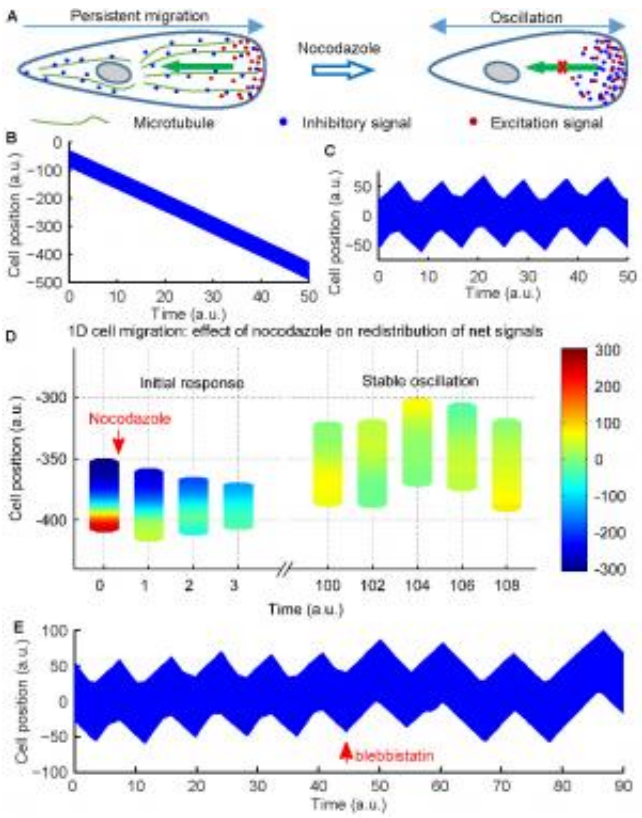
Going places: Microtubule-mediated transport of inhibitory signals critical in stabilizing cell migration polarity

December 9 2014, by Stuart Mason Dambrot



A modified LEGI model and computer simulation that reproduces both persistent cell migration and oscillations. A diagram of the modified LEGI model that involves the production of both excitation and inhibitory signals in the frontal region, followed by the transport of inhibitory signals away from the front to maintain frontal protrusions and cell polarity in control cells. In nocodazole-treated cells, the impairment of the transport and the resulting accumulation of inhibitory signals at the front act against the excitation signals and cause the head to switch into a tail (A). Computer simulation of this

mechanism for cells in 1D successfully reproduces both persistent migration (kymograph in B) and oscillations (kymograph in C). Diagrams of cell location and shape, colored to show the heat map of net signals, show the dynamics of net signals before and after the treatment of nocodazole (D). In addition, the period of oscillation increases upon the impairment of the generation of inhibitory signals (by increasing the delay), which mimics the effect of blebbistatin (kymograph in E). a.u. stands for an arbitrary unit used in computer simulation. See also Fig. S6 and Movies S4–S6. Credit: Zhang J et al. (2014) Microtubules stabilize cell polarity by localizing rear signals. *Proc Natl Acad Sci* USA 111(46):16383-16388.

(Phys.org)—Microtubules – tubular polymers of *tubulin* (a globular protein) that are a component of the cytoskeleton found throughout cell cytoplasm – are involved in a range of cellular functions, including the movement of secretory vesicles, organelles, and intracellular substances; cell division (mitosis and meiosis), including the formation of mitotic spindles; and cell polarity, which refers to spatial differences in cellular shape, structure and function. However, the nature of the role of microtubules in cell polarity has yet to be clarified. At the same time, cell migration plays an essential role in many important physiological processes, such as embryogenesis, wound healing, and immune responses; in engineering applications such as tissue regeneration; and, when defective, in causing severe problems such as birth defects, vascular disease and tumor metastasis. A key area for investigation in the linkage between cell polarity and cell migration is that directional cell migration requires a defined polarity, generated by an integrated network of signals, adhesions (the protein-based binding of a cell to a surface or substrate) and cytoskeleton.

Recently, scientists in the Department of Biomedical Engineering at Carnegie Mellon University, Pittsburgh obtained a series of analytical and experimental results showing that:

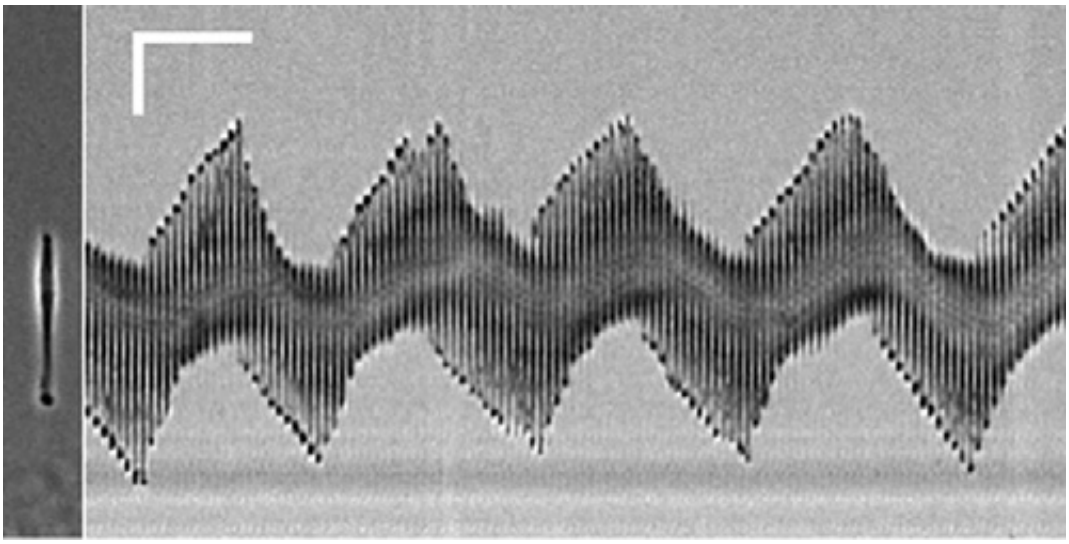
- Cells on micropatterned linear strips change from highly persistent migration into striking oscillations upon the disassembly of microtubules
- Positive feedback in the *local-excitation-global-inhibition*, or LEGI, mechanism – which is responsible for migration persistence, and in which the response to a stimulus (such as chemotaxis, the movement of an organism in response to a chemical stimulus) is mediated through the balance between a fast, local excitation and a slower, global inhibition process – might be converted into negative feedback to drive oscillations upon the disassembly of microtubules
- Microtubules facilitate the transport of inhibitory signals and their global distribution
- Feedback in the integrated control circuit may be either positive or negative, depending on the relative position of excitation and inhibitory signals

The scientists therefore concluded that these findings provide valuable insights into the role of microtubules in the control circuit of cell migration.

Prof. Yu-Li Wang discussed the paper that he, Graduate Research Assistant Jian Zhang and Project Scientist Wei-Hui Guo published in *Proceedings of the National Academy of Sciences*, starting with the challenges involved in the team finding that depolymerization of microtubules caused cells to change from persistent to oscillatory migration. The issue, Wang tells *Phys.org*, is that without confining cell migration along a one-dimensional (1D) strip, depolymerization of microtubules simply causes cells to lose directional migration without showing recognizable oscillations – so confinement to 1D is required for the highly persistent migration *before* microtubule depolymerization as well as the oscillatory migration *after* depolymerization. "This is most likely due to limiting cell migration to two opposite directions," he

explains. "Oscillations become difficult to observe when the cell is able to switch among many different directions. Moreover," he adds, "our observation was also facilitated by using the RPE-1 cell line, which has a relatively short oscillation period such that multiple cycles of oscillation may be observed before the cell enters division phase. RPE-1 cells therefore allowed unambiguous confirmation of the initial observation made with NIH/3T3 cells." The *retinal pigment epithelium*, or RPE, is the pigmented cell layer just outside the neurosensory retina that nourishes retinal visual cells, and is firmly attached to the underlying choroid and overlying retinal visual cells. 3T3 cells – from a cell line originally established in 1962, and published[1] in 1963, by George J. Todaro and Howard Green at New York University School of Medicine – has become the standard fibroblast cell line. (Fibroblasts are the most common connective tissue cell in animals.)

The scientists also faced a hurdle when applying computer modeling to understand how positive LEGI feedback might be converted into negative feedback to drive oscillations upon microtubule disassembly. "The main challenge was to model not only excitation and inhibitory signals at different locations, but also a transport process responsible for delivering the inhibitory signals away from the frontal region for global distribution," Wang explains, "because the oscillation phenomenon can be produced only when the model includes all the essential elements."



RPE-1 cells in 50 μM ciliobrevin D. Kymograph shows a representative RPE-1 cell treated with ciliobrevin D oscillating in a manner similar to those treated with nocodazole. (Scale bar, 50 μm, 60 min.) See also Figs. S1 and S7 and Movie S1.Credit: Zhang J et al. (2014) Microtubules stabilize cell polarity by localizing rear signals. See also Figs. S1 and S7 and Movie S1. *Proc Natl Acad Sci* USA 111(46):16383-16388.

Despite the complexity of their investigation, the researchers addressed these challenges in a number of ways. They showed that microtubules play a key role in maintaining the positive feedback in the LEGI mechanism, and as described above that positive feedback of the LEGI mechanism for persistent migration may be readily converted into a negative feedback for oscillations. "We found that, in order to generate oscillation, the production of inhibitory signals must lag behind the production of excitation signals," Wang tells *Phys.org*. "This is consistent with the general understanding that delay differential equations have the capability to generate oscillations." In addition, he continues, they used a highly reliable micropatterning method, developed in their lab, which grafts linear polyacrylamide on glass surfaces to block cell migration for confining cell migration to a 1D strip. "We also used a computer model

– also implemented in our lab – that simulates not only the control circuit, but also its effect on cell migration."

The paper presents two analytic conclusions, the first being that microtubules facilitate the transport of inhibitory signals and their global distribution. "Our reasoning was guided by the conversion from persistent migration – known to depend on LEGI-based positive feedback – to oscillation, which in general requires negative feedback," Wang says. "The simplest way for this conversion to occur involves the inhibition of global distribution of negative signals, which fits perfectly with the well-documented function of microtubules in intracellular transport." Wang points out that this type of reverse reasoning – where an intuitive hypothesis to provide a simple explanation of a biological phenomenon is made before seeking experimental evidence or computational verification – is widely practiced for understanding complex biological phenomenon that are too difficult to address through forward dissection. "Our results further imply that a cell is able to measure its own length based on the time required for end-to-end microtubule-mediated transport," Wang continues. "Other cellular processes may be similarly affected by the time for the transport of molecular signals along the cell length."

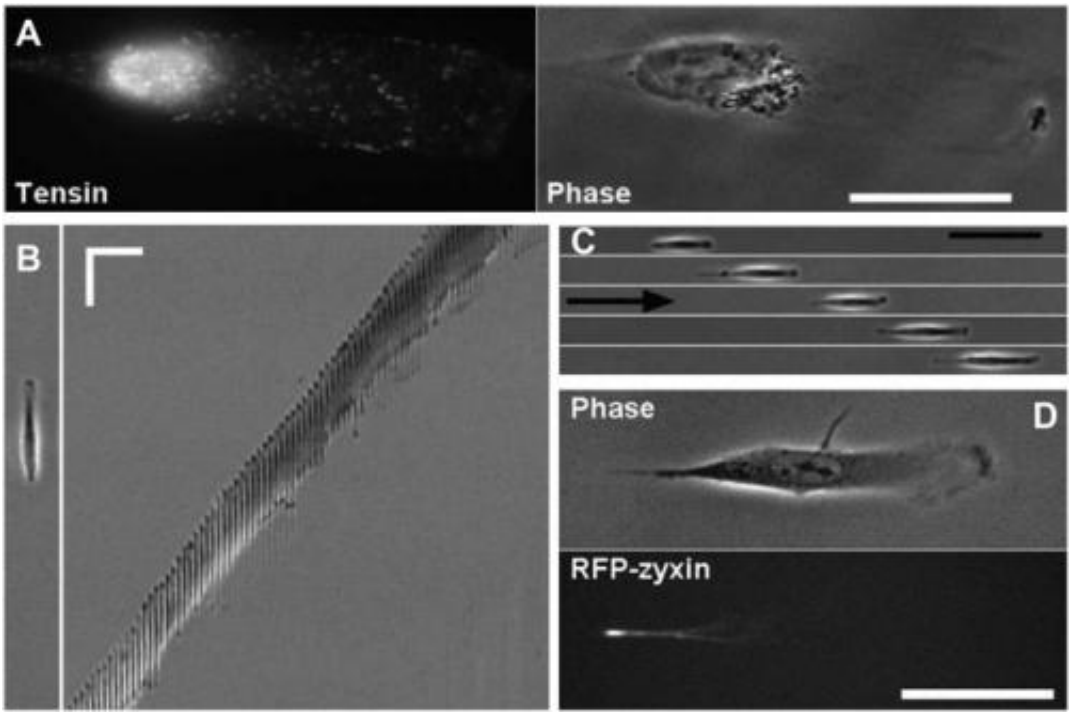
The second analytic conclusion was based on a primary difference between cells and man-made machines – the former involve dynamic localization of structures and signals, while the latter usually consist of components with a fixed location and geometrical relationship – so depending on the relative position of excitation and inhibitory signals in cells, the resulting feedback in the integrated control circuit may be either positive or negative. In contrast, the lack of dynamic localization in most man-made machines keeps them from exhibiting this conversion. "Although rarely realized in man-made machines, transport-dependent conversion between positive and negative feedback in the cell makes intuitive sense for regulating dynamically localized activities."

Wang points out. "When negative signals are transported away from positive signals, it creates a positive feedback by suppressing the activities elsewhere that may compete with the existing frontal end. Conversely, when negative signals are allowed to accumulate in the vicinity of positive signals, they cause negative feedback by overpowering positive signals."

The scientists suggest that microtubules are required not for the generation, but rather for the maintenance, of cell polarity by mediating the global distribution of inhibitory signals. "We found that after the depolymerization of microtubules, cells are still able to move directionally for more than an hour before reversing the direction," Wang says. "Therefore, cells without microtubules are able to establish a direction of migration but are not able to maintain the polarity for an extended period of time as control cells do. The defect may be explained by the inability to remove the accumulating inhibitory signals at the leading edge, which eventually overpower the excitation signals required for maintaining frontal protrusion activities."

A key finding discussed in the paper is that disassembly of microtubules induces cell oscillation by allowing inhibitory signals to accumulate at the front, which stops frontal protrusion and allows the polarity to reverse. "According to the LEGI model, frontal protrusion activities are determined by the balance between excitation and inhibitory signals," Wang explains. "Upon the loss of microtubules and the associated transport mechanism, the cell is no longer able to move inhibitory signals away from the leading edge where they are generated, and therefore cannot achieve their global distribution." Specifically, the accumulating inhibitory signals eventually overcome the excitation signal to stop frontal protrusion activities – and at the same time, the suppression of protrusion activities elsewhere also becomes compromised due to the defective global transport. "Spontaneously-generated excitation signals are then allowed to accumulate and cause the formation of a new front,"

Wang adds, "which in the case of cells confined to 1D translates into the reversal of polarity."



Persistent migration of cells on 1D strips and the corresponding localization of focal adhesion proteins. Related to Fig. 1. Immunofluorescence staining of tensin in a representative RPE-1 cell (A; scale bar, 25 μm) shows no apparent polarization. Kymograph (B; scale bar, 50 μm, 60 min) and time series (C; 90-min interval; scale bar, 100 μm; arrow indicates the direction of migration) of representative NIH 3T3 fibroblasts exhibiting persistent migration on 1D strips. RFP-zyxin in NIH 3T3 cells migrating on 1D strips localizes to the tail region (D). (Scale bar, 50 μm.) Credit: Zhang J et al. (2014) Microtubules stabilize cell polarity by localizing rear signals. *Proc Natl Acad Sci* USA 111(46):16383-16388.

The researchers also found that in addition to oscillation, their modified LEGI mechanism can create a range of possible cell behaviors as a result

of different relative dynamics of excitation and inhibitory signals – and thereby, the variable interplay between shape, mechanics, and polarity. "The magnitude of inhibitory signals is determined by the relative rate between the generation and transport-mediated depletion." Wang adds. "Our model accounts for the key elements between signal generation and transport – and while previous models, such as that created by Brian Camley and his colleagues[2], incorporate some of the relevant parameters such as cell adhesion and shape to generate oscillation, they are unable to generate as wide a range of activities."

Wang notes that their mathematical model is capable of generating testable, verifiable predictions. One of the primary predictions was that longer cells should oscillate with a longer period; another prediction was that inhibition of *dynein* (one of many proteins that bind to microtubules, in this case a motor protein responsible for the transports of cargos away from the cell front) should cause similar oscillations as those caused by microtubule disassembly. Wang emphasizes that both of these predictions were verified with experiments.

Moving forward, Wang tells *Phys.org*, the researchers would like to drill down the molecular mechanism and cellular structures for polarity control. "We found that a focal adhesion protein, *zyxin*, has some unique properties that parallel cell polarity, suggesting that it may be a key 'readout' of the LEGI mechanism as well as the control factor for cell polarity – and we're now in the process of elucidating its functional role in cell polarity. In addition," he continues, "while centrosomes have long been speculated to control cell polarity, we're trying to establish a logical connection between centrosomes and the LEGI mechanism." (The *centrosome* is an organelle that serves as the main microtubule organizing center, or MTOC, of the animal cell, where microtubules are nucleated.) Wang adds that they may also investigate fine regulation of cell migration based on the manipulation of microtubules or motor molecules without total depolymerization of microtubules, and a 'biological clock'

based on oscillating polarity.

Wang says that there are other areas of research that might benefit from their study. "Understanding the control mechanism of cell migration can greatly facilitate migration dependent processes such as tissue engineering and cancer treatment[3,4]. In addition, the elegant switch between positive and negative feedback may impact bio-inspired engineering, in which a man-made machine may use a biomimetic control mechanism to achieve innovative functions."

Specifically, *Phys.org* asked Wang if their findings might pertain to the observation that metastasis and neuroneogenic targeting have similar behavioral properties, and may share what has been termed an *ancient genetic toolkit*[5] that plays a role in cancer. "Yes," Wang affirmed. "Metastasis is driven by the migration of tumor cells. Our findings improve the understanding of the internal control circuit for cell migration, which will benefit the understanding and treatment of metastasis, – such that conditions that reduce the stability of tumor cell migration may be deployed for cancer treatment."

More information: Microtubules stabilize cell polarity by localizing rear signals, *Proceedings of the National Academy of Sciences* (2014) **111**(46):16383-16388, doi:10.1073/pnas.1410533111

Related:

[1]Quantitative Studies of the Growth of Mouse Embryo Cells in Culture and their Development into Established Lines, *Journal of Cell Biology* (1963) **17**(2):299–313, doi:10.1083/jcb.17.2.299

[2]Periodic migration in a physical model of cells on micropatterns, *Physical Review Letters* (2013) **111**(15):158102, doi:10.1103/PhysRevLett.111.158102

[3]Race to the top: Decoding metastasis, *Medical Xpress* April 29, 2011

[4]Probing the invasiveness of prostate cancer cells in a 3D microfabricated landscape, *Proceedings of the National Academy of Sciences* (2011) **108**(17):6853-6856, doi:10.1073/pnas.1102808108

[5]Cancer tumors as Metazoa 1.0: tapping genes of ancient ancestors, *Physical Biology* (2011) **8**:015001, doi:10.1088/1478-3975/8/1/015001