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Microreview

The metabolic control of schistosome egg production

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Summary

Schistosomiasis is a neglected tropical disease caused by infection with trematode parasites of the genus *Schistosoma*. Despite ongoing treatment programmes, the prevalence of schistosomiasis has failed to decline and the disease remains a cause of severe morbidity in millions of people. Understanding the biology of egg production by schistosomes is critical since eggs allow transmission of the infection, and when trapped in host tissues induce the immune responses that are responsible for the pathologic changes that underlie disease development. Unusually among trematodes, adult schistosomes exhibit sexual dimorphism and display a fascinating codependency in that the female is dependent on the male to grow and sexually mature. Thus, virgin females are developmentally stunted compared with females from mixed-sex infections and are unable to lay eggs. Moreover, fecund female schistosomes rapidly lose the ability to produce eggs when placed in tissue culture. Here we discuss the metabolic regulation of egg production in schistosomes, and in particular the critical role played by fatty acid oxidation in this process.

Introduction

Infection with trematode flatworms of the genus *Schistosoma* causes chronic and debilitating disease in over 200 million people worldwide (Chitsulo *et al*., 2004; King and Dangerfield-Cha, 2008). Adult *S. mansoni* worms live within the mesenteric veins producing eggs that are intended to pass into the intestinal lumen for release into the environment to continue the life cycle and allow transmission of the infection (Pearce and MacDonald, 2002). However, because blood within the portal vasculature flows away from the intestine, many eggs are carried to the liver, where they become trapped in sinusoids, and elicit strong Th2 cell-mediated immunopathology which is the cause of disease manifestations (Pearce and MacDonald, 2002). Since egg production is key for both transmission and pathogenesis, studying the mechanisms involved in schistosome reproductive development could lead to new methods of preventing or treating disease (LoVerde, 2002). Reproduction is a bioenergetically demanding process for female schistosomes, which each can produce 300 or more eggs per day (depending on species). Here we will focus on what is known about the metabolic regulation of egg production by these parasites.

Unusually among parasitic trematodes, adult schistosomes exhibit sexual dimorphism and display a fascinating codependency: the female resides in a groove (the gynecophoric canal) on the ventral side of the male, and ongoing physical pairing (but not sperm transfer; Basch and Basch, 1984) is necessary for proper sexual development (Armstrong, 1965; Michaels, 1969; Erasmus *et al*., 1982; Basch and Basch, 1984; Popiel *et al*., 1984b; Shaw, 1987; Kunz, 2001). Unmated adult female schistosomes, from female-only infections, are developmentally stunted compared with females from mixed-sex infections and are unable to lay eggs (Kunz, 2001; Grevelding, 2004). Furthermore, egg-laying females that are physically separated from their partners and are surgically implanted into a host in the absence of male worms cease egg production and regress reproductively to an immature state. Interestingly, this regression is reversible because normal reproductive activity is resumed when separated females are re-paired with males (Erasmus, 1973; Popiel and Basch, 1984a; Kunz, 2001). Much of the change in overall size of a female worm as it sexually matures or regresses is due to changes in the vitellarial tissues. The vitellarium is a proliferative tissue that occupies the posterior two-thirds of the female and produces cells that surround the ovum and provide the precursor proteins for eggshell formation and nutrients for the developing embryo. There is evidence that vitellarial involution in separated female parasites, and the failure of the vitellarium to develop in virgin females, is due to a lack of

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immature vitellocyte proliferation under these conditions (Den Hollander and Erasmus, 1984; Knobloch *et al*., 2002), or to profound increases in mature vitelline cell apoptosis (Galanti *et al*., 2012), or perhaps to a combination of both. While clearly sufficient to allow and sustain female development *in vivo*, male parasites are insufficient to prevent vitelline cell apoptosis, vitellarial atrophy or female sexual regression *in vitro*, suggesting that an additional factor(s) present in the host, but absent in tissue culture, is playing a critical role in female reproductive tract health (Galanti *et al*., 2012). This is an issue, since the absence of a culture system for schistosomes that fully supports the production of viable eggs by female worms represents a roadblock to the detailed molecular study of these parasites (Kunz, 2001).

Female schistosome maturation and fecundity: a matter of correct nutrition?

Mating-associated changes in vitelline cell proliferation and apoptosis are intriguing because differing metabolic pathways are argued to preferentially favour cellular proliferation versus cellular longevity in other systems (Pearce, 2010). There have been numerous suggestions that male parasites promote female maturation by 'providing' key nutrients (e.g. Gupta and Basch, 1987). The fact that starvation in planaria (free living flatworms) can lead to reversible reproductive system involution through apoptosis is consistent with the possibility that vitelline cell loss is the end result of nutritional deprivation in female parasites (Hyman, 1951; Pellettieri *et al*., 2010). This view of vitellarial involution is compatible with the observed effects of tissue culture on paired and unpaired females, since it is conceivable that regardless of the presence of male parasites, culture conditions are failing to provide key nutrients that would normally be available *in vivo*. This is consistent with the views of Paul Basch, who went to great lengths to create a complex medium that could support the development and maintenance of fecund female schistosomes (Basch, 1981) (success eluded him in this endeavour), that the inability of unpaired worms to produce eggs is a reflection of the fact that they are undernourished (Gupta and Basch, 1987). It seems reasonable that male parasites may serve to provide a signal that allows female parasites to access key nutrients, and that this process is of value only if the nutrients are present in the environment. (We will not specifically address the nature of this male-derived signal in this review.)

Fecund female worms from mixed-sex infections can produce eggs immediately *ex vivo*, but lose the ability to do so over the course of several days *in vitro*. We postulate that this reflects the use and final exhaustion of stored metabolic resources. The most likely metabolic stores in schistosomes are glycogen and fat. Glycogen is a source of glucose, but glucose is present in excess in tissue culture, and can be used for survival by worms in this setting (Schiller *et al*., 1975), and so the depletion of glycogen stores seems unlikely to be a critical factor *in vitro* (or *in vivo*). Moreover, < 3% of the dry weight of female schistosomes is made up of glycogen, but these worms take up three times their dry weight of glucose each day, supporting the view that glucose is used directly and not to establish energy stores in the form of glycogen (Skelly *et al*., 2014). Rather, lipid stores are most likely to be critical here. Schistosomes possess considerable triacylglycerol stores when recovered from mice (Brouwers *et al*., 1997), but the function of these stores has remained unclear (Berriman *et al*., 2009). Available evidence, discussed in detail below, indicates that these triacylglycerol stores are an essential intermediate in the metabolic pathway that supports egg production. Schistosomes are unable to synthesize fatty acids from other substrates (Meyer *et al*., 1970), but can take up fatty acids from the environment and store them as triacylglycerols (Rumjanek and Simpson, 1980; Young and Podesta, 1982). Female schistosomes ingest large amounts of lipid (50% of their body mass per day) (Skelly *et al*., 2014). Fatty acid uptake is believed to occur across the gut surface; saposin homologues have been identified in the gut lumen in schistosomes, supporting this as the major route of uptake (Don *et al*., 2008; Skelly *et al*., 2014). Intriguingly, the fecundity of schistosomes is dramatically increased when, experimentally, their hosts are fed with high-fat diets (Neves *et al*., 2007). Taken together, we believe that these observations support the view that fat is an essential nutrient for egg production by schistosomes.

Oxidative phosphorylation (OXPHOS) is a critical pathway for schistosome egg production, but not for schistosome survival

Glucose, fatty acids and amino acids are important macronutrients because they are used to fuel the production of ATP. The two major pathways that generate ATP are glycolysis, fuelled by glucose, and OXPHOS, which is coupled to the TCA cycle, which can be fuelled by glucose, fatty acids and glutamine (Fig. 1). ATP production by glycolysis can occur in low or absent oxygen, and under these conditions is referred to as Warburg metabolism (Warburg, 1956). In addition to generating energy, these pathways allow the production of key intermediates for biosynthesis. There is a general view that there is no appreciable lipid or amino acid catabolism in helminth parasites (Barrett, 2009) and glycolysis is considered to be the essential source of ATP in the intramammalian stages of schistosomes (Schiller *et al*., 1975; Barrett,

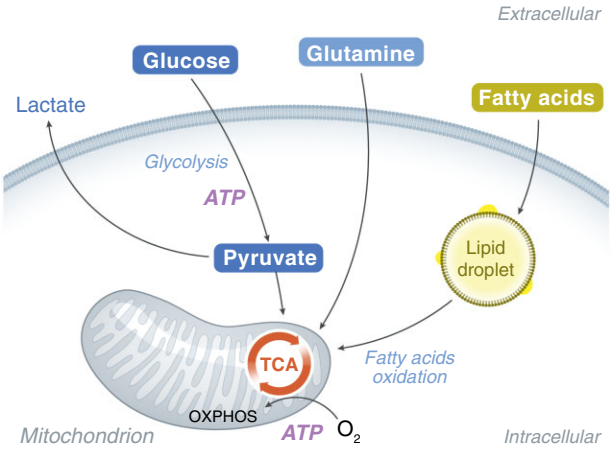

Fig. 1. Glycolysis allows the import of glucose and its conversion into pyruvate in the cytosol. Pyruvate has two possible main fates. The first is conversion into lactate. In this process, NAD^+ is produced which can be reused for the anaerobic production of ATP by glycolysis (Warburg metabolism). Alternatively, pyruvate enters mitochondria where it is converted into acetyl-CoA which enters the TCA cycle. Fatty acids and glutamine can also be utilized via the TCA cycle as indicated. Fatty acids are used for the synthesis of triacylglycerols, which can be stored in lipid droplets. Triacylglycerols are broken down by a regulated process of lipolysis to release fatty acids for oxidation. The TCA cycle fuels OXPHOS and the oxygen-dependent production of ATP.

2009) (although cercariae, the free living infectious stage, primarily use OXPHOS; Horemans *et al.*, 1991). Published data supporting the view that adult schistosomes are dependent on glucose are extensive, and include, for example, those in a paper by Schiller *et al.* (1975) which over 35 years ago reported that schistosomes can survive *in vitro* in anaerobic conditions as long as glucose is present. Under these conditions, mitochondrial OXPHOS cannot occur and the worms must be using Warburg metabolism to generate energy. Interestingly, Schiller *et al.* (1975) reported that worms placed under anaerobic conditions *in vitro* rapidly ceased egg production. This contrasts with aerobic cultures where female schistosomes continue to produce viable eggs for several days *ex vivo* (Michaels and Prata, 1968; Galanti *et al.*, 2012; Rinaldi *et al.*, 2012). One interpretation of these data is that schistosomes can survive using Warburg metabolism but need to use OXPHOS to produce eggs, a metabolic process that they cannot maintain in tissue culture. There are convincing data to support the view that fecund female schistosomes are using OXPHOS *in vivo*, and immediately *ex vivo* (van Oordt *et al.*, 1985; Huang *et al.*, 2012), but lose the ability to do so over time *in vitro* (Schiller *et al.*, 1975; Huang *et al.*, 2012). We speculate that this reflects the fact that schistosomes can survive by using Warburg metabolism, but are capable of using OXPHOS and that the process of egg production is dependent on this oxygen-dependent form of metabolism. In other words, there may be tissue-specific metabolic programming in female schistosomes, such that all cells use glucose through glycolysis and OXPHOS to a greater or lesser extent, but that among these cells, only mature vitellocytes have an additional and absolute requirement for OXPHOS to survive.

Fatty acid oxidation is required for egg production

Despite the ongoing belief that schistosomes cannot utilize fatty acid (or β-) oxidation to support OXPHOS (Barrett, 1981; Ferreira *et al.*, 2014a,b), the schistosome genome nevertheless encodes the enzymes of the β-oxidation pathway, through which fatty acids are catabolized into the TCA cycle (Berriman *et al.*, 2009), and mitochondrial oxygen consumption in schistosomes can be inhibited by etomoxir, a drug that blocks the transfer of activated fatty acids into mitochondria for subsequent oxidation (Huang *et al.*, 2012). Moreover, genetic loss of function of Acyl CoA synthase and Acyl CoA dehydrogenase, key enzymes in the β-oxidation pathway, also results in diminished mitochondrial oxygen consumption. Most importantly from the perspective of the topic under discussion here, all of these interventions, along with pharmacological approaches for blocking OXPHOS, have a marked inhibitory effect on egg production by female parasites recently placed in tissue culture (Huang *et al.*, 2012).

The understanding of how fatty acids are utilized by cells is developing rapidly. Following acquisition from the environment, fatty acids are converted into triacylglycerols and stored in cytoplasmic lipid droplets (LDs), from which they are released in a regulated fashion by lipolysis (Fig. 1) (Guo *et al.*, 2009; Zechner *et al.*, 2012). Fatty acids released in this way are used to fuel β-oxidation, but also act as endogenous ligands for nuclear receptors that induce the expression of genes encoding the β-oxidation pathway, and that regulate mitochondrial biogenesis (Palanker *et al.*, 2009; Haemmerle *et al.*, 2011). Thus, there is a link between LD and mitochondrial numbers and activity. Interestingly, greater than 40% of the lipid in adult schistosomes is in the form of triacylglycerol (Brouwers *et al.*, 1997) and stains for triacylglycerols have revealed that fecund female schistosomes possess a remarkable number of LD within their vitellarial tissues (Huang *et al.*, 2012). There are significantly fewer LDs in virgin females, and in previously fecund females that have ceased to produce eggs as a result of being cultured (Huang *et al.*, 2012). Moreover, mitochondrial oxygen consumption declines greatly as female schistosomes are maintained in tissue culture medium (Huang *et al.*, 2012). Thus, there are dynamic and kinetically coupled changes in LD and mitochondrial oxygen consumption that are related to

changes in female schistosome fecundity *ex vivo*. This is intriguing in the context of our understanding of the biology of the insect fat body (Arrese and Soulages, 2010). In insects, the fat body is an organ that partially surrounds the intestine and reproductive organ, and is a major site of triacylglycerol storage in cytoplasmic LD. The fat body plays a critical role in bioenergetically demanding processes such as flight and reproduction and in allowing survival during periods of starvation. While platyhelminths do not have fat body organs, it is interesting to speculate that the LD complex in vitellarial cells serves an analogous function to the fat body at least insofar as reproduction is concerned and that vitelline cell functions require them to utilize fatty acids, via a pathway that includes their storage in LD and presumably release by regulated lipolysis (Zechner *et al*., 2012).

In mammals, transcriptional activation of genes regulating fatty acid oxidation and controlling mitochondrial biogenesis is mediated to a considerable extent by the PPAR nuclear receptors (Plutzky, 2011). The identity of physiologic ligands for these receptors is intensely debated. However, an exciting recent publication has shown that the lipolysis of LD leads to the production of endogenous fatty acids that are ligands for PPARα (Haemmerle *et al*., 2011). Invertebrates lack PPARs, and work in *Drosophila* and *Caenorhabditis elegans* indicates that in these organisms, lipid mobilization and β-oxidation are regulated by a related nuclear receptor, HNF4. For example, in *Drosophila*, HNF4 null mutants are unable to use their lipid reserves even when starved, and exhibit reduced expression of genes controlling lipid catabolism and β-oxidation (Palanker *et al*., 2009). In this feedforward model, HNF4-induced increases in β-oxidation allow LD lipolysis to occur, and in the absence of HNF4, LD resources cannot be utilized even during starvation. The schistosome genome is recognized to encode at least 21 nuclear receptors including an HNF4 homologue (Wu and Loverde, 2008; Wu and LoVerde, 2011). Intriguingly, HNF4 expression is regulated through the intramammalian life stages, peaking in worms that are 5 weeks old (Wu and Loverde, 2008), which is the time at which females in mixed-sex infections begin to mature and lay eggs. HNF4 is therefore a candidate for a receptor that is able to regulate female worm mitochondrial respiration, vitellarial survival and/or fecundity.

Conclusions

Based on the available data, we have developed a model of the metabolic requirements of female schistosomes. We propose that glycolysis provides energy and intermediates for the majority of schistosome tissues, and is sufficient for survival. However, we believe that vitellocytes are highly dependent on OXPHOS and that they primarily use fatty acids acquired from their hosts to fuel this process via β-oxidation. Recent findings that female schistosomes infecting mice living on high-fat diets are fivefold more fecund than worms infecting mice being fed with regular mouse chow (Alencar *et al*., 2009) provide support, albeit indirect, for this view. It is feasible that, *in vivo*, in the absence of males, females either do not have access to, or are unable to ingest and/or absorb sufficient fatty acids to support vitellarial development. In the absence of sufficient fatty acids, the primordial vitellarial tissue could continue to create new vitellocytes by proliferation, but these cells might be unable to differentiate and survive due to a failure of β-oxidation. We hypothesize that the schistosome LD complex is functionally analogous to the insect fat body, and that *in vitro* the worms can continue to produce eggs until this reserve is depleted, after which egg production ceases. The regression of vitellarial tissue in cultured females even in the presence of male worms may reflect the fact that tissue culture medium is poor in key fatty acid nutrients that are available *in vivo* and which, in the form of short-chain and medium-chain fatty acids, are particularly well represented in portal blood versus peripheral blood (Dankert *et al*., 1981; Bergman, 1990). It is possible that beneficial effects of males on egg production during the initial stages of tissue culture (Michaels and Prata, 1968) may reflect their ability to help females utilize fatty acids that are present, although mechanistic details regarding how this might happen are unclear at present. Intriguingly, recent mass spectrometric analyses of host metabolic markers during infection have revealed highly significant declines in plasma short-chain fatty acid levels, which could be consistent with the extensive use of these fatty acids by the parasites (Wang *et al*., 2004; Balog *et al*., 2011). Future studies should aim to integrate vitellocyte metabolism with the role of the TGFβ signalling pathway, and other growth factor like signalling pathways, which have been shown to be important in schistosome reproduction (Freitas *et al*., 2007; Knobloch *et al*., 2007; LoVerde *et al*., 2009), and to explore the possible role in fatty acid oxidation of the schistosome RXR nuclear receptors, which are implicated in the regulation of expression of eggshell proteins (LoVerde *et al*., 2009), and homologues of which partner PPARs in vertebrates (Plutzky, 2011).

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