Identification and characterization of a novel isoform of β-chimaerins with nuclear localization

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INTRODUCTION

Chimaerins are a family of GTPase-activating proteins (GAPs) composed of four members: α1- α2- β1-and β2-chimaerin. All chimaerin isoforms have a catalytic GAP domain, with selectivity for the Rac GTPase, and a C1 domain with structural homology to those of protein kinase C isoforms (PKCs) that binds DAG and phorbol esters. α2- and β2-chimaerin also have an N-terminal SH2 domain involved in heteromolecular interactions with phosphotyrosine proteins. Chimaerins are generated by alternative transcription of two different genes; the *CHN1* gene which encodes α1- and α2-chimaerin, and the *CHN2* gene which encodes β1- and β2- chimaerin. The *CHN2* gene consists of 13 exons and has two start sites; one in exon 1 that renders β2-chimaerin, and one in intron 6 that renders the β1-chimaerin isoform. In addition, we have identified a new isoform generated by alternative splicing, that we named β1-Δ7p chimaerin. The functional characterization of this isoform suggests a role for this protein in the regulation of nuclear Rac.

METHODS

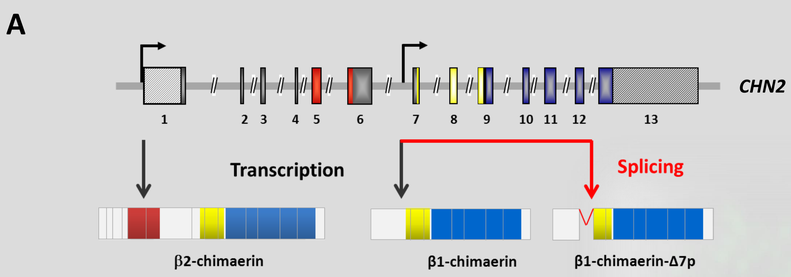
Immunofluorescence studies. COS1 cells were grown on glass coverslips, transfected with FuGene6 and fixed with 3.7% formaldehyde after 24-48 hours. For nuclear staining, cells were incubated with DAPI. Localization of chimaerins was analyzed using a laser scanning confocal microscope (Leica TCS SP5).

Mutational analysis. Point mutants were generated using the QuickChange Site-Directed mutagenesis kit using as template the plasmid encoding the EGFP-tagged version of β1-chimaerin.

Rac activation assay. Total COS cell lysates or nuclear fractions were lysed with buffer containing 10 µg of a GST fusion protein with the Rac binding domain of PAK1 (GST-PBD), followed by incubation with glutathione-sepharose beads (GE Healthcare) for 1 h at 4 C. The bound (Rac-GTP) was detected by immunoblotting using anti-Rac antibodies.

RESULTS

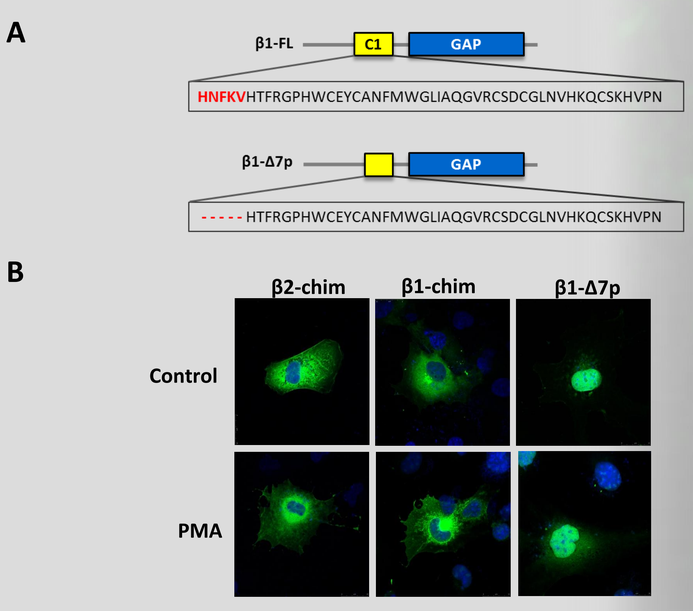
1 Identification of a novel β-chimaerin isoform with nuclear localization



Nuclear localization of the new chimaerin isoform β1-chimaerin-Δ7p.

A) Schematic representation of the *CHN2* gene The generation of β2- and β1-chimaerin from different start sites of the *CHN2* gene is indicated. β1-chimaerin-Δ7p is generated by alternative splicing. Numbers indicate the *CHN2* exons. Color indicate the different chimaerin domains: SH2 (red), C1 (yellow), and GAP (blue). **B)** Subcellular localization of β-chimaerin isoforms in transfected COS1 cells.. β2-chimaerin is cytosolic, β1-chimaerin is mainly cytosolic with some nuclear localization while β1-chimaerin-Δ7p is mostly localized in the nucleus.

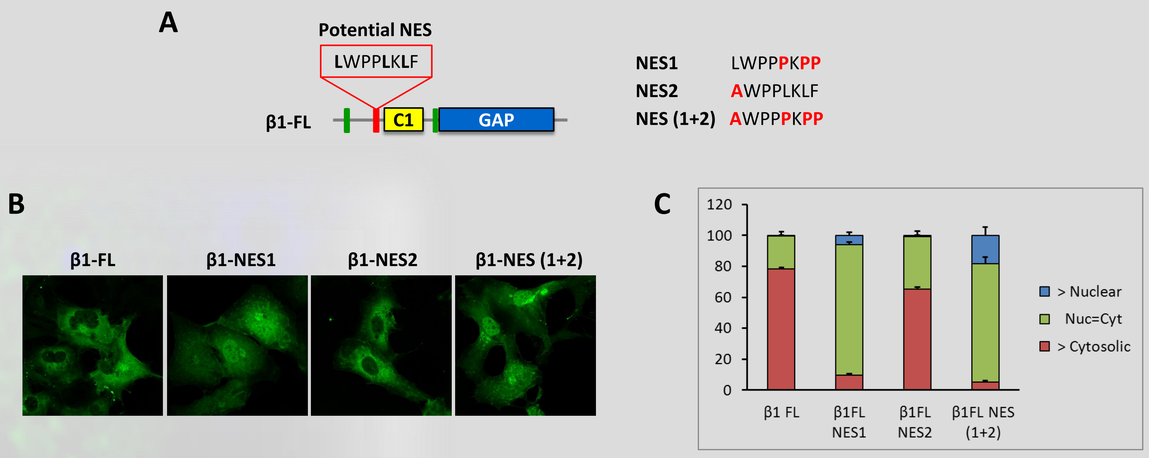
2 β1-chimaerin-Δ7p has a nonfunctional C1 domain



β1-chimaerin-Δ7p does not respond to PMA treatment.

A) Schematic representation of the functional domains of β1- and β1-chimaerin-Δ7p isoforms. The truncated isoform loses five aa of the C1 domain. **B)** Subcellular localization of the chimaerin isoforms in transfected COS1 cells treated with PMA. Translocation of β2- and β1-chimaerin, mainly to perinuclear regions, is observed. However, β1-Δ7p subcellular localization is not affected by PMA.

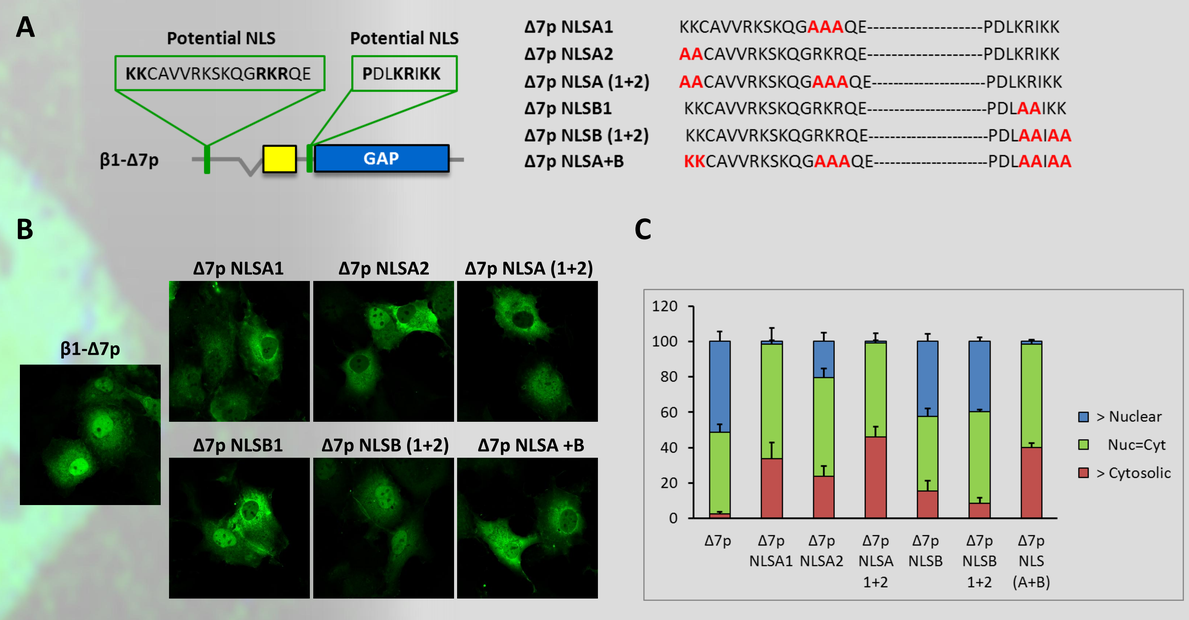
3 Loss of a Nuclear Export Signal (NES) results in nuclear accumulation of β1-chimaerin-Δ7p



β1-chimaerin has a functional NES that is lost in β1-chimaerin-Δ7p.

A) Computational analysis predicts one NES in β1-chimaerin that is lost in the β1-Δ7p isoform. Highlighted are the residues important for NES function. Point mutants generated on the β1-chimaerin potential NES are shown on the right. **B)** Representative confocal immunofluorescence images of COS-1 cells transfected with the EGFP-tagged mutants indicated. **C)** Subcellular distribution of each mutant was analyzed in ~100 cells scored in three different experiments. The result are given by >Nuclear, Nuc=Cyt, or >Cytosolic to indicate the predominant subcellular distribution of each mutant.

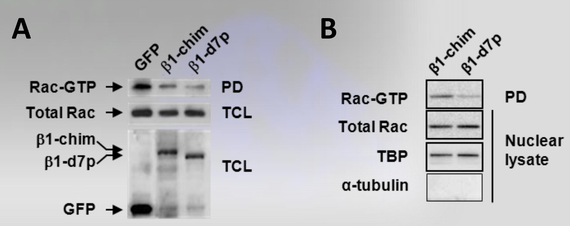
4 Nuclear import of β1-chimaerin-Δ7p is mediated by a Nuclear localization Signal (NLS)



β1- and β1-Δ7p chimaerins have a functional NLS.

A) Computational two NLS in β1- and β1-Δ7p chimaerins, one bipartite in the most N-terminal region and one classic NLS in the linker between the C1 and GAP domains. Point mutants generated on the β1-Δ7p potential NLSs are shown on the right. **B)** Representative confocal immunofluorescence images of COS-1 cells transfected with the EGFP-tagged mutants indicated. **C)** Subcellular distribution of each mutant was analyzed in ~100 cells scored in three different experiments. The result are given by >Nuclear, Nuc=Cyt, or >Cytosolic to indicate the predominant subcellular distribution of each mutant.

5 The nuclear chimaerin isoform β1-Δ7p regulates nuclear Rac activity



β1-chimaerin-Δ7p negatively regulates nuclear Rac

A) The levels of active Rac (Rac-GTP) in cells transfected with control (GFP), β1-chimaerin and β1-d7p were determined by a pull down (PD) assay in whole cell lysates. **B)** Rac-GTP levels were measured by PD assay in isolated nuclei from cells transfected with β1-chimaerin or β1-d7p. TBP was used as a nuclear loading control. α-tubulin was used to corroborate that nuclear fractions had no cytoplasmic contamination.

CONCLUSIONS

- ☑ **We have identified a new chimaerin isoform (β1-chimaerin Δ-7p) that is generated by alternative splicing.**
- ☑ **β1-chimaerin Δ-7p has a nuclear localization mediated by one NLS and the loss of one NESβ1-chimaerin Δ-7p has a nuclear localization mediated by one NLS and the loss of one NES.**
- ☑ **β1-chimaerin-d7p regulates the activation of nuclear Rac.**

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