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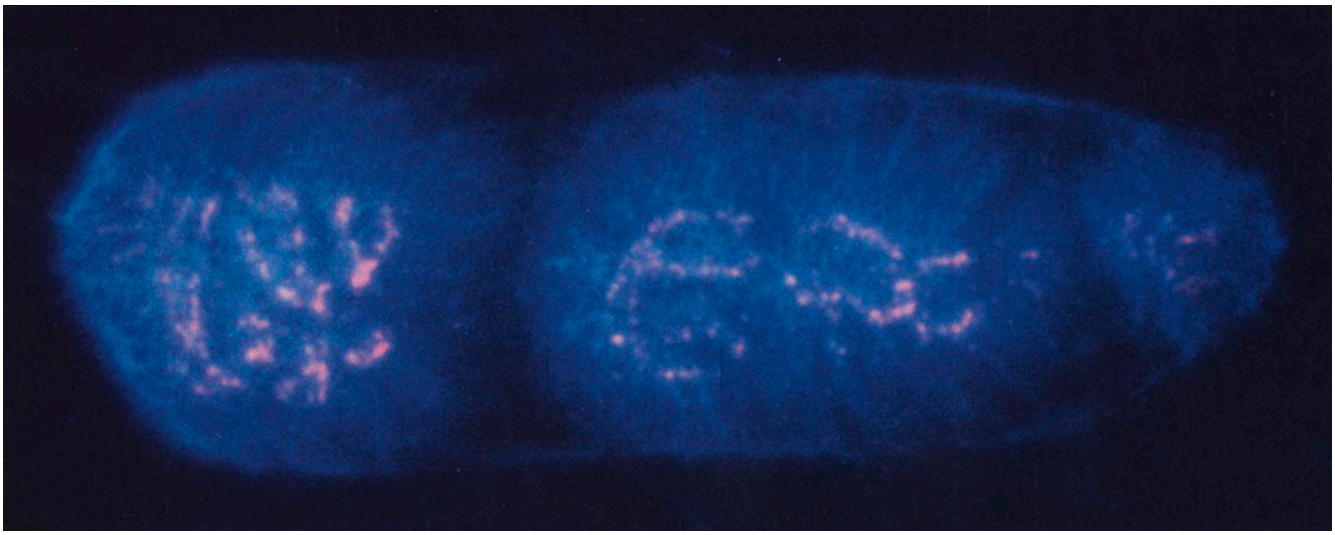
Building a case for the chromosome scaffold



EARNSHAW

Topo II (pink) is localized to the axes of mitotic chromosomes.

When William Earnshaw struck out from his postdoc with Ulrich Laemmli for his new lab at Johns Hopkins University (Baltimore, MD) in 1981, he took with him Laemmli's rather controversial idea that a chromosome scaffold of nonhistone proteins might be responsible for the radial loop structure of chromatin. Laemmli's studies of metaphase chromosomes, which had been depleted of histones, supported such a model (Paulson and Laemmli, 1977; Marsden and Laemmli, 1979).

William Earnshaw and Margarete Heck localize topo 2 to the base supports of the radial loops of chromatin.

At Hopkins, Earnshaw soon learned, "people used antibodies for everything," so he followed suit. After eight months spent isolating human chromosomes, digesting the DNA, then extracting away the more soluble proteins, he had three presumptive scaffold protein bands with which to immunize guinea pigs. Paranoid, he spray painted dots on the guinea pigs' backs so as not to lose track of them (it paid off when one ended up in someone else's cage).

He found a protein that reproducibly turned up on mitotic chromosomes and in subcellular fractions thought to hold the scaffold components, but he had no idea what the protein was. A fortunate lunch with Leroy Liu, who worked on topoisomerase 2, an enzyme known to untangle DNA strands by cutting and religation, led to some Western blot swapping that revealed Earnshaw's protein as topo 2 (Earnshaw et al., 1985). It was the first localization of a nonhistone protein to mitotic chromosomes.

Further investigation with his collaborator Margarete Heck localized topo 2 to the base supports of the radial loops of chromatin by immunofluorescence. The antibody did not cause global condensation of chromosomes in vitro as did other DNA-binding antibodies. Most bivalent DNA antibodies would bind along all lengths of chromatin and cause condensation through cross-linking. With anti-topo 2, only very localized condensation was seen along the axial regions of chromosomes, and Earnshaw argued that the protein must be stably localized there (Earnshaw and Heck, 1985).

"We now know that it probably doesn't have a structural role," says Earnshaw. Instead, topo 2 is required for untangling chromosomes and appears to be involved in compaction, perhaps in concert with the condensin complex. But the work opened the door for the scaffold hypothesis to flourish. At the time, critics said that the scaffold proteins might simply be a precipitation artifact. Now, the scaffold is envisioned as a protein core or network that regulates the higher-order structure of chromosomes—possibly by binding stretches of DNA called scaffold attachment regions that can be up to 100 kb apart. This creates a loop of histone-wound DNA (for review see Swedlow and Hirano, 2003). Recent work from the Earnshaw lab has shown that knocking out a key member of the condensin complexes can abolish the entire chromosome scaffold (Hudson et al., 2003).

An image from the 1985 study became, in January 1986, the first picture ever used on the cover of the *JCB*. Earnshaw says the paper's most lasting legacy may be the first use of antibodies to study the structure of chromosomes. JCB

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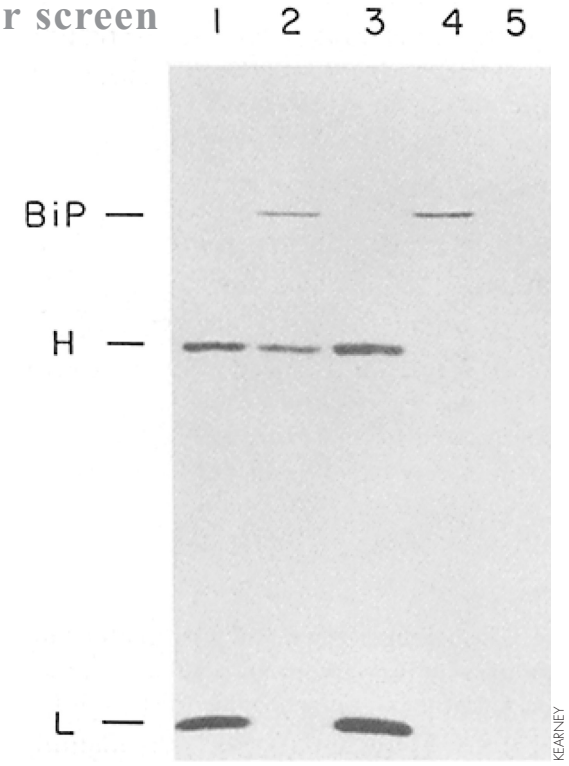
A big BiP on the radar screen

David Bole and John Kearney track BiP movements and gather evidence for its role as a chaperone.

The discovery of immunoglobulin heavy chain binding protein (BiP) in antibody-producing cells (Morrison and Scharff, 1975; Haas and Wabl, 1984) had researchers trying to assign an immune function to it. In one theory, BiP was thought to regulate allelic exclusion of heavy and light chain genes (Wabl and Steinberg, 1982). Part of the theory assumed that BiP neutralized a proposed heavy chain toxicity. If a cell was making heavy chains improperly from both alleles, then there would not be enough BiP to go around and the cells would die and be eliminated from the B lymphocyte pool.

But John Kearney (University of Alabama, Birmingham, AL) had been working with pre–B cell hybridomas that only produced heavy chains yet suffered no toxic effect, so he questioned the toxicity idea. He started by making a BiP antibody. The unexpected endpoint would be the competitive area of chaperone biology.



BiP association (top) disappears once light chain shows up (bottom).

A hybridoma expert, Kearney, along with graduate student David Bole, immunized rats with the mouse BiP-heavy chain complex and made a monoclonal antibody that recognized both free BiP and BiP bound to its target molecule. The team, eventually joined by postdoc Linda Hendershot, used the antibody to follow BiP in two cell lines—a nonsecretor and a secretor—to see how it interacted with Ig molecules at different stages of completion. In the cell line that produced only nonsecreted Ig heavy chains, BiP was stably associated with the heavy chains. But in the cell line that secreted completed Ig complexes of two light and two heavy chains, BiP dissociated from the Ig complex once heavy chains became associated with light chains (Bole et al., 1986). Furthermore, in the secreting line, BiP stayed associated with all of the Ig intermediates until the last light chain was added. They concluded that BiP prevented the premature secretion of incomplete Ig molecules.

The group also localized BiP to the rough ER. This, along with the lab's unpublished observations that BiP was showing up in every imaginable cell type and in all species tested (even lobsters with no immune system), catapulted the immunologists into a raging cell biology debate about protein transport from the ER. Did receptors carry proteins forward, or was there bulk flow with a retention mechanism for unfolded proteins?

"Here," says Hendershot, now at St. Jude's Children's Hospital (Memphis, TN), "we had an ER protein that associated with every intermediate, but not with the completely assembled complex," thus bolstering the idea of retention coupled with bulk flow. She recalls it being a very intimidating time for the "outsider" lab that had identified what soon turned out to be the first mammalian ER chaperone protein. They had lost the unstable anti-BiP hybridomas, so Hendershot was giving the antibody out in "dribs and drabs" to the "Who's Who of cell biologists" requesting it. She remembers a few investigators "just stopping by" Birmingham to drop in on the lab.

Just a few months later, the cloning of BiP from rat liver revealed its homology to the heat–shock proteins (Munro and Pelham, 1986)—a group of proteins that both Hugh Pelham and John Ellis had suggested might be involved in regulating protein folding and oligomeric assembly (Pelham, 1986; Ellis, 1987). The term "molecular chaperone" had been used as early 1978 to describe nucleoplasmin's role in overseeing histone–histone interactions. But Ellis expanded its use in 1987 to encompass this emerging family of proteins and their ability to supervise "improper interactions" between incompletely folded proteins.

Henderson continued to pursue BiP. She and colleagues showed that deletion of the BiP binding site on heavy chains resulted in secretion of intermediate Ig complexes (Hendershot et al., 1987), which "nailed down the idea of retaining proteins." At the same time, sequence mutations of BiP and other resident ER proteins defined the KDEL retention signal (Munro and Pelham, 1987). JCB

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