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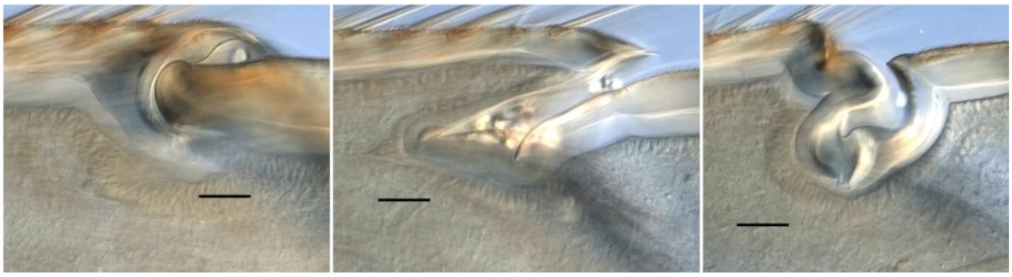
Triple-jointed: Notch signaling a key to insect joint diversity

March 1, 2012 – Insects show a remarkable variety of joint structures in their limbs, which despite their tiny size and simplicity and the rigid, external nature of their skeletons, rival our own in diversity of function. The basis for this articular range, however, remains something of a mystery.

Now, Reiko Tajiri and others in the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Group Director) have shown that, diversity of appearance notwithstanding, insect limb joint structures can be classed into one of three general forms, and that the developmental decisions that guide their morphogenesis are under the control of Notch signaling. Published in *Development*, this work goes a long way to building a framework for understanding how insect joints are formed.



Ball-and-socket, uniform and side-by-side joint types in insect limb (scale bar = 10 µm)

This new study built in previous work from the Hayashi lab, which showed that cell differentiation and movement are key processes in the sculpting of a specific type of joint – the ball-and-socket form – in the adult *Drosophila* leg, known as the tarsus. Taking this as a starting point, Tajiri began comparing tarsal joint morphologies in other insect species to gain a clearer understanding of the extent to which this model is conserved. The ball-and-socket type joint is not found in the tarsal limbs of primitive insects (Apterygota, Paleoptera), in which the joint cavity is covered entirely by cuticle with no underlying ball-socket distinction, which suggests that the cell differentiation needed to form this form of joint does not take place.

In other joints in the tarsus of several primitive species, however, the joint is formed by two cuticular components positioned side-by-side, without the encirclement seen in a true ball-and-socket joint. In other, more highly diverged insect lineages, such as Polyneoptera, Paraneoptera and Holometabola, ball-and socket-joints are common, in contrast to their basal relations. From these observations, the group realized that only three main morphologies account for all tarsal limb joint forms in insects: uniform, side-by-side, and ball-and-socket.

These findings additionally suggested that the formation of the most complex ball-and-socket joint relies generally on the two essential modular processes of cell differentiation and cell movement, and that gains or losses in either of these results in the failure of ball-and-socket formation. The evident diversity of joint forms in insect limbs presumably has evolutionary significance; each joint type may confer advantages under specific environmental conditions, and all may have evolved multiple times independently. But what Tajiri wished to know was, how might such diversity arise at the genetic level?

Previous reports have demonstrated the importance of the Notch signaling pathway in *Drosophila* limb development, so the group conditionally knocked down Notch at various stages in pupal development to watch for possible changes in joint structure. By altering the absence, presence and timing of Notch expression using a temperature-sensitive mutant, Tajiri was able to shift ordinarily ball-and-socket to take on the simpler uniform conformation, or a range of incomplete variations on its usual form. Labeling of a subset of cells in the developing joint showed that Notch knockdown affected cell motility, resulting in the failure of the cell movement step of joint formation.

But what of differentiation? Using a constitutively active form of its gene and an RNAi construct to interfere with its expression, Tajiri tested the role of differing levels of Notch expression role in determining the fate of cuticle cells. The model that developed was clear: higher levels of Notch signaling drive “ball” differentiation, while lower levels promote “socket.”

“What was fascinating about this study was that it showed that Notch signaling is not simply a switch driving differentiation, but a subtle regulator of the balance between differentiation and cell movement,” says Tajiri, who has since moved to the University of Tokyo. “It will be interesting to find out what specific factors Notch interacts with at each step in joint morphogenesis, which may lead to a better understanding of the links between development and evolution in this system.”