Given the stark contrast in lifestyle and habitat between Anelasma and its closest living relatives, this suggests there is a gap of currently unknown and extinct species that might be transitional forms between Anelasma and other stalked barnacles. Furthermore, Rees et al.’s analysis [5] points to an origin for Anelasma’s lineage that dates back to 120 million years ago in the Cretaceous period. They suggested that Anelasma may actually be a remnant species from a clade that was far more speciose in the past. Though Anelasma is only found on a few species of deep-water sharks, it might have once been part of a more diverse group of parasitic stalked barnacles that infected a wider range of marine animals.

But what of the coronuloids and other barnacles that live as epibionts? Given the lineage that led to Anelasma had successfully evolved to be parasitic from an ancestor which was most likely a rock-clinging filter-feeder, why have none of the coronuloids evolved to be parasitic since some species are already deeply embedded in the body of various animals? The results from Rees et al.’s study provide us with an additional perspective on what it takes for an organism to evolve from a free-living to a parasite lifestyle. It also raises more questions about why certain groups (such as the coronuloid barnacles) have not evolved to be parasitic, even though they seem to be in a prime position to do so. The discovery that Anelasma’s closest living relatives are intestinal rather than epibiont barnacles also reminds us that the most likely or plausible evolutionary scenarios we can come up with may not necessarily correspond with what actually happened. The evolutionary history of any organism is convoluted and complex, and does not always conform to our own expectations.

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Sensory Biology: It Takes Piezo2 to Tango

A trio of papers has resolved an outstanding controversy regarding the function of Merkel cells and their afferent nerve fiber partners. Merkel cells sense mechanical stimuli (through Piezo2), fire action potentials, and are sufficient to activate downstream sensory neurons.

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Effective tactile communication is crucial for the exquisite beauty and breathtaking dynamics embodied in dance and depends on sensory neurons embedded in the skin that vary in their size, shape, and sensitivity [1–3]. Mammals, including humans, have skin domains enriched in Merkel cell–neurite complexes needed for the discrimination of fine textures [4]. Merkel’s description of specialized cells associated with nerve endings in the skin in 1875 launched more than a century of speculation and investigation about the nature of the dance they might perform together with their sensory endings. Until now, researchers have been unable to decipher whether the Merkel cell, its sensory afferent, or both were responsible for touch sensation. A trio of recent papers [5–7] exploit the discovery of the Piezo proteins [8] and provide unprecedented clarity: Merkel cells rely on Piezo2 to transduce mild skin indentation and whisker deflection into electrical signals. Confirming speculation regarding the potential for a synapse-like connection between Merkel cells and their afferents, optical stimulation of Merkel cells engineered to express light-gated cation channels is sufficient to activate downstream sensory neurons [6].

Nestled at the inside border of both glabrous and hairy skin (Figure 1), Merkel cells aggregate in touch domes and are closely apposed to myelinated sensory nerves. In rodents, Merkel cells additionally cluster around guard hairs of the pelage and sinus hairs (i.e. vibrissae, whiskers) in facial skin. Though the cells and their nerves have been known since the 1880s, it was not clear that Merkel cells and their nerves were separate cells until the invention of the electron microscope (reviewed in [9]). Iggo and Muir [10] established that tactile stimulation elicits action potentials (spikes) from nerves associated with Merkel cells and classified such nerve fibers as slowly adapting type I (SAI) Aβ mechanosensory afferents according to their slow conduction velocity (Aβ fibers) and the observation that spike frequency adapted slowly during touch stimulation.

While this work established that the Merkel cell–Aβ afferent complex detects touch, it remained unclear which of the cell partners leads the dance. Over the years, evidence accumulated to both support and refute the idea that Merkel cells are like hair cells in the inner ear — non-neuronal cells that detect mechanical stimuli and signal to neurons. The alternative idea that tactile stimuli are sensed solely by the
afferents has also received experimental support. Finally, Merkel cells have also been proposed to provide critical trophic support for afferent outgrowth and maintenance (reviewed in [9]), but, since sensory afferents appear normal in Atoh1-deficient mice, which lack Merkel cells [6], this function appears unlikely. This issue has remained unresolved for a variety of reasons, including a lack of techniques for investigating the mechanosensitivity of Merkel cells in vitro and in situ. The new studies now report strategies to overcome these barriers, including Merkel cell purification [5,6] and in situ mechanical stimulation [7] paired with optical and electrophysiological techniques for measuring Merkel cell responses.

Gentle indentation of Merkel cells (in culture or when part of touch domes) elicits inward currents that depolarize the cell, activate voltage-gated calcium channels and trigger calcium-dependent action potentials [5–7]. Such mechanoelectrical transduction currents are carried by cations and are consistent with those reported for Piezo2 in heterologous expression systems. They feature fast activation (~1 ms), fast inactivation (6–8 ms), and inhibition by gadolinium ions and ruthenium red [6,7]. Such mechanoelectrical transduction currents are present in Merkel cells, but not other skin cells such as keratinocytes [7]. To assess whether Piezo2 is required for mechanoelectrical transduction in Merkel cells, the three groups deployed different but equally powerful strategies. Of paramount importance, all detected enriched Piezo2 expression in Merkel cells. Conditional knockout of the Piezo2 gene in Merkel cells [6] or reducing its expression or knockout of the Piezo2 gene in Merkel cells [6,7] abolished mechanoelectrical transduction currents in Merkel cells. Through these creative approaches to an outstanding problem, it is now clear that Merkel cells function as mechanoreceptors and that their ability to perform this function depends on the expression of Piezo2.

The conserved Piezo proteins are colossal, 500 kDa proteins thought to assemble into gigantic, tetrameric, mechanosensitive ion channels [11]. In addition to Merkel cells, Piezo2 is expressed in a subset of somatosensory neurons [8]. The similarity between mechanically-activated, Piezo2-dependent currents in heterologous cells [8] and those now reported in Merkel cells in vitro [5,6] or in situ [7] implies that Piezo2 forms mechanoelectrical transduction channels in Merkel cells. The current findings do not exclude the possibility that Piezo2 may require other protein partners. Little is currently known about how Piezo2-dependent channels are activated during indentation of a cell’s membrane, but the invention of a deformable micropillar substrate for cell culture reveals that such channels may be activated by nanometer-scale, local displacement [12]. It is also possible that Piezo2 senses changes in membrane tension, as found for the MscS and MscL channels of bacteria [13] and mammalian TRAAK and TREK1 K+ channels [14]. Direct answers to the question of how Piezo2-dependent channels are gated must wait for further study.

Having established that Merkel cells are bona fide mechanoreceptors, all three groups addressed the unresolved question of whether or not the afferent partners also functions as a mechanoreceptor. If Merkel cells are the sole mechanoreceptor, then their activation should be both sufficient and necessary to evoke afferent responses to tactile stimuli, and activating Merkel cells should be sufficient to evoke action potentials in afferents. Using an optogenetic approach, Maksimovic et al. [6] show unequivocally that Merkel cells are sufficient to generate or inhibit action potentials in SAI afferents. Based on their finding that calcium channel blockers decrease SAI activation in whiskers, Ikeda et al. [7] favor a model in which Merkel cells, but not their afferents, have the ability to perform mechanotransduction. Evidence against this single-site sensor model comes from mice lacking Merkel cells or expressing Merkel cells that lack Piezo2, however. In both cases, Aβ afferents retained the ability to detect and respond to the initial dynamic phase of mechanical stimulation [5,6]. This unexpected discovery inspired Lumpkin, Patapoutian and their co-authors to propose a model in which Merkel cells and nerve endings act together to create a slowly adapting response to skin indentation [5,6]. In this dual-site sensor model, dynamic changes in mechanical loads activate afferents, and Merkel cells confer longer-lived responses during static indentation. Together, they create a slowly adapting response to

Figure 1. Two dance partners needed to sense touch. Merkel cell–neurite complexes in vibrissae or facial whiskers (A) and in touch domes (B). Touch domes are present in both glabrous and hairy skin; for simplicity, only glabrous skin, such as that found on the rodent foot pad, is illustrated.
stimuli to generate touch sensation [1–3], making it terribly challenging to isolate the contribution of Merkel cell–afferent complexes to perception and behavior. Perhaps as a result of this complexity, the present studies focused on comparatively simple nociceptive and withdrawal reflexes. Investigating Merkel cell–Aβ afferent complexes in the hindpaw skin and stimulating the plantar surface of the hindpaw, Woo et al. [5] found that Piezo2 conditional knockout mice displayed withdrawal reflexes less frequently than control littermates at lower, but not higher force and that the conditional knockout mouse population contained more non-responders than the wild-type mouse population. Thus, Piezo2 in Merkel cells is essential for the detection of innocuous mechanical stimuli that cause withdrawal reflexes and may generate touch sensation in normal conditions.

By contrast, Ikeda et al. [7] investigated the function of rat Merkel cell–afferent complexes following capsaicin-induced sensitization of the face. Following capsaicin injection, normally innocuous mechanical stimuli are perceived as painful (allodynia) and cause nociceptive behaviors. In the present case, deflecting a whisker triggered biting, grabbing or avoidance of the filament following capsaicin injection. These nociceptive behaviors were drastically diminished by shRNA-mediated Piezo2 knockdown delivered to the whisker’s follicle [7]. This behavioral analysis reveals that Merkel cell–afferent complexes could contribute to the tactile allodynia that accompanies numerous inflammatory and neuropathic chronic pain conditions [15] and suggests that Piezo2 in Merkel cells could be a valuable target for the development of novel analgesics. These studies also suggest that it will be possible to probe the specific function of Merkel cell–afferent complexes in other rodent behavioral assays, such as those that evaluate texture discrimination [16], by silencing intact Merkel cells through Piezo2 conditional knockout or knockdown.

The Gu, Lumpkin, and Patapoutian groups overcame many obstacles to solve a century-long controversy regarding the function of Merkel cells and their afferent partners by combining genetic tools, challenging dissections, cell-type specific purification, and bold electrophysiological recordings. It is now clear that Merkel cells (in vitro and in situ) transduce mechanical stimuli into depolarization, generate action potentials, and signal to their closely apposed afferent neurons. Furthermore, they determined that Piezo2 is essential in the first step of mechanoelectrical transduction in Merkel cells and that the action potential firing relies on voltage-dependent calcium channels. These studies represent a giant paso in our understanding on how we feel and discriminate objects and raise several important questions. Does Piezo2 act by itself or cooperate with other partners in Merkel cells to sense mechanical stimuli? Is Piezo2 expressed in SAI Aβ afferents? What other molecules might confer mechanosensitivity to these fibers? What governs the rapid activation and adaptation of Piezo2-dependent mechanoelectrical transduction currents? How do Merkel cells communicate with the SAI afferents? Answering all these questions is not going to be easy and it will certainly take more than two to tango.

**Figure 2.** Two-site mechanosensor model in which both Merkel cells and the closely apposed Aβ sensory afferent function as mechanosensors.

Through the work of Gu, Lumpkin, Patapoutian and their research teams [5–7], we have learned that Merkel cells depend on the Piezo2 non-selective cation channel to convert mechanical stimuli into electrical signals.

Mechanical stimulation (Figure 2). Additional studies are needed to clarify whether all Merkel cell complexes follow either the single-site or dual-site sensor model or whether Merkel cell–neurite complexes are heterogeneous in this regard. These findings also suggest that the Aβ afferents innervating Merkel cells might express Piezo2 and rely on this protein to function as a second-site mechanosensor.

Merkel cell–afferent complexes respond to skin indentations with high spatial resolution, suggesting that they are particularly important for encoding objects’ texture and shape [3]. However, many other mechanosensory organs and afferents can be coincidentally activated by mechanical

**References**

A new study has identified a neural circuit that is responsible for increasing sleep in young fruit flies. Reduced dopamine signaling to the fan-shaped body during early life promotes sleep and is critical for proper brain development.

Kazuma Murakami and Alex C. Keene

When it comes to sleep, the needs of children and adults differ dramatically. Many animals sleep more in early life and a number of factors suggest this sleep is critical for proper brain development [1,2]. In a recent study published in Science, Kayser et al. [3] examine the neural and functional basis for enhanced sleep during early life. The authors demonstrate that reduced activity in a small population of wake-promoting dopamine neurons increases sleep of young flies, and that this early life sleep enhancement is critical for proper brain development.

While significant progress has been made towards understanding how sleep is regulated, the neural basis for interactions between sleep and brain development is less well understood. Sleep affects broad aspects of physiology, immunity and behavior, and sleep loss disrupts synaptic plasticity and memory in both flies and mammals. Animals sleep more during early life when the brain is developing, suggesting that enhanced sleep in young animals may be essential for proper brain development [1,2].

In Drosophila, as in mammals, sleep is regulated by neural networks that include sleep- and wake-promoting neurons. Dopamine is a key modulator of arousal, and both genetic and pharmacological manipulations of dopamine function support its role as a conserved wake-promoting transmitter [4,5]. In this new study, the authors find that dopamine levels are reduced in one day old flies, raising the possibility that a reduction in dopamine signaling underlies the early life increase in sleep [3].

Dopamine is expressed in only ~200 neurons in the fly brain, and these control diverse functions including memory, sleep, and courtship [6,7]. Subsets of arousal-promoting dopamine neurons target the dorsal Fan Shaped Body (dFSB), a brain region which expresses the dDA1 dopamine receptor [8,9]. Early life sleep deprivation causes memory defects that are rescued by blocking dDA1 receptor function, suggesting that dopamine signaling is particularly important for interactions between sleep and development [10]. Kayser et al. find that enhancing dopamine signaling through either genetic means or activation of dopamine neurons more potently suppresses sleep in one-day-old flies than older counterparts. The authors manipulated distinct classes of dopamine neurons to localize the relevant population of neurons underlying developmentally related changes in sleep. Selective activation of the wake-promoting dopamine neurons that project onto the dorsal dFSB prevents the increased sleep observed in one day old flies, suggesting that reduced dopamine release from dFSB-innervating dopamine neurons underlies the elevated sleep observed in young flies. A large genetic toolkit is available for analysis of neural function in Drosophila and three independent indicators of neural activity suggest the activity of the dFSB-innervating dopamine neurons is lower in one day old flies compared to 8–10 day old counterparts. Both Cre–Luciferase, an indicator of CREB activity, and the Ca2+-indicator CALexA (Ca2+-dependent nuclear import of LexA) revealed reduced activity in the dopamine neurons that target the dFSB. Furthermore, the authors use the DopR-Tango system to directly measure dopamine activity in postsynaptic neurons of the dFSB. DopR tango, which uses a stable fluorescent reporter as a readout for dopamine signaling, confirmed reduced dopamine signaling in the dFSB of one day old flies [3,11]. Therefore, both pre- and postsynaptic analysis of dopamine neuron function indicates that dopamine release from the wake-promoting neurons that target the dFSB is reduced in young animals.

The dFSB promotes sleep in Drosophila and the authors sought to manipulate neural function of this region to functionally validate its role in early life sleep enhancement [12]. Genetic activation experiments suggest that the dFSB, but not other sleep-promoting regions, are already near maximal activity levels in one day old flies, fortifying the notion that this brain region is less inhibited by dopamine neurons early in life. Indeed, detection of Ca2+ levels with CALexA confirmed enhanced activity in the dFSB in young flies [3]. Therefore, these findings provide physiological and behavioral evidence for a neural circuit where activity of dFSB-innervating dopamine neurons is reduced in one day old flies, enhancing activity of the dFSB and promoting sleep.

What is the function of enhanced sleep in young animals? In mammals, sleep during early life is thought to be