Identification of new medicines for novel pathogen protein kinases with substrate-dependent assays

Protein kinases (PKs) are enzymes that transfer a phosphate group, a process called phosphorylation, from the ATP to another protein, which acts as a phosphoacceptor (Fig. 1). Phosphorylation of proteins regulate their activity, and these reactions are necessary for signaling events within the cell. PKs represent a large class of enzymes, and drug discovery success targeting PKs for cancer has led to an interest in targeting PKs for infectious diseases.

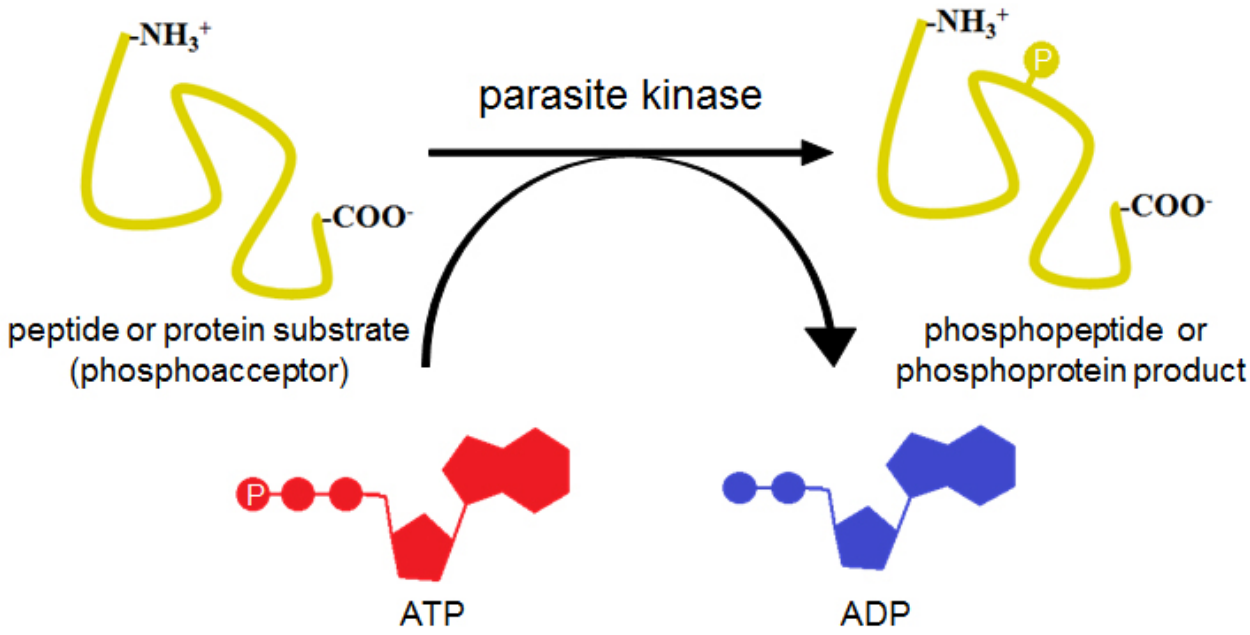

Fig. 1. The biochemical reaction catalyzed by a protein kinase (PK). A phosphate group is transferred from a molecule of ATP to an amino acid (Ser, Thr, Tyr) on phosphoacceptor protein (or peptide in an in vitro PK assay), to produce a phosphoprotein and a molecule of ADP. PK reactions are necessary for many processes in the cell including intracellular signaling.

Targeted drug discovery programs test molecules to be new potential drugs by measuring inhibition of an enzyme target (in this case PKs) as a first step towards identification of a new medicine. The therapeutic hypothesis is that inhibition of the PK by a specific molecule will be useful to treat the disease. PKs are a relatively large enzyme family in many parasites (Fig. 2). However, when targeting PKs from organisms that cause infectious diseases, a method to test inhibitors may not exist because the specific phosphoacceptor protein that is a substrate for the parasite protein kinase is not known. Methods to discover and acquire physiological phosphoacceptor proteins is costly and time-consuming, so, in infectious diseases, where the specific function of the PK is not

needed and resources are limited, alternative methods to test PKs is preferred. Several methods to overcome this obstacle in PK TDD have been used in our laboratory and others, including the use of autokinase assays, generic phosphoacceptors, genomically informed specific phosphoacceptors, cell lysates and library arrays.

In a process called autophosphorylation, several PKs can serve as their own phosphoacceptor protein. Inhibitors of PKs from parasitic organisms, including the causative agents of malaria and amoebiasis, have been discovered and tested by this method. The ability to undergo autophosphorylation eliminates the need for finding a substrate, however not all PKs are capable of auto-phosphorylation.

There are several proteins and portions of proteins called peptides generic phosphoacceptors/substrates, to substitute for physiological substrates in PK assays. Genetic data can be used to gauge the similarity of a new parasite PK to a known human PK. This information can be used to inform the choice of generic phosphoacceptor/substrates for a new uncharacterized PK. This strategy has been described for PKs from various organisms, including bacteria, protozoa, and worm pathogens. We have successfully employed this strategy with the substrates casein and myelin basic protein on two new kinases, TLK1 and MAPK6, from *Trypanosoma* brucei, the etiological agent of African sleeping sickness.These generic phosphoacceptor proteins are commercially available and relatively inexpensive. When generic substrate techniques do not work, the genetic similarity of the unknown PK to known PKs can identify active substrates which have worked in previous experiments with the related human PK.

When the aforementioned methods fail, the whole cells of a parasite can be lysed and used as a mixture to test activity of a new PK in lieu of substrate. The cells need to be deactivated so PKs that naturally occur in the cells do not interfere with the desired assay.

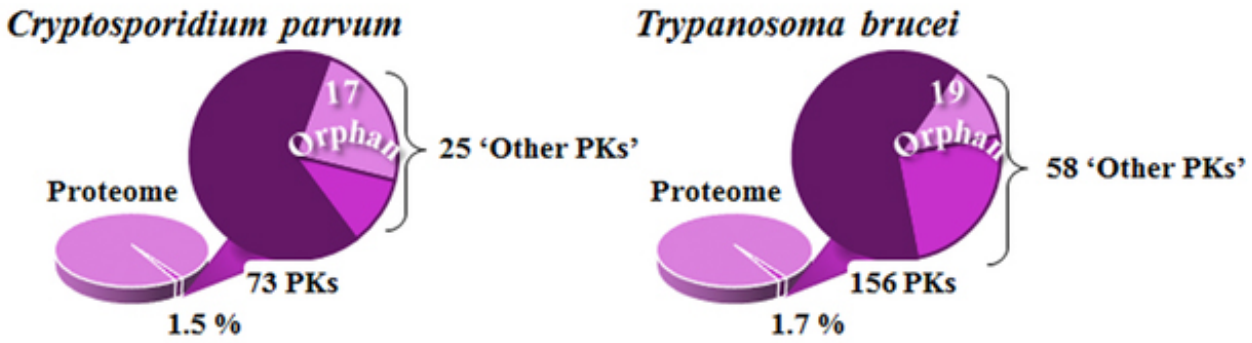

Fig. 2. Opportunities for targeting protein kinases (PKs) for medicines towards two protozoan parasites, Cryptosporidium parvum and Trypanosoma brucei. PKs are a comparatively large enzyme family in these parasites. However, a large portion of these do not fit into subfamilies of PKs (other PKs), and a portion of these do not have known genomic analogs (orphan PKs).

Commercial and custom-made arrays are available with protein and peptide libraries, which can aid in the discovery of active phosphoacceptors. This method has been used for many types of PKs, and was helpful to discover active substrates for orphan PKs of *Plasmodium falciparium*, the etiological agent of malaria, and FIKK kinases, which are only found in *P. falciparum* and other Apicomplexans in which similar human PKs do not exist.

There is no consensus way to identify active substrates, but these methods to develop PK enzyme assays can reduce costs for neglected diseases drug discovery.

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Publication

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