

more cell clusters. The more peripheral ones moved directionally and fairly steadily away from the embryo proper until each side of the yolk sac was dotted with more or less equally spaced black cell clusters. Curiously, movement ceased when the most peripheral ones reached the most ventral region, just short of the mid-ventral line.

There is much more to tell. Later, the cells of these scattered epithelial cell clusters transformed into mesenchymal dendritic melanocytes. The clusters disaggregated in a gradient, beginning with those closest to the embryo, and the melanocytes then migrated as individuals toward the pectoral fin bud on each side of the embryo. As they reached the fin bud, they penetrated it and eventually formed therein an intricate and characteristic pigment pattern, the very pattern I had seen when I first observed a *Blennius* embryo.

The following Spring I returned to Roscoff fully equipped optically and photographically and studied with passion all of this and more. I continued to collect eggs in an aquarium but, as the fish did not always cooperate, I had to supplement my supply with frequent trips to the vast intertidal zone (*la grève*) of Roscoff in the company of the *marins*. This was a happy sport, amid the rocks, seaweed and the eggs. Eventually, I published my studies of these remarkable developmental processes in two papers in the *Journal of Experimental Zoology*.

I tell this little story in an effort to convey some of the emotional impact of a simple scientific discovery and the importance of following one's curiosity. But, as is often the case, this discovery depended in large part on a stroke of good luck. If the cells of these spots were not stuffed with black melanin granules they would never have caught my eye.

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### The NB-ARC domain: a novel signalling motif shared by plant resistance gene products and regulators of cell death in animals

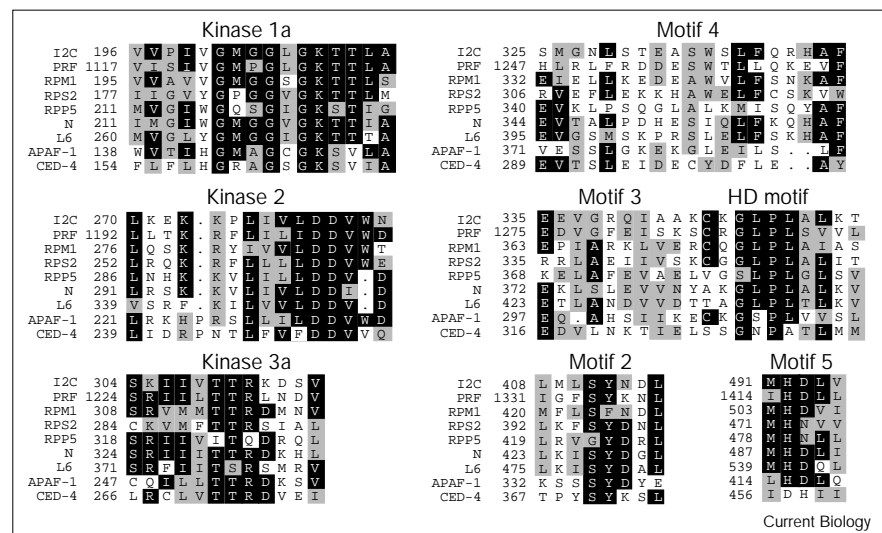
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Programmed cell death plays an important role in plant and animal development [1,2]. Extracellular death-inducing stimuli often lead in animal cells to the activation of cysteine proteases: the caspases [3]. Proteolytic cleavage of a number of substrates then results in distinctive morphological features, such as

condensation of cytoplasm and DNA fragmentation. Caspase activity is regulated in *Caenorhabditis elegans* by the cell death genes *ced-9* and *ced-4* [1]. The *ced-9* gene and its mammalian homologues, the *Bcl-2* family, encode negative regulators that protect cells from death. The *ced-4* gene and the recently identified human homologue, *Apaf-1* [4], encode controlling adaptors between the *ced-9/Bcl-2* death defenders and the caspase killers (encoded in *C. elegans* by *ced-3*).

Interestingly, database searches for proteins with homology to CED-4 [5] and APAF-1 identified several plant resistance (*R*) gene products. Using a region comprising amino acids 92–412 of APAF-1 [4] in a BLAST-P search [6] of the GenBank database, several *R* gene products were shown to have homology (~50% similarity) comparable to that of CED-4. This region includes a nucleotide-binding (NB) domain, consisting of kinase 1a (P-loop),

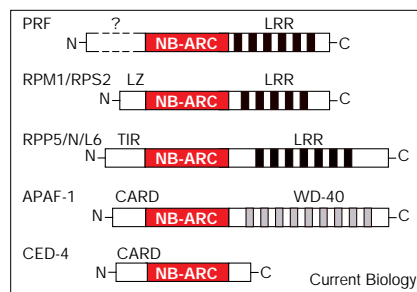
Figure 1



Eight motifs in the conserved region for nematode CED-4 (GenBank accession number X69016), human APAF-1 (AF013263), tomato PRF (U65391), and six plant *R* gene products: RPM1 (X87851), RPS2 (U14158) and RPP5 (U97106) from *Arabidopsis*, N (A54810) from tobacco, L6 (U27081) from flax, and I2C (AF004879) from tomato. To the left are shown the kinase

motifs that are part of the NB domain [7]. To the right are shown motifs that had been previously identified in the *R* gene products [8]. Black boxes indicate a majority of identical residues, grey boxes indicate similar residues. Numbers to the left of the sequences indicate the positions in the proteins of the first residue shown (amino acids are shown in the single-letter code).

Figure 2



Schematic representation of the structures of CED-4, APAF-1, PRF, and five plant *R* gene products: RPM1, RPS2, RPP5, N, and L6. The ~320 amino-acid region of homology between these proteins is shown in red and is designated the NB-ARC domain.

Abbreviations: LZ, leucine zipper; TIR, *Drosophila* Toll, mammalian interleukin-1 receptor and *R* gene products domain; LRR, leucine-rich repeat [8]; WD-40, WD-40 repeat; CARD, caspase recruitment domain [13]. The amino-terminal domain of PRF is shown fourfold smaller to fit into the figure and has no apparent homologues in the databases.

2 and 3a motifs [7], and several other short conserved motifs with unknown function (Figure 1). The *R* gene products are also similar to CED-4 and APAF-1 structurally (Figure 2), with an amino-terminal effector domain and carboxy-terminal leucine-rich repeats (LRRs) that are, like the WD-40 repeats in APAF-1 [4], often involved in protein–protein interactions [8].

*R* gene products are key components in plant defence — which appears macroscopically as rapid localised host cell death at the site of pathogen ingress [2]. This hypersensitive response (HR) is a form of programmed cell death that is thought to impede further infection. The HR is evoked only after specific recognition of pathogen-derived molecules (*avr* gene products) followed by a complex signal-transduction network comprising protein phosphorylation, reactive oxygen species and ion fluxes [2]. The tomato genes *Pto* and *Prf* are both required for induction of the HR after foliar infection with *Pseudomonas syringae* containing *avrPto* [8]. The AvrPTO peptide

physically interacts with the Ser/Thr protein kinase PTO, which phosphorylates another Ser/Thr protein kinase and binds to several defence-gene-related transcription factors [8]. The function of the NB-LRR protein PRF (Figure 2) remains elusive, however; it could be an effector of the defence response.

Are the *Prf* and *R* gene products functionally analogous to CED-4 and APAF-1? A clear model for CED-4 and APAF-1 function has emerged from recent analysis of the molecular interactions between the components of the cell-death machinery [9–12]. Attached at the mitochondrial membrane, CED-9/BCL-2 binds CED-4/APAF-1, which in turn binds the caspases (e.g. CED-3). These physical interactions keep the death complex — or apoptosome — in an inactive conformation and inhibit the release of cytochrome *c* from the mitochondria into the cytosol, which also prevents caspase activation. When the death programme is triggered, the apoptosome dissociates, cytochrome *c* is released and proteolytic cleavage activates the (now cytosolic) caspases. By analogy, the *R* gene products may function as controlling adaptors in a plant apoptosome which becomes activated by pathogen-derived *avr* signals. The LRRs of the *R* gene products could play a role in ensuring that activation of the protein complex is recognition dependent. The amino-terminal domains may be involved in activating downstream effectors following conformation-dependent ATP/GTP hydrolysis at the NB sites. These effectors could directly activate programmed cell death, or could activate other pathways. The TIR domain of the *R* gene products RPP5, N and L6 (Figure 2), for example, is shared with the cytoplasmic domains of the *Drosophila* Toll receptor and the mammalian interleukin-1 receptor [8]. Thus, the NB-ARC domain — a nucleotide-binding adaptor shared by APAF-1, certain *R* gene products

and CED-4 — links a protein–protein interaction module to an effector domain (Figure 2). Further research is needed to clarify the significance of these interesting homologies, which may indicate some conservation of the regulatory core of the cell-death programmes in plants, nematodes and mammals. Whatever the exact biochemical role, the NB-ARC domain is a novel protein motif shared by important plant and animal proteins whose activation results in cell death.

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