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REVIEW

Structural evidence for common functions and ancestry of the reovirus and adenovirus attachment proteins

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SUMMARY

The crystal structure of the reovirus attachment protein, σ1, reveals a fibre-like structure that is remarkably similar to that of the adenovirus attachment protein, fibre. Both proteins are trimers with head-and-tail morphology. They share unique domain structures and functional properties including defined regions of flexibility within the tail and an unusual symmetry mismatch with the pentameric viral capsid protein into which they are inserted. Moreover, the receptors for reoviruses and adenoviruses, junctional adhesion molecule 1 and coxsackievirus and adenovirus receptor, respectively, also share key structural and functional properties. Although reoviruses and adenoviruses belong to different virus families and have few properties in common, the observed similarities between σ1 and fibre point to a conserved mechanism of attachment and an ancient evolutionary relationship. Copyright © 2003 John Wiley & Sons, Ltd.

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INTRODUCTION

Specific attachment of viruses to cell-surface receptors is the initial step in viral infection. Although there exists substantial diversity in the types of receptors recognised by different viruses, all viruses must possess a strategy to penetrate cell membranes. Enveloped viruses achieve this through fusion of viral and cellular membranes, and many enveloped viruses employ a common strategy for this fusion process based on the formation of hydrophobic, triple-helical coiled-coil structures that are thought to insert into the cell membrane and initiate fusion. The identification of highly conserved trimeric coiled-coil structures in the glycoproteins of several enveloped viruses (e.g. Ebola virus, HIV and influenzavirus) has led to a model for their conserved mechanism of action (reviewed in [1]). In contrast to enveloped viruses, much less is known about the attachment and entry strategies used by nonenveloped viruses, a group that includes several important human pathogens. As is the case for enveloped viruses, identification of conserved structural features in nonenveloped viruses will likely facilitate an understanding of common mechanisms used by these viruses for attachment and cell entry.

The recently determined crystal structure of the reovirus attachment protein, σ1, [2] reveals unanticipated similarities to the structure of fibre, the adenovirus attachment protein [3]. Although both adenoviruses and reoviruses are icosahedrally shaped viruses without envelopes, they differ dramatically in capsid composition and particle architecture. Most importantly, adenoviruses have a double-stranded (ds) DNA genome, whereas reoviruses contain dsRNA. Both viruses, however, interact with cell-surface receptors using trimeric,

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Abbreviations used

CAR, Coxsackievirus and adenovirus receptor; ds, double-stranded; ICAM1, intercellular adhesion molecule 1; JAM1, junctional adhesion molecule 1; omp, outer membrane protein.

fibre-like molecules located at the 12 vertices of the virion icosahedron. The structural similarities between the attachment proteins of these viruses suggest conserved features of action and highlight a distant evolutionary relationship.

THE REOVIRUS ATTACHMENT PROTEIN, σ1

Reoviruses form nonenveloped, icosahedral particles that contain a segmented dsRNA genome [4]. The virions measure about 850 Å in diameter and are composed of eight structural proteins. The λ1, λ2, λ 3, μ2 and σ2 proteins form the 'core' or inner capsid particle, whereas the viral outer capsid is formed by μ1, σ1 and σ3. The μ1 and σ3 proteins comprise the bulk of the outer capsid. They are tightly associated, with μ1 providing the membrane penetration machinery and σ3 acting as a protective cap for μ1 [5]. The σ1 protein protrudes from the 12 vertices of the virion icosahedron [6,7] and serves as the viral attachment protein [8,9]. It is a long, fibre-like molecule with head-and-tail morphology and several discrete regions of flexibility [7]. The σ1 tail partially inserts into the virion via 'turrets' formed by the pentameric λ2 protein, while the σ1 head projects away from the virion surface [6,10].

Crystal structures of the reovirus core [11], isolated σ3 [12], the σ3/μ1 complex [5], and a *C*-terminal fragment of σ1 [2] have provided a detailed view of the organisation of the reovirus particle and the atomic structures of its key components. However, the structural changes that accompany reovirus attachment and entry, and its transition from the virion to subvirion intermediate and core particle [10], are poorly understood. The σ1 protein is key to precise delineation of these events as it facilitates viral attachment and undergoes major conformational changes during viral disassembly [6,10]. The σ1 protein binds two types of receptors and attaches to cells using an adhesion-strengthening mechanism [13]. A domain in the σ1 tail binds to cell-surface carbohydrate [8], which is known to be α-linked sialic acid for serotype 3 reoviruses [14–17], and the σ1 head binds to junctional adhesion molecule 1 (JAM1) [9]. The σ1 protein plays a pivotal role in determining strain-specific disease patterns in animal models [18,19], most likely by selective recognition of cell-surface receptors. It also is responsible for strain-specific differences in the efficiency of virus-induced apoptosis [20–22].

Structural analysis of the *C*-terminal half of σ1 (residues 246–455), which includes the JAM1-binding σ1 head, reveals an elongated, trimeric structure with maximum dimensions of 120 Å in length and 50 Å in width [2]. Each monomer contains two domains: a slender tail and a compact head (Figure 1). Residues 246–309 form the tail, a triple β-spiral that contains sequence repeats characterised by conserved hydrophobic and glycine or proline residues [2,3]. Each repeat consists of two short β-strands connected by a four-residue β-turn that has either a proline or a glycine residue at its third position. A surface-exposed, variable loop links successive repeats, and trimerisation generates the highly regular β-spiral structure. The remaining σ1 residues (310–455) assemble into a compact β-barrel that forms the head. The σ1 trimer features a distinct kink between the three-fold axes of the head and tail domains (Figure 1). Although this kink is most likely introduced by crystal packing forces, it indicates that the σ1 trimer possesses a high degree of flexibility between the second and third β-spiral repeats in the tail.

THE ADENOVIRUS ATTACHMENT PROTEIN, FIBRE

Adenoviruses are important human pathogens responsible for a variety of gastrointestinal, ocular and respiratory infections [23]. They contain dsDNA genomes and form particles with a diameter of about 1000 Å [24]. The adenovirus capsid includes 240 copies of the trimeric hexon protein and 60 copies of the pentameric penton protein, which is located at the 12 vertices of the icosahedral particle. The penton protein forms the penton base complex, which serves as the anchor for the trimeric attachment protein, fibre, an elongated structure that protrudes from the virion surface. The fibre can be subdivided into a thinner 'shaft' and a more globular 'knob'. Adenovirus serotypes 2, 5 and 12 bind to the coxsackievirus and adenovirus receptor (CAR) [25,26] by using a binding site located in the knob [27–29]. Crystal structures of the knob have been determined for serotypes 2 [30] and 5 [31], as well as for the non-CAR-binding serotype 3 [32]. Structures are also available for a complex between the serotype 12 knob and the *N*-terminal domain of CAR [33] and for a trimeric fragment that comprises the knob and a portion of the shaft of the serotype 2 fibre [3]. The latter

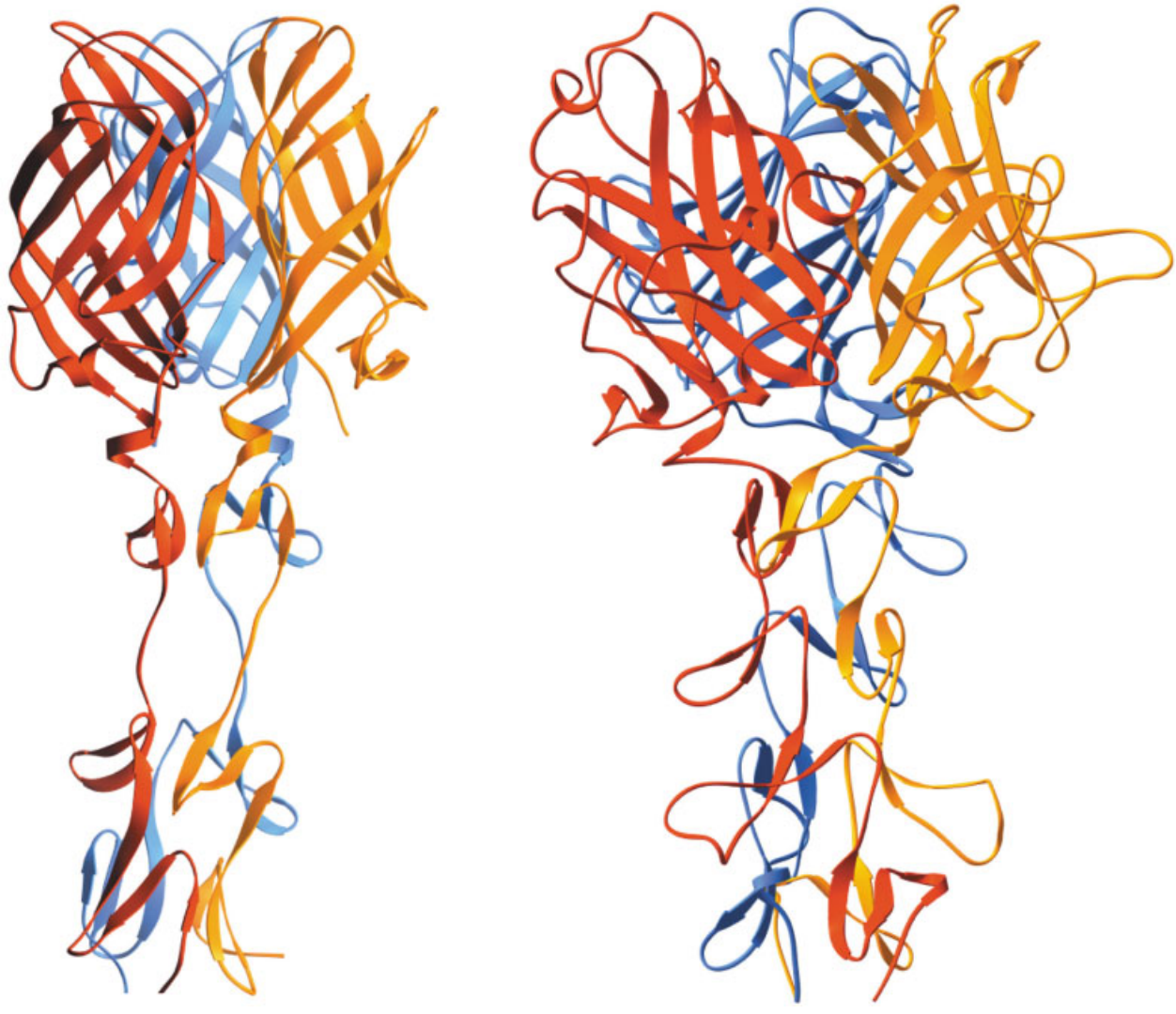

Figure 1. Ribbon tracings of reovirus σ1 (left) [2] and adenovirus fibre (right) [3]. The three monomers within each trimer are shown in red, orange and blue. Both proteins have head-and-tail morphology, with a triple β-spiral forming the tail and an eight-stranded β-sandwich domain forming the head. This and subsequent figures were prepared using the program RIBBONS [66]

structure revealed that the shaft forms a triple β-spiral (Figure 1). Although only a short β-spiral was seen in the crystallised fragment, residues 45 to 392 of the serotype 2 fibre are predicted to form a continuous 22-repeat β-spiral with residues 399–582 comprising the *C*-terminal knob [3].

PARALLELS IN THE STRUCTURES OF REOVIRUS σ1 AND ADENOVIRIUS FIBRE

The overall appearance of the reovirus σ1 protein is strikingly similar to that of the adenovirus fibre (Figure 1). Moreover, the two proteins share many architectural features. Both are trimeric, elongated fibrous structures of roughly equivalent length that contain multiple repeats of an *N*-terminal β-spiral followed by a globular domain with eight major β-strands (Figure 1). The σ1 tail is predicted to also contain an α-helical coiled-coil *N*-terminal to the β-spiral [2], whereas the fibre shaft is most likely composed entirely of β-spiral repeats [3]. The two proteins are to date the only structures known to contain such triple β-spiral motifs, and thus they use a unique design for trimer formation. Most trimeric protein structures are based on α-helical coiled-coils, in which three amphipathic α-helices are wrapped around each other

to form a supercoil structure. Sequences that form these amphipathic α-helices are characterised by a heptad repeat, in which non-polar residues are found at the first and fourth positions of the repeating unit [34]. Trimerisation motifs based on β-sheet structures are far less common and have to date been observed in very few cases. For example, the bacteriophage T4 gp5 and gp12 proteins each contain triple β-helices [35,36], trimeric structures formed by β-strands that are roughly parallel to the trimer axis. The bacteriophage T4 gp12 protein also contains an additional trimeric structure that somewhat resembles the β-spirals seen in reovirus σ1 and adenovirus fibre, although it is not a true β-spiral [35].

The surprising structural similarities between reovirus σ1 and adenovirus fibre also extend to their *C*-terminal domains, both of which are globular structures that contain eight antiparallel β-strands (Figure 2). A large number of domain folds are based on a core structure of eight antiparallel β-strands, ranging from the ubiquitous β-sandwich domains of the immunoglobulin (Ig) superfamily [37] to the circular β-barrel of the *E. coli* outer membrane protein (omp) A [38] and the jelly-roll motif found in many viral coat proteins [39]. The principal difference between these folds lies in the manner in which the various β-strands are connected (i.e. the 'topology' of each domain). Thus, analysis of the connectivity of a given fold serves to establish a structural relationship. The connectivity of β-strands in the σ1 head and the fibre knob is unique to these two proteins, demonstrating that they are more similar to each other than to any other protein for which structural information is available. This relationship is underscored by the observation that homology searches [40] with the coordinates of either domain yield the other as the only close structural homologue [2]. One difference between the two folds is that the β-strands of the σ1 head circularise to form a round β-barrel, whereas the fibre knob is a β-sandwich domain with two separate β-sheets facing each other. The only other known circular β-barrel featuring eight antiparallel strands is that of the ompA protein [38], a membrane-embedded domain with very different connectivity (Figure 2).

The remarkable homology between the σ1 head and the fibre knob extends to other structural features such as a conserved short helix in a loop between β-strands D and E (Figure 2) and the spatial relationship of the β-sandwich domains to the *C*-terminal repeat of the β-spiral. However, there exist differences between the two domains in the surface loops. The σ1 head features extremely short connections between most β-strands (except the D-E loop), which results in a highly compact structure. The fibre knob contains about 30 additional residues in comparison to the σ1 head, and these residues account for a more elaborate loop structure. This is not surprising as the two proteins bind different receptors.

REOVIRUS σ1 AND ADENOVIRUS FIBRE EXHIBIT FLEXIBILITY IN ANALOGOUS REGIONS

The σ1 trimer is a remarkably dynamic structure. A comparison of the three independent molecules present in the crystals reveals that a linear insertion into the β-spiral (residues 291–294 of T3D σ1) allows for substantial movement of the head with respect to the tail (Figure 3). This mobility is likely increased in the absence of constraining crystal packing forces, and it would allow the σ1 head to 'sway' above the reovirus capsid in a physiologic context. Electron microscopic images of full-length σ1 show flexibility in a region of the molecule that corresponds to this insertion and also in a second region close to the midpoint of the protein [7]. This latter region of flexibility, which is not included in the crystallised σ1 fragment, correlates with the transition from the predicted *N*-terminal α-helical coiled coil to the more *C*-terminal triple β-spiral [2]. The adenovirus fibre does not have an insertion similar to that of σ1 beneath the knob domain, and the four turns of β-spiral that precede the knob are uninterrupted. However, residues 394–396 of fibre, which follow the last spiral turn in sequence, have poor electron density and are presumed to be flexible [3]. These poorly ordered residues appear to act as a hinge that allows the knob to move with respect to the shaft. The three-fold axes of the fibre knob and shaft differ by 2° [3], and comparison of the three independent copies of the fibre molecule in the crystal structure demonstrates that this difference translates into significant mobility between the shaft and knob (Figure 3). Thus, the trimeric knob can move as a whole with respect to the spiral, although the movement is somewhat more modest than that observed for σ1. The fibre shaft

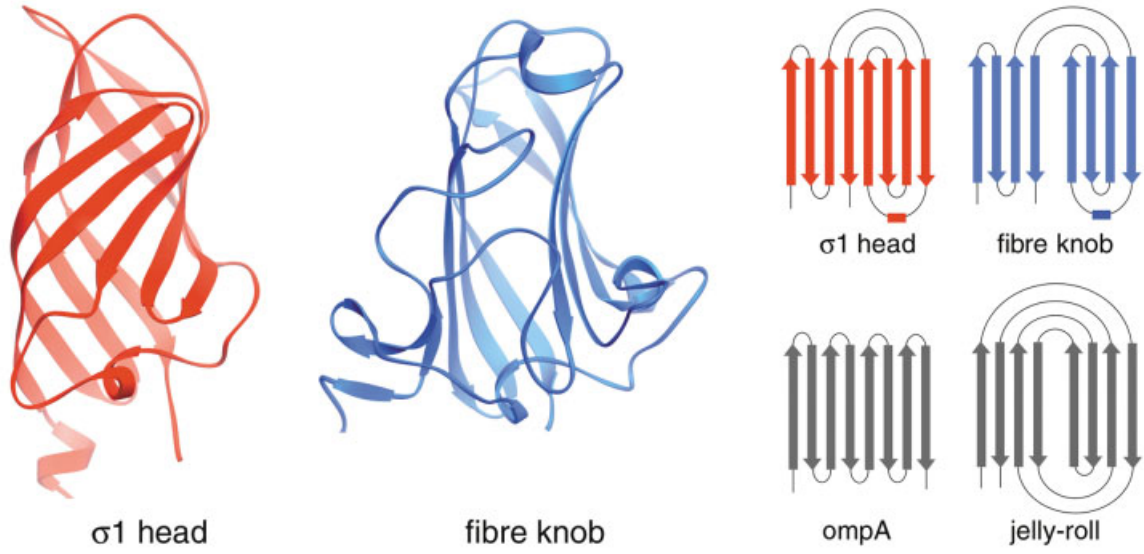

Figure 2. Ribbon tracings of the reovirus σ1 head (left) and the adenovirus fibre knob (right). In addition to the conserved β-sheet topology, the head and knob structures share a short helix in a long loop at the domain base. The β-sheet topologies for the two domains are shown on the far right, adjacent to the topologies for the unrelated jelly-roll and ompA structures, which also contain eight β-strands

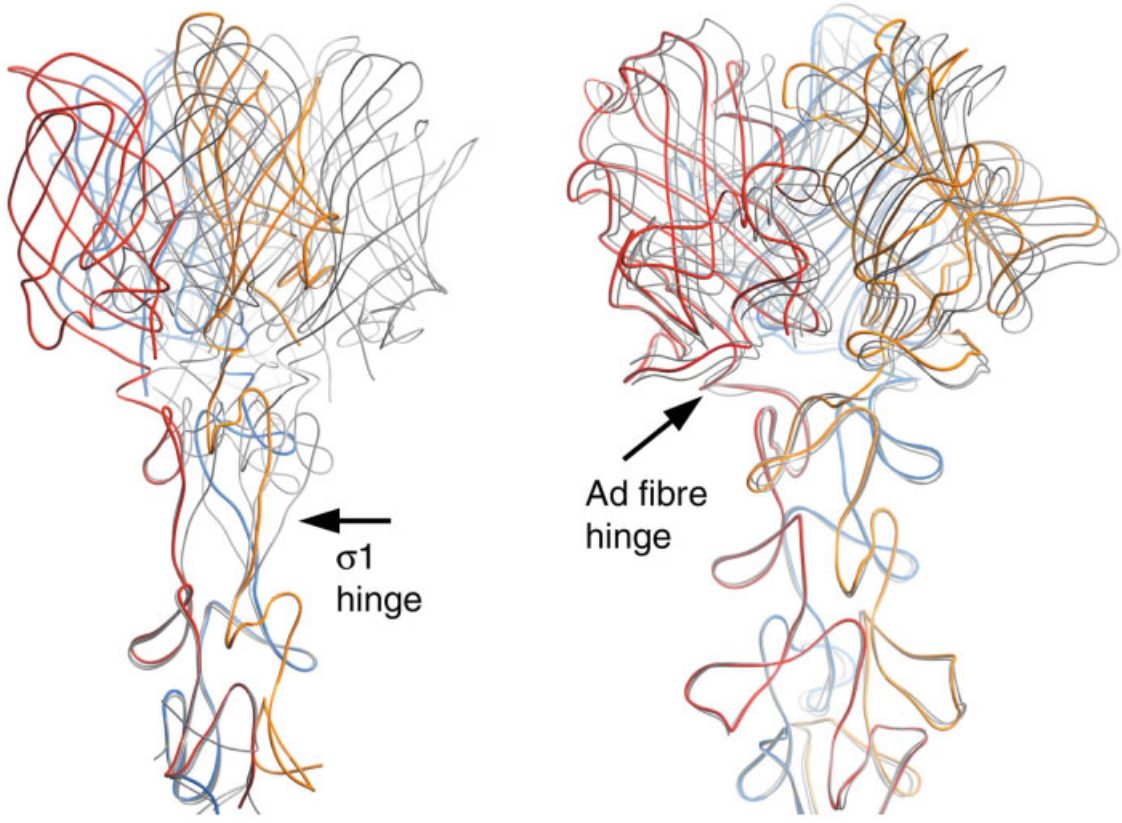

Figure 3. Reovirus σ1 (left) and adenovirus fibre (right) exhibit a high degree of flexibility. Both σ1 and fibre contain three crystallographically independent monomers that assume substantially different conformations. The superposition of the three monomers in each trimer reveals a defined region of flexibility with a hinge just below the head domain in each case. One trimer is shown in red, orange, and blue; the other two are shown in grey

also has several other kinks within its β-spiral [41,42], and these likely represent additional regions of flexibility.

Flexible regions in both proteins may serve a similar purpose by acting to facilitate interactions with receptors or structural rearrangements during viral disassembly. In the case of adenoviruses, shaft flexibility is a critical determinant of receptor binding as the rigid serotype 37 fibre fails to engage CAR despite the presence of the CAR-binding epitope in the knob [43]. Although similar analyses of σ1 have not been performed, it seems probable that mobility between the globular and fibrous regions of both σ1 and fibre is required for optimal positioning of the receptor-binding surfaces of these attachment proteins with their cell-surface receptors. It is striking that σ1 and fibre use functionally equivalent hinge regions just beneath their globular head domains for this purpose.

A SYMMETRY MISMATCH EXISTS IN THE INSERTION OF THE REOVIRUS AND ADENOVIRUS ATTACHMENT PROTEINS

Attachment proteins of reovirus and adenovirus are trimers that are anchored into the virion capsid via a pentameric structure, and therefore, they feature a highly unusual symmetry mismatch. The reovirus σ1 trimer is inserted into a slot formed by the λ2 pentamer, the structure of which is known [11]. The adenovirus fibre is inserted into the penton base, also a pentameric structure. Thus, in both cases a unique A_3B_5 complex is formed. While symmetry mismatches have been seen in other protein complexes such as the AB_5 toxins [44], the F1 ATP synthase [45], or the VP1/VP2 polyomavirus complex [46], they are not common. Such mismatches usually indicate interactions of limited strength or specificity, and proteins involved in these mismatches often undergo rearrangement. They also are often hydrophobic in nature. AB_5 toxins such as cholera toxin undergo dramatic conformational changes upon membrane binding [44], and this change involves exposure of the A subunit. The symmetry mismatch of three α, three β, and one γ subunit in F1 ATPase is crucial for its rotational mechanism of action, with the γ subunit forcing asymmetry onto the $\alpha_3\beta_3$ pseudohexamer and providing a hydrophobic contact area that allows for efficient rotation [45,47]. The structure of a polyomavirus VP1/VP2 complex, in which a single VP2 molecule engages a hydrophobic channel at the centre of the VP1 pentamer, suggests that some rearrangement must occur during viral entry in order to expose the myristylated VP2 *N*-terminus [46]. Thus, the conserved A_3B_5 mismatch in both reovirus and adenovirus indicates a strong potential for structural rearrangement, which may occur upon receptor binding or disassembly. Moreover, the A_3B_5 stoichiometry suggests that a conserved mechanism underlies these processes. To our knowledge, these structures are the only examples of A_3B_5 complexes.

COMMON REGIONS OF σ1 AND FIBRE ARE USED FOR BINDING TO STRUCTURALLY SIMILAR RECEPTORS

Both reovirus and adenovirus bind to receptors that belong to the Ig superfamily. In both cases, the *N*-terminal domain of the receptor is recognised by sequences in the globular *C*-terminal domain of the viral protein ([29] and Forrest and Dermody, unpublished). Crystal structures of the adenovirus receptor CAR [48] and the murine homologue of JAM1 [49] indicate that both proteins form physiologically relevant homodimers. The CAR homodimer features extensive interactions between the GFCC′ β-sheets of the *N*-terminal Ig-like (D1) domains [48] (Figure 4). The corresponding region of murine JAM1 also mediates homodimerisation, with an equally extensive contacting surface [49] (Figure 4). In addition, the relative orientations of the monomers in the CAR and JAM1 homodimers are strikingly similar (Figure 4), demonstrating a close structural relationship between the two receptors.

Although dimeric structures of Ig superfamily members are not uncommon [50–53], CAR and JAM1 are the only molecules for which structural information is available that form physiologically relevant homodimers via the GFCC′ β-sheet of D1. The recently published structure of a coronavirus receptor suggests that it also may use a similar mode of dimerisation [54]. We note, however, that for several receptors of the Ig superfamily the GFCC′ β-sheet is engaged by viral ligands for complex formation. For example, the HIV glycoprotein gp120 binds to residues on the GFCC′ face of the HIV-receptor CD4 [55]. Complexes of coxsackievirus B3 and rhinoviruses with their receptor intercellular adhesion molecule 1 (ICAM1) also demonstrate that residues at the top of D1 of

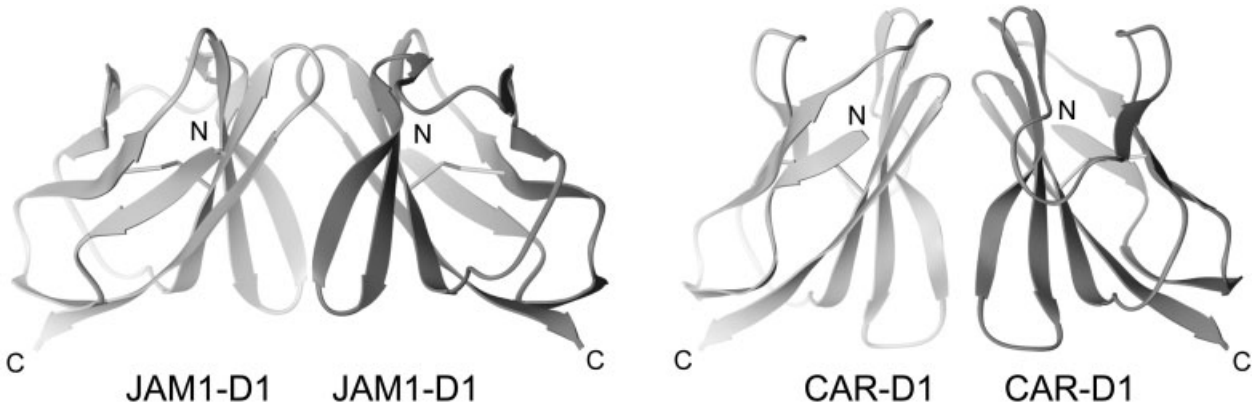

Figure 4. Comparison of reovirus receptor murine JAM1 (left) [49] and adenovirus receptor CAR (right) [48]. Both proteins form dimers using the same interface (formed by β-strands G, F, C, and C′) of the membrane-distal Ig-like domain. The adenovirus fibre knob binds to a monomeric version of CAR [33]

ICAM1, a region that includes part of the GFCC′ face, mediate virus binding [56,57]. The structure of a complex between adenovirus fibre knob and CAR shows that the heterodimeric knob–CAR interactions also involve the GFCC′ β-sheet of the CAR D1 domain [33]. In this last case, it appears that knob binding is incompatible with the homodimeric CAR structure shown in Figure 4. However, it is not known whether the knob is able to disrupt the CAR dimer or simply recognises monomeric versions of CAR at the cell surface.

REOVIRUS σ1 AND ADENOVIRUS FIBRE MAY SHARE COMMON ANCESTRY

The origins of viruses are poorly understood, and establishing evolutionary relationships between them is difficult because of their rapid divergence in sequence. Three-dimensional structures change much more slowly than amino acid sequences, and structural analyses can sometimes reveal a previously unsuspected relationship between viruses. However, the biological relevance of such a link may not be apparent. The jelly-roll motif, for example, has thus far been found in a large number of viruses, but it is not associated with a conserved function. It therefore appears to serve as a scaffold used by many viruses to construct their capsids, similar to the Ig-like domain that is the structural motif of choice for many cell-surface receptors.

For cases in which structural homologies parallel conserved function, an evolutionary relationship is more likely, and indeed several examples have been identified. The conservation of key structural and functional features between the alphavirus glycoprotein E1 [58,59] and the flavivirus glycoprotein E [60] suggests a common progenitor. Structural similarities between the adenovirus hexon protein and the P3 capsid protein of bacteriophage PRD1 also have led to the suggestion of an evolutionary relationship between the two proteins [61]. In both cases, the structural similarities extend beyond the conservation of a single domain and translate into common functional properties. The same is true for the reovirus and adenovirus attachment proteins, and thus we think that the similarities between them point to a conserved mechanism of action and a distant evolutionary relationship, although we cannot exclude the possibility that the similarities have arisen through convergent evolution.

Since reovirus and adenovirus face similar challenges in the initiation of an infectious cycle, it is perhaps not too surprising that these viruses have evolved highly similar fibre-like proteins for attachment to host cells. Particles of both viruses are relatively large, and the long fibre-like tail may be required to allow the head to reach cell-surface receptors, which in both cases contain only two Ig-like domains and thus are located close to the cell membrane. Viruses typically engage membrane-distal regions of their receptors, and in many cases these virus-binding epitopes are found on more protruding receptors with more than two domains [55,56,62,63]. Flexibility at a region just below the head domain may facilitate the engagement of receptors by allowing the head to position itself properly for a productive interaction. Thus, a globular head domain with

numerous protruding loops offers the opportunity to modulate this interaction. Finally, the triple β-spiral creates a highly stable, regular trimeric structure that nonetheless allows for sequence variations and insertions in its surface-exposed loops. This design feature includes added functionality that is missing in the more common α-helical coiled-coil trimerisation motif. In both reoviruses and adenoviruses, different serotypes are distinguished in part by insertions and deletions in the triple β-spiral region, and these differences lead to altered surface properties. For example, the β-spirals of serotype 1 and 3 reoviruses contain binding sites for carbohydrate receptors in two distinct regions [8], and differences in β-spiral loops between adenovirus serotypes are at least partially responsible for serotype-specific antigenic determinants [3]. In both cases, the loops emanating from the β-spiral structure are also likely to mediate contacts with other viral capsid proteins.

CONCLUSION

It is tempting to speculate that the numerous structural and functional parallels between the reovirus and adenovirus attachment proteins, which are reinforced by similarities between their receptors JAM1 and CAR, lead to conserved routes of entry and mechanisms of infection. We note that both receptor proteins are located at tight junctions. JAM1 was originally identified as an intercellular adhesion protein located at tight junctions and named accordingly [64]. CAR was shown recently to be a component of tight junctions between epithelial cells [65]. The fact that both viruses use tight junction proteins as receptors raises the possibility of conserved themes of receptor recognition, internalisation, tropism and disease. Thus, reovirus and adenovirus may have more in common than the striking structural similarities exhibited by their attachment proteins. Future studies will hopefully reveal the full extent of the fascinating relationship between these two pathogens.

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