

## **“Mr. Bax, Mrs. Bcl-2 and their dozen kids: A protein family on the edge of survival and death”**

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Members of the Bcl-2 family are crucial regulators of apoptosis acting predominantly at the mitochondrial outer membrane (MOM). In response to apoptotic stimuli, a subgroup of the family, the BH3-only proteins, are activated by transcriptional and posttranscriptional mechanisms and impinge on two other pro-apoptotic members of the family, Bax and Bak, on the MOM. Bax is localized in an inactive state in the cytosol of healthy cells while Bak is already inserted into the MOM. Both proteins seem to be stabilized in their inactive state by additional inhibitory binding partners. Bax and Bak activation is absolutely crucial for effective apoptosis, but how they are activated has remained enigmatic. It is thought that either their inhibitory binding partners are released and/or activating proteins shortly interact with Bax and Bak to change their conformational change leading to their MOM insertion, oligomerization and their capacity to perforate the MOM, an obligatory step for downstream caspase activation and apoptosis. In one model (the “direct” model), certain BH3-only proteins such as Bid, Bim and PUMA, directly bind to Bax and Bak, releasing inhibitory proteins which keep Bax in the cytoplasm or Bak in an inactive state on the MOM (e.g. VDAC-2). This would result in the liberation of Bax and Bak and spontaneous oligomerization. In another model (the “indirect” model), Bax and Bak bind to the pro-survival members of the Bcl-2 family, Bcl-2, Bcl-x<sub>L</sub>, Mcl-1 and are displaced from them by BH3-only proteins which bind to the pro-survival factors. I will present various proteomics techniques which led to the identification of real binding partners of Bax, Bcl-2 and Bcl-x<sub>L</sub> under endogenous conditions in both healthy and apoptotic cells. We find that only little Bak is bound to Bcl-2, Bcl-x<sub>L</sub> and Mcl-1 and is displaced from this survival factor in response to apoptotic stimuli. Most of the Bak is in high molecular mass complexes of unknown identity and can also be directly activated by Puma. Bax binds to other proteins than Bak, either in the cytosol or loosely attached to mitochondria, and I will present some new binding partners. Thus our findings support the “direct” model of Bak activation and a new model of Bax activation.