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# URJiit

Undergraduate Research Journal
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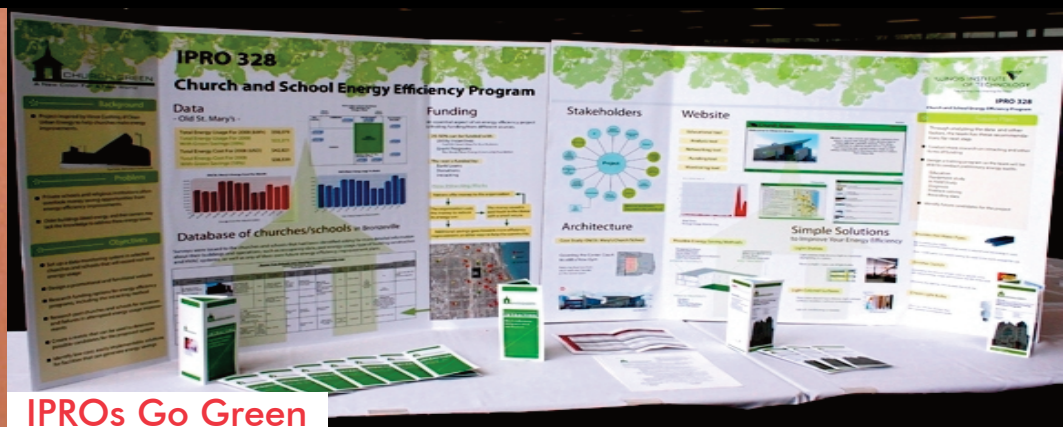
Architecture — Business — Engineering — Psychology — Science & Letters — Technology

## CSL Awards Summer Undergrad Research Stipends

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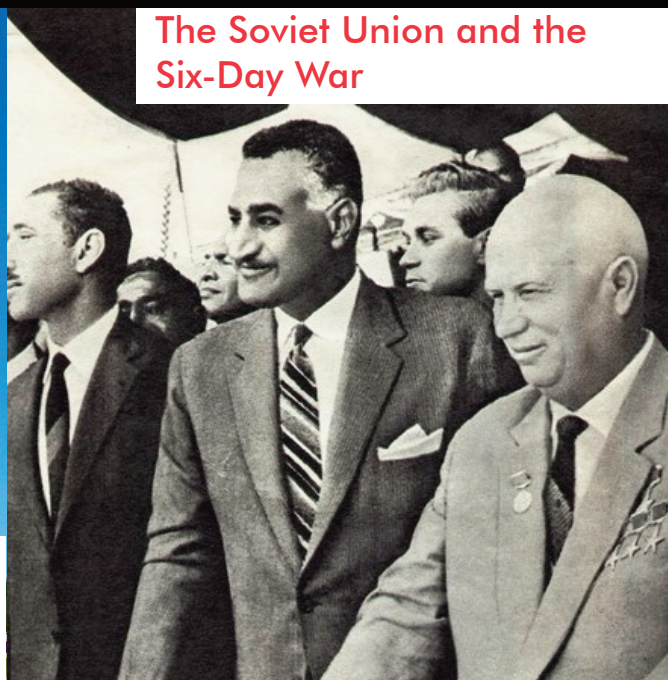

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URJiit is the official Undergraduate Research Journal of Illinois Institute of Technology, showcasing undergraduate research throughout all departments of IIT.

**Science & Letters Announces Undergraduate Summer Research Stipends**

The College of Science and Letters announced that it has awarded eight undergraduate students with 2010 College of Science and Letters Undergraduate Summer Research Stipends. Awardees will receive $5,000 to do 10 weeks of research with a faculty member on campus this summer.

Research helps students explore their interests, solve problems, advance knowledge, and prepare for their next step after IIT - such as graduate school, medical school, or the workplace.

As one of last year's recipients, Jesse Reinhardt, said of his experience, "I've been able to get in-depth, hands-on skills that are hard to get by just taking regular classes."

"Research helps to enrich the students' experience at IIT," said Ishaque Khan, CSL associate dean and professor of chemistry. "The financial support lets them focus on research between terms and catalyze their productivity."

This year's awardees and their faculty sponsors are:

* Keenan Gottschall (third-year PS) will work with Matthew Shapiro, assistant professor of political science, on original research about environmental policies and their political determinants in East Asia, with a goal to compare these countries with the United States.

* Jiang Lan (third-year CS), under the guidance of Gady Agam, associate professor of computer science, will develop a virtual clay software that will allow users to sculpt virtual objects - work that connects to a larger problem of perceptive user interfaces.

* Jeonghun Lee (fourth-year physics and applied mathematics) will help Grant Bunker, associate chair and professor of physics, to develop X-ray fluorescence imaging capability at IIT.

* Zhihe Liu (second-year applied mathematics) will build math models to find the signal pathways in cancer cells for Shuwang Li, assistant professor of applied mathematics, in a joint project with the research team of Jialiang Xiang, assistant professor of biology.

* Frank Lockom (third-year CS) will work with Matthew Bauer, senior lecturer of computer science, to redesign CS100 to improve its alignment to current needs, including the new Distinctive Education efforts.

* Nathan Majernik (second-year physics and applied mathematics) will improve the efficiency with which tomography can be done at MRCAT with Carlo Segre, professor of physics, associate dean for admissions, Graduate College, and deputy director of MRCAT.

* Sean Wallace (third-year CIS), under the guidance of Matt Bauer, assistant professor of linguistics, plans to develop a tool and protocol to measure hyperarticulated speech and rate its success at correct voice recognition.

* Julia Zaug (third-year chemistry) will work in the lab of Brant Cage, assistant professor of chemistry, to synthesize magnetic MRI contract agents and characterize them with NMR, with the data gained to be used to optimize NMR/MRI signals.

CSL emphasizes research experience for undergraduates and allows students to start doing it as early as second semester of their freshman year - unusual in most universities.

CSL started the paid summer research program to give students a chance to truly focus on their research work between terms without competition for their attention from other classes or a job.

This is the fourth year that CSL has awarded summer research stipends, which are funded primarily by the CSL board of overseers, CSL alumni, and the college.

Article from iit.edu

# BAX-induced cell death may not require interleukin 1b-converting enzyme-like proteases

Jialing Xiang
College of Science & Letters — Biology

*Expression of BAX, without another death stimulus, proved sufficient to induce a common pathway of apoptosis. This included the activation of interleukin 1bconverting enzyme (ICE)-like proteases with cleavage of the endogenous substrates poly(ADP ribose) polymerase and D4-GDI (GDP dissociation inhibitor for the rho family), as well as the f luorogenic peptide acetyl-Asp-Glu-Val-Aspaminotrifluoromethylcoumarin (DEVD-AFC). The inhibitor benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone (zVADfmk) successfully blocked this protease activity and prevented FAS-induced death but not BAX-induced death. Blocking ICE-like protease activity prevented the cleavage of nuclear and cytosolic substrates and the DNA degradation that followed BAX induction. However, the fall in mitochondrial membrane potential, production of reactive oxygen species, cytoplasmic vacuolation, and plasma membrane permeability that are downstream of BAX still occurred. Thus, BAXinduced alterations in mitochondrial function and subsequent cell death do not apparently require the known ICE-like proteases.*

## Introduction

Bax, a Bcl-2 family member, functions as a death agonist within a common apoptotic pathway (1). BAX forms homodimers and also heterodimerizes with death antagonists, BCL-2 and BCL-XL (1, 2). The ratio of BCL-2 family death agonists to antagonists dictates the susceptibility of cells to an apoptotic stimulus (3). When BAX is in excess, multiple death stimuli including withdrawal of survival factors, γ-irradiation, and dexamethasone result in apoptosis. Bax-deficient mice confirmed this role displaying an excess of lymphocytes, granulosa cells, spermatogonia, and select neurons that also demonstrate marked resistance to neurotrophic factor deprivation (4, 5). Important questions remained as to whether the BAX/BCL-2 ratio represents a passive checkpoint requiring an additional apoptotic signal or whether BAX itself could initiate death, and if so, how.

A family of interleukin 1ß-converting enzyme (ICE)-like cysteine proteases homologous to the ICE are clearly activated in apoptosis and appear required for certain aspects of cell death (6). The ICE homolog in Caenorhabditis elegans ced-3, is required for cell death in that extra cells accumulate in ced-3 mutants. In mammals the number of ICE-like proteases has expanded and includes CPP32/APOPAIN/YAMA, MCH2, MCH3/LAP-3, ICErel-III, ICH-1/NEDD-2, and ICH-2/TX/ICErel-II (6, 7). The activated ICE-like proteases have a unique recognition site cleaving at a novel P1 aspartic acid (8, 9). The subset of proteases most similar to the original ICE prefers Tyr-Val-Ala-Asp (YVAD), while the CPP32 subset prefers Asp-Glu-Val-Asp (DEVD). Substrates of ICE-like proteases include poly(ADP ribose) polymerase (PARP), D4-GDI (GDP dissociation inhibitor for the rho family), sterol regulatory element-binding proteins SREBP-1 and SREBP-2, GAS2, 70-kDa component of U1 small nuclear ribonucleoprotein, catalytic subunit of DNA protein kinase, and protein kinase Cδ (10). Some of the targeted proteins may prove to be death substrates that upon cleavage ensure the inevitability of death. In mammals, FAS-induced death appears to require ICE-like proteases, since protease inhibitors can prevent this cell death (11). Whether other pathways of apoptosis in mammalian cells absolutely require ICE-like proteases to induce death is less certain.

## Methods

### Inducible Expression System

Jurkat cells (clone E6-1, American Type Culture Collection) were cultured in medium RPMI 1640 supplemented with 10% fetal calf serum (FCS) and penicillin/streptomycin (100 units/ml). To establish the Jurkat-rtTA-Bax cell line, Jurkat cells were first transfected with rtTA transactivator plasmid (PUHD172-1). Stable transfectants (referred to Jt-1) were screened and selected as described (12). Murine Bax cDNA was cloned into the EcoRI site of pUHD10-3 and cotransfected into Jt-1 with PGK-Hygromycin B. For induction of BAX protein, doxycyline (Sigma) at 1 µg/ml was added to the culture for various periods of time as indicated.

### Antibodies and Immunoblot Analysis

Anti-human Fas antibody (IgM, clone CH-11) was purchased from Upstate Biotechnology. Anti-mouse BAX polyclonal antibody (651) was made against murine BAX amino acids 43–61. Anti-CPP32 and ICH-1L antibodies were from Transduction Lab (Lexington, KY). Anti-LAP3 antibody was a gift from V. M. Dixit (University of Michigan). Anti-ß-actin antibody was from Sigma. Anti-PARP antibody was a gift from N. A. Berger (Case Western Univ., Cleveland, OH).

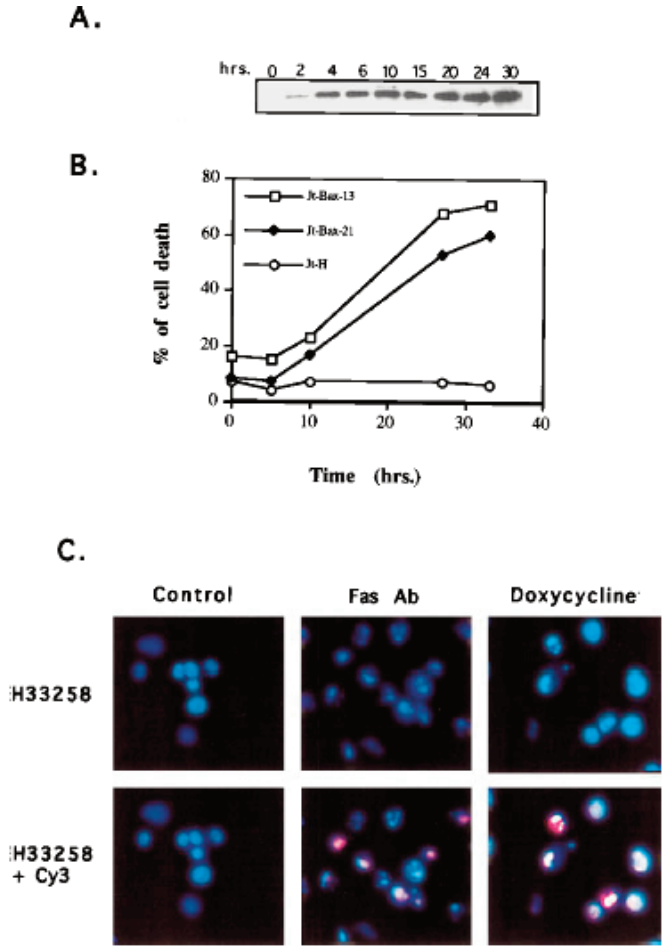


FIG. 1. BAX-induced apoptosis in Jt-Bax cells. (A) BAX levels were not substantially greater than that obtained in stable clones. (B) Jt-Bax-13 and -21 were two independent Jt-Bax clones, while Jt-H was an empty vector containing control clone. (C) The cells with blue or blue plus pink fragmented and condensed nuclei represent apoptotic cells.

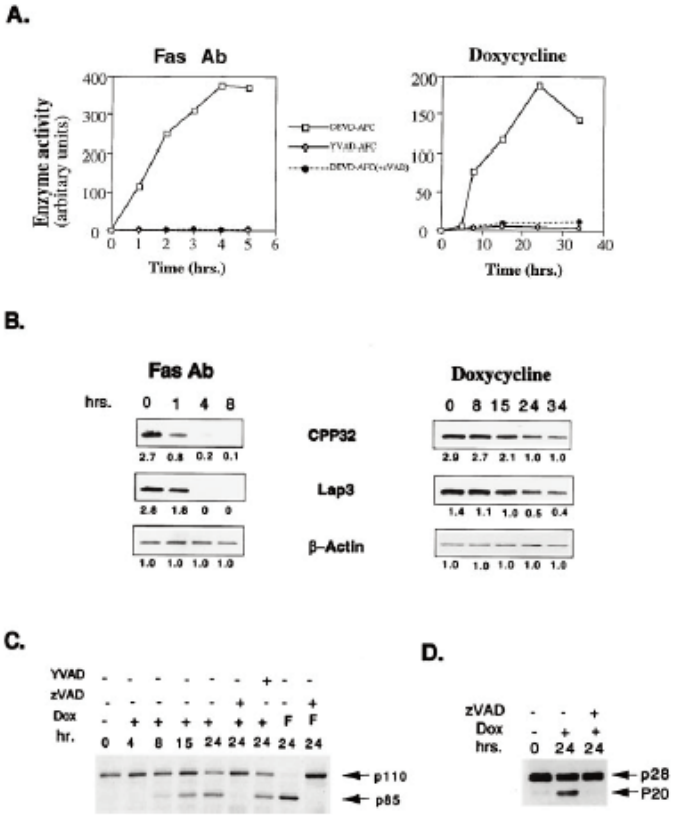


FIG. 2. Activation of ICE-like proteases in Jt-Bax cells. (A) ICE and CPP32-like activities were measured using fluorogenic substrates YVAD-AFC and DEVD-AFC, respectively. (B) The cleavage of ICE-like proteases was examined by immunoblot analysis with the antibodies indicated and quantitated by densitometer scanning (Ultroscan XL) using b-actin as a control. Apoptotic degradation of PARP (C) or D4-GDI (D) was analyzed by immunoblot. p85, the apoptotic fragment of PARP; p20, the apoptotic fragment of D4-GDI; Dox, doxycycline; F, anti-FAS antibody.

Anti-D4-GDI antibody was provided by G. M. Bokoch (Scripps Research Institute, La Jolla, CA). For Western blot analysis, cells were lysed in buffer containing 20 mM Hepes (pH 7.4), 0.25% Nonidet P-40, leupeptin at 10 μg/ml, aprotinin at 10 μg/ml, and trypsin inhibitor at 10 μg/ml. Thirty to 50 μg of protein was separated by SDS/PAGE and transferred to poly(vinylidene difluoride) membranes. The filters were first blocked with 2% milk, followed by 1-hr incubations with primary and secondary antibodies and finally developed with ECL (Amersham).

**CPP32 and ICE Activity Assay**

Cells were lysed in buffer A containing 25 mM Hepes (pH 7.4), 5 mM EDTA, 2 mM DTT, and 10 μM digitonin. The lysates were clarified by centrifugation and the supernatants were used for enzyme assays. Enzymatic reactions were carried out in buffer A containing 20 μg of protein and 50 μM acetyl-Asp-Glu-Val-Asp-aminotrifluoromethylcoumarin (DEVD-AFC) or 12 μM acetyl-Tyr-Val-Ala-Asp-aminotrifluoromethylcoumarin (YVAD-AFC). The reaction mixtures were incubated at 37°C for 15 min, and the fluorescent AFC formation was measured at excitation 400 nm and emission 505 nm using a FL500 microplate fluorescence reader (Bio-Tek, Burlington, VT). Sensitivity of the enzyme assay with AFC (Enzyme Systems Products, Livermore, CA) is equivalent to aminomethylcoumarin (8) in purified systems.

**Fluorescence and Electron Microscopy.**

Apoptotic nuclei were visualized by H33258 staining and the DNA fragmentation was detected by TdT-mediated dUTP-cyanine-3 nick end-labeling (TdT-TUNEL) (13). For electron microscopy, 5 × 106 cells were fixed with 3% glutaraldehyde in cocadylate buffer and analyzed by transmission electron micrographs.

**Mitochondrial Potential and Reactive Oxygen Species (ROS) Measurement**

For mitochondrial potential and intracellular ROS measurement, 5 × 105 cells were incubated for 15 min at 37°C with 3,3′-dihexyloxacarbocynine iodide [DiOC6(3), 40 nM], hydroethidine (2 μM), or 2′,7′-dichlorofluorescin diacetate (DCFH-DA, 5 μM; Molecular Probes), followed by FACScan (Becton Dickinson) analysis. The extracellular ROS was measured by electron paramagnetic resonance (EPR) using 5,5 dimethyl-1-pyrolline-N-oxide as the spin-trapping

agent (14, 15).

## Results

While Bax required an additional death stimulus to promote apoptosis in stably transfected cells, the toxicity of BAX appeared to limit its level of expression (1). A variety of apoptotic signals induce cell death by activating ICE-like cysteine proteases. ICE-like proteases are activated in a sequential cascade of cleavages from their inactive proforms (16, 17). To determine whether the ICE-like proteases were involved in BAX-induced apoptosis, the cleavage of specific fluorogenic peptide substrates YVAD-AFC for proteases most resembling ICE and DEVD-AFC for the CPP32-like subset was measured (Fig. (Fig.22A). Induction of BAX resulted in cleavage of DEVD, not YVAD, but in a delayed time course as compared with FAS-induced death. This enzymatic activity was blocked by pretreatment with the ICE-like protease inhibitor benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone (zVAD-fmk) (Fig. (Fig.22A). Western blot analysis identified CPP32 and LAP3 as participating proteases after BAX induction (Fig. (Fig.22B), while there was no change in the proenzyme levels of ICE or ICH-1L (data not shown). Activation of CPP32 and LAP3 was delayed and less complete in BAX than FAS-induced death.

## Discussion

Available evidence indicates that the BCL-2/BAX decisional point is proximal to the irreversible damage of cellular constituents. Genetic ordering in the nematode C. elegans argues that the ICE-like gene ced-3 is downstream of its BCL-2 homolog, ced-9 (26). In mammalian cells, the expression of BCL-2 blocks the activation of CPP32 (19, 27). Introduction of the baculovirus p35 gene into C. elegans inhibited CED-3 and blocked developmental cell death also arguing for an essential role of this protease in the nematode (28).

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## Acknowledgements

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Jialing Xiang

**Department of Biological, Chemical, and Physical Sciences**

The work in my laboratory is focused on the signaling network of cancer and its potential therapy. Cancer is characterized by uncontrolled cell growth, which results from unlimited proliferation and malfunction of programmed cell death.

We believe that understanding the apoptotic signaling mechanism of cancer cells should shed light on our understanding of cancer development and may provide information critical for treatment and prevention of cancers.