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Review

RUNX factors in development: Lessons from invertebrate model systems

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ABSTRACT

Runt-related (RUNX) transcription factors are evolutionarily conserved regulators of cell proliferation, differentiation and stem cell maintenance. They are critical for the correct development and function of a variety of human tissues, including during haematopoiesis. RUNX genes regulate various aspects of proliferation control, stem cell maintenance, lineage commitment and regulation of differentiation; disruptions in the correct function of RUNX genes have been associated with human pathologies, most prominently cancer. Because of the high context dependency and partial redundancy of vertebrate RUNX genes, invertebrate model systems have been studied in the hope of finding an ancestral function. Here we review the progress of these studies in three invertebrate systems, the fruit fly *Drosophila melanogaster*, the sea urchin *Strongylocentrotus purpuratus* and the nematode *Caenorhabditis elegans*. All essential aspects of RUNX function in vertebrates have counterparts in invertebrates, confirming the usefulness of these studies in simpler organisms. The fact that not all RUNX functions are conserved in all systems, though, underscores the importance of choosing the right model to ask specific questions.

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Introduction

The balance between cell proliferation and differentiation is of fundamental importance for all multicellular organisms. The two processes are usually thought of as mutually exclusive, but this view is an over-simplification. Every asymmetric cell division is a combination of proliferation, as the number of cells increases, and differentiation, as at least one daughter cell adopts a cell fate distinct from its mother's, thus starting its differentiation process. At the heart of differentiation lies the execution of a regulatory programme that is specific to the particular cell type in space and time. A core component of such regulatory programmes are transcription factors, regulating the transcription of a wide array of target genes. These genes can include factors that lead the cell to differentiate in a terminal fashion or components that influence the cell cycle and drive further cell proliferation. Crucially, the regulatory programmes generated by transcription factors can contain examples of both types, leading to cells that continually progress towards their ultimate, fully differentiated fate while still dividing, at least for a time.

Stem cells are of particular interest in the study of proliferation and differentiation. While they play important roles during development, their continued function is crucial for long-lived metazoans, maintaining and replacing a wide range of tissues composed of highly differentiated but short-lived cells, including blood. They depend on the correct regulation of classical, symmetrical proliferation and the mix of proliferation and differentiation of asymmetrical cell division. Disruptions of this regulation often lead to disease, the most obvious being cancer.

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This review will examine data concerning the influence of RUNX family transcription factors on the balance of cell proliferation and differentiation in mammalian haematopoiesis, and will then focus on how results from invertebrate model systems, including those lacking blood, can help us understand critical aspects of their function that, if disrupted, lead to serious disease in humans.

RUNX genes

RUNX genes form a family of transcription factors characterized by the presence of the highly conserved 128 amino acid runt domain [1,2]. The runt domain is required for DNA binding and protein-protein interactions as well as ATP binding. Its name is derived from the first member of the RUNX family to be described in molecular detail, *runt* in *Drosophila melanogaster* [3]. *runt* was first identified as a pair-rule gene in embryonic patterning and later found to play a role in sex determination and neural development [4,5]. *Drosophila* contains three other RUNX genes [6]. *lozenge* is involved in haematopoiesis and eye development [7] and will be discussed in more detail later while not much is known about the remaining two genes, *CG34145* and *CG42267*. The exception to this is the fact that RNAi against *CG42267* (also known as *CG15455*) led to an increase in apoptosis in a *Drosophila* blood cell line, Kc_{167} [8].

There are three RUNX genes in mammals, *RUNX1*, *RUNX2* and *RUNX3*. They were all identified several times independently and have thus a multitude of names, such as *AML1* for *RUNX1*, due to its role in acute myeloid leukaemia (AML). RUNX is the consensus name for the gene family and will be used throughout this article [9]. *RUNX1* is essential for definitive haematopoiesis and is discussed in more detail below. *RUNX2* is crucial for bone formation, with mouse knockout mutants failing to ossify the correctly formed cartilaginous skeleton precursor, dying shortly after birth [10,11]. *RUNX3* has been shown to be required for neuron development in the dorsal root ganglia, thymogenesis and gut development [12–16].

All three mammalian RUNX proteins interact with a binding partner, CBFβ, that increases the DNA binding affinity of the respective RUNX protein as well as protecting it from proteasome-mediated degradation [17]. The interaction between RUNX and CBFβ is mediated by the conserved runt domain, which explains why a single copy of *CBFβ* in the genome is sufficient. So far, only insects have been shown to possess two *CBFβ* genes, *brother* and *big brother* in *Drosophila* [18].

Mutations in all three human RUNX genes (and in *CBFβ*) have been shown to be involved with the onset of various cancers, including AML [19]. Interestingly, they have been shown to act as both oncogenes as well as tumour suppressors [20]. This is a good illustration of one of the fundamental characteristics of RUNX genes, their extreme context dependency. This makes them extremely versatile transcription factors, but complicates the analysis of their effects.

For the purpose of this review we will use the various documented functions of RUNX genes in definitive haematopoiesis to illustrate their functional range across the spectrum between proliferation and differentiation. We then investigate how studies in three invertebrate model systems, the fly *D. melanogaster*, the sea urchin *Strongylocentrotus purpuratus* and the nematode *Caenorhabditis elegans*, can help us understand these functions in more detail.

RUNX genes in haematopoiesis

It has been clear for a while that RUNX1 is essential for definitive haematopoiesis, the second wave of haematopoiesis. Haematopoietic stem cells (HSCs) that are born in the aorta/gonad/mesonephros (AGM) region of the embryo first settle in the foetal liver, then migrate to the stem cell niche in the bone marrow. These HSCs generate the whole blood organ of the body [21,22]. As all the work described below has been executed in mice, the respective nomenclature for gene names will be used. While primitive haematopoiesis is not affected in *Runx1* knockout mice, definitive haematopoiesis does not take place in these animals, and the embryos die before birth [23,24]. Very recent data indicates the exact point at which *Runx1* expression is essential. Several strands of evidence propose that HSCs are derived from haemogenic endothelium, forming intra-arterial clusters in the AGM [25–27]. It was now shown that *Runx1* expression is essential in vascular endothelial cells before they form the intra-arterial clusters and express the first known pan-haematopoietic marker *Vav1* [27]. *Runx1* expression is thus essential before the cells become *bona fide* HSCs.

In addition to this role in definitive HSC formation, *Runx1* plays roles in haematopoiesis at later stages, in stem cell maintenance and lineage maturation, mainly uncovered by studying conditional knockout mice. When *Runx1* is conditionally knocked out once the mice are born, a decrease in the platelets due to a maturation defect in megakaryocytes as well as a block in lymphocyte development (T- and B-cell differentiation) are observed [28,29]. This shows clearly that *Runx1* function is necessary for the correct differentiation of part of the haematopoietic lineage. Additionally, during the development of human T-cells, both RUNX1 and RUNX3 are critical for the determination of the CD4/CD8 lineage choice together with other transcription factors, including GATA3 [12,16,30].

While the data from several groups analysing conditional *Runx1* knockout mice confirm a positive regulatory role of Runx1 in the differentiation of several haematopoietic lineages, it is currently not entirely clear what effect Runx1 has on HSC populations. A comparison between wild-type and haploinsufficient *Runx1* mice concluded that in *Runx1* heterozygous mice the number of long-term repopulating HSCs was halved, but compensated for by an increase in multilineage progenitors [31]. Contrarily, the conditional *Runx1* knockout mice mentioned before showed an increase of their quiescent HSC population, indicating that maintenance of stem cells is not dependent on Runx1 expression, but rather limited by it [32].

In summary, vertebrate RUNX genes have a wide variety of functions, dependent on their context. All of these functions, plus some additional ones, are found in invertebrate model organisms, including the whole spectrum from proliferation control (sea urchin, nematode), stem cell maintenance (nematode) to differentiation and lineage commitment (fly, sea urchin). For the remainder of this review we will therefore look at which model is most suitable to address which question.

Invertebrate systems to study RUNX function

RUNX genes have been extensively studied in invertebrate model organisms, most notably in the fruit fly *D. melanogaster*, the sea urchin *S. purpuratus* and the nematode *C. elegans*. Apart from being less complex organisms than vertebrates, *S. purpuratus* and *C. elegans* in particular have the advantage of fewer (two and one, respectively) RUNX orthologues in their genomes which reduces problems with functional redundancy. This is not strictly the case in flies where, as in most insects analysed so far, gene duplications independent of the ones that led to three RUNX genes in vertebrates have resulted in four RUNX genes [6,33]. However, in all three invertebrate systems expression profiles of RUNX genes, if detected, showed no overlap, making functional redundancies unlikely.

Drosophila blood differentiation

D. melanogaster generate blood cells, haemocytes, in two distinct stages, reminiscent of the situation in vertebrates. In the embryo, prohaemocytes derived from the head mesoderm form two lateral clusters of cells that expand and differentiate into either plasmatocytes (~95%) or crystal cells (~5%). Plasmatocytes migrate along defined paths throughout the developing embryo and already enter