Portrait of a Stem Cell

There is great enthusiasm for the potential use of stem cells in treating tissue degenerative disorders, but little is known about the intrinsic molecular programs defining self-renewal and differentiation. New data sets produced by transcriptional profiling of purified stem cell populations begin to establish the nature of "stemness."

Stem cells (SC) can undergo asymmetric cell division (see Figure) in which one daughter adopts the mother SC fate and the other elects to differentiate. The ability of hematopoietic stem cells (HSC) to reconstitute the entire immune system of a lethally irradiated animal for life demonstrates the capacity of SCs for both self-renewal and differentiation. In 1961, Till and McCulloch proposed a stochastic model for the commitment of the hematopoietic system (Till and McCulloch, 1961), wherein the decision to self-renew or differentiate is random. In this model, the probability of this event is predictable only for an entire population, but not for an individual cell. A more modern model for SC fate suggests that SCs express a low level of lineage-affiliated genes, which are amplified or repressed upon differentiation (the "multi-lineage priming" model; May and Enver, 2001). Genetic experiments in *Drosophila* and mice suggest that SC number is controlled by microenvironments termed "niches" that regulate asymmetric division.

Many different genes have been implicated in the intrinsic and extrinsic programs leading to asymmetry. In fly neural stem cells (NSC), Numb, which is unequally partitioned in the dividing NSC, inhibits Notch activity in the inheriting daughter cell, thus allowing differentiation into a ganglion (reviewed in May and Enver, 2001). The environment is also known to regulate stem cell behavior by providing external cues (Hackney et al., 2002). Hedgehog, cytokine-induced JAK/STAT, and BMP signaling have been shown to regulate SC number (reviewed in Spradling et al., 2001). In mice, loss of the downstream Wnt effector, Tcf4, causes loss of intestinal SCs, suggesting that Wnt signals are required for SC maintenance (Korinek et al., 1998). Additionally, each organ SC population is imbued with lineage-specific transcription factors, such as the *scl* or *runx1* genes in blood that are required for HSC production (reviewed in Orkin and Zon, 2002). Despite these recent advances, the intrinsic program induced by environmental cues has not been molecularly described.

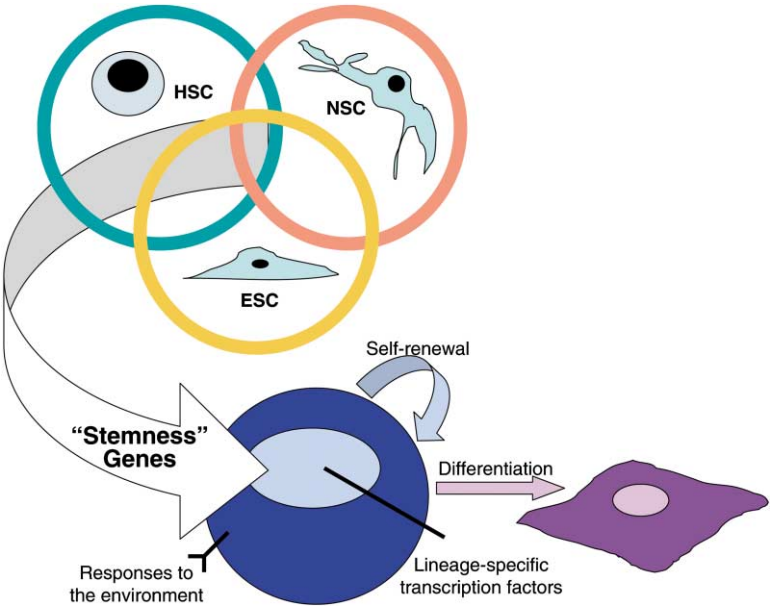
New papers published in *Science* (Ivanova et al., 2002; Ramalho-Santos et al., 2002) provide the first genome-wide transcript analysis of embryonic SCs (ESC), HSC, and NSC. Both efforts independently generated a list of approximately 200 genes that were upregulated in the tested SC populations. One finding from both groups was that most of the common enriched genes were not exclusively expressed in SCs. Thus, it may be the specific combination of genes rather than individual genes that endow the unique properties of SCs, priming them to accept specific environmental cues. Of the core 200 SC genes Ramalho-Santos et al. describe, 60 were mapped. Twelve of the 60 genes (20%) map to mouse chromosome 17. Such clustering of SC genes may suggest coregulation, which could be important for SC function.

Ivanova et al. also examined gene expression in the hematopoietic hierarchy. They found that both long-term (LT) and short-term HSCs express many known HSC markers, such as *Kit*, *Tie1*, and *Gata2*. Moreover, approximately 50% of the enriched genes from these HSC populations were the same as those found in a previous subtractive hybridization screen for HSC specific gene products (Phillips et al., 2000). Cell-cell communication genes, such as EGF family members, and many ligand-receptor pairs, such as *Wnt10A*/*Frizzled*, were specifically enriched in LT-HSCs. Consistent with previous studies implicating Hox genes in HSC regulation (reviewed in Lawrence et al., 2001), specific Hox transcripts were found upregulated in different hematopoietic classes.

Comparative analyses provided further insights. The authors found that fetal and adult HSCs share ~70% of the enriched transcripts, defining a more select list of HSC genes and linking the programs used during embryogenesis and adulthood. Human and mouse HSCs share at least ~40% of enriched genes. Although this number may seem small, the purity of mouse and human HSC populations may be quite different. Impure SC samples increase the number of background transcripts detected, thus clouding potentially relevant SC programs shared by both mouse and human. The ~40% overlap may thus be a conservative estimate. Syntenic relationships could provide an additional approach for assessing shared genes.

Both papers present overwhelming gene lists potentially relevant to SC biology. Although their goals of defining a "stem cell molecular signature" were similar, there were some important differences in methodology. Ivanova et al. hybridized stem cell probes to three Affymetrix mouse genome chips (~36,000 genes), while Ramalho-Santos et al. hybridized to one of these three chips (~12,000 genes). Additionally, the manner in which SC populations were isolated, the purity of those populations, and the computational algorithms used to measure changes in gene expression differ between the groups. A comparison of the lists of "stemness" enriched transcripts derived by each group yielded only 15 genes in common. This lack of similarity underscores both the variability in isolated SC populations and the difficulty in assigning computational parameters. While the concept of "stemness" that these groups have developed is important, the variance in the core "stemness" gene list raises some questions as to whether the analyses are truly comparable or simply a parceling of data. A comparison of any three cell populations would likely lead to overlapping gene sets. Therefore, before we assign true "stemness" genes, the analysis of many more tissues would be required.

Despite these caveats, the two papers do provide a first glimpse of the networks of pathways defining "stemness". Further analysis is clearly required to ascertain the relevance of the identified core SC genes. Because there are no "stemness" assays available, the functionally important genes will likely be uncovered by



Cartoon Depicting Stem Cell Asymmetric Division

Theoretically, all stem cells, represented here by ESC, HSC, and NSC, may share a core set of "stemness" genes that enable both self-renewal and differentiation. These internal programs may be regulated by environmental cues. Each individual SC also expresses a set of individual lineage-specific genes. The specific combination of common and lineage-specific transcripts and the biased distribution of internal factors may lead to SC asymmetric division.

comparing SCs in different lineages and species. Our current impressionistic portrait of a stem cell may then transform into realism.

Caroline Erter Burns and Leonard I. Zon
Department of Hematology/Oncology
Howard Hughes Medical Institute
Children's Hospital of Boston
320 Longwood Avenue, Enders 650
Boston, Massachusetts 02115

Selected Reading

Hackney, J.A., Charbord, P., Brunk, B.P., Stoeckert, C.J., Lemischka, I.R., and Moore, K.A. (2002). Proc. Natl. Acad. Sci. USA *99*, 13061–13066.

Ivanova, N., Dimos, J., Schaniel, C., Hackney, J., Moore, K., and Lemischka, I. (2002). Science *298*, 601–604.

Korinek, V., Barker, N., Moerer, P., van Donselaar, E., Huls, G., Peters, P.J., and Clevers, H. (1998). Nat. Genet. *19*, 379–383.

Lawrence, H.J., Sauvageau, G., Lagman, C., and Humphries, R.K. (2001). In Hematopoiesis: A Developmental Approach, L.I. Zon, ed. (New York, Oxford University Press), pp. 402–416.

May, G., and Enver, T. (2001). In Hematopoiesis: A Developmental Approach, L.I. Zon, ed. (New York, Oxford University Press), pp. 61–81.

Orkin, S.H., and Zon, L.I. (2002). Nat. Immunol. *3*, 323–328.

Phillips, R.L., Ernst, R.E., Brunk, B., Ivanova, N., Mahan, M.A., Deanehan, J.K., Moore, K.A., Overton, G.C., and Lemischka, I.R. (2000). Science *288*, 1635–1640.

Ramalho-Santos, M., Yoon, S., Matsuzaki, Y., Mulligan, R., and Melton, D. (2002). Science *298*, 597–600.

Spradling, A., Drummond-Barbosa, D., and Kai, T. (2001). Nature *414*, 98–104.

Till, J., and McCulloch, E. (1961). Radiat. Res. *14*, 213–222.