Commentary & View

Wech proteins

Roles in integrin functions and beyond

Birgit Löer and Michael Hoch*

Life & Medical Sciences (LIMES)-Institute; Program Unit Development; Genetics & Molecular Physiology; Laboratory for Molecular Developmental Biology; University of Bonn; Bonn Germany

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Members of the integrin family of cell adhesion receptors are pivotal to the formation of complex tissues and organs in animals. They mediate cell adhesion by interacting with the extracellular matrix and by binding to intracellular linker proteins that connect to the cytoskeleton. We have recently identified a new and evolutionarily conserved component of the linker complex, the Drosophila Wech protein. Wech is essential for embryonic muscle attachment. It belongs to the RBCC/TRIM family of cytoplasmic multidomain proteins and contains a carboxyterminal NHL domain. Wech protein is specifically localized to the embryonic muscle attachment sites and wech mutant embryos show muscle detachment from the body wall. In β-integrin or talin mutants Wech is mislocalized, as the localization of Integrin-linked-kinase (ILK) depends on Wech. Biochemical data indicate that Wech is associated with the head domain of Talin and the kinase domain of ILK suggesting that Wech may be involved in the linkage of both core proteins of the linker complex. We discuss that Wech proteins may be crucial and evolutionarily conserved regulators of cell-type specific integrin functions and that their activities may underlie complex regulation by microRNAs.

Tissue and organ formation in animals involves adhesion between different cell layers and the extracellular matrix which is often mediated by members of the integrin family of heterodimeric transmembrane receptors.1 In Drosophila, the functional role of integrins in adhesion and signalling has been extensively studied for the attachment of somatic and visceral muscles, morphogenesis of the gut and in adhesion and signalling has been extensively studied for the attachment of somatic and visceral muscles, morphogenesis of the gut and the tumor suppressor brain tumor (Brat),14 the meiotic protein Mei-P26,15 and the newly identified Another B-Box Affiliate (ABBA) protein whose function is still unknown.16 Single copy genes of wech orthologs are found in other invertebrates and in mammals, including mice, rats and humans. The C. elegans Wech ortholog is named Lin-41 and is involved in the regulation of the progression from L4 to the adult developmental program.17 Phenotypic analysis of Drosophila embryos carrying a homozygous wech allele in which both the wech transcript and protein levels are strongly reduced, reveals that muscles are detached from the...
body wall in late embryonic stages\(^\text{11}\) (\textit{wech} is a German Rhineland dialect for “detached” or “gone”). This muscle detachment phenotype is remarkably similar to mutants for \(\beta\)-\textit{integrin} or \textit{talin} (Fig. 1A and B).\(^\text{7,18,19}\) During early stages of embryonic development Wech protein is expressed ubiquitously in all epithelial cells, and after germband retraction it increasingly accumulates very specifically in muscle attachment sites where it is found in a cortical localization in both the epidermal tendon cells and the muscle cells.\(^\text{11}\) \(\beta\)PS Integrin and components of the cytoplasmic integrin-linked complex, Talin, ILK and Tensin, which bind to the cytoplasmic tail of \(\beta\)PS Integrin, co-localize with Wech. Both \(\beta\)PS Integrin and Talin are required for Wech localization. In contrast, Wech is required for ILK and Tensin localization at the attachment sites. PINCH, which was shown to modulate ILK function by direct binding or by recruitment of an ILK-modifying factor,\(^\text{9}\) is still localized properly in \textit{wech} mutants. Altogether, the data suggest that Wech may be required to link an ILK-containing multiprotein complex to Talin, thereby providing a link between integrins and the cytoskeleton in the muscle attachment sites. PINCH apparently acts in parallel to Wech and may contribute to the assembly of an ILK-containing complex (Fig. 1C). Biochemical analysis further supports this hypothesis since it was found by immunoprecipitation that Wech is associated with the head domain of Talin and the kinase domain of ILK. It is not yet known whether the interactions of Wech with ILK and Talin are direct or whether additional proteins are involved. The finding that the \textit{wech} mutant phenotype is stronger than the one of ILK mutants suggests that other unknown factors, in addition to ILK, may depend on Wech function during muscle attachment.

Is Wech a general regulator of integrin-dependent processes? Many components of the integrin-cytoskeleton linker complex, including Talin, PINCH and Tensin have been identified and characterized in the Drosophila wing.\(^\text{20}\) In the wing, loss of integrin functions cause the separation of the two layers of the wing, resulting in a wing blister phenotype.\(^\text{21}\) Although \textit{wech} mRNA expression has been observed in the wing disc,\(^\text{16}\) our unpublished observations indicate that Wech protein is not significantly expressed in the wing. Furthermore, we have not observed wing blister phenotypes in \textit{wech} RNAi knock down animals. It is therefore possible that Wech may not be functionally involved in integrin-dependent adhesion of the wing layers. Furthermore, other phenotypes of \(\beta\)-\textit{integrin} or \textit{talin} mutants, such as impaired germband retraction or a failure of adhesion of the visceral mesoderm to the gut endoderm, are not evident in \textit{wech} mutants. Thus it is likely that Wech may be involved in cell-specific integrin functions rather than being a general regulator of integrin-dependent processes. Tissue-specific integrin functions have also been demonstrated for other components of the integrin pathway, including Tensin which was demonstrated to be essential for integrin-dependent adhesion of the wing layers, but which is not pivotal for integrin-dependent muscle attachment.\(^\text{10}\) These data are in support of the “toolkit” hypothesis put forward by Delon and Brown\(^\text{22}\) which suggests that the components of the link are differentially used in various tissues and developmental situations, like a “toolkit.” In the muscle attachment sites, Wech may be required to specifically strengthen the adhesive junctions between muscles and tendon cells or assemble yet unknown factors that are functionally dependent on integrin-mediated adhesion in their control function during muscle attachment. A similar scenario may also apply to the single Wech orthologs in vertebrates for which, however, no functional studies are available yet. The murine, the human and the chicken Wech ortholog (named TRIM71/Lin41) also possess a B-box, a coiled-coil domain, and a carboxyterminal NHL-domain which shows a sequence similarity of more than 60% as compared to the Drosophila Wech protein.\(^\text{11,23}\) In mouse and chicken, the mRNA of the wech orthologs is expressed in the developing limb buds, brachial arches, the eyespot, the developing muscles and the developing brain.\(^\text{23-25}\) These tissues represent a subset of tissues for which integrin-functions have been described which may also point towards a more tissue and cell-specific function of vertebrate Wech proteins in integrin-dependent processes.

Apart from the putatively conserved function of Wech proteins in the integrin-cytoskeleton link, there are a number of particularities about the Drosophila \textit{wech} gene locus and its regulation that may point to additional roles of Wech proteins in organ development and physiology. We know from Drosophila \textit{wech} that its mRNA and protein expression patterns are not identical. \textit{wech} mRNA is strongly expressed, for example, in the developing peripheral and central nervous system, however, we have not observed significant Wech protein expression in these tissues in the embryo.\(^\text{11}\)
A similar phenomenon is known for Drosophila Talin, for which a discrepancy of embryonic mRNA and protein expression was also described. talin mRNA is also strongly expressed in the PNS and CNS, however protein is not significantly expressed in these tissues. Whether a micro RNA (miRNA) control mechanism may contribute to the regulation of integrin-dependent processes is not known. However, for the lin41/wech/dappled gene family, miRNA control is well studied. In C. elegans, the lin41 gene is a target of the let-7 miRNA. Drosophila wech/dappled also contains three let-7 regulatory sites present in the 3’-region of its transcript, and it has been suggested that the strict regulation of wech/dappled mRNA levels by let-7 miRNAs may be crucial for normal eye development. Reciprocal expression of the mouse Wech ortholog TRIM71/Lin-41 and the microRNAs let-7 and mir-125 has been observed during mouse embryogenesis and computational analysis indicates that the let-7 regulatory binding sites are conserved in the mammalian wech orthologs. This suggests that also mammalian Wech expression may be regulated by miRNAs which may cause a discrepancy between mRNA and protein expression patterns.

Another significant feature of Wech proteins is their C-terminal NHL domains. In Drosophila, NHL domains are only found in Wech and three additional proteins: the Drosophila tumor suppressor proteins Brat and Mei-P26 and the newly identified ABA protein which is also expressed in muscle tissue. Mutations in brat cause brain tumors and mei-P26 mutations lead to ovarian tumors. It has recently been shown that Brat acts as a growth regulator and inhibits self-renewal in one of the two daughter cells of neural stem cells by posttranscriptionally inhibiting dMyc and thereby controlling ribosome biogenesis and protein translation. Interestingly, the Drosophila NHL-domain protein Brat is also annotated as being regulated by the miRNA let-7 (miRBase, Sanger Institute). The activation of mei-P26 causes overproliferation of germline stem cells, most likely due to the lack of proliferation control. The molecular characterization of brat mutations suggests that the NHL domain carries the tumor suppressor function of Brat and that this domain is involved in regulating proliferation of stem cells. Functions of Brat and Mei-P26 in integrin-dependent adhesion or vice versa, of Wech in growth regulation of stem cells, are not known. However, it is of note that Wech is expressed both in the female germline and in the larval nervous system (Löer and Hoch, unpublished results). Hence, it will be interesting to determine whether Wech interacts with other NHL-domain proteins and if Wech has additional functions beyond integrin signalling in the regulation of cell growth and differentiation.

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