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# Setting a new standard for kinetochores

Two studies reassess the number of proteins at yeast kinetochores and centromeres.

To really understand how a biological structure works, we need to know not just which, but also how many, molecules are present in it. For kinetochores—the structures that link centromeric DNA to spindle microtubules—protein components have been tallied by comparing the fluorescence of GFP-tagged kinetochore proteins to fluorescent versions of the centromeric protein CENP-A, two copies of which are believed to assemble at every budding yeast centromere. Two studies now report that CENP-A is actually much more abundant at yeast centromeres, meaning that kinetochore protein numbers have previously been underestimated (1, 2).

CENP-A—known as Cse4 in budding yeast—is a variant of histone H3 that assembles into specialized nucleosomes that mark the position of centromeric chromatin. *S. cerevisiae* chromosomes have very small "point" centromeres that are thought to incorporate a single centromeric nucleosome containing two copies of Cse4 (3). This assumption has been used to estimate the numbers of kinetochore proteins that assemble on point centromeres and attach each budding yeast chromosome to a single spindle microtubule (4). Although fission yeast and vertebrates have much larger "regional" centromeres that attach to multiple microtubules, the Cse4 standard has been used to quantify kinetochore proteins in these cell types as well (5, 6).

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Jian-Qiu Wu, Valerie Coffman, and their colleagues, from The Ohio State University in Columbus, wanted to count the number of proteins in cytokinesis nodes, precursors of the cytokinetic contractile ring. But when they compared fluorescent node proteins to Cse4-GFP, their results didn't make sense. "For some proteins, there was too few, or even less than one molecule, in each node!" Wu explains. At the same time, Kerry Bloom and Ted Salmon, whose labs at the University of North Carolina, Chapel Hill had developed Cse4 as a standard for counting kinetochore proteins, became aware that several research groups were having similar problems.

Both sets of researchers decided to directly count the number of Cse4 molecules at centromeres using several alternative standards, ranging from individual GFP molecules in vitro (1) to an *E. coli* motor complex that contains 22 copies of a GFP-tagged protein (1, 2). Using these new references, the researchers found that there was more Cse4 in the vicinity of each budding yeast centromere than previously thought—5 or 6 copies according to Bloom, Salmon, and their colleague Josh Lawrimore, or even 8 molecules according to Coffman et al.

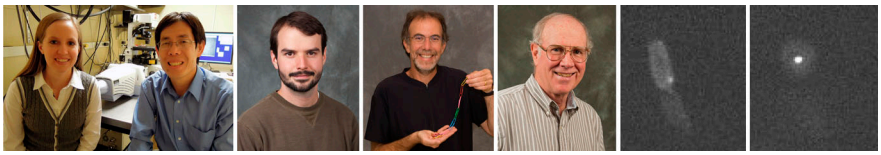
"We sat down and asked: What's the data underlying the dogma that there's one CENP-A nucleosome [at budding yeast centromeres]?" Bloom recalls. Chromatin immunoprecipitation experiments had suggested that a single CENP-A nucleosome occupies budding yeast point centromeres (3). But computer simulations showed that this approach would miss additional CENP-A nucleosomes located at random chromatin positions on either side of the centromere (1).

Meanwhile, Coffman et al. found that fission yeast have around 40 times more CENP-A at their centromeres than expected (2); previous estimates had suggested that, although *S. pombe* centromeres are much larger than budding yeast point centromeres, they only incorporate 2–3 CENP-A nucleosomes to nucleate attachments to 2 or 3 spindle microtubules (5). "But there's actually many more CENP-A nucleosomes in fission yeast," says Coffman, "which makes them more similar to human centromeres."

Budding and fission yeast kinetochore protein quantities also increased when recounted using the new standards, which helps explain how kinetochores hold on to dynamic spindle microtubule ends. "[The kinetochore] needs many transient attachment sites so that it rarely lets go," Salmon explains. Previous estimates had suggested that kinetochore microtubule-binding proteins such as Ndc80 and *S. pombe* Dam1 were too few in number to maintain robust dynamic attachments to a growing or shrinking microtubule, but the new figures indicate these proteins are present in sufficient quantities.

"We've learned a great deal about kinetochores in recent years," Salmon continues. "Now we have to build more detailed mechanistic models of protein function."

## Focal Point



Two groups of researchers (left to right), one led by Valerie Coffman and Jian-Qiu Wu and the other comprising Josh Lawrimore, Kerry Bloom, and Ted Salmon, demonstrate that the histone variant CENP-A is more abundant at budding yeast centromeres than previously thought. By comparing the fluorescence of GFP-tagged CENP-A (far right) to fluorescent standards such as an *E. coli* motor complex containing 22 GFP molecules (second from right), the researchers estimate that there is 3–4 times more CENP-A than previously assumed. Because CENP-A itself has been used as a standard to count kinetochore proteins, the new data also increase the abundance of proteins in these structures, boosting efforts to model kinetochore protein organization and function.

COFFMAN AND WU PHOTO COURTESY OF HU LEE; BLOOM PHOTO COURTESY OF SUSAN WHITFIELD; OTHER PHOTOS COURTESY OF THE AUTHORS

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