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Live imaging of stem cells: answering old questions and raising new ones

Sangbum Park[1], Valentina Greco[1,2] and Katie Cockburn[1]

Stem cells are essential for both tissue maintenance and injury repair, but many aspects of stem cell biology remain incompletely understood. Recent advances in live imaging technology have allowed the direct visualization and tracking of a wide variety of tissue-resident stem cells in their native environments over time. Results from these studies have helped to resolve long-standing debates about stem cell regulation and function while also revealing previously unanticipated phenomena that raise new questions for future work. Here we review recent discoveries of both types, with a particular emphasis on how stem cells behave and interact with their niches during homeostasis, as well as how these behaviours change in response to wounding.

Addresses
[1] Department of Genetics, Yale School of Medicine, New Haven, CT 06510, USA
[2] Departments of Dermatology & Cell Biology, Yale Stem Cell Center, Yale Cancer Center, Yale School of Medicine, New Haven, CT 06510, USA

Corresponding authors: Greco, Valentina (valentina.greco@yale.edu) and Cockburn, Katie (katherine.cockburn@yale.edu)

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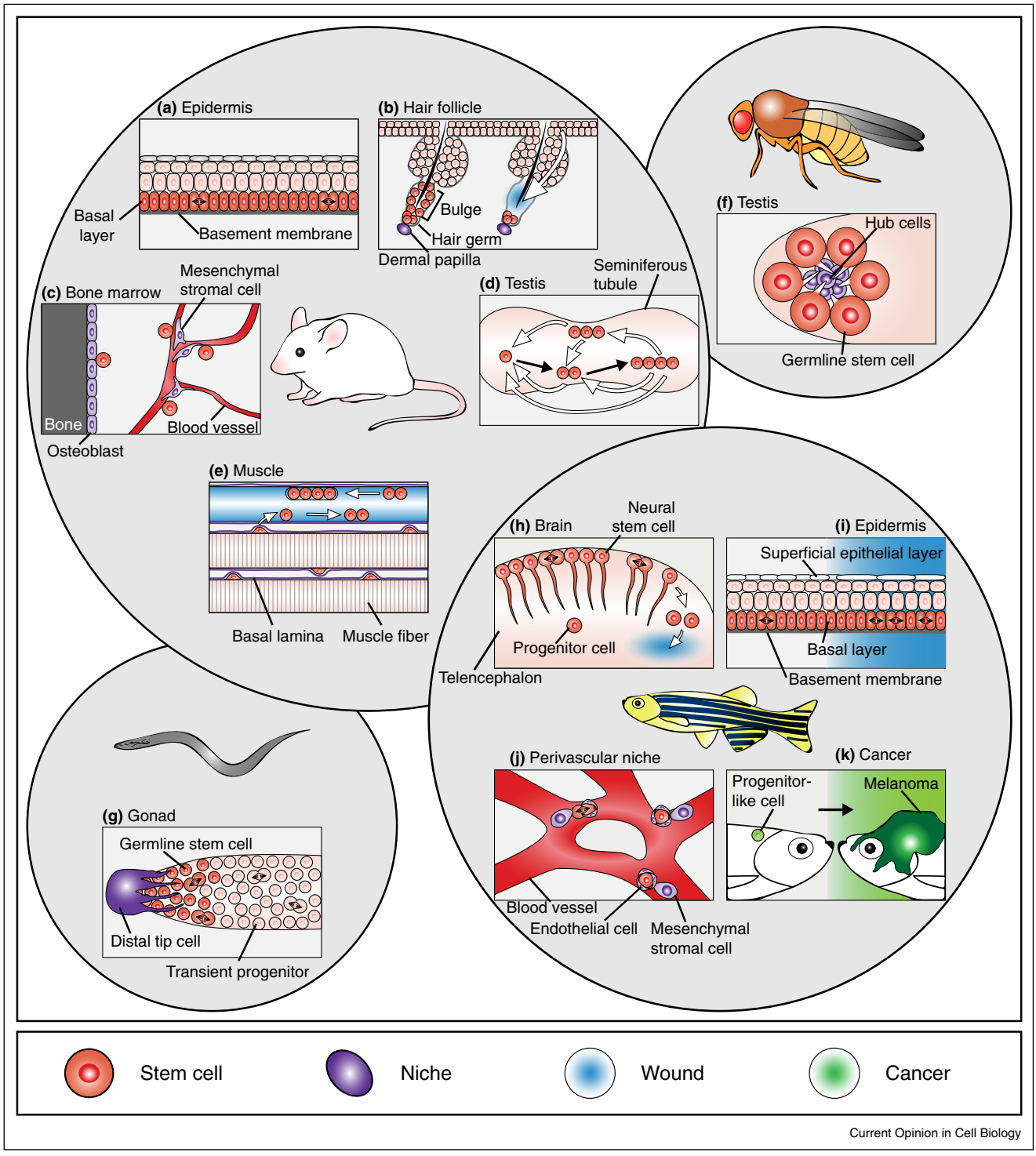
Introduction

Since their initial identification, stem cells have emerged as the key units fuelling tissue maintenance and repair. Over the years, efforts to characterize these unique cell types and to understand how they sustain lifelong tissue turnover have benefitted from a wide variety of experimental technologies. Of particular significance in recent years has been the advent of various live imaging approaches: the arrival of new imaging modalities such as multiphoton and light sheet microscopy, the improved brightness and versatility of genetically encoded fluorophores, and the development of quantitative strategies for digital image analysis. Together, these technologies have greatly improved our ability to visualize and follow living tissues, cells, and even single molecules over time. The resident stem cells within different tissue types and in many model organisms can now be imaged over time in their natural environments (Figure 1). As with any technological advances, the application of live imaging approaches to the study of stem cells provides two fundamental advantages: the ability to address questions in the field that have previously been unanswerable, and the capacity to discover entirely novel phenomena whose existence have not even been hypothesized. Here we review recent insights of both flavours provided by live imaging of tissue-resident stem cells.

Following stem cell behaviours over time

One of the main ways that live imaging has contributed to the stem cell field is also one of the most conceptually straightforward: it has allowed the behaviour of individual stem cells to be followed over time during the process of tissue turnover. Stem cells have two fundamental roles during tissue maintenance: to replenish the differentiated cell types that are lost during normal turnover, and to renew themselves over time. How these two tasks are achieved, and how they are balanced at the tissue-wide level to achieve homeostasis remain an outstanding question in the field. Traditionally, these problems have been addressed by labelling groups or individual stem cells of interest, followed by fixation and visualization of resulting progeny at later timepoints [1,2]. More recently, live imaging studies have extended these approaches by enabling the behaviours of individual stem cells to be not just inferred but directly observed as they generate differentiated progeny and self-renew. For example, lineage analysis of fixed samples in the brain has led to conflicting hypotheses about whether individual neural stem cells (NSCs) can self-renew indefinitely [3] or whether they might become depleted over time [4,5]. In the adult zebrafish brain however, multiphoton imaging has recently allowed individual NSCs to be followed for periods of up to one month [6,7••] (Figure 1h). Because of morphological differences between radial NSCs and their more differentiated non-radial progeny, simple changes in cell shape, number and position can be used to determine how these cells both self-renew and contribute to neurogenesis. Strikingly, such imaging has revealed that while the majority of proliferative NSCs sustain their numbers via asymmetric divisions, other NSCs differentiate directly into neural progenitors [7••], supporting the hypothesis that individual NSCs can become

Figure 1



Recent insights gained from live imaging of tissue-resident stem cells. **(a)** The underlying basal layer of the mouse epidermis is composed of an equipotent population of stem cells that move upwards to replace differentiated cells lost during homeostasis. **(b)** In the mouse hair follicle, stem cell behaviours such as proliferation and apoptosis are localized in gradients in relation to the underlying mesenchymal niche. When these stem cells are ablated, they are functionally reconstituted by neighbouring epithelial cells. **(c)** In the mouse bone marrow, HSCs may exist in a peripheral environment surrounded by osteoblasts and endothelial cells, as well as in additional niches deeper inside the marrow. **(d)** In the mouse germline, spermatogonial stem cells interconvert between single cell and syncytial states, both of which have the potential to differentiate or self-renew. **(e)** Upon injury in the mouse muscle, quiescent stem cells become activated and divide and migrate along the longitudinal axis of ECM remnants from previous muscle fibres. **(f)** In the Drosophila male germline, nanotubes extend from GSCs to their neighbouring niche cell, and are