or Aub localize to the 42AB and other dual-strand clusters. A physical interaction between Piwi and HP1a has been observed in somatic cells of *Drosophila* (Brower-Toland et al., 2007), where Piwi might recruit HP1a to transcriptionally silence these regions. In the fly ovaries, Rhino may take the place of HP1 in a similar kind of interaction to promote the expression of dual-cluster RNAs and the generation of piRNAs to silence transposons. An intriguing possibility is that another HP1 protein, HP1e, which is preferentially expressed in the male germline (Vermaak et al., 2005), promotes dual-cluster RNA expression in sperm. Future studies on the interplay between Rhino, other HP1 proteins, and piRNA generation should provide further insights into this fascinating area of genome biology.

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p53: A New Kingpin in the Stem Cell Arena

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Identifying new regulators of the stem cell state offers potential for future gains in biomedicine. Evidence that the tumor suppressor p53 is a key regulator of the stem cell state (Cicalese et al., 2009) suggests a broad role for this protein and its pathways in the control of normal tissue homeostasis and tumor formation.

"Self-renewal potential" is the usual answer to the topical question of what distinguishes stem cells from other cell types. Interestingly, a clear definition of "self" remains enigmatic. Stem cells are unique in their possession of a latent readiness to differentiate into many cell types that can be propagated through many cell divisions without being lost. The fact that tumors represent rare perturbed clonal outgrowths of normal tissue has led to the concept that their continuous propagation may rely on an analogous subset of cancer stem cells whose self-renewal and differentiation fate decisions are deregulated by mutation or epigenetic changes. Our understanding of the molecular machinery that governs the self-renewal behavior of both normal and cancer stem cells remains ephemeral. A recent stimulus to addressing this challenge has come from a growing expectation of major benefits for regenerative medicine and cancer therapy. In this issue of *Cell*, Cicalese et al. (2009) provide a further link between normal and cancer stem cells with their study of self-renewal divisions in a mouse model of breast cancer. Their study provides new evidence that the tumor suppressor protein p53 may serve as a kingpin guardian of the normal stem cell state.

Many adult tissues are thought to be sustained by hierarchies of differentiating cells that ultimately depend on the balanced turnover and loss of "self" status of a specific stem cell compartment. Yet, for only a few has a biologically distinct stem cell population been definitively identified. The hematopoietic system was one of the first of these and has served as a paradigm for subsequent analyses of other tissues, including the normal mammary gland of the breast (Shackleton et al., 2006; Stingl et al., 2006).

Stem cells can execute either a symmetric or asymmetric self-renewal division, thereby giving rise to either two daughter stem cells or one daughter stem cell plus one that is destined to differentiate within a few cell divisions. A favored mechanism for the acquisition of functional asymmetry by the

two daughter cells is the acquisition of physical asymmetry during cell division. Notable candidates that may be asymmetrically distributed include mRNA transcripts or other cytoplasmic components such as the Notch, Numb, and Par proteins. Potential consequences of cell stress or genome damage on cell division outcomes have been less well explored. Recently, five different laboratories independently identified p53 as an important checkpoint during the multifactor reprogramming process in which induced pluripotent stem (iPS) cells are derived from differentiated adult cells (Hong et al., 2009; Kawamura et al., 2009; Li et al., 2009; Marion et al., 2009; Utikal et al., 2009). In each case, absence of functional p53 enhanced the yield of iPS cells, suggesting that p53 may be a major gatekeeper of self-renewal.

Key to delineating the properties of normal and cancer stem cells is the specificity of the method used to detect and measure them. For normal mammary epithelial stem cells, the specificity is obtained by demonstrating the ability of a single cell to reconstitute an entire mammary gland structure. The prospective isolation of cells with such properties (called mammary repopulating units or MRUs) has now been defined for normal human (Eirew et al., 2008) as well as mouse mammary tissue (Shackleton et al., 2006; Stingl et al., 2006). In the mouse, highly enriched MRU-containing populations are obtained by removal of non-mammary cells (hematopoietic and stromal cells that heavily contaminate fresh breast tissue) and positive selection for cells expressing the highest levels of CD49f and CD29 (an integrin complex found on the surface of most basal mammary cells) as well as intermediate levels of CD24. However, the MRU purities of these fractions are still insufficient (<10%) to allow meaningful inferences about MRUs to be made from direct characterization of such cell suspensions.

Mammospheres are three-dimensional multilayered structures that form when disaggregated, unseparated normal mammary epithelial cells are cultured under conditions that inhibit cell attachment to the culture dish surface. When the culture medium contains appropriate supplements, primitive mammary cells capable of further proliferation and differentiation can be detected in these structures for several weeks (Dontu et al., 2003). Terminal differentiation appears to be less well supported under these conditions, so some apparent enrichment in the content of primitive cell subtypes is seen. These features, and the obvious practical advantages of in vitro systems for analyzing cellular events, have made mammosphere cultures popular for investigating mechanisms that regulate the biology of primitive mammary cells, as Cicalese et al. now demonstrate. However, as these authors point out, mammosphere cultures also have notable limitations. For example, definitive evidence of a clonal origin of MRUs from cells that generate the other cells present in the mammosphere has not yet been reported. In addition, normal epithelium mammosphere cultures do not yield net increases in either sphere-forming cells or MRUs.

Notwithstanding these caveats, Cicalese et al. sought to compare the growth properties of normal and cancer stem cells in cultured mammospheres. The mammospheres were derived from the mammary tissue of wild-type mice, mice lacking p53, and a transgenic mouse model of breast cancer in which the cells overexpress the ERBB2 oncogene. They report that $CD49f^{hi}CD24^{+}$ cells (the putative MRU-enriched fraction) in the mammary gland of mice lacking p53 display a much greater replicative potential in mammosphere cultures than do their normal counterparts. Moreover, they found that the frequency of MRUs (detected in transplantation assays) in mammary tissue from p53-deficient mice was 10-fold higher than in wild-type mammary tissue. Taken together, these findings point strongly to a key role for p53 in the control of MRU compartment size. In a parallel series of experiments, they found that the sphere-forming activity of mouse mammary tumor cells overexpressing ERBB2 was perpetuated in vitro beyond that typical of premalignant mammary tissue expressing ERBB2. Although p53 was not apparently mutated in the tumor cells, evidence that their p53 activity was reduced was shown by abrogated responses to DNA damage. Furthermore, reactivation of p53 by nutlin3 (through inhibition of MDM2) both normalized the sphere-forming activity of the ERBB2-induced mammary tumor cells and concomitantly reduced their tumor-initiating activity. These results identify p53 as a participant in processes regulating malignant as well as normal epithelial stem cell populations.

But how does p53 regulate the stem cell state? One possibility, hinted at in the Cicalese et al. study, is that p53 may control whether or not a stem cell division is asymmetric or not. This idea is based on the authors' observations of the heterogeneous rates of dilution of PKH26 (a membrane intercalating dye) in dividing pairs of cells in early developing mammospheres. The ERBB2 tumor cells showed predominantly symmetric losses of PKH26 fluorescence, in marked contrast to normal mammospheres in which an asymmetric label dilution pattern was commonly seen. The investigators propose that breast cancer stem cells have a deregulated ability to execute symmetric self-renewal divisions as compared to their normal counterparts. Follow-up studies to define the functional properties of the daughter cells of the first and second divisions of these cells and to determine if and how the distribution of p53 (or other members of the pathway) may be affected will be important to put the PKH26 data into a more precise cellular and molecular context. The observations of Cicalese et al. and those implicating p53 in iPS cell generation beg for further studies to determine if and how this new kingpin may play a general role in regulating stem cell states.

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Reducing the Mystery of Neuronal Differentiation

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In the developing nervous system, neural progenitors exit the cell cycle and differentiate on a precise schedule, yet the mechanisms driving this process remain poorly defined. Yan et al. (2009) now identify a thiol-redox reaction mediated by the membrane protein GDE2 and the peroxiredoxin protein Prdx1 that promotes neurogenesis.

During embryogenesis, neural circuits are formed through the precise spatial and temporal production of functionally distinct classes of neurons by undifferentiated stem and progenitor cells. Although significant progress has been made in identifying the morphogen signals that spatially organize the developing nervous system (Ulloa and Briscoe, 2007), much less is known about the mechanisms that control the temporal pattern of neuronal differentiation. In this issue of *Cell*, Yan et al. (2009) identify a thiol-redox signaling cascade mediated by the transmembrane protein GDE2 and the antioxidant protein Prdx1 that controls the timing of motor neuron differentiation in the spinal cord.

Spinal motor neurons have long served as a model for studying neurogenesis. Motor neuron progenitors are first specified by the patterning actions of Sonic hedgehog and retinoic acid signaling, which culminate in the induction of the essential motor neuron determinant Olig2 (Briscoe and Novitch, 2008). Once formed, these motor neuron progenitors divide a limited number of times before exiting the cell cycle and differentiating at a characteristically early time in development. Although retinoid signaling plays an essential role in this process, the mechanisms of its actions are not well understood. In their previous work investigating the downstream effectors of the retinoid pathway, Sockanathan and colleagues had identified the six-transmembrane glycerophosphodiester phosphodiesterase domain protein GDE2 as a retinoid-induced factor expressed by motor neurons. They demonstrated that the catalytic activity of GDE2 is both necessary and sufficient to promote the differentiation of Olig2-positive neural progenitors (Rao and Sockanathan, 2005). Although this study implicated glycerophospholipid metabolism in neuronal development, it remained unclear how GDE2 carries out this function. In the current study, Yan et al. use an innovative proteomic screening approach to demonstrate that the peroxiredoxin protein Prdx1 directly binds to GDE2 and serves as an activating cofactor that promotes neuronal differentiation (Figure 1).

Peroxiredoxins are widely expressed hydrogen peroxide (H_2O_2) scavenger proteins best known for their role in detoxifying reactive oxygen species, protecting against oxidative stress, DNA damage, and cancer, but they have also been suggested to act in cellular signaling and as molecular chaperones (Hall et al., 2009; Jang et al., 2004). Here, the authors identify a new role for peroxiredoxins in regulating neuronal differentiation. Newly born neurons in the intermediate zone and mantle zone of the spinal cord broadly express *Prdx1*, such that *Prdx1* expression overlaps with *Gde2* as motor neurons are differentiating. Yan et al. provide a comprehensive demonstration that Prdx1 and GDE2 functionally interact in the same signaling pathway. Embryos lacking Prdx1 function recapitulate the phenotype of *Gde2* mutant embryos; progenitors have a reduced capacity to exit the cell cycle and differentiate into motor neurons. Their gain-of-function analysis shows that, although misexpression of Prdx1 alone has little effect on neuronal development, Prdx1 synergistically promotes motor neu-