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Structural Characterisation of the Bcl-2 protein A1 bound to BH3-domain peptides

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Bcl-2 family proteins play a central role in regulating the intrinsic apoptotic pathway. Pro-survival family members such as Bcl-2, Bcl-x_L, Bcl-w, Mcl-1 and A1 protect cells from apoptosis. Pro-apoptotic BH3-only proteins such as Bim, Puma, Noxa, Bad, Bmf, and Bid promote apoptosis by interacting with and inactivating pro-survival proteins. It was originally thought that each pro-apoptotic BH3-only protein could interact with any pro-survival protein. However, it has recently become clear that there is selectivity within the pathway suggesting functional groupings [1]. Bim and Puma behave as originally predicted and can interact with all pro-survival proteins and are potent killers. In contrast, Noxa and Bad interact with distinct subsets of pro-survival proteins. Noxa only interacts with Mcl-1 and A1, while Bad interacts with Bcl-2, Bcl-x_L and Bcl-w. As a result, either Noxa or Bad acting alone is a weak killer, but together they are potent. Other BH3-only proteins are not as promiscuous as Bim and Puma, but not as selective as Noxa or Bad. This work aims to structurally characterise interactions between A1 and various BH3-domain peptides, enabling the determinants of selective binding to be identified. Crystals have been obtained of A1 in complex with several BH3-domain peptides. Progress on structural characterisation will be reported.

[1] Chen L., Willis S.N., Wei A., Smith B.J., Fletcher J.I., Hinds M.G., Colman P.M., Day C.L., Adams J.M., Huang D.C.S., *Mol. Cell*, 2005, 17, 393-403.

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Structural basis of yeast aminoacyl-tRNA synthetase complex formation

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The yeast aminoacyl-tRNA synthetase (aaRS) complex is formed by the methionyl- and glutamyl-tRNA synthetases (MetRS and GluRS, respectively) and the tRNA aminoacylation and nuclear export cofactor Arc1p. It is considered an evolutionary intermediate between mostly monomeric prokaryotic aaRS and the multi-aaRS complex found in higher eukaryotes. While a wealth of structural information is available on the enzymatic domains of single aaRS, insight into complex formation between eukaryotic aaRS and associated protein cofactors is missing. We report crystal structures of the binary complexes between the interacting domains of Arc1p and MetRS as well as those of Arc1p and GluRS at resolutions of 2.2 and 2.05 Å, respectively. The data provide a complete structural model for ternary complex formation between the interacting domains of MetRS, GluRS and Arc1p. The structures reveal that all three domains adopt a GST-like fold and that simultaneous interaction of Arc1p with GluRS and MetRS is mediated by the use of a novel interface in addition to a classical GST dimerization interaction. The results demonstrate a novel role for this fold as a heteromerization domain specific to eukaryotic aaRS, associated proteins and protein translation elongation factors.