Tales of a 24th grade nothing:

A survivor's guide to graduate school

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Graduate school is hard

- 4-6 year commitment (for PhD)
  - Classes and teaching
  - Research, writing, and presenting
- The journey is a tortuous, non-linear path
- Lack of literature dealing with the ‘grad student journey’
- Construct a map that helps current & future grad students navigate the high seas of grad school

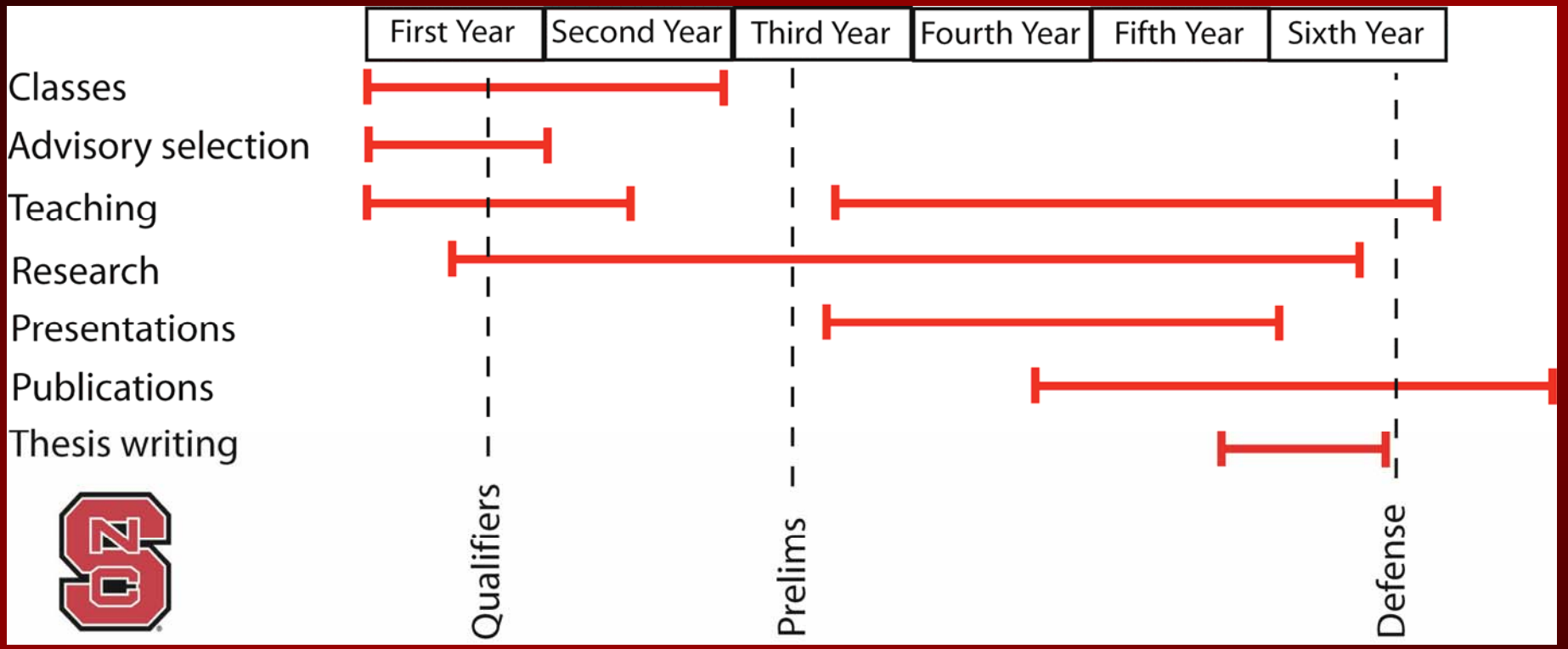
Q: How do you cram 6 years of advice into a 10 page paper and 15 minute talk?

A: You don't

- Why grad school?
- The first year
- Conducting research
- The advisor/student relationship
- Maintaining a personal life
- Grad school potpourri
- Publishing and presenting

- Teaching
- Managing undergraduates
- Running a lab
- Qualifying Exams
- Preliminary Exams
- Writing the thesis
- Defense
- Etc

Choose your own adventure. . . .



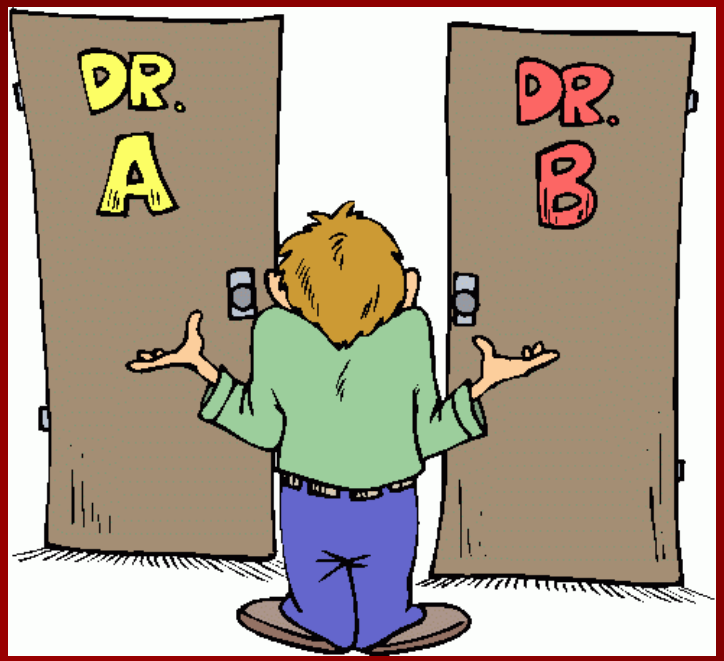
- North Carolina State University Graduate School
- Enrolled in the PhD program in CHE (Fall 2004)
- Defended thesis (March 2010) and graduated (May 2010)

Why should I attend graduate school?

- Industry vs. Academia
- My motivation:
  - Undergraduate research (REU programs)
  - Passion for teaching
- When applying to graduate school
  - Shoot for the stars
  - Keep an open mind
- Make sure you really want that degree

Surviving the first year

- Can possibly include:
  - Taking classes
  - Selecting an advisor
  - Teaching
  - Getting started in the lab
  - Qualifying exams



- Make friends with your classmates
- When choosing an advisor:
  - Ask lots of questions
  - Don't hesitate to take the initiative
  - Discuss your top choices with your classmates
  - Always have a back-up plan

Stand back, I'm about to try science!

- Don't be afraid to screw up
- Don't be shy about asking for help
- Branch out and try new methods and techniques
- Even negative results can still be useful

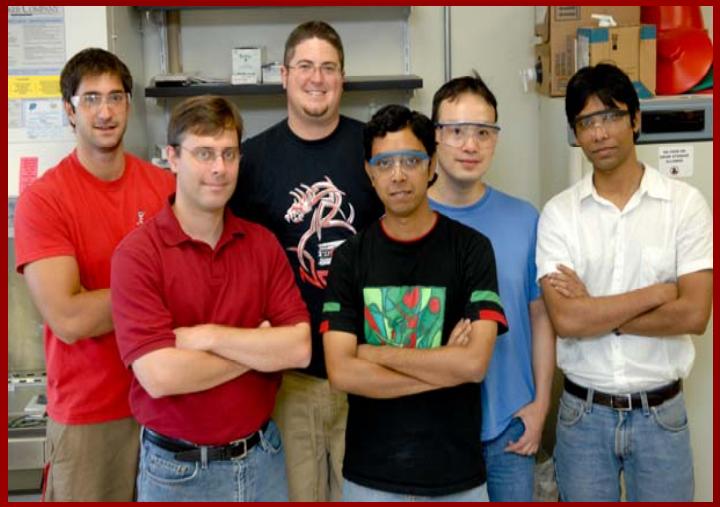
Student-advisor paradox

Problems

- You can spend days to weeks in the lab without a purpose, producing nothing

Solutions

- Talk to your advisor and utilize their knowledge



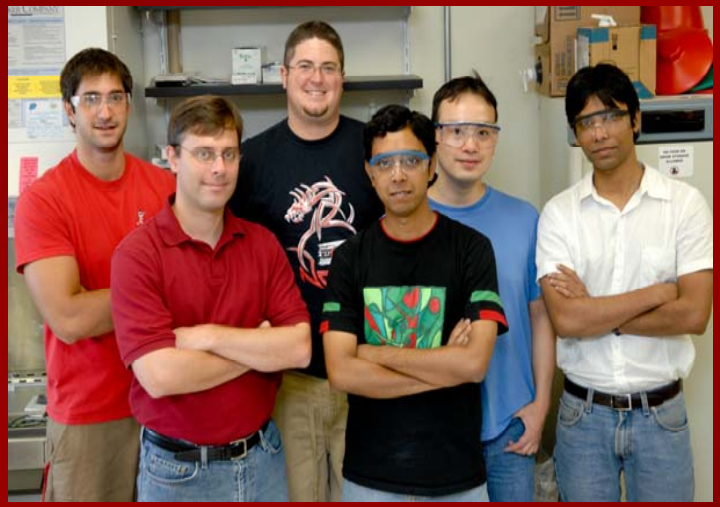
Student-advisor paradox

Problems

- Projects can get cancelled
- Funding can dry up

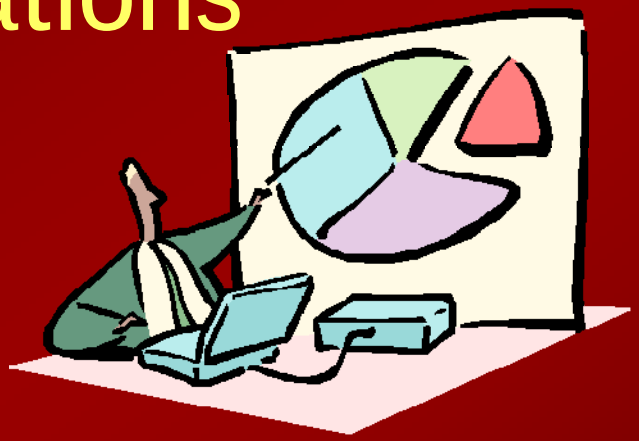
Solutions

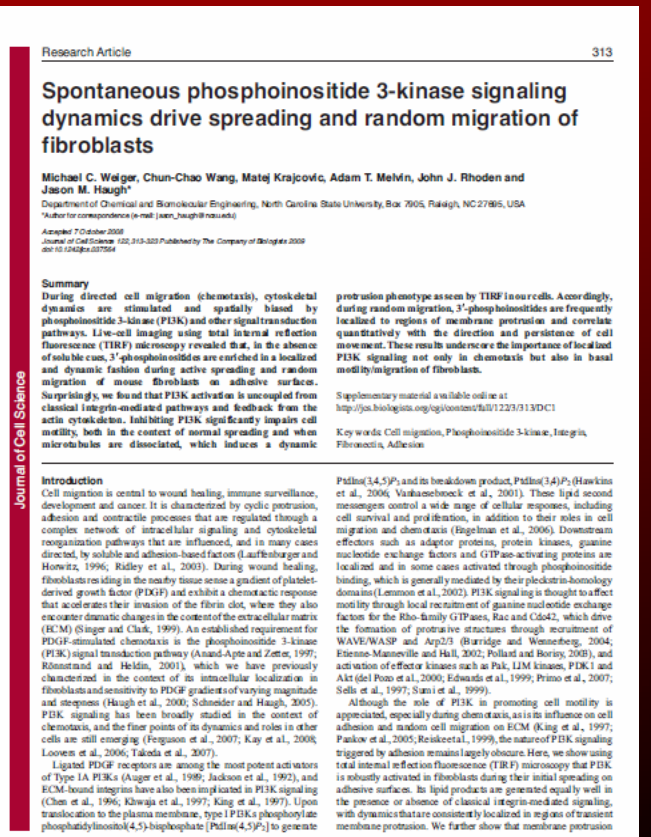
- This is how research goes
- Get over it and move forward



Publications and presentations

- Attend conferences
  - Great networking
  - Get advice from peers
  - Opportunities to showcase your research
- Write at least one first author publication
- Believe in your writing and research
  - Be proud of what you've done
  - Keep finding new joy in your work
  - Write papers outside of your thesis research





Research Article 313

Journal of Cell Science

Spontaneous phosphoinositide 3-kinase signaling dynamics drive spreading and random migration of fibroblasts

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Summary

During directed cell migration (chemotaxis), cytoskeletal dynamics are stimulated and spatially biased by phosphoinositide 3-kinase (PI3K) and other signal transduction pathways. Live-cell imaging using total internal reflection fluorescence (TIRF) microscopy revealed that, in the absence of soluble cues, 3′-phosphoinositides are enriched in a localized and dynamic fashion during active spreading and random migration of mouse fibroblasts on adhesive surfaces. Surprisingly, we found that PI3K activation is uncoupled from classical integrin-mediated pathways and feedback from the actin cytoskeleton. Inhibiting PI3K significantly impairs cell motility, both in the context of normal spreading and when microtubules are dissociated, which induces a dynamic protrusion phenotype as seen by TIRF in our cells. Accordingly, during random migration, 3′-phosphoinositides are frequently localized to regions of membrane protrusion and correlate quantitatively with the direction and persistence of cell movement. These results underscore the importance of localized PI3K signaling not only in chemotaxis but also in basal motility/migration of fibroblasts.

Supplementary material available online at http://jcs.biologists.org/cgi/content/full/122/3/313/DC1

Keywords: Cell migration, Phosphoinositide 3-kinase, Integrin, Fibronectin, Adhesion

Introduction

Cell migration is central to wound healing, immune surveillance, development and cancer. It is characterized by cyclic protrusion, adhesion and contractile processes that are regulated through a complex network of intracellular signaling and cytoskeletal reorganization pathways that are influenced, and in many cases directed, by soluble and adhesion-based factors (Lauffenburger and Horwitz, 1996; Ridley et al., 2003). During wound healing, fibroblasts residing in the nearby tissue sense a gradient of platelet-derived growth factor (PDGF) and exhibit a chemotactic response that accelerates their invasion of the fibrin clot, where they also encounter dramatic changes in the content of the extracellular matrix (ECM) (Singer and Clark, 1999). An established requirement for PDGF-stimulated chemotaxis is the phosphoinositide 3-kinase (PI3K) signal transduction pathway (Anand-Apte and Zetter, 1997; Rönnstrand and Heldin, 2001), which we have previously characterized in the context of its intracellular localization in fibroblasts and sensitivity to PDGF gradients of varying magnitude and steepness (Haugh et al., 2000; Schneider and Haugh, 2005). PI3K signaling has been broadly studied in the context of chemotaxis, and the finer points of its dynamics and roles in other cells are still emerging (Ferguson et al., 2007; Kay et al., 2008; Loovers et al., 2006; Takeda et al., 2007).

Ligated PDGF receptors are among the most potent activators of Type IA PI3Ks (Auger et al., 1989; Jackson et al., 1992), and ECM-bound integrins have also been implicated in PI3K signaling (Chen et al., 1996; Khwaja et al., 1997; King et al., 1997). Upon translocation to the plasma membrane, type I PI3Ks phosphorylate phosphatidylinositol(4,5)-bisphosphate [PtdIns(4,5)P_2] to generate PtdIns(3,4,5)P_3 and its breakdown product, PtdIns(3,4)P_2 (Hawkins et al., 2006; Vanhaesebroeck et al., 2001). These lipid second messengers control a wide range of cellular responses, including cell survival and proliferation, in addition to their roles in cell migration and chemotaxis (Engelman et al., 2006). Downstream effectors such as adaptor proteins, protein kinases, guanine nucleotide exchange factors and GTPase-activating proteins are localized and in some cases activated through phosphoinositide binding, which is generally mediated by their pleckstrin-homology domains (Lemmon et al., 2002). PI3K signaling is thought to affect motility through local recruitment of guanine nucleotide exchange factors for the Rho-family GTPases, Rac and Cdc42, which drive the formation of protrusive structures through recruitment of WAVE/WASP and Arp2/3 (Burridge and Wennerberg, 2004; Etienne-Manneville and Hall, 2002; Pollard and Borisy, 2003), and activation of effector kinases such as Pak, LIM kinases, PDK1 and Akt (del Pozo et al., 2000; Edwards et al., 1999; Primo et al., 2007; Sells et al., 1997; Sumi et al., 1999).

Although the role of PI3K in promoting cell motility is appreciated, especially during chemotaxis, as is its influence on cell adhesion and random cell migration on ECM (King et al., 1997; Pankov et al., 2005; Reiske et al., 1999), the nature of PI3K signaling triggered by adhesion remains largely obscure. Here, we show using total internal reflection fluorescence (TIRF) microscopy that PI3K is robustly activated in fibroblasts during their initial spreading on adhesive surfaces. Its lipid products are generated equally well in the presence or absence of classical integrin-mediated signaling, with dynamics that are consistently localized in regions of transient membrane protrusion. We further show that membrane protrusion

Graduate school is like a box of chocolates

- More than classes and research
- Take advantage of endless opportunities
  - Teach a class
  - Get a summer internship
  - Apply for a fellowship
  - Mentor undergraduates
  - Initiate collaboration with other faculty, departments, and universities
  - Tutor
  - Perform education outreach
  - Get involved in recruiting

Wait. . . . I’m supposed to have a personal life?

- Ensuring that you have a personal life is probably the most important aspect of graduate school
- Maintain an equilibrium between school work and your personal life

A Call to Arms!

- Grad school is difficult, but not impossible
- This paper was written as a baseline for all new, current, and past graduate students – a starting point for all of you to tell your story
- Expand and improve on what I've written, pass along YOUR knowledge and experience to help better prepare the next round of grad students
- Ultimately, if we can survive getting our PhD – we can survive anything!

Questions?