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Extended Abstract
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Bacteriology 2019: Identification of novel P. falciparum Kelch13 interacting partners via Co-immunoprecipitation (Co-IP) and mass spectrometry- Atul- National Institute of Malaria Research, IndiaPramathadhip Paul

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The emergence of resistance against the frontline antimalarials- Artemisinin (ART) combination therapies (ACTs) is of great concern for South East Asia region and in light of recent reports from India, too. Phenotypically, the delayed parasite clearence represents ART resistance and the association of *P. falciparum* Kelch 13 (*Pf*K13) gene mutations with ART resistance have drawn focus of researchers towards delineating the role of *Pf*K13 in malaria life cycle and in emergence of ART resistance. The kelch motif of *Pf*K13 protein is well conserved among all the *Plasmodium* spp. We have identified three novel proteins through co-immunoprecipitation of *Pf*K13-interacting partners with anti-*Pf*K13 antibodies and further analysis *via* mass spectrometry. These proteins have been previously implicated in critical life processes of malaria parasite viz. 1) thioredoxin-like mero protein (PF3D7_1104400)- reported for a crucial role in erythrocyte invasion; 2) pyridoxal kinase (PF3D7_0616000)- potential involvement in detoxification of reactive oxygen species and 3) trafficking protein particle complex subunit 3, (PF3D7_0418500)- putative role in vesicular trafficking. Homology modeling and protein-protein docking results have also identified highly conserved residues in the shallow pocket of the kelch domain of *Pf*K13, as a hot-spot for binding with other interacting proteins. Understanding the functional importance of these protein-protein interactions (PPIs) would provide significant molecular insights in delineating the role of *Pf*K13, in emergence of ART resistance.

Types of immunoprecipitation

Individual protein immunoprecipitation

Involves using an antibody that is specific for a known protein to isolate that particular protein out of a solution containing many different proteins. These solutions will often be in the form of a crude lysate of a plant or animal tissue. Other sample types could be body fluids or other samples of biological origin.

Protein complex immunoprecipitation

Immunoprecipitation of intact protein complexes (i.e. antigen along with any proteins or ligands that are bound to it) is known as co-immunoprecipitation (Co-IP). Co-IP works by selecting an antibody that targets a known protein that is believed to be a member of a larger complex of proteins. By targeting this *known* member with an antibody it may become possible to pull the entire protein complex out of solution and thereby identify *unknown* members of the complex.

This works when the proteins involved in the complex bind to each other tightly, making it possible to pull multiple members of the complex out of solution by latching onto one member with an antibody. This concept of pulling protein complexes out of solution is sometimes referred to as a "pull-down". Co-IP is a powerful technique that is used regularly by molecular biologists to analyze protein–protein interactions.

- A particular antibody often selects for a subpopulation of its target protein that has the epitope exposed, thus failing to identify any proteins in complexes that hide the epitope. This can be seen in that it is rarely possible to precipitate even half of a given protein from a sample with a single antibody, even when a large excess of antibody is used.
- As successive rounds of targeting and immunoprecipitations take place, the number of identified proteins may continue to grow. The identified proteins may not ever exist in a single complex at a given time, but may instead represent a network of proteins interacting with one another at different times for different purposes.
- Repeating the experiment by targeting different members of the protein complex allows the researcher to double-check the result. Each round of pull-downs should result in the recovery of both the original known protein as well as other previously identified members of the complex (and even new additional members). By repeating the immunoprecipitation in this way, the

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researcher verifies that each identified member of the protein complex was a valid identification. If a particular protein can only be recovered by targeting one of the known members but not by targeting other of the known members then that protein's status as a member of the complex may be subject to question.

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) is a method used to determine the location of DNA binding sites on the genome for a particular protein of interest. This technique gives a picture of the protein–DNA interactions that occur inside the nucleus of living cells or tissues. The in vivo nature of this method is in contrast to other approaches traditionally employed to answer the same questions.

The principle underpinning this assay is that DNA-binding proteins (including transcription factors and histones) in living cells can be cross-linked to the DNA that they are binding. By using an antibody that is specific to a putative DNA binding protein, one can immunoprecipitate the protein–DNA complex out of cellular lysates. The crosslinking is often accomplished by applying formaldehyde to the cells (or tissue), although it is sometimes advantageous to use a more defined and consistent crosslinker such as DTBP. Following crosslinking, the cells are lysed and the DNA is broken into pieces 0.2–1.0 kb in length by sonication. At this point the immunoprecipitation is performed resulting in the purification of protein–DNA complexes. The purified protein–DNA complexes are then heated to reverse the formaldehyde cross-linking of the protein and DNA complexes, allowing the DNA to be separated from the proteins. The identity and quantity of the DNA fragments isolated can then be determined by PCR. The limitation of performing PCR on the isolated fragments is that one must have an idea which genomic region is being targeted in order to generate the correct PCR primers. Sometimes this limitation circumvented simply by cloning the isolated genomic DNA into a plasmid vector and then using primers that are specific to the cloning region of that vector. Alternatively, when one wants to find where the protein binds on a genome-wide scale, ChIP-Sequencing is used and has recently emerged as a standard technology that can localize protein binding sites in a high-throughput, cost-effective fashion, allowing also for the characterization of the cistrome. Previously, DNA microarray was also used (ChIP-on-chip or ChIP-chip).

RNP Immunoprecipitation

Similar to chromatin immunoprecipitation (ChIP) outlined above, but rather than targeting DNA binding proteins as in ChIP, an RNP immunoprecipitation targets ribonucleoproteins (RNPs). Live cells are first lysed and then the target protein and associated RNA are immunoprecipitated using an antibody targeting the protein of interest. The purified RNA-protein complexes can be separated by performing an RNA extraction and the identity of the RNA can be determined by cDNA sequencing or RT-PCR. Some variants of RIP, such as PAR-CLIP include cross-linking steps, which then require less careful lysis conditions.

Tagged proteins

One of the major technical hurdles with immunoprecipitation is the great difficulty in generating an antibody that specifically targets a single known protein. To get around this obstacle, many groups will engineer tags onto either the C- or N-terminal end of the protein of interest. The advantage here is that the same tag can be used time and again on many different proteins and the researcher can use the same antibody each time. The advantages with using tagged proteins are so great that this technique has become commonplace for all types of immunoprecipitation including all of the types of IP detailed above. Examples of tags in use are the Green Fluorescent Protein (GFP) tag, Glutathione-S-transferase (GST) tag and the FLAG-tag tag. While the use of a tag to enable pull-downs is convenient, it raises some concerns regarding biological relevance because the tag itself may either obscure native interactions or introduce new and unnatural interactions.