1. Wilson, J. R., Kuehn, R. E. & Beach, F. A. *J. Comp. Physiol. Psychol.* **56,** 636–644 (1963).
2. Adler, N. T. in *Biological Determinants of Sexual Behaviour* (ed. Hutchison, J. B.) 656–695 (Wiley, New York, 1978).
3. Magurran, A. E. & Seghers, B. H. *Proc. R. Soc. Lond. B* **258,** 89–92 (1994).
4. Houde, A. E. *Sex, Color, and Mate Choice in Guppies* (Princeton Univ. Press, New Jersey, 1997).
5. Liley, N. R. *Behaviour* **13** (suppl.), 1–197 (1966).
6. Milinski, M., Külling, D. & Kettler, R. *Behav. Ecol. Sociobiol.* **27,** 17–21 (1990).
7. Dugatkin, L. A. & Wilson, D. S. *Rev. Fish Biol. Fish.* **3,** 368–372 (1993).
8. Griffiths, S. W. & Magurran, A. E. *Anim. Behav.* **56,** 689–693 (1998).
9. Griffiths, S. W. & Magurran, A. E. *Anim. Behav.* **53,** 945–949 (1997).
10. Griffiths, S. W. & Magurran, A. E. *Proc. R. Soc. Lond. B* **264,** 547–551 (1997).
11. Heinrich, W. & Schröder, J. H. *Biol. Zbl.* **105,** 491–502 (1986).
12. Farr, J. A. *Evolution* **31,** 162–168 (1977).
13. Hughes. K. A. *et al. Anim. Behav.* **58,** 907–916 (1999).

Apoptosis

Searching for FLASH domains

During programmed cell death (apoptosis), a protein named FLASH is required to regulate the proteolytic cascade that ends in the death of the cell. Imai and co-workers have reported[1] that FLASH appears to be a functional analogue of two other apoptotic proteins, mammalian Apaf-1 and its nematode homologue CED-4, and that FLASH contains an amino-acid sequence motif that is homologous to the ATPase domain of Apaf-1, to the CED-4 sequence, and to a family of plant stress-resistant proteins that are apoptotic ATPases[2]. Furthermore, FLASH contains two other domains (DRD) that are apparently related to the death-effector domain (DED)[1], an adaptor sequence that mediates interactions between proteins of the apoptosis machinery[2]. These findings should help to explain the mechanism of action of this important protein. However, we have been unable to confirm the existence of these domains after re-examining the FLASH sequence.

We could identify no sequence similarity between FLASH and the Apaf-1/CED-4 or DED domains by searching the non-redundant protein sequence database at the NCBI using the gapped BLAST or PSI-BLAST programs[3,4], and over 1,000 sequences in the database were found to be more similar to the 'CED-4 homology'[1] and 'DED homology' regions of FLASH than were CED-4 or Apaf-1.

Searching databases, however, may only detect less than half of all similarities between sequences in proteins that are considered to be homologues on the basis of structural comparisons[5,6]. Further analysis is needed, for example by direct comparison of functionally analogous proteins. We compared FLASH with Apaf-1/CED-4 and with DED-containing proteins by using the MACAW program[7], but failed to detect any blocks of statistically significant sequence similarity (data not shown). We also used the PHI-BLAST program to assess the importance of the ATP-binding (P-loop) signature in FLASH (this program screens for similarity only those sequences that contain the specified signature), but found no similarity to the apoptotic ATPases even in this reduced search space. The other four motifs typical of the apoptotic ATPases[2] are not conserved in the published alignment of FLASH with these ATPases.

To confirm the presence of DED-related domains in the FLASH sequence, we searched it with the DED profile by using the SMART system[8] and an independent method based on the PSI-BLAST program that detects all known DED domains[9], but we were unable to find any similarity to DED.

These tests cannot rule out a subtle similarity, although we believe that structure predictions for FLASH and phylogenetic analysis may effectively do so, at least with regard to the purported ATPase domain. Compositional complexity analysis using the SEG program[10] indicates that FLASH is largely a non-globular protein (Fig. 1). The entire 'CED-4 homology' region of FLASH is predicted to be non-globular, which is incompatible with the compact structure based on a parallel β-sheet with inserted α-helices that is typical of ATPase domains[11]. The P-loop in ATPases and GTPases is preceded by a hydrophobic β-strand, but this feature is lacking in FLASH.

The argument against structural similarity is supported by phylogenetic evidence. We have cloned and partly sequenced a human homologue of FLASH which has 67% amino-acid identity with FLASH in an alignment of 1,250 residues; the P-loop signature, however, is not conserved (data not shown; GenBank accession no. AF165161).

The structural and evolutionary evidence thus indicates that FLASH contains no globular domains with predictable functions and is not homologous to its functional analogues. FLASH does contain a predicted coiled-coil domain (Fig. 1) which may mediate functionally important protein–protein interactions[12] and so is probably the best available lead we have from the sequence for further experiments.

Eugene V. Koonin*, L. Aravind†, Kay Hofmann‡, Jurg Tschopp§, Vishva M. Dixit¶

**NCBI, National Library of Medicine, National Institutes of Health, Bethesda, Maryland 20894, USA*
†Department of Biology, Texas A&M University, College Station, Texas 70843, USA
‡Bioinformatics Group, MEMOREC Stoffel GmbH, Stoeckheimer Weg 1, 50829 Köln, Germany
§Institute of Biochemistry, Lausanne Branch, University of Lausanne, Epalinges, Switzerland
¶Department of Molecular Oncology, Genentech Inc., 1 DNA Way, South San Francisco, California 94080, USA
e-mail: dixit@gene.com

1. Imai, Y. *et al. Nature* **398,** 777–785 (1999).
2. Aravind, L., Dixit, V. M. & Koonin, E. V. *Trends Biochem. Sci.* **24,** 47–53 (1999).
3. Altschul, S. F. *et al. Nucleic Acids Res.* **25,** 3389–3402 (1997).
4. Aravind, L. & Koonin, E. V. *J. Mol. Biol.* **287,** 1023–1040 (1999).
5. Park, J. *et al. J. Mol. Biol.* **284,** 1201–1210 (1998).
6. Brenner, S. E., Chothia, C. & Hubbard, T. J. *Proc. Natl Acad. Sci. USA* **95,** 6073–6078 (1998).
7. Schuler, G. D., Altschul, S. F. & Lipman, D. J. *Proteins* **9,** 180–190 (1991).
8. Schultz, J., Milpetz, F., Bork, P. & Ponting, C. P. *Proc. Natl Acad. Sci. USA* **95,** 5857–5864 (1998).
9. Chervitz, S. A. *et al. Science* **282,** 2022–2028 (1998).
10. Wootton, J. C. & Federhen, S. *Methods Enzymol.* **266,** 554–571 (1996).
11. Hubbard, T. J. P., Murzin, A. G., Brenner, S. E. & Chothia, C. *Nucleic Acids Res.* **25,** 236–239 (1997).
12. Lupas, A., Van Dyke, M. & Stock, J. *Science* **252,** 1162–1164 (1991).

Kimura, Imai and Yonehara reply — We do not consider that our inferences about FLASH function are misleading because they were deduced from functional analyses[1]. We originally identified the death-effector domain (DED)-binding activity of the DED-recruiting domain (DRD) of FLASH before analysing the structural homology between the DED and DRD domains. Moreover, we deduced the self-association activity of FLASH through its central region from functional analysis of several deletion mutants of mouse FLASH. We note that self-association activity of

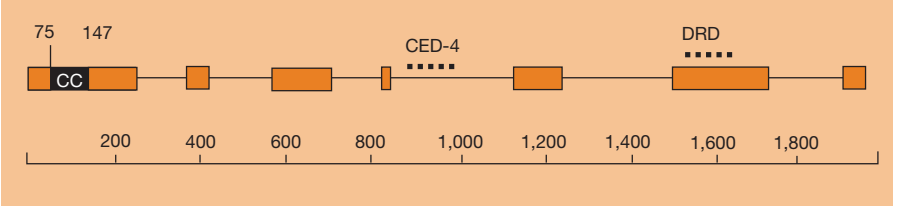

Figure 1 Diagram of the predicted domain organization of FLASH (roughly to scale). The numbered bar indicates amino-acid residue positions. Boxes, predicted globular regions; lines, predicted non-globular domains; CC, coiled-coil. Regions of alleged similarity[1] to the apoptotic ATPases (CED-4) and DED domains (DRD, or DED-related domains) are indicated by broken lines. Predicted non-globular domains were detected by using the SEG program[10], with the following parameters optimized for partitioning protein sequences into globular and non-globular domains: window length, 45; trigger complexity, 3.4; extension complexity, 3.7. Coiled-coil domains were predicted using the COILS2 program[12]; boundaries of the strongly predicted coiled-coil domain are indicated.

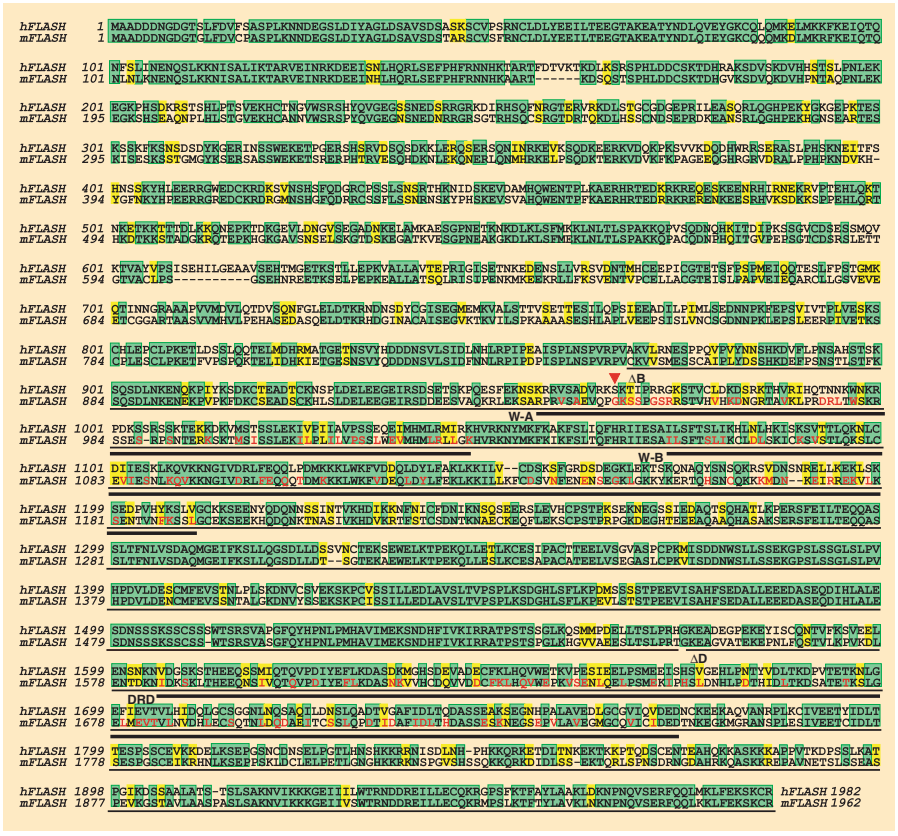

Figure 1 Comparison of deduced amino-acid sequences of mouse and human FLASH. Green and yellow boxes indicate identical and similar residues, respectively. Deletion mutants ΔB and ΔD containing 'CED-4-homologous' and DRD domains, respectively, which showed self-association and DED-recruiting activity, respectively[1], are underlined. Walker's A- and B-box-containing regions are underlined (W-A and W-B), as are the DRD domains. The amino-acid residues of mouse FLASH (red) in W-A, W-B and DRD domains are identical or similar to residues in CED-4-homologous and DED-containing proteins, as indicated previously[1]. Human FLASH was cloned by using a cDNA library of human KT cells[6] (a gift from S. Nagata). The cDNA library was screened by using fragments of human FLASH cDNA (EST clones N68740 and H50582) as probes and by standard colony hybridization procedures. The nucleotide sequence corresponding to the amino-acid sequence of human FLASH has been deposited in the GenBank database (accession no. AF154415).

CED-4-homologous proteins is required for the activation of caspases[2].

To determine the significance of the conserved amino-acid residues in mouse FLASH and in other apoptotic proteins, we cloned human FLASH to see whether these residues are still conserved in human FLASH (Fig. 1). Human FLASH complementary DNA showed 80% identity in the nucleotide coding sequence with mouse FLASH cDNA; in the amino-acid sequence, human FLASH shares 66% identity and 78% similarity with mouse FLASH.

In human FLASH, however, the ATP/GTP-binding motif homologous to the Walker's A-box consensus sequence[3] is not conserved, with a serine residue being substituted for an essential glycine in the ATP/GTP-binding motif (arrowhead in Fig. 1). In addition, amino-acid residues that are conserved between mouse FLASH and CED-4-homologous proteins in the Walker's A-box-containing region are not conserved in human FLASH (W-A in Fig. 1). These results indicate that the ATP/GTP-binding motif of mouse FLASH is not essential and that FLASH is not a CED-4-homologous protein. As Koonin *et al.* suggest, we could have overestimated the structural homology of FLASH with CED-4, even though these proteins are functionally homologous[1]. In the self-association region (ΔB in Fig. 1), there are some well conserved domains outside the W-A region, and it needs to be determined which of these conserved domains are required for the self-association activity of FLASH.

Contrary to the inference of Koonin *et al.* (a misunderstanding that may have arisen as the result of our calling DRD a DED-related domain to indicate a DED-recruiting domain), we reported that the DRD domain of FLASH is functionally different from the DED domain[1] in that the DRD domain is unable to self-associate, whereas the DED domain can. However, there could be some conservation of amino-acid residues in the FLASH DRD domain with those in the DED domain, given that these residues are conserved between the DRD domain of mouse FLASH and the DED domain of human FLASH (Fig. 1). Moreover, Koonin *et al.* show that the DRD domain is a globular domain, so we presume that the DRD domain has a structure that is quite similar to the DED domain.

Although the EST database has been exclusively searched using the BLAST program for DED-containing proteins, this method has never been able to identify FLASH. Having established that FLASH can bind to the DED domain[1], we cloned FLASH by using this function to detect it. Such an approach complements cloning methods based on searches of the EST database using BLAST, which has been successful for many other important apoptotic proteins, such as DED-containing FLICE and FLIP[4,5].

To address a more general concern regarding the specificity of our antibody, we cloned human FLASH and expressed recombinant Flag-tagged human FLASH in 293T cells. Following immunoprecipitation of human FLASH with anti-Flag antibody, our affinity-purified anti-mouse FLASH antiserum[1] was able to detect human FLASH on a western blot. Furthermore, we have shown that our antibody, which was raised against the peptide LSPNSDRNGDAHR (from mouse FLASH), can bind the peptide PTQDSCENTEAHQ (from human FLASH), albeit with a lower affinity than to mouse FLASH. We have also obtained monoclonal antibodies that recognize both mouse and human FLASH with associated characteristics to our affinity-purified polyclonal antibodies, and we shall report these results elsewhere.

Takaharu Kimura, Yuzuru Imai, Shin Yonehara*

Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan

e-mail: syonehar@virus.kyoto-u.ac.jp

1. Imai, Y. *et al. Nature* **398,** 777–785 (1999).
2. Walker, J. E. *et al. EMBO J.* **1,** 945–951 (1982).
3. Yang, X., Chang, H. Y. & Baltimore, D. *Science* **281,** 1355–1357 (1998).
4. Muzio, M. *et al. Cell* **85,** 817–827 (1996).
5. Irmler, M. *et al. Nature* **388,** 190–195 (1997).
6. Itoh, N. *et al. Cell* **66,** 233–243 (1991).

Materials

Transformation of diamond to graphite

Despite almost forty years of trying, no one has managed to transform diamond into graphite under pressure[1], or find out what the pressure limit for diamond might be[2]. If diamond were to behave like other group IV elements, such as silicon, germanium or tin, it would transform under compressive indentation to the β-tin structure[3], but it does not[2,4]. Here we use micro-Raman spectroscopy to determine what happens to diamond when it is subjected to high contact compression as a result of pressing a