FUNCTIONAL PROTEOMIC ANALYSIS OF NUCLEAR CALMODULIN

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Calmodulin (CaM) is the major Ca^{2+} acceptor protein in eukaryotic cells. It acts as a mediator of the Ca^{2+} signal, thus regulating a number of protein functions. It is mainly located in the cytoplasm but it has also been reported in the nucleus. Nevertheless, its role in this specific cellular localization is still mostly unknown. Thus, we aimed to analyze the nuclear roles of CaM by using a functional proteomics approach. Purified recombinant CaM was coupled to a Sepharose 4B matrix, and affinity chromatography columns were generated with these beads. Then, nuclear extracts from rat liver nuclei were loaded onto a CaM-column using a Ca^{2+}-containing buffer. After extensive washes, CaM-binding proteins (CaMBPs) were eluted using a Ca^{2+}-chelator (EGTA)-containing buffer. After SDS-PAGE separation, the different CaMBPs were cut, digested and identified using a LTQ VELOS ORBITRAP. From these studies, 228 CaMBPs were identified. Then, these proteins were functionally grouped by using the Gene Ontology program. Results revealed that 94 of the CaMBPs were involved in pre-mRNA processing and splicing whereas other 83 proteins participate in transcription. Interestingly, we identified a subgroup of more than 20 CaMBPs that specifically belong to the spliceosome complex C, a ribonucleoprotein structure involved in pre-mRNA splicing. We subsequently analyzed which of these 20 proteins contained putative CaM-binding domains. Results revealed that only 4 of these proteins are putative direct targets of CaM. Altogether, these results indicate that CaM could regulate pre-mRNA splicing by a direct Ca^{2+}-dependent interaction with a specific subset of proteins of the spliceosome complex C.