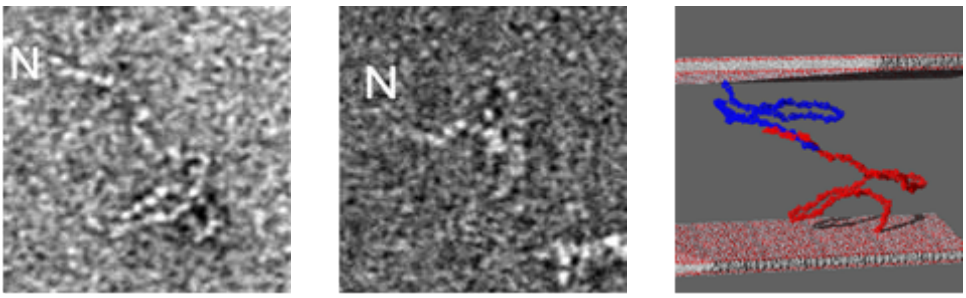
RIKEN Center for Developmental Biology (CDB)
2-2-3 Minatojima minamimachi, Chuo-ku, Kobe 650-0047, Japan

Strategy of bending by giant cadherins

February 5, 2015– The cadherin superfamily of transmembrane proteins play an important role in mediating cell-cell adhesion, which is fundamental for maintaining organization of multicellular organisms. They require calcium ions (Ca^{2+}) to function, and also regulate many developmental processes such as tissue and neural circuit formation through their intercellular interactions. Cell-cell adhesion is achieved through homophilic or heterophilic binding between cadherins of adjacent cells through their filamentous extracellular domains (ectodomains). The so-called "classical" cadherins have ectodomains consisting of five repetitive units called, extracellular cadherin (EC) domains, which are joined together with a segment containing a Ca^{2+}-binding motif (CBM), an amino acid sequence that can bind Ca^{2+}. When Ca^{2+} binds to the CBM, it activates the cadherin by causing the ectodomain to elongate into a filamentous morphology and interacts with its partner molecule in cell adhesion. But when Ca^{2+} is depleted, the ectodomain loses its elongated morphology, and consequently, cell-cell adhesion is also lost. The ectodomains of "non-classical" cadherins such as protocadherins, Fat, and Dachsous have varying numbers of EC domains, ranging from five up to 34. However, these larger cadherins have also been found to occupy intracellular spaces with spans smaller than the linear length of its ectodomain. Thus, this raises the question of how larger cadherin molecules are able to fit into relatively narrow intercellular spaces.

Now, a new study by research scientist Yoshikazu Tsukasaki of the Laboratory for Cell Adhesion and Tissue Patterning (Masatoshi Takeichi, Team Leader) and other colleagues demonstrates a strategy of bending adopted by the giant cadherins, Fat and Dachsous, which allows them to squeeze into narrow intercellular spaces despite their large size. Published in the *Proceedings of the National Academy of Sciences (PNAS)*, their findings indicate that some segments of the large ectodomain of Fat and Dachsous have been modified to prevent Ca^{2+}-binding, and facilitate the folding of these domains to assume a more compact form.



TEM images of Fat4 (left) and Dachsous1 (middle). In both molecules, the N-terminal end had an elongated configuration, while the C-terminal end had a bent configuration. The right panel shows a simulated 3D structure of Fat4 (red) and Dachsous1 obtained from the elastic network model.

The mammalian Fat4 and Dachsous1 form a heterophilic binding that regulates planar cell polarity and proliferation. Fat4 is the largest molecule within the cadherin superfamily with 34 EC domains, and Dachsous1 is also large with 27 EC domains. Tsukasaki et al. first examined purified ectodomain structures of both cadherins under transmission electron microscopy (TEM), and found that while the N-terminal end of ectodomains, the region furthest from the cell membrane, displayed a relatively linear morphology, the C-terminal end which is close to the membrane had multiple locations where the filament was sharply bent. When the amino acid sequences of the CBMs were analyzed, they found modifications of amino acid residues in several of the CBMs near the C-terminal end, suggesting the possibility that these modifications prevent Ca^{2+}-binding.

The group generated mutants of the classical cadherin, E-cadherin, by replacing one of its CBMs with a modified CBM from Fat4. TEM revealed that these E-cadherin mutants displayed a bent arrangement in the location where the CBM was switched. Gel filtration experiments measuring elution speed showed that both wildtype E-cadherin run in Ca^{2+}-free conditions and the CBM-replaced E-cadherin mutants moved slower than the wildtype E-cadherin run in presence of Ca^{2+}; these results supported their hypothesis that the modified CBM found in Fat and Dachsous is unable to bind Ca^{2+}, thus leading to a nonlinear ectodomain.

To further understand the morphological changes that occur in CBM-replaced E-cadherin, the group carried out computer simulations using the elastic network model based on the known X-ray crystal structure of E-cadherin. The simulations showed that, in CBM-replaced E-cadherin, the segments with modified CBM exhibited a wide range of bent configurations, consistent with TEM observations. They applied the same method for Fat4 and Dachsous1, and successfully created atomic models that matched morphologies seen under TEM. In a separate series of experiments, they also found that the four most N-terminal ectodomains appeared to be sufficient for Ca^{2+}-mediated heterophilic binding between Fat4 and Dachsous1, and that this binding is unaffected even when the C-terminal end is bent.

Tsukasaki et al. next analyzed the morphology of Fat4 and Dachsous1 in vivo, by transfecting a cell line with Fat4 or Dachsous1 cDNAs. When these separately transfected cells were co-cultured, they observed the formation of Fat4-Dachsous1 bindings at the cell boundaries between the two cell types just above the tight junctions. Closer analysis with TEM revealed that the intercellular spaces in which the Fat4-Dachsous1 binding complexes were found were approximately 47 nm wide, matching the dimensions of the molecules calculated from the elastic network model. Observations of Fat4-Dachsous1 binding in the mouse embryonic cortex also showed the span between cell boundaries were similar to those observed in the co-culture of transfected cells.

“It is a mystery as to why giant cadherins have gone as far as losing their Ca^{2+}-binding ability to squeeze into intracellular spaces by folding,” says Takeichi. “What is interesting is that the size of Fat and Dachsous, and the positions of modified CBMs within the ectodomain have been conserved across species. No doubt the bending strategy revealed in our study is closely linked to the physiological function of these molecules.”