Structural Basis for Semaphorin Signalling through the Plexin Receptor
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LETTER

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Structural basis for semaphorin signalling through the plexin receptor

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Semaphorins and their receptor plexins constitute a pleiotropic cell-signalling system that is used in a wide variety of biological processes, and both protein families have been implicated in numerous human diseases[1–4]. The binding of soluble or membrane-anchored semaphorins to the membrane-distal region of the plexin ectodomain activates plexin's intrinsic GTPase-activating protein (GAP) at the cytoplasmic region, ultimately modulating cellular adhesion behaviour[5]. However, the structural mechanism underlying the receptor activation remains largely unknown. Here we report the crystal structures of the semaphorin 6A (Sema6A) receptor-binding fragment and the plexin A2 (PlxnA2) ligand-binding fragment in both their pre-signalling (that is, before binding) and signalling (after complex formation) states. Before binding, the Sema6A ectodomain was in the expected 'face-to-face' homodimer arrangement, similar to that adopted by Sema3A and Sema4D, whereas PlxnA2 was in an unexpected 'head-on' homodimer arrangement. In contrast, the structure of the Sema6A–PlxnA2 signalling complex revealed a 2:2 heterotetramer in which the two PlxnA2 monomers dissociated from one another and docked onto the top face of the Sema6A homodimer using the same interface as the head-on homodimer, indicating that plexins undergo 'partner exchange'. Cell-based activity measurements using mutant ligands/receptors confirmed that the Sema6A face-to-face dimer arrangement is physiologically relevant and is maintained throughout signalling events. Thus, homodimer-to-heterodimer transitions of cell-surface plexin that result in a specific orientation of its molecular axis relative to the membrane may constitute the structural mechanism by which the ligand-binding 'signal' is transmitted to the cytoplasmic region, inducing GAP domain rearrangements and activation.

Both semaphorins and plexins contain, at the amino terminus of their ectodomain, a ~500-residue sema domain followed by a short (~50 residues) plexin–semaphorin–integrin (PSI) domain. Those regions corresponding to the sema plus PSI segment of Sema6A ($Sema6A_{SP}$, residues 19–570) and PlxnA2 ($PlxnA2_{SP}$, residues 38–561) that mediate ligand–receptor interaction were first expressed in mammalian cell lines, and then purified and crystallized (Supplementary Fig. 1). Structures of $Sema6A_{SP}$ and $PlxnA2_{SP}$ were determined at 2.5 Å and 2.1 Å resolution, respectively (Fig. 1a, b, Supplementary Tables 1 and 2, and Supplementary Results). In both proteins, the sema domain displays a seven-bladed β-propeller fold very similar to previously determined structures of Sema3A[6], Sema4D[7] and Met[8]. In addition to the long 'extrusion' within blade 5 described previously[7] (hereafter called extrusion 2), we noted a second insertion between blades 1 and 2 that proved characteristic to all sema domains (hereafter called extrusion 1) (Fig. 1e).

In the $Sema6A_{SP}$ crystal, monomers make contact with one another using the upper rim of the β-propeller, thereby assuming a 'face-to-face' dimer configuration (Fig. 1a). This dimeric configuration is essentially identical to that seen in the crystal structures of the Sema3A and Sema4D sema domains (Supplementary Fig. 2a). The location of the loops involved in the dimerization is precisely conserved among the three semaphorins, with the exception of the N-terminal region's participation in Sema6A (Supplementary Fig. 2b). Surprisingly, $PlxnA2_{SP}$ also assumes a dimeric configuration in the crystal, albeit with a markedly different mode compared to that observed in the semaphorin sema domains (Fig. 1b). The two $PlxnA2_{SP}$ fragments in the asymmetric unit are related by a non-crystallographic two-fold axis and interact with each other by using a flat surface located at the side of the β-propeller, exhibiting a 'head-on' configuration twisted orthogonally, in contrast to the face-to-face configuration observed in the known semaphorin structures. All the key residues involved in the dimerization are well conserved among the A-type plexin family (Supplementary Results and Supplementary Fig. 3), indicating the physiological relevance of the dimerization. Analytical ultracentrifugation sedimentation velocity experiments performed on the $Sema6A_{SP}$ protein confirmed that it does indeed form a dimer in solution with a dissociation constant (K_d) value of 3.5 μM (Supplementary Fig. 4). The dimerization affinity for $PlxnA2_{SP}$, however, was extremely low (K_d > 300 μM) and could not be definitively determined (Supplementary Fig. 5).

We next crystallized the Sema6A–PlxnA2 complex by mixing $Sema6A_{SP}$ and $PlxnA2_{SP}$ at an equimolar concentration, obtaining a structure at 3.6 Å resolution (Supplementary Results, Supplementary Table 3 and Supplementary Fig. 6). The $Sema6A_{SP}$ and $PlxnA2_{SP}$ molecules constitute a 2:2 complex in the crystal, which contained a crystallographic two-fold symmetry (Fig. 1c). The two $Sema6A_{SP}$ molecules in the complex formed the same face-to-face dimer as was observed in the plexin-free state (Supplementary Fig. 7a). On the other hand, the $PlxnA2_{SP}$ head-on homodimer was no longer present in the complex, and the two plexin molecules independently docked onto the two Sema6A monomers with their carboxy-terminal PSI domains emanating away diagonally. Despite their participation in different molecular interactions, there were no major changes in the structure of individual $Sema6A_{SP}$ and $PlxnA2_{SP}$ monomers, including the conformation of the loops at the interface, upon the complex formation (root mean squared deviation of 0.7Å for $Sema6A_{SP}$ and 0.8Å for $PlxnA2_{SP}$, respectively; Supplementary Figs 7 and 8). At the interface, the Sema6A side showed positively charged surface potentials whereas the PlxnA2 side was negatively charged, indicating that complex formation is driven mainly by electrostatic interactions (Fig. 1d).

We subsequently mutated a select number of interface residues on Sema6A to see whether these mutations disrupt plexin binding. The H212N mutation is expected to create a novel N212-D213-S214 glycosylation sequon and place a large carbohydrate obstacle at the heart of the interface (Fig. 1d and Supplementary Fig. 9). Another mutation, K393E, is expected to convert the electrostatic interaction between

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The following is a comment on the published paper shown on the preceding page.

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Introduction

Cells receive various 'signals' from outer environment, and change their location, behavior, and fate by these 'signals'. Many of these 'signals' come as a form of extracellular protein, while the 'sensors' are proteins located on cell surface generally called receptor. Although numerous "ligand-receptor" systems that play fundamental roles in the intercellular signal transduction are known, the mechanisms for the information transmission from extracellular to intracellular space remain poorly understood. Semaphorins ('signals') are a large family of cell surface or secreted proteins that are implicated in development and homeostasis of various tissues by regulating cellular migration behavior[1-4]. Semaphorins are divided into 8 distinct classes according to their domain structure, of which five (classes 3 to 7) are present in mammals[5]. Among mammalian semaphorins, classes 4-6 are type I transmembrane proteins, class 7 is GPI-anchored, and class 3 is secreted. Each semaphorin binds to and signals through specific receptors belonging to the plexin family transmembrane proteins ('sensors')[1-4]. At the intracellular side, plexins have a split GTPase-activating protein (GAP)-homology domain intervened by a Rho-binding domain. It is believed that ligand-activated plexin GAP domain deactivates R-Ras, leading to the down-modulation of integrin-mediated cell adhesion. The mechanistic linkage between the extracellular semaphorin binding and the cytoplasmic GAP activation, however, remains unclear. Class 6 semaphorins (Sema6s) comprise of 4 members (A-D), and are regarded as "prototype" semaphorins, because they show the greatest structural similarity to invertebrate semaphorins and directly bind to type A plexins as is the case for invertebrate semaphorins. Signal transduction by Sema6s-plexin A system has been implicated in the control of neuronal migrations, cardiogenesis, navigation of neural crest cells, bone homeostasis, and immune responses. Furthermore, Sema6s and their receptors have been implicated in the pathology of various kinds of human diseases including autoimmunity, osteopetorosis, inflammation, and neurodegenerative diseases.

Therefore, the structural information about the Sema6-plexin A interaction as well as the elucidation of the signalling mechanism would not only set the basis for the mechanistic understanding of semaphorin-plexin systems utilized in various biological contexts, but also contribute to define the potential therapeutic targets for human diseases.

In the present study, we report the crystal structures of Sema6A receptor-binding fragment and plexin A2 ligand-binding fragment, in their pre-signalling state as well as in the signalling state (i.e., after complex formation). The structures provide a detailed picture of the interaction between the two proteins, as well as a possible signalling mechanism involving a "partner switch" at cell surface.

Crystal structures of Sema6A and PlxnA2 and their oligomeric state in solution

Structures of Sema6A and PlxnA2 were determined at 2.5Å and 2.1Å resolution, respectively (Figs. 1a and b). In both proteins, the sema domain displays a seven-bladed β-propeller fold very similar to previously determined structures of Sema3A, Sema4D and Met. In the Sema6A crystal, monomers make contact with one another using the upper rim of the β-propeller, thereby assuming a 'face-to-face' dimer configuration (Fig. 1a). This dimeric configuration is essentially identical to that seen in the crystal structures of the Sema3A and Sema4D sema domains. Surprisingly, PlxnA2 also assumes a dimeric configuration in the crystal, albeit with a markedly different mode compared to that observed in the semaphorin sema domains (Fig. 1b). The two PlxnA2 fragments in the asymmetric unit are related by a non-crystallographic two-fold axis and interact with each other by using a flat surface located at the side of the β-propeller, exhibiting a 'head-on' configuration twisted orthogonally, in contrast to the face-to-face configuration observed in the known semaphorin structures.

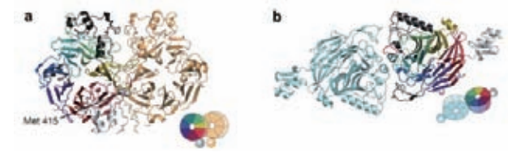

Fig.1 Crystal structure of Sema6A and PlxnA2 ectodomain fragments in pre-signalling states. (a) Structures of the Sema6A face-to-face homodimer and (b) PlxnA2 head-on dimer. Individual propeller blades are coloured differently in one monomer. Arrangement of the toroidal propeller domains within the structure is schematically depicted in the cartoon next to each ribbon presentation.

Analytical ultracentrifugation sedimentation velocity experiments performed on the Sema6A protein confirmed that it does indeed form a dimer in solution with a dissociation constant (Kd) value of 3.5μM (Fig. 2). The dimerization affinity for PlxnA2, however, was extremely low (Kd>300 μM) and could not be definitively determined.

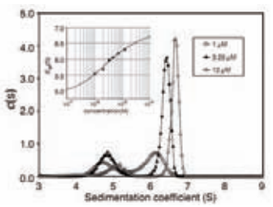

Fig.2 C(s) distribution from sedimentation velocity analytical ultracentrifugation experiments performed at three different concentrations of Sema6A. The concentration-dependent change observed in the sedimentation coefficient distribution confirms the presence of a monomer-dimer equilibrium. The weight-average sedimentation coefficients were plotted against protein concentration, yielding a Kd value of 3.5 μM (inset),