inside blood

pathways and biologic activities of eosinophils stimulated with IL-5. The results are quite compelling in that the ras/ERK pathway appears to be selectively affected. Furthermore, the survival effect of IL-5 on eosinophils is severely compromised, allowing the authors to offer a strong, direct link between signaling and function.

Primary cells and, in particular, short-lived cells such as eosinophils are notoriously difficult to transduce in a way that maintains their morphologic and functional integrity. The fact that this can now be achieved offers the potential of inhibiting discrete steps in the eosinophil signaling machinery to analyze functional outcomes. The possibility therefore arises of establishing a cause-and-effect link between signaling pathways and function. This work should also encourage more work on primary eosinophils and less emphasis on transduced cell lines, which often do not offer the same range of functions or exhibit their own signaling machinery, which makes extrapolation to the eosinophil rather risky. Given the role of not only eosinophils but also myeloid cells in general in serious inflammatory conditions such as asthma, there is the further potential of clinically applying the protein transduction approach to selectively inhibit myeloid cell function in vivo. Clearly reducing myeloid cell survival would offer significant benefits in asthma. The proof of principle has been established. The challenge remains of how best to apply this approach to dissect signaling pathways and ultimately to translate these findings into the clinical setting.

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Simple math for the β-globin locus control region

The only universally agreed upon property attributable to the locus control region (LCR) is its absolute requirement for elevated transcriptional activity of the vertebrate β-type globin genes. Previously, the Groudine group reported that targeted deletion of hypersensitive sites (HSs) 2, 3, or 5/6 from the LCR affected the level, but not the developmental timing, of globin gene transcription. Here, Bender and colleagues (page 2022) report deletions of the final 2 murine β-globin LCR HSs, HS1 and HS4, thus closing one chapter in this saga. Because the mouse has 2 genetically distinguishable alleles (Hbb^S and Hbb^D), the wild-type allele serves as an internal control for nicely distinguishing quantified expression of the mutant. Additionally, since mutation of the endogenous locus cannot suffer from position of integration effects (as can transgenes), analysis of mutant expression leads to clear assessment of the transcriptional activity from individual HS loss of function. The data show that deletion of HS1 or HS4 reduces adult (definitive) gene transcription by approximately 20%, while there is little effect on embryonic transcription. In summarizing the results of all individual murine LCR HS deletions, Bender and colleagues conclude that each HS site contributes additively to LCR transcriptional stimulatory function.

But there is almost certainly another chapter to follow. Bender and colleagues' conclusions are in marked contrast to the inferences from some transgenic human β-globin locus studies, which suggest that the LCR stimulates transcription as a synergistic holocomplex. As reviewed in the paper, these contrasting conclusions could arise from transspecies issues (human genes expressed in the mouse; possible), from position of integration effects (likely), or from the nature and extent of the different mutations that have been examined to date (also likely). Aspects of these potential complications may be resolved by examining finer mutations in the endogenous locus, since the basis for a synergistic LCR model was established by examining small deletions in human β-globin YAC transgenes, in contrast to the larger targeted deletions such as those reported here.

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Another SHIP on the horizon

It is now well established that the phosphatidylinositol-3-kinase (PI-3K) pathway plays a central role in regulating many biologic processes. A key second messenger within this pathway is the plasma membrane–associated PI-3,4,5-P_3. This phospholipid is present at low levels in resting cells but is rapidly synthesized from PI-4,5-P_2 by PI-3K in response to growth factors, cytokines, and chemokines and attracts pleckstrin homology (PH)–containing proteins to the plasma membrane to mediate its effects. To ensure that the activation of this pathway is appropriately repressed/terminated, the tumor supressor PTEN hydrolyzes this phospholipid back to PI-4,5-P_2 while the hemopoietic-specific SH2-containing inositol 5-phosphatase (SHIP) and the ubiquitously expressed SHIP2 break it down to PI-3,4-P_2. The full-length 145-kd SHIP translocates to the plasma membrane and becomes both tyrosine phosphorylated and associated with the adaptor protein Shc following stimulation. It prevents the overproduction of myeloid progenitors and the activation of mature B cells, platelets, and mast cells.

Tu and colleagues (page 2028) have now identified a 104-kd form of SHIP (s-SHIP, for stem cell SHIP) that, unlike full-length SHIP, is expressed in embryonic and hemopoietic stem cells but not in lineage-committed or mature hemopoietic cells. Interestingly, s-SHIP, which is the murine homolog of the human SIP-110, is generated by transcription from a promoter within the intron between exons 5 and 6 of the *SHIP* gene. It thus lacks the SH2 domain of full-length SHIP and is not tyrosine phosphorylated nor associated with Shc following stimulation. But it does bind constitutively to Grb2 and may be recruited via Grb2's SH2 domain to the plasma membrane to regulate PIP_3 levels and thus the activation of primitive stem cells. It will be interesting to determine what regulates the switch from s-SHIP to full-length SHIP and the ramifications of this switch.

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