NON-CANONICAL INTERACTIONS BETWEEN SEPTINS: THE STUDY BETWEEN SEPT3, A SEPTIN FROM GROUP I, AND SEPTINS FROM GROUP II.

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Septins are eukaryotic proteins that form hetero-filaments, interact with membranes and take part in many cellular processes such as cell division and vesicle trafficking. Humans express 13 septins, which are divided into 4 homologous groups: Group I (SEPT3, SEPT9, SEPT12), Group II (SEPT6, SEPT8, SEPT10, SEPT11, SEPT14), Group III (SEPT1, SEPT2, SEPT4, SEPT5) and Group IV, which contains only SEPT7. A canonical filament, characterized in HeLa cells, is based on the polymerization of SEPT7-SEPT6-SEPT2-SEPT6-SEPT7 hexamers, and is the basis for the rules of filament assembly proposed by Kinoshita. However, this model does not account for septins from group I. On the other hand, we have recently reported non-canonical interactions based on yeast two-hybrid studies which suggest interactions between group I and group II septins. The aim of this project is specifically to verify and characterize non-canonical interaction between SEPT3 (from Group I) and the septins of Group II. We have co-expressed SEPT3-SEPT6, SEPT3-SEPT8, SEPT3-SEPT10, SEPT3-SEPT11 and SEPT3-SEPT14 using the bicistronic vector pETDuet-1 in the *E.coli* strain Rosetta-1. In all cases SEPT3 carries a histidine tag. Co-purification was performed using cobalt metal-affinity chromatography followed by gel filtration in Superdex 200. Our results from the metal affinity chromatography clearly show that SEPT8 interacts with SEPT3. However when either SEPT10 or SEPT14 are co-expressed with SEPT3 the former are found to be insoluble whilst SEPT3 is soluble and elutes in the imidazole gradient suggesting that the two components do not interact. On gel filtration the SEPT3-SEPT8 pair eluted with an apparent molecular weight corresponding to a dimer, together with an excess of monomeric SEPT3 and high Mw aggregates. Sedimentation velocity for the dimers SEPT3-SEPT8 is currently being performed. Interactions between Group I and Group II appear to be weak and may vary depending on the specific combination.

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