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Identification of a functional nuclear export sequence in diacylglycerol kinase-ζ

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Diacylglycerol kinases (DGKs) phosphorylate diacylglycerol (DG) to yield phosphatidic acid. Therefore, they act as key modulators of DG levels. Ten DGK isoforms have been identified in the mammalians. Among the 10 DGK isoforms, DGK-ζ and -ι are the only isozymes which contain a nuclear localization signal. Here, we demonstrate that DGK-ζ also displays a functional independent nuclear export signal (NES) sequence between the amino acid residues 362-370. Mutation of DGK-ζ NES was performed by replacing leucines with alanines using site-directed mutagenesis. The NES mutant forms of DGK-ζ accumulated in the nucleus of C2C12 myoblasts to a much greater extent than wild type DGK-ζ, as documented by both immunofluorescence analysis and western blot. Moreover, treatment with leptomycin B, an inhibitor of leucine-rich type NES, resulted in accumulation of both endogenous and ectopically expressed DGK-ζ in the nucleus, demonstrating that nuclear export of DGK-ζ is chromosome regional maintenance protein 1 (CRM1) -dependent. To test if the leucine-rich sequence within DGK-ζ is a functional independent NES, C2C12 cells were transfected to overexpress either the DGK-ζ NES peptide LSTLDQLRL (NES-GFP) or the mutated DGK-ζ ΔNES peptide ASTADQARA (ΔNES-GFP) as GFP fusion proteins. While wild type-GFP localized throughout the nucleus and the cytoplasm, as expected, NES-GFP localized predominantly to the cytoplasm. Instead, the ΔNES-GFP fusion protein displayed the same cellular distribution as WT-GFP. These results documented that the DGK-ζ NES peptide was able to restrict GFP distribution to the cytoplasm, therefore acting as a functional independent NES. Previously, we reported that nuclear DGK-ζ is a negative regulator of cell cycle progression in C2C12 mouse myoblasts (Evangelisti et al. FASEB J 21:3297, 2007). Here we show that enhancement of DGK-ζ nuclear localization by NES sequence mutation, increases G0/G1 block in C2C12 cells. Overall, our data demonstrate that DGK-ζ export from nucleus to cytoplasm is regulated by a leucine-rich NES through the exportin CRM1 and suggest that the nuclear localization of DGK-ζ finely tunes its function as a regulator of G1/S cell cycle transition.

Key words
DGK-ζ, myoblasts, nucleus, nes