Scientists develop novel technique to map protein interactions leading to better understanding of disease mechanisms

March 15 2012

Scientists have developed a powerful new technique, named BioID, to screen for both interacting and neighboring proteins in their native cellular environment. Elucidating protein interactions is key to better understanding disease mechanisms and developing therapies. This work, headed by Dr Brian Burke, Principle Investigator at the Institute of Medical Biology (IMB) under the Agency for Science Technology and Research (A*STAR) and Dr Kyle Roux, Associate Scientist at the Sanford Children's Health Research Centre, was published in the *Journal of Cell Biology* on March 12.

The scientists chose to use the protein Lamin-A (LaA) to demonstrate the utility of BioID, as LaA is encoded by the LMNA gene, which holds the record for the greatest number of mutations associated with multiple human diseases, including muscular dystrophy, lipodystrophy, and premature aging syndromes. Being able to identify lamin-interacting proteins is crucial to better understand the mechanisms of these rare genetic disorders caused by mutations in LMNA.

Unfortunately, it is extremely difficult to identify LaA interacting proteins using currently existing methods for probing protein-protein interactions such as biochemical approaches or yeast two-hybrid screens. Current biochemical approaches are limited by protein solubility and can miss weak or transient interactions while existing yeast two-hybrid screens assess protein interactions in a non-native cell environment

which can lead to erroneous and incomplete data.

The scientists developed this novel BioID technique and successfully applied it to LaA. The advantages of this technique over existing methods is evident, given that BioID was not only able to detect many proteins already known to associate with LaA, the scientists also discovered a previously uncharacterized protein, which they identified as a novel constituent of the nuclear envelope. This is because BioID uniquely combines two important attributes; it detects potential interactions in their normal cellular context and sidesteps issues associated with protein solubility.

BioID is based on a mutant biotin protein ligase (BirA*) that promiscuously biotinylates (adds a biotin 'label') proteins in a proximity-dependent fashion. This means that the mutant BirA* is very unselective and will biotinylate all the proteins it comes into contact with. The scientists fused BirA* to LaA (the protein of interest), forming a fusion protein, and then introduced the fusion protein into mammalian cells where it biotinylated (labeled) proteins that are proximate to and/or interact with LaA. These biotinylated proteins can then be separated from the complex mixture of proteins very easily as biotin binds very strongly to a protein called Streptavidin. This literally allows scientists to pick the biotinylated proteins out like a magnet picking out iron pieces amongst non-magnetic materials. This easy, single purification step (usually a major difficulty), is another benefit of the BioID technique.

"Our findings highlight the use of BioID as a valuable complement to the earlier studies and confirms the use of BioID as an effective proximity-based tool to screen for neighbor and potentially interacting proteins. Beyond mammalian cells, BioID can be applied to any cell type and organism as well as to any target protein, not just LaA. We will continue to explore the advantages intrinsic to the BioID system which will aid us in better understanding complex biological processes and disease

mechanisms," said Dr Brian Burke, lead author of the paper.

Prof Birgitte Lane, Executive Director of IMB, said, "Mapping protein interaction networks is important because it allows us to identify new protein targets for therapeutics development. However, unraveling the protein 'interactome' remains one of the biggest challenges in cell and molecular biology. We believe that BioID will be a valuable addition to existing analytical methods, and we expect it will be widely adopted in cell and molecular biology labs as a tool to help understand disease mechanisms and develop innovative healthcare strategies."

More information: The research findings described in this news release can be found in the 12 Mar 2012 issue of *Journal of Cell Biology* under the title, "A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells" by Kyle J. Roux et al. jcb.rupress.org/content/early/ … 6/jcb.201112098.full

Provided by Agency for Science Technology and Research (A*STAR)