Parasite Biochemistry

Lecture 4

Hemoglobin metabolism in parasites

- Hemoglobin is a tetrameric globular protein consisting of two alpha and two beta chains
- Each of the 4 chains of hemoglobin encloses an iron-containing heme co-factor responsible for oxygen binding
- The main function of hemoglobin is to transport oxygen in the blood
- Hemoglobin is a critical molecule during infection, as many microbes rely on it to grow within their hosts
- Humans attempt to conceal hemoglobin from invading pathogens but microbes employ elaborate mechanisms to acquire hemoglobin during infection.

Hemoglobin as source of Iron

- Like in pathogenic bacteria, some eukaryotic pathogens, including *Leishmania*, *Entamoeba*, and *Trypanosoma*, have evolved mechanisms of iron acquisition from hemoglobin.
- These protozoan parasites capture hemoglobin through either specific surface receptors or phagocytosis.
- Upon phagocytosis, the protein portion of hemoglobin is digested to release heme-iron.
- Surface hemoglobin receptors have been studied as potential targets for vaccine and drug development.

Hemoglobin as source of amino acids

- While many microorganisms target hemoglobin to acquire iron, others e.g. *Plasmodium*, digest the protein as a source of amino acids.
- Hemoglobin plays a central role during the blood stage of *Plasmodium* infections
- Hemoglobin consumption is achieved through several distinct mechanisms depending on the stage of parasite development.
- The critical importance of hemoglobin digestion is illustrated by the fact that blocking hemoglobin proteolysis prevents parasite development.
- Inhibitors of hemoglobin proteases have been suggested as potential therapeutic agents against parasites that utilize hemoglobin as a source of amino acids.

Hemoglobin catabolism in *Plasmodium*

- Reactive heme group is released from the globin portion of the protein.
- In *Plasmodium*, free heme is toxic hence it is detoxified by polymerization into crystals known as hemozoin.
- Hemozoin crystals are generated by polymerization of heme through the formation of a bond between the iron atom of one heme molecule and carboxylate of another (Slater *et al*., 1991).
- Dimers further polymerize through the formation of hydrogen bonds between propionates (Pagola *et al*., 2000).
- Hemozoin formation during chronic infection manifests itself in the blackening of the spleen and liver due to the accumulation of hemozoin within these organs.
- Antimalarial drugs such as chloroquine and possibly artemisinin inhibit hemoglobin detoxification by *Plasmodium*, underscoring the importance of this process for malarial viability

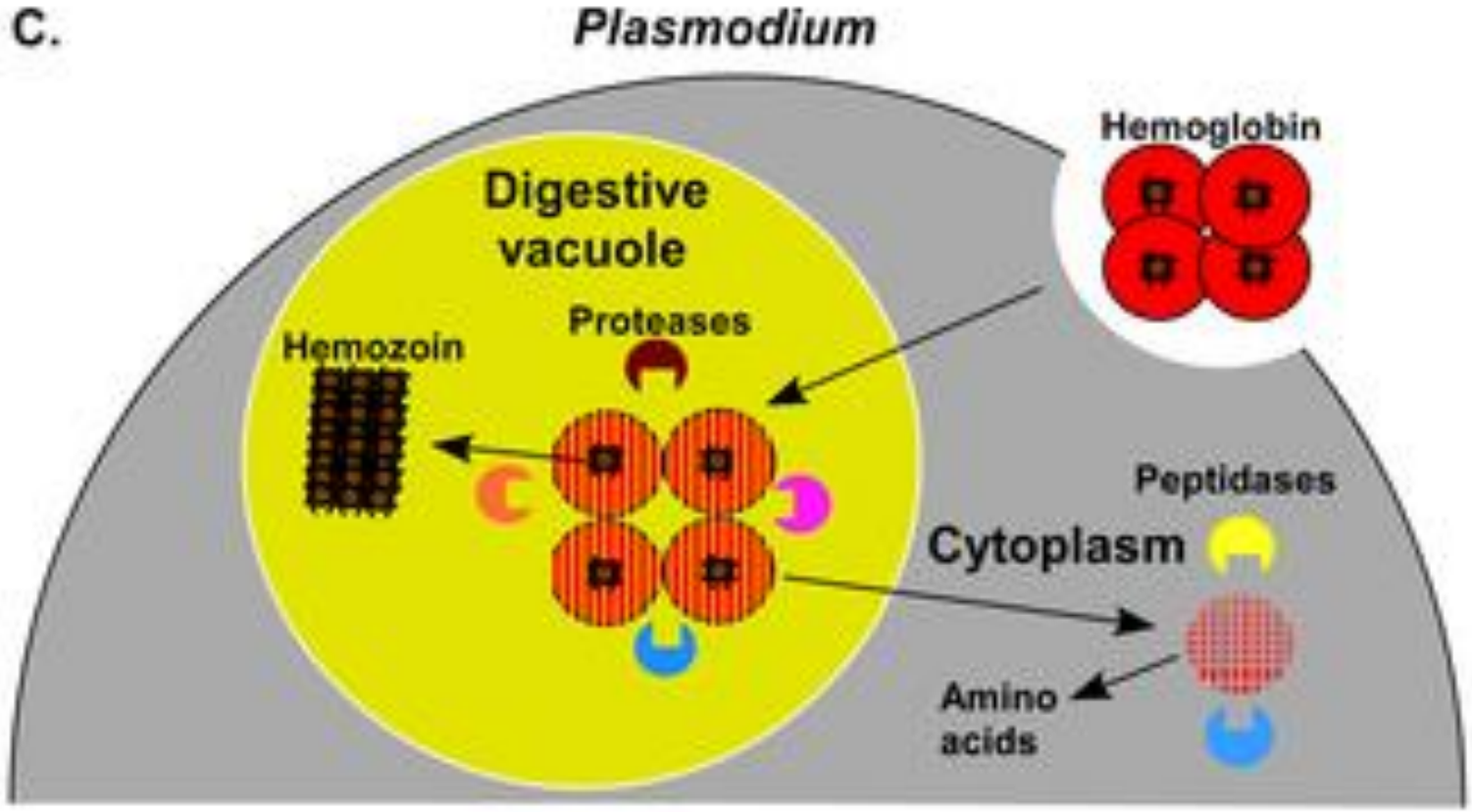
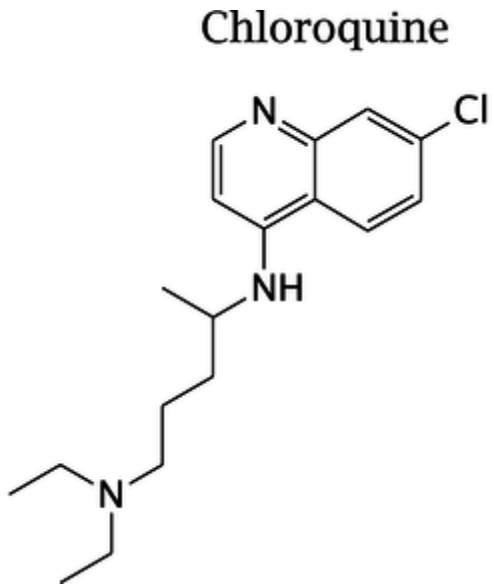
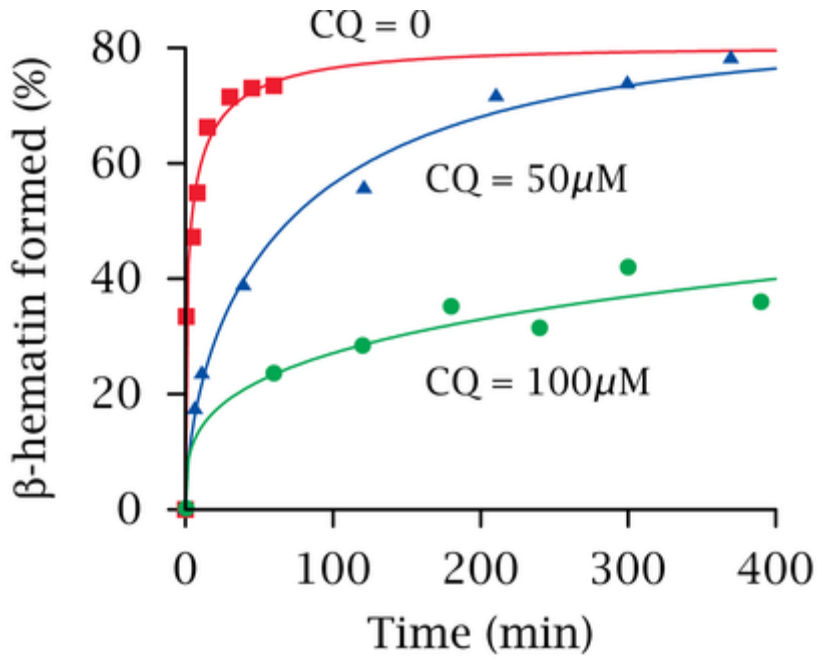


Figure 4.1. Mechanism of hemoglobin utilization by Plasmodium.

Pishchany G, Skaar EP (2012) Taste for Blood: Hemoglobin as a Nutrient Source for Pathogens. PLOS Pathogens 8(3): e1002535. https://doi.org/10.1371/journal.ppat.1002535
http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1002535

Chloroquine inhibits formation of β-hematin (hemozoin)

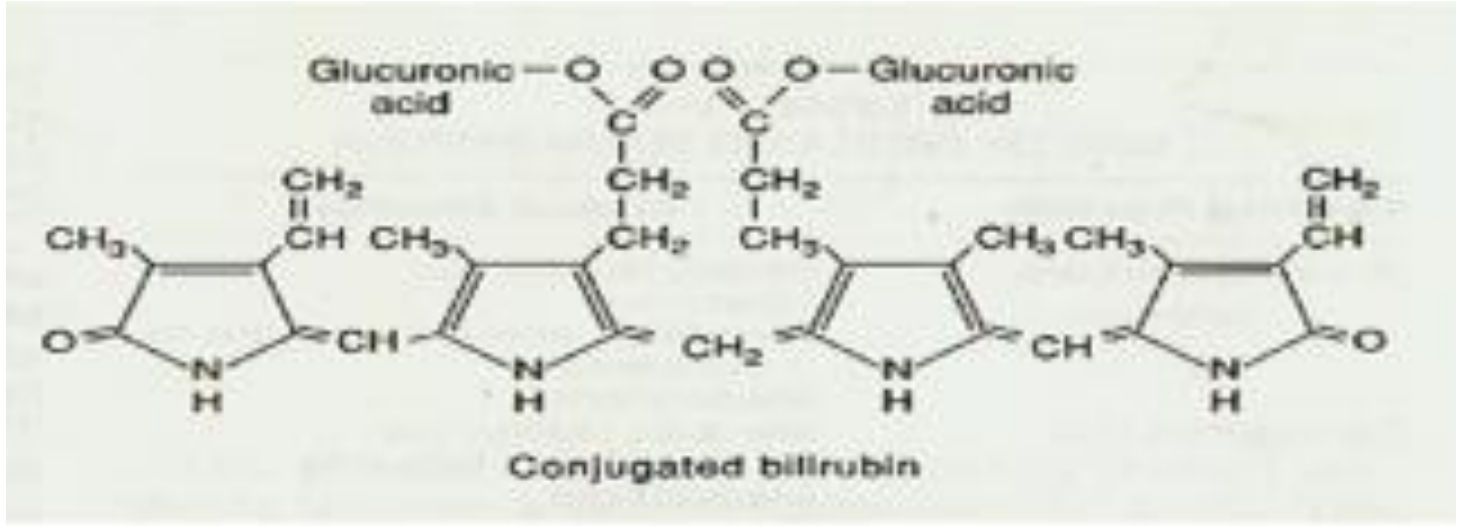


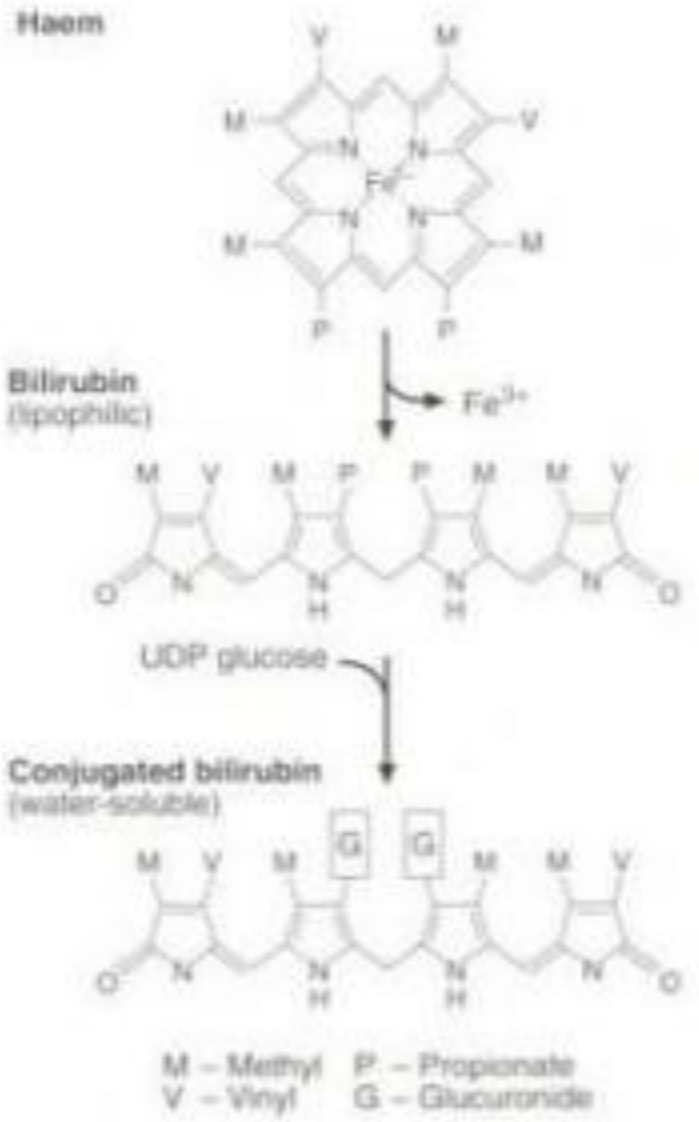


- After the parasite removes globin, free heme is left over and is toxic to the parasites, because it catalyzes the formation of reactive oxygen species from molecular oxygen.
- To combat heme toxicity, malaria parasites induce its crystallization; these β-hematin or hemozoin crystals are visible in blood smears as the so-called malaria pigment.
- Chloroquine interacts with the surfaces of growing β-hematin crystals, inhibits deposition of further heme molecules and thereby indirectly promotes formation of toxic reactive oxygen species.
- Considering the immutable nature of its target, it is clear that resistance to chloroquine must be mediated by removal or inactivation of the drug.
- Indeed, the most important mechanism of resistance consists in extrusion from the plasmodia by ABC type transporters.

Hemoglobin catabolism in Human

- Hemoglobin is first broken down to globin and heme (an iron containing protoporphyrin)
- Heme is then converted to Bilirubin which is insoluble in water and is transported in plasma almost totally bound to albumin.
- Bilirubin is taken up by liver cells and conjugated to form mono- and di-glucuronides which are more soluble in water. The conjugated bilirubin is excreted into the bile.



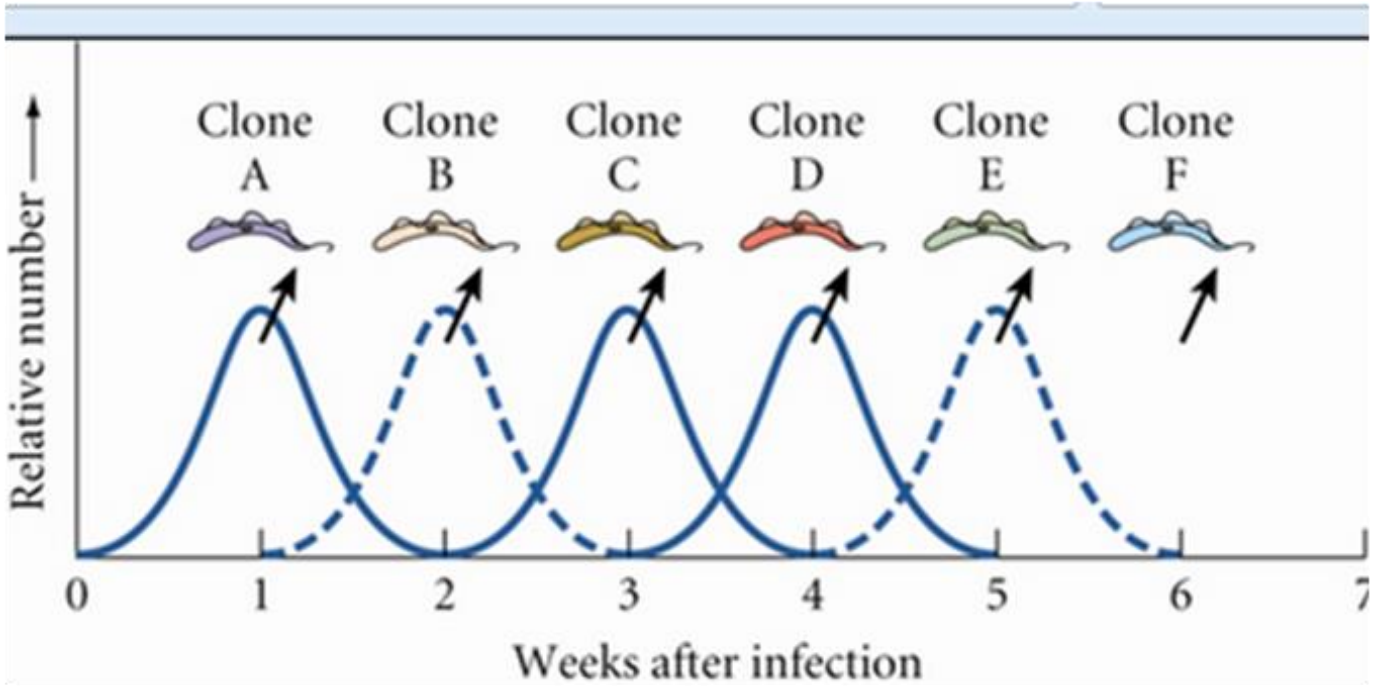


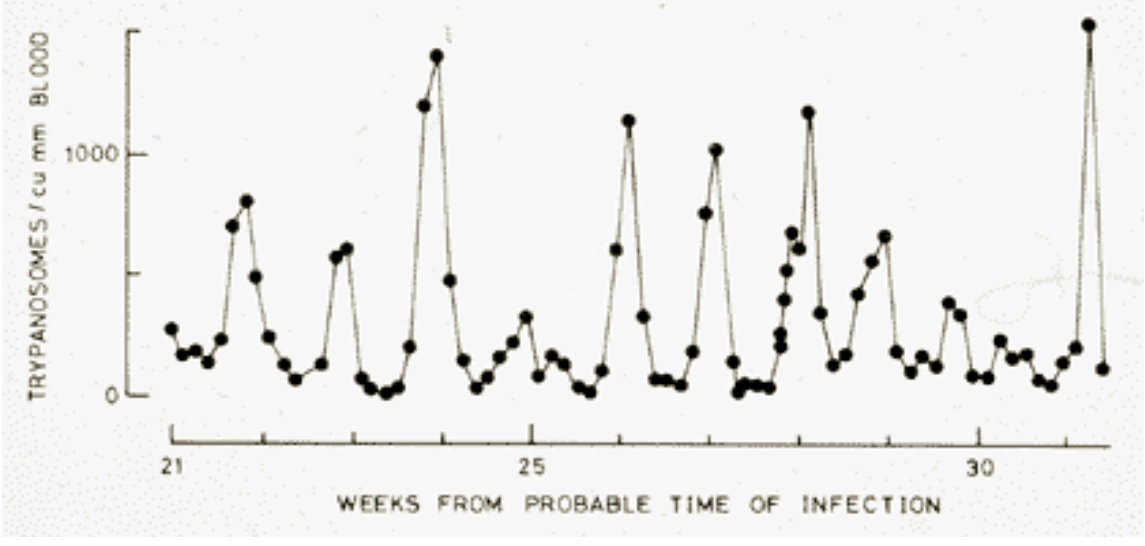
Antigenic variation and antigenic diversity in protozoan parasites

Trypanosomes

- The African trypanosome *Trypanosoma brucei* is best known for its antigenic variation of a protective variant surface glycoprotein (VSG)
- VSGs are a type of proteins coating the surface of some infectious microorganisms (e.g. *Trypanosoma brucei*) and helping them to evade the host's immune system by means of antigenic variation.
- The VSG protein is a key molecule for immune escape and parasitic success.
- Trypanosomes continually arise that have switched to antigenically different VSGs, prolonging a chronic infection that can last for years.
- VSG protein is continually varied, protects against antibody mediated lysis by the adaptive immune system and correlates with new peaks of parasitaemia.

VSG switching in trypanosomes





VSG switching

- An individual bloodstream-form trypanosome expresses a single VSG gene, in a mutually exclusive fashion from one of ~20 telomeric bloodstream-form VSG expression site transcription units
- Switching the active VSG can involve activation of another VSG expression site
- Alternatively, DNA rearrangements including gene conversion or telomere exchange can insert one of many hundreds of silent VSG genes into an active VSG expression site
- The genome sequence has revealed that *T. brucei* has a repertoire of at least 1250 to 1500 VSG genes (available at Trypanosome database, TriTrypDB, http://www.tritrypdb.org)
- Majority of these VSGs (at least 1250) are present in several tandem arrays located at subtelomeres

Plasmodium

- One of the most remarkable biological features of *P. falciparum* is an exceptionally polymorphic parasite antigen expressed on the surface of infected erythrocytes, known as P. falciparum erythrocyte membrane protein 1 (PfEMP1)
- PfEMP1 is encoded by a family of hypervariable genes known as *var*, each representing a different antigenic form, and the parasite is able to vary its antigenic profile by switching expression between different *var* genes
- This allows the parasite to evade the human immune system
- Each parasite genome contains approximately 60 *var* genes distributed in clusters across most of the 14 chromosomes

Mechanism of *var* Gene Switching

- unlike *T. brucei*, the mechanism by which *var* switching occurs in *P. falciparum* is thought to be purely transcriptional (at the level of transcriptional initiation or post-transcriptional).
- *Var* switching has been shown to take place soon after invasion of an erythrocyte by a *P. falciparum* parasite.
- The antigenic variation mechanism is carried out by successive switching of expression of *var* gene family members
- This allows parasites to modify the antigenic and functional properties of infected erythrocytes (IE) resulting in modified adhesive phenotypes and possibly altered virulence
- Antigenic variation allows parasites to establish persistent chronic infections.

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