MS9-O5 The architecture of the muscle filament myomesin by hybrid structural biology Matthias Wilmanns,[a] Petr Konarev,[a] Dmitri Svergun,[a] Gabriel Zoldak,[b] Matthias Rief,[b] Fabienne Beuron,[c] Ed Morris,[c] & Spyros D. Chatziefthimiou,[a] [a]*European Molecular Biology Laboratory Hamburg Unit, Hamburg, Germany,* [b]*Institute for Biophysics and Munich Center for Integrated Protein Science, Technical University of Munich, Munich, Germany,* [c]*Section of Structural Biology, The Institute of Cancer Research, Chester Beatty Laboratories, London, United Kingdom.*
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Some of the largest proteins identified to date are found in muscle sarcomeres. Their principle function is to form a stable overall architecture to support contraction/relaxation by the actomyosin system under substantial mechanical forces. We are investigating two important filament systems in terms of structural/functional relationships: the first one is titin, a protein of almost 4 MDa in molecular weight and composed of about 300 single protein domains; it extends through one half sarcomere over a distance of about 2 nm. The second one is myomesin, a protein of about 150 kDa, which elastically links titin and the thick filaments (myosin) in the central M-band of sarcomeres. At the molecular level, myomesin has a domain structure related to the one of titin and, hence, has been described as "mini titin". Recently, we have unraveled the filament architecture of the complete C-terminal part of myomesin, involving domains My9-My10-My11-My12-My13. We have chosen a "divide-and-conquer" approach by combining electron microscopy, small angle X-ray scattering and high-resolution X-ray crystallography. Our data revealed a dimeric tail-to-tail filament structure that is folded into an irregular superhelical coil of almost identical domain modules, separated by highly exposed á-helical linkers [1]. Unfolding of these linkers, when tension is applied, can stretch this myomesin segment to about 2.5 times, returning to its original state when the tension is removed. To gain further insight into the myomesin molecular architecture and identify the borders of its elasticity we have solved an additional crystal structure of the myomesin domains My8-My11, in which My8 is folded as a fibronectin type III domain. In contrast to the helical linkers connecting the My9-My13 [1], the My8-My9 interface is tight and unrelated to the following ones, indicating that the established mechanism of myomesin filament elasticity is strictly confined to the C-terminal domain array My9-My13. We have generated an extended myomesin model My8-My13 with an overall length of about 38 nm, thus approaching the measured M4-M4' distance of 44 nm, which characterizes the distance of the distal myomesin anchoring sites in the central M-band of muscle sarcomeres [2]. We confirmed its dimensions and overall structural arrangement by complementary small angle X-ray scattering data and electron microscopy. Additional atomic force spectroscopy data indicate that helix-mediated elasticity in myomesin is strictly limited to the My9-My13 segment of myomesin. Our new data present an important step to dissect different functions of myomesin along its highly repetitive domain structure.

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MS10-O1 Type Ve secretion: a novel autotransport mechanism. Jack C. Leo,[a] Phillip Oberhettinger,[b] Monika Schütz,[b] Ingo B. Autenrieth,[b] Dirk Linke[a] [a]*Protein Evolution, Max Planck Institute for Developmental Biology, Germany*, [b]*Interfaculty Institute for Microbiology and Infection Medicine, University Clinics Tübingen, Germany*
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Type V secretion is a prevalent mechanism for protein secretion in Gram-negative bacteria encompassing classical autotransporters (type Va secretion), two-partner secretion systems (type Vb) and trimeric autotransporter adhesins (type Vc) [1]. Recently, a fourth category, type Vd secretion, was added to this list [2]. Though it is now clear that other factors, such as periplasmic chaperones and the Bam complex, are involved in the biogenesis of type Va proteins, the term 'autotransporter' can still be considered valid. The C-terminal transmembrane β-barrel domain is responsible for the translocation of the N-terminal extracellular or passenger domain of these proteins across the bacterial outer membrane. The transport most probably proceeds through a hairpin intermediate, with the passenger being exported C-to-N. We have recently proposed that proteins of the Intimin-Invasin family form a novel subclass of type V secretion: type Ve [1]. Type Ve proteins are widely distributed within the γ-proteobacteria and form an important class of virulence factors. Many lines of evidence suggest these proteins are autotransporters, but with an inverted topology compared to type Va systems; the passenger is C-terminal and the â-barrel translocator is N-terminal, so the passenger would be secreted with an N-to-C polarity. We present data to confirm this topology model for the β-barrel domains of Intimin and Invasin [3], and show that, as for other type V systems, the Bam complex and periplasmic chaperones play a role in type Ve biogenesis. We also show that the small, N-terminal LysM motif-containing periplasmic domain of Intimin – but not Invasin – mediates binding to peptidoglycan and dimerisation.

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