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Molecular basis of pathogenic parasitic infections: insights from parasite kinome

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1. ABSTRACT

Infectious diseases caused by numerous parasitic pathogens represent a global health conundrum. Several animal and plant pathogens are responsible for causing acute illness in humans and deadly plant infections. These pathogens have evolved a diverse array of infection strategies and survival methods within the host organism. Recent research has highlighted the role of protein kinases in the overall virulence and pathogenicity of the pathogens. Protein kinases (Pks) are a group of enzymes known to catalyse the phosphorylation of a wide variety of cellular substrates involved in different signalling cascades. They are also involved in regulating pathogen life cycle and infectivity. In this review, we attempt to address the role of parasite kinome in host infection, pathogen survival within the host tissue and thereby disease manifestation. The understanding of the parasite kinome can be a potential target for robust diagnosis and effective therapeutics.

2. INTRODUCTION

Protein phosphorylation is a reversible post-translation modification mediated by kinases. This process plays a crucial role in gene regulation as many receptors and activators of signalling pathways are activated/deactivated upon phosphorylation/dephosphorylation. Kinases phosphorylate numerous proteins which further act as factors regulating the expression of certain specific genes. Protein kinases (PKs) represent a large family of homologous proteins having a conserved catalytic domain containing ~ 250-300 amino acid residues (1). PKs are involved in numerous cellular activities as they control and

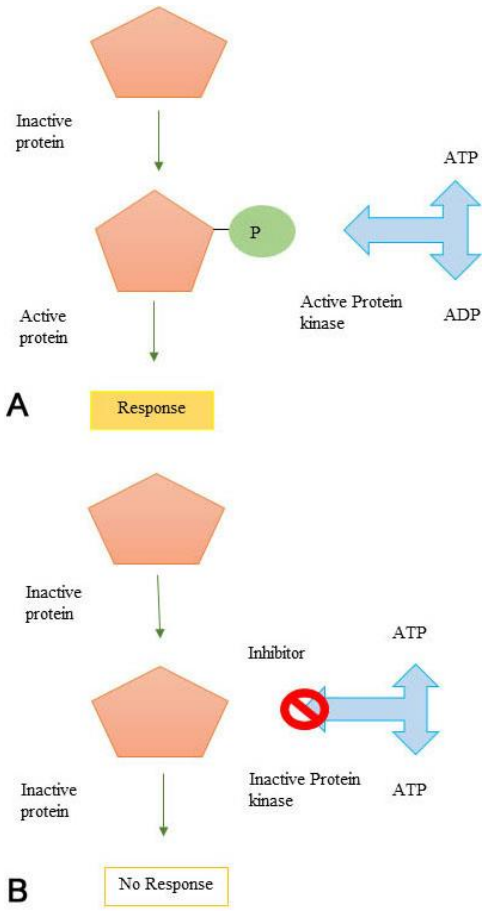

Figure 1. a. In the absence of an inhibitor, protein kinase phosphorylates the inactive protein and convert them into active forms. B. In the presence of an inhibitor molecule protein kinase is not able to phosphorylate the protein, thus the protein remains in its inactive form.

regulate a wide array of signal transduction cascades. PKs chemically modify other molecules, mostly proteins, by adding a phosphate group from nucleoside triphosphate like ATP/GTP to the hydroxyl group of the specific amino acids. Hence, PKs are often referred to as phosphorylating enzymes. Depending upon the residue which they phosphorylate, kinases are grouped into different categories. The kinases which act on both serine and threonine are known as Serine/Threonine kinases (Ser/Thr kinases) while those acting on tyrosine residues are referred to as tyrosine-kinases (Tyr kinases) and the kinases which act on all three residues are known as dual-specificity kinases (2). PKs acting on histidine residues are known as histidine kinases (3). Eukaryotic protein kinases (ePKs) are divided into several groups like casein kinases (CK1), CMGC group (CDK [cyclin-dependent kinases], MAPK [mitogen-activated protein kinases], GSK3 [glycogen synthase kinase 3] and CLKs [CDK-like kinases]), Tyrosine-kinase-like (TKL), AGC PKA [cyclic-adenosine-monophosphate-dependent protein kinase], PKG [cyclic-guanosine mono-phosphate-dependent protein kinase], PKC [protein kinase C] and related proteins), Calcium/calmodulin-dependent kinases (CamK), STE (PKs acting as regulators of MAPKs and first identified in a genetic screen of sterile yeast mutants), TyrK (tyrosine kinases). ePKs which do not fit into the above seven groups are referred to as other protein kinases (OPKs) (12).

The activity of PKs is a highly regulated process where they can interact with the secondary messengers as well as other kinases to regulate different cellular responses and the signalling pathways (4). About 2% of the human genome constitute for genes encoding PKs i.e. there are approximately 518 protein kinase encoding genes in the human genome. Protein kinases also play a crucial regulatory role in bacteria and plants and belong to pseudokinase subfamily which shows unusual activities like atypical nucleotide-binding and weak or no catalytic activities (5). For instance, innate immunity in plants like *Arabidopsis sp* is based upon non-functional pseudokinase ZED1 which acts a decoy to trap HopZ1a effector protein of *Pseudomonas syringae* in the host resistance protein ZAR1 to induce an effective immune response (6). With technical advancements in molecular biology, we now have access to information regarding the complete set of PKs encoded by an organism. Recent studies on the kinome of pathogenic/parasitic organisms have been crucial in understanding the role of kinases in pathogen/parasite survival within the host and how such kinases assist in disease manifestation. Here, we discuss the role of different kinases and how they act as key molecular components in pathophysiology of some of the deadly disease conditions caused by pathogenic parasites both in humans and plants. We have also discussed how inhibition of these kinases using certain compounds canhalt disease progression in the host (Figure 1). In the present review, we have tried to provide an overview of the different classes of PKs like mitogen-activated

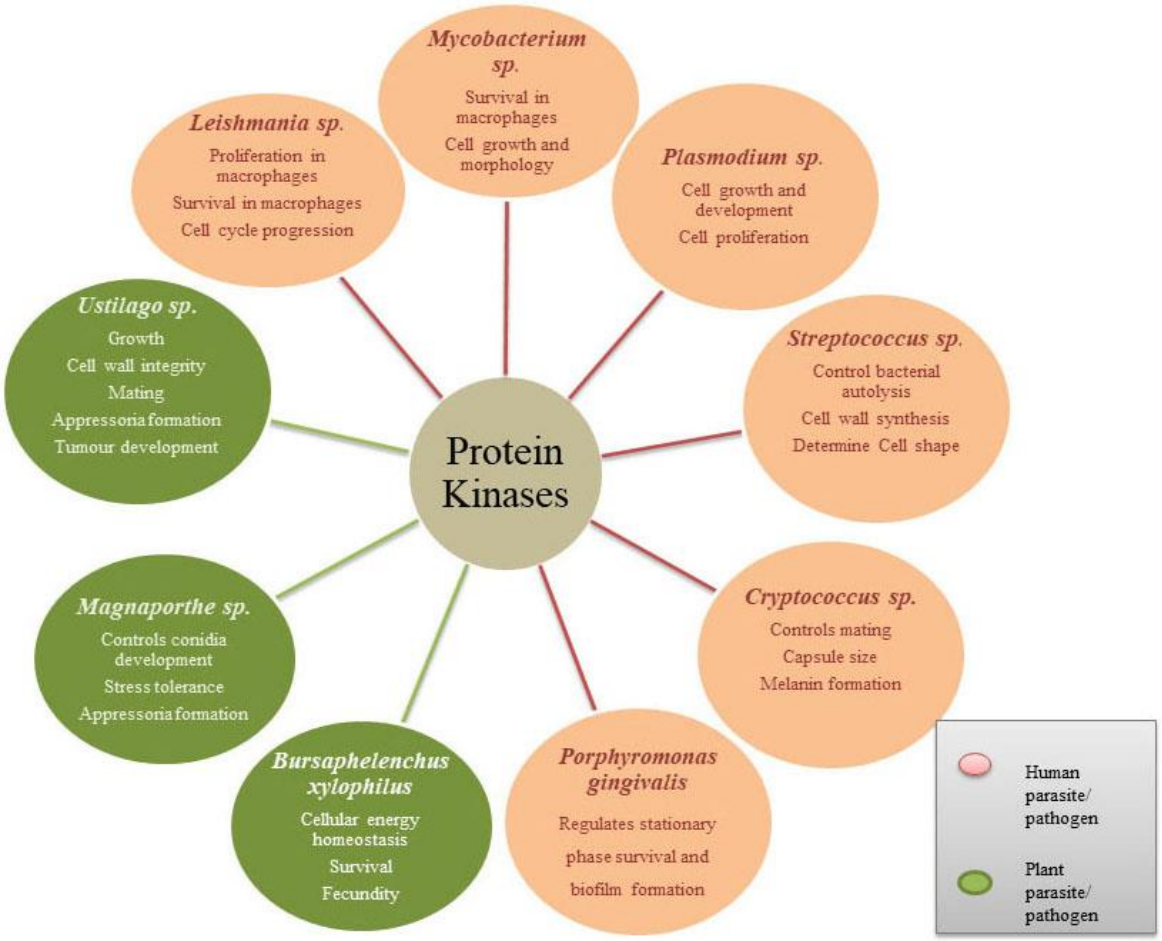

Figure 2. Role of Protein kinases in parasite/ pathogen life-cycle.

protein kinases (MAPK), Ser/Thr kinases, cyclin-dependent kinases etc. with respect to life cycle and pathogenicity of diverse animal and plant pathogens/parasites like *Plasmodium sp., Mycobacterium sp., Leishmania sp., Streptococcus sp., Cryptococcus sp., Porphyromonas sp., Magnaporthe sp., Ustilago sp* and *Bursaphelenchus sp* as outlined in Figure 2 and Table 1. Such kinome studies governing the virulence and pathogenesis of diverse parasitic pathogens holds a promising target for effective and robust therapeutic approaches in future.

3. ROLE OF PKS IN MALARIAL PARASITE, *PLASMODIUM SP.*

Malaria is a life-threatening disease caused by the bite of female Anopheles mosquito infected with *Plasmodium sp.*. Annually, it takes a heavy toll on human life and has become a global health problem especially in tropical countries (7). As per the latest reports of WHO, during the period of 2015-2017, no progress was observed as far as the global spread of Malaria is concerned (7). Development of drug resistance by the parasite against the conventional drug regime was the prime cause of the increased prevalence of the disease in recent years (7). Therefore, it is the need of the hour to develop more efficient therapeutic strategies to curtail the spread of such a deadly disease. *Plasmodium falciparum,* the most severe strain responsible for causing malaria, completes its life-cycle in two different hosts, human beings and mosquito. Human beings are the intermediate host, where the asexual

Table 1. Function of PKs in different pathogens/parasites

Protein Kinase	Function	Disease	Parasite	Reference
Parasite protein kinase 5 (PfPK5)	Activation and maintenance of S-phase of the parasite cell cycle Sensitive to cdk inhibitors like flavopiridol and olomoucine	Malaria	Plasmodium falciparum	12
P. falciparum ménage a trios 1 (PfMAT1) and *P. falciparum* MO15 related kinase (Pfmrk)	Regulate parasite cell-cycle	Malaria	Plasmodium falciparum	13
Mitogen activated protein kinases (MAPK) Pfmap-1 and Pfmap-2	Participate in cell proliferation and differentiation in response to external stimuli. Pfmap-2 is crucial for the completion of the asexual cycle of the parasite Pfmap-1 play a compensatory adaptation for Pfmap-2	Malaria	Plasmodium falciparum	14, 15
Glycogen synthase kinase (GSK-3) (PfGSK3)	Expressed during the trophozoite stage. Also transferred to erythrocyte cytoplasm where it gets associated with vesicles.	Malaria	Plasmodium falciparum	16
Calcium/calmodulin-dependent kinases (CamK) and cyclin-dependent protein kinase (cdks/ CDPK)	Involved in ookinete motility, microgamete formation and hepatocyte invasion at the sporozoite stage	Malaria	Plasmodium berghei	17, 18, 19, 20
Plasmodium falciparum cyclin-dependent protein kinases (PfCDPKs)	A major role in the asexual lifecycle of the parasite in erythrocytes CDPK inhibitors arrest the merozoite egress and proliferation of the parasite in host	Malaria	Plasmodium falciparum	21
Serine/threonine-protein kinase G (PKnG)	Block the lysosome degradation pathway. Survival and replication within the phagosomes	Tuberculosis	Mycobacterium sp.	23
Protein kinase A (PKnA) and protein kinases B (PKnB)	Play an important role in cell shape and morphology	Tuberculosis	Mycobacterium sp.	26
11 Serine/threonine-protein kinase (STPKs)	Phosphorylation of several proteins like transport proteins, lipid metabolic enzymes, chaperones along with autophosphorylation of STPKs (PKnA, PKnB, PKnD and PKnG) is crucial for pathogen survival in the host and disease progression	Tuberculosis	Mycobacterium sp.	28
MAP kinases	Responsible for the proliferation of amastigotes inside host macrophages.	Leishmaniasis	Leishmania mexicana	31
A signal-related kinase (ERK)- p38 mitogen-activated protein (MAP) kinases	Survival inside the host by hampering the host immune response against the parasite	Leishmaniasis	Leishmania sp.	32
Cyclin-dependent kinase CRK3	Regulate G2/M phase transition during cell-cycle in promastigotes. Sensitive to inhibitor, flavopiridol	Leishmaniasis	Leishmania mexicana	33
Serine/threonine kinases (StkP)	Check autolysis of bacteria inside the host and development of cell envelope. Involved in lung infection and blood stream invasion Gives Streptococcus its characteristic round shape	Pneumonia	Streptococcus pneumoniae	37,38,39,42,43,44
Protein Kinase A (PKA)	Regulates pathogenicity by controlling Melanin and capsule formation	Cryptococcal meningitis	Cryptococcus neoformans	63-65

contd...

Table 1. Contd...

Protein Kinase	Function	Disease	Parasite	Reference
Polyphosphate-kinase (PPK)	Regulates stationary phase survival and biofilm formation.	Periodontitis	Porphyromonas gingivalis	68
Kss 1 MAPK	Appressoria formation	Rice blast	*Magnaporthe oryzae*	75
MAP kinase Pmk-1	Cell to cell invasion by the pathogen. Suppression of host immune response. Prevention of plasmodesmatal callose deposition and generation of reactive oxygen species	Rice blast	*Magnaporthe oryzae*	76
MAPKs, Mps1 and Osm1	Infection and virulence	Rice blast	*Magnaporthe oryzae*	77,78
Magnaporthe oryzae Actin regulating kinase (MoArk1)	Regulation of endocytosis. Conidia development Stress tolerance	Rice blast	*Magnaporthe oryzae*	79
AGC Ser/Thr Kinase Aga1	Cell wall integrity Mating Appressoria formation Actin dependent endocytosis	Corn smut	*Ustilago maydis*	92
Protein Kinase A (PKA)	Tumour development	Corn smut	*Ustilago maydis*	94
DNA damage response kinaseAtr1 and Chk	Appressoria formation	Corn smut	*Ustilago maydis*	95, 96
Arginine Kinase (AK)	Cellular energy homeostasis Survival Fecundity	Pine wild disease	*Bursaphelenchus xylophilus*	101,104

reproduction stage occurs while the mosquito is the definitive host where sexual reproduction occurs. As the infected mosquito takes the blood meal, it delivers sporozoites from its salivary glands into the human bloodstream which then reaches the hepatocytes. Within the hepatocytes, sporozoites transform into trophozoites and with further mitotic and meiotic divisions, it forms schizont which releases numerous merozoites into the human bloodstream. The merozoites further invade the erythrocytes and manifest the disease symptoms (8). With the current advancement in the field of parasite kinome, it is evident that protein phosphorylation and dephosphorylation events play a crucial role in the pathogenicity. Further studies are needed to unravel the role of specific kinases and their substrates in the manifestation of the disease, which can be explored as a target for drugs and thus disease control.

Recent research on parasite kinome has revealed that it plays a major role in disease pathophysiology, which can be exploited as novel drug targets to combat malaria. There are studies which have revealed the role of protein kinases in the development and proliferation of *Plasmodium sp.* in the host cell (9-11). In a study by Graeser et al. (1996), the role of *Plasmodium falciparum* protein kinase 5 (PfPK5) which is a member of cyclin-dependent protein kinase (cdks) was investigated (12). It was observed that PfPK5 plays an active role in parasite nuclear divisions as well as the activation and maintenance of S-phase of the parasite cell cycle. Thus, PfPK5 can be a potential drug target corroborated by the fact that inhibitors of cdk (flavopiridol and olomoucine) used in this study could inhibit *in-vitro* DNA synthesis in parasites. Although the inhibition of other cdk-like protein kinases cannot be ruled out, the involvement of PfPK5 was justified by restricting the time of treatment of inhibitors to the time of nuclear division cycles during which PfPK5 was active in the cell (12). Such insights into the *Plasmodium* kinome have unveiled the role of different kinases in proliferation and development of parasite inside the host cell. Waters and associates

(2006) also characterised an effector molecule *P. falciparum* ménage a trios1 (PfMAT1) and *P. falciparum* MO15 related kinase (Pfmrk) whose activity is regulated by cyclins and play a major role in cell-cycle of the parasite (13).

Numerous PKs are involved in signal transduction pathways thereby regulating diverse cellular functions. One such class of PKs is Mitogen-activated protein kinases (MAPK), which are known to participate in cell proliferation and differentiation in response to external stimuli (14). Doerig et al. (2007) conducted functional studies on rat malaria model and reported two MAPK homologs Pfmap-1 and Pfmap-2 in *Plasmodium falciparum.* Using a reverse genetics approach they demonstrated that Pfmap-2 is crucial for the completion of the asexual cycle of the parasite and further Pfmap-1 exhibits a compensatory adaptation for Pfmap-2. Thus, Pfmap-1 can be a potential target for chemotherapy against malaria (15). Glycogen synthase kinase (GSK-3) is another class of kinase known for phosphorylating glycogen synthase. Drouchaeu and group studied the location, function and activity of recombinant PfGSK-3. It is an intracellular protein expressed during the trophozoite stage which is then transferred to erythrocyte cytoplasm where it gets associated with vesicles (16). They further suggested PfGSK-3 as a potential drug target to treat the disease as its activity was inhibited by certain specific inhibitors. CamK and CDPK are the most common kinases known to control numerous regulatory functions in a living system. Gene knockout experiments for these kinases were performed in *P. berghei* and they were found to be involved in ookinete motility, microgamete formation and hepatocyte invasion at the sporozoite stage (17-20). PfCDPK plays a major role in the asexual lifecycle of the parasite in erythrocytes. Treatment with inhibitors of CDPK was found to halt the merozoite egress and proliferation of the parasite in the host (21). Emerging drug resistance of the parasite against the frontline antimalarial drug regime has become a key hindrance in the global malaria eradication goals. Therefore, it is extremely important to develop a novel treatment strategy that not only cures the disease but also prevent its transmission. Alam et al. (2019) recently identified a protein kinase from the *P. falciparum* CLK3 (cyclin-dependent-like kinases) family which can be a potential drug target in the treatment of malaria (22).*Pf*CLK3 is an essential regulator of RNA processing and therefore inhibiting this key enzyme would kill the parasite in asexual stages in the blood and liver as well as halt the development of the sexual-stage gametophyte thereby blocking further transmission. They screened 24,619 compounds for their inhibitory property against the parasite kinase. TCMDC-135051 showed the highest inhibitory potency for *Pf*CLK3 and also lower activity against closely related human kinase CLK2 which shows 29% sequence similarity with *Pf*CLK3. Despite current clinical research in the field of discovering molecules against parasite kinases, there is limited progress in targeting parasite protein kinases in developing antimalarial drugs. Therefore, additional research in this field would be helpful in designing novel and more efficient therapeutic strategy against malaria.

4. ROLE OF PKS IN *MYCOBACTERIUM SP.* RESPONSIBLE FOR CAUSING TUBERCULOSIS

M. tuberculosis is the causative agent of tuberculosis (TB). The pathogen is present in the environment and is responsible for causing infectious disease affecting lungs and other parts of the body. TB is a deadly disease affecting millions of people globally (23). This disease spreads when the pathogen from the infected person is spread into the air by coughing, sneezing or talking. Despite the immaculate defence system of the host, the pathogens have evolved several mechanisms to surpass the immunity barrier of the host and successfully manifest the disease. Under normal condition, the phagocytosed pathogens are transferred to lysosomes where they are usually degraded. Such mycobacteria containing lysosomes possess certain extracellular markers like lysosomal associated membrane proteins (LAMP) and mature lysosomal hydrolases (23). However, in the case of *Mycobacterium tuberculosis* and *Mycobacterium bovis,* the pathogen resides and multiplies inside the macrophage and escapes the degradation by

lysosomes. This was explained in a study conducted by Walburger et al. (2004) where they proved that a eukaryotic-like serine/threonine-protein kinase G (PKnG) is responsible for pathogen survival within the macrophage. Using gene deletion experiments they reported that strains in which PKnG gene was deleted were non-pathogenic while strains in which the PKnG gene was present were pathogenic as they were able to block the lysosome degradation pathway and could survive and replicate within the phagosomes (24). PKnG does not play any role in normal bacterial growth, therefore in non-pathogenic bacterial strains these genes have been lost (25). Thus, the expression of eukaryotic-like serine/threonine kinases has been maintained by pathogenic *Mycobacterium sp.* for their survival by blocking fusion and degradation of phagosomes and lysosomes. In another study by Kang et al. (2005), it was demonstrated that the genome of *Mycobacterium tuberculosis* possesses 11 serine/threonine kinase genes; two among those code for protein kinase A (PKnA) and protein kinases B (PKnB) which play important role in cell shape and morphology (26). Such vast knowledge about the role of kinases and phosphorylation in mycobacterial infections has increased our understanding regarding its pathophysiology. In silico experiments conducted by Prisic and group (2010) identified 11 serine/threonine protein kinases (STPKs). They reported that phosphorylation of several proteins like transport proteins, lipid metabolic enzymes, chaperones along with autophosphorylation of STPKs (PKnA, PKnB, PKnD and PKnG) are crucial for pathogen survival within the host and disease progression (27). Therefore, with increased cases of drug-resistant tuberculosis, we need to develop novel compounds which can target the bacterial kinases and reduce our dependency on conventional drug regime.

5. ROLE OF PKS IN *LEISHMANIA SP.* RESPONSIBLE FOR CAUSING LEISHMANIASIS

In many tropical and sub-tropical regions, diseases caused by protozoan *Leishmania sp.* pose a grave medical threat. There are more than 20 species of *Leishmania* responsible for causing the disease known as Leishmaniasis or Kala-azar, which can manifest itself in three forms namely cutaneous (skin ulcers), mucocutaneous (ulcers of skin, mouth and nose), or visceral leishmaniasis (involving skin ulcers followed by fever, low red blood cell count and enlarged spleen and liver) (28). The parasite responsible for causing Leishmaniasis is transmitted through the bite of infected female phlebotomine sand-flies. In order to reduce the prevalence of this disease, early diagnosis and effective therapeutics are necessary. The life cycle of *Leishmania sp.* involves two developmental stages, promastigotes in the pharyngeal valve of the flies where they undergo division to form metacyclic promastigotes which are transferred to humans by the fly during blood meals. Once inside the human body, these metacyclic promastigotes are phagocytosed by macrophages where the parasite transforms into infectious amastigotes and multiplies within the host blood cells thereby manifesting the disease. These physiological, biochemical and morphological changes during the life-cycle of *Leishmania* involve changes in gene expression followed by complex signal transduction pathways. Such changes in gene expression involve extensive protein phosphorylation, important for differentiation and proliferation of cells (29). With the advanced high-throughput technologies we have access to the parasitic kinome which revealed the role of kinases in cell cycle progression (30). Thus, parasitic kinases can be a potential target for designing better diagnostic tools and anti-parasitic chemotherapy. Weise (1998) reported an *L. mexicana* gene which codes for a protein that shares a strong homology to yeast and eukaryotic MAP kinases. The deletion mutation in promastigotes used in this study had *lmsap* gene deleted. Such mutants were able to infect the macrophages *in vitro* similar to wild-type promastigotes and were able to differentiate into amastigotes. However, they were unable to proliferate and thereby, failed to develop lesions in Balb/c mice. This revealed that MAP kinases like protein are responsible for the proliferation of amastigotes inside host macrophages and thus helping in disease manifestation (31). Feng et al. (1999) further reported that in case of *Leishmania sp.*, the lipophosphoglycan help the parasite to survive inside the host by stimulating extracellular signal-related kinase (ERK)- p38 mitogen-activated protein (MAP) kinases which hamper the host immune response against the parasite (32). In another development,

Hassan et al. (2001) reported the role of cyclin-dependent kinase CRK3 in cell cycle progression of *L. mexicana.* They worked with CRK3 null mutant cell lines and reported that the cell cycle was arrested at the G2/M phase transition in promastigotes. They further treated the cells with cyclin-dependent kinase inhibitor, flavopiridol which inactivated the CRK3 and thereby reducing parasite cell growth (33). Recently, Borba et al. (2019) conducted an *in -silico* study to classify and compare the kinome of *L. infantum* and *L. braziliensis* responsible for causing visceral and cutaneous Leishmaniasis. The aim of the study was to elucidate and thoroughly classify the kinomes of *L. infantum* and *L. braziliensis* at subfamily level and to further see if they can be used as potential drug targets in order to design better drug regimen for Leishmaniasis (34). The bioinformatics pipeline used in this study enabled the authors to classify the kinomes of *L. infantum* and *L. braziliensis* and catalogue their functions. The study predicts 30 protein kinases which can be used as potential leishmanicidal drug targets. Using drug repurposing pipeline, the authors report few drugs targeting kinases which are currently being used against Cancer, that also have the potential to be repositioned for the treatment of leishmaniasis. Trametinib and the compounds NMS-1286937 and RG-1530 inhibited the development of *L. infantum* and *L. braziliensis* promastigotes and amastigotes, therefore the compounds can be further studied in-depth to design drugs for effective treatment of leishmaniasis. This corroborates the fact that kinases play a crucial role in the parasite life-cycle and therefore, this class of proteins is crucial for pathogenesis. With our current knowledge and understanding about role of pathogen kinases, novel compounds should be tested which will inhibit the activity of these kinases thereby restricting the pathogen growth and proliferation.

6. ROLE OF PKS IN *STREPTOCOCCUS PNEUMONIAE* RESPONSIBLE FOR CAUSING PNEUMONIA

Streptococcus pneumoniae is a non-motile non-spore forming group of gram-positive bacteria responsible for causing pneumonia infections in elderly and young children (35). *S. pneumoniae* resides in respiratory tract and nasal passages asymptomatically in healthy individuals, however in susceptible individuals with weaker immune system the bacterium becomes pathogenic causing infections in lungs and other respiratory organs. The disease is contagious as it gets transmitted from person-to-person through respiratory droplets (36). Similar to above human pathogens, genomic and proteomic studies of *S. pneumoniae* has brought into view the potential role of PKs in bacterial life-cycle and in disease manifestation by modifying host cellular machinery. Based on biochemical and molecular experiments it has been revealed that serine/threonine kinases play a vital role in checking autolysis of bacteria inside the host and development of cell envelope which are crucial for virulence of the pathogen. In a study, it was proved that strains with mutated *stkP* genes were not virulent when tested against the wild-type strains (37). The study revealed the role of StkP as a crucial signalling molecule involved in lung infection and blood stream invasion. This study also revealed the role of StkP in determining bacterial autolysis rate and establishing virulence. According to the authors, StkP is required for expression of central competence operon *comCDE* and positively regulate the bacterial transformability which was validated by measuring the levels of *comCDE* in cells and transformants recovery in cultures. During exponential growth of the bacteria in alkaline medium, competence development occurs where StkP plays a positive role to avoid bacterial autolysis and also sense low concentrations of cell wall inhibitors (38). The results demonstrated that in *S. pneumoniae* StkP, a membrane associated protein, has found to assist the bacterium to sense and respond to changes in environmental conditions and also control genetic transformability, thereby playing a crucial role in virulence. Nova´kova´ et al. (2005) performed differential phosphoproteome analysis of wild-type and stkP null mutant which revealed that StkP, a serine/threonine kinase with a cognate protein phosphatase P, PhP are functional enzymes where PhpP dephosphorylates specifically phosphorylated StkP in presence of manganese ions. The study further proved the phosphorylation of phosphoglucoseamine mutase GlmM by StkP, which is the enzyme catalysing the first step of formation of UDP-*N*-acetylglucoseamine, an important component of cell envelope. Thus, StkP is important for cell-wall development and thereby plays a pivotal role in *Streptococcus* survival and progression inside

the host (39). Thus, there are several StkP substrates which play a crucial role in cell-wall synthesis and cell division, namely phosphoglucoseamine mutase GlmM and cell-division proteins DivIVA and FtsZ (39, 40, 41). Later, Beilharze et al. (2012) conducted protein-labelling and automated fluorescent time-lapse microscopic studies to establish the role of StkP and PhpP during cell-cycle leading to not only cell-wall synthesis, but specific cell-shape (42). The StkP consist of cytoplasmic kinase domain and extracellular C-terminal region comprising of many penicillin-binding proteins and Ser/Thr- kinase associated (PASTA) domains which binds to peptidoglycan (PG) (43, 44). This study gave *in vivo* evidence that growing cells carry uncross-linked PG, likely *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) linked to pentapeptide (NAG/NAM-pp) which binds to PASTA domain and thereby acts as a signal for StkP autophosphorylation. According to Beilharze et al., StkP is recruited to cell-division site just after assembly of early cell-division protein FtsA, but before, DivIVA (a cell-division protein). StkP remains in midcell for a longer duration than FtsA till cell division is complete and thus helps in septal wall synthesis. This gives *Streptococcus* its characteristic round shape while *stkP*- null mutants remain elongated. To conclude, the study proves that StkP acts as a "molecular switch" which through a cascade of phopshorylation events of certain key substrates signals the shifts from peripheral to septum wall progression.

7. ROLE OF PKS IN OPPORTUNISTIC HUMAN PATHOGEN *CRYPTOCOCCUS NEOFORMANS*

Cryptococcus neoformans is a known opportunistic fungal pathogen responsible for lethal infections of the human central nervous system (45, 46). Although cryptococcal meningitis is more common in immunocompromised patients who have undergone HIV, cancer or any steroid treatments (47-49), a small percentage of patients can be affected by a hyper-virulent strain of this pathogen without having any immune dysfunction (50-52). *C. neoformans* is a basidiomycetous encapsulated yeast which is ubiquitous in the environment. The infection spreads through inhalation of its desiccated spores followed by hematogenous dissemination to the brain (53, 54). The pathogenicity of *C. neoformans* has been linked to the production of two inducible factors: a polysaccharide capsule which ensheaths the fungal cells and melanin deposition in the fungal cell wall (55-57). The polysaccharide capsule is induced in the infected host by iron limitation and carbon dioxide which is required to enhance intracellular pathogen survival within the macrophages along with protection from phagocytosis (58-60). Melanin synthesis is mediated by an enzyme laccase and provides resistance to the pathogen against oxidative and nitrosative challenges offered by the macrophages (61, 62). In the case of *C. neoformans* different components of cyclic-AMP protein kinase A (cAMP-PKA) signalling pathway are known to regulate melanin formation, capsule size and overall virulence (63-65). For instance, Gα protein (Gpa1) is known to regulate mating and virulence in *C. neoformans* in response to diverse environmental cues like nitrogen limitation. It was observed that *gpa1* mutants exhibit reduced melanin and capsule production, lower virulence and sterility (63). Interestingly, the mutants of adenylate cyclase have a similar phenotype as that of *gpa1* mutants and exogenous supply of cAMP restores both mating and virulence in *gpa1* mutants. This suggests that adenylate cyclase function downstream of Gpa1protein (64). In a study by D'Souza et al (2001), it was found that the mutants lacking *PKA1* gene which encodes a catalytic subunit of protein kinase A are sterile and avirulent as they fail to produce capsule or melanin. Furthermore, mutants lacking *PKR1* gene which encodes a regulatory subunit of protein kinase A are hyper-virulent and overproduce capsule (65). Moreover, *pka1 pkr1* double mutants exhibit phenotype similar to that of *pka1* mutants further supporting the fact that Pka1 does functions downstream of the Pkr1 regulatory subunit (65). Thus, the vital role of protein kinases in *C. neoformans* infections and disease manifestation cannot be ignored and this points towards exploiting protein kinases as potential targets for drug development.

8. ROLE OF PKS IN PERIODONTAL BACTERIA *PORPHYROMONAS GINGIVALIS*

Periodontitis commonly known as the 'Gum disease' in humans is an inflammatory disease of the gums and other periodontal tissues supporting the

teeth. This disease is primarily caused by a gram-negative anaerobe *Porphyromonas gingivalis* which is capable of biofilm formation in the oral cavity commonly known as dental plaque. Polyphosphate kinase (PPK) is an essential enzyme associated with polyphosphate (PolyP) synthesis and have been implicated in a wide variety of bacterial functions like motility, virulence and quorum sensing (66) along with overall membrane stability and desiccation tolerance (67). In order to assess the role of PPK gene in the pathophysiology of *P. gingivalis*, a *ppk* gene mutant, CW120 of this pathogen was constructed and it was found that the mutant was attenuated in stationary-phase survival as well as *in-vitro* biofilm formation (68) indicating the indispensable role of PPK in the pathogen survival and infectivity. This suggests that protein phosphorylation is also important for the survival and infectivity of the pathogens even in case of chronic inflammatory diseases like periodontitis.

9. ROLE OF PKS IN RICE BLAST FUNGUS *MAGNAPORTHE ORYZAE*

Protein kinase-mediated pathways are known to integrate diverse external and internal cues to regulate important events in the fungal life cycle. These include sexual/asexual development, polar growth and stress response. Fungal plant pathogens are known to affect a large number of agriculturally important crops resulting in huge economic losses. Although fungal phytopathogens have a wide variety of infection strategies, the underlying molecular events like cycles of protein phosphorylation and de-phosphorylation are remarkably conserved. *Magnaporthe oryzae* or the rice blast fungus is a phytopathogenic fungus which is known to cause the rice blast disease. Besides rice, it also infects a wide range of grasses, including millet and barley. Blast disease poses a grave danger to global food security and destroys around 30% of the rice crops annually (69). Several regional outbreaks of the rice blast disease have affected the livelihood of millions of people in Asia, Africa and America (70). Infection of the host plant by this fungal pathogen is dependent upon the formation of a dome-shaped melanised structure known as the appressorium. The appressorium uses a pressure-driven mechanism to penetrate the tough plant cuticle and enter into the underlying plant cells (71). Upon successful entry into the plant tissues, the pathogen extends its invasive pseudohyphae into the living host cells, secreting a wide spectrum of effector molecules. The main purpose of the effector molecules is to suppress the host immunity and allow the further proliferation of the pathogen from one cell to another through a cluster of plasmodesmata known as pit fields. Among different plant mechanisms to prevent the spread of infection, the most important of all is the closure of intercellular plasmodesmata by callose deposition (72, 73) and the production of reactive oxygen species (74). One of the earliest mutational analysis of a MAPK in *M. oryzae* was reported by Xu et al. (1996) in which deletion of the MAPK gene resulted in the absence of appressoria formation leading to a complete loss of pathogenicity (75).

In a genetic chemical inhibition study by Sakulkoo et al. (2018), it has been shown that a single fungal MAP kinase Pmk-1 is essential for the cell to cell invasion by the pathogen. Also, inhibition of this kinase post appressorium formation blocked the penetration of cuticle by inhibiting septin ring assembly at the appressorium pore (76). Furthermore, this study reported that Pmk1 regulates the expression of pathogen effector proteins associated with suppression of host immune response, prevention of excessive plasmodesmatal callose deposition and generation of reactive oxygen species. In addition, *M. oryzae* has two other important MAPKs: Mps1, which regulates cellular integrity and is necessary for infection (77) and Osm1 which is crucial for its virulence (78). Apart from this conserved MAPKs, *M. oryzae* also contains an actin-regulating kinase homologue MoArk1which has been associated with the regulation of endocytosis and it is required for virulence, conidia development and stress tolerance (79). In *M. oryzae,* the intracellular levels of cAMP are known to mediate the recognition of plant surface and appressorium formation during pathogenesis which is further corroborated by the fact that exogenous application of cAMP or its analogues to germinating conidia or vegetative hyphae triggers appressorium formation even on non-inductive surfaces (80). Furthermore, molecular studies involving different components of cAMP-PKA pathways associated with cAMP biosynthesis (Adenylate cyclase) or cAMP hydrolysis (cAMP-

phosphodiesterase) confirms the role of the signalling pathway involved in recognition of host surface and appressorium formation in *M. oryzae* (81, 82). Moreover, using gene manipulation studies it has been shown that the cell cycle-regulating kinase-like Cdc7 and NIMA (never in mitosis gene A), are crucial for the initiation and maturation of appressorium respectively (83). Therefore, current research must be focused on exploiting and targeting the PKs for designing better diagnostic tools and effective therapy regimes.

10. ROLE OF PKS IN CORN SMUT FUNGUS *USTILAGO MAYDIS*

Ustilago maydis is a phytopathogenic fungus which is known to cause the smut disease in maize. Smut disease is characterised by stunted growth, yellowing of tissues (chlorosis) and tumour formation. Although this pathogenic fungus is dimorphic in nature, it exhibits three different life forms: (a) A saprophytic, unicellular and uninucleate haploid form known as sporidium; (b) A dikaryotic, filamentous pathogenic form; (c) Diploid form known as teliospore which is only produced inside the tumours. All these morphological transitions are mainly governed by 'a' and 'b' mating-type loci: 'a' is diallelic and encodes a receptor and a pheromone; 'b' is multiallelic and encodes homeodomain proteins associated with pathogenicity and tumour formation (84-86). Cell fusion usually occurs on the leaf surface between haploid cells having different alleles. The dikaryon thus formed can exhibit hyphal growth only if the cells carry any two different b alleles. In case of the filamentous dikaryon, only the tip cells contain the cytoplasm, which then differentiates into appressoria leading to penetration of the host cuticle and intracellular proliferation of the dikaryon within the plant tissues. This penetration and proliferation of dikaryon results in tumour formation. Within the tumour, the dikaryotic hyphal cells round up to become diploid teliospores which can be spread by wind or rainfall when tumours crack open at maturity. The teliospores do not show any vegetative growth and undergo reduction division or meiosis to produce haploid progeny at the onset of favourable conditions (87). In contrast to the melanised appressoria of *M. oryzae* which uses high turgor pressure to penetrate the plant cuticle (71), the appressoria in case of *U. maydis* are not melanised and are supposed to penetrate the host surface with the aid of plant cell wall degrading enzymes (88). The corn smut fungus *U. maydis* contains two Kss 1 type MAPKs having functional redundancy supported by the fact that only double mutant strains are fully impaired in pathogenicity (89-91). Using gene deletion experiment (Berndt et al. 2010) showed that Aga1, a conserved Ser/Thr kinase of the AGC kinase family, is essential for growth, cell wall integrity, mating and appressoria formation along with actin-dependent endocytosis in *U. maydis* (92). In *U. maydis* intracellular cAMP levels fluctuate according to the nutrient conditions and regulate the morphogenetic transition between filamentous growth and budding. This involves nutrient limitation or exposure to low pH triggers filamentous growth while high glucose concentration increases intracellular cAMP and triggers bud formation (93). Additionally, different cAMP-PKA signalling components like adenylate cyclase, cPKA (catalytic subunit) and rPKA (regulatory subunit) of PKA are all crucial for tumour development in corn, independent of their roles in sexual development (94). In *U. maydis* the formation of an infectious filament containing a G2 arrested dikaryotic hypha is crucial for appressoria formation (95). The G2 arrest is mediated by the inhibitory phosphorylation of Cdk1 by Cdc25 phosphatase. This inhibitory phosphorylation is mediated by the cytoplasmic localisation of Cdc25 by Bmh1 protein assisted by DNA damage response kinases like Atr1 and Chk (95, 96), this is further supported by the fact that *atr1* and *chk1* mutants of this pathogen lose their pathogenicity and are avirulent in nature.

11. ROLE OF PKS IN PINE WILT DISEASE NEMATODE, *BURSAPHELENCHUS XYLOPHILUS*

Pine wilt disease (PWD) is one of the serious diseases affecting the native pine tree species caused by the nematode *Bursaphelenchus xylophilus* and it spreads from one tree to another by the cerambycid beetle *Monochamus spp* (97). PWD affects *Pinus spp* globally and is known to cause significant timber loss in the affected countries (98) along with huge pecuniary losses in terms of disease management. Arginine kinase (AK) which belongs to the category of phosphotransferase is exclusively

found in the tissues of invertebrates (99,100) and plays an indispensable role in their cellular energy metabolism (101). Since AK is only present in the invertebrates and completely absent from the vertebrates especially mammals, therefore, it can be a potential chemotherapeutic target for invertebrate pest management (102, 103). In a study by wang et.al 2012, one arginine kinase gene from *B. xylophilus* (*Bx* AK1) was cloned and a dsRNA targeting this gene was developed and tested for its RNAi (RNA interference) effect on the nematode (104). RNAi not only reduced the nematode survival rate but also reduced its fertility and the fecundity rate suggesting that RNAi based targeting of arginine kinase genes like *Bx* AK1 can be used as an effective strategy to control nematode pests.

12. SUMMARY AND FUTURE PERSPECTIVES

With the increasing prevalence of some severe infectious diseases and development of drug-resistant strains of pathogenic parasites, we need to develop some robust diagnostic tools for timely diagnosis and advanced therapeutic approach for the cure of such diseases. Little research has been done on the role of host and parasite kinome in disease occurrence and spread. This review clearly sheds light on the role of kinases in controlling different aspects of pathogen life-cycle including mating, proliferation and survival within the host organism contributing to overall virulence of the pathogen and disease pathophysiology (Figure 2 and Table 1). It is evident from this article that different classes of PKs like Ser/Thr kinases, MAPK PKnA etc. and their associated proteins play a crucial role in bacterial and fungal life-cycle thereby aiding in disease manifestation and spread. Thus, in-depth knowledge of the role of pathogen kinases underlying the molecular basis of these infections and disease breakdown opens up a new horizon for research endeavours focusing on designing better diagnostic tools and therapeutic compounds which would help in controlling the outbreak and spread of some of the deadly infectious diseases of both plants and animals.

The crucial role of kinases in signal cascades makes them a potential centre for drug research. Drugs targeting kinases which play a key role in cell cycle have already been developed to treat deadly diseases like Cancer (e.g. Gleevec). However, these drugs are highly specific to the binding domain of the associated kinases (105). Gleevec binds to tyrosine kinase, Abl but does not have an affinity towards other tyrosine kinases, including the closely related Src tyrosine kinase. Both Abl and Src tyrosine kinases have identical structures including N and C- terminal lobes and similar drug-binding pocket. The selectivity of the drug is based on two alternative conformations of a conserved segment in the activation loop, DFG- motif (for Asp-Phe-Gly). DFG- out position is the Gleevec binding-competent while the DFG- in position is binding-incompetent (106). Thus, choosing protein kinases as potential drug targets will require an in-depth study of the structure and functions of the signalling cascades and signalling molecules. Thus, parasite kinases represent a domain which still remains unexplored and further research will definitely give a new direction to clinical therapeutics of many infectious diseases caused by parasitic pathogens.

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14. REFERENCES

1. Hanks SK, Hunter T.: Protein kinases 6. The eukaryotic protein kinase super-family: kinase (catalytic) domain structure and classification. The FASEB journal. 9(8):576-96 (1995)
DOI: 10.1096/fasebj.9.8.7768349

PMid:7768349

2. Dhanasekaran N, Reddy EP.: Signaling by dual specificity kinases. Oncogene.-17(11):1447 (1998)
DOI: 10.1038/sj.onc.1202251
PMid:9779990

3. Besant PG, Tan E, Attwood PV.: Mammalian protein histidine kinases. Intl J Biochem Cell Biochem Cell Biol 35(3):297-309 (2003)
DOI: 10.1016/S1357-2725(02)00257-1

4. Van Linden OP, Kooistra AJ, Leurs R, De Esch IJ, De Graaf C.: KLIFS: a knowledge-based structural database to navigate kinase-ligand interaction space. J Med Chem 57(2):249-77 (2013)
DOI: 10.1021/jm400378w
PMid:23941661

5. Reiterer V, Eyers PA, Farhan H.: Day of the dead: pseudokinases and pseudophosphatases in physiology and disease. Trends in Cell Biol 24(9):489-505 (2014)
DOI: 10.1016/j.tcb.2014.03.008
PMid:24818526

6. Lewis JD, Lee AH, Hassan JA, Wan J, Hurley B, Jhingree JR, Wang PW, Lo T, Youn JY, Guttman DS, Desveaux D.: The Arabidopsis ZED1 pseudokinase is required for ZAR1-mediated immunity induced by the Pseudomonas syringae type III effector HopZ1a. Proce Natl Acad Sci USA ;110(46):18722-7 (2013)
DOI: 10.1073/pnas.1315520110
PMid:24170858 PMCid:PMC3831984

7. WHO | World Health Statistics 2018: Monitoring health for the SDGs [Internet]. WHO. [cited 2019 Mar 7]. Available from: http://www.who.int/gho/publications/world_health_statistics/2018/en/

8. Garcia JE, Puentes A, Patarroyo ME.: Developmental biology of sporozoite-host interactions in Plasmodium falciparum malaria: implications for vaccine design. Clin Micro Rev19(4):686-707 (2006)
DOI: 10.1128/CMR.00063-05
PMid:17041140 PMCid:PMC1592691

9. Anamika, S.N. and Krupa, A.: Proteins, 58, 180-189 (2005)
DOI: 10.1002/prot.20278
PMid:15515182

10. Ward, P., Equinet, L., Packer, J. and Doerig, C.: BMC Genomics,5, 79(2004)
DOI: 10.1186/1471-2164-5-79
PMid:15479470 PMCid:PMC526369

11. Schneider, A.G. and Mercereau-Puijalon, O.: BMC. Genomics,6, 30 (2005)
DOI: 10.1186/1471-2164-6-30
PMid:15752424 PMCid:PMC1079819

12. Graeser R, Wernli B, M. Franklin R, Kappes B.: Plasmodium falciparum protein kinase5 and the malarial nuclear division cycles.. 82, 37 (1996)
DOI: 10.1016/0166-6851(96)02716-8

13. Chen Y, Jirage D, Caridha D, Kathcart AK, Cortes EA, Dennull RA, Geyer RA, Prigge ST, and Waters NC : Identification of an effector protein and gain-of-function mutants that activate Pfmrk, a malarial cyclin-dependent protein kinase. Mol Biochem Parasit. 149(1):48-57 (2006)
DOI: 10.1016/j.molbiopara.2006.04.004
PMid:16737745

14. Raman, M., Chen W and Cobb MH: Differential regulation and properties of MAPKs. Oncogene 26, 3100-3112(2007)
DOI: 10.1038/sj.onc.1210392
PMid:17496909

15. Dorin-Semblat D, Quashie N, Halbert J, Sicard A, Doerig C, Peat E, Ranford-Cartwright L and Doerig C.: Functional characterization of both MAP kinases of the human malaria parasite Plasmodium falciparum by reverse genetics. Mol Microbiol 65(5):1170-80 (2007)
DOI: 10.1111/j.1365-2958.2007.05859.x
PMid:17651389

16. Kato K, Sugi T, Iwanaga T.: Roles of Apicomplexan protein kinases at each life cycle stage. Parasitology Intl 61(2):224-34 (2012)
DOI: 10.1016/j.parint.2011.12.002
PMid:22209882

17. Ishino T, Orito Y, Chinzei Y, Yuda M.: A calcium-dependent protein kinase regulates Plasmodium ookinete access to the midgut epithelial cell. Mo Microbiol 59(4):1175-84 (2006)
DOI: 10.1111/j.1365-2958.2005.05014.x
PMid:16430692

18. Siden-Kiamos I, Ecker A, Nybäck S, Louis C, Sinden RE, Billker O.: Plasmodium berghei calcium-dependent protein kinase 3 is required for ookinete gliding motility and mosquito midgut invasion. Mol Microbiol 60(6):1355-63 (2006)
DOI: 10.1111/j.1365-2958.2006.05189.x
PMid:16796674 PMCid:PMC1513514

19. Billker O, Dechamps S, Tewari R, Wenig G, Franke-Fayard B, Brinkmann V.: Calcium and a calcium-dependent protein kinase regulate gamete formation and mosquito transmission in a malaria parasite. Cell. 117(4):503-14 (2004)
DOI: 10.1016/S0092-8674(04)00449-0

20. Coppi A, Tewari R, Bishop JR, Bennett BL, Lawrence R, Esko JD, Billker O, Sinnis P.: Heparan sulfate proteoglycans provide a signal to Plasmodium sporozoites to stop migrating and productively invade host cells. Cell host & microbe. 2(5):316-27 (2007)
DOI: 10.1016/j.chom.2007.10.002
PMid:18005753 PMCid:PMC2117360

21. Kato N, Sakata T, Breton G, Le Roch KG, Nagle A, Andersen C, Bursulaya B, Henson K, Johnson J, Kumar KA, Marr F.: Gene expression signatures and small-molecule compounds link a protein kinase to Plasmodium falciparum motility. Nature chemical biology. 4(6):347 (2008)
DOI: 10.1038/nchembio.87
PMid:18454143

22. Alam MM, Sanchez-Azqueta A, Janha O, Flannery EL, Mahindra A, Mapesa K, Char AB, Sriranganadane D, Brancucci NM, Antonova-Koch Y, Crouch K.: Validation of the protein kinase PfCLK3 as a multistage cross-species malarial drug target. Science. 365(6456):-eaau1682 (2019)
DOI: 10.1126/science.aau1682
PMid:31467193

23. Sturgill-Koszycki S, Schaible UE, Russell DG.: Mycobacterium-containing phago-somes are accessible to early endosomes and reflect a transitional state in normal phagosome biogenesis. The EMBO journal.15(24):6960-8 (1996)
DOI: 10.1002/j.1460-2075.1996.tb01088.x
PMid:9003772 PMCid:PMC452522

24. Walburger A, Koul A, Ferrari G, Nguyen L, Prescianotto-Baschong C, Huygen K, Klebl B, Thompson C, Bacher G, Pieters J.: Protein kinase G from pathogenic mycobacteria promotes survival within macrophages. Science 04(5678):1800-4 (2004)

DOI: 10.1126/science.1099384
PMid:15155913

25. Ogawara H, Aoyagi N, Watanabe M, Urabe H.: Sequences and evolutionary analyses of eukaryotic-type protein kinases from Streptomyces coelicolor A3(2) Microbiology. 145 (Pt 12):3343-52 (1999)
DOI: 10.1099/00221287-145-12-3343
PMid:10627033

26. Kang CM, Abbott DW, Park ST, Dascher CC, Cantley LC, Husson RN.: The Mycobacterium tuberculosis serine/-threonine kinases PknA and PknB: substrate identification and regulation of cell shape.Genes Dev. 19(14):1692-704 (2005)
DOI: 10.1101/gad.1311105
PMid:15985609 PMCid:PMC1176007

27. Prisic S, Dankwa S, Schwartz D, Chou MF, Locasale JW, Kang CM, Bemis G, Church GM, Steen H, Husson RN.: Extensive phosphorylation with overlapping specificity by Mycobacterium tuberculosis serine/threonine protein kinases. Proc Natl Acad Sci USA 107(16):7521-6 (2010)
DOI: 10.1073/pnas.0913482107
PMid:20368441 PMCid:PMC2867705

28. Okagbue HI, Adamu MO, Owoloko EA, Opanuga AA.: Correspondence analysis of the global epidemiology of cutaneous and Visceral Leishmaniasis. IJONS.-6(31):8634-52 (2015)

29. Treisman R.: Regulation of transcription by MAP kinase cascades. COCEBI.-8(2):205-15 (1996)
DOI: 10.1016/S0955-0674(96)80067-6

30. Naula C, Parsons M, Mottram JC.: Protein kinases as drug targets in trypanosomes and Leishmania. BBA-PROTEINS PROTEOM. 1754(1-2):151-9 (2005)
DOI: 10.1016/j.bbapap.2005.08.018
PMid:16198642 PMCid:PMC1452262

31. Wiese M.: A mitogen-activated protein (MAP) kinase homologue of Leishmania mexicana is essential for parasite survival in the infected host. The EMBO Journal. 17(9):2619-28 (1998)
DOI: 10.1093/emboj/17.9.2619
PMid:9564044 PMCid:PMC1170603

32. Feng GJ, Goodridge HS, Harnett MM, Wei XQ, Nikolaev AV, Higson AP, Liew FY: Extracellular signal-related kinase (ERK) and p38 mitogen-activated protein (MAP) kinases differentially regulate the lipopolysaccharide-mediated induction of inducible nitric oxide synthase and IL-12 in macrophages: Leishmania phos-phoglycans subvert macrophage IL-12 production by targeting ERK MAP kinase. J. Immunol... 163(12):6403-12 (1999)

33. Hassan P, Fergusson D, Grant KM, Mottram JC.: The CRK3 protein kinase is essential for cell cycle progression of Leishmania mexicana. Mol Biochem Parasit..113(2):189-98 (2001)
DOI: 10.1016/S0166-6851(01)00220-1

34. Borba JV, Silva AC, Ramos PI, Grazzia N, Miguel DC, Muratov EN, Furnham N, Andrade CH.: Unveiling the Kinomes of Leishmania infantum and L. braziliensis Empowers the Discovery of New Kinase Targets and Antileishmanial Compounds. CSBJ ;17:352-61 (2019)
DOI: 10.1016/j.csbj.2019.02.005
PMid:30949306 PMCid:PMC6429582

35. Ryan KJ, Ray CG.: Medical microbiology. McGraw Hill.4:370 (2004)

36. Manual I. QuickStripe™ Streptococcus pneumoniae.

37. Echenique J, Kadioglu A, Romao S, Andrew PW, Trombe MC.: Protein serine/threonine kinase StkP positively controls virulence and competence in Streptococcus pneumoniae. Infect Immun. 72(4):2434-7 (2004)
DOI: 10.1128/IAI.72.4.2434-2437.2004
PMid:15039376 PMCid:PMC375209

38. Berry AM, Lock RA, Hansman DA, Paton JC: Contribution of autolysin to virulence of Streptococcus pneumoniae. Infect Immun 57(8):2324-30 (1989)
DOI: 10.1128/IAI.57.8.2324-2330.1989
PMid:2568343 PMCid:PMC313450

39. Novakova L, Saskova L, Pallova P, Janeček J, Novotna J, Ulrych A, Echenique J, Trombe MC, Branny P: Characterization of a eukaryotic type serine/threonine protein kinase and protein phosphatase of Streptococcus pneumoniae and identification of kinase substrates. The FEBS journal. 272(5):1243-54 (2005)
DOI: 10.1111/j.1742-4658.2005.04560.x
PMid:15720398

40. Nováková L, Bezoušková S, Pompach P, Špidlová P, Sasková L, Weiser J, Branny P: Identification of multiple substrates of the StkP Ser/Thr protein kinase in Streptococcus pneumoniae. J BACTERIOL. 192(14):3629-38 (2010)
DOI: 10.1128/JB.01564-09
PMid:20453092 PMCid:PMC2897338

41. Giefing C, Jelencsics KE, Gelbmann D, Senn BM, Nagy E: The pneumococcal eukaryotic-type serine/threonine protein kinase StkP co-localizes with the cell division apparatus and interacts with FtsZ *in vitro*. Microbiology. 156(6):1697-707 (2010)
DOI: 10.1099/mic.0.036335-0
PMid:20223804

42. Beilharz K, Nováková L, Fadda D, Branny P, Massidda O, Veening JW.: Control of cell division in Streptococcus pneumoniae by the conserved Ser/Thr protein kinase StkP. Proc Natl Acad Sci USA 109(15):E905-13 (2012)
DOI: 10.1073/pnas.1119172109
PMid:22431591 PMCid:PMC3326482

43. Yeats C, Finn RD, Bateman A.: The PASTA domain: a β-lactam-binding domain. Trends Biochem Sci.27(9):438-40 (2002)
DOI: 10.1016/S0968-0004(02)02164-3

44. Jones G, Dyson P.: Evolution of transmembrane protein kinases implicated in coordinating remodeling of gram-positive peptidoglycan: inside versus outside. J BACTERIOL.. 188(21):7470-6 (2006)
DOI: 10.1128/JB.00800-06
PMid:16936012 PMCid:PMC1636289

45. Casadevall A, Perfect JR.: Cryptococcus neoformans. Washington, DC: ASM press; (1998)
DOI: 10.1128/9781555818241

46. Mitchell TG, Perfect JR.: Cryptococcosis in the era of AIDS--100 years after the discovery of Cryptococcus neoformans. CMR. 8(4):515-48 (1995)
DOI: 10.1128/CMR.8.4.515-548.1995
PMid:8665468 PMCid:PMC172874

47. Chuck SL, Sande MA.: Infections with Cryptococcus neoformans in the acquired immunodeficiency syndrome. NEJM321(12):794-9 (1989)
DOI: 10.1056/NEJM198909213211205
PMid:2671735

48. Kontoyiannis DP, Peitsch WK, Reddy BT, Whimbey EE, Han XY, Bodey GP, Rolston KV: Cryptococcosis in patients with cancer. Clin Infect Dis 32(11):e145-50 (2001)
DOI: 10.1086/320524
PMid:11340547

49. Goldstein E, Rambo ON.: Cryptococcal infection following steroid therapy. Ann. Intern. Med..56(1):114-20 (1962)
DOI: 10.7326/0003-4819-56-1-114
PMid:13899889

50. Aberg JA, Mundy LM, Powderly WG.: Pulmonary cryptococcosis in patients without HIV infection. Chest.115(3):734-40 (1999)
DOI: 10.1378/chest.115.3.734
PMid:10084485

51. Núñez M, Peacock Jr JE, Chin Jr R.: Pulmonary cryptococcosis in the immunocompetent host: therapy with oral fluconazole: a report of four cases and a review of the literature. Chest.-118(2):527-34 (2000)
DOI: 10.1378/chest.118.2.527
PMid:10936151

52. Patel P, Ramanathan J, Kayser M, Baran Jr J.: Primary cutaneous cryptococcosis of the nose in an immunocompetent woman. J Am Acad Dermatol.43(2):344-5 (2000)
DOI: 10.1067/mjd.2000.100961
PMid:10901718

53. Neilson JB, Fromtling RA, Bulmer GS.: Cryptococcus neoformans: size range of infectious particles from aerosolized soil. Infect Immun. 17(3):634-8 (1977)
DOI: 10.1128/IAI.17.3.634-638.1977
PMid:332630 PMCid:PMC421174

54. Sukroongreung S, Kitiniyom K, Nilakul C, Tantimavanich S.: Pathogenicity of basidiospores of Filobasidiella neoformans var. neoformans.mycology Med Mycol. 36(6):419-24 (1998)
DOI: 10.1046/j.1365-280X.1998.00181.x
PMid:10206753

55. Kwon-Chung KJ, Rhodes JC.: Encapsulation and melanin formation as indicators of virulence in Cryptococcus neoformans. Infect Immun . 51(1):218-23 (1986)
DOI: 10.1128/IAI.51.1.218-223.1986
PMid:3079732 PMCid:PMC261090

56. Chang YC, Kwon-Chung KJ.: Complementation of a capsule-deficient mutation of Cryptococcus neoformans restores its virulence. Mol Cell Biol.14(7):4912-9 (1994)
DOI: 10.1128/MCB.14.7.4912
PMid:8007987 PMCid:PMC358863

57. Kwon-Chung KJ, Polacheck I, Popkin TJ.: Melanin-lacking mutants of Cryptococcus neoformans and their virulence for mice. J BACTERIOL.-150(3):1414-21 (1982)
DOI: 10.1128/JB.150.3.1414-1421.1982
PMid:6804444 PMCid:PMC216368

58. Feldmesser M, Kress Y, Novikoff P, Casadevall A.: Cryptococcus neoformans is a facultative intracellular pathogen in murine pulmonary infection. Infect Immun.68(7):4225-37 (2000)
DOI: 10.1128/IAI.68.7.4225-4237.2000
PMid:10858240 PMCid:PMC101732

59. Granger DL, Perfect JR, Durack DT.: Virulence of Cryptococcus neoformans. Regulation of capsule synthesis by carbon dioxide. J CLIN INVEST..76(2):508-16 (1985)
DOI: 10.1172/JCI112000
PMid:3928681 PMCid:PMC423853

60. Vartivarian SE, Anaissie EJ, Cowart RE, Sprigg HA, Tingler MJ, Jacobson ES.: Regulation of cryptococcal capsular polysaccharide by iron. J Infect Dis.167(1):186-90 (1993)
DOI: 10.1093/infdis/167.1.186
PMid:8418165

61. Jacobson ES, Tinnell SB.: Antioxidant function of fungal melanin. J BACTERIOL.175(21):7102-4 (1993)
DOI: 10.1128/JB.175.21.7102-7104.1993
PMid:8226653 PMCid:PMC206840

62. Wang Y, Casadevall A.: Susceptibility of melanized and nonmelanized Cryptococcus neoformans to nitrogen-and oxygen-derived oxidants. Infect Immun.62(7):3004-7 (1994)
DOI: 10.1128/IAI.62.7.3004-3007.1994
PMid:8005689 PMCid:PMC302912

63. Alspaugh JA, Perfect JR, Heitman J.: Cryptococcus neoformans mating and virulence are regulated by the G-protein α subunit GPA1 and cAMP. Genes Dev.11(23):3206-17 (1997)
DOI: 10.1101/gad.11.23.3206
PMid:9389652 PMCid:PMC316752

64. Alspaugh JA, Pukkila-Worley R, Harashima T, Cavallo LM, Funnell D, Cox GM, Perfect JR, Kronstad JW, Heitman J.: Adenylyl cyclase functions downstream of the Gα protein Gpa1 and controls mating and pathogenicity of Cryptococcus neoformans. Eukaryot. Cell.1(1):75-84 (2002)
DOI: 10.1128/EC.1.1.75-84.2002
PMid:12455973 PMCid:PMC118042

65. D'Souza CA, Alspaugh JA, Yue C, Harashima T, Cox GM, Perfect JR, Heitman J.: Cyclic AMP-dependent protein kinase controls virulence of the fungal pathogen Cryptococcus neoformans. Mol Cell Biol.. 21(9):3179-91 (2001)
DOI: 10.1128/MCB.21.9.3179-3191.2001
PMid:11287622 PMCid:PMC86952

66. Rashid MH, Rumbaugh K, Passador L, Davies DG, Hamood AN, Iglewski BH, Kornberg A.: Polyphosphate kinase is essential for biofilm development, quorum sensing, and virulence of Pseudomonas aeruginosa. Proc Natl Acad Sci USA15;97(17):9636-41 (2000)
DOI: 10.1073/pnas.170283397
PMid:10931957 PMCid:PMC16917

67. Fraley CD, Rashid MH, Lee SS, Gottschalk R, Harrison J, Wood PJ, Brown MR, Kornberg A.: A polyphosphate kinase 1 (ppk1) mutant of Pseudomonas aeruginosa exhibits multiple ultrastructural and functional defects. Proc Natl Acad Sci USA 104(9):3526-31 (2007)
DOI: 10.1073/pnas.0609733104
PMid:17360677 PMCid:PMC1803759

68. Chen W, Palmer RJ, Kuramitsu HK.: Role of polyphosphate kinase in biofilm formation by Porphyromonas gingivalis. Infect Immun. 70(8):4708-15 (2002)
DOI: 10.1128/IAI.70.8.4708-4715.2002
PMid:12117989 PMCid:PMC128176

69. Wilson RA, Talbot NJ.: Under pressure: investigating the biology of plant infection by Magnaporthe oryzae. Nat. Rev. Microbiol.7(3):185 (2009)
DOI: 10.1038/nrmicro2032
PMid:19219052

70. Zeigler, R.S., Leong, S.A., and Teng, P.S., Eds. Rice Blast Disease. (Wallingford, UK: CAB International) (1994)

71. Howard RJ, Ferrari MA, Roach DH, Money NP.: Penetration of hard substrates by a fungus employing enormous turgor pressures. Proc Natl Acad Sci USA88(24):11281-4 (1991)
DOI: 10.1073/pnas.88.24.11281
PMid:1837147 PMCid:PMC53118

72. Cheval C, Faulkner C.: Plasmodesmal regulation during plant-pathogen interactions. New Phytol.. 217(1):62-7 (2018)
DOI: 10.1111/nph.14857
PMid:29083038

73. Faulkner C, Petutschnig E, Benitez-Alfonso Y, Beck M, Robatzek S, Lipka V, Maule AJ.: LYM2-dependent chitin perception limits molecular flux via plasmodesmata. Proc Natl Acad Sci USA110(22):9166-70 (2013)
DOI: 10.1073/pnas.1203458110
PMid:23674687 PMCid:PMC3670346

74. Torres MA, Jones JD, Dangl JL.: Reactive oxygen species signaling in response to pathogens.. Plant Physiol. 141(2):373-8 (2006)
DOI: 10.1104/pp.106.079467
PMid:16760490 PMCid:PMC1475467

75. Xu JR, Hamer JE. 1996.: MAP kinase and cAMP signaling regulate infection structure formation and pathogenic growth in the rice blast fungus Magnaporthegrisea. Genes Dev. 10:2696-706 (1996)
DOI: 10.1101/gad.10.21.2696
PMid:8946911

76. Sakulkoo W, Osés-Ruiz M, Garcia EO, Soanes DM, Littlejohn GR, Hacker C, Correia A, Valent B, Talbot NJ.: A single fungal MAP kinase controls plant cell-to-cell invasion by the rice blast fungus. Science. 359(6382):1399-403 (2018)
DOI: 10.1126/science.aaq0892
PMid:29567712

77. Xu JR, Staiger CJ, Hamer JE.: Inactivation of the mitogen-activated protein kinase Mps1 from the rice blast fungus prevents penetration of host cells but allows activation of plant defense responses. Proc Natl Acad Sci USA95(21):12713-8 (1998)
DOI: 10.1073/pnas.95.21.12713
PMid:9770551 PMCid:PMC22896

78. Dixon KP, Xu JR, Smirnoff N, Talbot NJ.: Independent signaling pathways regulate cellular turgor during hyperosmotic stress and appressorium-mediated plant infection by Magnaporthegrisea. Plant Cell. 11(10):2045-58 (1999)
DOI: 10.1105/tpc.11.10.2045
PMid:10521531 PMCid:PMC144108

79. Wang J, Du Y, Zhang H, Zhou C, Qi Z, Zheng X, Wang P, Zhang Z.: The actin-regulating kinase homologue MoArk1 plays a pleiotropic function in Magnaportheoryzae. MOL PLANT PATHOL..14(5):470-82 (2013)
DOI: 10.1111/mpp.12020
PMid:23384308 PMCid:PMC3642230

80. Lee YH, Dean RA.: cAMP regulates infection structure formation in the plant dpathogenic fungus Magnaporthegrisea. Plant Cell..5(6):693-700 (1993)
DOI: 10.2307/3869811
PMid:12271080 PMCid:PMC160306

81. Choi W, Dean RA.: The adenylate cyclase gene MAC1 of Magnaporthegrisea controls appressorium formation and other aspects of growth and development. Plant Cell. 9(11):1973-83 (1997)
DOI: 10.1105/tpc.9.11.1973
PMid:9401122 PMCid:PMC157051

82. Ramanujam R, Naqvi NI.: PdeH, a high-affinity cAMP phosphodiesterase, is a key regulator of asexual and pathogenic differentiation in Magnaporthe oryzae. PLOS PATHOG.6(5):e1000897 (2010)
DOI: 10.1371/journal.ppat.1000897
PMid:20463817 PMCid:PMC2865543

83. Saunders DG, Aves SJ, Talbot NJ.: Cell cycle-mediated regulation of plant infection by the rice blast fungus. Plant Cell. 22(2):497-507 (2010)
DOI: 10.1105/tpc.109.072447
PMid:20190078 PMCid:PMC2845407

84. Puhalla JE.: Compatibility reactions on solid medium and interstrain inhibition in Ustilago maydis. Genetics.60(3):461 (1968)

85. Holliday R.: The genetics of Ustilago maydis. GENET RES.. 2(2):204-30 (1961)
DOI: 10.1017/S0016672300000719

86. Rowell JB.: Genetics of Ustilagozeae in relation to basic problems of its pathogenicity. Phytopathology.44:356-62 (1954)

87. Banuett F.: Genetics of Ustilago maydis, a fungal pathogen that induces tumors in maize. Annu. Rev. Genet. 29(1):179-208 (1995)
DOI: 10.1146/annurev.ge.29.120195.-001143
PMid:8825473

88. Schirawski J, Böhnert HU, Steinberg G, Snetselaar K, Adamikowa L, Kahmann R.: Endoplasmic reticulum glucosidase II is required for pathogenicity of Ustilago maydis. Plant Cell. 17(12):3532-43 (2005)
DOI: 10.1105/tpc.105.036285
PMid:16272431 PMCid:PMC1315386

89. Brachmann A, Schirawski J, Müller P, Kahmann R.: An unusual MAP kinase is required for efficient penetration of the plant surface by Ustilago maydis. The EMBO journal.22(9):2199-210 (2003)
DOI: 10.1093/emboj/cdg198
PMid:12727886 PMCid:PMC156070

90. Mayorga ME, Gold SE.: A MAP kinase encoded by the ubc3 gene of Ustilago maydis is required for filamentous growth and full virulence. Mol Microbiol.34(3):485-97 (1999)
DOI: 10.1046/j.1365-2958.1999.01610.x
PMid:10564490

91. Müller P, Aichinger C, Feldbrügge M, Kahmann R.: The MAP kinase kpp2 regulates mating and pathogenic development in Ustilago maydis. Mol Microbiol.34(5):1007-17 (1999)
DOI: 10.1046/j.1365-2958.1999.01661.x
PMid:10594825

92. Berndt P, Lanver D, Kahmann R.: The AGC Ser/Thr kinase Aga1 is essential for appressorium formation and maintenance of the actin cytoskeleton in the smut fungus Ustilago maydis. Mol Microbiol.78(6):1484-99 (2010)
DOI: 10.1111/j.1365-2958.2010.07422.x
PMid:21143319

93. Gold S, Duncan G, Barrett K, Kronstad J.: cAMP regulates morphogenesis in the fungal pathogen Ustilago maydis. Genes Dev. 8(23):2805-16 (1994)
DOI: 10.1101/gad.8.23.2805
PMid:7995519

94. Dürrenberger F, Wong K, Kronstad JW.: Identification of a cAMP-dependent protein kinase catalytic subunit required for virulence and morphogenesis in Ustilago maydis. Proc Natl Acad Sci USA 95(10):5684-9 (1998)

DOI: 10.1073/pnas.95.10.5684
PMid:9576944 PMCid:PMC20439

95. Mielnichuk N, Sgarlata C, Pérez-Martín J.: A role for the DNA-damage checkpoint kinase Chk1 in the virulence program of the fungus Ustilago maydis. J. Cell Sci.122(22):4130-40 (2009)
DOI: 10.1242/jcs.052233
PMid:19861497

96. de Sena-Tomás C, Fernández-Álvarez A, Holloman WK, Pérez-Martín J.: The DNA damage response signalling cascade regulates proliferation of the phytopathogenic fungus Ustilago maydis in planta. Plant Cell. 23(4):1654-65 (2011)
DOI: 10.1105/tpc.110.082552
PMid:21478441 PMCid:PMC3101559

97. Kulinich O, Mota M, Vieira P, Ryss A.: A synopsis of the genus Bursaphelenchus Fuchs, 1937 (Aphel-enchida: Parasitaphelenchidae) with keys to species. Nematology. (3):393-458 (2005)
DOI: 10.1163/156854105774355581

98. Mamiya Y.: Pine wilt disease in Japan. The pine wood nematode. :9-20. (2004)

99. Chamberlin M.: Mitochondrial arginine kinase in the midgut of the tobacco hornworm (Manduca sexta). JEB. 200(21):2789-96 (1997)

100. Kucharski R, Maleszka R.: Arginine kinase is highly expressed in the compound eye of the honey-bee, Apis mellifera. Gene. 211(2):343-9 (1998)
DOI: 10.1016/S0378-1119(98)00114-0

101. Newsholme EA, Beis IS, Leech AR, Zammit VA.: The role of creatine kinase and arginine kinase in muscle. Biochem. J.172(3):533-7 (1978)
DOI: 10.1042/bj1720533
PMid:210761 PMCid:PMC1185728

102. Brown AE, Grossman SH.: The mechanism and modes of inhibition of arginine kinase from the cockroach (Periplaneta americana) Arch. Insect Biochem. Physiol.57(4):166-77 (2004)
DOI: 10.1002/arch.20026
PMid:15540275

103. Wu QY, Li F, Zhu WJ, Wang XY.: Cloning, expression, purification, and characterization of arginine kinase from Locusta migratoria manilensis. Comparative Biochemistry and Physiology Part B: Biochem Mol Biol 1;148(4):355-62. (2007)
DOI: 10.1016/j.cbpb.2007.07.002
PMid:17689280

104. Wang XR, Cheng X, Li YD, Zhang JA, Zhang ZF, Wu HR.: Cloning arginine kinase gene and its RNAi in Bursaphelenchus xylophilus causing pine wilt disease. Eru J Plant Biolo.134(3):521-32 (2012)
DOI: 10.1007/s10658-012-0035-0

105. Lin YL, Meng Y, Jiang W, Roux B.: Explaining why Gleevec is a specific and potent inhibitor of Abl kinase. Proc Natl Acad Sci USA110(5):1664-9 (2013)
DOI: 10.1073/pnas.1214330110
PMid:23319661 PMCid:PMC3562763

106. Seeliger MA, Nagar B, Frank F, Cao X, Henderson MN, Kuriyan J.: c-Src binds to the cancer drug imatinib with an inactive Abl/c-Kit conformation and a distributed thermodynamic penalty. Structure. 15(3):299-311 (2007)
DOI: 10.1016/j.str.2007.01.015
PMid:17355866

Abbreviations: Pks: protein kinases, Ser/Thr kinases: Serine/Threonine kinases, Tyr kinases: tyrosine-kinases, ePKs: eukaryotic protein kinases, ck1: casein kinases, CDK: cyclin-dependent kinases, MAPK: mitogen-activated protein kinases, GSK3: glycogen synthase kinase 3, CLKs: CDK-like kinases, TKL: tyrosine-kinase-like, AGC PKA: cyclic-adenosine-monophosphate-dependent protein kinase, PKG: cyclic-guanosine mono-phosphate-dependent protein kinase, PKC: protein kinase C and related proteins, CamK: calcium/calmodulin-dependent kinases,

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