New gels shed light on cancer-related protein

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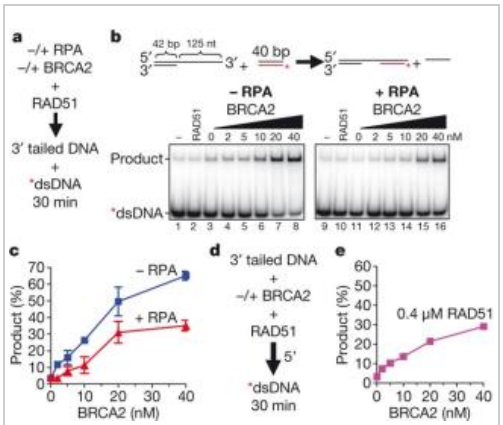
After years of trying, Ryan Jensen has successfully purified BRCA2, the DNA repair protein associated with breast cancer cases.

Ryan B. Jensen, a post-doctoral fellow at the University of California, Davis (UC Davis), has purified BRCA2, a protein notorious for both its instability and its connection to breast and ovarian cancers, opening the door for greater understanding of cell regulation and possible cancer therapies.

While most cancers are associated with mutations in multiple genes, BRCA2 is associated with over 50% of hereditary breast and ovarian cancers. “It’s kind of rare to have a single gene lead to such a high risk for cancer,” Jensen told *BioTechniques*. “That’s why people have been trying so hard to purify BRCA2 for the past fifteen years.”

Although many laboratories have tried to purify BRCA2 to ascertain its particular biochemistry in an attempt to understand why this protein is implicated in so many hereditary cases of breast cancer, none have been successful until now. Previous to Jensen’s work, the closest any group has come to purification was the identification of the C-terminus, barely one third of the protein.



BRCA2 preferentially binds tailed and ssDNA strands over dsDNA and stimulates DNA strand exchange promoted by RAD51. Source: Nature.

BRCA2 is difficult to purify for several reasons, according to Jensen. One reason is its large size; it’s one of the largest proteins in the cell, consisting of 3418 amino acids, which makes it almost a 400-kDa protein. Also, the endogenous protein is expressed at very low levels in human cell lines, making BRCA2 difficult to detect. “Another bonus is that it tends to degrade very easily,” says Jensen. “We think it likes to be bound to other proteins; it doesn’t like to be by itself.” Despite these difficulties, researchers from around the world continued to try purifying the protein because the potential benefits were too great.

After three years and many unsuccessful expression systems, Jensen decided to rely on his background in mammalian cell cultures and attempted to express the protein in human cells. Even though making a large molecule even larger was counterintuitive, Jensen added two maltose binding proteins (MBP) tags, each 40-kDa, before the N-terminus; with this addition, Jensen was finally able to visualize the protein on a Western blot.

Jensen’s next hurdle was to track the purification of the BRCA2 protein on SDS-PAGE. In addition to its large size, BRCA2 does not have any intrinsic biochemical activity that can be used to track the protein, and Jensen was having difficulty pouring a gel that was sensitive enough to detect it. As if it were dictated by fate, Bio-Rad contacted him to beta test their new mini-PROTEAN TGX (Tris-Glycine eXtended) precast gels. Not only are these precast gels sensitive enough to detect BRCA2, they provided Jensen with a higher resolution than hand-poured gels could provide. “I used TGX gels for all of the figures in my paper, which was a little bit challenging because I wanted nice resolution of BRCA2, which is 470-kDa, and also RAD51, which is [only] 37kDa. The gels worked great for that.”

After successfully purifying BRCA2, Jensen was able to characterize its biochemistry. BRCA2 aids RAD51 in the repair of double-stranded DNA (dsDNA) breaks. BRCA2 also blocks RAD51 from binding to dsDNA, thereby focusing its repair activity on 3’ tailed, 5’ tailed, or ssDNA in need of attention.

Jensen and his coworkers in Stephen Kowalczykowski’s lab at UC Davis have many plans for future BRCA2 assays. They hope to use electron microscopy (EM) or small-angle x-ray scattering (SAXS) to take a picture and determine the structure and folding pattern of BRCA2. Additionally, they hope to use their lab’s specialty, single molecule analysis, to fluorescently tag BRCA2 and watch it interact with RAD51 and DNA in real time.

In terms of cancer, researchers can now alter the BRCA2 protein to reflect the cancer-associated mutations seen in patients. These mutated proteins can then be purified to assess how their biochemistry differs from the wild type protein. Since BRCA2 is so large, its applications to gene therapy are limited; however, purification has led to a greater understanding of its functions, which can themselves be targeted to enhance the benefits of chemotherapy and radiation

therapy from killing cells.

“[The cancer cells] are already having trouble repairing DNA damage from chemotherapy and radiation therapy; now, let’s just push them over the edge so that they have no chance of repairing the damage. It’s a tumor-selective killing strategy, which is what we want to do for cancer therapy.”

The paper, “Purified human BRCA2 stimulates RAD51-mediated recombination,” was published 22 August 2010 at *Nature*.

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