# Under Tension: Kinetochores and Basic Research

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THE Genetics Society of America's Edward Novitski Prize recognizes an extraordinary level of creativity and intellectual ingenuity in the solution of significant problems in genetics research. The 2015 winner, Sue Biggins, has made significant contributions to our understanding of how chromosomes attach to the mitotic spindle, a process essential for cell division and frequently impaired in cancer. Among other achievements, Biggins was the first to demonstrate that the Aurora B protein kinase is a key regulator of kinetochore function and that chromatin composition and centromere identity can be regulated by histone proteolysis. In 2010, Biggins and her colleagues were the first to purify kinetochores and, using this system, have already made several groundbreaking discoveries about the function and structure of these crucial components of the segregation machinery.

It is easy to forget that basic research on a simple model organism has led to many fundamental insights about how cells work and what goes wrong in disease, especially with the continual pressure from funding agencies and institutions to perform translational research. It is also easy to make the mistake of thinking that all major discoveries using model organisms have already been made.

In his Novitski prize essay last year, Charlie Boone noted that the yeast *Saccharomyces cerevisiae* is better understood than any other cell. This year, I am honored to receive the same award for work that exploited yeast's powerful combination of relative simplicity and strong conservation of function. In a collaborative effort with Chip Asbury's lab, we reconstituted kinetochore–microtubule attachments that withstand tension *in vitro* for the first time (Akiyoshi *et al.* 2010). Our work is an example of how yeast can provide unexpected insights into conserved processes, and why it is important to support scientists in exploring new directions.

Many key discoveries about cell division were initially made using budding yeast. Centromeres were first identified and cloned from yeast, and this information was critical to constructing the first artificial chromosome (Clarke and Carbon 1980; Murray and Szostak 1983; Bloom 2015). Over the years, yeast genetic screens have identified most kinetochore components as well as the key pathways that regulate chromosome segregation (for reviews, see Biggins 2013; Malvezzi and Westermann 2014). Cell-cycle checkpoints were first demonstrated in this organism (Weinert and Hartwell 1988), and the majority of conserved spindle checkpoint genes were identified in two seminal genetic yeast screens (Hoyt *et al.* 1991; Li and Murray 1991).

**Isolating intact kinetochores was an intellectual and technical tour-de-force that laid the groundwork for mechanistic and proteomic analysis of kinetochore proteins. Sue Biggins' perseverance and intellectual creativity in pursuing this question produced extraordinary insights into how kinetochores interact with microtubules.**

**— Needhi Bhalla, University of California, Santa Cruz**

It has been known for decades that chromosome segregation in all organisms relies on the tension-dependent stabilization of proper kinetochore–microtubule attachments (for review, see Nicklas and Ward 1994). This behavior was attributed to a protein kinase-mediated error correction mechanism that destabilizes incorrect attachments because they lack tension (for review, see van der Horst and Lens 2014). To ultimately understand how tension regulates the kinase, I decided that we needed a system for directly manipulating tension on kinetochore–microtubule attachments *in vitro*. However, kinetochores had never been isolated from any organism; we were far from testing the regulation of error correction *in vitro*.


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I was trained as a geneticist, not a biochemist. However, the supportive culture at the "Hutch" (Fred Hutchinson Cancer Research Center) helped our lab to take a risk on something new. Bungo Akiyoshi (now at the University of Oxford) developed the first technique with which to purify the core yeast kinetochore (Akiyoshi *et al.* 2010). We got a lot of advice and support from colleagues at the Hutch with biochemistry expertise, especially from Toshi Tsukiyama. Once Bungo had optimized a protocol by which to purify kinetochores, our next step was to develop a technique for binding these kinetochores to microtubules and putting them under tension. Fortunately, we are located near Chip Asbury's lab at the University of Washington, which pioneered laser-trapping techniques to study kinetochore proteins (Asbury *et al.* 2006; Franck *et al.* 2007; Powers *et al.* 2009). Together, our labs used the purified kinetochores to reconstitute kinetochore–microtubule attachments under tension. Our reconstitution system does not include the error correction kinase or any additional cellular factors, so we were surprised to find that the kinetochore–microtubule attachments were stabilized *directly* by tension (Akiyoshi *et al.* 2010). Discoveries come from unexpected places—this was preliminary work intended as the foundation for analyzing how tension regulates the error-correction kinase.

This finding helps to explain one aspect of the long-standing observation that attachments under tension *in vivo* are stable. We do not yet know whether and how often the aneuploidy that is a hallmark of so many cancers is due to alterations in kinetochore function, but this reconstitution system can now be applied to understanding the properties of kinetochores in other organisms and in cancer cells. We have also started to use our purification technique to address other aspects of kinetochore function and structure (Gonen *et al.* 2012; London *et al.* 2012; Sarangapani *et al.* 2013; London and Biggins 2014; Sarangapani *et al.* 2014).

**It is [. . .] easy to make the mistake of thinking that all major discoveries using model organisms have already been made.**

**—S.B.**

When I first started this work, my grant renewal application did not get a fundable score because of the risky nature of the project and the lack of convincing preliminary data. Luckily, I had colleagues at the Hutch who supported our attempts to do something new despite our lack of expertise. Funding agencies often dismiss applications when the investigator isn't well versed in the necessary skills, and it is difficult for investigators to obtain money to initiate pilot projects. The current movement of the National Institute of General Medical Sciences and other institutes at the National Institutes of Health to fund investigator-initiated research as well as project-based research is a step in the right direction. We also need to promote collaborations that can help move fields forward, to integrate genetics with other disciplines, and to foster an environment where scientists can try something new. Research in model organisms will continue to provide unpredictable insights into biological processes, especially if we stay open minded to the research we fund and we continue to support investigators who take on new endeavors.

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