

## Review Article

# The Ins and Outs of Nuclear Trafficking: Unusual Aspects in Apicomplexan Parasites

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Apicomplexa is a phylum within the kingdom Protista that contains some of the most significant threats to public health. One of the members of this phylum, *Toxoplasma gondii*, is amenable to molecular genetic analyses allowing for the identification of factors critical for colonization and disease. A pathway found to be important for *T. gondii* pathogenesis is the Ran network of nuclear trafficking. Bioinformatics analysis of apicomplexan genomes shows that while Ran is well conserved, the key regulators of Ran—Regulator of Chromosome Condensation 1 and Ran GTPase activating protein—are either highly divergent or absent. Likewise, several import and export receptor molecules that are crucial for nuclear transport are either not present or have experienced genetic drift such that they are no longer recognizable by bioinformatics tools. In this minireview we describe the basics of nuclear trafficking and compare components within apicomplexans to defined systems in humans and yeast. A detailed analysis of the nuclear trafficking network in these eukaryotes is required to understand how this potentially unique cellular biological pathway contributes to host–parasite interactions.

### Introduction

**A**PICOMPLEXAN PARASITES ARE a major health concern, causing disease to humans and animals worldwide. In addition to their medical relevance, apicomplexans should also be appreciated as divergent eukaryotes with unique cellular biology. Members of this phylum that are medically important to humans and of common research interest include *Plasmodium* spp., *Cryptosporidium* spp., and *Toxoplasma gondii*. Between 350 and 500 million people worldwide become infected by *Plasmodium* annually. Most of the over one million people who succumb to the *Plasmodium* infection each year are children in sub-Saharan Africa. *Cryptosporidium* causes severe diarrhea in children of developing countries and immunocompromised individuals worldwide. *T. gondii* is a very successful colonizer of humans, with an estimated one third of the world's human population infected (Tenter *et al.*, 2000). While normally dormant for the duration of the host's lifetime, *T. gondii* can become an opportunistic pathogen that causes encephalitis in immunocompromised patients, and can be passed vertically to developing fetuses during gestation (Dubey, 1994; Dubey, 1998).

*T. gondii*, like other Apicomplexa, is a single-cell eukaryote with many unique organelles, including micronemes, rhop-

tries, dense granules, and the apicoplast. Along with these distinct organelles, *T. gondii* contains the typical organelles found in other eukaryotes such as the Golgi, endoplasmic reticulum, a single mitochondrion, and a nucleus. However, the typical organelles do not always contain standard components as defined by model eukaryotes. In particular, recent studies have identified a highly divergent ortholog of the Regulator of Chromosome Condensation 1 (RCC1) that is critical for parasite pathogenesis (Frankel *et al.*, 2007) and functions as a guanine nucleotide exchange factor (GEF) for the GTPase Ran (Frankel and Knoll, 2008). These studies have opened questions about the sequence divergence and apparent absence of key components of nuclear transport in apicomplexans.

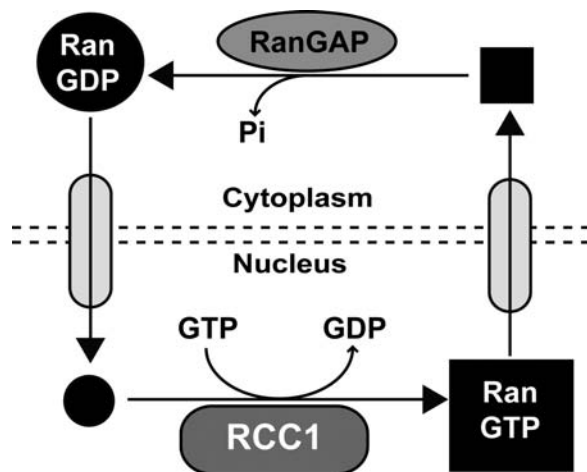
Here we review the basics of nucleocytoplasmic transport, highlighting the potential differences in transport machinery present in apicomplexan parasites. We have performed bioinformatics analyses and compiled a comprehensive table that includes the orthologs present or absent in the three human pathogens, *T. gondii*, *Plasmodium falciparum*, and *Cryptosporidium parvum*, compared to human nuclear trafficking components. The apparent absence of sequence similarity to many factors of nuclear transport requires an investigation to determine whether unique nucleocytoplasmic transport machinery exists in apicomplexans or if

there are simply fewer components. If a simplified system does exist in apicomplexans, due to the increasing genetic and proteomics tools available for *T. gondii*, we propose the possible advantages of using *T. gondii* as a model system to study the complex mechanisms of nuclear transport.

### The Master Switch, Ran

The first step of understanding nuclear transport is to identify the main protein driving it, Ran, a member of the Ras GTPase superfamily. All Ras family members are bound to GTP or GDP, serving as an “on/off” switch. Structural changes occur in Ran depending on its nucleotide-bound state, which results in different substrate specificity. Ran has a high level of sequence identity between eukaryotic organisms, including the protozoa, representing its well-conserved function among species. Along with driving nuclear transport, there are two other described functions for Ran: re-assembly of the nuclear envelope during mitosis and spindle formation (Di Fiore *et al.*, 2004). The transport of cargo between the nucleus and cytosol is tightly controlled by Ran and its nucleotide-bound state. Using nonhydrolyzable analogs of GTP and GDP, as well as dominant-negative Ran mutants, it was determined that the GTP-bound form of Ran is the sole energy source for nucleocytoplasmic transport (Melchior *et al.*, 1993; Carey *et al.*, 1996; Palacios *et al.*, 1996; Murphy *et al.*, 1997; Richards *et al.*, 1997). There is a clear asymmetry of the two bound nucleotide states of Ran, with Ran-GTP being primarily located in the nucleus and Ran-GDP in the cytoplasm. This differential localization of Ran requires the spatial separation of its two opposing regulators, RCC1 and Ran GTPase activating protein (RanGAP) (reviewed in Moore, 1998).

RCC1 is an essential nuclear protein bound to chromatin that serves as a GEF for Ran, replacing RanGDP with GTP



**FIG. 1.** The Ran cycle. Ran cycles between the nucleus and cytoplasm bound to either GDP or GTP. RanGDP enters the nucleus and encounters RCC1. GDP is then replaced with GTP by RCC1, which creates a predominance of RanGTP. In the cytoplasm, RanGTP quickly interacts with RanGAP, and the GTP is hydrolyzed to GDP. The actions of RCC1 and RanGAP create a steep gradient of RanGDP in the cytosol and RanGTP in the nucleus (represented by a large circle or box, respectively).

(Fig. 1) (Bischoff and Postngl, 1991). RCC1 has been shown to bind to histones H2A and H2B, which enhances its GEF activity (Nemergut *et al.*, 2001). RCC1 proteins contain seven tandem repeats of 50–60 amino acids termed RCC1 repeats. These tandem repeats form a seven blade propeller structure with one side binding to Ran for exchange activity and the other bound to chromatin (Azuma *et al.*, 1996, 1999; Renault *et al.*, 1998). Surprisingly, none of the sequenced protozoa contain typical RCC1 proteins with seven tandem RCC1 domains.

Due to the low intrinsic GTPase activity of Ran alone, RanGAP is located in the cytosol and enhances the hydrolysis of RanGTP to RanGDP approximately 1000-fold. This GTPase activity is aided by Ran binding proteins 1 and 2 in mammalian systems; however, yeast encodes only Ran binding protein 1 (Bischoff and Gorlich, 1997). RanGAP possesses structural symmetry, consisting of 11 leucine-rich repeats that form a crescent shape (Hillig *et al.*, 1999). Mammalian RanGAP functions as a dimer, whereas yeast forms do not. RanGAP also contains nuclear export signals (NES) to ensure that if any protein enters the nucleus, it is quickly exported. Similar to the divergence seen with RCC1, bioinformatics analyses show that none of the sequenced protozoa contain any protein with similarity to RanGAP. With RCC1 exclusively nuclear and RanGAP cytosolic, it creates an essential gradient of RanGDP in the cytoplasm and RanGTP in the nucleus. Perturbations to the Ran system, either by altering the cellular localization of RCC1 (Akhtar *et al.*, 2001) or by introducing dominant negative Ran mutants (Carey *et al.*, 1996), are lethal. It is perplexing how protozoa regulate such an essential process as nuclear trafficking with divergent Ran regulators.

### The Ins and Outs of Nuclear Trafficking

In eukaryotes, the nuclear and cytoplasmic compartments are divided by the nuclear envelope. Cells use this division to regulate numerous functions, such as transcription and translation, by controlling the movement of molecules between the nucleus and the cytosol. Proteins smaller than approximately 40 kDa are able to passively diffuse through the nuclear pore complex (NPC), whereas larger proteins require active transport through the assistance of karyopherins, specific transport receptors that shuttle between the nucleus and cytosol (reviewed in Macara, 2001). Karyopherins can be subdivided into those that transport molecules into the nucleus (importins) and those that transport molecules out of the nucleus (exportins). Karyopherins are able to distinguish between the diverse proteome to target specific cargo molecules for transport. In yeast, it is estimated that over 2000 proteins are shuttled between the nucleus and the cytoplasm, yet the receptors and signals are known for only a few.

### Characterized Import Components

Importin  $\beta$  is responsible for transporting a large number of cargo molecules into the nucleus. The importin  $\beta$  superfamily consists of 14 members in yeast and more than 20 in mammals. Most substrates are identified by their nuclear localization signal (NLS), which is encoded in either a mono- or a bi-partite sequence characterized as stretches of basic amino acids

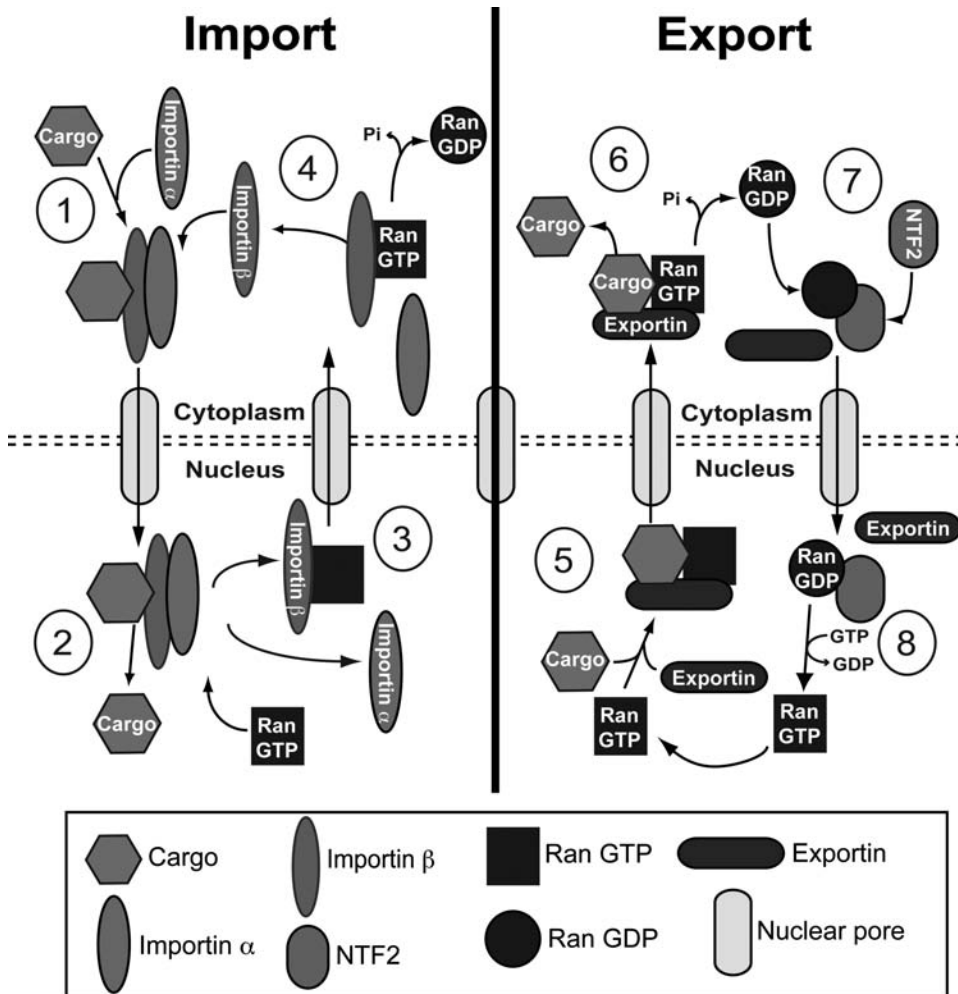
(Hodel *et al.*, 2001). The identification of NLS sequences using bioinformatics methods can be difficult because nonfunctional NLSs can be buried within the tertiary structure, or functional NLS sequences can be missed if they are short or if protein folding brings together basic amino acids. Additionally, with growing knowledge of import and export receptors, new binding sites and sequences required for nuclear transport will likely be discovered. Imported proteins will most often bind to an importin  $\alpha$  molecule, although direct binding to importin  $\beta$  does occur. Importin  $\alpha$  serves as a middleman, to identify cargo destined for the nucleus and then interact with importin  $\beta$ , which is needed for binding and movement through the NPC (Fig. 2) (reviewed in Fried and Kutay, 2003). There are at least six identified importin  $\alpha$  isoforms in humans, two in *Schizosaccharomyces pombe*, and only one in *Saccharomyces cerevisiae* (SRP1). In addition, transportin is another importin that does not require an adaptor (importin  $\alpha$ ) to serve as a receptor for nuclear import. Transportin recognizes a 38-residue signal termed the M9 domain, which does not contain basic amino acids and is best characterized for hnRNP1 (Bogerd *et al.*, 1999).

Once the cargo–receptor complex makes its way into the nucleus, it encounters Ran bound to GTP. RanGTP binds to importin  $\beta$ , which causes a conformational change and subsequent dissociation of the complex that releases the nuclear cargo (reviewed in Cook *et al.*, 2007). Importin  $\beta$ ,

coupled to RanGTP, shuttles back to the cytoplasm where it quickly encounters RanGAP. This leads to the hydrolysis of RanGTP to GDP to release Ran from importin  $\beta$ . For cargo requiring importin  $\alpha$ , a specific transport receptor termed CAS binds with a second RanGTP, and then the ternary complex shuttles back to the cytosol to interact with RanGAP and start the cycle over. RanGDP in the cytosol is transferred back to the nucleus by its exclusive transporter, NTF2, to be exchanged to its GTP-bound state by RCC1 (Ribbeck *et al.*, 1998). The energy required to fulfill a complete cycle is the hydrolysis of GTP on Ran.

**Characterized Export Components**

Protein export from the nucleus takes a similar route as import. The most prominent exportin is CRM1, named Xpo1p in yeast. It recognizes proteins with a short leucine-rich region, which acts as a NES. To exit, cargo forms a ternary complex with an exportin receptor and RanGTP. This complex traverses the NPC to the cytosol where RanGAP induces hydrolyses of GTP on Ran. The conformational change induced by the nucleotide change causes the exported cargo to be released. Empty exportin and RanGDP are then recycled back to the nucleus. There are additional cofactors that aid in either the binding of cargo to CRM1 or the release of cargo from its receptor. Examples include



**FIG. 2.** Nucleocytoplasmic transport. For import of molecules, cytoplasmic cargo is identified by importin  $\alpha$ , which then binds to importin  $\beta$  (1). This ternary complex translocates through the nuclear membrane and into the nucleus. Once there, RanGTP binds to importin  $\beta$  and causes a dissociation of the complex, which releases cargo to the nucleus (2). Import receptors are then recycled back to the nucleus (3) through binding of RanGTP and export to the cytosol. RanGTP is then hydrolyzed to the GDP-bound state and causes the release of the import receptors (4) and the cycle starts over again. Export of cargo undergoes a similar mechanism. Exported molecules will bind to the export receptor with RanGTP and exit the nucleus (5). Next RanGTP is hydrolyzed to cause release of cargo into the cytoplasm (6). NTF2 specifically identifies RanGDP and returns it to the nucleus (7) for RCC1 to then exchange it to RanGTP (8).

RanBP3, Nxt1, RanBP1, and, possibly, eIF-5A. RanBP3 binds directly to CRM1 and enhances its affinity for RanGTP- and NES-containing cargo (Lindsay *et al.*, 2001). Nxt1 binds to RanGTP to aid in cargo-receptor complex dissociation at the NPC (Black *et al.*, 2001).

In addition to CRM1, there are at least six other export receptors in humans. The yeast exportin Msn5 (closest ortholog is exportin 5 in humans) is interesting as it also serves as an importin for at least one cargo (RPA) (Yoshida and Blobel, 2001). Exportin 6 is responsible for the export of profilin-actin complexes (Stuven *et al.*, 2003), and exportin 7 performs general export using a CRM1-independent export signal (Mingot *et al.*, 2004). Exportin-t (called Los1p in yeast) is responsible for tRNA export (Kutay *et al.*, 1998), while TAP (called Mex67 in yeast) performs mRNA export (Segref *et al.*, 1997). There are likely other import and export receptors yet to be elucidated.

### Components of Nuclear Trafficking in Apicomplexans

No predicted proteins in any sequenced apicomplexan encode seven tandem RCC1 repeats and are predicted to be

nuclear. MAL7P1.38, the *P. falciparum* ortholog with the highest *E*-value against human RCC1, is 688 amino acids, contains only three repeats, and is predicted to be nuclear (Table 1). The reported RCC1 ortholog in *P. falciparum* is 1327 amino acids, much larger than the conventional RCC1 proteins of ~420 amino acids (Ji *et al.*, 1998). This *P. falciparum* protein contains six RCC1 repeats and is localized to the nucleus, but has not yet been confirmed to be a functional GEF. These RCC1 repeat-containing proteins may have redundant GEF activity or may be active during different stages of the lifecycle. *T. gondii* encodes 13 proteins that contain RCC1 repeats. The *T. gondii* RCC1 ortholog (TgRCC1) has the highest similarity to human RCC1 and localizes to the parasite nucleus (Frankel *et al.*, 2007). The *C. parvum* ortholog with the highest sequence identity is *cgd6\_2770*. This 487 amino acid protein has seven tandem RCC1 repeats, but no obvious NLS to direct the protein to the nucleus. In *S. cerevisiae*, the Ats1p protein contains four RCC1 repeats and is localized to the cytosol, yet this protein does not appear to function as a GEF and is not an essential protein (Kirkpatrick and Solomon, 1994). It is possible that the apicomplexan parasites encode a protein similar to Ats1p, whose function(s) is yet to be determined.

TABLE 1. COMPARISON OF NUCLEAR TRANSPORT COMPONENTS

Protein name	Accession no.	<i>S. cerevisiae</i>	<i>T. gondii</i> (E-value)	<i>P. falciparum</i> (E-value)	<i>C. parvum</i> (E-value)
RCC1	BAA00469	Prp20	31.m00904 (7.5e-27)	MAL7P1.38 (9.4e-24)	<i>cgd6_2770</i> (2.1e-23)
RanGAP	CAA57714	RNA1	None	None	None
Ran	P62826	GSP1	50.m00042 (4.8e-84)	PF11_0183 (4.6e-79)	<i>cgd7_220</i> (3.0e-84)
Importin alpha	NP_002255	Srp1p	52.m00001 (5.9e-127)	PF08_0087 (1.2e-110)	<i>cgd8_3260</i> (8.4e-116)
Importin beta	NP_002256	Kap95	83.m01307 (e-100)	PF08_0069 (4.1e-77)	<i>cgd7_3030</i> (3.1e-106)
Transportin 1	NP_002261	Kap104	35.m00891 (e-118)	PFF1345w (3.9e-88)	<i>cgd8_3440</i> (5.3e-98)
Transportin 2	NP_038461	Kap104	35.m00891 (e-121)	<i>PFF1345w</i> (4.1e-83)	<i>cgd8_3440</i> (1.2e-88)
Transportin SR1	AAD38537	Mtr10	57.m01705 (6.0e-09)	None	None
Transportin SR2	NP_036602	Kap122p	57.m01705 (7.0e-09)	None	None
Importin 4	NP_078934	Yrb4p	44.m02667 (2.0e-24)	<i>PFE1195w</i> (4.0e-07)	<i>cgd7_3270</i> (2.1e-05)
Importin 5/RanBP5	CAA70103	Pse1p	44.m02525 (4.0e-83)	<i>PFE1195w</i> (3.3e-98)	<i>cgd7_3270</i> (1.8e-97)
Importin 7	NP_006382	Nmd5p	42.m03288 (9.0e-32)	MAL7P1.202 (2.0e-17)	<i>cgd7_790</i> (4.1e-07)
Importin 8	NP_006381	Kap119p	42.m03288 (2.0e-25)	<i>MAL7P1.202</i> (3.4e-16)	<i>cgd3_1790</i> (3.4e-06)
Importin 9	NP_060555	Kap114p	44.m02525 (0.003)	None	None
Importin 11/RanBP11	AAF21936	Lph2p	52.m01606 (4.0e-04)	PF14_0304 (5.4e-08)	<i>cgd3_1790</i> (5.4e-05)
Importin 13	NP_055467	Pdr6p	50.m03311 (5.0e-05)	<i>PFC0135c</i> (0.0045)	None
NTF2	NP_005787	Ntf2	44.m00054 (7.0e-20)	PF14_0122 (8.0e-17)	<i>cgd4_590</i> (1.4e-23)
Nxt1	AAD54942	Nxt1	44.m00054 (8.6e-07)	<i>PF14_0122</i> (1.8e-06)	<i>cgd4_590</i> (1.4e-08)
CRM1/Exportin 1	NP_003391	Xpo1p	50.m03311 (6.0e-189)	<i>PFC0135c</i> (1.8e-123)	<i>cgd3_3060</i> (7.4e-117)
TAP	NP_006353	Mex67p	None	None	None
CAS	NP_001307	Cse1p	52.m01606 (6.0e-88)	PFI1590c (3.0e-33)	<i>cgd3_1790</i> (3.4e-35)
Exportin-t	NP_009166	Los1p	50.m03311 (3.0e-06)	MAL13P1.83 (1.6e-23)	<i>cgd7_1780</i> (2.0e-16)
Exportin 4	NP_071904	None	41.m00005 (1.6e-07)	<i>PF10490c</i> (2.6e-08)	<i>cgd7_1670</i> (6.2e-08)
Exportin 5	NP_065801	Msn5p	50.m03311 (7.0e-12)	<i>PFC0135c</i> (9.1e-13)	<i>cgd3_3060</i> (2.7e-08)
Exportin 6	NP_055986	None	50.m03311 (3.7e-10)	<i>PFC0135c</i> (1.8e-07)	<i>cgd3_3060</i> (7.2e-05)
Exportin 7	NP_055839	None	41.m00005 (e-161)	PF10490c (4.9e-112)	<i>cgd7_1670</i> (1.7e-134)
RanBP1	CAG30442	Yrb1p	83.m01206 (7.0e-24)	PFD0950w (8.1e-24)	<i>cgd5_3950</i> (4.2e-31)
RanBP3	Q9H6Z4	Ranbp3	None	<i>PFD0950w</i> (0.025)	<i>cgd5_3950</i> (0.0042)
RanBP6	AAC14260	Kap121p	44.m02525 (6.0e-76)	<i>PFE1195w</i> (8.3e-89)	<i>cgd7_3270</i> (7.6e-73)
RanBP17	NP_075048	None	41.m00005 (e-121)	<i>PF10490c</i> (5.3e-105)	<i>cgd7_1670</i> (2.7e-131)

Human nuclear transport constituents (accession numbers listed in the second column) were used to BLAST search the annotated genomes of *T. gondii*, *P. falciparum*, and *C. parvum*. The *S. cerevisiae* orthologs are listed in the third column. The annotated parasite protein is listed with the *E*-value given in parentheses. To ascertain that the absence of protein orthologs was not due to inaccurate protein annotations, we used tblastn and blastn searches of the parasite genomes with the mRNA nucleotide sequence of human genes as the query. These searches did not uncover any additional orthologs. For *E*-values in italics, the annotated protein appeared in the BLAST search but has a higher *E*-value to another protein.



At 1155 amino acids, TgRCC1 is unusually large and has been proven to be involved in parasite pathogenesis (Frankel *et al.*, 2007). It was shown that TgRCC1 mutant parasites were growth defective in serum-starved host cells, and thus it was speculated that the rate of nuclear trafficking is important for eukaryotic pathogens under the stress of nutrient limitation *in vivo* (Frankel *et al.*, 2007). Even though TgRCC1 contains only five RCC1 domains, it is able to complement a temperature-sensitive mammalian RCC1 mutant cell line (TsBN2) (Frankel and Knoll, 2008). The ability of TgRCC1 to complement the TsBN2 cell line creates a potential model for the functional analysis of parasite RCC1 proteins. TgRCC1 contains a zinc finger motif that is found in the nucleoporins Nup153 and Nup358 (RanBP2). This zinc-finger motif from RanBP2 has been shown to interact directly with Ran (Yasun and Blobel, 1999). While we speculated that the zinc-finger aids in Ran binding in the absence of the typical seven RCC1 repeats, alteration of the TgRCC1 zinc-finger motif did not ablate function (Frankel and Knoll, 2008). *C. parvum* also contains cgd4\_1350, a 958 amino acid protein that is predicted to be nuclear with four RCC1 repeats and the same zinc-finger motif present in TgRCC1. It is likely that a function for this conserved zinc-finger motif will be uncovered with further analysis of these RCC1 orthologs.

Sequence similarity searches have been unable to identify a RanGAP ortholog in any protozoan. Keyword searches among annotated proteins in the *T. gondii* genome database identified one candidate, but sequence analysis shows a strong similarity to RanBP1. Perhaps the RanGAP function in apicomplexans is performed by a single protein with multiple cellular responsibilities (i.e., a fusion of Ran binding protein 1 and RanGAP). It is also possible that a completely unique protein possesses the RanGAP function. Another possibility is that *T. gondii* Ran has a higher intrinsic GTPase activity than other homologs, removing the need for RanGAP. Higher intrinsic GTPase activity would require additional cofactors in the nucleus to prevent this activity, and would reveal a novel Ran network. We have observed that TgRan displays similar kinetics of GTPase activity to human Ran (Frankel and Knoll, 2008), suggesting the presence of an unidentified TgRanGAP. It will be critical to determine what protein is performing the RanGAP function.

Apicomplexans encode Ran and NTF2 orthologs to recycle Ran from the cytosol to the nucleus. While *T. gondii* Ran shares 71% amino acid identity to human, there are clear differences in Ran properties from described systems. For example, TgRan is found throughout the parasite cell, as opposed to the predominantly nuclear localization in all other eukaryotes examined (Frankel and Knoll, 2008). In addition, *T. gondii* is able to stably express dominant negative Ran mutants that are lethal in other systems. Purified human Ran T24N, which has been shown to have essentially no affinity for GTP and bound to either GDP or no nucleotide at all, binds to RCC1 and inactivates it, thus acting as a dominant negative (Kornbluth *et al.*, 1994). Ran G19V is a gain-of-function lethal mutant because Ran becomes insensitive to RanGAP activation and is locked in the GTP-bound state (Lounsbury *et al.*, 1996). Expression of TgRan T24N recapitulated phenotypes observed for TgRCC1 mutant parasites, specifically attenuation in mice and a growth defect in serum-starved host cells (Frankel and Knoll, 2008). An elucidation of the Ran network in *T. gondii* will help us to

understand why these mutant forms of Ran can be expressed and which enzyme is capable of RanGAP activity.

### Import Components in Apicomplexans

An importin  $\alpha$  ortholog has previously been reported in *T. gondii* (Bhatti and Sullivan, 2005). While BLAST searches of the *T. gondii* database with both human and the *T. gondii* importin  $\alpha$  identified multiple proteins with similarity, only the annotated protein identified by Bhatti *et al.* contains the characteristic importin  $\beta$  binding domain (Bhatti and Sullivan, 2005). This importin  $\beta$  binding domain is sufficient and essential for binding to importin  $\beta$ . It will be important to determine if there are more importin  $\alpha$  family members in *T. gondii*. Importin  $\beta$  is conserved in all sequenced apicomplexans, with relatively higher similarity (Table 1). While orthologs of both importin  $\alpha$  and  $\beta$  have been reported for *P. falciparum*, functional analysis has not yet been performed (Mohammed *et al.*, 2003).

In addition to importin  $\beta$ , apicomplexans contain orthologs of importin  $\beta$  family members, including transportins and importins 4, 5, and 7. However, we were unable to identify orthologs for either human or yeast importin 4b, 8, 9, 11, and 13. Interestingly, while *T. gondii* encodes both importin 4 and 5 orthologs, *P. falciparum* and *C. parvum* each encode only one, which has a higher sequence identity to importin 5 (the *E*-values compared to either importin 4 or 5 are shown on Table 1). While humans contain 20 members of the importin  $\beta$  family and *S. cerevisiae* contains 13, *T. gondii* appears to contain only 5. If this is true, it will be the most minimal system for transport reported. It will be interesting to understand how *T. gondii* is able to perform the tremendous task of shuttling proteins between the nucleus and cytoplasm using so few receptors. Without the redundancy and complexity in other systems such as yeast or *Xenopus*, the apicomplexans may serve as an excellent model to determine the mechanism of protein import into the nucleus. No studies have looked experimentally at the number of karyopherin members in protozoa. Parasites may contain unique means of receptor-mediated transport or simply encode a small number of proteins to transport a large number of cargos.

### Export Components in Apicomplexans

The majority of nuclear export is supported by CRM1. All of the apicomplexans contain a CRM1 ortholog with high sequence conservation (Table 1). CAS, which is required for cycling importin  $\alpha$  back to the nucleus, and exportin 7 (RanBP16) are also well conserved. The NES for exportin 7 does not use the typical leucine-rich region, but instead includes folded motifs with basic residues (Mingot *et al.*, 2004). Exportin 7 has broad substrate specificity, which may be used widely in apicomplexans in the absence of other exportins. There are no orthologs for other reported export receptors such as exportin 4, 5 (discussed below), and 6 (RanBP20). In addition, there are no identifiable orthologs for the CRM1 cofactors RanBP3 and Nxt1 in apicomplexan parasites. While *P. falciparum* and *C. parvum* contain orthologs of eIF-5A, it appears that *T. gondii* does not. The karyopherin Msn5, which functions in both import and export in yeast, is not conserved in apicomplexans.

Apicomplexans appear to be missing the receptor for mRNA transport, TAP (Mex67 in yeast). Interestingly, *P. falciparum* encodes a protein with weak similarity to the yeast Mex67 (annotated protein PF14\_0305; e-value of  $4.4e^{-05}$ ). How do apicomplexans transport mRNAs? Cells may survive by passive diffusion of the RNA molecules, but given the large size of many of the *T. gondii* mRNAs, it is unlikely that these RNAs merely diffuse into the cytosol. Perhaps the *T. gondii* export receptors that share similarity to human and yeast equivalents share additional functions beyond what is described in other systems. In *Trypanosoma brucei* and *T. cruzi*, CRM1 functions to also export mRNAs (Cuevas *et al.*, 2005). Thus, CRM1 in apicomplexans may be shuttling RNAs out to the cytosol in addition to other cargo.

*T. gondii* does not encode a protein with similarity to exportin-t, which is responsible for export of tRNAs, while both *P. falciparum* and *C. parvum* contain orthologs. Studies using *Xenopus* oocysts show that inhibition of exportin-t reduces the trafficking of tRNAs by 80% (Arts *et al.*, 1998). This study illustrated that there may be additional mechanisms responsible for the transport of tRNAs. Exportin 5 is able to bind to tRNAs in a Ran-dependant fashion to transport them (Bohnsack *et al.*, 2002); however, apicomplexans do not contain an exportin 5 ortholog. *T. gondii* may possess an unknown export receptor for tRNA, as the annotation of the *T. gondii* genome has not been confirmed for many proteins. Unraveling the mechanisms of how these parasites are able to perform all of these critical functions with proteins of unknown sequence similarity will undoubtedly bring forth new knowledge to the fields of parasitology and cell biology. These studies may reveal novel machinery and transport mechanisms.

### Conclusions and Future Directions

The study of nuclear transport in apicomplexans is an understudied area of research; however, the availability of tools for the manipulation of apicomplexans makes them excellent models (Kim and Weiss, 2004). Multiple selectable markers are available to perform targeted disruption of various genes of interest. Fluorescent tags (e.g., GFP) have been used for photobleaching to monitor the movement of parasite proteins in viable cells (Hu *et al.*, 2002; Klonis *et al.*, 2002). The tet-inducible system allows induction or repression of genes of interest (Meissner *et al.*, 2001, 2002). For instance, the tet-inducible expression system was used to evaluate the key role of a microneme protein (MIC2) during the invasion process of *T. gondii* (Huynh and Carruthers, 2006). It was recently reported that fusion of a ligand-controlled destabilization domain (ddFKBP) to a protein of interest would allow for rapid degradation (Herm-Gotz *et al.*, 2007). Importantly, this action is as quick as 20 min and reversible. These tools will enable researchers to replace native copies of components of nuclear trafficking with inducible genes to determine their role in transport and their kinetics of movement, as well as to identify additional transport machinery.

Sequence analysis with human and yeast components of nuclear trafficking to the sequenced genomes of *T. gondii*, *P. falciparum*, and *C. parvum* has revealed several unexpected findings. Apicomplexans appear to be either missing several

key nuclear transport proteins compared to other model organisms, or significant genetic drift has occurred in these nuclear transport proteins such that they are no longer recognized by BLAST searches. It is possible that these parasites encode minimal transport components that perform multiple, diverse functions. These unique parasites may also have mechanisms for nucleocytoplasmic transport that have not been previously described. In particular, questions arise about how these parasites transport RNA molecules out to the cytoplasm, and what protein functions as a RanGAP. For such a critical process as nuclear transport, late branching eukaryotes have multiple transport mechanisms, which apicomplexans appear to lack. This dearth of functional redundancy may enable a simplified analysis of this complex process. While the study of nuclear trafficking mechanisms in apicomplexans will answer many important cellular biological questions, these studies can potentially unveil new drug targets in these medically relevant pathogens.

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### Disclosure Statement

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