functions (tapers) before the Fourier transformations (**Fig. 1b**). The resulting smoothing of the signal reduces the impact of the irregularities that affect the densities of footprints at individual positions. It also tests for the consistency of the decomposed frequencies and estimates the statistical significance of the observed triplet periodicity. The authors demonstrated the general applicability of this approach by testing it on data obtained in different organisms. They confirmed the translation of a subset of their predicted sequences by detecting the presence of corresponding protein products with mass spectrometry[2].

We are aware that there is variability among the genomes of individuals from the same species. We are also familiar with transcriptome diversity among different tissues in higher eukaryotes. Therefore, the need to assemble genomes and transcriptomes *de novo* is well appreciated. It is usually assumed, however, that a single RNA molecule always produces the same protein products, and thus reference annotations of protein-coding regions are used to interpret translationally active regions in different samples obtained from the same organism. The observation that not only the levels of protein synthesis but also the sequences of produced proteins change in response to external stimuli is more recent[8]. Therefore, there is a clear need for *de novo* translatome annotation that is not biased by our previous assumptions and is immune to errors that may have already propagated across many sequence databases.

RiboTaper can be used for *de novo* translatome annotation even though it has a number of limitations. It is computationally expensive and is limited to the identification of only those coding regions where translation initiates at standard AUG codons. Also, it cannot identify recoding events, and it struggles when actively translated regions overlap. Despite these limitations, it is an important step toward the objective data-driven characterization of translational activity in the cell. Triplet periodicity is not the only property of the ribosomal profiling signal that can be used to detect translation[9], and methods that take the multiple signatures of translated regions into account are also being developed[10]. We expect a surge in the number of different approaches for *de novo* translatome characterization in the near future. The challenge will be to benchmark their performance and reliably verify their predictions.

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Hidden in the mist no more: physical force in cell biology

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To drive its migration through a fibrillar matrix—and thus to spread, invade or metastasize—a cancer cell must exert physical forces. The first visualization of these forces in three dimensions reveals surprising migration dynamics.

How does a cancer cell invade and migrate through surrounding tissue? In this issue, Steinwachs *et al.*[1] help us to understand this process by mapping for the first time the physical forces exerted by a cell migrating in a three-dimensional (3D) fibrillar collagen matrix. Moreover, they report the surprising finding that in the case of breast cancer cell lines, cellular forces within such a matrix are insensitive to changes in collagen concentration and bulk material properties. This finding stands in contrast to cellular forces measured previously in less physiological systems, including cell migration on a two-dimensional (2D) linearly elastic material such as polyacrylamide gel or PDMS (polydimethylsiloxane), or even within a 3D linearly elastic hydrogel such as PEG (polyethylene glycol). In the case of collagen matrices, by contrast, material viscoelasticity and nonlinearity severely complicate the problem of force recovery, but these complications represent merely the tip of the iceberg. This is because some collagen fibers straighten, extend and stiffen in response to an imposed force, whereas other fibers in the very same region simultaneously compress and buckle. All can change local orientation and spacing. As a result, local deformations on the fiber scale do not follow deformations on the bulk scale, and are therefore said to be non-affine. Steinwachs *et al.*[1] now take these factors into

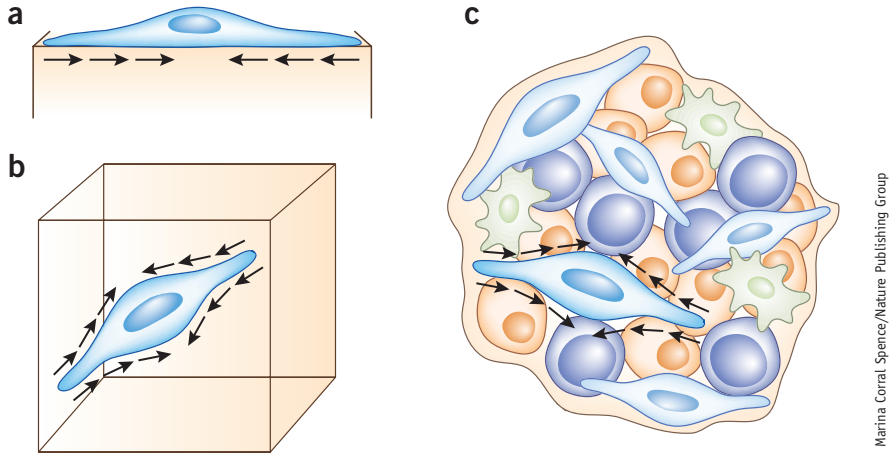

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Figure 1 | Traction stresses generated by a single cell. (**a**–**c**) Arrows denote stresses for a cell on a 2D matrix (**a**), within a 3D fibrillar collagen matrix (**b**) and in a multicellular 3D cellular cluster or tissue (**c**).

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account for a single cell migrating through a fibrillar matrix.

Physical forces at the cellular scale have remained unmeasurable for a long time, even though for at least 100 years we have known that these forces have a central role in biological growth, form, adaptation, wound healing and remodeling. For example, the classical work of Thompson[2] emphasizes the relationship between form and function, Wolff's law[3] describes the adaptation of bone structure to the load that the bone must support, and Murray's law[4] describes how fluid shear stress exerted on the endothelial wall regulates the adaptation of vessel diameter to the blood flow through the vessel. McMahon's principle of elastic similarity[5] explains how variations in energy metabolism, muscle mass and bone size scale across species as a function of body mass. Much more recently we learned that physical forces can direct stem cell fate[6].

To infer the presence of physical forces at the cellular scale, Harris *et al.*[7] used wrinkles generated by a single adherent cell contracting upon a thin silicone rubber sheet, but it was Dembo and Wang[8] who first formalized the problem of traction microscopy, making such forces visible, quantifiable and mappable (**Fig. 1a**). Their breakthrough was followed in rapid succession by computational streamlining[9], refining[10], extension to multicellular clusters[11,12] and sensing by fluorescence resonance energy transfer. To extend traction microscopy to 3D systems, Legant *et al.*[13] reconstructed cellular force fields in PEG hydrogel matrices, but deformations in such matrices are linearly elastic and affine, whereas those in fibrillar collagen matrices are not.

In their efforts to overcome the conceptual obstacles presented by traction microscopy in 3D fibrillar collagen matrices, Steinwachs *et al.*[1] recognized that the central problem was not so much the nonlinear elastic behavior of such matrices as how that nonlinear behavior could be understood in terms of non-affine fiber deformations. With a continuum description capturing that behavior in hand, they went on to use confocal reflectance microscopy to quantify local matrix deformations and then to compute the distribution of forces exerted by an MDA-MB-231 breast carcinoma cell migrating through a fibrillar collagen matrix (**Fig. 1b**).

In the case of a cell migrating upon a flat, linearly elastic 2D matrix, physical forces increase with increasing matrix stiffness, but it has now been shown that for a cell migrating within a 3D fibrillar collagen matrix, this sort of relationship does not hold. Rather, a cell migrating in 3D generates roughly similar forces regardless of matrix stiffness. The reasons for this surprising behavior, at least for now, remain a matter of speculation. Steinwachs *et al.*[1] did not independently control for matrix stiffness, ligand density or pore size, so a simple explanation might be that a cell in a stiff matrix with small pores is unable to spread and extend enough to generate the amount of force it would have in a similarly stiff but more porous matrix. Another open question now concerns the manner by which a cell seems to glide within the 3D collagen matrix, in contrast to the 'inchworm' migration commonly observed on a 2D substrate. Nor do we understand how the cell perceives and responds to the difference between 2D and 3D microenvironments, or how cell navigation is altered by associated differences in cell-matrix adhesion and cytoskeletal dynamics.

Where does traction microscopy go from here? Shortcomings of the present approach are clear. Difficult but nevertheless important issues not yet taken on include improving spatial resolution and accuracy and better defining the roles of time-dependent mechanical properties of the matrix such as viscoelastic relaxation processes, cross-linking dynamics, interstitial fluid flows and active matrix remodeling[14].

The greater challenge, however, will be to devise a strategy for measuring physical forces exerted by a cell embedded in a 3D cell cluster or organoid *in vitro*, or even in a living tissue (**Fig. 1c**). For a cell in a 2D cellular collective, such as an endothelial or epithelial layer *in vitro*, this problem has already been solved[11,12]. This success derives in part from the fact that the properties of the substrate material upon which such a layer migrates are controlled by the experimentalist; the substrate is typically homogeneous and elastic, as well as passive and well characterized. But for a cell in a 3D cellular collective, the surrounding material comprises mainly neighboring cells, and thus the material properties are defined not by the experimentalist but by the cells themselves. Accordingly, these properties tend to be not only heterogeneous and inelastic but also active and poorly characterized. For a cell in a 3D cellular collective, therefore, the traction microscopy strategies described above are fundamentally inapplicable; they cannot be made to work through either refinement or extension. Thus no strategy for force mapping in a 3D cell cluster is yet in sight.

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