function, however, the data are complex and investigators are often at the mercy of the experimental limitations of their particular system. Overlapping, multifunctional roles for RUNX genes in stem cell biology are a very likely scenario, especially in the case of hematopoiesis. However, the hypothesis that RUNX genes may be an emerging class of "stemness" factor certainly warrants further investigation; *C. elegans* offers simplicity of interpretation and ease/speed of genetic manipulation, coupled with lack of RUNX gene redundancy. Most importantly, the completely described and invariant cell lineage offers the opportunity to study stem cell biology at single cell resolution. However, *C. elegans* does not maintain a somatic stem cell population during adult life, so its value as a model system for analyzing "stemness" may be questioned by some. In terms of being able to probe the molecular basis of RUNX function in promoting stem cell proliferation, however, it has much to offer. Similarly, the *C. elegans* seam stem cell system provides a powerful opportunity to dissect the genetic basis of the transition from quiescence to proliferation and back again (at terminal differentiation). It will be fascinating to test whether or not other molecular components of HFSC activation have a role in seam stem cell proliferation in the worm, and vice versa. Likewise, the investigation of similarities, and differences, between Runx1 function in HSCs and HFSCs promises to be an exciting avenue for future investigation. Studying several different systems in parallel will continue to add enormous value to our understanding of RUNX and stem cell biology.

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