of thymocyte development, the E47 gene products form heterodimers with yet another bHLH protein, HEB. The DNA binding activity of this heterodimeric complex is further modulated at two developmental checkpoints (Engel and Murre, 2001). This has raised the question as to how HLH dimerization is regulated. One form of regulation is well established and involves members of the Id gene family. For example, the antagonist HLH protein, Id3, is induced during thymocyte maturation by pre-TCR and TCR-mediated signaling. High levels of Id3 readily form heterodimers with the E47 and HEB, to inactivate their DNA binding activity. Another level of regulation has been suggested by studies that showed that hypophosphorylation promotes the formation of E47 homodimers in mature B lineage cells (Sloan et al., 1996). An alternative model for the formation of E47 homodimers has been suggested by studies demonstrating an intermolecular disulphide bond in E47, which was detected only in B lineage cells (Benezra, 1994).

Now Firulli et al. (2003) provide new data that go to the heart of HLH regulation. Their studies have focused on the activities of the bHLH proteins, HAND1 and HAND2. HAND proteins are expressed in the developing heart, neural crest cells that occupy the brachial arches, lateral mesoderm, and trophoblasts. Hand1 functions to promote the terminal differentiation of trophoblasts. Additionally, ectopic expression of HAND gene products in the limbs of both chickens and mice results in duplication of digits. These and other studies have suggested that the HAND proteins play a key role in the growth and patterning of the limb bud along the anteroposterior axis (Johnson and Tabin, 1997). Firulli et al. (2003) demonstrate that during trophoblast differentiation the activity of HAND proteins is modulated by phosphorylation. Specifically, they show that residues present in the HLH domain are diffentially phosphorylated during trophoblast differentiation. They show that both PKA and PKC have the ability to modify these residues and further they identify a phosphatase, PP2A, that specifically interacts with the N- and C-terminal portion of HAND1, to modulate phosphorylation. Finally, they show that mutations in amino acids within the HLH region that are differentially phosphorylated alter the ability of HAND proteins to dimerize and severely affect the ability to induce polydactylily in the chick hindlimb. Together, these experiments are the first to demonstrate that HLH dimerization is regulated, at least in part, by phosphorylation. Since HAND1 and HAND2 are expressed in multiple tissues, it will be particularly interesting to mutate the relevant residues in the mouse germline. Analysis of such mutants should provide further insight into how HLH dimerization is controlled during distinct stages of embryonic development and how it affects developmental progression.

Although some progress has been made with regard to the regulation of bHLH activity, little insight has been gained into how dimerization actually is regulated. The findings by Firulli et al. are an important step toward addressing this problem. Will other HLH proteins also utilize phosphorylation or other forms of modification as a means to regulate dimerization? Modulation of HLH dimerization by posttranslational modification provides an efficient mechanism to regulate transcription factor activity, and it is likely that it will be used in a wide variety of biological systems.

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A Two-Tiered Transcription Regulation Mechanism that Protects Germ Cell Identity

In the November issue of *Developmental Cell*, Schaner and colleagues (2003) describe remarkable versatility in how the embryonic germ lineage blocks differentiation: in early *C. elegans* germ cell precursors transcription is silenced but chromatin remains open, and then after lineage restriction a conserved inhibitory chromatin architecture appears.

Germ cells are specialized cells that have the potential to develop into any type of tissue in the body. Mechanisms that protect germ cells from inappropriate differentiation are therefore crucial to reproduction, and may serve as models for understanding how pluripotency and identity are also maintained in somatic stem cell lineages. In various organisms, early embryonic germ cell precursors are protected from somatic differentiation cues by mechanisms that transiently and globally silence mRNA transcription (Leatherman and Jongens, 2003; Tomioka et al., 2002). In these cell lineages, early differentiation steps are guided by translational control of stored mRNAs until a germline transcription program takes over.

In the nematode *C. elegans*, the identity of early germline precursors is initially maintained through successive

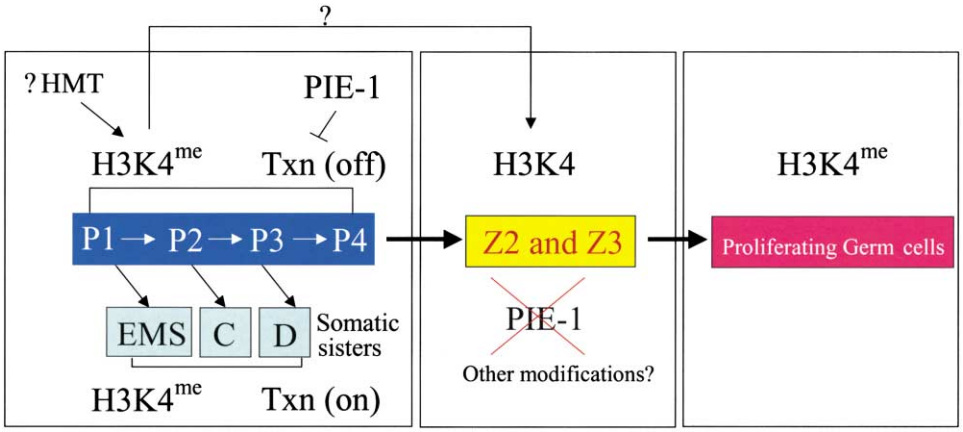

Figure 1. Transcriptional Silencing in *C. elegans* Germ Cells

During *C. elegans* early embryogenesis, the germ cell precursors P1, P2, and P3 each divide asymmetrically to give rise to germ cell and somatic lineage. Although the chromatin structure in these germ cells appears to be open as suggested by methylation at histone H3 lysine 4 ($H3K4^{me}$), transcription is inhibited by PIE-1. P4 divides to give rise to Z2 and Z3 (Z2/3), which become dedicated to the germ cell lineage. PIE-1 is absent in Z2/3, and these germ cell precursors appear to have adopted a chromatin-based inhibitory mechanism that is associated with loss of H3K4 methylation through an unknown mechanism. The histone methytransferase that methylates H3K4 in the P lineage germ precursors is yet to be identified. Txn, transcription; HMT, histone methyltransferase.

asymmetric divisions that each give rise to a somatic lineage (Figure 1). Somatic transcription starts at the four-cell stage, but is delayed in the germline due to transcriptional silencing by the germ cell-specific, maternally provided zinc finger protein PIE-1 (Mello et al., 1996; Seydoux and Dunn, 1997; Seydoux et al., 1996). Remarkably, analysis of RNA polymerase II (RNAP) phosphorylation patterns suggests that early transcription initiation steps occur in these transcriptionally silent germline precursors (Walker et al., 2001), and functional experiments indicate that PIE-1 prevents the later elongation steps of transcription (Zhang et al., 2003). These findings predict that in early PIE-1-expressing germline precursors chromatin is likely to be permissive for transcription, a model that has not been tested previously.

Schaner and colleagues have addressed the role of chromatin in germline transcriptional silencing by examining embryonic germ cell precursors for the presence of chromatin modifications important for transcriptional repression and activation (Jenuwein and Allis, 2001; Schaner et al., 2003). In both the P lineage germ cell precursors and their somatic sisters, they have detected multiple histone modifications associated with transcriptionally competent chromatin (such as histone H3 lysine (K) 4 methylation) (Schaner et al., 2003). This suggests that chromatin structure in the P lineage germ cells is indeed likely to be "open," even though transcription is blocked by PIE-1. This strategy makes sense because the open chromatin structure would allow immediate transcriptional engagement in the newly born somatic cells after they are segregated from their germline sisters (Figure 1).

Later during embryogenesis, P4 divides to give rise to Z2 and Z3 (Z2/3), which in contrast to the P cells are dedicated germ cell precursors. Although RNAP modifications associated with transcription are readily detectable in Z2/3, few mRNAs are known to be produced in these cells (Seydoux and Dunn, 1997; Schaner et al., 2003). This suggests that Z2/3 may maintain widespread if not complete transcriptional silence until larval stages, when they begin to proliferate in order to generate a large population of germ cells. Consistent with this model, Schaner and colleagues show that the active chromatin marker H3K4 methylation is lost in Z2/3, and then reappears later in development coinciding with germ cell proliferation (Schaner et al., 2003).

As in *C. elegans*, in *Drosophila* germ cell precursors also undergo a period of transcriptional quiescence during early embryogenesis (Leatherman and Jongens, 2003). An important distinction between *C. elegans* and *Drosophila* early germ cell precursors is that the latter (pole cells) are dedicated to the germline fate. Thus a more stable, chromatin-based silencing mechanism would appear to be better suited for the pole cells. This is indeed what Schaner and colleagues have found: the pole cells lack methylation at H3K4 but are characterized by extensive H3K9 methylation, a marker for silenced chromatin (Schaner et al., 2003; Zhang and Reinberg, 2001). Accordingly, in *Drosophila* PIE-1 is not present. Transcriptional silencing in the *Drosophila* pole cells instead involves a protein termed germ cell-less (GCL) (Leatherman and Jongens, 2003). The relationship between GCL and chromatin remodeling in the pole cells is unclear and clearly warrants further investigation. Taken together, although different organisms may tailor their strategies to protect germ cell identity, chromatin remodeling appears to be a conserved germline silencing mechanism in both *C. elegans* and *Drosophila*.

An interesting mechanistic question arising from these studies is how H3K4 methylation is lost in Z2/3. Other histone modifications are readily detectable in Z2/3, arguing against cleavage of histone tails. The simplest model is that loss of H3K4 methylation is due to the action of a histone demethylase, although to date there is no experimental evidence for the existence of

this class of enzymes. Alternatively, H3K4 histone methyltransferase (HMT) activity or expression may be lost, followed by replacement of histone H3. Identification of the HMT and the mechanisms that control H3K4 methylation and demethylation during *C. elegans* germline development will significantly enhance our understanding of chromatin remodeling in development.

Another intriguing observation of this study is that H3K4 methylation is inappropriately present in Z2/3 cells in worms lacking the *C. elegans nanos* homologs, *nos-1* and *nos-2* (Schaner et al., 2003), suggesting that translational control mechanisms may also be involved. Significantly, in *Drosophila* pole cells *nanos* is required for transcription silencing and absence of H3K4 methylation (Leatherman and Jongens, 2003). Understanding how nanos proteins are integrated into regulatory pathways that lead to germ cell chromatin remodeling represents an immediate challenge.

It will now be of interest to determine whether similar mechanisms are utilized in other germline settings, and in somatic stem cell differentiation. The studies described by Schaner and colleagues have provided a perfect starting point for these exciting future explorations, which will bring us closer to a complete understanding of mechanisms that protect germ/stem cell identity.

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"Getting It On"—GDI Displacement and Small GTPase Membrane Recruitment

Understanding the mechanisms by which signaling events are localized and the physiological consequences of spatial restriction are major questions in cell biology. A recent issue of *Nature* reports the identification of an endosomal factor that can directly recruit Rab GTPases from the cytosol onto membranes (Sivars et al., 2003). Together with work describing the structure of prenylRab with Rab-GDI (Rak et al., 2003), this provides us both with new avenues and important insights into long-standing questions of Rab GTPase signaling and membrane traffic.

Ras superfamily members have proven to be critical players in a variety of fundamental cellular processes, which they influence by modulation of their GTP binding and hydrolysis cycle. These small GTPases are commonly found to cycle between two pools, a membrane-associated and a cytosolic pool. Because numerous studies have made it apparent that membrane attachment is a prerequisite for the signaling roles of these proteins, it is clear that reversible membrane translocation offers cells a means to regulate the location of the activation event. However, there is a serious handicap to such physical cycling for small GTPases of the Rho and Rab subfamilies. In contrast to the moderate hydrophobicity of other lipid-modified Ras superfamily members, the highly hydrophobic geranylgeranyl moieties of Rho and Rab proteins render them energetically unfavorable to partition into the cytosol as individual monomers. Posttranslationally modified Rho and Rab proteins can only detach from membranes if they are assisted by a "chaperone" that shields the bulky lipid moieties from the aqueous environment of the cytosol. For Rho and Rab proteins, the chaperone role is played by a Guanine nucleotide Dissociation Inhibitor (GDI) protein, Rho-GDI and Rab-GDI, respectively. These two proteins have no sequence or structural relationship, but share several biochemical features. First, each GDI binds with high affinity to only the lipid-modified form of its partner. Second, each GDI is a pleiotropic factor capable of interacting with many different members of the same subfamily; in the "minimal eukaryote" *S. cerevisiae*, a single gene encodes Rab-GDI function for every Rab protein, and likewise, a single gene provides Rho-GDI function for all Rho proteins. Third, the interaction of each GDI with its partner slows the dissociation of nucleotide from the GTPase. The physiological consequence of these characteristics is that the dimeric GDI/GTPase complexes represent a cytosolic reservoir of the GTPase. For the cell to draw upon this reservoir, GDI must first be induced to release its GTPase. Because of the high (nM) affinity of GDIs for their cognate GTPases, GDI release is unlikely to be a spontaneous event. Factors that promote GDI displacement and help recruit GTPases onto membranes are of tremendous importance, because these factors will determine where and when the Rho and Rab GTPases are activated.