the sites of normal tissue toxicity. These studies therefore appear to contradict those of Deverman et al. who suggest that activation of p53 would in fact protect normal cells from apoptosis induced by genotoxic stress.

The multifaceted paper by Deverman et al. provides novel insight into the regulation of Bcl-X_L function by posttranslational modification and indicates that sequestration of BH3-only proteins by Bcl-2/Bcl-XL is important for the inhibition of genotoxic stress-induced apoptosis. In addition, the paper raises thought-provoking questions regarding the possible prosurvival roles of Rb and p53 and provides molecular clues as to how these famous tumor suppressors may, in certain circumstances, suppress apoptosis. However, unlike a classic whodunit, not all of the loose ends have been tied up, and the importance of the proposed apoptosis regulatory pathway involving p53, Rb, BH3-only proteins and deamidation of Bcl-X_L in various physiological and/or pathological situations remains to be determined.

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Targeting and Beyond: New Roles for Old Signal Sequences

Signal sequences, once considered degenerate hydrophobic elements whose sole task is to target proteins to the secretory pathway, are increasingly being recognized as playing roles beyond targeting. Recent work is beginning to shed light on some of the ways the cell decodes and exploits additional functions encoded within signal sequences.

The existence of short (~15–40 amino acids) removable extensions on some polypeptides was invoked over 25 years ago to help explain how secretory proteins could be distinguished from cytoplasmic proteins to facilitate their sorting (Blobel and Dobberstein, 1975). These extensions, termed signal sequences, target nascent polypeptides to translocation sites at the endoplasmic reticulum where they are subsequently removed by an enzyme called signal peptidase. The limited sequencing of the first few signals appeared to reveal homology between them and suggested that a common motif may facilitate the targeting of all secretory proteins. However, it quickly became apparent that while signals did share certain general features, most notably a hydrophobic domain of ~6–15 residues, there were extremely few if any absolute constraints on their specific sequence (von Heijne, 1985). While this observation stimulated much investigation into the central question of how the specificity for signal recognition could be encoded, a more subtle implication of this remarkable flexibility remains largely ignored.

The ability to tolerate tremendous diversity could make signal sequences, in principle, an excellent substrate for evolution. That is, sequence elements that do not disrupt the targeting function of a signal but are useful for some other purpose could arise and be maintained by selection over time. But what kind of functions might these cryptic sequences serve? Two qualitatively different possibilities can be envisioned. First, signal sequences, after targeting but before their removal by signal peptidase, could participate in the folding and/or maturation of the protein to which they are attached. Although there is no coherent framework yet for understanding such a post-targeting signal sequence function, the few existing examples (Li et al., 1996; Chen et al., 2001; Kim et al., 2002) serve to illustrate the signal's potential for multifunctionality.

The second, equally provocative idea is that the signal sequence, after its liberation by signal peptidase, can serve functions unrelated to the protein from which it was derived. The remarkable versatility of small peptides, with functions as varied as hormones, neurotransmitters, or self-antigens, combined with the wide diversity of sequences that can be encoded within a targeting signal, certainly allows for this theoretical possibility. For this to be achieved, one would not only need functions for which this type of regulation is useful, but also mechanisms to encode, decode, and liberate the relevant peptides from the signal sequence. The recent convergence of two lines of study is beginning to provide support for the existence, utility, and mechanisms of postcleavage functions for signal sequences.

The first key observation was the discovery that a portion of the signal sequences from certain polymor-

phic MHC class I molecules is presented on the cell surface by the nonclassical MHC class I molecule HLA-E (Braud et al., 1997). The picture that has emerged is one in which cell surface HLA-E is able to report on the intracellular expression of other MHC class I molecules to prevent killing by natural killer cells (Braud et al., 1998). The second observation came from experiments attempting to define the pathway of disposal after signal sequence removal from the mature domain (Lyko et al., 1995). This approach led to the identification of a proteolytic event, by an activity termed signal peptide peptidase (SPP), that occurs within the hydrophobic domain of the signal peptide. Two new studies (Weihofen et al., 2002; Lemberg and Martoglio, 2002) now provide mechanistic insight into how SPP, rather than (or perhaps in addition to) promoting the destruction of signal peptides, facilitates their involvement in regulatory functions such as the HLA-E-mediated antigen presentation described above.

The purification, cloning, and identification of SPP as a novel member of a family of intramembrane proteases (Weihofen et al., 2002) and the delineation of some of the features that give SPP its substrate specificity (Lemberg and Martoglio, 2002) define a novel pathway for the production of functionally useful peptides. The most recent work by Lemberg and Martoglio, published in this issue of *Molecular Cell*, not only demonstrates that some but not other natural signal sequences are substrates for SPP, but also identifies three key structural features that distinguish between them. First, a helix-breaking residue within the hydrophobic domain substantially facilitates SPP cleavage. Second, positive charges downstream of the hydrophobic domain can inhibit cleavage. And finally, release of the signal sequence from the mature domain by signal peptidase is a prerequisite for SPP cleavage. While the molecular basis for these observations awaits future structural studies, it seems plausible, as the authors speculate, that all three requirements influence the access of SPP to a suitable peptide bond within the hydrophobic core of the signal peptide. Moreover, the prerequisite for signal peptidase-mediated release of the signal prior to further cleavage by SPP probably mitigates inappropriate cleavage of other hydrophobic segments such as transmembrane domains.

These findings have two major implications. First, the similarity of SPP to other intramembrane proteases (discussed in Wolfe and Selkoe, 2002) may provide important insights into the mechanism of action of presenilins, enzymes implicated in the pathogenesis of Alzheimer's disease. And second, the observation that not all signal sequences are substrates for SPP raises the intriguing possibility that SPP is not simply a signal-disposal enzyme, but one involved in the regulated generation of bioactive peptides. This is supported by the observation that yeast not only do not have a SPP homolog, but appear to lack a comparable activity (Weihofen et al., 2002), suggesting that clearance of signal sequences may not require intramembrane cleavage. Furthermore, the key structural features involved in SPP cleavage (a helix-breaking residue and charges flanking the hydrophobic domain) are highly variable among signals (von Heijne, 1985) and usually do not influence the signal's primary role in protein targeting. Thus, the latest study by Lemberg and Martoglio (2002) reveals that multiple layers of information (in this case, targeting, cleavage by SPP, and antigenic sequences for HLA-E presentation) can be simultaneously encoded in and decoded from a signal sequence to maximize its functionality.

While it may be tempting to think that all signals that are processed by SPP serve a common function (such as antigen presentation), it is perhaps more likely that this is an instance of divergent evolution where different signal-derived peptides serve unique functions. Given that intramembrane proteolysis is gaining increased recognition as a common regulatory mechanism, that there exists a large family of SPP-like proteins with unknown function (Weihofen et al., 2002), and that signal peptides are tremendously diverse in sequence (von Heijne, 1985), postcleavage functions for signal sequences may be a far more common phenomenon than is presently appreciated. This "proof-of-principle" demonstration that the degeneracy of the signal sequence (from a targeting standpoint) can be elegantly exploited for additional roles is a reminder that a single polypeptide should not always be equated with a single function.

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