Looking for protein partners of nebulin SH3

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Nebulin - a large protein (600-800 kDa) located in the thin filament of striated vertebrate muscle - is assumed to bind and stabilize F-actin. Complete sequence determination of human nebulin has only recently been accomplished showing a uniform modular structure along the whole length of the molecule. Up to 97 % of the sequence is assembled from repeats of a sequence motif 35 amino acids long. At the C-terminus, an SH3 domain is found whose function and partner have not been identified so far. It is known however that the SH3 is localized in the Z-disk of the muscle sarcomere and is thought to play an important role in the assembly of this zone. SH3 domains are known to recognize proline-rich sequences, that can bind in the so-called type I and type II orientations. We have addressed the problem of identifying possible partners of nebulin by an integrated approach which combines bioinformatic and experimental tools. Owing to the large number and the size of t!
he muscular proteins, the choice of a partner sequence is not feasible by hand. Accordingly, we resorted to SPOT (Brannetti et al., 2000), a program that matches proline-rich peptides to a given SH3 partner. This program was used for screening the whole SWISSPROT (release 37) database as well as a subdatabase of muscle proteins to identify possible partners. Of the resulting sequences, only those present in muscle proteins which could realistically have the possibility to colocalize with nebulin in the Z-disk were chosen. Two peptide sequences, belonging to the zyxin and titin proteins, score in the first positions of the class I and class II binding orientations. They were produced as synthetic peptides and their affinity in the binding to nebulin SH3 were explored both by fluorescence and NMR studies. Their affinities and binding orientations were determined showing that while the titin peptide has relatively high affinity and binds in the class II orientation (in agreement!
with the SPOT prediction), the zyxin peptide does not bind with sufficient affinity.

References

B.Brannetti, A.Via, G.Cestra, G.Cesareni and M.Helmer Citterich. SH3-SPOT (2000): an algorithm to predict preferred ligands of different members of the SH3 gene family. J.Mol.Biol, 298, 313-328.