A molecular switch that governs mitochondrial fusion and fission mediated by the BCL2-like protein CED-9 of Caenorhabditis elegans

Yun Lu, Stéphane G. Rolland1, and Barbara Conradt1,2

Department of Genetics, Norris Cotton Cancer Center, Dartmouth Medical School, Hanover, NH 03755

AUTHOR SUMMARY

Mitochondria, most commonly known as the powerhouse of the cell, are highly dynamic. Their morphology constantly changes under the control of two opposing processes: mitochondrial fusion (whereby two mitochondria fuse into one mitochondrion) and mitochondrial fission (whereby one mitochondrion divides into two mitochondria). B-cell lymphoma 2 (BCL2)-like proteins have long been known for their key roles in apoptosis, a process that allows multicellular organisms to eliminate damaged or unwanted cells. In recent years, BCL2-like proteins have also been found to be important regulators of mitochondrial morphology because they can promote both mitochondrial fusion and fission. However, the factor that determines which of these two opposing processes BCL2-like protein will promote remains unknown. We addressed this question by using the nematode Caenorhabditis elegans, a model organism known for pioneering studies on apoptosis (1). Here, we propose a mechanism by which the BCL2-like protein CED-9 of C. elegans is converted from a protein that promotes mitochondrial fusion (i.e., profusion activity) to the one that promotes mitochondrial fission (i.e., profission activity). Specifically, we demonstrate that the C. elegans BH3-only protein EGL-1, another crucial regulator of apoptosis, acts as a molecular switch that converts CED-9 into a component of the mitochondrial fusion machinery, thereby “switching” the profusion activity of CED-9 to a profission activity.

Mitochondrial fusion and fission are under the control of a conserved family of proteins known as the dynamin-like guanosine triphosphatases (GTPases). Specifically, the dynamin-like GTPase Drp1 of mammals and DRP-1 of C. elegans are required for mitochondrial fission. Conversely, the GTPases Mfn1,2 and Opa1 of mammals and FZO-1 and EAT-3 of C. elegans are required for mitochondrial fusion. The mechanisms by which the functions of these proteins are regulated are currently under intense investigation.

Approximately a decade ago, BCL2-like proteins, central regulators of apoptosis, were discovered to play an important role in the regulation of mitochondrial morphology. For example, a member of the BCL2 family, BAX, controls mitochondrial morphology by promoting Drp1-dependent mitochondrial fission during apoptosis. BAX also interacts with Mfn1,2 and promotes mitochondrial fusion in healthy cells. Thus, the same BCL2 family member is able to promote mitochondrial fission and mitochondrial fusion, depending on the cellular context (2). Similarly, we previously found that CED-9 promotes DRP-1–dependent mitochondrial fission in apoptotic cells but promotes FZO-1- and EAT-3–dependent mitochondrial fusion in healthy cells (3, 4). In the present study, we identified a molecular switch that determines whether CED-9 promotes mitochondrial fission or fission.

By using an in vitro approach, we showed that the CED-9 protein alone or in a complex with CED-4 (a protein bound to CED-9 in healthy cells) can interact with the protein FZO-1, which is required for mitochondrial fusion, as well as the protein DRP-1, which is required for mitochondrial fission. Interestingly, when the CED-9 protein was associated with the BH3-only protein EGL-1, another key regulator of apoptosis, it preferentially interacted with DRP-1. Hence, the association of EGL-1 with CED-9 may displace CED-9 from the mitochondrial fusion protein FZO-1, allowing CED-9 to interact with the mitochondrial fission protein DRP-1. To determine the effect of these interactions in vivo, we overexpressed CED-9 alone or with EGL-1 in C. elegans embryos. The overexpression of CED-9 resulted in FZO-1- and EAT-3–dependent mitochondrial fusion [as we previously described (4)], whereas the overexpression of CED-9 and EGL-1 caused DRP-1–dependent mitochondrial fission. Because DRP-1 is largely localized in the cytoplasm (the aqueous interior of the cell containing the mitochondria and chondrial fission during apoptosis), BAX also interacts with Mfn1,2 and promotes mitochondrial fusion in healthy cells. Thus, the same BCL2 family member is able to promote mitochondrial fission and mitochondrial fusion, depending on the cellular context (2). Similarly, we previously found that CED-9 promotes DRP-1–dependent mitochondrial fission in apoptotic cells but promotes FZO-1- and EAT-3–dependent mitochondrial fusion in healthy cells (3, 4). In the present study, we identified a molecular switch that determines whether CED-9 promotes mitochondrial fission or fission.

By using an in vitro approach, we showed that the CED-9 protein alone or in a complex with CED-4 (a protein bound to CED-9 in healthy cells) can interact with the protein FZO-1, which is required for mitochondrial fusion, as well as the protein DRP-1, which is required for mitochondrial fission. Interestingly, when the CED-9 protein was associated with the BH3-only protein EGL-1, another key regulator of apoptosis, it preferentially interacted with DRP-1. Hence, the association of EGL-1 with CED-9 may displace CED-9 from the mitochondrial fusion protein FZO-1, allowing CED-9 to interact with the mitochondrial fission protein DRP-1. To determine the effect of these interactions in vivo, we overexpressed CED-9 alone or with EGL-1 in C. elegans embryos. The overexpression of CED-9 resulted in FZO-1- and EAT-3–dependent mitochondrial fusion [as we previously described (4)], whereas the overexpression of CED-9 and EGL-1 caused DRP-1–dependent mitochondrial fission. Because DRP-1 is largely localized in the cytoplasm (the aqueous interior of the cell containing the mitochondria and

---

Fig. P1. Regulation of mitochondrial morphology in C. elegans by the BCL2 family of proteins. In healthy cells, a balance of CED-9, CED-9–CED-4, and CED-9–EGL-1 leads to a balance of mitochondrial fusion and fission. The signal acting upstream of this pathway in healthy cells remains to be elucidated. In cells programmed to undergo apoptosis, apoptotic signals trigger up-regulation of EGL-1, leading to a shift in the balance toward mitochondrial fission.

Author contributions: Y.L., S.G.R., and B.C. designed research, performed research, analyzed data, and wrote the paper.

The authors declare no conflict of interest.

This Direct Submission article had a prearranged editor.

1Present address: Department Biology II, Ludwig-Maximilians-Universität, 82152 Planegg-Martinsried, Germany.

2To whom correspondence should be addressed. E-mail: conradt@bio.lmu.de.

See full research article on page E813 of www.pnas.org.

Cite this Author Summary as: PNAS 10.1073/pnas.1103218108.
other cellular components) and needs to be recruited to the mitochondrial surface to promote mitochondrial fission, we next determined where DRP-1 was localized by performing cellular fractionation. Overexpression of CED-9 did not affect DRP-1 localization, but overexpression of CED-9 and EGL-1 caused translocation of DRP-1 to the mitochondrial surface. Therefore, we propose that, when bound to EGL-1, CED-9 acts as a receptor for DRP-1 on the mitochondrial surface.

Other researchers and we have previously shown that ced-9–mutant animals do not show any obvious defects in mitochondrial morphology (4). In contrast, as evident in our study, egl-1–mutant animals showed a defect in mitochondrial morphology. Specifically, we found that EGL-1 is present at a low level in most cells. We therefore reasoned that, under normal cellular conditions, CED-9–dependent mitochondrial fusion and fission might take place and be in balance in most cells. If this model is correct, one would expect to observe a defect in mitochondrial morphology in egl-1–mutant animals, as, in the absence of EGL-1 protein, CED-9–dependent mitochondrial fission, but not CED-9–dependent mitochondrial fusion, would be blocked. Analysis of mitochondrial morphology in the muscle cells indeed revealed that the mitochondria in egl-1–mutant animals are 20% longer than those in WT animals, confirming that CED-9–dependent mitochondrial fusion and fission are in balance in most cells and that egl-1 is specifically required for CED-9–dependent mitochondrial fission.

In conclusion, we propose that, in “healthy” (i.e., non-apoptotic) cells, a balance of CED-9, CED-9–CED-4, and CED-9–EGL-1 complexes leads to a balance in CED-9–dependent mitochondrial fusion and fission. In contrast, in cells programmed to die, apoptotic signals lead to the up-regulation of EGL-1, and thereby a shift in the balance toward mitochondrial fission (Fig. P1). The physiological role of CED-9–dependent mitochondrial fusion and fission in healthy cells remains to be elucidated. Mitochondrial fusion/fission cycles have been shown to be temporarily linked and are proposed to help in maintaining mitochondrial functionality (5). However, how mitochondrial fusion and fission are coupled during these cycles remains unclear. As CED-9 can promote both processes, we speculate that, in healthy cells, CED-9 plays a role in the coupling of mitochondrial fusion and fission during the fission/fusion cycles and thereby helps in maintaining mitochondrial integrity. Finally, our work implicates BH3-only proteins in the control of mitochondrial dynamics. Specifically, we propose that BH3-only proteins act as modulators of the function in mitochondrial dynamics of BCL2-like proteins.