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Trends in Parasitology

Spotlight

Converting and hoarding driven by protein phosphorylation in *Toxoplasma gondii*

Martin Blume [1,*] and Deborah Maus [1]

Successful parasitism relies on the evasion of adversarial host responses. Wang *et al*. have recently shown that *Toxoplasma gondii* relies on the protein phosphatase 2A (PP2A) to cause persisting infections. The phosphatase controls the development of dormant parasite stages and the accumulation of sugar supplies.

T. gondii is a eukaryote that has the ability to chronically infect animals and humans. To do so, it switches from the fast-replicating and disseminating tachyzoite form into bradyzoites which reside mostly dormant within tissue cysts in skeletal muscle and the brain. Using this strategy, *T. gondii* parasites evade not only the response of the host immune system but also medical treatments: no current antimicrobial eradicates bradyzoites. As proliferation and dormancy require distinct metabolic capabilities, converting between these different lifestyles involves extensive remodeling of the cellular protein equipment and a concomitant reshaping of the parasite's metabolism. As a result, bradyzoites employ distinct isoforms of some metabolic enzymes and also accumulate large quantities of the storage carbohydrate amylopectin. However, exactly how bradyzoite formation is induced in response to external clues, and how it is coordinated with the necessary metabolic rewiring, is not well understood, but the process includes transcriptomic, translational, and epigenomic adjustments [1].

Now, in their recent work, Wang *et al.* [2] found that post-translational regulation of protein function also plays an important role by investigating the parasite's PP2A. In eukaryotic cells, PP2As constitute more than half of the serine/threonine protein phosphatase activity. These enzymes maintain cellular homeostasis by controlling the phosphorylation of proteins involved in fundamental functions, including proliferation, cell cycle, survival, apoptosis, DNA damage response (DDR), metabolism, cell–cell communication, and cytoskeleton dynamics [3]. PP2As fulfil these diverse roles by working as heterotrimeric complexes consisting of a scaffolding subunit A, a catalytic subunit C, and a single variant of regulatory subunits B that determines substrate specificity. As stage conversion strategies are widely cultivated among parasites, PP2As were naturally found to participate in their regulation. In *Trypanosoma brucei*, *Plasmodium falciparum*, and *Giardia lamblia*, PP2As regulate differentiation into amastigotes, gametocytes, and cyst formation, respectively [4]. In the Chinese liver fluke *Clonorchis sinensis*, PP2As control the development of metacercariae into adult flukes [4]. In *T. gondii,* however, the cellular functions of PP2As have not been studied so far.

Wang *et al.* combined gene similarity searches, epitope tagging, and protein pull-down experiments and found that all three PP2A subunits form a heterotrimer in both tachyzoites and early bradyzoites. They investigated PP2A importance for both stages using gene-knockout mutants of all three subunits. PP2A-null mutants were viable but grew much slower than wild-type parasites and were avirulent in mice. Interestingly, a closer investigation of the parasite phenotype revealed that the PP2A-null tachyzoites also accumulated amylopectin granules in their cytosol, which is a hallmark of bradyzoites. However, when the authors tried to induce the formation of bradyzoites, they were unable to observe other typical bradyzoite markers such as the development of a cyst wall, the expression of the bradyzoite antigen BAG1, or a characteristic stage-specific transcriptome. Consequently, mice infected with mutants lacking PP2A subunits did not develop tissue cysts in their brains. These results show that PP2A is a major player in both bradyzoite development and metabolic regulation and in the right position to coordinate these processes.

To dissect the mechanisms underlying PP2A's function, the authors compared the changes in the phosphoproteome caused by the absence of PP2A with those caused by a lack of the calcium-dependent protein kinase 2 (CDPK2). CDPK2 does not influence the ability to stage convert but also controls amylopectin metabolism, in particular through phosphorylation [5] of the parasite's glycogen phosphorylase enzyme [6]. Surprisingly, many proteins were actually less phosphorylated in PP2A-null parasites, which led the authors to conclude that PP2A acts mainly through control of other kinases. Interestingly, however, CDPK2 itself was hyperphosphorylated in PP2A-null mutants, but corresponding phosphomimetic and phosphoablative mutations of the kinase failed to restore normal amylopectin levels. While these results point towards a phosphorylation-dependent control of CDPK2, they also show that this is not a dominant mechanism through which PP2A controls amylopectin storage (Figure 1). Instead, the levels and turnover rates of amylopectin likely reflect the broader state of the parasite's central carbon metabolism. For example, amylopectin synthesis depends on gluconeogenesis [7], and it is also linked to optimal glucose utilization and growth of tachyzoites [8]. It will be exciting to learn how PP2A overall shapes parasite metabolism. In this context, the ability of human PP2A to crosstalk with the metabolic

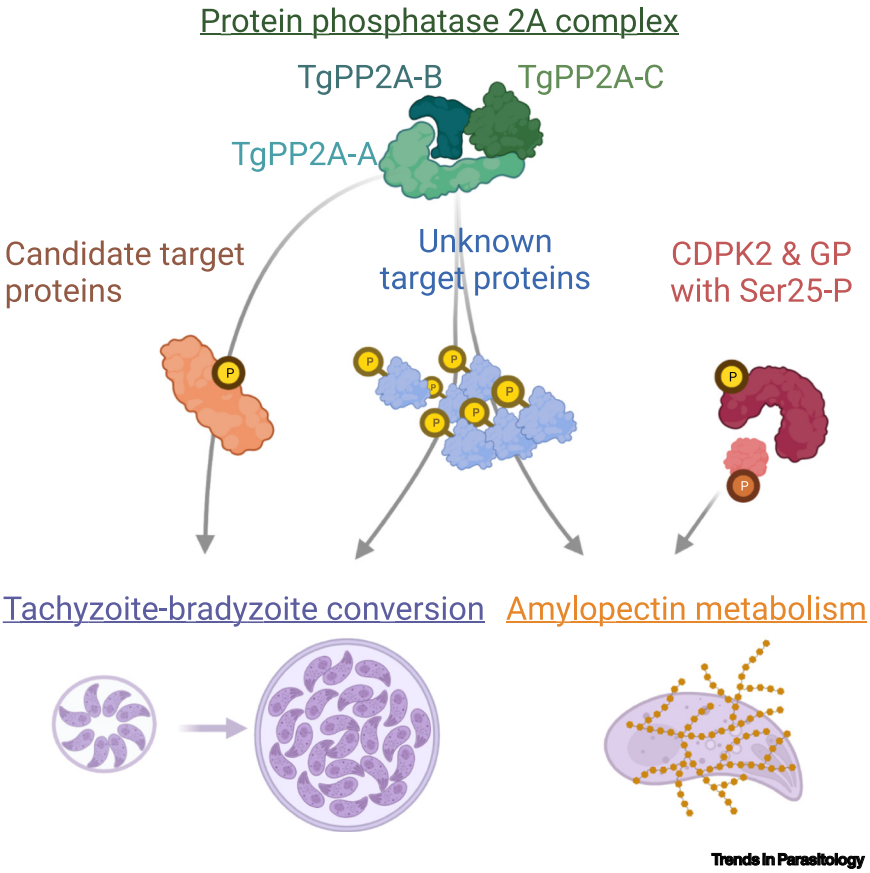

Figure 1. *Toxoplasma gondii* protein phosphatase 2A regulates stage conversion and amylopectin metabolism. *T. gondii* PP2A acts as a heterotrimer consisting of TgPP2A-A, TgPP2A-B, and TgPP2A-C (shown in green) to regulate stage conversion through hypothetically identified protein factors (shown in brown) including TgBFD2, TgME49_298610, TgME49_224260, TgME49_237520, and a pool of unknown proteins (shown in blue). PP2A also cooperates via a pool of unknown phosphatase substrate proteins (blue) with the calcium-dependent protein kinase 2 (CDPK2)-dependent glycogen phosphorylase (GP) (shown in purple) to modulate amylopectin metabolism. Regulation of amylopectin metabolism by CDPK2 depends on the phosphorylation status at serine 25 of GP. This figure was created using BioRender.

master regulator AMP kinase in human cells is intriguing [9].

To identify proteins that are PP2A-dependent mediators of stage conversion, Wang *et al.* [2] focused on phosphoproteins that are exclusively altered in PP2A-C-deficient parasites but not in CDPK2-null mutants. Indeed, the authors found a number of hyperphosphorylated proteins in the PP2A-C-null mutant and tested the role of four hits (Figure 1) in stage conversion by gene knockout. While three proteins were also important for tachyzoite growth, all four were required to develop an early cyst wall 3 days after induction. Interestingly, among the four proteins was the bradyzoite-formation-deficient 2 protein (BFD2) that, together with BFD1, induces stage conversion [10].

Although exact mechanisms of how PP2A regulates parasite metabolism remain unknown, this important work by Wang *et al.* [2] suggests that PP2A may act as a hub to integrate metabolic regulation with stage conversion. With this knowledge, it is now possible to study the regulation of PP2A itself and how it responds to the diverse stresses that are known to induce stage conversion [1]. It will also be interesting to interrogate its role during other environment-dependent stage-conversion events, such as differentiation, into sexual stages in the feline intestinal epithelium. Interestingly, outside of parasitology, PP2A is also known as an oncogene and has been the target of immense drug development efforts that promisingly address quiescent cancer cells [3] in addition to proliferating ones. The discoveries in the current work [2] advance and consolidate our understanding of the mechanics of *T. gondii*'s evasion strategies and may lead to new intervention strategies.

Acknowledgments

We thank Frank Seeber for his comments on the manuscript. M.B. is funded by the Robert Koch-Institute, and D.M. is funded by the Federal Ministry of Education and Research (BMBF) under project number 01KI1715 as part of the 'Research Network Zoonotic Infectious Diseases'.

Declaration of interests

The authors declare no competing interests.

[1]P6: Metabolism of Microbial Pathogens, Robert Koch Institute, 13353, Berlin, Germany

*Correspondence:
blumem@rki.de (M. Blume).

https://doi.org/10.1016/j.pt.2023.02.002

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