to track the movements of the body wall, and on the inside, the trachea provide clear landmarks to track movements of the viscera. This allowed Simon *et al.* [8] to combine X-ray images with conventional cameras to examine the internal and external motions of crawling caterpillars.

Unlike worms, caterpillars crawl by using a series of stubby, non-articulated structures attached to each body-wall segment. Each stubby structure is called a 'proleg' (Figure 1A). In a wave of activity that starts at the tail and moves to the head, each proleg is first swung forward until it contacts the ground, and then the ground contact is used to propel the animal forward. You might assume that each section of viscera would move synchronously with the body wall that surrounds it. Surprisingly, however, this is not the case, as X-ray images showed that near the abdominal prolegs, the viscera move out of phase with the body wall. While the abdominal prolegs are in stance, the viscera move through the body wall like a piston, and then, after stance, the abdominal prolegs swing forward and slide back along the viscera (Figure 1B) [8].

Motions of the viscera are ubiquitous in many animals, but are generally associated with digestion, not locomotion. In caterpillars, however, visceral motion is a critical part of the step cycle. Thus, analysis of the internal and external body motions of the caterpillar has demonstrated a new kind of legged locomotion, one in which the body wall and viscera are acting as two separate components, a container and the contained. With each step, the contained first slides like a piston within the container, and then the container slides forward along the contained. The consequence of this type of locomotion is that the mechanical properties of the viscera are as important to locomotion as the mechanical properties of the body wall.

It is still not known, however, exactly what advantage this new form of locomotory pistoning locomotion gives the caterpillar. Simon *et al.* [8] suggest that it may minimize impacts to the viscera, facilitate different kinds of locomotion, add stability, aid respiration, or allow better digestion. Even though the advantages of visceral-body wall pistoning are unclear, this work used a novel imaging technique to show a new kind of legged locomotion, i.e. locomotion that both involves pistoning movements of the legs and the viscera. Napoleon famously said "An army marches on its stomach"; Simon *et al.* [8] have shown that an army of caterpillars marches (partially) with their stomachs.

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Cell–Matrix Adhesion: Slip and Immobilization under Force

When force is applied to cell–matrix adhesion complexes, they respond by growing larger and stronger. It emerges that strengthening involves transient motion of the transmembrane integrin receptors and their eventual immobilization to the extracellular matrix.

Alexander B. Verkhovsky

Focal adhesions are discrete sites at the cell periphery where the actin cytoskeleton connects to the extracellular matrix (ECM) through transmembrane integrin receptors and numerous specialized proteins that link actin filaments to the intracellular domains of integrins [1,2]. Focal adhesions transmit forces from the actin cytoskeleton to the substrate, allowing the cell to migrate and remodel the ECM. At the same time, focal adhesions are the sites where the cell probes the environment and generates signals that control important aspects of its behavior, such as migration and proliferation. The composition, assembly and regulation of focal adhesions have been studied intensely for several decades, but the biophysical properties of the mechanism of force transmission have only recently become accessible for experimental quantitative studies. This is thanks largely to the development of high-resolution traction-force microscopy — a method capable of resolving forces applied to the ECM at the level of a single focal adhesion [3]. The study of Aratyn-Schaus and Gardel [4], published in a recent issue of *Current Biology*, combines traction force and fluorescence confocal imaging to provide an informative and esthetic visual account of how focal adhesions grow and remodel under applied force. The authors discover a previously unidentified phase in the life history of focal adhesions: a frictional slip of the integrin molecules before they become affixed to the ECM.

Focal adhesions have unusual physical properties. The most curious and counter-intuitive feature is probably their ability to grow and strengthen in response to a force. Focal adhesions appear at the cell periphery as small isotropic clusters

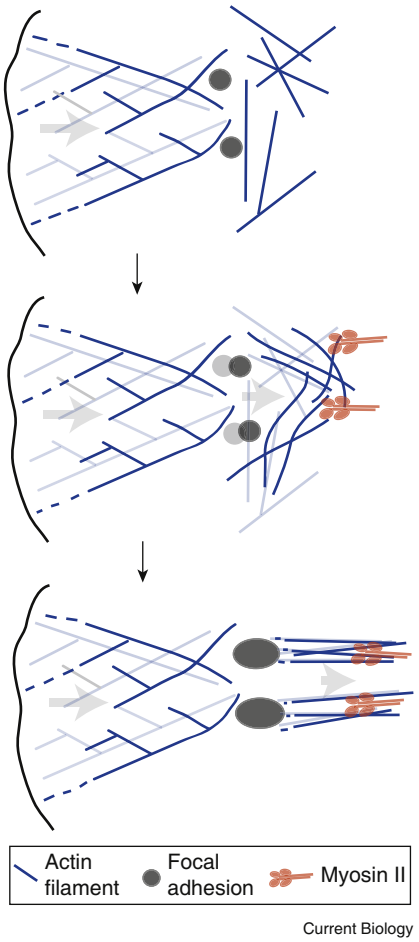

Figure 1. Schematic representation of dynamics of focal adhesions and the actin network during adhesion maturation.

Top: nascent adhesions (grey circles) arrest the fast flow of the lamellipodial actin (blue lines) network and form the boundary between the lamellipodium (left) and the lamellum (right). Middle: tension generated by myosin II (red figures) pulls actin network in the lamellum and produces frictional slip of nascent adhesions. Bottom: adhesions immobilize and grow under myosin-generated tension. Transparent color represents the state at a previous time point, while dashed lines show newly assembled actin filaments.

of integrins and other specific proteins, such as vinculin, talin, paxillin, and focal adhesion kinase. Over time, they grow, become elongated in the direction of applied tension, acquire additional components and become associated with the tips of contractile bundles of actin filaments and myosin II motor proteins. This process of growth and polarization has been termed maturation and is dependent on force, which is either developed by the cell through actomyosin contraction or applied externally [5]. In an apparent paradox, force that may be expected to tear focal adhesions apart instead makes them stronger. It was speculated that force induces a conformational change or reorganization of adhesion components that may enhance their assembly, either directly or through signaling cascades [6,7]. However, the exact nature of the force-dependent events remains unclear.

Another intriguing feature of focal adhesions is that they transmit forces between cellular components that are not permanently linked, but instead move with respect to each other. The actin network in the cell is engaged in a continuous retrograde flow from the periphery to the center, powered by actin assembly against the membrane and actomyosin contraction in the middle of the cell [8,9]. In contrast, the ECM and the focal adhesion sites are mostly stationary. It was proposed that adhesion works like a clutch, fixing the actin network to the substrate and converting network flow into cell migration [10,11]. However, adhesion to the ECM does not arrest the actin flow completely. Focal adhesions are localized at the transition between two zones of the actin network: the peripheral lamellipodium, which exhibits a fast flow of branched actin network, and a more central lamellum, which contains contractile actomyosin bundles and displays slower actin flow than in the lamellipodium. Recent studies [12–14] indicated that nascent focal adhesions first appear within the lamellipodium, and it is their emergence that arrests the fast lamellipodial flow and triggers the transformation of the actin network into the lamellar type. The specific mechanism may involve a build-up of mechanical stress in the vicinity of the adhesions, which may cause breakdown and disassembly of the lamellipodium network [15]. At the same time, the slower actin flow that is characteristic of the lamellum persists at the focal adhesion sites, indicating that the connection between actin filaments and the ECM is not rigid, but rather allows for relative movement of the components.

How are the forces transmitted through such a labile connection? Movement of the components of the adhesions with respect to each other may involve their deformation and/or dissociation and re-formation of the bonds between them [16,17]. In the first scenario, the force would depend on the extent of deformation, while in the second on the velocity of the respective motion (analogous to viscous friction). Recent studies of ECM dynamics under tension from neuronal filopodia [16], as well as the analysis of the traction stress/actin velocity relationship in fibroblasts [18] and keratocytes [19], are consistent with the possibility that both mechanisms could participate in force transmission. The next question is where exactly the movement within the focal adhesion takes place. Within mature focal adhesions, transmembrane integrin molecules are stationary in relation to the ECM, while intracellular adhesion components move in the same direction as actin filaments, albeit at a slower speed [20]. Thus, formation of the focal adhesions reduces the velocity of actin flow, but the residual flow in turn engages intracellular adhesion components that move with respect to the integrins and the ECM.

What was notably missing from this picture of adhesion dynamics is what happens during adhesion maturation. Does the pattern of movement of the adhesion components and force transmission change? Is maturation just an increase in the number of adhesive bonds or does a specific type of bond become selectively stronger? Finally, what is the event that is induced by the applied force in the nascent adhesions that results in their maturation? The new work by Aratyn-Schaus and Gardel [4] addresses some of these questions.

The authors observed the dynamics of focal adhesions in a human osteosarcoma cell line cultured on a compliant polyacrylamide gel substrate and noted that nascent focal adhesions moved transiently towards the center of the cell before starting to enlarge and elongate. To investigate the biophysical parameters of this movement and to see whether it is a part of the maturation process, the authors developed an assay in which the maturation of focal adhesions was synchronized all over the cell. The cells were first treated with

blebbistatin, an inhibitor of actomyosin contractility, which rendered all adhesions small and isotropic, resembling nascent adhesions. Blebbistatin was then removed to restore contractility, and the adhesion dynamics were observed and analyzed with confocal and traction force microscopy.

It turned out that, upon restoration of actomyosin contractility, all adhesion components, including transmembrane integrin molecules, moved away from the cell edge for a few seconds and for a distance of a few tenths of a micrometer. The extent of movement depended on the compliance of the substrate, with greater movement on softer substrates, and no detectable movement on a rigid glass substrate. Several lines of evidence indicated that the movement represented a genuine slip of the integrin molecules with respect to the ECM. The extent of motion was always much larger than the deformation of the substrate measured in the same experiments, and also larger than could be accounted for by the deformation of the integrins or other adhesion molecules. Nor could it be explained by integrin assembly at one side of the adhesion and disassembly at the other side because fluorescence recovery after photobleaching (FRAP) experiments indicated that, during the movement, integrin molecules did not turn over but remained stably associated with the adhesion.

During the slip, the force exerted at the ECM steadily increased, while the velocity of the motion decreased until eventually integrins became stabilized with respect to the ECM. The relationship between slip velocity and stress at the adhesions was similar to the force/velocity relationship of myosin-driven contraction. This is consistent with the idea that myosin-generated force was driving the slip against increasing frictional resistance at the adhesion sites. While stress at the adhesions increased during the slip, the number of integrin molecules did not change significantly, indicating an increase in the force per integrin bond rather than in the number of bonds. Finally, the authors demonstrated that the slipping motion arrested at an approximately constant tension value, irrespective of the time and the extent of the slip.

Thus, sophisticated imaging by Aratyn-Schaus and Gardel [4] has identified a novel early event in adhesion maturation: a force-dependent strengthening of the integrin–ECM bonds. In the nascent adhesions, integrins are engaged with both the cytoskeleton and the ECM, as manifested by the arrest of fast actin flow (Figure 1, top), but the integrin–ECM bonds are not yet strong enough to withstand myosin-generated forces, resulting in the slip of focal adhesions with respect to the ECM (Figure 1, middle). During the slip, forces are transmitted to the ECM in a frictional manner, and tension builds up, eventually producing an as yet unidentified change in either integrin conformation or the clustering and stabilization of integrin–ECM bonds. Stabilization is triggered by tension, rather than by integrin motion *per se*, since adhesions also mature on the rigid substrate, where integrin motion is not detectable. Substrate rigidity controls the extent and timing of adhesion slip probably through the kinetics of tension build-up, which may be important for mechanosensitivity [6]. After immobilization of the integrins, the position of the slip interphase within the adhesion changes (slip occurs between integrins and the rest of adhesion components [20] rather than between integrins and the ECM) and focal adhesions continue to grow and build up tension (Figure 1, bottom).

The major challenge for future studies is to elucidate the exact mechanism of the stabilization of the integrin–ECM bonds. Even if Aratyn-Schaus and Gardel [4] do not answer this question, by identifying the key physical events involved in adhesion maturation, they significantly narrow the search field for the molecular mechanisms.

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