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### Structural basis of the interaction between integrin β4 and plectin at the hemidesmosomes

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Hemidesmosomes are adhesive structures that mediate stable anchoring of epithelial cells to the basement membrane by connecting the extracellular matrix to the intermediate filament cytoskeleton. The integrin α6β4 (a laminin receptor) and the cytoskeletal protein plectin are key components of hemidesmosomes. The binding of the cytoplasmic moiety of integrin beta4 subunit to plectin is essential for the stability of hemidesmosomes. We have crystallized the integrin β4-plectin primary complex, form by the first pair of fibronectin type III (FnIII) domains of integrin β4 and the actin binding domain (ABD) of plectin. The structure has been solved by using molecular replacement methods and has been refined against data to 2.75 Å resolution. Most of the contacts occur between the second FnIII domain of β4 and the first calponin homology (CH) domain of the plectin ABD. The integrin β4 binding interface includes Arg1225 and Arg1281, two residues that are mutated in non-lethal forms of epidermolisis bullosa (a skin blistering disorder), linking the fragility of epithelia in these patients to the disruption of the integrin β4-plectin interface. Additionally, we have solved the crystal structure of the aforementioned region of integrin β4 in a free form. Comparison of the free and plectin-bound structures reveals a conformational change of the segment downstream the second FnIII sequence upon binding to plectin. In summary, the structure of the integrin β4-plectin primary complex provides the first detailed insight into the macromolecular interactions responsible of hemidesmosome assembly. Our data paves the way to a better understanding of hemidesmosome disassembly during processes such as wound healing and carcinoma invasion.

Keywords: cell adhesion, protein-protein interactions, conformational change

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### X-ray structural studies of Salmonella FlgA, a periplasmic chaperone for flagellar P-ring assembly

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The bacterial flagellum is a macromolecular complex composed of the filament, the hook and the basal body. The basal body is divided into three sub-structures, the LP-ring, the MS-ring and the rod. FlgH and FlgI are the subunit proteins of the LP-ring and FlgA is involved in the P-ring assembly. In the absence of FlgA, basal bodies lacking LP-ring are assembled. Three proteins, FlgH, FlgI, and FlgA, have signal sequences in their N-termini for secretion into the periplasm. They are secreted not by the flagellar specific type III secretion but by the Sec protein translocation machinery. FlgA might associate with FlgI after secretion into the periplasm and act as a modulator protein for the P-ring assembly. Structural information is required to understand how FlgA works. We have cloned the full-length flgA gene from Salmonella typhimurium and overproduced it in E.coli. The protein was isolated from the periplasmic fraction. Initial crystallization screening was performed by the sitting-drop vapor diffusion method with commercially available screening kits. Clusters of thin needle-shaped crystals were grown at 289K. We optimized the crystallization condition by varying the pH of the crystallization buffer and the concentration of the precipitant. We finally succeeded to obtain the rod-like crystals with approximate dimensions of 0.05 × 0.05 × 0.5 mm by the hanging-drop vapor diffusion method within a week. Heavy-atom derivative crystals were prepared by soaking method. All diffraction data were collected at BL41XU SPring-8. Crystals were picked up with nylon loops and flash-cooled in the liquid nitrogen. Native FlgA crystals diffracted to 2.0 angstrom resolution. MAD data collection of a Pt-derivative crystal was also successful. Data processing is under way.

Keywords: bacterial chemotaxis, molecular chaperones, macromolecular assemblies

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### Structure determination of human cytokines and extracellular domains of receptors

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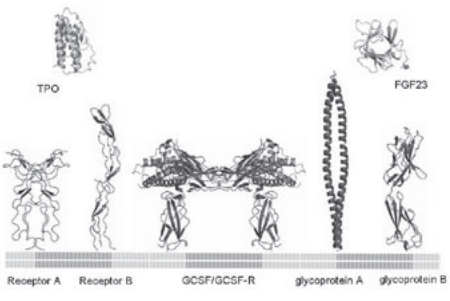
The interaction between cytokines and their receptors regulates diverse biological processes controlling growth, development, homeostasis, and immune function. The ligand-receptor interaction in the extracellular environment is essential for signal transduction. We have focused on the structure/function relationships of these proteins. Granulocyte colony-stimulating factor (GCSF) has become an important cytokine for medical treatment of patients suffering from granulopoenia through regulating the maturation, proliferation, and differentiation of the precursor cells of neutrophilic granulocytes. We determined a crystal structure of the signaling complex between human GCSF and a ligand binding region of GCSF receptor (GCSF-R). This signaling structure is consistent with thermodynamic and mutational analyses, and elucidated that the previously reported GCSF/GCSF-R complex structure had an artificial conformation caused by crystal packing. We also determined additinal structures of human cytokines and extracellular regions of cytokine receptors, where the complexation with an antibody fragment (Fab) was used for crystallization and structure determination.




Keywords: antibodies, cytokines, receptors

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