Cell Death and Differentiation (1999) 6, 823–824

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Letter to the Editor

Genes with homology to DFF/CIDEs found in *Drosophila melanogaster*

Dear Editor,

Fragmentation of genomic DNA is an evolutionary conserved event associated with the execution of apoptosis. The DNA fragmentation factor (DFF) was identified and purified as a factor that induces fragmentation of genomic DNA in mammalian cells.[1,2] DFF is composed of two subunits, DFF40 (also called caspase-activated DNase or CAD and caspase-activated nuclease or CPAN) and DFF45 (also called inhibitor of CAD or ICAD).[1–5] DFF40 has a nuclease domain that can cleave naked and chromosomal DNA, whereas DFF45 is a regulatory subunit that suppresses the nuclease activity of DFF40.[1–6] Cleavage of DFF45 by caspase-3 induces the nuclease activity of DFF40.[1–7] Human and mouse DFF proteins are highly homologous at the amino acid level.[1,2] The fruit fly *Drosophila melanogaster* also has a DFF45 homologue, DREP-1 (for DFF45-related protein-1), suggesting that the mechanism by which DFF regulates nuclear condensation and DNA fragmentation is highly conserved during evolution.[8]

CIDE proteins were identified as DFF45-homologous proteins, that include CIDE-A, CIDE-B, and Fsp27–a 27 kDa fat cell-specific protein whose function is unknown.[8] Unexpectedly, overexpression of CIDE proteins induced not only nuclear condensation and DNA fragmentation, but also membrane blebbing and cytosolic fragmentation.[8] CIDE proteins are highly homologous and have an amino terminal CIDE-N domain with significant homology to the regulatory domains of DFF40 and DFF45.[6,8] To identify additional genes encoding products with homology to DFF/CIDE proteins, we searched EST public databases using TBLASTN[9] with mammalian CIDE-N domain sequences. The analysis revealed that *Drosophila melanogaster* has three additional DFF-related proteins (Figure 1A). We designated these proteins DREP-2 (GeneBank accession number AF149795), DREP-3 (GeneBank accession number AF149796), and DREP-4 (GeneBank accession number AF149795). Search of the *Drosophila* genome database with DREP cDNAs mapped the *drep-1* and *drep-3* loci to chromosome 2 where they are located in tandem (*Drosophila* Genome Sequencing Project, accession number AC007475). We sequenced the DREP-2 and DREP-3 cDNAs. Analysis of their nucleotide sequence and that of the truncated DREP-4 cDNA (GeneBank accession number AF149795) revealed that the homology of DREP-2, -3 and -4 to DREP-1, DFF40/CAD, or other CIDE/DFF family members was restricted to the CIDE-N peptide module (Figure 1B). Thus, all these DFF/CIDE-related proteins share a CIDE-N domain (Figure 1A). To assess physical interactions between *Drosophila* DREP proteins, we co-expressed tagged DREPs in human 293T cells and detected protein associations by immunoprecipitation and immunoblotting. The analysis showed that DREP-1 and DREP-3 can interact with DREP-2 (Figure 1C). We could not detect any nuclease activity in DREP-1, DREP-2, or DREP-3 (and their complexes) in the absence or presence of human caspase-3, which has a substrate specificity similar to that of *Drosophila melanogaster* downstream caspases (data not shown). These findings suggest that DREP-2 and DREP-3 are not nucleases, but they might regulate the activity of DREP-1. The CIDE-N peptide sequence of the DFF40 (CAD) nuclease and CIDE proteins acts as a regulatory domain.[6,8] Thus, CIDEs and DFF40 appear to share a common mechanism by which the CIDE-N domain regulates the effector domain of these molecules (CIDE-C in CIDEs and nuclease domain in DFF40). Similarly, the activity of DREP-2, DREP-3 and DREP-4 might be regulated by their corresponding CIDE-N domains. Genetic analyses of DREPs in *Drosophila* should provide insight into their role *in vivo*.

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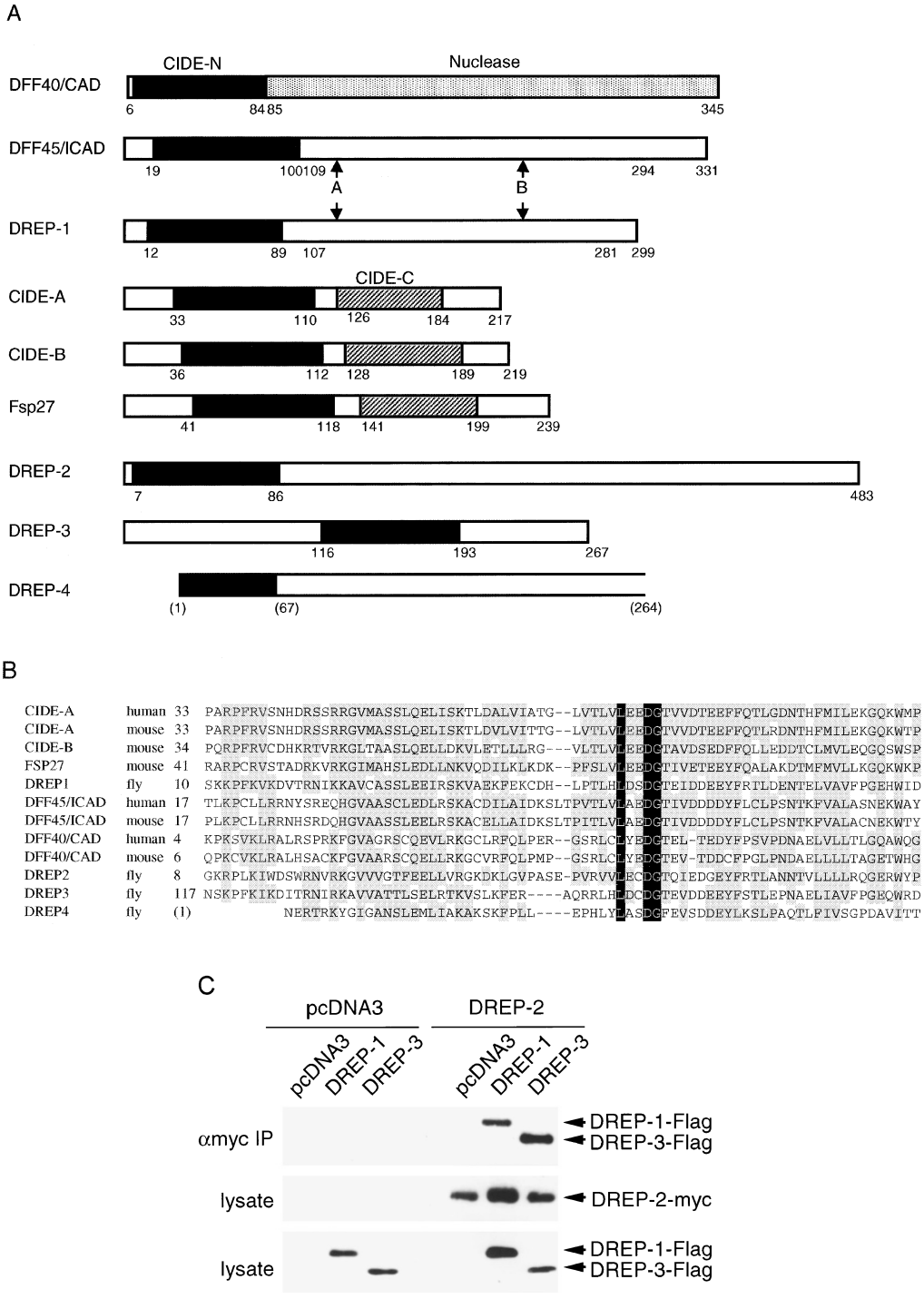

Figure 1 *Drosophila* DREP proteins shared homology with mammalian DFF and CIDE proteins. (**A**) Schematic structure of DFF, CIDE and DREP proteins. All proteins have a conserved domain, CIDE-N (shown as a black bar). DFF45 has two sites that are cleaved by caspase-3 and conserved in DREP-1 (arrows A and B), whereas DFF40 contains a nuclease domain (dotted bar). CIDE-A, CIDE-B, and Fsp27 belong to the CIDE subfamily that contains a conserved CIDE-C domain (crossed bars) necessary and sufficient to induce apoptosis.[8] DREP-1 is a *Drosophila melanogaster* homologue of DFF45.[8] For DREP-4, a partial structure is shown because the full sequence is not available. (**B**) Sequence alignment of CIDE-N domains of CIDE/DFF family members. The completely conserved residues and partially conserved residues are indicated by black and gray highlights, respectively. The homology between the CIDE-N domain of DREP-2, DREP-3 or DREP-4 and that of human DFF45 was statistically significant (P=4.3 × 10^{-5}, P=0.005 and P=0.012, respectively). (**C**) Interaction between DREP-1, DREP-2 and DREP-3. Interaction between DREP proteins was tested by co-immunoprecipitation assay.[7] 293T cells were co-transfected with vector control pcDNA3, Flag-tagged DREP-1, or DREP-3 expression plasmid and Myc-tagged DREP-2 expression plasmid. DREP-2 complexes were co-immunoprecipitated with anti-Myc antibody, and associated proteins were detected with anti-Flag antibody (upper panel). As a control, total lysates were blotted with anti-Myc (middle panel) or anti-Flag (lower panel) antibody