further careful investigation. For example, although the authors found that Ang1 increased the proportion of quiescent MyoD$^-$ cells in differentiating myogenic cultures, this effect was most marked when the stimulus was added at later time points. In contrast, asymmetric mitoses that produce both MyoD$^+$ and MyoD$^-$ daughters have been observed mainly during the initial cell divisions of satellite cell cultures (Zammit et al., 2004; Kuang et al., 2008). It may be that the Ang1/Tie2-induced entry into quiescence is operating on cells in which the decision to stay in the stem cell pool has already been made during the earlier asymmetric division. Another possibility is that Ang1 induces the MyoD$^-$ quiescent state in both preordained stem cells and cells that are on the path to terminal differentiation.

The demonstration that nonmyogenic cells regulate myogenic cells via shared mechanisms raises the possibility of reciprocity between the myogenic and nonmyogenic populations. Our practical interest in the mechanisms of repair of skeletal muscle is largely powered by the desire to mitigate the suboptimal outcome seen in chronic conditions of muscle degeneration and repair, as in the muscular dystrophies. Derangement of the web of mechanisms described in this article surely plays some central role in the progressive failure of regeneration and loss of normal muscle structure and function that occur in these chronic conditions. It has been suggested, for example, that the progressive fibrosis that characterizes severe dystrophies is inimical to muscle regeneration. This inhibition is commonly attributed to straightforward physical obstruction, but the observation that Ang1 secreted by fibroblasts can induce satellite cell quiescence identifies this population, and the pathway, as candidates for targeted therapeutic strategies. One might also ponder whether factors from the satellite cells impinge reciprocally on the behavior of their smooth muscle, fibroblast, and endothelial neighbors, not to mention their previously identified interactions with patrolling cells of the monocyte macrophage series (Chazaud et al., 2003).

Plainly, effective function of a stem cell requires exquisite attunement to its environment, particularly when, as in the case of the skeletal muscle satellite cell, it is obliged to spend most of its time soundly asleep while remaining responsive to sporadic "rude awakenings" evoked by muscle damage. We should not be surprised, therefore, at the subtlety and complexity of intercellular and autologous signaling activity that is being revealed by the investigations of the Chazaud group in consequence of an inclusive approach that goes beyond simply examining the actions of stem cells in isolation and that embraces the multifarious influences of the cellular community.

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Growth Signaling at the Nexus of Stem Cell Life and Death

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Stress can activate tumor-suppressive mechanisms, causing the loss of adult stem cell function with age. In this issue of *Cell Stem Cell* and in *Nature*, Castilho et al. (2009) and Harrison et al. (2009) highlight the importance of mTOR signaling in stem cell exhaustion and mammalian aging, respectively.

The process of aging is characterized by the reduced capacity to maintain tissue homeostasis and repair damaged tissues after injury. Physiological aging appears to be due, in part, to a decline in the regenerative capacity of adult tissue stem cells. Loss of stem cell function with age can result from cell-intrinsic changes as well as environmental factors. For example, the accumulation of DNA damage in melanocyte stem cells (MSCs) triggers their differentiation, leading to the

depletion of the MSC pool and the graying of hair (Inomata et al., 2009). Similarly, the accumulation of DNA damage as well as potential epigenetic changes in hematopoietic stem cells (HSCs) reduce their function with age, a reduction that is related not only to changes in HSC number but also to changes in their mobilization, homing, and differentiation (Rossi et al., 2008). Finally, the aging of satellite cells of muscle can be reversed by exposure to a young blood supply, suggesting that cell-extrinsic, microenvironmental factors may also play a role in the impaired function of certain stem cell populations with age (Conboy et al., 2005).

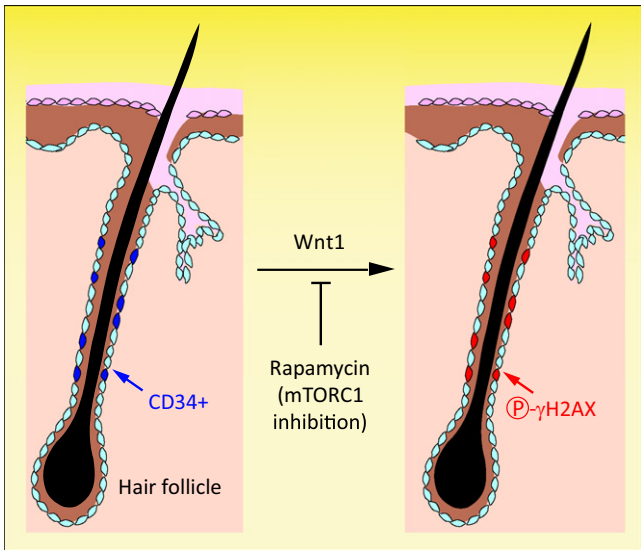

Figure 1. Simplified Schematic Depicting Hair-Follicle Stem Cell Exhaustion Mediated by Wnt1 Overexpression

Overexpression of Wnt1 causes rapid hair-follicle hyperproliferation (not shown) followed by the loss of $CD34^+$ hair-follicle stem cells (blue) and the appearance of nuclear phosphorylated γH2AX foci (red) indicative of DNA double-strand breaks, a marker of senescence. Wnt1 overexpression also promotes the expression of endogenous β-galactosidase at pH 6, another characteristic of senescent cells, and progressive hair loss. Hair-follicle hyperproliferation, the loss of $CD34^+$ hair-follicle stem cells, and the appearance of senescence markers can be largely reversed by pharmacological inhibition of mTORC1 with rapamycin.

Recent evidence suggests that stress resulting from DNA damage, oxidative stress, telomerase dysfunction, or persistent growth signaling can lead to the loss of stem cell function by activating tumor-suppressive mechanisms that lead to senescence (Rossi et al., 2008). It is known that stem cells from multiple tissues express senescence markers with age. Further, studies addressing the functional role of tumor-suppressive mechanisms in aging demonstrated in HSCs, neural stem cells (NSCs), and pancreatic islet stem cells that not only do levels of $p16^{INK4a}$ (a known mediator of senescence) increase with age but also that deficiency of this tumor suppressor partially attenuated the age-induced replicative failure of these tissues (Janzen et al., 2006; Krishnamurthy et al., 2006; Molofsky et al., 2006). Addressing the role of oncogenic growth signaling in stem cell function, Yilmaz and colleagues demonstrated that deletion of *Pten*, which negatively regulates proliferation and survival through the phosphatidylinositol-3-OH kinase [PI(3)K] pathway, led to the short-term expansion of HSCs followed by their depletion. Most of these effects were dependent on downstream signaling through the mammalian target of rapamycin (mTOR), given that pharmacological inhibition of mTOR with rapamycin prevented the phenotypic reduction in HSCs and rescued most of their in vivo functions (Yilmaz et al., 2006). Although the mechanisms responsible for stem cell depletion observed in the above examples were not conclusively elucidated, and may have involved senescence as well as altered self-renewal or differentiation, these studies nevertheless suggest a general theme wherein tumor-suppressive mechanisms play an important role in regulating stem cell function with age.

In this issue of *Cell Stem Cell*, Castilho and colleagues expand on this theme by demonstrating that persistent growth signaling (mediated by Wnt1 overexpression) in hair follicles (HFs) causes aggressive HF growth followed by epithelial cell senescence, loss of the epidermal stem cell compartment, and progressive hair loss (Castilho et al., 2009). Interestingly, although Wnt1 expression led to activation of both β-catenin and the mTOR pathway, hair-follicle hyperproliferation and stem cell loss could be largely reversed with rapamycin, suggesting that mTOR plays a dominant role in this process (Figure 1). This work extends the principle that sustained mTOR signaling can lead to the loss of stem cell function to a tissue outside of the hematopoietic system. Further, although the authors cannot conclusively rule out the role of other processes in contributing to the observed loss of hair-follicle stem cells, their results suggest that senescence plays a prominent role as a protective mechanism to prevent tumor formation in the face of persistent mTOR activation.

Together, this body of work suggests a delicate balance between the process of aging, which is mediated in part through tumor-suppressive mechanisms that lead to the loss of stem cell function, and cancer, which occurs in the absence of such tumor-suppressive mechanisms. Moreover, it also suggests a particularly important role for the mTOR pathway in aging and stem cell exhaustion. This begs the question of whether the blockade of senescence-inducing signals such as those produced by persistent mTOR activation can lead not only to the preservation of individual tissue stem cell populations but also to the slowing of organismal aging. This hypothesis is bolstered by previous observations that TOR inhibition can lead to lifespan extension in invertebrates including yeast, nematodes, and fruit flies (Schieke and Finkel, 2006). Addressing this question for the first time in mammals, a recent study in *Nature* found that rapamycin, when fed to mice beginning at 600 days of age, extends median and maximal lifespan in both males and females (Harrison et al., 2009). On the basis of age at 90% mortality, rapamycin led to a 14% increase in female lifespan and a 9% increase in males. Similarly, when rapamycin treatment began at 270 days, increased survival was also observed in both genders. These highly significant findings reveal a role for mTOR signaling in the regulation of mammalian lifespan as well as demonstrate pharmacological lifespan extension in both genders.

These findings raise many important new questions. For example, was the observed lifespan extension in mice caused by delaying deaths from cancer, delaying the mechanisms of aging, or both? Does mTOR inhibition decrease stem cell senescence or increase the function of various tissue stem cell populations in comparison with age-matched controls? Perhaps most importantly, what are the molecular mechanisms connecting mTOR and aging? Potential clues to this question come from our understanding of mTOR biology. The mTOR kinase nucleates two distinct signaling complexes, mTORC1 and mTORC2, one of which (mTORC1) is allosterically inhibited by rapamycin. The activation of mTORC1 in response to a broad range of progrowth signals is known to regulate mitochondrial activity, which itself has been shown in several studies to be involved in the regulation of lifespan. Mitochondria may exert this influence through the generation of intracellular reactive oxygen species (ROS) (Schieke and Finkel, 2006), which, in previous work, have been suggested to contribute to HSC exhaustion (Tothova et al., 2007). The connection between mTOR and ROS production is far from understood at the molecular level, but these types of findings point to a potential direction for future research into the role of mTOR signaling in aging and stem cell function.

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Lineage Commitment: Cytokines Instruct, At Last!

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Instructive and permissive models of commitment have been proposed for hematopoietic cytokines. In recent issues of *Science* and *Cell*, Rieger et al. (2009) and Sarrazin et al. (2009) together show that cytokines can instruct lineage choice.

Multipotent hematopoietic stem cells (HSCs) with the ability to reconstitute the blood and lymphoid lineages long term and to self-renew are predominantly quiescent, dividing only in response to demand for blood cells (Wilson et al., 2008). Both multilineage and lineage-specific cytokines synergistically regulate the proliferation and differentiation of these long term (LT)-HSCs that express receptors for several of the lineage-specific cytokines. A major area of conjecture in the HSC field has been whether cytokines can "instruct" lineage commitment (Metcalf, 1998) or whether their actions are simply "permissive" (Enver et al., 1998), allowing the survival and expansion of already committed cells. Although both cell-autonomous transcription factors (Laiosa et al., 2006) and the activation of ectopically expressed cytokine receptors (Kondo et al., 2000) have been shown to instruct lineage choice, it has been technically difficult to demonstrate an instructive action of cytokines in nonengineered cells. Two recent papers that used novel approaches (Rieger et al., 2009; Sarrazin et al., 2009) convincingly demonstrate that cytokines can instruct.

Macrophage CSF (M-CSF), also known as colony stimulating factor-1 (CSF-1) and granulocyte CSF (G-CSF), generate clones from cultured hematopoietic cells that are composed almost exclusively of macrophages (Ms) or granulocytes (Gs), respectively. Using novel bioimaging approaches that permit continuous long-term observation at the single-cell level (Eilken et al., 2009), Rieger et al. (2009) used time-lapse movies of cultures of purified bipotent granulocyte/monocyte progenitors (GMPs) to follow the fate of unselected progenitors as they differentiate to either Ms or Gs. GMPs, which do not express lysozyme, were obtained from Lys::GFP mice in which enhanced green fluorescent protein expression is