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Poster Presentation

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N-terminally acetylated tropomyosin generated in *E. coli* by coexpression of the *S. cerevisiae* NatB acetylation complex shows functional properties *in vitro*

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Background

One of the most significant differences in proteins produced in eukaryotes is their chemical modification, which does not occur in prokaryotes. These post-translational modifications of certain amino acid residues are often essential for protein function. Therefore, generating systems that allow them to be made in bacterially expressed proteins is of great fundamental interest and of potential benefit in biotechnological applications. We have developed a system that allows the production of one kind of protein modification in bacteria, N-terminal acetylation [1], by coexpressing the yeast NatB acetylation complex and the target protein tropomyosin (TM).

Results

Tropomyosin is a protein that plays a role both in controlling the contraction of muscle and also a much more general role as part of the scaffolding matrix within cells, known as the actin cytoskeleton. Without an essential acetylation of one of its ends, TM no longer binds to the cytoskeleton and the cells are severely impaired or even die. A pair of proteins, of which similar versions are found in all eukaryotic cells, does this modification. In yeast, these proteins are Nat3 (195 amino acids) and Mdm20 (796 aa), together making up the NatB acetylation complex [2]. Nat3 is the acetylase core, and Mdm20 the TM binding protein. Yeast and mammalian TM can be easily overproduced in bacteria. However, since bacteria do not have proteins similar to NatB, TM does not work correctly when produced in this way. We generated an *E. coli* coexpression system for TM plus the two NatB components based on the Novagen pET Duet vectors that allow cloning of two genes in tandem under identical T7 promoters.

The coding regions of the two proteins were cloned directly from *S. cerevisiae* genomic DNA via PCR. Expression trials of these proteins in isolation showed good overproduction of Nat3, with a clear band visible in total cell lysate separated by SDS-PAGE. This band has an apparent size of around 28 kDa, somewhat larger than the predicted molecular mass of 22.9 kDa. However, no band was visible for Mdm20 at around its predicted 92.8 kDa. The two coding sequences were then transferred into the Duet expression vector, and the resulting vector was transformed into *E. coli* BL21(DE3)pLys. For TM coexpression, the cells were additionally transformed with pJC20 expression vectors containing either yeast TM1 or vertebrate skeletal TM that we had characterized previously [3-5]. Vectors were maintained by selection by ampicillin (pJC20) plus either kanamycin (pRSFDuet) or spectinomycin (pCDFDuet). TM was purified and its binding to rabbit F-actin measured by cosedimentation studies as described [3]. The yeast TM1 species was found to behave similar to the one carrying a short N-terminal extension, such as Ala-Ser, or Ala-Gly-Ser-Ser-Ser, to mimic the acetylation present in native TM from eukaryotic sources, thus indicating the presence of N-terminal acetylation as a prerequisite for high-affinity binding of TM to actin.

Conclusion

Our data show that all three proteins can be produced via coexpression. However, since their relative quantities proved to be very variable, more work is needed to optimize the expression conditions to produce balanced quantities of the acetylating proteins (Nat3/Mdm20) and a larger amount of the acetylation target (TM).

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