Anchorage of the contractile actomyosin apparatus to the plasma membrane at discrete sites in muscle and non-muscle cells enables the transmission and conversion of force into work, such as muscle contraction and membrane deformation to regulate cell and tissue shape. Assembly, stabilization, and turnover of adhesion sites are complex processes that involve structural components, a variety of signalling and adapter molecules, diverse kinases and phosphatases, and phospholipids. The dynamic turnover of adhesions also requires the frequent interaction with other filament systems of the cytoskeleton, in particular with microtubules. How the delivery and activation of all the required components is co-ordinated, however, remains to be fully understood. In the current issue of *Biochemical Journal*, Sun et al. provide evidence that a specific exon that is exclusively present in the α variant of the type IV intermediate filament protein synemin interacts directly with the focal adhesion protein vinculin in its active state. Interaction of adhesion components with intermediate filaments could serve as a general mechanism to regulate cell- and tissue-specific cytoskeleton-membrane attachment.

Key words: alternatively spliced exon, focal adhesion, synemin, vinculin.

De novo formation of myofibrils and costameres during the development and repair of contractile tissues such as skeletal muscle are initiated at the sites of membrane-cytoskeleton interactions that mediate contact with the extracellular matrix. Costamere formation progresses from specific protein assemblies at focal adhesion-type complexes that gradually elongate and mature into the aligned sarcomeric arrangements that are typical for striated muscle [1]. This process requires the co-ordinated recruitment of adhesion components and the establishment of contractile events via cytoskeleton filaments and myosin motor proteins.

Vinculin is a multifunctional, ubiquitously expressed component of cell-matrix and cell-cell adhesions. Recent insights into the structure of vinculin have substantially improved our understanding of the molecular events that lead to focal adhesion assembly [2]. The crystal structure of the entire vinculin molecule identifies five, mostly α-helical, structural domains (D1–D5, where D1–D4 represent the globular head of vinculin, and D5 equals the C-terminal tail). Substantial molecular rearrangements switch the molecule from an open (active) to a closed (inactive) state. Cytoplasmic vinculin is in its soluble, inactive form and the conformation and activity status is altered upon arrival at focal adhesions. Only the activated form of vinculin is able to interact with its numerous binding partners, including the dimeric actin cross-linking protein α-actinin, focal adhesion components such as talin, and actin filaments, and if vinculin is not rapidly activated in nascent focal complexes these primordial structures turn over rapidly [3].

Activation of vinculin requires the release of the strong inhibitory head-to-tail interaction, and the potential of a single molecular interaction (e.g. with the vinculin-binding sites of either talin or α-actinin) to release these bonds is questionable [2,4]. It appears that helix interconversion at the site of the first helix in the N-terminal D1 domain is involved in disturbing the head-to-tail binding and in stabilizing the activated conformation of vinculin. Although peptide studies indeed suggest that α-actinin can contribute such a helix from its fourth dimerization-mediating spectrin repeat [5], this helix is probably not accessible in the native, dimeric α-actinin molecule. Hence combinatorial activation models have been presented where two or more vinculin-binding partners are required for full unfolding of the tail (D5) domain [2]. In addition to protein–protein interactions, the molecular unfolding is also influenced by interaction with phospholipids, and by phosphorylation. However, in contrast with Vav (a nucleotide-exchange factor for Rho GTPases that also uses an intramolecular domain interaction to adopt an autoinhibitory conformation), phosphorylation alone does not suffice to fully unfold and activate vinculin.

The single vinculin gene produces a second, muscle-specific splice variant, metavinculin, which carries an additional 68-residue insert in the D5 domain. Both isoforms co-localize in adhesive structures in muscle, such as the smooth muscle dense plaques, the intercalated discs in cardiac muscle and the costameres of striated muscle. Notably, vinculin and metavinculin form heterodimers upon binding of PtdIns(4,5)P2 to vinculin via their tail domains [6]. The specific use of alternatively spliced exons and selective homo- and hetero-dimerization are powerful tools to increase molecular specificity in complex biological systems, and are well suited to allow the assembly of cell-type-specific adhesion complexes.

It has been proposed that the recruitment of vinculin to focal adhesions may require actomyosin contraction, rather than active actin polymerization. Although attractive, this model poses a problem with respect to the ill-developed contractile forces at primordial focal complexes, and other factors such as membrane- and filament-bound adapters, or delivery by molecular motor-based processes, may rather contribute to vinculin accumulation. Vinculin binds not only to a set of adhesion molecules including talin, α- and β-catenin, and paxillin [7], but also to the actin microfilament system and to the promiscuous type IV IF (intermediate filament) protein synemin [8]. Syneimin in turn interacts directly with a variety of actin-associated molecules such as dystrophin, utrophin and α-actinin [9]. Syneimin (which is identical to desmuslin) is special among the IF proteins, as it cannot

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form homotypic filaments and requires one of the type III IFs (mainly desmin or vimentin) for the formation of heteropolymeric filaments. Synemin thus co-purifies with desmin and vimentin, and they co-localize together at the cell periphery in striated muscle cells, as well as in vinculin-rich adhesions of hepatic stellate cells [8]. The concept of synemin linking these hetero-polymetric IFs to adhesions has been explored already in the late 1990s [10].

In this issue of the Biochemical Journal, Sun et al. [11] have now investigated the molecular basis for interaction of vinculin with human synemin, and identified that the C-terminal 104 residues of the 312-amino-acid-spanning, alternatively spliced insert of α-synemin (termed SNT III), which is absent from the shorter β-synemin molecule, is both necessary and sufficient for mediating the direct interaction with vinculin and metavinculin tails. This interaction is promoted by PtdIns(4,5)P2 binding, and, as found for most other molecular interactions, the closed conformation of vinculin abolishes synemin interaction. Using a synemin-specific antibody the authors also show co-localization with vinculin at adhesion sites in cross-sections of muscle tissue, and SNT III-dependent targeting to focal adhesions in transfected cultured smooth muscle cells. Aside from the specific interaction of the alternatively spliced SNT III domain, this investigation also shows that the metavinculin-specific 68-residue insert impacts negatively on the affinity for α-synemin. The present study by Sun et al. [11] touches upon the important issue of vinculin recruitment and activation, and on the role of cytoskeleton-membrane adhesions in the formation and maturation of costameres. It points towards the biological significance of heteropolymeric intermediate filaments to generate functional and structural asymmetry in cells, and suggests a specific role for IFs in the regulation of cell–matrix adhesions in muscle tissue. This latter process might entail the physical strengthening of the muscle architecture and the temporal stabilization of membrane adhesions. Perhaps even more importantly, the work raises a series of important questions for the future. Which heterotypic intermediate filament polymer is responsible for the interaction with vinculin in vivo in different cell and tissue types? What role do focal adhesion-resident ‘cytolinkers’ such as plectin (which can simultaneously interact with the IF and actin cytoskeletons) have in vinculin recruitment and adhesion turnover? What is the role of the metavinculin insert in the vinculin–synemin interaction? Does α-synemin interact with monomeric vinculin, with the vinculin–vinculin homodimer, or with vinculin–metavinculin heterodimers, and does this interaction stabilize any of these? The potential mode of interaction between the two molecules raises another important point. Similarly to what has been discussed above for the antiparallel, homodimeric α-actinin molecule, synemin heterodimerization with the type III IFs desmin and vimentin requires clarification of the mechanism by which the SNT III site becomes available for interaction with vinculin in the diverse heteropolymers, and if indeed the common helix insertion mode, or the newly described helix addition mechanism [12], which does not require substantial conformational changes in the helical bundle structure, applies to the synemin–vinculin interaction. For a better understanding of these processes, it appears to be imperative to solve the structure of the vinculin–synemin SNT III co-crystal. Synemin can also act as an anchoring protein for PKA (protein kinase A) [13]. Notably, PKA phosphorylates desmin IFs, leading to their disassembly. The specific recruitment of PKA to adhesion sites can potentially clear the immediate vicinity of adhesions of pure vimentin and desmin IFs, and thus increase the concentration of α-synemin-containing IF heteropolymers for improved interaction at the adhesion site.

In summary, the findings by Sun et al. [11] allow a refinement of the current hypotheses on the regulation of adherens-type junctions in muscle, and illustrate the potential of alternative splicing for achieving functional diversity.

REFERENCES