# Correspondence

## Reply to: Are β-thymosins WH2 domains?

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WH2 (WASP homology 2) domain is a ubiquitous actin monomer binding motif that is present in a wide variety of regulators of the actin cytoskeleton. The article by John Edwards presents an analysis suggesting that β-thymosins (including proteins containing tandem β-thymosin-like repeats) and other WH2 domains would not have a common ancestry. As already pointed out in our review article [1], the WH2 domain is a very short protein motif and thus reliable phylogenetic analysis of this domain is difficult. Thus, we agree that it is not possible to conclude whether β-thymosins and other WH2 domains have a common ancestor. However, all available biochemical and structural data indicate that β-thymosins and other WH2 domains belong, from a structural and functional point of view, to the same family of actin-binding motifs.

Alignments of β-thymosins and other WH2 domains show that their most highly conserved regions are the LKK motif and region N-terminal of it. In contrast, the C-terminal regions of β-thymosins display high sequence identity to each other, whereas the C-terminal regions of other WH2 domains are very heterogeneous [1,2]. Recent structural studies on β-thymosin and ciboulot provide a plausible explanation for the conservation of these sequence features. The most critical actin-binding region is located in the LKK motif and in the α-helical region preceding this motif. These regions interact with the subdomains 1 and 3 at the 'barbed-end' of the actin monomer. The highly conserved C-terminal region of β-thymosins forms an additional α-helix, which interacts with the subdomain 2 at the 'pointed-end' of actin monomer. This C-terminal helix is present in actin filament sequestering β-thymosins but it is absent from ciboulot, which promotes actin filament assembly. Interestingly, β-thymosin can be changed from an actin filament sequestering to assembly promoting protein by disrupting this α-helix by site-directed mutagenesis [3,4]. Together, these data provide a plausible explanation for the lack of sequence conservation at the C-terminal regions of those WH2 domains that do not display actin monomer sequestering activity. Consequently, sequence conservation between β-thymosins and other WH2 domains is expected to be limited in the N-terminal actin-binding region.

It is also important to note that β-thymosins and other WH2 domains have very similar actin-binding properties to each other. All WH2 domains and β-thymosins tested so far bind ATP-actin monomers with much higher affinity than ADP-actin monomers [5–7]. Furthermore, mutagenesis studies suggest that β-thymosins and other WH2 domains interact with actin through a similar interface, indicating that WH2 domain is a structurally conserved actin-binding motif [6,8–10]. However, further structural analysis of other WH2 domain proteins than β-thymosins and ciboulot will be required to reveal if β-thymosins and other WH2 domains indeed interact with actin through a conserved structural mechanism.

Based on these data, we suggest that from a structural and functional point of view WH2 domains and β-thymosins most likely belong to a single actin-binding motif family. However, whether they have a common ancestry or if they are a result of a convergence cannot be concluded at the moment. Reliable phylogenetics of this actin-binding motif will thus require identification and analysis of a large number of new WH2 domains/β-thymosin family proteins.

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