A Munc13/RIM/Rab3 Tripartite Complex: From Priming to Plasticity?

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Bacterial expression vectors.

Plasmids for bacterial expression of RIM2α, Munc13-1 and Rab3A fragments were generated by polymerase chain reaction (PCR) with custom-designed primers and subcloned into pGEX-KG (Guan and Dixon, 1991), pGEX-KT (Hakes and Dixon, 1992) or pET-21 (Novagen) vectors. The longest RIM2α fragment (residues 1-165) in pGEX-KG vector was flanked by *Bam*HI and *Hind*III restriction sites. RIM2α fragments (residues 22-81, 22-155, 82-142 and 82-155) were amplified on a RIM2$\alpha_{1\text{-}165}$ template using corresponding pairs of the following primers that incorporated either *Bam*HI or *Eco*RI restriction sites: (1) 5'-AGGGATCCCCGCAGCCCGAGATGCCG-3', (2) 5'-CAGG ATCCCAGCAAGAGCAGAAGGGCGA-3', (3) 5'-GCGAATTCTACTGTTGTGAT TCCTCTCCCA-3', (4) 5'-GTGAATTCTACCCACTATTGTAAAACCACG-3' and subcloned into pGEX-KT vector. Site-directed mutagenesis of RIM2$\alpha_{82\text{-}142}$ fragment was performed using QuickChange™ Site-Directed Mutagenesis Kit (Stratagene).

The plasmid for bacterial expression of the recombinant rat Munc13-1 fragment (residues 3-317) in pGEX-KG vector was described earlier (Betz *et al*, 2001). The shorter Munc13-1 N-terminal fragments (residues 3-132, 3-150, 3-209, 3-228 and 3-252) were generated by PCR on the Munc13-$1_{3\text{-}317}$ template using sense primer 5'-GGGCTGGCAAGCCACG TTTGGT- 3' that initiates in the pGEX vector sequence 5'- to the MCS region and the following antisense primers introducing a *Hind*III sequence: (1) 5'-GTAAGCTTTCAG TCCAAAGGCAGCTCAAAA -3', (2) 5'-CTAAGCTTAGGCATTCAGCTGCTCCA-3', (3) 5'-GTAAGCTTATGGGATGCTGTTGCTCGTCT-3', (4) 5'-AGAAGCTTACCG

CACGGAGTACTGGTG-3', (5) 5'-ACAAGCTTACCGCGGCTCAGAGAACTC-3', and subcloned in pGEX-KG vector using the *Eco*RI and *Hind*III cloning sites. To produce T7-tagged Munc13-1 fragments, the corresponding *Eco*RI/*Hind*III fragments were subcloned from pGEX-KG into the pET-21 bacterial expression vector (Novagen).

Plasmids for bacterial expression of Rab3A fragments (residues 15-217) were generated by PCR with the following primers: 5'-AGGGATCCGACCAGAACTTCGACTATATG -3' and 5'-GCGAATTCAATCCTGATGAGGTGGCG -3' using earlier described constructs (Schluter *et al*, 2002) encoding mouse full-length Rab3A (either Q81L or T36N mutant) as template. PCR fragments were subcloned in pGEX-KT vector using *Bam*HI and *Eco*RI restriction sites. All constructs were verified by sequencing.

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