Sporozoite invasion

SIR — Our understanding of the molecular basis of the invasion and subsequent development of malaria sporozoites in hepatocytes has been improved by recent reports that two sporozoite surface proteins, CS (ref. 1) and TRAP (ref. 2), both contain a sequence, region II, similar to the cell adhesion domain of thrombospondin[3]. Cox in News and Views[4] reviewed work[5,6] suggesting that recombinant CS protein containing region II binds to the sinusoidal face of hepatocytes and to the human hepatoma cell line HepG2, and that anti-region II antibodies significantly reduce sporozoite development in HepG2 cells. Cox suggested that these experiments show how malaria sporozoites invade hepatocytes directly from liver sinusoids, and that region II could be used as a target for immunization or chemotherapy.

However, region II probably binds to sulphated glycoproteins of the extracellular matrix. It is unlikely that this is sufficient in itself to mediate hepatocyte invasion specifically and subsequent development, which may, as suggested[5,6], require additional regions of CS or other sporozoite proteins. We have previously demonstrated that a peptide KLKQPGDGNPDP (N1), spanning region I of CS protein, binds to HepG2 cells and specifically recognizes two HepG2 proteins of relative molecular mass 55,000 (55K) and 35K. Because anti-N1 antibodies inhibit sporozoite invasion of HepG2 cells[7], region I is also likely to be involved in sporozoite invasion. When purified human hepatocyte membranes are crosslinked to sporozoites, two 55K and 20K proteins are identified which, when purified, each inhibit sporozoite invasion into human hepatocytes[8]. The sporozoite ligand(s) for these receptors remains to be identified.

A series of complex molecular interactions between sporozoite and hepatocyte molecules is therefore critical for sporozoite invasion and subsequent intrahepatic development. Region II-mediated adhesion is likely to be involved in the early stage of hepatocyte recognition. *In vitro* culture of malaria parasites in HepG2 cells alone is consistent with the suggestion that sporozoites invade hepatocytes in the space of Disse. However, the fact that hepatocytes rather than endothelial or Kupffer cells are labelled by region II does not indicate that sporozoites directly travel from the sinusoid to the space of Disse. Molecular interactions other than those in region II could be involved in endothelial or Kupffer cell recognition.

The relevance of these results to the development of anti-malarial vaccines based on CS protein remains an open question. CS vaccines containing non-repeat regions including region II are undergoing clinical trials. Although there are potential adverse pathological effects resulting from anti-region II antibodies that might recognize human thrombospondin, it is probably fortuitous that CS region II is naturally so poorly immunogenic. Other CS domains, or other sporozoite or liver stage proteins, must also be considered as candidate vaccines. In this context it is interesting to note that it is now becoming clear that entry of viruses into cells requires several ligands[9]. If there are parallels in malaria, then development of a vaccine based on inhibition of hepatocyte invasion will probably be difficult.

Michael R. Hollingdale
Malaria Department,
Biomedical Research Institute,
Rockville,
Maryland 20852, USA
Robert E. Sinden
Jos van Pelt
Imperial College of Science, Technology and Medicine,
London SW7 2BB, UK

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Missing link in ion channels

SIR — Na^+ and Ca^{2+} channels comprise four homologous domains, each of which is thought to contain six transmembrane segments. K^+ channels, on the other hand, have a single such domain, and appear to function as tetramers. Based on voltage-clamp data from phylogenetically diverse organisms and on sequence similarities of each domain of the Na^+ channel to the corresponding domain of the Ca^{2+} channel, Hille[1] has proposed that an ancestral single-domain channel gene gave rise to a primordial four-domain Ca^{2+} channel by two successive intragenic duplications; subsequent gene duplication and divergence then gave rise to the first Na^+ channel. Our phylogenetic analyses[2] support this scheme, which implies the historical existence of functional two-domain channels.

Two recent reports may illuminate these historical events. First, the predominant isoform of Ca^{2+} channel in newborn rabbit muscle appears to consist of a two-domain structure, seemingly the product of an alternate splicing event of a transcript derived from a four-domain gene[3]. Although Isacoff *et al.*[4] have constructed and expressed a fusion protein consisting of two *Shaker* K^+-channel domains, this is the first evidence for a naturally occurring channel with a two-domain structure, the product of a putative splice event resulting in the precise joining of homologous sequences in domains II and IV. Although the genomic structure has not yet been determined, this suggests that an intron was present at this position in the two-domain ancestor of the modern Ca^{2+} channel gene, evidence for which may yet be found in this or related genes.

Second, two recently identified Drosophila genes, *transient receptor potential* (*trp*) and *transient receptor potential-like* (*trpl*), encode putative Ca^{2+} channels involved in photoreception. Both proteins consist of a single domain of six putative transmembrane segments which show significant sequence similarity to known Ca^{2+} and Na^+ channels[5–7]. This single-domain channel may provide a missing link in the evolution of the ion channel superfamily, suggesting that the acquisition of Ca^{2+} selectivity preceded both of the duplication events which gave rise to the modern four-domain Ca^{2+} and Na^+ channels. The question of whether K^+ or Ca^{2+} selectivity represents the more ancient property of single-domain channels remains open.

Michael Strong
Department of Physiology and Biophysics,
George A. Gutman
Department of Microbiology and Molecular Genetics,
University of California,
Irvine,
California 92717, USA

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