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A new model for myxosporean (Myxozoa) development explains the endogenous budding phenomenon, the nature of cell within cell life stages and evolution of parasitism from a cnidarian ancestor

D.J. Morris *

Institute of Aquaculture, University of Stirling, Stirling, Scotland FK9 4LA, UK

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ABSTRACT

The phylum Myxozoa is composed of endoparasitic species that have predominately been recorded within aquatic vertebrates. The simple body form of a trophic cell containing other cells within it, as observed within these hosts, has provided few clues to relationships with other organisms. In addition, the placement of the group using molecular phylogenies has proved very difficult, although the majority of analyses now suggest that they are cnidarians. There have been relatively few studies of myxozoan stages within invertebrate hosts, even though these exhibit multicellular and sexual stages that may provide clues to myxozoan evolution. Therefore an ultrastructural examination of a myxozoan infection of a freshwater oligochaete was conducted, to reassess and formulate a model for myxozoan development in these hosts. This deemed that meiosis occurs within the oligochaete, but that fertilisation is not immediate. Rather, the resultant haploid germ cell (oocyte) is engulfed by a diploid sporogonic cell (nurse cell) to form a sporoplasm. It is this sporoplasm that infects the fish, resulting in the multicellular stages observed. Fertilisation occurs after the parasites leave the fish and enter the oligochaete host. The nurse cell/oocyte model explains previously conflicting evidence in the literature regarding myxosporean biology, and aligns phenomena considered distinctive to the Myxozoa, such as endogenous budding and cell within cell development, with processes recorded in cnidarians. Finally, the evolutionary origin of the Myxozoa as cnidarian parasites of ova is hypothesised.

1. Introduction

The phylum Myxozoa is composed entirely of endoparasites, including some that cause diseases which substantially impact on aquaculture and fisheries around the world (Kent et al., 2001). Whilst the vast majority of species infect fish, both in freshwater and marine environments, myxozoans have been identified as parasites of all other vertebrate classes (Garner et al., 2005; Lom and Dyková, 2006; Prunescu et al., 2007; Bartholomew et al., 2008; Sitjà-Bobadilla, 2009). Invertebrate hosts are in the superphylum Lophotrochozoa and belong to the phyla Bryozoa, Annelida, Mollusca and Platyzoa (Štolc, 1899; Canning et al., 1996; Yokoyama and Masuda, 2001; Freeman and Shinn, 2011).

The placement of the Myxozoa within the tree of life has proved unusually obstinate (Evans et al., 2010). The simple trophic body form found in most vertebrate hosts, consisting of a plasmodium with single cells growing within it, has provided very few clues to ancestry or phylogenetic relationships (Canning and Okamura, 2004). Early debates were focused on morphological and developmental attributes regarding their small size, amoebic nature, reproduction, multicellular stages and the possession of specialised stinging cells, referred to as polar capsules. This led to an extended consideration of affinities with either the Protista or Metazoa and, in particular, the Cnidaria (Kent et al., 2001). The controversy was partially resolved by a genetic study that confirmed their metazoan origin (Smothers et al., 1994). Repeated attempts at a more definitive placement using molecular phylogenies have not been consistent, identifying relationships to either the Cnidaria or basal Bilateria (Siddall et al., 1995; Schlegel et al., 1996; Siddall and Whiting, 1999; Zrzavý and Hypša, 2003; Jimenez-Guri et al., 2007; Evans et al., 2010). This has been attributed to the relatively rapid evolutionary rate of the group, resulting in exceptionally long branch lengths, both leading to the Myxozoa, and separating the major clades identified within it (Evans et al., 2010).

The morphological, functional and biochemical similarities between myxozoan polar capsules and cnidarian nematoblasts have repeatedly been highlighted and commented upon (Weill, 1938; Siddall et al., 1995; Cannon and Wagner, 2002; Ringuette et al., 2011; Reft and Daly, 2012). Recent identification of a myxozoan

* Present address: Department of Mathematics and Statistics, University of Strathclyde, Livingstone Tower, 26 Richmond Street, Glasgow, Scotland G1 1XH, UK. Tel.: +44 (0) 141 5483372; fax: +44 (0) 141 548 3345.
E-mail address: morrisdj8@gmail.com

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gene with homology to a taxon-specific cnidarian nematocyst minicollagen has further indicated a relationship between these structures (Holland et al., 2011). If the Myxozoa are bilaterian, how they obtained nematocysts remains open to debate, but several possibilities have been proposed (Canning and Okamura, 2004); the alternative hypothesis, that they are cnidarian, represents a significant increase in the diversity of this phylum as endoparasites of freshwater, marine and terrestrial hosts (Morris, 2010). Both scenarios however, do not address how the Myxozoa evolved from multicellular organisms to parasites that have undergone a substantial morphological simplification in the vertebrate host.

The Myxozoa is divided into two classes: the Malacosporea and Myxosporea (Canning et al., 2000). Four species of malacosporeans and over 2,310 species of myxosporeans have been formally described (Canning et al., 2008; Morris, 2010). The myxozoan life cycle is believed to be indirect. Where life cycles have been completed, myxosporeans have been found to require a fish and an annelid, and malacosporeans require a fish and bryozoan (Wolf and Markiw, 1984; Morris and Adams, 2006a). Instances of direct transmission have been identified but these are regarded as facultative rather than obligate (Diamant et al., 2006; Morris and Adams, 2006b). The focus of this paper is on the Myxosporea, as this class represents the overwhelming majority of recorded species.

Most published reports on the Myxosporea relate to new species identified within vertebrate hosts and usually include detailed descriptions of the myxospore (the spore type released from a vertebrate host), this being the main taxonomic feature of the class (Lom and Arthur, 1989). However it has long been recognised that the classification of Myxozoa based primarily on this criterion is artificial and phylogenetic analyses have highlighted that myxospore morphology is not a reliable systematic characteristic for resolving within group relations (Fiala, 2006; Fiala and Bartšova, 2010). Phylogenetic analyses using different actinospore (i.e. myxosporean spores released from annelid hosts) morphotypes have also indicated a lack of congruence between spore type and phylogeny, reiterating that this is not a reliable character (Holzer et al., 2004). Lom et al. (1982) suggested that incorporation of additional developmental characteristics would be very helpful when considering myxozoan systematics. However, new species are still described primarily based on myxospore characteristics as presporogonic stages can be difficult to identify with certainty, especially in wild fish where infections comprised of multiple myxosporean species may be occurring. This has led to substantial gaps in our knowledge regarding fundamental aspects of myxosporean biology. Such information, however, is critical if morphological and developmental data are to be used to assist molecular approaches examining evolutionary relationships both within and without the group.

Despite the relative scarcity of information available in the literature regarding intra-vertebrate biology, four distinct developmental lineages for the Myxosporea, which approximately correspond to the main clades previously identified in molecular phylogenies, have been recognised. These clades are referred to as: sphaerosporid, marine, kudoid and freshwater (Fiala, 2006; Morris and Adams, 2008). The life cycles of relatively few species of the marine and freshwater clades have been completed, with sphaerosporid and kudoid extra-piscine development remaining totally unknown. For species of the marine clade, polychaetes appear to be the predominant host, while for species of the freshwater clade, oligochaete hosts are predominant (Picon-Camacho et al., 2009). It is obvious that combining intra-annelid development with intra-vertebrate studies would substantially aid systematics and provide a major contribution to our knowledge of myxosporean biology. However, although considered the definitive host, intra-annelid development has only been studied in a handful of cases. The interpretation of these, within the freshwater clade, has been further hampered by well-established factoids relating to key areas of myxosporean biology and the existence of confounding co-infections (Morris and Adams, 2008; Morris, 2010; Morris and Freeman, 2010). The result is that significant parts of myxosporean intra-oligochaete development need re-evaluation.

Without a detailed knowledge of myxosporean development, understanding the evolutionary origins and relationships of the Myxozoa will remain problematic. The objective of this study was to document an actinosporean stage of a member of the freshwater clade of Myxosporea, and form a developmental framework upon which other studies into the myxosporean life cycle can build. The overall goal of the study was to aid the understanding of myxozoan ontogeny and evolution.

2. Materials and methods

Archival myxozoan-infected oligochaete material collected in 2007 was used for this study, consisting of two individual *Tubifex tubifex* fixed and embedded in Spurr's resin for electron microscopy. This material has been used in two previous studies examining binucleate stages and sporoplasmogenesis (Morris, 2010; Morris and Freeman, 2010). The oligochaetes had been collected from the same fish pond and were infected with the same aurantiactinomyxon-type myxosporean. Previous examination provided no evidence of co-infection and has confirmed, beyond reasonable doubt, that the parasites studied represent a single species, with phylogenetic analysis of the 18 small subunit ribosomal gene region confirming placement within the freshwater myxosporean clade (Morris and Freeman, unpublished data). Ultrathin sections were stained using uranyl acetate and lead citrate and examined at 120 kV using a Technai Spirit G^2 electron microscope. In total, 32 grids were examined, each holding a minimum of two ultrathin sections, each section containing two separate pieces of worm. Sections contained on a grid were serial, while between each grid the cut was advanced by 10 μm.

3. Results

Both oligochaetes were heavily infected with the myxosporeans. The developmental stages observed were binucleate proliferating stages, immature and mature pansporocysts.

3.1. Pre-pansporocyst development

Numerous binucleated cells were present, primarily underlying the vascular lamina of the supra-intestinal vessel. Interactions between these cells were noted, with filopodial extensions connecting neighbouring parasites (Fig. 1). However, it could not be determined whether these represented the final stages of division, initiation of cell fusion or just the result of chance encounters. The characteristic sporoplasmosome organelles present in the binucleate cell cytoplasm were not observed to be overtly involved in any cellular interactions.

No tetranucleate cells, as detailed in other studies (e.g. Janiszewska, 1957; Marques, 1987; El-Matbouli and Hoffmann, 1998; Özer and Wootten, 2001), could be unambiguously identified within the tissues examined. However, a single multi-nucleated parasite cell was observed that appeared to be undergoing plasmotomy, with a notable protrusion partially enclosing the original cell (Fig. 2). This protrusion was the only part of the cell that contained sporoplasmosomes. A young pansporocyst was noted; this was composed of four cells in section, two of which were envelope cells. The nuclei of the envelope cells were opposite each other, and the cells connected with adherens junctions, forming