Ngn3 is both necessary and sufficient to induce endocrine islet cell differentiation from endodermal progenitor cells during embryogenesis. Because robust *Ngn3* expression has not been detected in hormone-expressing pancreatic islet cells, Ngn3 is utilized as an endocrine progenitor marker and regarded dispensable for the function of differentiated islet cells. Thus, detection of *Ngn3* expression in the adult pancreatic cells was interpreted as evidence of the presence of endocrine progenitors or stem cells. Here we utilized *Ngn3-CreER* knock-in reporter mice and mRNA/protein-based assays to examine *Ngn3* expression in hormone-expressing islet cells. We showed that Ngn3 mRNA and protein are detected in hormone-producing cells at both embryonic and adult stages. Significantly, inactivating Ngn3 in insulin-expressing β cells at embryonic stages or in Pdx1-expressing islet cells in the adults impairs endocrine function, a phenotype that is accompanied by a reduced expression of several Ngn3 target genes that are essential for islet cell differentiation, maturation, and function. These findings demonstrate that Ngn3 is required for not only initiating endocrine cell differentiation, but also islet cell maturation and functional maintenance, and Ngn3 production in the adult pancreatic cells cannot be utilized as an endocrine progenitor marker.

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Program/Abstract # 406
Erythroid development in the absence of hemoglobin
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The mammalian erythrocyte is a highly specialized blood cell that differentiates via an orderly series of committed progenitors in the bone marrow in a process termed as erythropoiesis. In mature red blood cells over 95% of the protein is hemoglobin (Hb) that consists of two alpha and two beta globin polypeptide chains. What happens during erythropoiesis in the absence of hemoglobin? To answer this question, we generated homozygous alpha and beta globin knockout (Null Hb) embryos, adult chimeric mice using novel Null Hb embryonic stem cells (Null Hb ES), and an in vitro ES cell derived erythroid progenitor (ES-EP) culture system. Null Hb embryos died at ~12.5 d in utero. Committed Null Hb erythroid progenitors were present, but did not differentiate beyond the basophilic erythroblast stage. EKLF was tagged by EGFP to track Null Hb ES cells derived from erythroid cells in chimeras. Analysis of adult chimeric bone marrow revealed that Null Hb derived white blood cells developed normally, but the erythroid lineage was again blocked at the basophilic erythroblast stage. In vitro Null Hb ES-EP cultures could support the growth and expansion of Null Hb proerythroblasts; however, upon terminal differentiation Null Hb ES-EP cells undergo apoptosis and cell death. Expression of human myoglobin targeted to the beta globin locus in Null Hb ES cells could rescue erythroid development in the bone marrow of chimeras. These experiments demonstrate that Hb is not necessary for erythroid lineage commitment, is required for terminal erythroid differentiation, and that human myoglobin can rescue erythroid development in the absence of Hb.

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Program/Abstract # 407
Basal cells as stem cells of the mouse trachea and human conducting airways
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The epithelial cells of the respiratory epithelium of mice and humans, constantly exposed to inhaled toxins and pathogens, are maintained over the long term via controlled division of adult tissue stem cells. We have demonstrated that basal cells (BCs) of the mouse trachea give rise to both Clara and ciliated cells by in vivo lineage tracing. Furthermore, we have developed a novel culture system to assay the self-renewal and differentiation of BCs. To identify mechanisms that regulate these behaviors, we have purified BCs by fluorescence activated cell sorting and performed microarray analysis. Using mutant mice and in vitro assays, we are currently testing the hypothesis that genes expressed at high levels in BCs, including transcription factors, signaling molecules, and cytoskeletal components, control their proliferation, differentiation, and motility both at a steady-state and in response to epithelial injury. Finally, we have determined that p63-expressing cells are present even within the smallest conducting airways of humans. Characterization of this stem cell population in mice and humans should enhance our understanding of pathological conditions of the airways including chronic asthma, chronic obstructive pulmonary disease, and cancer.

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Program/Abstract # 408
Bone marrow-derived macrophages fuse with intestinal epithelium in the stem cell niche after injury
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Adult bone marrow-derived cells (BMDCs) can engraft into damaged intestinal epithelium of mice representing a potential avenue for facilitating tissue regeneration. The underlying mechanism for this BMDC engraftment occurs by cell fusion, analogous to cell fusion that occurs during development. We previously identified the intestinal stem cell as the fusion target, but the marrow-derived fusion partner remains unknown. Here we identified the macrophage population as the primary BMDC fusion partner by isolation and transplantation of discrete hematopoietic lineages into recipient mice. Transplantation of isolated macrophages supported robust intestinal epithelial fusion at levels equivalent to whole bone marrow. Additionally, a close examination of the time course for cell fusion reveals that macrophages are among the first cell types recruited to the intestine after injury and surround the stem cell niche. Interestingly, the fusion hybrid cells are not multinucleate, indicating they may be reprogrammed. Indeed expression of macrophage genes was sustained in long-lived fusion hybrids. These studies are the first to illustrate the critical temporal window for visualization of cell fusion after injury. Importantly, understanding the timing and cellular players involved in cell fusion establishes the foundation for further investigations into the molecular mechanism. Establishing the temporal dynamics of fusion and subsequent genetic reprogramming in cell fusion hybrids may provide insight into the physiologic impact of cell fusion in regeneration and susceptibility to disease.

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Program/Abstract # 409
Identifying gene regulatory networks that control adult regeneration in zebrafish
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