We conclude that, although $p34^{cdc2}$ activation accompanies some forms of apoptosis (*1*) and may be an essential requirement in these instances, it is not obligatory for apoptosis in general. This implies that Cdc2 is not a part of the apoptosis machinery, but rather an upstream regulator of this machinery under certain conditions. Thus, the idea that apoptosis is essentially a form of mitotic catastrophe is not supported by these findings.

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8. Cells were incubated with 10 μg/ml propidium iodide (PI) dye at room temperature for 5 min and were then analyzed on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) for discrimination of live (PI-negative) from dead (PI-positive) cells, as previously described [S. J. Martin, P. M. Matear, A. Vyakarnam, *J. Immunol.* **152**, 330 (1994)].
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Response: The cytotoxic T lymphocyte (CTL) protease granzyme B (also called fragmentin-2), which is a molecular mediator of apoptosis by CTL, prematurely induces Cdc2 kinase activity and requires Cdc2 protein expression to mediate apoptosis (*1*). Cells transiently transfected with and overexpressing $p50^{Wee1}$ kinase, the kinase that phosphorylates and maintains Cdc2 in an inactive form during the cell cycle until mitosis (*2*), are rescued from granzyme B by preventing Cdc2 dephosphorylation (*3*). These data provide evidence that granzyme B uses a Cdc2-dependent apoptosis pathway. It is now clear that this Cdc2 pathway is not peculiar to granzyme B. Li *et al.* (*4*) recently identified the activation of cyclin A–associated Cdc2 in HIV-1 Tat–induced apoptosis, and they were able to inhibit the apoptotic effects of Tat on T cells with antisense cyclin oligonucleotides. Fotedar *et al.* (*5*) find that cross-linking the T receptor of the A1.1 hybridoma with anti-CD3 antibodies results in apoptosis and elevated Cdc2 and cyclin B activity, and that the apoptosis can be blocked by cyclin B antisense oligonucleotides.

Cdc2 kinase, a protein that is expressed in all cycling mammalian cells and that induces mitotic catastrophe when prematurely activated (*6*), seems a possible effector mechanism for apoptosis. The potential link between a universal regulator mitosis and a cell death pathway might also satisfy the need to link the control of cellular proliferation and apoptosis. However, the observation of programmed cell death in postmitotic neurons (*7*) would argue that it cannot be a universal regulator as noncycling cells do not express Cdc2. Although drugs are often able to induce Cdc2 kinase activity during apoptosis (*8*), it is not evident from these studies that it is necessary. Indeed, Martin *et al.* (*9*) in the accompanying technical comment report that some drugs induce apoptosis in the face of reduced expression of Cdc2 in the TS Cdc2 mutant FT210.

It is becoming evident that Cdc2 is not the only member of the CDK family that is activated during apoptosis. For example, cyclin A–associated Cdk2 is induced after treatment with HIV-1 Tat (*4*) and several types of drugs (*8*). In the experiments described by Martin *et al.*, the expression of Cdk2 is unaffected in the FT210 Cdc2 TS mutant at restrictive temperatures, and its activation and participation in apoptosis cannot be excluded. Recently, another CDK-related kinase, p58 PITSLRE, was found to initiate apoptosis when overexpressed, and was activated during Fas-induced apoptosis (*9*). The possibility that other CDKs are responsible for the "Cdc2-independent" apoptosis would be consistent with the multiplicity of apoptotic effectors (and suppressors) seen in the ICE and bcl-2 families. The idea that there exists a biochemical event that is necessary and sufficient for apoptosis with a single pathway and a single limiting effector molecule, such as that found in the nematode *Caenorhabditis elegans*, might be an oversimplification in higher eukaryotic cells. The variety of unique proteins in the ICE and bcl-2 families argues for the existence of different pathways each using one or more family members to regulate induction of apoptosis within a given tissue or for a particular differentiation program. This is supported by the observation that homozygous null mutants of single members of these families produces a variable phenotype in which programmed cell death is altered in only certain tissues (*10–22*). Thus, it is possible that parallel apoptotic pathways exist which ultimately converge at a late postcommitment stage to produce the phenotype of apoptosis. Cdc2, as a member of a larger family of CDK effectors, participates in some of these apoptotic pathways, although it is unknown whether all pathways would necessarily involve a CDK effector.

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Response

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