

**PLASMODIUM FALCIPARUM  
CIRCUMSPOROZOITE VACCINE GENE  
OPTIMIZATION FOR SOLUBLE PROTEIN  
EXPRESSION**

CROSS-REFERENCE TO RELATED  
APPLICATION

**[0001]** This application claims the benefit of priority from U.S. Provisional Patent Application No. 61/394,048 entitled “*Plasmodium Falciparum* Circumsporozoite Vaccine Gene Optimization for Soluble Protein Expression” filed on Oct. 18, 2010, which is incorporated by reference in its entirety.

RIGHTS IN THE INVENTION

**[0002]** The present invention was made with support from the United States Government and, specifically, the Walter Reed Army Institute of Research, and, accordingly, the United States government has certain rights in this invention.

TECHNICAL FIELD

**[0003]** The present technology is directed generally to recombinant *Plasmodium falciparum* circumsporozoite proteins, to methods for production of those proteins, and to vaccines including those proteins, among other aspects.

BACKGROUND

**[0004]** A malaria parasite infected mosquito can inject approximately 100-200 *Plasmodium sporozoites* under the human skin during a blood meal. These sporozoites travel a considerable distance through several layers of tissue to reach the liver. The sporozoite journey from skin to the liver can take several minutes, during which it is exposed to the host immune system. Each successful sporozoite invasion can yield ~30,000 blood stage merozoites, each capable of invading an RBC seconds after its release (Blum-Tirouvanziam, Servis et al. 1995). Hence immune interventions that block sporozoite invasion can be the most effective strategy to induce sterile immunity in humans (Blum-Tirouvanziam, Servis et al. 1995). The most abundant sporozoite surface protein of *P. falciparum* is the 397 amino acid long Circumsporozoite protein (CSP). A comparison of amino acid sequences of CSP across the genus *Plasmodium* revealed a highly conserved gene structure (Doolan, Saul et al. 1992). The central region of the gene consists of a species specific repeat sequence flanked by a N-terminal region that contains a conserved stretch of a five amino acid sequence called “region I” and the C-terminal that contains a conserved cell-adhesive motif similar to one found on thrombospondin.

**[0005]** The functional role of CSP in the life cycle of the parasite is multifaceted. Genetic knockout studies with CSP show its involvement in development of sporozoites from mosquito oocysts (Menard, Sultan et al. 1997). CSP also binds specifically to salivary glands and is involved in the movement of sporozoites from oocysts to the salivary glands (Wang, Fujioka et al. 2005) (Myung, Marshall et al. 2004). Genetic replacement of *P. berghei* CSP with the corresponding CSP from the avian malaria parasite *P. gallinaceum* showed a failure to invade mosquito salivary glands and to infect mice (Tewari R, Rathore D, et al. 2005). Once on the surface of the infective sporozoite stage, the N- and C-terminal regions of CSP have an adhesive function that along with the thrombospondin-related adhesive protein allow the sporozoite binding to heparan sulfate proteoglycans on the liver

cell (Frevort 1999). CSP is also known to shield the sporozoite as it traverses through several layers of host tissues including professional phagocytes (Usynin, Klotz et al. 2007) and inhibits host cell protein synthesis. After invasion CSP gets exported into the hepatocyte cytoplasm and the nucleus where it can alter the gene expression profile of the host cell to protect the parasite and promote its growth and maturation (Singh, Buscaglia et al. 2007).

**[0006]** RTS,S is a human malaria vaccine grown in yeast cells and comprises the central repeats and the C-terminal cysteine rich region of *Plasmodium falciparum* CSP, fused to the S antigen of hepatitis B virus. The expressed protein self-assembles into a particle and is formulated with the proprietary adjuvant system ASOX (GlaxoSmithKline, Belgium) that contains immune stimulants MPL and QS21 (Cohen, Nussenzweig et al. 2010). Among the malaria naive individuals, RTS,S vaccination protects approximately 40% of vaccinees against experimental sporozoite challenge (Cohen, Nussenzweig et al. 2010). In a phase 2b trial the efficacy of RTS,S was estimated to be ~35% against first clinical episode and ~49% against severe malaria during an 18-month period among 1- to 4-year-old African children (Alonso, Sacarlal et al. 2004). In another phase II trial among 5-17 month old children, vaccine efficacy of RTS,S against clinical episodes was found to be 53% during an average 8 month period of observation (Bejon, Lusingu et al. 2008).

SUMMARY OF THE INVENTION

**[0007]** The present technology provides novel recombinant *Plasmodium falciparum* circumsporozoite proteins (rCSP), along with nucleotide sequences that express the recombinant *P. falciparum* CSP in bacterial cells, such as *E. coli*, as a soluble protein. The present technology also provides processes of expressing and purifying a soluble recombinant CSP protein without denaturing or refolding the protein. The purified protein produced by the present technology can be greater than 95% pure (that is, the soluble protein present in the composition is greater than 95% rCSP by weight) and contain low levels of endotoxin and low or undetectable levels of host cell proteins when analyzed by current techniques.

**[0008]** As one aspect of the present technology, novel recombinant *Plasmodium falciparum* circumsporozoite proteins are provided. The recombinant *P. falciparum* circumsporozoite proteins are characterized by an N-terminal region that lacks twenty to twenty-five N-terminus amino acid residues of native *P. falciparum* circumsporozoite protein; a reduced number of NANP repeats compared to native *P. falciparum* circumsporozoite protein; and at least 85% homology to SEQ ID NO:2, alternatively at least 90% homology to SEQ ID NO:2, alternatively at least 95% homology to SEQ ID NO:2. Preferably the recombinant *P. falciparum* circumsporozoite proteins comprise the peptide sequence of SEQ ID NO:2 or SEQ ID NO:8. In some embodiments, the protein lacks Met<sub>1</sub> to Cys<sub>25</sub> of the N-terminal region of native *P. falciparum* circumsporozoite protein. In some embodiments, the protein has 18 or 19 NANP repeats, preferably 19 NANP repeats, and/or has 0 to 3 NVDP repeats, preferably 3 NVDP repeats. In some embodiments, the recombinant *P. falciparum* CSP has a C-terminal region, preferably one that lacks ten to fourteen C-terminus amino acid residues of native *P. falciparum* circumsporozoite protein, more preferably, the protein ends at Ser<sub>383</sub>.

**[0009]** As another aspect of the present technology, nucleotide sequences are provided which encode a recombinant *P.*