ing is not the source of internal stabilization for $3H^+$, we will have to invent a new physicochemical phenomenon to explain the basicity of compound 3.

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$p34^{cdc2}$ and Apoptosis

Recently, Lianfa Shi *et al.* (*1*) reported on the involvement $p34^{cdc2}$ in two instances of apoptotic cell death. As apoptosis exhibits several features reminiscent of mitosis, it has been suggested that some of the cell cycle components that drive cells into mitosis may also be responsible for triggering apoptosis. The serine-threonine kinase, $p34^{cdc2}$, is essential for entry into mitosis and, if prematurely activated, can induce a process resembling apoptosis that has been termed "mitotic catastrophe" (*2*).

Fragmentin-2, a cytotoxic cell granule serine protease, can trigger apoptosis in cells exposed to this protease in combination with perforin, a pore-forming cytotoxic granule protein (*3*). Apoptosis induced by fragmentin-2 and perforin was shown by Shi *et al.* to be accompanied by a dramatic increase in $p34^{cdc2}$ kinase activity and to be inhibited by a $p34^{cdc2}$ substrate peptide (*1*). Also, FT-210 cells, which carry a temperature-sensitive $p34^{cdc2}$ that prematurely degrades at 39°C (*4*), resisted apoptosis in the presence of fragmentin-2 and perforin or staurosporine after pre-incubation at this temperature (*1*). These and other recent observations (*5*) suggest that $p34^{cdc2}$ may play a key role in several forms of apoptosis.

To explore whether $p34^{cdc2}$ activity is a general requirement for apoptosis, we used the FT-210 cell line, in combination with a diverse array of apoptosis-inducing stimuli, to ask whether these cells generally died less rapidly after culture at the restrictive temperature of 39°C ($p34^{cdc2}$ degraded), as compared with the kinetics of cell death after culture at 32°C (normal $p34^{cdc2}$ content).

FT-210 cells degraded their $p34^{cdc2}$, but not another cell cycle–associated kinase ($p33^{cdk2}$), when incubated at 39°C versus 32°C (Fig. 1), as previously reported (*4*). No changes in amounts of Cdc2 were detected in parental FM-3A cells under the same conditions (Fig. 1). Decreases in $p34^{cdc2}$ were also reflected in changes to the cell cycle profile of FT-210 cells incubated at 39°C, as these cells could not traverse the G_2/M boundary and arrested in late G_2 (*6*), whereas FM-3A cells traversed the cell cycle at either temperature (*6*).

Actinomycin D (Act D; an RNA synthesis inhibitor), hydrogen peroxide (H_2O_2), ultraviolet (UV) B radiation, VP-16 (a topoisomerase II inhibitor), cycloheximide (Chx; an inhibitor of protein synthesis), and C_2-ceramide (a sphingolipid implicated as a second messenger in apoptosis induced by TNFR and Fas ligation, as well as other forms of apoptosis) are all potent inducers of apoptosis (*7*). FT-210 cells, pre-incubated for 18 to 24 hours at 32°C or 39°C, were exposed to a range of concentrations of each agent. To minimize potential kinetic differences between the rate of apoptosis at 32°C versus 39°C, exposure to the various agents was conducted at 37°C (*1*). Cell death was quantitated 18 to 24 hours later.

Rather than being resistant to the induction of apoptosis after pre-incubation under conditions that degraded $p34^{cdc2}$, FT-210 cells proved to be equally susceptible to several of the apoptosis-inducing stimuli tested, whether pre-incubated at 32°C or 39°C (Fig. 2). In each case, the cell deaths observed were accompanied by typical features of apoptosis, such as condensation and fragmentation of the nucleus as well as DNA cleavage (*6*). G2-arrested FT-210 cells actually proved to be more susceptible to undergoing apoptosis in response to UV irradiation and H_2O_2, but the reasons for this are unknown.

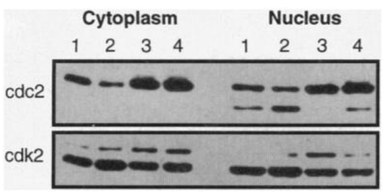

Fig. 1. Western blot detection of $p34^{cdc2}$ (top panel) and $p33^{cdk2}$ expression in FT-210 (columns 1 and 2) versus FM-3A cells (columns 3 and 4) after a 24-hour incubation at the permissive (32°C; columns 1 and 3) or restrictive (39°C; columns 2 and 4) temperature.

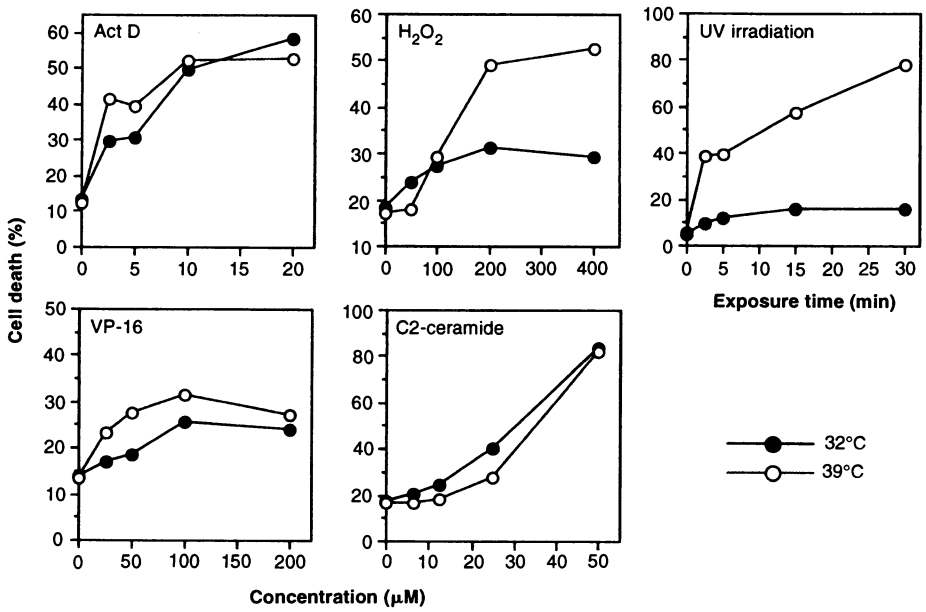

Fig. 2. Cell viability, as assessed by PI dye uptake (*8*) of FT-210 cells pre-incubated at either the permissive or restrictive temperature, followed by a further 18-hour incubation (at 37°C) in the presence or absence of the indicated stimuli. For UV B irradiation, culture dishes were placed on a 302 nM UV transilluminator and were irradiated from below for the indicated periods of time, as previously described (*7*). Each data point is derived from counts performed on 5000 cells. All treatments were carried out in triplicate. Results shown are representative of six independent experiments.

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 post-commitment 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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