

20.109 Module 2

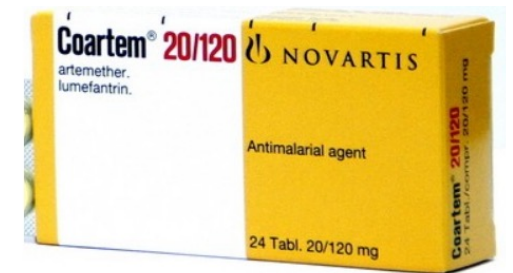
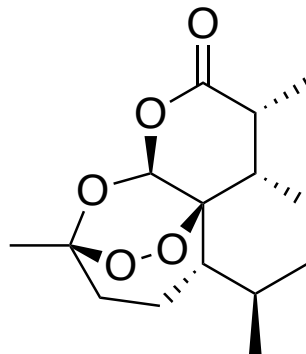
Lecture #5: **Introduction to screening: concepts & principles II:** *Phenotypic Screens*

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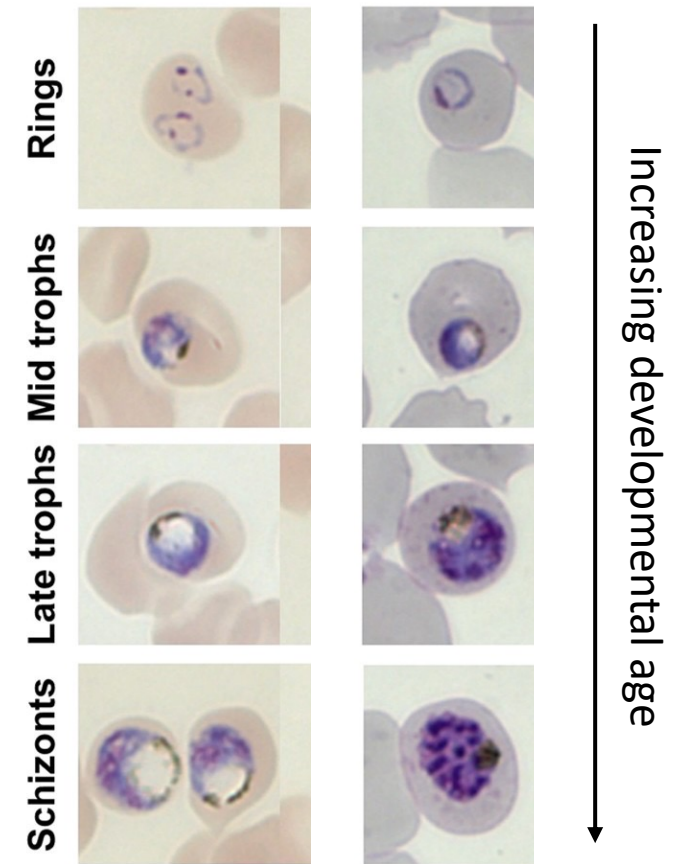
Learning Objectives

- A. Discovering compounds (“hits”) using phenotypic screens
 - A. What are phenotypic screens
 - B. Requirements for a good phenotypic screen
 - C. Constraints placed on small molecules during phenotypic screens

- B. Comparing target-based and phenotypic screens
 - A. Is one approach better than the other?

What do we mean by phenotype?

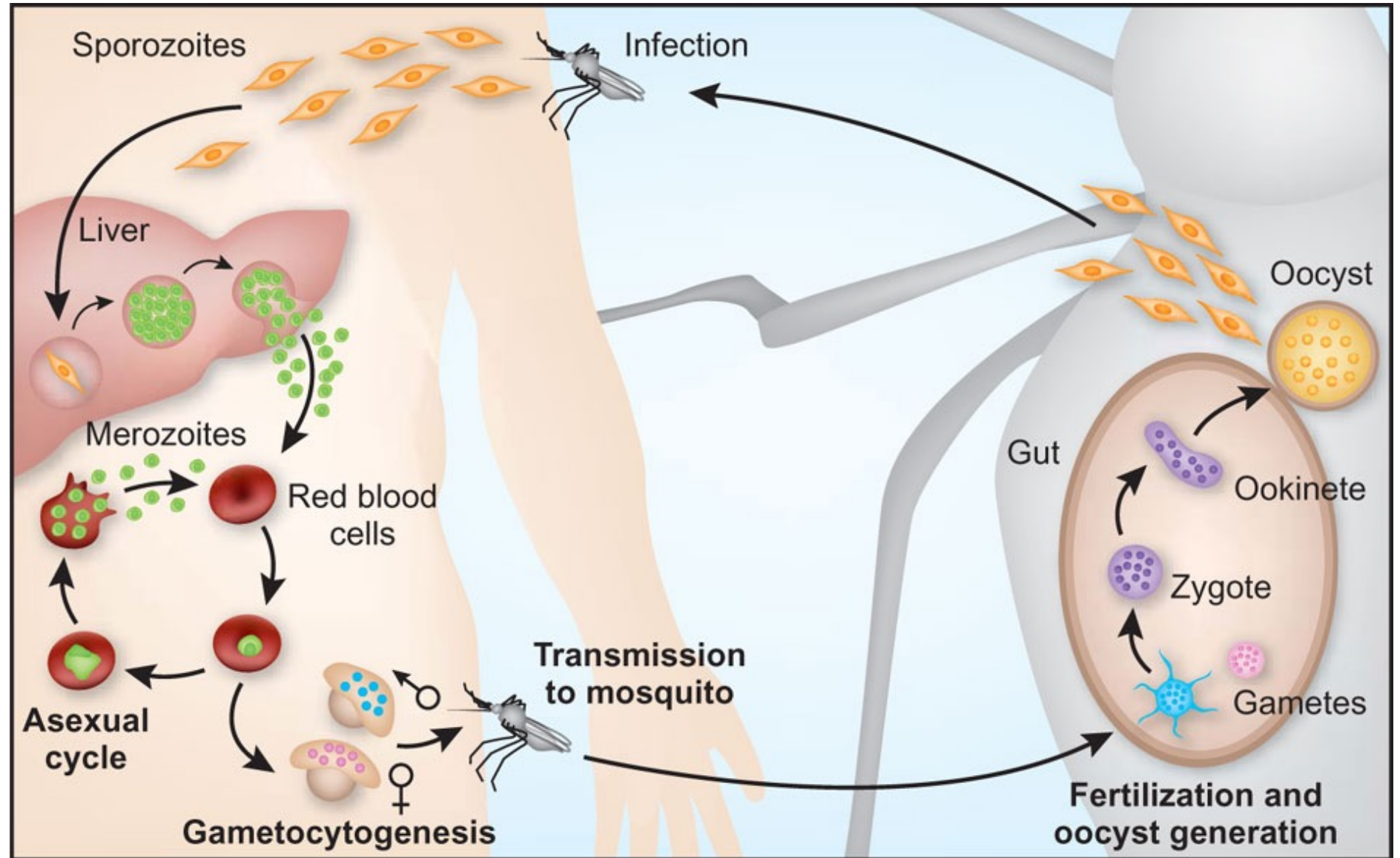
- Observable characteristics resulting from the interaction between genotype and environment
- Many different phenotypes to consider:
 - Cellular
 - Growth/ survival
 - Subcellular
 - Cell size, morphology
 - Organelle size, shape, distribution
 - Others?



How do you choose a phenotype for screening purposes?

P. vivax – a dormant form (hypnozoite) persists in the liver

All malaria symptoms associated with red blood cell infection



Devise some potential phenotypic assays based on the parasite biology described above

Pasvol, Nature Genetics (2010)

How do you choose a phenotype for screening purposes?

1. What biology do you want to manipulate:
 1. Infectious microbe?
 2. Vector?
 3. Host organism?

How do you choose a phenotype for screening purposes?

1. What biology do you want to manipulate:
 1. Infectious microbe?
 2. Vector?
 3. Host organism?
2. Can rigorously connect alterations in the selected phenotype with disruption of the disease process (e.g., infection/ disease pathogenesis)
 1. Direct adverse effects on the parasite;
 2. Indirect effects: altered phenotype disrupts critical interactions with host to prevent disease pathogenesis
 1. For example, cell death >> decreased risk of infection and disease;
 2. Non-lethal phenotype (e.g., altered cell surface protein display), BUT interferes with disease pathogenesis >> may still be reasonable.
3. Should be assayable ... preferably in high throughput [Input – operation --- output]
 1. *In vitro* cell culture (tractable)
 2. Animal model (... probably not!)
 3. Target organism (NOT!)

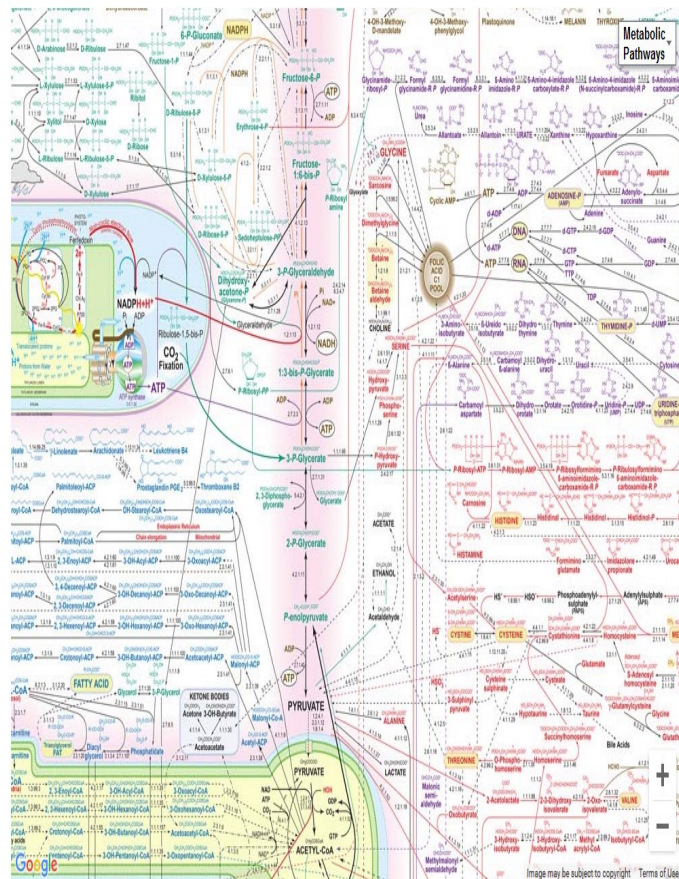
What question(s) are being asked in a phenotypic screen?

- Are there *compounds that can enter the target cell* to cause a measurable change in phenotype?
- Is there a process(es) that can be targeted by these compounds to disrupt cellular phenotype (survival)

Compounds



Phenotypic Outcome
(e.g., cell death)



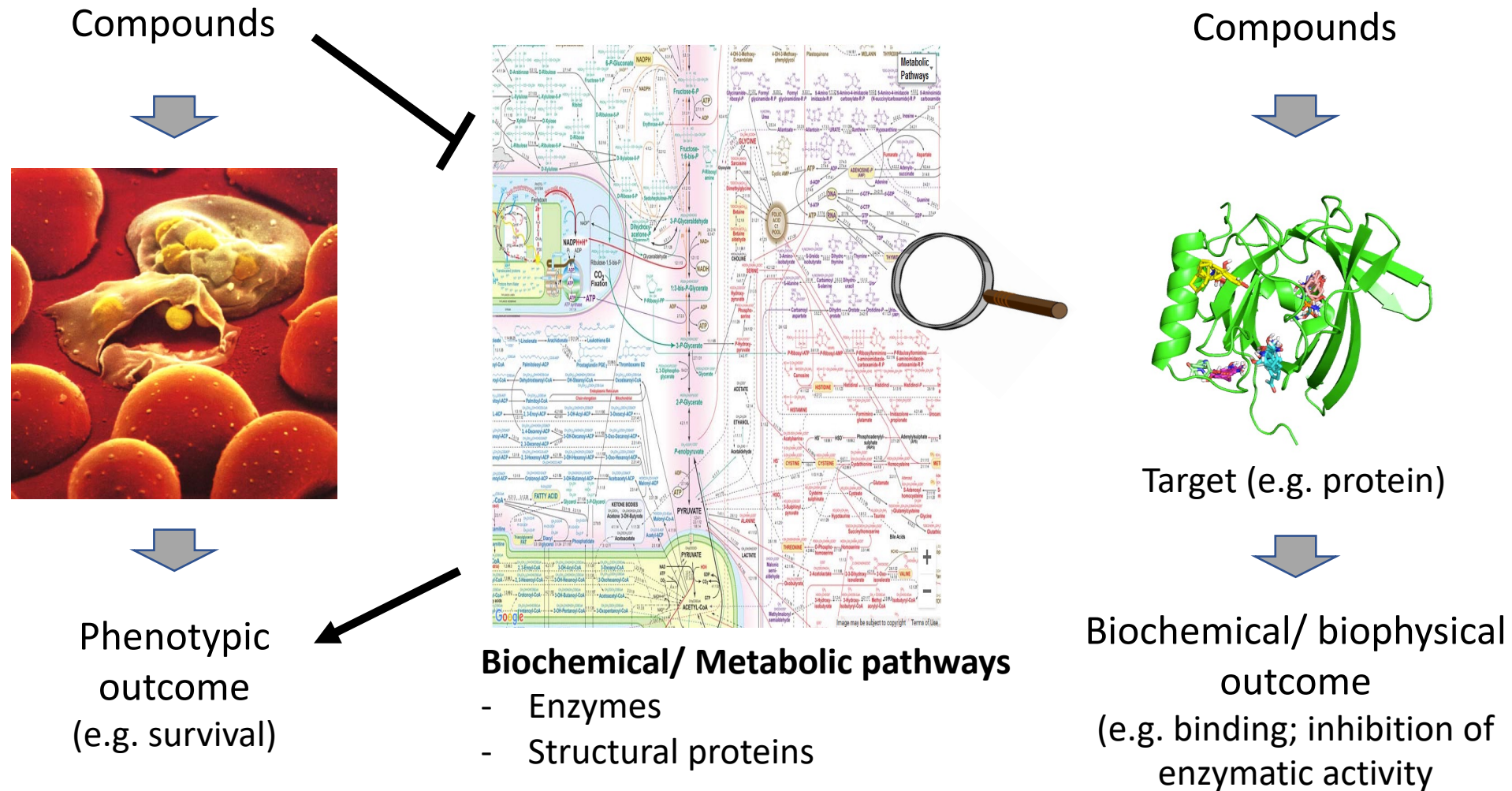
Biochemical/ Metabolic pathways

- Enzymes
- Structural proteins

What don't we immediately know from this screen?

- The molecular pathways perturbed by phenotypically active compounds
- Single or multiple molecular target(s)?

Comparing target-based and phenotypic screens



1. What are some assumptions made in target-based versus phenotypic screens?
2. When would you select one option over the other?

Comparing target-based and phenotypic screens

	Target-based Screen	Phenotypic Screen
Biological		
Prior specification of precise molecular target required	Yes	No
Assumption/ knowledge of underlying biology required	Yes	No
Requires correlate between assay/ screening outcome and disease biology	Yes	Yes
Immediate requirement for biological components (e.g. cells) during initial screening process	No	Yes
Compounds		
Cellular permeability immediately required	No	Yes
Compound can be modified to enhance cell permeability, metabolic stability, etc.	Yes	Yes

Summary

- Small molecule therapeutic candidates can be identified using screens:
 - Target-based
 - Phenotypic
- Both types of screens require assays that:
 - Provide reliable and reproducible readouts of the effects of tested compounds
 - Are scalable to accommodate exploration of large chemical space to identify relatively rare 'hits'
- These screening modes have non-overlapping pros and cons
 - Can be used in complementary ways

