rates of each of the clinical steps involved. The treatment would require IVF, blastomere biopsy, temporary cryopreservation of biopised embryos, derivation of ESC lines and their cryopreservation, and then selective thawing and uterine transfer of embryos with genetically matching hESC lines. There is currently no demonstrated advantage over cord blood banking—a much less invasive and easier procedure.

The political motivation is also problematic. In the United States, federal funding of hESC derivation is prohibited unless "no harm" is done to the embryo. It is unclear whether this new technique will satisfy the ethical criterion of no harm, and statements from the administration that followed online publication of the article were equivocal at best. Paradoxically, it is the same no harm stimulus that may scupper federal approval. Thus, the same political motivation is also driving the high investment in efforts to derive induced pluripotent stem cells (iPSCs) from skin cells. This technology is fraught with dangers, including oncogenesis, but its promotion as a potential alternative to the use of human embryos may provide a potent disincentive to approve use of this new technique.

Perhaps it is time that consumer societies faced the fact that IVF, PIGD, and associated reproductive technologies (ARTs) all involve research on and destruction of human embryos. Attempts like those above, and others involving genetically disabling an embryo (Meissner and Jaenisch, 2006) or attempting rescue of living blastomeres from otherwise "moribund" human embryos (Lerou et al., 2008), may have been ingenious responses to political pressures, but these approaches basically ignore these central facts about ART. If these new ARTs are acceptable ethically as well as clinically, then banning hESC derivation seems ethically inconsistent. Perhaps it would be more honest to think through, and even to question, the biological basis of the belief in the early embryo's status, which rests on the view that human identity is fundamentally genetic (Johnson, 2001). In the age of epigenetics, this view is becoming increasingly difficult to defend both scientifically and ethically.

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A New Role for an Old Friend: NFAT and Stem Cell Quiescence

Anthony E. Oro[1,*]
[1]Program in Epithelial Biology, School of Medicine, Stanford University, CCSR 2145, 269 Campus Drive, Stanford, CA 94305, USA
*Correspondence: oro@stanford.edu
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NFAT proteins are calcium-regulated transcription factors that play a critical role during the timing and activation of many vertebrate tissues. A recent paper in *Cell* (Horsley et al., 2008) demonstrates a role of the calcineurin-NFAT-CDK4 pathway in maintaining hair follicle stem cell quiescence.

"Knowledge of the hair cycles and their control will undoubtedly give needed insight into many complicated growth processes of other body structures which are not at present well understood."

—Earl O. Butcher (Butcher, 1934)

Despite their architectural and functional diversity, epithelial organs or appendages share common developmental strategies, including periodic self-renewal through the reactivation of multipotent progenitor cells. As Butcher foretold, the study of organ stem cell activation and regeneration has been a central issue in developmental biology and medicine for nearly a century. The hair is an ideal system to study the regulation of stem cell quiescence because stem cell identity and location (Morris et al., 2004; Tumbar et al., 2004) are well-characterized and

the cells can be purified in large quantities. Hair follicles are easily visualized and possess an internal clock that allows them to cycle with fidelity about every 2 weeks in the mouse (about 2 years in humans) (Stenn and Paus, 2001).

Seventy-four years after the observations of Butcher, a paucity of information exists about the regulators of hair follicle quiescence. Intensive study of hair cycling has shown that proper growth and patterning require key morphogens such as Sonic hedgehog, Wnt, and bone morphogenetic protein (BMP) at the right times and places. One recent clue revealed that the BMP pathway plays a critical role in maintaining stem cell quiescence. Previous work by a number of labs demonstrated that loss of the BMP receptor or overexpression of BMP inhibitors can activate stem cells prematurely, although how BMP signaling regulates quiescence was not understood (Botchkarev and Sharov, 2004). A paper in a recent issue of *Cell* identifies the NFAT-calcium-CDK4 signaling pathway as a central regulator of stem cell quiescence and unifies several long-studied aspects of hair cycling (Horsley et al., 2008).

NFAT proteins are calcium-regulated transcription factors related to the Rel family that play a critical role during the timing and activation of many vertebrate tissues (Wu et al., 2007). The four major NFATc proteins are kept inactive in the cytoplasm by phosphorylation of their nuclear import signals by regulatory kinases such as Dyrk1, GSK3B, or PKA. Stimuli that raise intracellular calcium levels activate the trimeric calcium/calmodulin-dependent phosphatase calcineurin to remove phosphatases from NFATc. Dephosphorylated NFATc is able to move into the nucleus where it dimerizes with various transcriptional partners (NFATn proteins) to induce growth factors, cytokines, and adhesion molecules. The relationship between calcineurin and NFAT signaling in the immune system has been exploited clinically to develop calcineurin inhibitors such as cyclosporine or FK506 that are potent immunosuppressive agents (Schreiber and Crabtree, 1992).

The role of NFATc as a signal integrator of widely varied pathways comes from at least three mechanisms. First, a wide variety of stimuli can raise intracellular calcium and activate calcineurin, including growth factor receptors, voltage-dependent ion channels, and gap junctions. Second, the DNA binding domain structure of NFATc requires interaction with other DNA binding domains for high-affinity binding. This requirement allows interactions with other signaling pathways at the level of DNA, thus connecting NFAT/calcium signaling to pathways such as AP1. Third, although NFAT activation occurs in response to growth factor signals, NFAT in turn induces a myriad of growth factors and receptors itself to amplify the initial signals.

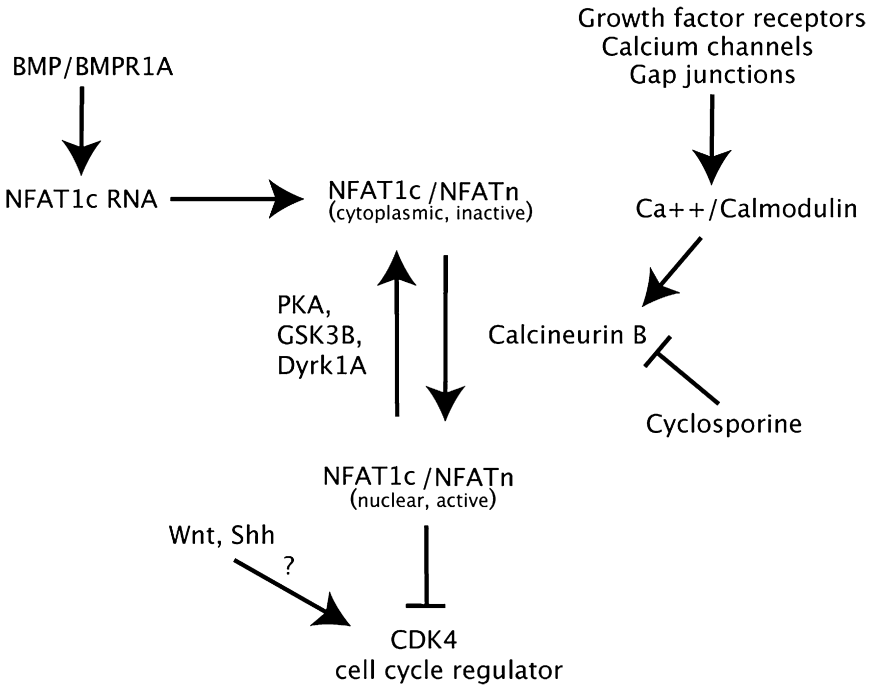

Figure 1. NFATc1 Signaling at the Nexus of Stem Cell Quiescence
NFATc1 RNA accumulates in response to BMP signaling, whereas NFATc1 localization and activity are controlled by intracellular calcium levels regulating the serine phosphatase calcineurin. NFATc1 maintains hair follicle stem cells in a quiescent state in part by repressing the cell-cycle regulator CDK4. Although overexpressions of Wnt and Shh are known to induce follicle cycling, they do so without altering the localization of NFATc1, suggesting an alternative mechanism.

Recent work culminates 2 decades of experimentation on the role of NFAT/calcineurin in hair follicle activation. Initial interest in calcium/calcineurin signaling in the epidermis came from the observation that increased calcium triggers epidermal differentiation but appears to inhibit hair follicle cycling. This calcium switch idea drew additional support from the initial observations that cyclosporine caused hair cycling apart from its effects on the immune system in mice and humans (Sawada et al., 1987). Further studies using conditional mutants implicated NFAT signaling in hair cycling by the demonstration that *calcineurin* B mutants showed cycling alopecia (Mammucari et al., 2005).

The present work shows that NFATc1 is the key NFAT regulating hair follicle stem cell quiescence. The epidermis lacking NFATc1 develops normally, but after initiating telogen, it prematurely enters the next anagen. The observation that stem cell and differentiation markers are not affected in the mutant mice suggests that NFATc1 acts specifically on hair cycling. The relationship of NFAT signaling to other hair follicle regulators is elucidated, as NFAT transcription appears to lie downstream of BMP signaling. Loss of BMP receptor results in loss of NFAT accumulation in the CD34+ stem cells. One difference between BMP and NFAT mutants is that, in *BMPR1A* mutants, the epithelium not only loses quiescence but also fails to differentiate. This suggests that BMP-mediated differentiation does not require NFATc1. Finally, the authors show that treatment with cyclosporine results in dramatic decreases in NFATc1 nuclear levels, consistent with its predicted mechanism of action. This elegant work shows that the NFAT-calcineurin pathway

lies at the nexus of a variety of previously known hair follicle regulators (Figure 1).

Butcher predicted that knowledge of hair cycling would provide insight into the workings of other organs. Studies of NFAT, a well-characterized signaling pathway in the immune system, have revealed important details regarding hair cycling and have also stimulated many additional questions. Future studies will need to identify other environmental influences that effect pathway-mediated quiescence. In particular, because NFAT nuclear activity depends on intracellular calcium levels, a greater understanding is needed of calcium regulation in stem cells. Moreover, the role of other known hair cycle regulators, such as Sonic hedgehog or Wnt, in regulating stem cell cycling needs to be further elucidated. Wnt-induced stem cell activation occurs in the presence of nuclear NFAT, suggesting the existence of an NFAT-independent pathway for overcoming quiescence. Finally, BMP-dependent NFAT expression affects cycling, but not differentiation, arguing for the existence of an unidentified NFAT-independent differentiation pathway downstream of BMP. With the current pace of advances in understanding how hair follicle stem cells are regulated, studies of hair cycling will likely contribute greatly to studies of how other stem cells are regulated as well.

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How Do Mesenchymal Stromal Cells Suppress T Cells?

Armand Keating[1,*]
[1]Princess Margaret Hospital, 610 University Avenue, Suite 5-303, Toronto, ON M5G 2M9, Canada
*Correspondence: armand.keating@uhn.on.ca
DOI 10.1016/j.stem.2008.01.007

Accumulating information indicates that mesenchymal stem or stromal cells (MSCs) are immunomodulatory, but the data to explain the observations are frequently conflicting. In this issue of *Cell Stem Cell*, Ren et al. (2008) provide evidence for a possible underlying mechanism of MSC-mediated T cell suppression. A perspective for considering these interesting observations is discussed.

Mesenchymal stem cells, or as they have been termed more recently, mesenchymal stromal cells (MSCs) (Horwitz et al., 2005) are a fascinating component of the microenvironment in the bone marrow and other tissues, and for many years they were thought to have a predominantly supportive role. The focus of studies with MSCs over the past decade, however, has shifted dramatically, first to their possible role in tissue regeneration via broad, multilineage differentiation potential, and more recently, to a further characterization of their immunomodulatory properties (Keating, 2006; Nauta and Fibbe, 2007).

A better understanding of the mechanisms underlying the immunomodulatory effects of MSCs is important on two counts. First, it may uncover a poorly understood arm of the immune system, and second, it carries profound therapeutic implications. MSCs have the potential to effectively treat immune-mediated diseases refractory to front-line medical therapy, including acute graft-versus-host disease (GvHD), a frequently serious condition that occurs after allogeneic hematopoietic cell transplantation. This area of investigation, however, and the basis of MSC-mediated T cell suppression in particular, has been bedeviled by conflicting data.

The study by Ren et al. (2008) in this issue provides a timely and important contribution to the ongoing debate about how MSCs suppress T cells and enables a clearer picture of the mechanism to emerge. Previous work by several groups had established that MSCs inhibit the proliferation of T cells induced by alloantigens and nonspecific mitogens and that the inhibition is not genetically restricted (reviewed in Keating, 2006). Moreover, a number of studies show that the suppression is variably sustained in transwell experiments, suggesting that a soluble factor(s) is involved, although other investigators claim a requirement for MSC-T cell contact (Krampera et al., 2003). Results have also been divergent in other in vitro experiments: one group implicated transforming growth factor-β (TGF-β) and