complete abandonment. Ocean-atmosphere interactions may produce a salt oscillator by driving variations in, for example, net precipitation patterns, water vapor transport, or export of ice from the Arctic Ocean to the subpolar North Atlantic. Variation in any of these can affect the surface salinity and, hence, deep-water production (*9*, *11*). Bond and his colleagues present the first chemical evidence of deep-water reduction associated with one of the earlier and more prominent Holocene cool events, but as yet, no direct evidence has been found for recurring oscillations in deep-water production associated with Holocene climate oscillations. Circumstantial evidence, however, does exist.

In a subtropical North Atlantic core, Keigwin identified carbonate minima accompanying the two most recent of Bond *et al.*'s cold events and the Little Ice Age (*5*). During the last glacial cycle, carbonate oscillations in this region were associated with chemical evidence for deep-water variability (*12*); thus, Holocene carbonate variations may also reflect deep-water oscillations. Furthermore, the very finding of coeval oscillations in the subtropical and subpolar North Atlantic may suggest deep-water variability, because it is unlikely that cold water from the Greenland and Iceland seas penetrated as far south as 34°N to cause the coolings. Instead, with a reduction in deep-water production, surface cooling might occur because less warm water was drawn northward over the subtropical site to replace sinking waters at higher latitudes. Alternatively, the subtropical coolings may be a consequence of the expanded polar atmospheric circulation, which apparently coincided with the subpolar cool events. But even such atmospheric changes may involve changes in deep-water production. If millennial-scale climate cycles are indeed paced by internal oscillations of the ocean-atmosphere system, then the presence of large ice sheets may provide the amplifying mechanism as originally envisioned (*1*) and recently modeled (*13*).

Several of the Holocene cold events, especially the one 8200 years ago and the Little Ice Age, have been recognized outside of the circum–North Atlantic region (*4*, *14*), suggesting that like glacial millennial oscillations they were almost global in extent. Modeling studies suggest that the occurrence in the North Pacific of the larger deglacial and glacial oscillations may be explained by an atmospheric response to variable North Atlantic deep-water production (*15*), but the mechanisms for transmitting the signal almost globally have not been worked out. The importance of deep-water variability in the Holocene must still be confirmed. First, convincing evidence for deep-water variability must be found. Second, the difficulty of explaining the quasi-global nature of large millennial climate oscillations during the last glacial cycle reminds us that explaining the presence of subtle, widespread climate fluctuations during the Holocene will be even more daunting.

References and Notes

1. W. S. Broecker, G. Bond, M. Klas, G. Bonani, W. Wolfi, *Paleoceanography* **5**, 469 (1990).
2. G. Bond *et al.*, *Nature* **365**, 143 (1993).
3. D. A. Meese *et al.*, *Science* **266**, 1680 (1994).
4. S. R. O'Brien *et al.*, *ibid.* **270**, 1962 (1995).
5. L. D. Keigwin, *ibid.* **274**, 1504 (1996).
6. G. Bond *et al.*, *ibid.* **278**, 1257 (1997).
7. P. A. Mayewski *et al.*, *J. Geophys. Res.*, in press.
8. A. McIntyre and B. Molfino, *Science* **274**, 1867 (1996).
9. W. S. Broecker, *Oceanography* **4**, 79 (1991).
10. E. A. Boyle and L. Keigwin, *Nature* **330**, 35 (1987); E. A. Boyle and P. Rosener, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **89**, 113 (1990); S. J. Lehman and L. D. Keigwin, *Nature* **356**, 757 (1992); D. W. Oppo and S. J. Lehman, *Paleoceanography* **10**, 901 (1995); L. Vidal *et al.*, *Earth Planet. Sci. Lett.* **146**, 29 (1997).
11. C. Mauritzen and S. Hakkinen, *Newsl. Atl. Clim. Change Program* **4**, 6 (1997).
12. L. D. Keigwin and G. A. Jones, *J. Geophys. Res.* **99**, 12397 (1994).
13. K. Sakai and W. R. Peltier, *J. Clim.* **10**, 949 (1997).
14. R. B. Alley *et al.*, *Geology* **25**, 483 (1997).
15. U. Mikolajewicz, T. J. Crowley, A. Schiller, R. Voss, *Nature* **387**, 384 (1997).

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APOPTOSIS

A Myc-Induced Apoptosis Pathway Surfaces

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Why is cancer so rare? Simple probability should dictate that in every large complex animal at least one of the huge number of dividing cells will inevitably transform and lead to cancer. Conventional wisdom has it that redundant molecular checks on cellular function, via tumor suppressors, keep the real rates of oncogenesis at low levels. But an emerging view, pioneered by G. Evan at the Imperial Cancer Research Fund Laboratories in London, suggests an additional process at work; that is, that the basic mechanisms of cellular proliferation and transformation are tied to the process of apoptosis: The default for all proliferating cells is to die unless specifically told not to do so.

This concept of an active suppression of death (via specific survival signals) arose from the observation that c-Myc drives apoptosis in fibroblasts unless survival factors are present (*1*). Likewise, the anti-apoptotic effects of Bcl-2 are mediated by the diversion of c-Myc signals toward cell proliferation rather than cell death (*2*, *3*).

c-Myc, in association with its partner Max, functions as a transcription factor to drive apoptosis when low amounts of survival factors, such as IGF-1, are present (*4*, *5*). But what does Myc-Max induce (or repress) that is responsible for the death of cells? In this issue, Hueber *et al.* on page 1305 (*6*) show that c-Myc–induced cell death in fibroblasts is mediated by the cell surface interactions of Fas (CD95) with its ligand, FasL (CD95L). This effect is due, at least in part, to the ability of c-Myc to sensitize the cells to Fas-mediated apoptosis, an effect of c-Myc that is also seen in tumor necrosis factor–induced apoptosis (*7*, *8*). The death pathway induced by c-Myc meanders to the cell surface, where the binding of Fas by its ligand generates a critical apoptotic signal, leading ultimately to the activation of caspase proteases and the death of the cell (see figure).

This "surfacing" of a c-Myc–dependent cell death pathway via Fas-FasL interaction has been described before only in T lymphocytes, during activation-induced apoptosis. Ligation of the T cell receptor (TCR) on previously activated or transformed T cells induces the expression of FasL, which then binds Fas, either on that cell itself or on the surface of a neighboring cell (*9*–*11*). This induction is dependent on c-Myc expression in the T cells, as demonstrated by the use of antisense or dominant-negative approaches (*12*, *13*). That is, activation-induced cell death in T cells occurs by c-Myc–dependent, Fas-FasL–mediated apoptosis.

Perhaps it should not have been surprising to immunologists that the activation-induced cell death pathway in T cells surfaces to allow the engagement of a second receptor-ligand interaction in order for the process to proceed. After all, this is how the immune system often works: Clonal selection occurs when TCR signals affect transcriptional activation of cytokine and cytokine receptor genes, so that the activation pathway again surfaces in the form

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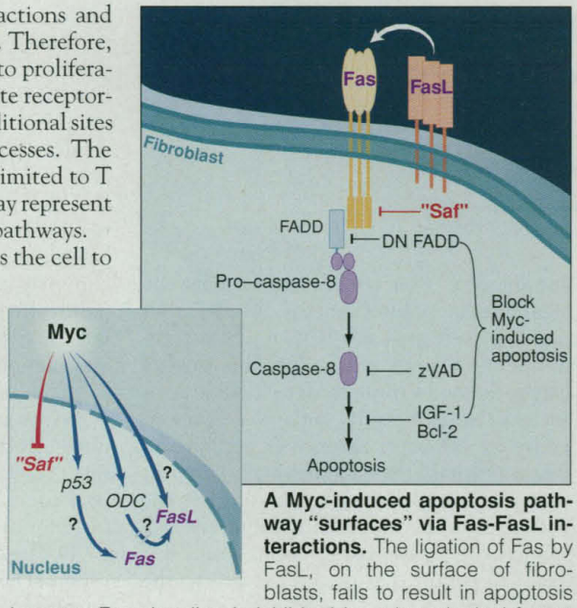
of growth factor–receptor interactions and leads to entry into the cell cycle. Therefore, in T cells, the pathways leading to proliferation or death involve intermediate receptor-ligand steps that may provide additional sites for regulation of these key processes. The surprise is that this effect is not limited to T cells, suggesting that surfacing may represent a general principle in signaling pathways.

In fibroblasts, c-Myc sensitizes the cell to Fas-mediated apoptosis by an unknown mechanism. This process is shown in the figure as the inhibition of a factor (directly or indirectly by Myc) that is itself postulated to inhibit Fas-mediated death. This suppressor of apoptosis by Fas (Saf) is hypothetical, but its existence is likely because in many cells resistance to Fas-mediated apoptosis can be removed by treatment with cycloheximide or actinomycin D, which presumably eliminate this short-lived inhibitory factor. A prime candidate for c-Myc-regulated Saf is an inhibitor of Fas function that currently goes by many names, for example, Flip (*14*). Whether such sensitization (by removal of Saf) is also involved in activation-induced apoptosis in T cells is unclear, but there are hints that it may be (*14–16*). Although this does not exclude effects of Myc on Fas or FasL expression, it does ensure that only those cells expressing Myc will die; neighboring cells may contact FasL on the Myc-expressing cell but will not have been sensitized for death unless they too express c-Myc.



A Myc-induced apoptosis pathway "surfaces" via Fas-FasL interactions. The ligation of Fas by FasL, on the surface of fibroblasts, fails to result in apoptosis because Fas signaling is inhibited by a hypothetical factor, Saf (suppressor of apoptosis by Fas). Myc expression sensitizes fibroblasts for Fas-mediated apoptosis, perhaps by removal of Saf. Meanwhile, Myc also induces expression of genes that may up-regulate Fas, FasL, or both (**inset**), thus enhancing the Fas-mediated death signals. Ultimately, ligation of Fas triggers the association of FADD/Mort-1 and pro–caspase-8, and the latter is activated. The activation of caspase-8 is blocked by dominant-negative FADD (DN FADD) or by peptide caspase inhibitors (zVAD), both of which block Myc-induced apoptosis but do not rescue proliferation in these cells. Activated caspase-8 triggers downstream apoptotic events that can be inhibited by survival factors (such as IGF-1) and Bcl-2, which also block Myc-induced cell death.

Other molecules have been implicated in c-Myc–driven apoptosis, although it is not known whether these participate in the Fas-FasL–mediated cell death described here. In one hematopoietic cell line, expression of c-Myc sensitizes cells to undergo rapid apoptosis upon growth factor withdrawal. This effect appears to be mediated by the expression of ornithine decarboxylase (ODC), because ectopic expression of ODC is able to substitute for c-Myc, whereas inhibitors of ODC neutralize the effect of c-Myc (*17*). Could ODC function in the induction of, or sensitization to, Fas-FasL interactions? ODC effectively alters intracellular nucleotide pools, an effect that can trigger apoptosis via FasL up-regulation and Fas-FasL interactions in a colon carcinoma line (*18*).

The coordinate regulation and interaction between apoptosis and proliferation is further illustrated by the observation that p53 is up-regulated upon c-Myc expression, a critical event implicated in c-Myc–induced apoptosis in fibroblasts (*19*). p53 is able to transcriptionally down-regulate the expression of the IGF-I receptor, thereby limiting the input of survival and proliferative signals in favor of those that promote death (*20*). Whether p53 plays any role in c-Myc–induced, Fas-FasL–mediated apoptosis is unclear, although activation of p53 does induce Fas in several cell lines, probably via transcriptional activation (*21*).

The absence of Fas, blockade of FasL, or a disruption of Fas signaling via FADD all suppress c-Myc–induced apoptosis. This result might suggest that such defects could permit the selective expansion of c-Myc–expressing cells and represent a critical step in the progression to oncogenesis. So, are Fas and FasL tumor-suppressor genes? Transgenic expression of L-Myc in lymphocytes results in tumors only in animals defective for Fas expression (*22*). In general, however, defects in Fas or FasL do not lead to tumors the way that defects in p53 do. But if Fas-FasL interactions are required for c-Myc–induced apoptosis, then why don't defects in this process predispose to cancer? The answer to this question may provide important clues to the control of transformation by apoptosis.

Potentially, this answer may lie in a paradox presented by c-Myc–induced apoptosis. Apoptosis initiated by ligation of Fas and signaled via the FADD molecule proceeds through a caspase protease, caspase-8, as a necessary step in this death pathway (*23*). (A second Fas-mediated apoptosis pathway present in some cells is not inhibited by dominant-negative FADD (*24*) and therefore probably does not concern us here.) Caspase inhibitors not only block this pathway to apoptosis, but eliminate all of the signals leading to cell death (the cells survive and can grow after Fas ligation if caspases are blocked) (*25*). However, caspase inhibitors do not prevent the death of fibroblasts in which c-Myc is expressed (*26*). Either inhibition of caspases fails to block Fas-mediated death in these cells, which seems unlikely given our current knowledge, or else there is at least one other caspase-independent mechanism that ensures that cells die when c-Myc is expressed under conditions of limiting survival factors. Indeed, blockade of Fas signaling or of Fas-FasL interactions fails to allow proliferation of c-Myc–driven cells; another mechanism is apparently at work. This other mechanism might commit the cells to die, even when Fas or FasL are nonfunctional. Does this mechanism surface as well?

References

1. G. I. Evan *et al.*, *Cell* **69**, 119 (1992).
2. R. P. Bissonnette, F. Echeverri, A. Mahboubi, D. R. Green, *Nature* **359**, 552 (1992).
3. A. Fanidi, E. A. Harrington, G. I. Evan, *ibid.*, p. 554.
4. B. Amati, T. D. Littlewood, G. I. Evan, H. Land, *EMBO J.* **12**, 5083 (1993).
5. E. A. Harrington, M. R. Bennett, A. Fanidi, G. I. Evan, *ibid.* **13**, 3286 (1994).
6. A. O. Hueber *et al.*, *Science* **278**, 1305 (1997).
7. J. Klefstrom *et al.*, *EMBO J.* **13**, 5442 (1994).
8. R. U. Janicke, F. H. Lee, A. G. Porter, *Mol. Cell. Biol.* **14**, 5661 (1994).
9. T. Brunner *et al.*, *Nature* **373**, 441 (1995).
10. J. Dhein, H. Walczak, C. Baumler, K. M. Debatin, P. H. Krammer, *ibid.*, p. 438.
11. S. T. Ju *et al.*, *ibid.*, p. 444.
12. Y. Shi *et al.*, *Science* **257**, 212 (1992).
13. R. P. Bissonnette, A. McGahon, D. R. Green, *J. Exp. Med.* **180**, 2413 (1994).
14. M. Irmler *et al.*, *Nature* **388**, 190 (1997).
15. C. Klas, K. M. Debatin, R. R. Jonker, P. H. Krammer, *Int. Immunol.* **5**, 625 (1993).
16. T. Brunner, N. J. Yoo, D. LaFace, C. F. Ware, D. R. Green, *ibid.* **8**, 1017 (1996).
17. G. Packham and J. L. Cleveland, *Curr. Top Microbiol. Immunol.* **194**, 283 (1995).
18. J. A. Houghton, F. G. Harwood, D. M. Tillman, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 8144 (1997).
19. H. Hermeking and D. Eick, *Science* **265**, 2091 (1994).
20. H. Werner, E. Karnieli, F. J. Rauscher, D. LeRoith, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 8318 (1996).
21. L. B. Owen Schaub *et al.*, *Mol. Cell. Biol.* **15**, 3032 (1995).
22. M. Zornig, A. Grzeschiczek, M. B. Kowalski, K. U. Hartmann, T. Moroy, *Oncogene* **10**, 2397 (1995).
23. J. P. Medema *et al.*, *EMBO J.* **16**, 2794 (1997).
24. X. Yang, R. Khosravi Far, H. Y. Chang, D. Baltimore, *Cell* **89**, 1067 (1997).
25. V. L. Longthorne and G. T. Williams, *EMBO J.* **16**, 3805 (1997).
26. N. J. McCarthy, M. K. Whyte, C. S. Gilbert, G. I. Evan, *J. Cell Biol.* **136**, 215 (1997).