iBiology.org Teaching Tools
Mary Beckerle's Lecture Part 3:

Teaching Tools were prepared by Julie Kadrmas and Mary Beckerle.

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1. Review Questions

1. Define homeostasis in the context of a biological organism.
2. Give some examples of organs sensitive to mechanical force.
3. True or False? Excessive mechanical load on the heart leads to cardiac hypertrophy via an increase in the number of cardiomyocytes.
4. What is the term for the structure found at concentrated sites of integrin-based adhesion to the substratum?
5. Upon uniaxial cyclic stretch of cultured fibroblast cells, what changes are observed in the actin cytoskeleton?
6. Which focal adhesion constituent shows a dramatic change in subcellular distribution after mechanical stimulation?

7. The vinculin protein serves as a stable component/marker of what subcellular structure, independent of mechanical load?
8. True or false? Zyxin is required for cytoskeletal reinforcement after mechanical stimulation, but is not required for reorientation of the actin cytoskeleton.
9. What is the general function of Rho-GTPase?
10. What is the function of proteins in the Ena/VASP family?

2. Answers to Review Questions

1. Homeostasis is the process of maintaining a stable internal environment while being subject to a wide variety of external stimulation.
2. bone, muscle, lung, vasculature, and heart were presented, but there are others as well
3. False
4. focal adhesion
5. Actin stress fibers become more robust and reorient perpendicular to the direction of stretch.
6. Zyxin moves from focal adhesions to actin stress fibers.
7. focal adhesions
8. True
9. Rho is required to build actin stress fibers.
10. enhancing the assembly of actin

3. Discussion Questions

1. Why is it important that uniaxial stretch be used in the experiments described here?

2. Hypothesize why the actin cytoskeleton reorients perpendicular to the direction of stretch.
3. Why was the focal adhesion protein zyxin identified as a good candidate for a stretch sensor?
4. From the stretch experiments presented, how do we know that stress fiber reorientation and reinforcement occur by two distinct pathways?
5. Actin stress fibers are made from bundles of filamentous actin, which are themselves polymers of actin monomers. What are some potential molecular mechanisms by which actin stress fiber thickening could occur?

4. Answers to Discussion Questions

1. If stretch were occurring in multiple different directions, presumably in each cell the actin stress fibers would reorient and reinforce, but unless we could measure the stretch vector at every location, the data would be impossible to interpret. Uniaxial stretch is a way in which to subject the cell population to a controlled and measurable force.
2. This is a question for which we do not yet have a defined “answer”. One idea is that the perpendicular orientation minimizes the stress on individual actin stress fibers. Consider a stress fiber oriented parallel to the stretch vector. Stretch of this sort might be capable of compromising structural integrity and could ultimately tear the fiber apart—not so with a fiber oriented perpendicularly.
3. Zyxin in a focal adhesion constituent. It is therefore well positioned to act as a sensor, being linked to both ECM contacts and to the actin cytoskeleton. It has long been known that zyxin can be localized to both focal adhesions and actin stress fibers. Our observation that zyxin mobilizes from focal adhesions to stress fibers upon mechanical stimulation makes it an even more attractive candidate for a sensor.
4. There are conditions under which reorientation takes place without actin stress fiber reinforcement—in the zyxin null cells. Likewise there are conditions in which stress fiber reinforcement is observed without reorientation—when treated with the Rho

kinase inhibitor. If the two processes were in a single, linear pathway, this would not be possible.

5. Thickening of actin stress fibers could occur by recruitment of pre-formed filamentous actin, actin fiber bundling, preventing the depolymerization of filamentous actin, or de novo polymerization of actin monomers.

5. Explain or Teach These Concepts to a Friend

1. Explain how muscle size is responsive to physiological cues.
2. Explain how a uniaxial cyclic stretch experiment is conducted, being sure to include all of the appropriate controls.
3. Explain how integrins, zyxin and Ena/VASP are involved in cytoskeletal reinforcement upon mechanical stretch, with an emphasis on constructing a linear pathway of events.

6. Research the Literature on Your Own

In addition to Ena/VASP, alpha-actinin is a well-established binding partner of zyxin, with connections to actin regulation. Research the molecular functions of alpha-actinin and develop a hypothesis of how zyxin might participate in reinforcement of the actin cytoskeleton in conjunction with alpha-actinin.

The experiments described here utilize a uniaxial cyclic stretch device. There is another class of cell-stretching apparatus that uses vacuum pressure to apply multi-directional cyclic stretch to cells cultured on flexible-bottomed culture plates (Flex Cell). Compare and contrast the kinds of data capable of being generated in these two different systems.

Proteins of the p130Cas family of signal transducers have also been implicated as cellular stress sensors. What is the mechanism by which p130Cas is thought to participate in mechanotransduction? Members of the p130Cas family have been shown to bind directly to zyxin family members. How might you determine whether p130Cas

and zyxin are working together in a single stress sensing pathway, or whether they are functioning independently?

7. Papers for Journal Club

The first two papers contain many of the studies that are featured in the lecture.

- Yoshigi M, Hoffman LM, Jensen CC, Yost HJ, Beckerle MC. Mechanical force mobilizes zyxin from focal adhesions to actin filaments and regulates cytoskeletal reinforcement. J Cell Biol. 2005 Oct 24;171(2):209-15.
- Hoffman LM, Jensen CC, Kloeker S, Wang CL, Yoshigi M, Beckerle MC. Genetic ablation of zyxin causes Mena/VASP mislocalization, increased motility, and deficits in actin remodeling. J Cell Biol. 2006 Feb 27;172(5):771-82.

These next two papers were published after the iBioseminars lecture was taped, and expand upon the role of zyxin in mechanotransduction.

- Hiroaki Hirata, Hitoshi Tatsumi and Masahiro Sokabe. Mechanical forces facilitate actin polymerization at focal adhesions in a zyxin-dependent manner. Journal of Cell Science 121, 2795-2804 (2008)
- Hiroaki Hirata, Hitoshi Tatsumi, and Masahiro Sokabe. Zyxin emerges as a key player in the mechanotransduction at cell adhesive structures. Commun Integr Biol. 2008 Oct–Dec; 1(2): 192–195.