mechanisms different from those that are secreted, it is still mediated by discrete peptide segments which are not cleaved off from proteins. These signals, in fact, can target heterologous proteins into peroxisomes or nuclei.

In my perception, a very important aspect of Blobel's work is the demonstration that the intricate process of protein sorting in cells can be studied by *in vitro* reconstitution of various components of the cellular transport machinery.

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# Progress in genetic manipulation of *Plasmodium**

Parasites were the focus of a recently concluded conference. The parasites covered in this molecular and cell biology meeting were mainly protozoan parasites such as *Plasmodium*, *Trypanosoma*, *Leishmania*, *Toxoplasma*, *Giardia* and *Entamoeba* with a few papers on helmynths and other parasites. In this report the latest developments in the genetic manipulations of *Plasmodium*, and the functional implications of these studies will be discussed.

Even though transient transfections of *Plasmodium* had been reported earlier, the first successful report of stable transformation of the human malarial parasite *P. falciparum* and the murine malarial parasite *P. berghei*, using homologous recombination technique, appeared in 1996 (refs 1, 2). In the meeting this year, the effect of this technique was visible. Answers to functions and vitality of malarial antigens were emerging. Important functions were being assigned to protein domains or specific amino acid residues using deletions, chimeras or point mutations in the corresponding genes. In many cases divergent gene equivalents from different species of *Plasmodium* or other *Apicomplexan* organisms such as *Toxoplasma* were swapped to study the minimum essential domains of the proteins in question.

The targets of drug resistance of *P. falciparum* to pyrimethamine, cycloguanil and sulfadoxine, are known to be the enzymes dihydrofolate reductase (DHFR), thymidylate synthase (TS) and dihydropteroate synthase (DHPS), respectively. The antifolate resistance has already been fine-tuned by looking at the mutations at the active site of these enzymes. Mutations created *in vitro* as well as those obtained in the field have been analysed at the molecular level over several years. By transforming the parasites, the residues crucial for drug resistance could now be defined. For instance, it has been shown by transfection that mutations of DHPS can confer resistance to sulfadoxine in *P. falciparum*[3]. Hayton *et al.* from David Walliker's group reported data on the DHPS resistance of *P. chabaudi*, and postulated the resistance to be linked to Asp-106. To dissect out the mutations of DHFR-TS, Fohl *et al.* from David Roos' group at University of Pennsylvania, USA, first replaced the *Toxoplasma* DHFR-TS (ref. 4), and then generated mutant lines and assayed them for drug-resistance. This year they reported studies, in which they looked at competition between mutant and wild type alleles in culture and in mice. They showed that pyrimethamine-resistant double mutants Arg-59 + Asn-108 show no difference in growth *in vitro* but show loss of fitness *in vivo*. Their work demonstrated that parasites adapted to *in vitro* cultivation exhibit a profound loss of *in vivo* fitness. This provides a cautionary note for extrapulating studies on lab-adapted strains to the field.

The search for gene(s) responsible for chloroquine resistance is still on. While the groups of Alan Cowman, WEHI, Australia, have attributed chloroquine resistance to the functions of the *pfmdr* gene, Tom Wellems' group at NIH, USA, had demonstrated distinct linkage of chloroquine resistance to a 36 kb region of chromosome 7 of *P. falciparum*[5]. The putative gene, *cg2*, which was earlier assigned the possible role for chloroquine resistance, was disproved by transfection experiments, as it did not confer resistance to the chloroquine sensitive strain. David Fidock from Wellems' group reported this result in the 1998 meeting. This year Fidock presented data on *pftcr*, a novel and complex polymorphic gene from the same chromosomal segment, as the candidate for chloroquine resistance. The gene *pftcr* contains 13 exons and the deduced protein sequence is consistent with a ten transmembrane domain integral membrane protein. The point mutations in the *pftcr* gene of the resistant strains from the laboratory and the field were shown to be consistent. The transfection of this gene from the resistance strains into chloroquine sensitive strain did confer resistance. The same group also reported homologues of this gene for *P. vivax* and *P. berghei*, with 73% identity and 12% similarity. However, the resistance to chloroquine is unlikely to be due to a single gene. Reed and Cowman from WEHI, Australia, showed that the introduction of *pfmdr1* mutations could also modulate the susceptibility of *P. falciparum* to chloroquine and mefloquine.

The gene disruption, or the knock-out technique, is carried out at the asexual stages of the parasite. As a result, the study of essential genes expressed in stages other than the erythrocitic stages is facilitated. A large number of gene disruptions were reported for genes expressed in the sexual and mosquito stages. Earlier morphological and molecular studies have established a chronology for the genes expressed during the sexual development[6]. Eksi, Fanning and Williamson

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from Chicago, showed that the disruption of the *Pfs*230 gene, which codes for a surface protein in the sexual stages, caused a 94% reduction in the gametocytes produced. Surprisingly, this block appeared much earlier than was expected. Nirbhay Kumar from Johns Hopkins University, USA, reported the disruption of the gene for an internal protein Pfg27, and demonstrated that the development was blocked quite early in the sexual stage. The largest number of studies was reported on the gene equivalent to *Pfs25*, a leading candidate for a transmission blocking vaccine. The structurally conserved members to Pfs25, in the murine malarial parasite *P. berghei*, are Pbs21 and Pbs25 proteins. Koning-Ward *et al.* from Waters' group at Leiden, Netherlands, showed that individual knock-outs of either *Pbs21* (21KO) or *Pbs25* (25KO), or a double knock-out (21/25 dKO), showed no detectable difference in the gamete development, fertilization or maturation of the ookinetes in these mutants. There was a 3-, 2- and 1000-fold reduction in the number of oocysts formed in these 21KO, 25KO and 21/25 dKO mutants, respectively. However, infective sporozoites were still produced by each of these, and could be transmitted into mice within the normal range of time.

Various functions have been ascribed to the circumsporozoite (CS) protein, the major surface protein on the sporozoite stage of the *Plasmodium* species. The predominant of these have been (i) to signal the homing of sporozoites to the salivary glands of mosquito, and (ii) homing of sporozoites to hepatocytes in the vertebrate. The 1997 knock-out of the CS-protein from *P. berghei* had demonstrated that the CS-protein has an indispensable structural requirement in the formation of sporoblasts at the oocyst stage itself[7]. Further transformation studies with modifications on the 3′-UTR region of the CS-gene of *P. berghei* were presented by Thathy and Robert Menard from Victor Nussenzweig's group, New York University Medical Center, USA. The deletion of a part of the 3′-UTR region of the CS-gene, resulted in a lower production of CS-protein, and in the formation of short sporozoites. This emphasized once again the structural requirement of CS-protein for the sporoblast budding, which was also supported by EM results. Natarajan from the same group also presented results on sporozoites expressing GFP under the control of CS-promoter. The idea is to be able to track the fluorescent sporozoites as they migrate from the ruptured oocysts to the salivary glands in the mosquito. This group is actively engaged in methods of complementation of mutations. Sultan *et al.* reported the design of using two selection markers, the usual parasite *dhfr-ts* gene which confers resistance to pyrimethamine, and the human *dhfr* gene which confers resistance to the new antifolate drug WR99210 (ref. 8). Mamoun *et al.* from Dan Goldberg's group at Washington University, St. Louis, reported the successful use of the genes *BSD* and *NEO*, as the second marker for selection, with resistance towards blasticidin and G418, respectively.

The other protein of the sporozoite stage that has been studied extensively is the thrombospondin-related anonymous protein (TRAP). It has been shown earlier that TRAP knock-outs are viable, but lose their gliding motility and *in vivo* infectivity[9]. Kappe *et al.* from New York University Medical Center, USA, showed that TRAP mutations, carrying deletion of the cytoplasmic domain of this protein, do not invade mosquito salivary glands, are not infective when injected into rats and do not show normal gliding motion. It has been postulated that the lack of gliding motion is a major cause of lack of infectivity of sporozoites. Kai Matuschewski from the same NYU group, demonstrated that deletions in the A-domain of TRAP resulted in nearly $10^5$-fold reduction in the invasion of salivary glands, as also in rats. However, there was no detectable change in the gliding phenotype of these mutants, indicating that there were more parameters needed other than gliding motility for invasion. Clearly, the integrin A-domain like motif plays a definite role in the invasion of sporozoites into the salivary glands of the mosquitoes. It was also shown by Kappe *et al.* that the cytoplasmic domain of a similar MIC2 protein of *Toxoplasma* could replace the cytoplasmic domain of the TRAP protein. Wengelnik *et al.* from A. Crisanti's group at Imperial College, London, also presented replacement and mutational studies of the TRAP molecule. To begin with, the *P. berghei* TRAP was replaced by *P. falciparum* TRAP (PfTRAP), and it was found that such tansgenic *P. berghei* containing *P. falciparum* TRAP developed absolutely normally with no difference in infectivity to the vertebrate host. Transgenic *P. berghei* sporozoites containing PfTRAP with mutations in the A-domain, could not invade mosquito salivary glands. However, these were motile, and could still infect the host. Deletion of the thrombospondin (TSP) motif from such PfTRAP, resulted in the loss of motility in these transgenic sporozoites. Thus, the A-domain seems to play a role in the invasion of salivary glands, while both the cytoplasmic tail and the TSP are apparently required for the gliding movements of the sporozoites. Another molecule CTRP, which also has the TRAP features of A-domains and TSP domains, is expressed in ookinetes. Knock-out results of this molecule were presented by Templeton *et al.* from NIH, as well as Dessens *et al.* from Bob Sinden's group at Imperial College, London. The CTRP knock-outs showed an arrest of *Plasmodium* at the ookinete stage. This strengthened the postulation that CTRP is involved in the invasion of the ookinetes into the insect midgut epithelium, in a manner similar to TRAP invasion of salivary glands.

Amongst the asexual stages, transgenes for two erythrocytic stage proteins were reported this year. The disruption of rhoptry associated protein (RAP1) was reported by Baldi *et al.* from Alan Cowman's group at WEHI, Australia. O'Donnell *et al.* from B. Crabb's group, also from WEHI, reported the replacement of the C-terminal region of the merozoite surface protein 1 ($MSP1_{19}$) with the corresponding sequence from the rodent parasite *P. chabaudi*. The RAP1 deletion mutant was just as viable as the wild type *P. falciparum*, indicating that there are multiple invasion pathways. The *P. chabaudi* $MSP1_{19}$ domain is quite divergent from the *P. falciparum* sequence antigenically. Yet this chimeric mutant did not show (i) any difference in the growth or invasion capability of *P. falciparum*, (ii) any difference in the processing of the $MSP_1$ protein. Heterologous trans-species expression of *P. falciparum* apical membrane antigen in the murine malarial parasite *P. berghei*, has recently been reported[10]. The expression and localization of the *P. falciparum* protein in *P. berghei* was found to be normal.

As more results of gene manipulations in *Plasmodium* pour in, it appears that

the parasites can tolerate very large protein changes. Total knock-outs of important candidate vaccine antigens such as RAP1 and Pbs25, showed no effect on the growth of the parasite. Replacement of candidate vaccine domains from one species to another, e.g. TRAP and $MSP1_{19}$, also did not have any apparent effect on the parasite survival. Antigenically the rodent and the human malarial parasites are quite different from one another. It has been documented that cross-protection on immunization with vaccine candidate antigens is not very effective even on heterologous strains of a particular species[11,12]. The parasite tolerance to large divergence of certain candidate vaccine antigens and to the total lack of some has serious implications on the design of a malaria vaccine. The parasite seems to operate with multiple pathways, and therefore immune responses to some of these components may not be effective. Also the acceptance of antigenically diverse proteins of one species into another indicates the possibility of extensive antigenic diversity arising in response to selection pressures caused by an effective vaccine.

So far, protozoan parasites have evaded the efforts towards a successful vaccine. The challenge is to understand the mechanisms employed by the parasites to get around the host and vector immune systems. Genetic manipulation of *Plasmodium* is a powerful tool, which will eventually help in uncovering and understanding the functions of proteins and pathways that are crucial in the host–parasite interaction.


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# Swarnajayanti Fellowships for 1998–99 announced

In one of his first statements in the Thirteenth Lok Sabha, Minister for S&T, Murli Manohar Joshi announced on 26 October 1999 the names of the six young scientists awarded the Swarnajayanti Fellowships for 1998–99. They are: Debajyoti Choudhury and D. Prasad of the Mehta Research Institute of Mathematics and Mathematical Physics, Allahabad; V. V. Ranade of the National Chemical Laboratory, Pune; and N. Kumar Sivarajan, S. Umapathy, and R. Varadarajan of the Indian Institute of Science, Bangalore.

While announcing the names Joshi added: 'I wish to congratulate all these young scientists for receiving the Swarnajayanti Fellowships and would like the House to join me in supporting this initiative for the Swarnajayanti Fellowships for the Young Scientists in the years to come so that many more young scientists could join the programme and contribute towards making Indian science internationally competitive.'

# India and the US to cooperate in achieving Kyoto goals through environment-friendly energy technologies

Bill Richardson, the US Energy Secretary, arrived at the head of a delegation to confer on 26 October 1999 with an Indian side led by Jaswant Singh, Minister of External Affairs. In a 'Joint Statement on Cooperation in Energy and related Environmental Aspects', the two sides recalling '. . . past cooperation that established the framework for several joint initiatives between the two Governments and their agencies for research and development in the energy sector, as well as stimulated private cooperation in conventional energy projects in India', decided '. . . to further enhance their cooperation in the energy sector such as conventional energy projects, renewable energy, clean coal technology, energy efficiency and related environmental aspects', and resolved '. . . to work closely together and with other countries, in keeping with the principle of common but differentiated responsibilities, to advance the goal of protecting the people of the world from the threat of climate change, while promoting economic growth . . .'.