

## **Divergent protein kinases: an Achilles' heel of malaria parasites and other protozoa?**

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Parasitic protists are responsible for a number of diseases of enormous medical and veterinary importance, affecting mostly (but not exclusively) tropical regions. For example, almost half the world population lives in countries where malaria, caused by Apicomplexans of the genus *Plasmodium*, is endemic, and several hundred million cases (leading to 1-3 million deaths) are reported yearly. Other protozoan scourges of the developing world are species of the genus *Trypanosoma*, causing sleeping sickness and Chagas disease, and of the genus *Theileria*, responsible for a cattle disease that bears a considerable socioeconomic impact in Old World tropical regions.

The lecture will be focussed mostly on malaria parasites, which is the main research subject in our laboratory, although the illustration of some specific points will draw on the literature concerning other parasites. It will be divided in three parts:

1. Discussion of the phylogenetic distance between parasitic protists and the eukaryotes used as models in biology, and of how this distance is reflected by vast divergences in host versus parasite protein kinases.
2. Presentation of a specific example of parasite-host divergence, namely the MAPK pathways.
3. An overview of the strategies that are being followed in the context of antiparasitic drug discovery research based on protein kinase inhibition, exploiting the divergence between the host and parasite enzymes

### **Part 1. Divergences between host and parasite protein kinases.**

The major parasitic protists (*Plasmodium*, *Trypanosoma*, *Leishmania*) belong to various taxonomic groups that are phylogenetically vastly distant from the Opisthokonta branch, which includes animals and fungi (Baldauf, 2003). Over the recent years, evidence has accumulated that this phylogenetic distance is reflected by important divergences, in comparison with yeast or mammalian cells, in the properties of protein kinases (PKs). Divergences exist at the levels (i) of the complement of protein kinase genes encoded in the genome (the kinome); (ii) of the organisation of signalling pathways, and (iii) of individual enzyme orthologues (when these can be identified!).

***Divergences at the kinome level.*** The first kinome of a parasitic protist to be described was that of *Plasmodium falciparum*, the species responsible for the lethal form of human malaria (Anamika et al., 2005; Ward et al., 2004). Phylogenetic analyses showed that most established eukaryotic PK groups have members in the plasmodial genome, with the notable absence of TyrK and STEs. Conversely, many “orphan” PKs were identified,

which do not cluster with any of the PK families established from the yeast and mammalian kinomes. A particularly striking example of orphan kinases is that of a novel group of 20 enzymes, members of which are found only in Apicomplexan parasites. Finally, several plasmodial PKs do indeed cluster within established families, but branch off near the base of the group cluster, precluding the assignment of any precise orthology to specific mammalian enzymes --we call such PKs “semi-orphans”. The general observations on the *Plasmodium* kinome (presence of orthologues, orphans and semi-orphans) are also valid for the kinomes of other parasites, such as *Trypanosoma* ssp (Parsons et al., 2005).

***Divergences in the organisation of signalling pathways.*** Malaria parasites lack signalling kinases that are present in most other eukaryotes, such as PKC homologues. In contrast, they possess several genes encoding calcium-dependent kinases, a PK family found in Ciliates and Plants but not in mammalian cells. Two atypical MAP kinases are present in *P. falciparum*, but the absence of typical MAPKKs in the malarial kinome indicates that their mode of activation differs from that of MAPKs of other eukaryotes (Dorin et al., 2005).

***Divergences in the properties of individual PKs.*** In addition to enzymes that do not cluster with established PK families (and hence which cannot be assigned a precise orthology), many “orphan” or “semi-orphan” plasmodial PKs (which clearly belong to known families, see above) nevertheless display atypical characteristics, such as large insertions/extensions or variant regulatory sites (Doerig, 2004). Examples include a MAPK lacking the usual TxY motif (Dorin et al., 1999), and a PKG homologue with an atypical regulatory region (Deng et al., 2003). Furthermore, several plasmodial PKs are « composite » enzymes, displaying primary structure features from distinct PK groups in a single molecule.

## **Part 2. A specific example of parasite-host divergence: the MAPK pathways.**

As mentioned above, the *Plasmodium* kinome includes two MAPKs : one clusters with the ERK7/8 subfamily, while the other is a “semi-orphan” branching off near the base of the MAPK cluster. Interestingly, there are no orthologues of the ERK1/2, p38 or JNK subfamilies. Although no plasmodial PK clusters within the STE family, we identified an enzyme, which we called PfPK7, that displays maximal homology to MAPKK3/6 in its C-terminal lobe. As a recombinant enzyme, PfPK7 is able to phosphorylate a variety of proteins, but the two plasmodial MAPKs are not among them, making it unlikely that PfPK7 is a MAPKK functional homologue. This is confirmed by a reverse genetics study, which shows that parasites lacking either of the MAPKs or PfPK7 display distinct phenotypes, indicating that these enzymes do not function in the same pathways. It is of interest to note that none of these PKs is required for sexual differentiation (gametocytogenesis), in contrast to the established role of MAPK pathways in yeast mating type differentiation.

### **Part 3. Strategies for anti-parasitic chemotherapeutic intervention based on PK inhibition.**

**1. Targeting PKs of the parasite.** Many PKs from protozoan parasites display activity *in vitro* as recombinant enzymes, and can be used in medium/high throughput screening operations. We will present an example (GSK3) showing that parasite versus host specific inhibition can be obtained, even in the case of PKs that have orthologues in both organisms. Another approach consists of identifying potential targets of known PK inhibitors in parasite extracts using affinity chromatography on immobilised molecules (Knockaert and Meijer, 2002). Rational drug design based on structural differences between parasite and host orthologues is another possible avenue. The 3D structure of two plasmodial PKs has been solved (in collaboration with Jane Endicott, Oxford): a CDK, PfPK5 (Holton et al., 2003), and the atypical MAPKK-like enzyme PfPK7 (see above). Detailed examination of the structures reveals possible avenues for the rational design of parasite-specific inhibitors.

**2. Targeting PKs of the host cell.** Many of the most important protozoan pathogens are obligate intracellular parasites, and in some instances an essential role for host cell PKs in the establishment of the infection has been identified (Hall and Pereira, 2000; Heussler et al., 2002). The possibility of interfering with infection through inhibition of such host cell PKs will be discussed, and illustrated by selected examples, including preliminary data from our laboratory suggesting that signalling pathways of the infected erythrocyte may be required for *Plasmodium* survival. Targeting host kinases would allow a piggy-back approach benefiting from the heavy investment that the pharmaceutical industry has put into inhibitors of human PK, and would significantly hinder development of drug resistance by the parasites.

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