The Effects of Nanoscale Topography on Palladin Dynamics in Human Pancreatic Stellate Cells

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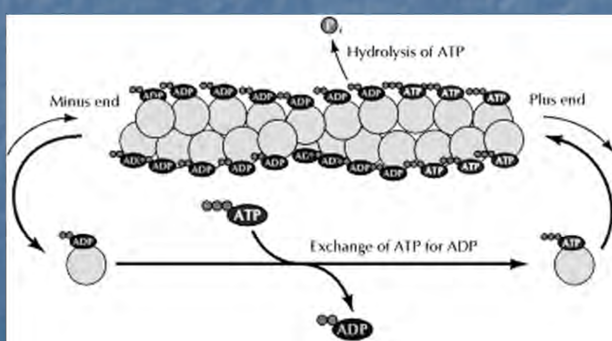
TREND 2012

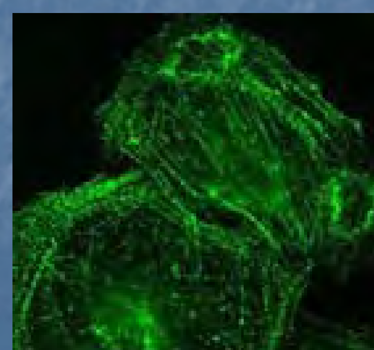
Training and Research Experiences in Nonlinear Dynamics

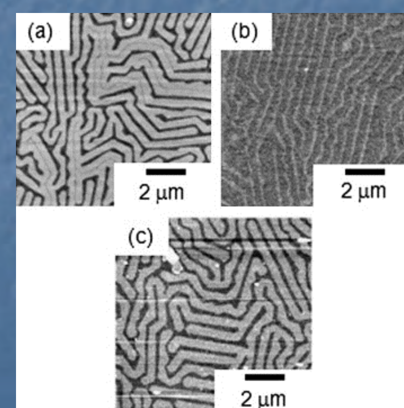
Actin, Palladin, and Nanopatterns

- Actin is one of the primary proteins responsible for the structural and mechanical properties of cells
- Palladin is an actin binding protein (ABP) and helps to modulate the actin cytoskeleton
- How does nanoscale topography of the environment influence cell shape and actin organization?
- Recent advances in nanotechnology have allowed us to create detailed patterns on polymer resin



Actin polymerization

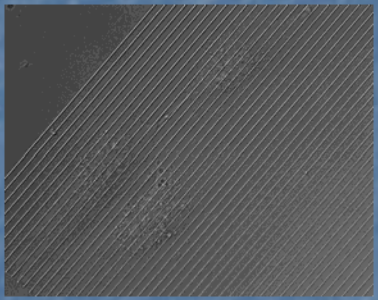

GFP Palladin in cells



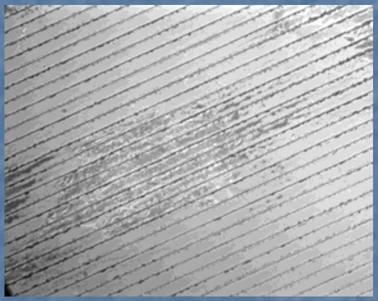
Wantanabe et al. 2009

Experimental Method

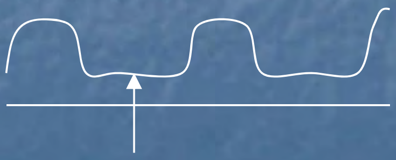
- Cells are grown using standard protocol in cell culture medium.
- We use two different substrates: glass and polymer resin. The polymer resin has nanopatterns.
- We image the cells spreading using different microscopy techniques, including Differential Interference Contrast (DIC), Interference Reflection Microscopy (IRM), and Total Internal Reflection Fluoroscopy (TIRF).



3 µm wide grooves

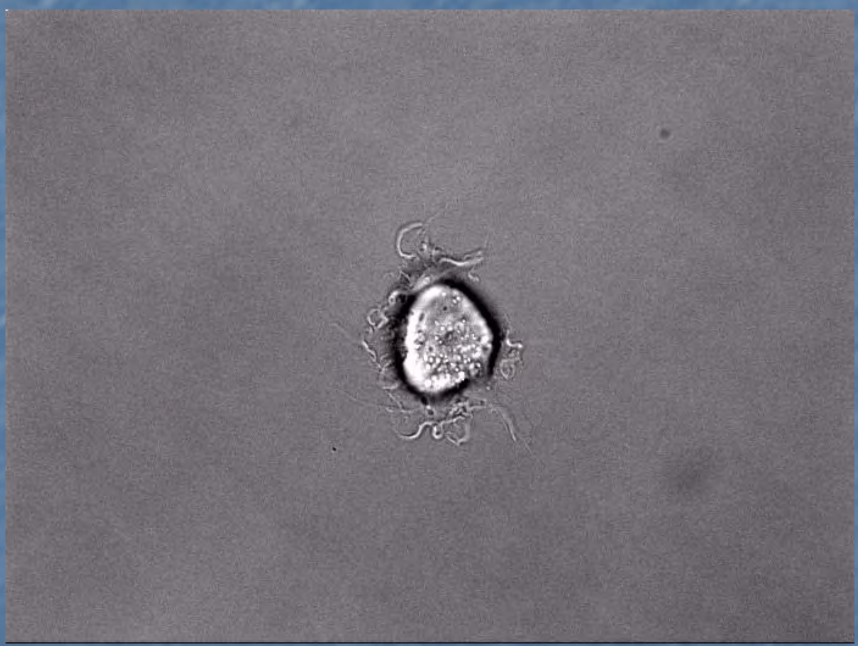


5 µm wide grooves



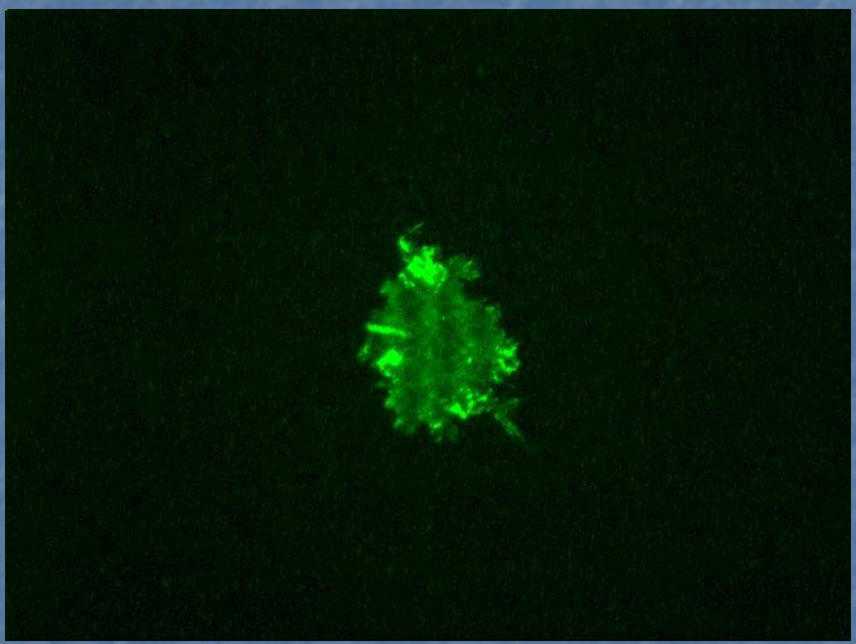
Cells Spreading on Glass

Bright field



75 µm

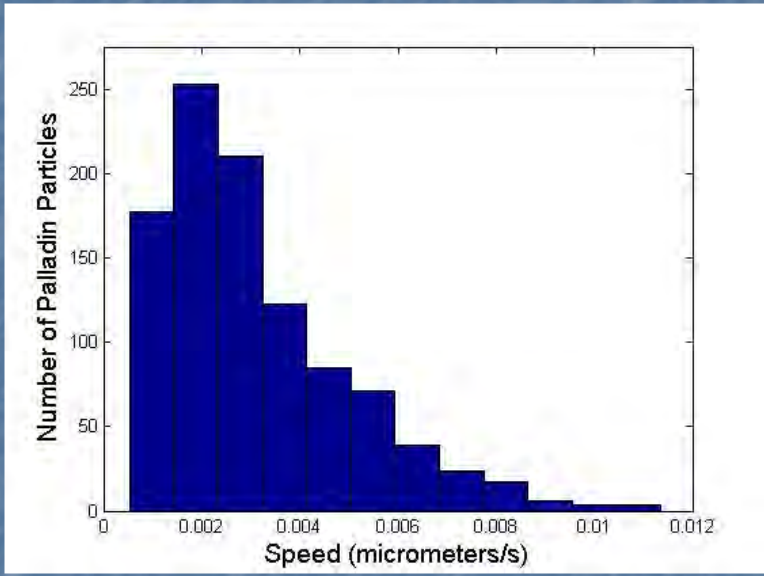
TIRF of GFP Palladin



Time elapsed: 64 minutes

Cells on Glass

Distribution of palladin speeds on glass



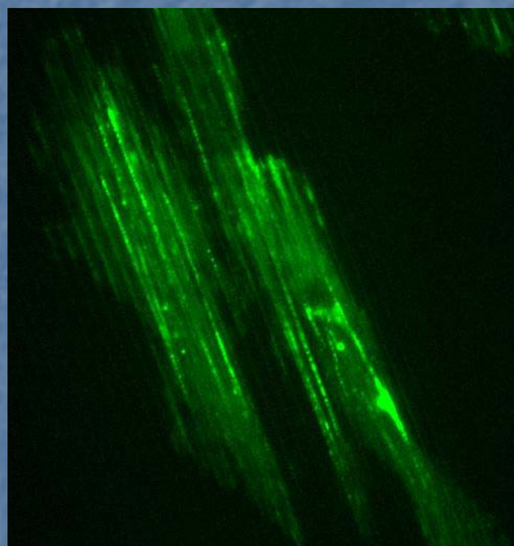
- The speed distribution of palladin particles is left-tailed. The mean speed is 0.0031 ± 0.0020 µm/s.

Cells on Grooved Substrate

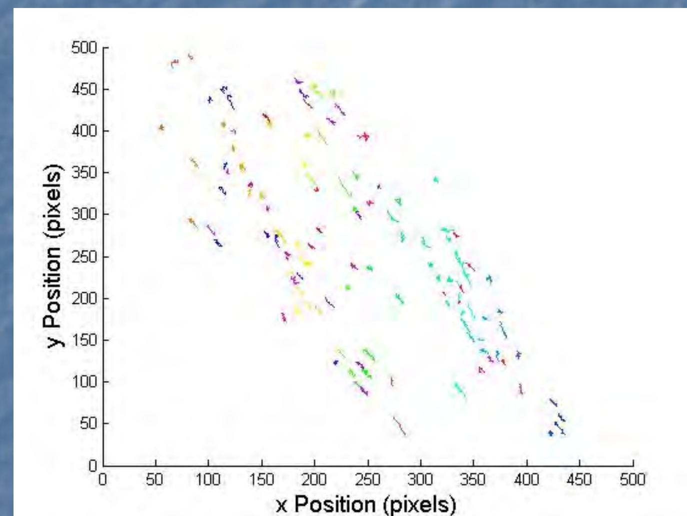
Cell on 3 µm grooves

55 µm

Time elapsed: 31 minutes



Tracks of palladin in the same cell



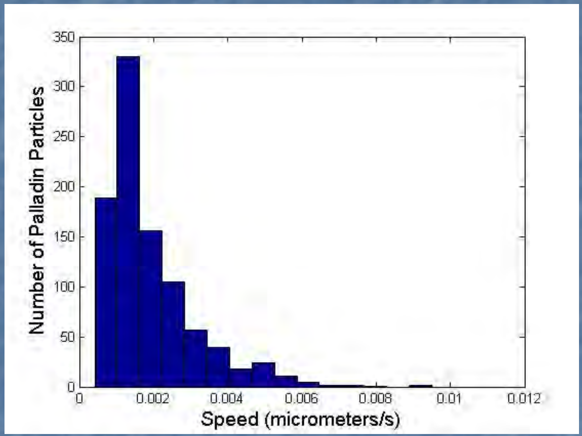
•When cells are plated on the grooved substrate, the cells spread along the direction of the grooves.

•Stress fibers also form along the direction of the grooves, and in many cases, inside the grooves themselves.

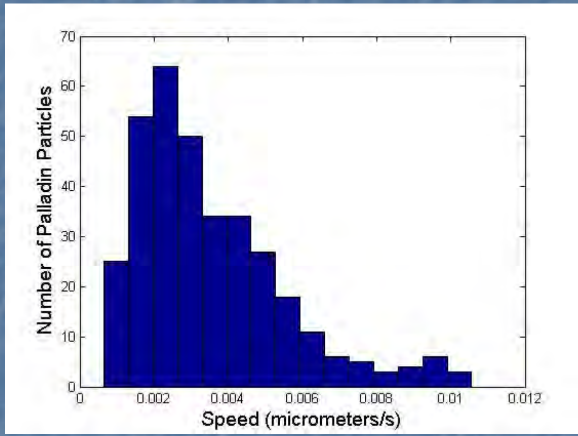
•Palladin structures, which appear like punctate spots, predominantly move in the direction of the grooves, and often stay confined within the grooves.

Cells on Grooved Substrate

Distribution of palladin speeds on 3 µm grooves



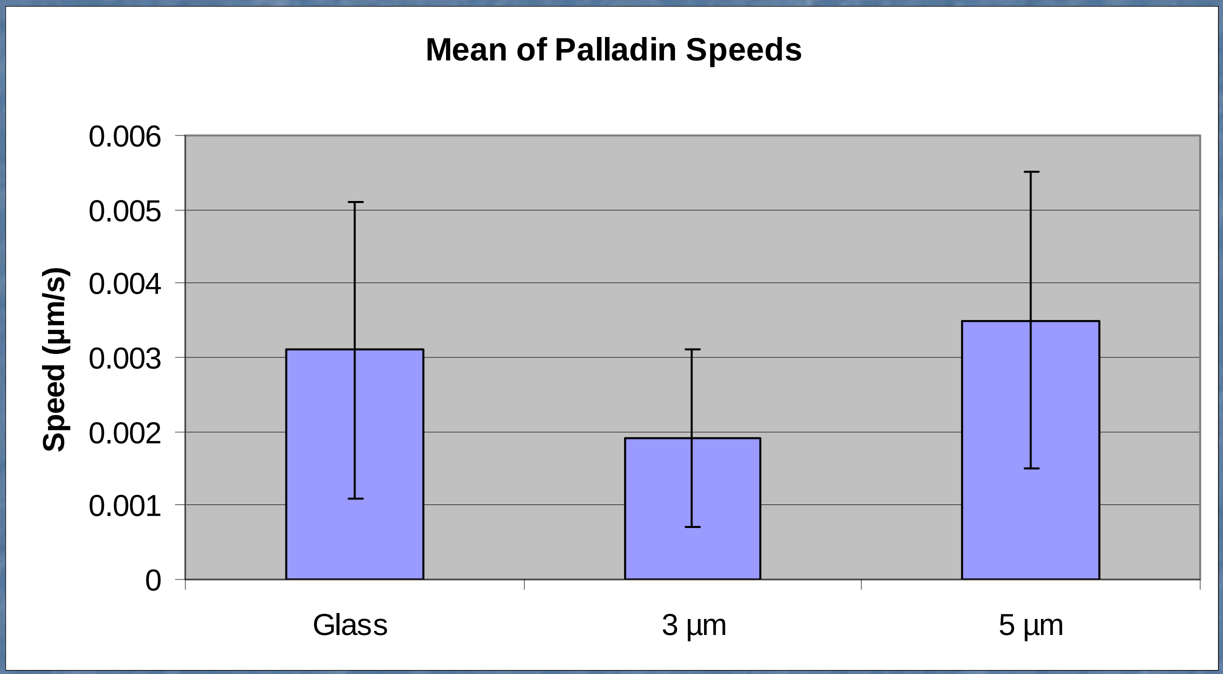
Distribution of palladin speeds on 5 µm grooves



•The speed distribution of palladin particles moving in 3 µm grooves is narrower than the speed distribution in 5 µm grooves. They are both left-tailed.

•The mean for palladin speeds on 3 µm grooves is 0.0019 $\pm$ 0.0012 µm/s while the mean for palladin speeds on 5 µm grooves is 0.0035 $\pm$ 0.0020 µm/s.

Comparison



The average speed of palladin structures is significantly reduced in 3 µm grooves compared to a glass surface or 5 µm grooves (Students' t-test, P-value = .000019)

Future Work

- We must study nanogrooves of different widths to fully understand the influence of topography
- Additional aspects of palladin dynamics and stress fiber formation should be researched
- Matlab-based tracking software needs to be improved

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  - Mike Azatov
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