CHAPTER 7

Multirepeat β-Thymosins

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Abstract

Multirepeat β-thymosins contain multiple copies of the β-thymosin actin binding module. This family is mainly distributed within lower metazoan species and, with one exception, absent in mammals in which the classical single repeat β-thymosins appear dominant. The repeated nature in combination with sequence variation in the consecutive modules renders these proteins different actin modulating capacities as compared to the classical β-thymosins. These properties are discussed in function of recent structural models indicating how these proteins contact actin. The importance of the multirepeat β-thymosins is underscored by their crucial role in neuronal development and reproduction.

Introduction

Actin filament turnover forms the basis of essential cellular properties ranging from cell division to cell migration. Consequently actin dynamics are crucial during embryogenesis and morphogenesis of eukaryotic organisms. The dynamic equilibrium between actin monomers (Globular)-actin and polymeric filamentous (F)-actin can be viewed as a cyclic process in which actin monomers, loaded with ATP and a divalent cation, associate with the fast growing (barbed or plus) end of an actin filament. Subsequently associated monomers undergo ATP-hydrolysis and finally dissociate at the other (pointed or minus) filament end as ADP-actin. At equilibrium, the kinetic and structural differences present at both filament ends drive this cyclic process in a unidirectional fashion also called treadmilling. It is evident that changes in the number of free barbed filament ends or changing concentrations of polymerization competent actin monomers will shift the G/F-equilibrium. In cells, this system is strongly regulated by the activity of actin binding proteins with diverse functions such as monomer sequestration, barbed end elongation or barbed end capping.

The multirepeat β-thymosins were first reported in 1999 and more members are detected as genome sequencing projects are being pursued. As their name suggests they share homology with β-thymosins and thus are actin binding proteins. We here present their distribution within the eukaryotic kingdoms and their structural and biochemical properties in relationship to other actin binding proteins such as the single repeat β-thymosins and WH2-domain containing proteins. We discuss in more detail ciboulot from fruit fly, the amoebal actobindin, tetraThymosinβ from nematode and CSP(condition stimulated phosphoprotein)-24 from sea slug since these have been extensively studied both biochemically and/or in vivo.

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The Multirepeat β-Thymosin Family: Evolutionary Relationships and Structural Characteristics

The multirepeat β-thymosin proteins contain multiple copies of the β-thymosin actin binding module. This characteristic, together with functional features (see below), sets them apart from the classical β-thymosins that consist of just one of these modules. The number of copies or repeats varies from two to no less than twenty-eight in the recently discovered and highly intriguing thypedin protein from the primitive metazoan *Hydra vulgaris.¹³*

Figure 1A illustrates the taxonomic coverage of this family compared to the one of the classical β-thymosins. Two double repeat members in amoeba and one in *Dictyostelium discoideum* represent the multirepeat β-thymosins in Protista. The other proteins are found in Metazoa in six out of the eight phyla (Fig. 1A, left). Multirepeat β-thymosins appear absent from Fungi and plants, although nonannotated EST-sequences with significant similarity to actobindin are found in *Chlamydomonas*, soybean and sorghum. The β-thymosin distributions (Fig. 1A) are in strong contrast to that of the in sequence partly similar WH2-repeat proteins that are ubiquitously expressed in all eukaryotic kingdoms. Based on current datasets the multirepeat proteins are the only beta-thymosin homologs in Cnidaria (thypedin, 28 repeat-protein), in flat and round worms (two and four repeat proteins respectively, the latter termed tetraThymosinβ)¹¹ and in Arthropoda (typified by ciboulot,³ three repeats) (Fig. 1A, left). The single repeat β-thymosins are not represented in these phyla (Fig. 1A, right). Different species of Mollusca however possess either a single or double repeated variant.¹⁴ The repeat β-thymosins are largely absent from Echinodermata and Chordata (Fig. 1A, left) whereas classical β-thymosins are broadly expressed here (Fig. 1A, right). We only found a five repeat protein in the sea squirt *Ciona intestinalis*, which belongs to the Urochordata, the most atypical of the Chordata subphyla. In addition, a neuroblastoma like β-thymosin and its nearly perfect duplication are together present in mice. We isolated the transcript of this double repeat in several types of adult mouse tissue (Dhaese et al, unpublished). As it is not present in related species, even not in other rodents, it probably arose recently in evolution. The latter example also illustrates that the occurrence of multirepeat and single repeat proteins is not necessarily mutually exclusive.

For some multirepeat β-thymosins, splice variants are present (see alignment discussed below). Alternative splicing of the transcript coding for the three repeat protein ciboulot from *Drosophila melanogaster*, results in a two-repeat variant with a longer second repeat that differs carboxyterminally. Based upon exon skipping, a four repeat and five repeat protein, respectively termed CSP-24 and CSP-29, are present in *Hermissenda crassicornis*. In *Apis mellifera* two different splice variants with three and four repeats are found.

The evolutionary path that resulted in this distribution of the different β-thymosins is still elusive: the multirepeat β-thymosins appear more frequently in lower eukaryotes and in protista; the single forms are clearly more typical for Echinodermata and vertebrates (Fig. 1A). On the other hand, single repeats are already present in primitive eukaryotes such as *Porifera* (sponges).¹⁵ Taken together, this suggests that after ancient gene multiplication events the multirepeat proteins evolved and that the single repeat β-thymosins in mammals were subsequently generated by partial deletion from these longer variants.

Both from an evolutionary and from a functional viewpoint, it is important to clearly define the features of the β-thymosin module (InterPro-domain 001152 or Pfam-domain PF01290) to allow distinguishing it from the shorter WH2-domain (InterPro 003124, Pfam domain PF02205). The latter are present in a very large group of eukaryotic proteins and in a few cases also as repeated domains. We illustrate the features of the β-thymosin module in Figure 1B using human thymosinβ4.¹⁸ The central part of the module is a strongly conserved tetrapeptide sequence (17-LKKT-20 in thymosinβ4)¹⁸ starting and ending with a hydrophobic or noncharged residue and containing one or two positively charged residues.¹⁹

This consensus motif is preceded by a stretch of amino acids forming an amphipathic α-helix