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Abstract

Cell-Free Synthesis and Reconstitution of Bax in Nanodiscs: Comparison between Wild-Type Bax and a Constitutively Active Mutant †

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Abstract: Bax is a major player in the mitochondrial pathway of apoptosis, by permeabilizing the Outer Mitochondrial Membrane (OMM) to various apoptogenic factors, including cytochrome c. In order to get further insight into the structure and function of Bax when it is inserted in the OMM, we attempted to reconstitute Bax in nanodiscs. Cell-free protein synthesis in the presence of nanodiscs did not allow to obtain Bax-containing nanodiscs, but it provided a simple way to purify full-length Bax without any tag. Purified wild-type Bax (BaxWT) and a constitutively active mutant (BaxP168A) displayed structural and functionnal properties that were in line with previous characterizations following their expression in yeast and human cells followed by their reconstitution into liposomes, showing that the mutant BaxP168A was more active than BaxWT. Both Bax variants were then reconstituted in nanodiscs by co-formation. Size exclusion chromatography, dynamic light scattering and transmission electron microscopy showed that nanodiscs formed with BaxP168A were larger than nanodiscs formed with BaxWT. We calculated that nanodiscs containing BaxP168A displayed a pore having a diameter of about 4.8 nm. This was consistent with the hypothesis that BaxP168A was reconstituted in nanodiscs as an active oligomer, corresponding to an intermediate step between the initial insertion of active Bax monomers/dimers (forming a pore having a diameter of 3.5 nm) and the formation of large pores observed during late apoptosis (having a diameter above 40 nm).

Keywords: apoptosis; B-cell lymphoma-2 (Bcl-2) family; Bax; nanodiscs; liposomes; electron microscopy (EM)

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