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The role of DNA microarrays in *Toxoplasma gondii* research, the causative agent of ocular toxoplasmosis

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Abstract Ocular toxoplasmosis, which is caused by the protozoan parasite *Toxoplasma gondii*, is the leading cause of retinochoroiditis. *Toxoplasma* is an obligate intracellular pathogen that replicates within a parasitophorous vacuole. Infections are initiated by digestion of parasites deposited in cat feces or in undercooked meat. Parasites then disseminate to target tissues that include the retina where they then develop into long-lived asymptomatic tissue cysts. Occasionally, cysts reactivate and growth of newly emerged parasites must be controlled by the host's immune system or disease will occur. The mechanisms by which *Toxoplasma* grows within its host cell, encysts, and interacts with the host's immune system are important questions. Here, we will discuss how the use of DNA microarrays in transcriptional profiling, genotyping, and epigenetic experiments has impacted our understanding of these processes. Finally, we will discuss how these advances relate to ocular toxoplasmosis and how future research on ocular toxoplasmosis can benefit from DNA microarrays.

Keywords Microarrays · Uveitis · Retinitis · Parasitology · Host–pathogen interaction · Bioinformatics

Introduction

Toxoplasma gondii is an obligate intracellular Apicomplexan parasite that can infect a wide range of warm-blooded animals including humans [1]. This pathogen is one of the most common in humans due to many contributing factors that include: (1) its complex life cycle allows it to be transmitted both sexually via felid fecal matter and asexually via carnivorism. (2) *Toxoplasma* has an extremely wide host cell tropism that includes most nucleated cells. (3) In humans and other intermediate hosts, *Toxoplasma* develops into a chronic infection that cannot be eliminated by the host's immune response or by currently used drugs. In most cases, chronic infections are largely asymptomatic unless the host becomes immune compromised. Together, these and other properties have allowed *Toxoplasma* to achieve infection rates that range from ~23% in the USA [2] to 50–70% in France [3, 4].

In humans and other intermediate hosts, infections are the result of digesting parasites shed in felid feces or present in undercooked meat [4]. Both infection routes result in the infection of intestinal cells after which the parasites develop into tachyzoites, which are the fast-growing, disseminating form of the parasite. Tachyzoites replicate within intestinal cells where they stimulate recruitment of neutrophils and dendritic cells. The parasite can then infect these immune cells and use them to disseminate throughout their hosts [5, 6]. Once parasite reach their target tissue they respond to the resulting IFNγ-based T_h1 response by transforming into bradyzoites. Ultimately, bradyzoites will form quiescent tissue cysts that do not cause any significant disease [7]. Bradyzoite conversion is a critical step in the parasite's life cycle since bradyzoites are impervious to immune-mediated destruction, are relatively non-immunogenic, and are the infectious form of the parasite during horizontal transmission (e.g. digestion of undercooked meat). Thus, it is critical that tachyzoites evade IFNγ-induced death while they convert to bradyzoites. The molecular details underlying each of

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these processes are largely unknown but are important because these data could lead to the development of new drugs to treat the infection.

The past decade has seen important developments in the molecular tools to study *Toxoplasma gondii*. These include the development of transfection technologies [8–10], sequencing of both host and parasite genomes (see www.toxodb.org), increasing use and refinement of high-throughput genomic and proteomic technologies [11–17], sensitive whole animal imaging [18–20], and large-scale mutagenesis-based screens [21, 22]. These technologies and approaches have been instrumental in increasing our understanding of *Toxoplasma* replication within its host cell, bradyzoite development, and virulence mechanisms. In this review, we will focus our discussion on how the use of DNA microarrays and other high-throughput transcriptome analysis contributed to these developments and the implications these findings have for ocular disease.

The role of host cell transcription in *Toxoplasma gondii* growth

A common requirement for intracellular pathogens is they must scavenge nutrients from their hosts while avoiding innate host defense mechanisms [23]. *Toxoplasma* is no different and how it replicates within a host cell has been the focus of intense investigation by several laboratories. Biochemical- and cell-biological-based assays demonstrated that parasites modify host microtubule and intermediate filament organization [24–26], inhibit host cell apoptosis [27, 28], upregulate pro-inflammatory cytokines [29–32], and scavenge purine nucleosides, cholesterol, and other nutrients from their host cells [33, 34].

To examine the molecular basis for these changes, we and others used DNA microarrays to analyze changes in host gene expression following infection [11, 17, 35]. These studies indicated that changes in host transcription were extremely widespread. These changes came in at least two distinct waves with the first wave being induced within 2 hours and included a large number of pro-inflammatory response genes [11]. The significance of the expression of these genes will be discussed later in this review. Besides the inflammatory response genes, the first wave of gene expression also included genes (EGR1, EGR2, c-jun, and jun-B) that encode transcription factors commonly activated in response to cellular stresses. These data suggests that activation of these genes helps the infected host cell withstand the stress of a *Toxoplasma* infection. In support of this hypothesis, upregulation of these genes is not a general feature of a cell's response to infection since these genes were not modulated in host cells infected with either *Trypanosoma cruzi* [36] or the closely related Apicomplexan parasite, *Neospora caninum* [37]. This result indicated that parasite activation of these transcription factors is accomplished through a *Toxoplasma*-derived molecule that interacts with a specific host protein. One mechanism by which *Toxoplasma* can specifically signal to its host cell is by the release of proteins from the rhoptries, which are specialized secretory organelles that contain proteins secreted into the host cytoplasm and nucleus, in a manner analogous to bacterial Type III secretion systems [38]. Consistent with rhoptries being key regulators of host cell functions, upregulation of EGR2 and, likely the other immediate early response host transcription factors, is mediated by a rhoptry factor [37].

The second wave of gene expression included genes that encode proteins that function in a diverse set of cellular processes. Most striking from these studies was the finding that glucose, mevalonate, and iron metabolic genes were upregulated specifically by *Toxoplasma* [11]. This was intriguing because these genes function in pathways related to some of *Toxoplasma*'s auxotrophies. Thus, their upregulation may be necessary to increase levels these nutrients for the parasite to scavenge. Expression of the glycolytic and iron genes (as well as other genes also observed in the microarray experiments) is regulated by a common transcription factor named hypoxia inducible factor 1 (HIF1) [39, 40]. HIF1 is a heterodimer composed of α and β subunits and stabilization of HIF1α is the rate-limiting step to its activation. Consistent with the array data, *Toxoplasma* increased HIF1α proteins levels, activated HIF1-dependent transcription, and required HIF1α for parasite growth [41].

Host cell cycle modulation is a common cellular target for many types of pathogens [42]. When both proliferating and non-proliferating cells are infected with *Toxoplasma*, genes commonly associated with cell growth are differentially modulated [11, 43]. These data suggested that like other intracellular pathogens, *Toxoplasma* is actively modulating the host's cell cycle. This hypothesis was tested by several groups who demonstrated that parasite infection leads to changes in host cell cycle progression and causes cells to arrest at the G2/M border [16, 44, 45]. *Toxoplasma*'s effect on the host cell cycle was cell type independent, which was consistent with the microarray data noting differential expression of these genes in various types of infected host cells. Surprisingly, both replicating and senescent cells were similarly affected. The specific parasite factor(s) that regulate the host cell cycle is unknown but at least one appears to be a secreted factor larger than 10 kDa [45]. The importance of such an extrinsic-acting factor is unknown but could be to optimize neighboring cells for infection.

Besides regulating expression of metabolic and cell cycle genes, anti-apoptotic transcripts are a third major class of genes upregulated in *Toxoplasma*-infected cells. Given that apoptosis of cells infected with viruses and

bacteria is one host defense against infection [46, 47], it is logical that *Toxoplasma* would actively prevent host cell apoptosis and appears to do so by interfering with both extrinsic and intrinsic induction of apoptosis (reviewed in [48]). The extrinsic pathway is activated by death signals such as TNFα and FAS and is dependent on Caspase 8. *Toxoplasma* interferes with this process by blocking Caspase 8 activity [49]. In contrast, parasite modulation of the intrinsic pathway, which is activated by intracellular stress and the subsequent release of cytochrome C from the mitochondrion, is dependent on the host cell transcription factor NF-κB [50]. NF-κB is a family of five different proteins and several of these have been observed to be activated in parasite-infected host cells [51–53]. The NF-κB-regulated genes required for parasite inhibition of host cell apoptosis are unknown but candidates include the anti-apoptotic gene bcl2 as well as several members of the IAP family.

Turning on and off the switches that regulate bradyzoite development

Bradyzoite development is a complex process in which the parasite expresses enzymes that allow it to form a cyst wall while dramatically altering its metabolism and immunological characteristics [7]. These changes are important because bradyzoite-containing cysts are impervious to host defenses and currently prescribed anti-toxoplasmotic drugs. In addition, bradyzoite differentiation is a critical step in the parasite's life cycle since cysts are a transmissible form of the parasite during horizontal transfer. Molecular characterization of the genes encoding bradyzoite-specific antigens indicated that their stage-specific expression was due, in large part, to increasing the abundance of the transcripts that encode them [54–56]. It was not until the transcriptomes of bradyzoites and tachyzoites were directly compared by either comparative EST sequencing or SAGE analysis that the extent of these changes were realized [57, 58]. Although these studies were critical in allowing us to appreciate the complexity of the transcriptional changes that take place during development, the laborious nature of preparing and analyzing EST and SAGE libraries limited their ability to assess the dynamic nature of bradyzoite development.

In contrast, an important advantage of DNA microarrays is that they can readily examine multiple time points and conditions [59]. As a first step, microarrays spotted with the cDNAs used for the bradyzoite EST sequencing project [58] were generated and used to compare the transcriptional responses that take place at various time points following induction of differentiation [13]. Although these first generation microarrays were spotted with fewer than 650 unique genes, they demonstrated that the microarrays could be used to discover additional bradyzoite-specific genes. Besides gene discovery, DNA microarrays can also be used to map transcriptional pathways. As an example, the transcriptional response of wild-type parasites and bradyzoite differentiation mutants were compared after stimulating the parasites to undergo differentiation. The resulting microarray data demonstrated that the transcriptional pathways induced during development were hierarchal [60, 61].

The full complexity associated with differentiation was demonstrated using full-genome *Toxoplasma* microarrays that compared the transcriptional responses of three distinct *Toxoplasma* strains to a drug that induces bradyzoite development [62]. Analysis of the 5' proximal promoters of some bradyzoite-specific genes identified a short 6–8 bp sequence that conferred stage-specific expression. Electrophoretic mobility shift assays indicated that a parasite nuclear factor binds this promoter element [62]. This was a significant finding because *Toxoplasma*'s genome (and those of other Apicomplexan parasites) lacked genes that shared homology with most known transcription factors. Recently, however, a novel transcription factor family (ApiAP2) was identified in *Plasmodium falciparum* [63] and subsequent sequence analysis indicated that *Toxoplasma*'s genome possesses approximately 40 ApiAP2 family members [64]. Given the lack of other known transcription factors and the large number ApiAP2 proteins, it is likely that one or more members of this family will be involved in bradyzoite development.

Post-translational modifications of histones (e.g. acetylation and methylation) are a widespread epigenetic mechanism critical for regulating gene expression [65]. Due to a lack of experimentally confirmed bradyzoite-inducing transcription factors, investigators began testing whether bradyzoite-specific gene expression could be regulated by epigenetic remodeling. These experiments demonstrated that the promoters of bradyzoite-specific genes in parasites growing under tachyzoite conditions have low levels of acetylated histones and become more extensively modified after exposure to bradyzoite-inducing conditions [66, 67].

Histone acetylation is a reversible modification controlled by enzymes that add (histone acetyltransferases (HATs)) or remove (histone deacetylases (HDACs)) acetyl groups from specific lysine residues in the various histones. The importance of histone acetylation was demonstrated by inhibiting HDAC3, a histone H4 deacetylase. Treatment of parasites with a HDAC3 inhibitor (FR235222) induced tachyzoite to bradyzoite differentiation. DNA complexes co-immunoprecipitated using α-acetyl-H4 antibodies and hybridized to a high-resolution *Toxoplasma* DNA microarray demonstrated that the promoters of many but not all bradyzoite genes were hyper-acetylated on histone H4 [68]. But the fact that not all bradyzoite-specific genes harbored this modification suggests that other histone modifications

may be important for regulating these genes. Altogether, these data paint a picture in which differentiation is controlled transcriptionally by both DNA binding proteins and by epigenetic-based histone modifications. But how all of these changes come together to convert a tachyzoite to a bradyzoite remains unclear.

Besides epigenetic control of *Toxoplasma* gene expression during bradyzoite differentiation, HATs are expressed in tachyzoites [69, 70] and the expression of some tachyzoite-specific genes are epigenetically regulated [67, 71]. An extremely high-resolution DNA oligonucleotide *Toxoplasma* microarray representing a well-annotated region of Chromosome Ib was used in CHIP-on-chip assays to characterize the organization of active and silent promoters in tachyzoites [72]. This study demonstrated that the location and organization of specific modifications of acetylated and methylated histones within the genome could predict not only whether a promoter was active but the 5′–3′ orientation of the gene it was regulating.

As an intracellular pathogen, the interplay between the parasite and host cell is likely to have an impact on all aspects of the parasite life cycle including bradyzoite development. An example of this interplay came from the observation that a novel drug named Compound 1 stimulated bradyzoite development in specific *Toxoplasma* strains [73]. Multifactorial microarray analysis of RNA isolated from Compound-1-treated, mock-infected, or parasite-infected host cells led to the discovery that overexpression of a human gene named CDA1 was sufficient to promote bradyzoite development. CDA1 encodes a protein whose overexpression leads to cell cycle arrest suggesting the status of the host's cell cycle determines if a parasite will undergo bradyzoite development. This is an intriguing hypothesis given the observations that bradyzoites appear to preferentially develop in cells such as neurons and muscle cells that have exited from the cell cycle [74–76].

Virulence

The population structure of *Toxoplasma* is extremely clonal and the genotypes of the majority of *Toxoplasma* strains isolated in North America and Europe group into one of three clonal lineages (types I, II, and III) [77, 78]. In mice, type I strains are highly virulent while the other two are significantly less so [79]. Although all *Toxoplasma* strain types can cause disease in human infections, type II strains are more commonly associated with congenital infections and toxoplasmic encephalitis while type I and other atypical strains are more commonly associated with postnatally acquired infections that lead to ocular disease [80, 81]. Understanding the basis for differences between *Toxoplasma* strain types is important for two reasons. First, optimal treatment options to either prevent or cure reactivated infections may be dictated by the parasite's genotype. Second, optimal vaccine design necessitates identifying non-polymorphic antigens.

Toxoplasma virulence is a multi-step, complicated process comprised of transmission, dissemination, host immune evasion, encystation, and reactivation. Although it was commonly accepted that multiple parasite genes would be important for virulence, the first experimental data that this was true came from the finding that a cross between either two avirulent genotypes (types II and III) resulted in virulent progeny [82]. Quantitative trait locus (QTL) mapping of these progeny identified five virulence loci [83]. Thus far, two of these virulence genes have been identified as ROP16 and ROP18. These virulence genes encode rhoptry kinases that are secreted into infected host cells. In an independent study, QTL mapping of progeny from a type I/III cross also identified ROP18 as a virulence gene [84].

One way that the different strains may affect virulence is by differentially modulating host gene expression [12]. Based on human DNA microarray analysis, over 3,000 host genes were differentially expressed in cells infected with progeny from the type II/III cross [12]. Expression QTL mapping of these differences in host gene expression indicated that at least one locus on each parasite chromosome is responsible for differential expression of a portion of these host genes. Modulation of the largest number of these genes (~1,100 genes) mapped to a single locus on Chromosome VIIb and ROP16 was determined to be the gene responsible for these differences in host gene expression. Pathway analysis indicated that many ROP16-modulated genes were targets of the STAT3/STAT6 transcription factors. How ROP16 specifically regulates STAT3/STAT6-dependent expression is unknown but infection with parasites harboring the type III allele of ROP16 (which is identical to the allele in type I strains) leads to sustained activation of STAT3/STAT6 [12]. Given ROP16's role in virulence it is therefore tempting to speculate that sustained STAT3/STAT6 activation causes an overproduction of pro-inflammatory cytokines that can induces immune-mediated tissue destruction.

NK and T-cell-derived IFNγ is the critical cytokine in protection against infections with all *Toxoplasma* strains [85]. This cytokine protects against *Toxoplasma* infections by upregulating the expression of inducible nitric oxide synthase, indoleamine dioxygenase, and a family of IFNγ-regulated GTPases that degrade the parasitophorous vacuole (reviewed in [86]). Regardless of its effectiveness, some parasites can evade IFNγ-mediated killing and develop into bradyzoites. One possible mechanism by which the parasite avoids IFNγ is to disable IFNγ-induced signaling. Indeed, microarray and cell biological assays demonstrated that IFNγ-induced transcription is abrogated in cells previously

infected with *Toxoplasma* [87, 88]. In contrast to the polymorphic ROP16 and ROP18 virulence factors, *Toxoplasma's* effects on IFNγ-dependent transcription are strain independent [87]. The mechanism underlying parasite abrogation of IFNγ-stimulated transcription is still unclear but does not appear to involve blocking nuclear localization of STAT1, which is a key IFNγ-regulated transcription factor. However, infection upregulates the expression of members of the suppressor of cytokine signaling (SOCS) family that function by preventing sustained STAT1 activation or levels [89]. Thus, the parasite may utilize a feedback regulatory mechanism to reduce IFNγ-dependent signaling. It should be noted that others have failed to find evidence of SOCS-mediated inhibition of IFNγ signaling since they did not detect differences in STAT1 protein levels in mock- or parasite-infected cells [87, 90]. Instead, these studies suggest that the parasite affects the ability for STAT1 to bind DNA.

Implications for ocular toxoplasmosis

It is critical that the retina be protected from inflammation-induced damaged because photoreceptors and other neuroretinal cells cannot be replaced or regenerated. One way the retina does this is by maintaining its immune-privileged state that is characterized by a lack of lymphatics, few endogenous antigen-presenting cells, and the blood-retinal barrier [91]. Regardless, retinal infections do occur and proper regulated protective immune responses are necessary.

Toxoplasma is one of the most common infections of the retina and normally resides in quiescent tissue cysts. Two lines of evidence indicate that in the retina *Toxoplasma* cysts occasionally reactivate (reviewed in [92]). First, parasite-induced retinal scarring is often observed in *Toxoplasma*-infected individuals. In many cases, active lesions lie adjacent to older ones and are noted as satellite scars. This satellite scarring is caused by the short migration of some parasites after they emerge from a reactivated cyst. These migrated parasites then re-encyst and the new lesion begins after this cyst reactivates. Second, immune suppression of a chronically infected animal leads to ocular toxoplasmosis. Together, these data indicate that *Toxoplasma* tissue cysts are normally found in the retina, they sporadically reactivate, and efficient immune responses are required to limit parasite growth and stimulate them to re-encyst.

CD8 T cells, and to a lesser extent CD4 T cells and B cells, are required for protection against *Toxoplasma* in the peripheral tissues and the retina [93–95]. In addition, the early recruitment of blood-derived monocytes and neutrophils is also critical for protection [96, 97]. Thus, a key question is what signals the recruitment of these cells to the infected retina. DNA microarray analysis of several types of parasite-infected retinal cells (Müller and endothelial cells) [98, 99] demonstrated that several chemokines, whose primary function are the recruitment of immune cells, were upregulated by infection. These chemokines included those that recruit neutrophils—(CXCL1 (GROα), CXCL2 (GROβ), CXCL8 (IL-8), and CXCL6 (GCP2)); monocytes—CCL2 (MCP1), CCL5 (RANTES), and CX3CL1 (fractalkine); and T cells—CXCL10 (IP10) and CXCL11 (I-TAC). These data are consistent with the finding that neutrophils, monocytes, and T cells are the major types of leukocytes present in *Toxoplasma*-infected retinas (E. Charles and I. J. Blader, manuscript in preparation). These data are also largely consistent with the responses of other types of *Toxoplasma*-infected host cells suggesting that parasite-induced chemokine expression is largely independent of the type of infected host cell or tissue.

While these experiments have helped shed light on what directs immune cells to parasite-infected retinas, they still leave many questions unanswered. First, how are reactivated infections recognized in the retina? In the periphery, various TLR receptors (e.g. TLR2, TLR4, and TLR11) recognize *Toxoplasma*-derived factors [100–102]. But whether specific TLRs or other pattern recognition molecules are required in the retina is unclear [103]. While it is established that toll receptors are expressed in the retina it is not known how they contribute to the mobilization of a memory T-cell-based retinal immune response [104, 105]. Second, which antigen presenting cells are responsible for activation of memory $CD8^+$ and $CD4^+$ T cells? This is a particularly interesting problem for the retina since the existence of professional antigen-presenting cells in the retina has not been formally established. Thus, does infection induce a resident retinal cell (e.g. retinal microglia or astrocyte) to develop these properties or is antigen presentation a function only fulfilled by an infiltrating leukocyte? Third, which type of memory (effector vs. central) T cell is activated during a reactivated retinal infection? Finally, do the T cells that gain access to the infected retina do so because of a breach in blood–retinal barrier integrity or because the retina selectively permits specific types of T cells to gain entry? Obviously, these issues are not unique to ocular toxoplasmosis but are also relevant to other types of infection of the retina and other immune-privileged tissues. But given the experimental manipulability of *Toxoplasma*, it is likely that important progress will soon be made.

In addition to the immunological aspects of ocular toxoplasmosis, more parasite-centric questions regarding ocular toxoplasmosis need to be addressed and DNA microarrays are poised to have an important role in addressing them. For example, what parasite virulence factors act in the retina? As discussed above, ROP16 and

ROP18 were identified as virulence factors using QTL-based approaches. But, how these two proteins function is not known nor is it clear whether they would have a role in the retina. Besides these two proteins, the SAG1 parasite surface protein was proposed to have an important role in *Toxoplasma*-induced intestinal damage and loss of SAG1 had a slight effect on subsequent retinal damage after parasites were injected into the anterior chamber of the eye [106, 107]. But, when parasites were directly injected into the posterior chamber, there were no apparent differences in retinal damage between wild-type and SAG1 knockout parasites [108]. These data suggested that SAG1 was not acting as a virulence factor per se in the retina but rather may help the parasite cross anatomical barriers (e.g. traffic from the anterior to posterior chambers).

Another question that has not been addressed is how parasite gene expression is influenced by the host cell it is growing within. This could be important since different host cells present different environments and challenges that the parasite must overcome. For example, macrophages may release higher levels and be more responsive to anti-toxoplasmotic host defense molecules such as oxygen radicals, IFNγ, and TNFα. To survive in these conditions, parasites have developed several strategies. For example, they express an efficient set of antioxidant proteins that protect the parasite from oxidative damage [109]. But the parasite also express novel stress response/immune evasion factors as was demonstrated by the discovery that a novel parasite protein containing a patatin-like phospholipase domain was required for survival in activated macrophages [110]. It is likely that additional stress response/immune evasion factors remain to be discovered. As they have in bacterial and fungal pathogens, DNA microarrays are poised to have an important role in the identification of these factors and the proteins they interact with [111, 112].

As a parasite with a potentially devastating clinical outcome, an important goal of ocular toxoplasmosis research is the development of new drugs and treatments. There are two major reasons that new drugs are needed to treat *Toxoplasma* infections. First, the drugs currently used to treat *Toxoplasma* infections are poorly tolerated, have severe side effects, and cannot act against bradyzoites [113, 114]. Second, there are reports that *Toxoplasma* is developing resistance to the current generation of drugs [115, 116]. How resistance to these drugs has developed is not known but is critical to understand because it will lead to improved drug design and will increase our understanding of the biological functions of these drug targets. One way to understanding mechanisms of resistance is to compare the transcriptional profiles of wild-type and resistant parasites grown in the absence or presence of the drug. Such studies in bacterial resistance have demonstrated that pathogen responses to antibiotics are multifactorial and complex [117]. Whether the same will be true in *Toxoplasma* is unclear, but data from these types of experiments will likely impact new anti-*Toxoplasma* drug design.

Ocular toxoplasmosis is more severe and prevalent in Brazil than in Europe or North America suggesting that either parasite strains or host responses are responsible for these differences [118]. Genotyping of strains from the Brazilian patients have identified parasite strains that are highly divergent from the types I, II, and III strains found in Europe and North America [119]. But what properties of these strains confer their ability to cause severe ocular disease is not known. Given the significantly reduced costs of whole genome sequencing, it is likely that genome sequences of these novel strains will provide important clues. But, these studies will need to be followed by others that examine the transcriptome and proteome of these strains in order to understand how these changes in sequence correspond to biologically distinct phenotypes.

Summary

Over the past decade, the application of host and parasite microarrays have allowed *Toxoplasma* researchers to make great strides in understanding how *Toxoplasma* grows, differentiates, and causes disease. The majority of these experiments have thus far focused on tissue culture-based experimental systems or death-as-endpoint virulence studies. Relative to these systems, our understanding of how *Toxoplasma* interacts with and causes disease in the retina has been lagging. But the techniques and technologies that these other studies have pioneered (e.g. microarrays, QTL screening, and epigenetic mapping) coupled with high-throughput DNA sequencing and proteomics, will allow ocular toxoplasmosis researchers to make important and rapid advances in the very near future.

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