Summary of *Pneumocystis* Research Presented at the 7th International Workshop on Opportunistic Protists

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Twenty years have passed since the first startling cases of *Pneumocystis carinii* pneumonia were reported in gay men. During the intervening time, an explosion of knowledge occurred that focused on clinical diagnosis, therapy, and prophylaxis is HIV-infected individuals and in other immunosuppressed individuals. Concurrently, major advances were made in understanding the cellular and molecular biology of the organism, and in understanding the lung's responses to it. To focus on the current state of knowledge in this field, and to present new experimental data, the International Workshops on Opportunistic Protists were initiated in Bristol, England in 1988 [1]. These workshops have been reconvened every few years since that time, and this summary focuses on the 7th International Workshop on Opportunistic Protists, held from June 14-16, 2001 in Cincinnati, Ohio.

In the last 10 years, much of the conventional scientific and clinical knowledge about *Pneumocystis* has been challenged (Table 1). Using molecular approaches, *Pneumocystis* was reclassified from protozoan to fungus. While in the pre-1990 era, infections with *P. carinii* were very common in HIV-infected individuals, these infections are now less common in developed nations, as a result of the use of highly active antiretroviral therapy directed against HIV. However, these potent antiretroviral therapies remain unavailable to millions of HIV-infected individuals in the developing world. Previously, most clinical infections with *P. carinii* were thought to represent reactivation of latent infections. With molecular biologic approaches, it has now become clear that most cases represent a new acquisition, although the source remains uncertain. Environmental reservoirs seem unlikely, and the bulk of recent evidence implicates person-to-person transmission. Previously, diagnosis depended on morphologic identification of the organism in respiratory specimens, but detection of DNA by modern methods is supplanting this approach. Therapy and prophylaxis have also undergone striking changes in the last 10 years. Sulfa remains the therapy of choice for infections, despite the high incidence of side effects in HIV-infected individuals. However, the use of adjunctive corticosteroids for patients with moderate to severe disease has decreased morbidity and mortality, probably by blocking the host's inflammatory responses directed against the organism. While aerosolized pentamidine had been a mainstay of prophylaxis, unacceptable rates of failure led to a return to sulfa use. More recently, modern antiretroviral therapy has led to recommendations to discontinue primary and secondary prophylaxis in those individuals who respond to the antiretrovirals with significant immunologic reconstitution. While the prognosis for HIV-infected individuals with respiratory failure has improved, partially because of corticosteroids, the prognosis for other immunosuppressed individuals remains poor. Finally, 10 years ago questions about drug susceptibility and treatment failure were unanswerable. More recently, studies of mutations in the *Pneumocystis* genes that serve as drug targets, have produced provocative new information. While it is becoming clear that long-term exposure to sulfa results in increased frequencies of mutant organisms, conclusive proof that these mutations change clinical outcomes remains elusive. All of these aspects were areas of active discussion and debate at the workshop.

In the previous Workshop held in 1999, about 70 abstracts were presented dealing with basic and clinical aspects of the *Pneumocystis* organism and the infections it causes [1]. During the current Workshop, nearly 100 abstracts were presented as platform sessions or posters. Additionally, roundtable discussions focused on international cooperative clinical studies, and the *Pneumocystis* genome project. Investigators from North America, South America, Europe and Japan presented their data and participated in the discussions. Brief summaries of the various research areas are presented below.

NOTE: During a roundtable discussion on the nomenclature of *Pneumocystis*, the community as a whole decided to begin speciation of the distinct populations found in mammalian hosts. This summary does not reflect those changes from the trinomial to the suggested binomial since the process has just begun.

Table 1. Significant changes in approach to *Pneumocystis* infections over the last 10 years.

	Before 1990	1990-2001
Classification	Protozoan	Fungus
Frequency in HIV-infected individuals	Common (zidovudine)	Uncommon (highly active antiretroviral therapy)
Source of infection	Reactivation	Acquistion
Diagnosis	Morphologic	DNA detection by PCR
Therapy	Sulfa	Sulfa and adjunctive corticosteroids
Prophylaxis	Aerosolized pentamidine	Sulfa or discontinuation after response to antiretroviral therapy
Intensive care unit survival for HIV-infected individuals	Dismal	Moderate
Drug failure	Unexplained	Mutations in DHPS genes?

CLINICAL ASPECTS OF INFECTION

With the advent of PCR to detect *Pneumocystis* DNA in respiratory specimens, *Pneumocystis* seems to be present during a wide variety of clinical illnesses, although its pathologic contribution remains uncertain. Data were presented demonstrating that *Pneumocystis* may be associated with bronchiolitis in immunocompetent infants, and may be implicated in the pathogenesis of bronchiolitis obliterans-organizing pneumonia in adults. *Pneumocystis* DNA has been detected in HIV-infected individuals during episodes of bacterial pneumonia, associated with the use of corticosteroids. Additionally, some populations of otherwise healthy elderly individuals demonstrate an increased prevalence of *Pneumocystis* in their respiratory secretions, suggesting increased colonization.

Surveillance of immunosuppressed individuals without HIV infection, such as heart transplant recipients, suggests acquisition of organisms long after transplant, indicating a need for continued prophylaxis.

GENOTYPING AND EMERGING DRUG RESISTANCE?

Studies conducted in the San Francisco area using the DHPS (dihydropteroate synthase gene) and mtLSU (mitochondrial large subunit ribosomal RNA) loci for typing, confirmed new acquisition rather than reactivation of latent organisms, as the source of PcP (*Pneumocystis carinii* pneumonia). In the same study it was found that 86% of the patients harbored a mutant DHPS genotype, including 72% who never received prophylaxis. Moreover, in one area, 89% of the cases produced a mutant phenotype compared to 74% outside the area. These data argue strongly for person to person transmission of PcP and a positive selection for the mutant phenotype. Although this study

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indicated that the mutations were not associated with previous exposure to sulfa, other studies presented at the meeting provided evidence that these mutations arose independently in multiple strains and that exposure to the drug selected for them. These data are not necessarily in conflict. It is possible that exposure to sulfa selects for the mutants which are then transmitted throughout the human population, infecting those that have or have not had previous treatment. What remains unclear is the effect of the DHPS mutants on clinical outcome. Some studies seem to suggest there is no correlation with the presence of the mutants and severity or response of the infection to treatment. In another genotyping study, there was no correlation between virulence and ITS genotypes.

Genotyping techniques used by 2 different groups showed that different types of *P. carinii* were distributed throughout the lobes of the lungs. In some cases, areas contained single infections, while others a mixed infection. These data raise questions concerning the trafficking of the types within the lung and the possibility that infections can occur from multiple exposures.

CLINICAL COOPERATIVE STUDIES

As detailed above, considerable controversy persists regarding the relationship to genetic mutations in *P. carinii* and clinical outcomes. Several groups reported their results, but the studies used different definitions of exposure to sulfa-containing regimens, and measured different clinical endpoints. Additionally, numbers of clinical cases seen in individual centers have decreased significantly with the advent of highly active antiretroviral therapy for HIV. Therefore, a series of discussions were held to discuss methods in which to begin cooperative studies. These issues, including standardization of definitions, handling of specimens, target genes of interest, and mechanisms for cooperation and publication, are discussed in a separate summary in this volume.

IMMUNE RESPONSES AND PATHOGENESIS

Studies of host defenses in humans and in animal models have demonstrated that immune responses against *Pneumocystis* involve a large number of cells and their associated mediators. The advent of potent antiretroviral therapy has significantly altered our understanding of defense against *Pneumocystis* in HIV-infected individuals. Most importantly, current recommendations support discontinuation of prophylaxis against *Pneumocystis* once antiretroviral therapy produces immunologic reconstitution. Major questions remain concerning when to restart prophylaxis after antiretroviral therapy. For example, preliminary data were presented to suggest that proliferative responses of peripheral blood mononuclear cells to *Pneumocystis* antigens could provide a clinically useful test to predict the risk of pneumonia. Study of genomic differences in host responses against *Pneumocystis* is just beginning, and preliminary data were presented showing that variability in the tumor necrosis factor-α gene promoter does not alter the outcome of pneumonia. However, further population-based investigations are clearly needed to define genetic differences in host responses.

Focus on the alveolar macrophage as a major effector cell in defense against *Pneumocystis* continues. In an experimental model, numbers of alveolar macrophages decrease during infection, but it unclear whether macrophages move from the lung or die. New information demonstrates that the phagocytic capabilities of alveolar macrophages are mediated, in part, by regulation of specific transcription factors, such as GATA-2 and NF-κB. Additionally, bronchoalveolar lavage fluid from infected rats inhibits phagocytosis of *Pneumocystis* by uninfected and immunologically intact alveolar macrophages, although the identity of the active moiety is not known. Modulation of transcription factors or lavage constituents could provide an important method to alter host response against *Pneumocystis*.

Lung lymphocytes and their products also remain areas of active investigation. Distinct advantages of animal models include the ability to modulate immunity by adoptive transfer or removal of specific cell populations, and to supplement or block specific cytokines and chemokines. While much of the adoptive transfer work has been performed in mice, studies were presented demonstrating that adoptive transfer experiments can be extended to rats. In mouse models, significant age-related differences were demonstrated in the kinetics of chemokine and adhesion molecule expression, by comparing responses in adult and neonatal mice. The mechanisms by which lymphocytes signal and proliferate during *Pneumocystis* infections remain quite controversial. While it is clear that costimulatory pathways must be important in the clonal expansion of lymphocytes for host defense, the relative importance of costimulatory molecules and their receptors, such as CD40 and CD40 ligand, require further investigation. Additionally, it is becoming increasingly apparent that models using environmentally acquired infection may differ substantially from models using intra tracheal inoculation of organisms.

Finally, experimental attention is being directed toward additional host defense cells. Because alveolar macrophages are known to have poor antigen-presentation capabilities, preliminary investigations have examined the role of dendritic cells. In vitro, however, it does not appear that *Pneumocystis* induces the maturation of dendritic cells for antigen presentation. Further investigation of the role of the alveolar epithelial cell, and its products, in control of infection is needed. For example, surfactant protein A enhances clearance of *Pneumocystis* from mouse lungs, raising the possibility that supplementation with exogenous surfactant could improve clearance. New data were also presented demonstrating that rat alveolar epithelial cells release macrophage inflammatory protein-2 after stimulation with *Pneumocystis*, indicating that the alveolar epithelium plays an important role in inflammatory cell recruitment during pneumonia.

ANIMAL MODELS

Because of the inherent difficulty in performing in vitro experiments with *Pneumocystis*, animal models are necessary to study most aspects of the organisms' biology and especially, host responses. Inoculation of nude rats with very low numbers of organisms produced a near clonal population of organisms as assessed by PCR amplification of the UCS MSG (Major Surface Glycoprotein) junction, similar to a previously reported study using corticosteroid immunosuppressed rats. In contrast naturally acquired latent infections were non-clonal and produced complex patterns of products representing the expression of different MSG genes. Inoculation of low numbers of organisms can be used to study MSG expression over time with a clinically relevant, in vivo system. Primate models, using animals immunosuppressed with simian immunodeficiency virus, are being developed to study *Pneumocystis* and host defense in higher animals. Rhesus macaques with *Pneumocystis* infections demonstrated significant increases in the numbers of CD8+ lymphocytes in their lungs, and further characterization of this model could be used to develop immunotherapies for humans.

In a survey of several commercial rat colonies conducted to identify *Pneumocystis*-free colonies, no colony was shown to be free of the organism upon chronic immunosuppression. Instead, 3 new karyotype forms of the predominant species in rats, *P. carinii* f. sp. *carinii*, were identified and characterized by CHEF profile and gene localization studies. These studies also showed that while *P. carinii* f. sp. *carinii* displayed extensive chromosomal length polymorphisms, there were no sequence differences in 3 genetic regions. Moreover, unlike any other colony previously surveyed, it appeared that rats could be co infected with 2 forms of *P. carinii* f. sp. *carinii*.

TRANSMISSION AND LIFE CYCLE

A full understanding of the life cycle of *Pneumocystis*, including how the organism enters the host; what developmental cycles ensue in the lung milieu; and how the organism exits the host remain high priorities Within the lung, those signals that regulate transition from the trophi

to cyst stages (or initiation of sex), and nutrient sensing are poorly understood and critical to define if we are to develop novel interdiction strategies.

It is well accepted that *Pneumocystis* is transmitted by an airborne route. Yet, the agent of transmission has not been demonstrated. Would it be the cyst- using 8 daughter forms to quickly initiate the infection, or the trophic form- a micron or so sized spore capable of remaining suspended in the air for long periods of time, or an as yet unknown form? Two studies presenting the putative infectious agent showed cyst- like organisms by electron microscopy and by cresyl-echt violet staining. In the latter study, the forms were isolated by magnetic beads linked to monoclonal antibodies produced to an MSG protein, with a subsequent PCR step targeting regions of the genome specific for *Pneumocystis*, as verification of its identity. The presence of intact surface antigens and genomic material together with appropriate staining properties were highly suggestive of intact organisms and may provide the material necessary to fulfill Koch's postulates.

Previous studies of commercial rat colonies have shown that *Pneumocystis* organisms were harbored by non-immunosuppressed animals. Neonatal rats are routinely housed with adults in commercial colonies and may act as reservoirs for the organisms by virtue of their immature immune systems. In separate studies performed on neonatal populations, it was found that humans and rats acquired the organisms early after birth. In the rat studies, a combination of oral swabs and *Pneumocystis*- specific PCR showed the presence of the organism in the oral cavities as early as 1 hour after birth. Studies of dams and their pups showed no evidence of vertical transmission, suggesting that the pups acquired the organisms through direct contact or through the air. Likewise, genotyping of a human mother and her baby (both of whom had the infection) supported transmission between the 2, or through a common source. A separate study showed the presence of the organism in a large number of non-immunosuppressed infants, supporting a role for the neonate as one reservoir of infection.

Two separate modes of transmission for infants and adults were suggested by molecular epidemiological studies. Genotyping of organisms targeting the DHPS gene from infants that died from causes other than an immunodeficiency disorder and adults with AIDS with documented PcP showed a single genotype in the infants, the "wild type", and 4 genotypes in the adults. The DHPS double mutant, correlated with sulfonamide exposure and prophylaxis failures, was present in 50% of the adult samples and in none of the infant samples.

The period of potential transmission associated with non-immunocompromised, immunocompromised, or infants is completely unknown. Genotyping studies indicated that hospital employees caring for HIV-infected individuals may have carriage of the organism versus colonization, but the length of time of carriage remains uncertain. A number of studies examined "outbreaks" of *P. carinii* pneumonia in hospital settings, in attempts to identify case clusters and causally relate the organism genotypes. Although some studies were suggestive of transmission, low numbers of cases prevented a definitive answer.

Once *P. carinii* are resident within the lung alveoli, sexual reproduction occurs, with the presence of synaptonemal complexes and recently identified meiosis- and mating type- specific genes confirming the presence of this phase. One type of pheromone receptor was cloned and sequenced, with convincing homology to the *Ste3* receptor of *Saccharomyces cerevisiae*. However, the receptor of the other putative mating type, *Ste2*, has yet to be identified, as well as the pheromones associated with these receptors. The other mode of replication which the organism uses is asexual binary fission. Several genes with strong homology to cell cycle control genes in yeast and other fungi were detected in the EST database, providing insights into this process. The cdc42 gene, which plays a role in nutrient sensing and/or filamentation/invasion in yeast was cloned and sequenced.

In an interesting study designed to evaluate encystment triggers, lungs infected with *P. carinii* removed from rats were shown to increase in cyst to trophic form ratio 24 hrs after removal from the animal. The cysts were 80% viable after 24 hr as determined by RT-PCR. One isolate, I-1444, was shown to consistently produce higher cyst ratios upon a series of inoculations into recipient rats. These studies indicate there may be chemical triggers within the lung milieu signaling changes in morphogenesis.

Analysis of the influence of environmental factors on the prevalence of the 2 species of rat *Pneumocystis, P. carinii* f. sp. *carinii* and *P. carinii* f. sp. *ratti*, showed that relative humidity, temperature, and rat census were associated with the predominance of one species over another. Seasonal and geographic studies of the incidence of human PcP suggested that infections were more common in winter months, when individuals spend more time indoors.

In a separate study, geographical region was associated with the number of shrews harboring *Pneumocystis* in regions in California. The role of extrinsic factors or animal behavior on the level of infection was not determined.

PHYLOGENY AND EVOLUTION

It has long been known that *Pneumocystis* organisms exhibit exquisite host specificity, and that experimental infections with organisms from one host cannot be established in the lungs of a different host. For example, even with the use of sensitive molecular detection techniques, *Pneumocystis* infection cannot be established in mice with severe combined immunodeficiency after inoculation with human-derived organisms. The first detailed analysis of organisms obtained from a dog indicates that like other *Pneumocystis* from different mammalian hosts, this organism was genetically distinct from all others. Using molecular analysis of the mitochondrial large subunit ribosomal RNA gene of *Pneumocystis* obtained from various primates, it has become apparent that *Pneumocystis* is even more tightly linked to its hosts than was previous suspected. Phylogenetic analyses indicate that that the genetic divergence in primate-derived *Pneumocystis* varies with the phylogenetic divergence of the primate host. Furthermore, *Pneumocystis* organisms isolated from a wide variety of mammals tend to cluster according to the phylogeny of the host, suggesting that *Pneumocystis* may have co-evolved with its mammalian hosts. Finally, preliminary data were presented suggesting that *Pneumocystis* infects an extended range of mammalian hosts, perhaps even dolphins.

PHYSIOLOGY AND CULTURE

A recurrent theme throughout the platform presentations and posters was the need for long-term culture system that does not depend upon the use of feeder cells. Although such a culture system has been developed and published, many investigative groups around the world have had difficulty achieving success with this method. Most investigators continue to use freshly isolated organisms obtained from the lungs of animal hosts, but recent advances have been made in cryopreservation of organisms while retaining their viability and pathogenicity. Several abstracts focused on the metabolic capabilities of *Pneumocystis*. Recent data demonstrate that *Pneumocystis* takes up most amino acids by facilitated diffusion; but aspartic acid does not have a carrier. One of two glucose transporters functioned by an active transport mechanism. *Pneumocystis* synthesizes four ubiquinone homologues in two separate cellular compartments. Atovaquone, but not stigmatellin, inhibited the biosynthesis of all homologs in only one compartment, thus the effectiveness of the drug may depend on targets in addition to mitochondrial cytochrome b. *Pneumocystis* appears to deplete S-adenosylmethionine in vivo and in vitro, and this capacity was proposed as a method to detect clinical episodes of pneumonia. *Pneumocystis* does not appear to salvage thymidine, suggesting inhibition of de novo thymidine synthesis as a potential drug target. Metabolic and cellular processes inferred by identification of gene orthologs in the Expressed Sequence Tag database created as a result of the *Pneumocystis* Genome Project include sterol biosynthesis with many of the *ERG* genes present; heme biosynthesis; cell cycle control;

mating, including a pheromone receptor gene; and signal transduction pathways for nutrient and environmental sensing. The *erg6* and *erg7* genes were cloned, sequenced and found to be functionally active in a heterologous system.

MOLECULAR BIOLOGY

Two cosmids from the pWEB library constructed for the *Pneumocystis* Genome Project containing subtelomeric and telomeric regions were sequenced, assembled and analyzed in detail. Each cosmid contained arrays of MSG-, MSR- and PRT1 genes that differed significantly from one another. Most of one of the arrays , 1B2, resembled a previously described array, suggesting that this particular gene cluster may have a propensity for being copied into different sites in the genome. The 1B2 cosmid mapped to chromosome band 9 on a CHEF gel blot, while another cosmid resembling it mapped to band 4, indicating that the same or similar arrays can be found on different chromosomes. These data provide evidence that movement of these arrays may be through gene conversion. The other cosmid, 3G5, mapped to chromosome band 15, and contained the first example of a *Prt1* gene upstream of MSR and MSG genes. Of note were the putative gene sequences found at the repeated array-unique sequence junction. These two open reading frames encoded genes related in part to MSG genes. In the case of *Orf2*, hybridization to a northern blot revealed a 1.2 kb band which did not hybridize to an MSG probe, suggesting only a distant relationship with the MSG family.

Analysis of a third pWEB cosmid, 3C5, showed the presence of a single ribosomal RNA locus, supporting previous techniques that inferred the presence of 1 to 2 copies of the locus. A single copy of the rRNA locus sets *Pneumocystis* apart from other fungi (which usually contain hundreds of a copies) and also most other eukaryotes.

Few antigens besides the MSG family have been characterized. One such antigen gene, p55, was previously identified in the rat *Pneumocystis* genome. Four additional p55 antigen gene variants were cloned from the rat organism genome and shown to vary in the number and size of the hepta-peptide motif region containing glutamic acids. A fragment of a gene with homology to a rat *P. carinii* p55 variant was cloned from mouse *Pneumocystis*. Characterization of this family of antigens and its variants may provide useful targets for immunotherapy.

Primers targeting the single copy DHPS gene in the mouse *P. carinii* genome were used in combination with real time quantitative PCR, to determine the levels of *P. carinii*. This technique should be useful in determining organism burdens and perhaps, response to therapy.

NOMENCLATURE

Pneumocystis species. A roundtable discussion was held at the conclusion of the meeting at which a consensus was reached to discontinue use of the trinomial nomenclature for *Pneumocystis* organisms. Instead, the population (s) of *Pneumocystis* harbored by each mammalian host will be considered different species. The naming of the *Pneumocystis* populations will follow the Botanical Code of Nomenclature. A detailed report of this discussion is presented in a separate summary in this volume. Human-derived organisms will be termed *Pneumocystis jiroveci*, based upon Jirovec's description of plasma cell pneumonia in 1951 [2], submitted according to the Code by Dr. Jack Frenkel.

Gene names. Discussants at the Genome Project Update roundtable (see below and in this volume) urged investigators to use a standard gene naming convention; gene identification in genomic sequence; protein names; and to identify the animal origin of all sequences submitted to public databases. The gene naming convention should follow that suggested by Stringer and Cushion, 1998 [3] and will be discussed in detail in the Genome Project Summary in this volume.

GENOME PROJECT

At the 5th International Workshop on Protists, held in Lille, France, September, 1997, consensus was sought and obtained for a community-based *Pneumocystis* Genome Project [4]. The members of the *Pneumocystis* Community present voted to embark on an international genome project with rat (*Pneumocystis carinii* f. sp. *carinii* Form 1) and human (*P. carinii* f. sp. *hominis*) *P. carinii* populations as the focus. The strategy approved by the community was to first produce a physical map (in vitro reconstruction of the chromosomes) of *P. carinii* f. sp. *carinii* followed by directed or shotgun sequencing. Expressed sequence tags (ESTs) would be produced from an existing cDNA library (A.G. Smulian) to provide a gene inventory and hybridized to the cosmid clones to aid in physical mapping. The project was funded in April, 1999 by NIH under the RO1 mechanism. Limits in funding reduced the scope of the grant to mapping and sequencing of the rat *P. carinii* genome, and if possible a 1-2X shotgun library coverage of the human organism.

The EST project has been completed and is discussed in a separate summary in this volume. Standard assembly tools were found to inaccurately assemble the MSG-, MSR-, PRT and other multiple gene families and must be modified. Compression of the 5,000+ ESTs using CAP3, which provided the most accurate assemblies, produced a condensed database of approximately 1700 genes. Based on estimations of gene density from sequenced cosmids, this number likely represents a little over half of the gene content of the genome. All but about 12 of the total number of *P. carinii* genes submitted to the NCBI GenBank were present in the EST database. After BLASTN and BLASTX analyses of the ESTs, 13% of the sequences were identified as known *P. carinii* genes; 72% were orthologous to genes of other organisms (e<-10) or had no identifiable orthologs (e>-10); 15% of the sequences represented contaminants that were mostly rat in origin. Of the orthologs, most were fungal (91%) and of those, most had highest homology with genes from *Schizosaccharomyces pombe* (~65%). The presence of low amounts of *P. carinii* f. sp. *ratti* in the cDNA library were detected by sequence analysis and verified by PCR. All cDNA clones from the library constructed by A.G. Smulian are available from the ATCC (www.atcc.org).

Assembly of the physical map and directed sequencing using assembled cosmids are being performed concurrently. Chromosomes 3, 7, 10/11 and 15 have been partially assembled and shotgun sequencing of these cosmids at 5X coverage has been initiated. Two dimensional pulsed field gel electrophoresis revealed that chromosomes 7, 10/11, 15 and perhaps 1 have more than 1 chromosome in the band separated by CHEF. These data imply that *P. carinii* f. sp. *carinii* form 1 is an aneuploid organism. Such information will be essential for proper assembly of the chromosomes.

Once genes of interest are identified, function is usually shown by knockout or mutational analysis. Since *P. carinii* cannot be cultured in a robust fashion, establishment of a transformation system has been difficult. Heterologous systems offer an alternative, but do not provide the definitive answer of a homologous system. Progress towards a transformation system was reported using a blasticidin resistance cassette under the MSG promoter. The system used short term in vitro selection and in vivo amplification. Evidence for integration into the *P. carinii* genome was shown. Further development of the system is ongoing and will provide an essential tool for the field.

Reconstruction of a shotgun library of a pWEB cosmid containing approximately one-third the *P. carinii* mitochondrial genome revealed typical fungal mitochondrial genes, but differences in size and content to the mitochondrial genome of its closest phylogenetic relative, *S. pombe*. Migration on CHEF gels at a consistent band size suggests the genome may be linear.

Data produced by the Genome Project is currently available at:
http://gene.genetics.uga.edu
and in part at:
http://www.biology.uky.edu/Pc.
These data as well as interactive search tools will soon be available at:
www.pneumocystis.org.
Genes of *Pneumocystis* currently deposited in public databases, e.g. GenBank and those identified by the Genome Project will be annotated and entered into a Bioknowledge library as a member of the MycoPathPD group, an information center produced by Proteome Inc. and Incyte, Inc. Academic licenses are free and registration and access are available via
www.proteome.com

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