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Trafficking and developmental signaling: Alix at the crossroads

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Abstract

Alix is a phylogenetically conserved protein that participates in mammals in programmed cell death in association with ALG-2, a penta-EF-hand calciprotein. It contains an N-terminal Bro1 domain, a coiled-coil region and a C-terminal proline-rich domain containing several SH3- and WW-binding sites that contribute to its scaffolding properties. Recent data showed that by virtue of its Bro1 domain, Alix is functionally associated to the ESCRT complexes involved in the biogenesis of the multivesicular body and sorting of transmembrane proteins within this specific endosomal compartment. In *Dictyostelium*, an *alx* null strain shows a markedly perturbed starvation-induced morphogenetic program while ALG-2 disruptants remain unaffected. This review summarizes *Dictyostelium* data on Alix and ALG-2 homologues and evaluates whether known functions of Alix in other organisms can account for the developmental arrest of the *alx* null mutant and how *Dictyostelium* studies can substantiate the current understanding of the function (s) of this versatile and conserved signaling molecule.

Keywords: Alix; ALG-2; ESCRT; Development; Endocytic trafficking; *Dictyostelium*

Introduction

The multimodular adaptor protein Alix/AIP1 (Apoptosis-linked gene-2 interacting protein X/Apoptosis-linked gene-2 interacting protein 1) and its binding partner ALG-2 (the product of apoptosis-linked gene-2) are actors of programmed cell death (PCD) processes (Missotten et al., 1999; Vito et al., 1996, 1999). More recently, data have demonstrated a direct connection between Alix and the multivesicular body (MVB), an endosomal intermediate on the way to lysosomal degradation of transmembrane cargo (Babst, 2005; Katzmann et al., 2002). While the interaction of Alix with the MVB-associated ESCRT (Endosomal sorting complex required for transport) machinery is a major advance in our understanding of the cellular function of Alix, it may not reflect all aspects of it. In this review, we report on *Dictyostelium* homologues of Alix and ALG-2 and discuss how these data contribute to the general understanding of the molecular mechanisms underlying the biological processes they are linked to.

A role for the death-linked proteins Alix and ALG-2 in *Dictyostelium*?

Alix and ALG-2, partners in cell death programs

Alg-2 encodes a penta-EF-hand calciprotein that plays a critical role in T-cell receptor, Fas- and glucocorticoid-induced apoptosis, as its depletion in T-cells blocks apoptosis whereas its overexpression promotes apoptosis induced by a variety of signals

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(Vito et al., 1996). Intriguingly, apoptosis evoked in the same experimental conditions with T-cells from *alg-2* null mutant mice is not significantly impaired, results that indicate a more complex situation than initially thought (Jang et al., 2002). In Ca^{2+}-dependent ER stress-induced apoptosis, ALG-2 participates in the activation of caspase 12 via an Apaf-1-independent pathway (Rao et al., 2004). Besides these pro-apoptotic effects, ALG-2 was shown to be up-regulated in numerous cancer tissues suggesting also a role in cell proliferation (la Cour et al., 2003), an alternate explanation being that the increase in ALG-2 level is a consequence of the induction of apoptotic pathways that are, however, unable to stop proliferation. Unfortunately, some other properties have been attributed to ALG-2 erroneously because of the use of non-ALG2-specific commercial antibodies and should therefore be discarded (Mollerup et al., 2003). A number of partners of ALG-2 have been identified including peflin (Kitaura et al., 2001) and annexins VII and XI (Satoh et al., 2002) that may modulate the function of ALG-2 in calcium signaling.

Alix/AIP1 has been added in 1999 to the catalogue of pro-apoptotic proteins when it was independently described by two groups to interact with the calciprotein ALG-2 (Missotten et al., 1999; Vito et al., 1999). Alix is a 92-kDa multimodular protein, very close by sequence to Bro1, a yeast protein involved in the pkc1p-MAP kinase cascade (Nickas and Yaffe, 1996). It possesses an N-terminal Bro1 signature domain separated from its C-terminal proline-rich domain by a coiled-coil domain. Overexpression of the C-terminus of Alix in HeLa and COS cells induces a significant protective effect against apoptosis elicited by growth factor withdrawal and staurosporine or etoposide treatment (Vito et al., 1999). Along the same line, human Alix has been shown to negatively regulate the G1-to-S phase progression (Wu et al., 2001) and to promote detachment-induced cell death (anoikis) of HeLa cells (Wu et al., 2002). A direct link between Alix and cell adhesion has been demonstrated (Schmidt et al., 2003). Overexpression of Alix in neurons activates the caspase cascade and induces neuronal death independently of JNK- or p38 MAPK-signaling pathways, whereas its C-terminal half protects neurons from potassium withdrawal-induced death (Trioulier et al., 2004) and induces cytoplasmic vacuolization (Chatellard-Causse et al., 2002). Neurodegeneration induced by nitropropionic acid or kainate is followed by an increased Alix immunoreactivity in the degenerating regions (Blum et al., 2004; Hemming et al., 2004). Alix and ALG-2 have been shown to bind the SH3 domain-containing adaptor, SETA/Ruk/CIN85 (Chen et al., 2000). SETA is expressed in astrocytomas and gliomas, and expression of at least its N-terminal SH3 domain sensitizes astrocytes to apoptosis in response to UV irradiation (Chen et al., 2000).

Altogether, Alix and ALG-2 appear as regulators of several types of death programs, both caspase-dependent and -independent. Given their Ca^{2+}-dependent interaction, they are likely to function in a Ca^{2+}-signaling pathway (Missotten et al., 1999; Trioulier et al., 2004; Vito et al., 1999).

The *Dictyostelium* genome harbors genes encoding Alix and ALG-2 homologues

Dictyostelium grows as a unicellular organism that switches to multicellularity when nutrient source declines (for review, Aubry and Firtel, 1999). Chemotactic aggregation of individual cells takes place in response to pulses of cAMP secreted by starving cells. Once formed, the mound undergoes a series of morphogenetic and differentiation steps leading to the culmination of a mature fruiting body, consisting of a mass of resistant spores supported by a stalk. The cells that make up the stalk are vacuolated and dead. *Dictyostelium* developmental cell death can be mimicked in a monolayer assay exposing starving cells to appropriate morphogen treatments (Kay, 1987; Levraud et al., 2001). The chlorinated alkyl phenone DIF (differentiation-inducing factor-1) is essential to trigger cell death in starved cells. Morphological changes induced by DIF include a massive cytoplasmic vacuolization, a condensation of the cytoplasm and of the chromatin (though without DNA fragmentation) and late membrane lesions (Cornillon et al., 1994; Levraud et al., 2003). These features are characteristic of the mammalian autophagic/vacuolar cell death and justify *Dictyostelium* as a model to study non-apoptotic cell death programs (Golstein et al., 2003).

Dictyostelium is a haploid organism, the genome of which is now completely sequenced (Eichinger et al., 2005). This allowed an informed approach, looking in *Dictyostelium* for homologues of molecules involved in PCD in other organisms. Key PCD effectors known in higher eukaryotes (such as the receptors Fas, adaptors FADD or TRADD, members of the Bcl-2 family) are not present in the *Dictyostelium* genome. Exceptions are the mitochondrial components: cytochrome c, apoptosis inducing factor (AIF), voltage-dependent anion channel (VDAC), adenine nucleotide translocase (ANT), and cyclophilin D, that are known to participate primarily in essential metabolic functions (for review, Brenner and Kroemer, 2000). Among these proteins, the flavoprotein AIF has been shown to translocate into the cytosol during *Dictyostelium* cell death induced by conditioned medium (Arnoult et al., 2001). *Dictyostelium* bears neither caspase nor metacaspase genes, and a null-mutant of the only paracaspase gene shows unaltered cell death, an indication that *Dictyostelium* developmental PCD is independent of any