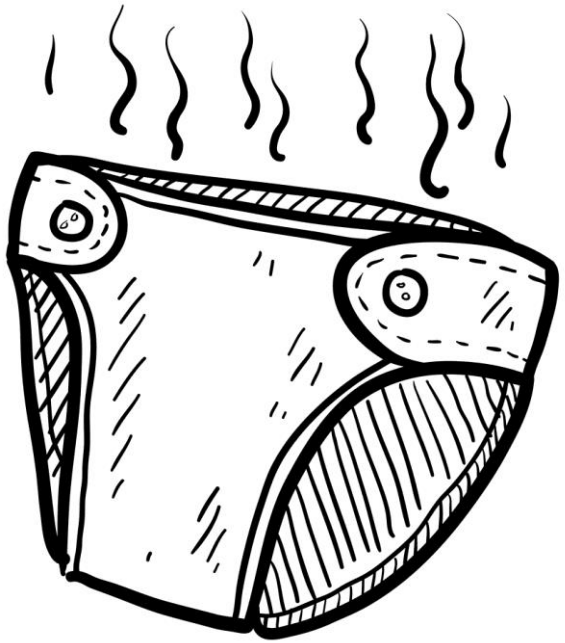
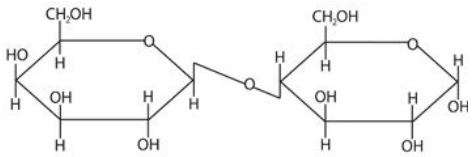
WISCONSIN
UNIVERSITY OF WISCONSIN–MADISON

Congenital Lactase Deficiency (LCT)

Hayley Stoneman

What is congenital lactase deficiency (CLD)?





CLD patients cannot digest lactose, resulting in diarrhea

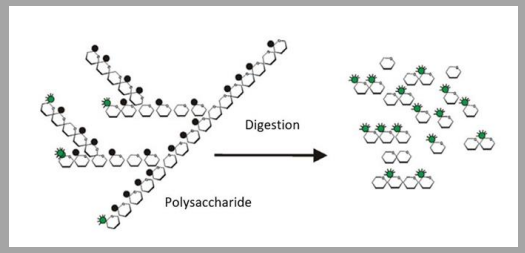
LCT is associated with CLD

Signal sequence

Glycosyl hydrolase family 1

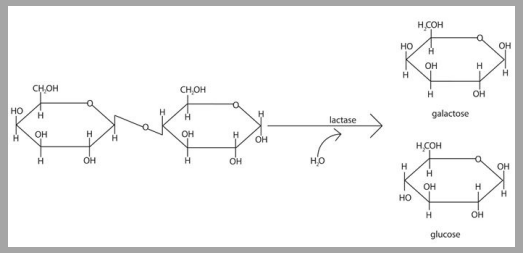
Transmembrane region

Biological Process



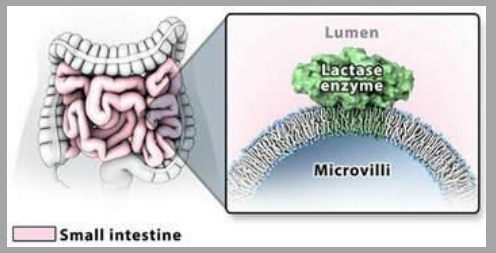
Polysaccharide digestion

Molecular Function



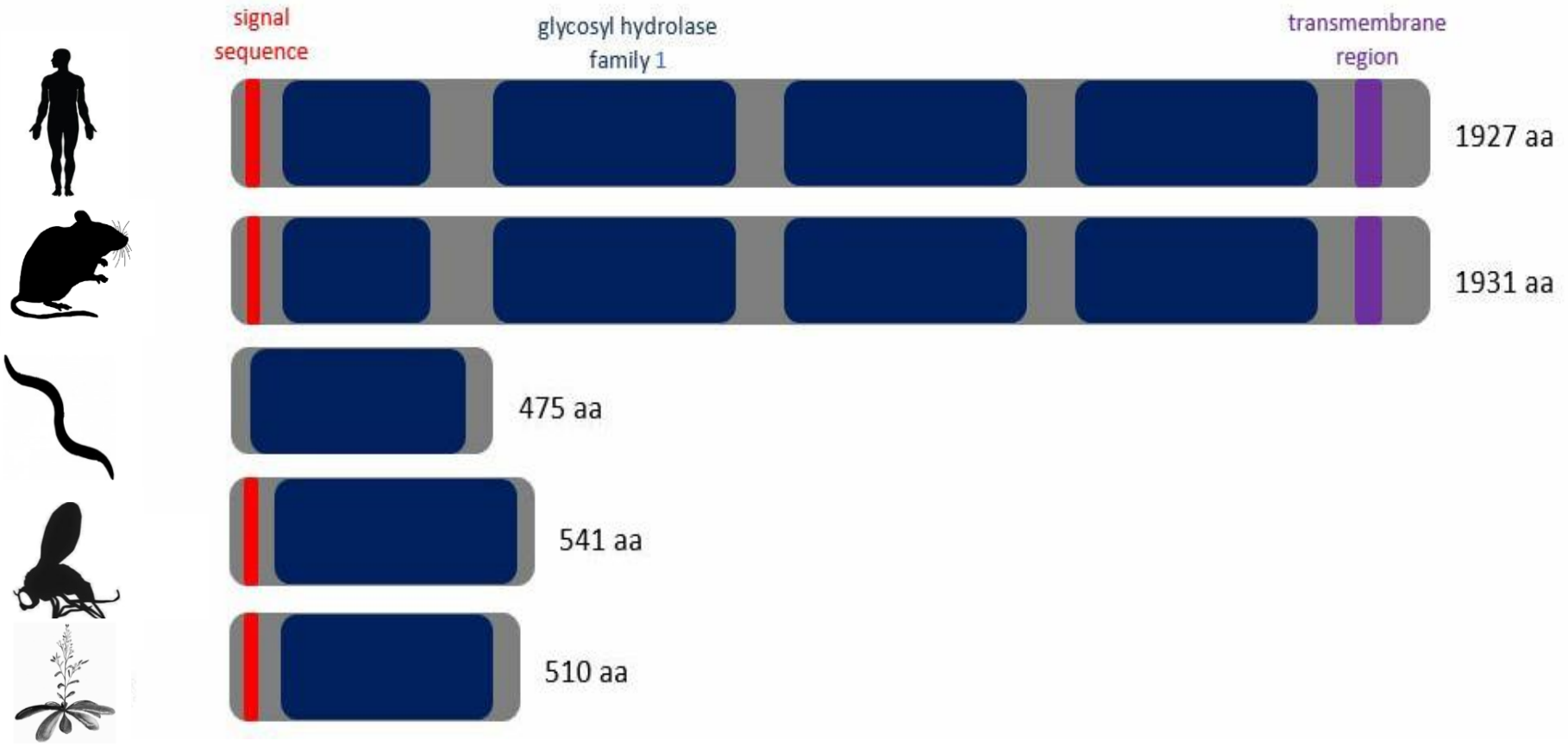
Lactase activity

Cellular Component



Intestinal tract

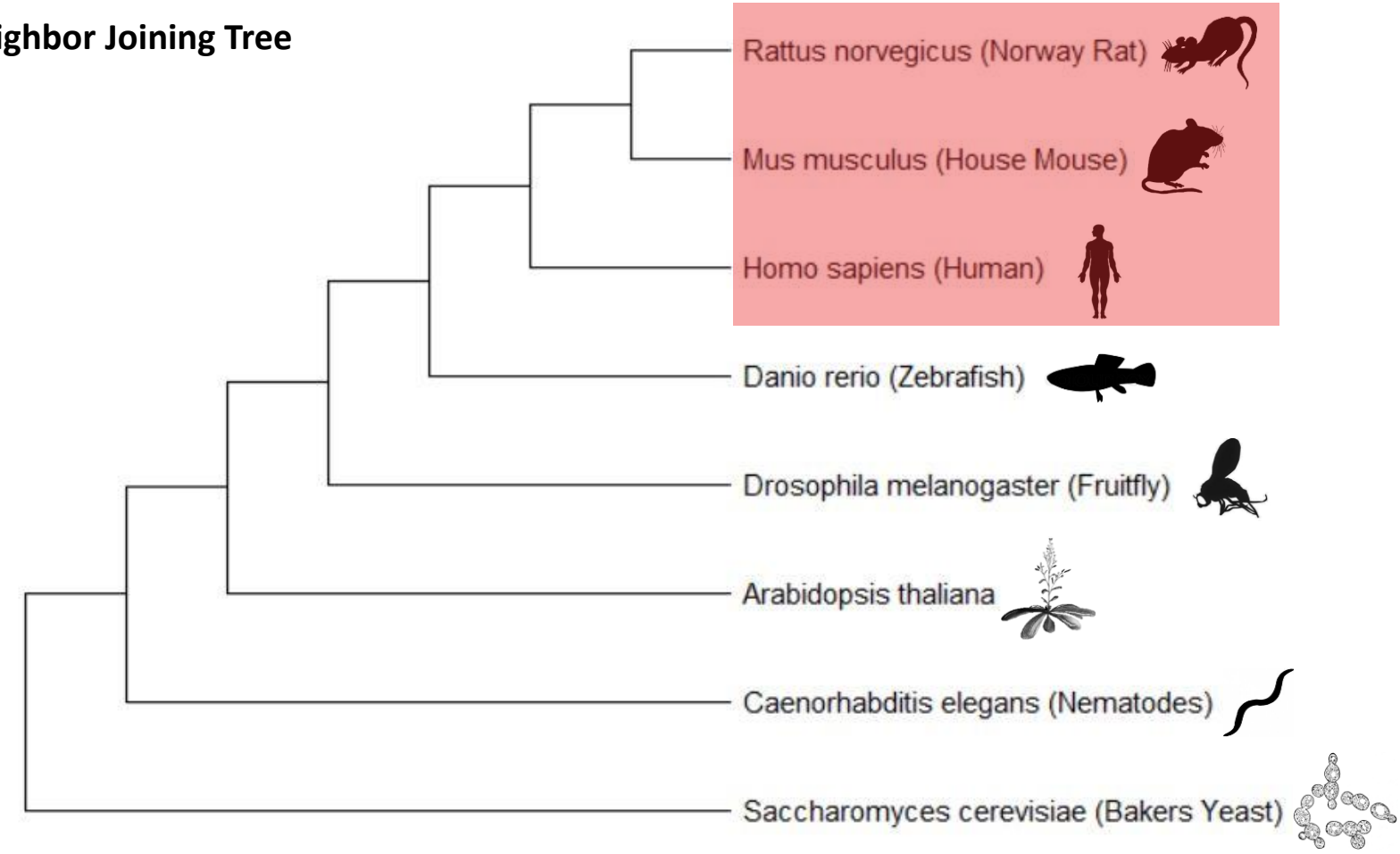
LCT is well conserved in mammals



All homologs have glycosyl-hydrolase family 1 domains

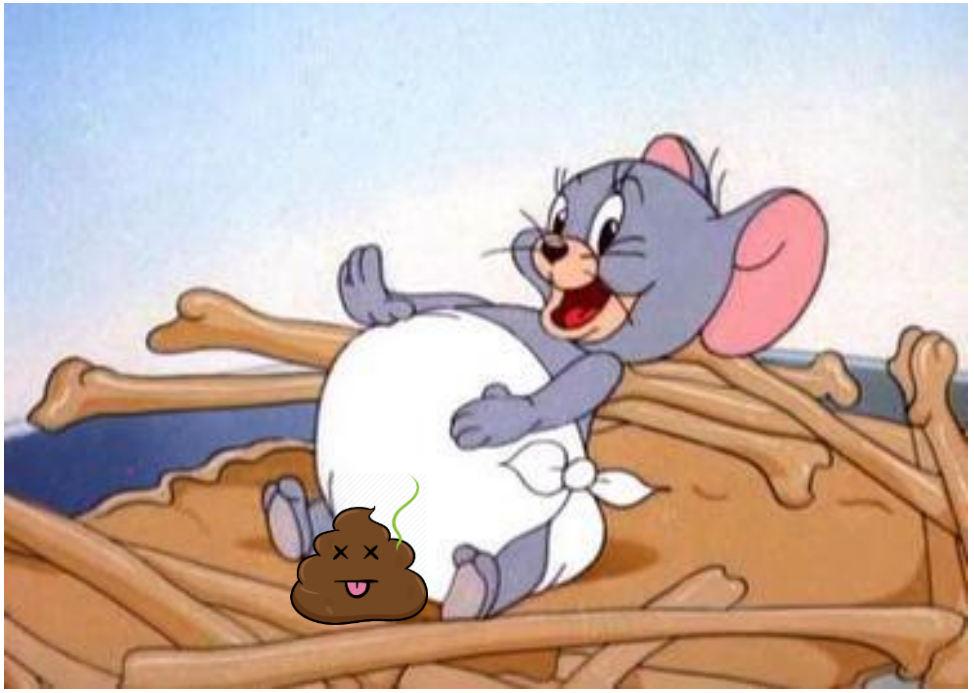
What is LCT phylogeny?

Neighbor Joining Tree



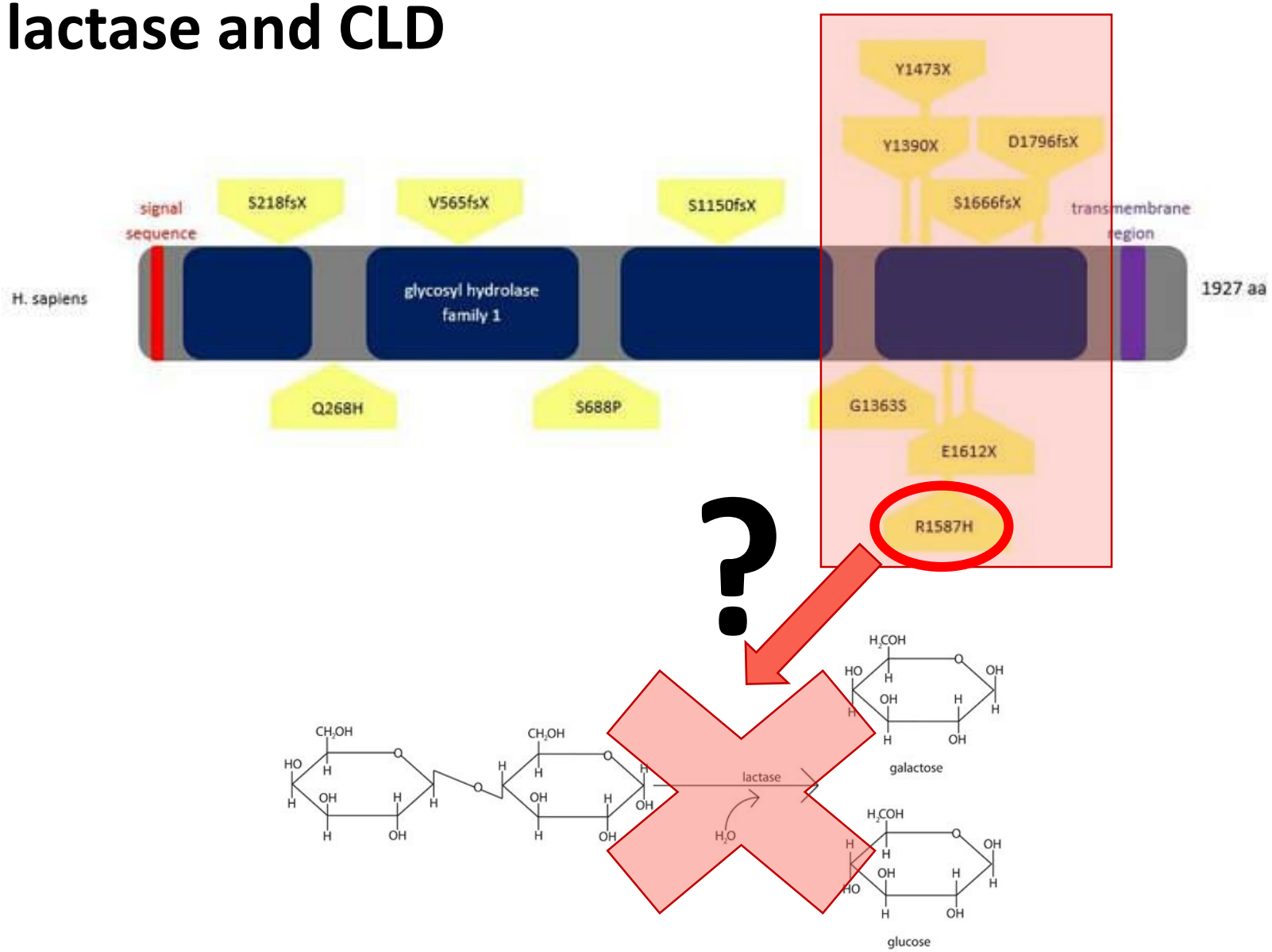
Milk-consuming animals have closest related LCT proteins

What model organism should be used to study CLD?



Mouse diet is easily manipulated and diarrhea is easily observed

Unknown how R1587H causes loss of functional lactase and CLD



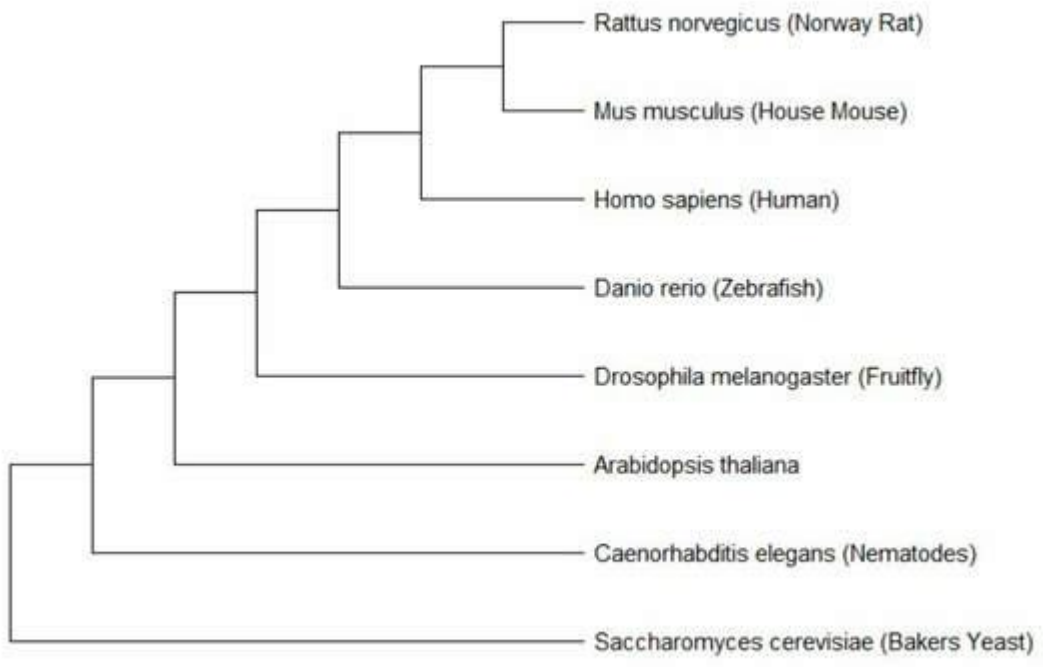
What is the **primary goal**?

To understand how a single amino acid substitution, R1587H, affects the structure, function, and interaction of LCT to determine how it leads to CLD

Aim 1	Aim 2	Aim 3
Understand the evolutionary history of LCT and conservation of the C-terminus and R1587	Determine differentially expressed genes and their functions in WT and R1587H mutant mice	Experimentally determine protein-protein interactions of WT and R1587H mutant mice LCT

Aim 1: Understand evolutionary history of LCT and conservation of R1587 and the C-terminus

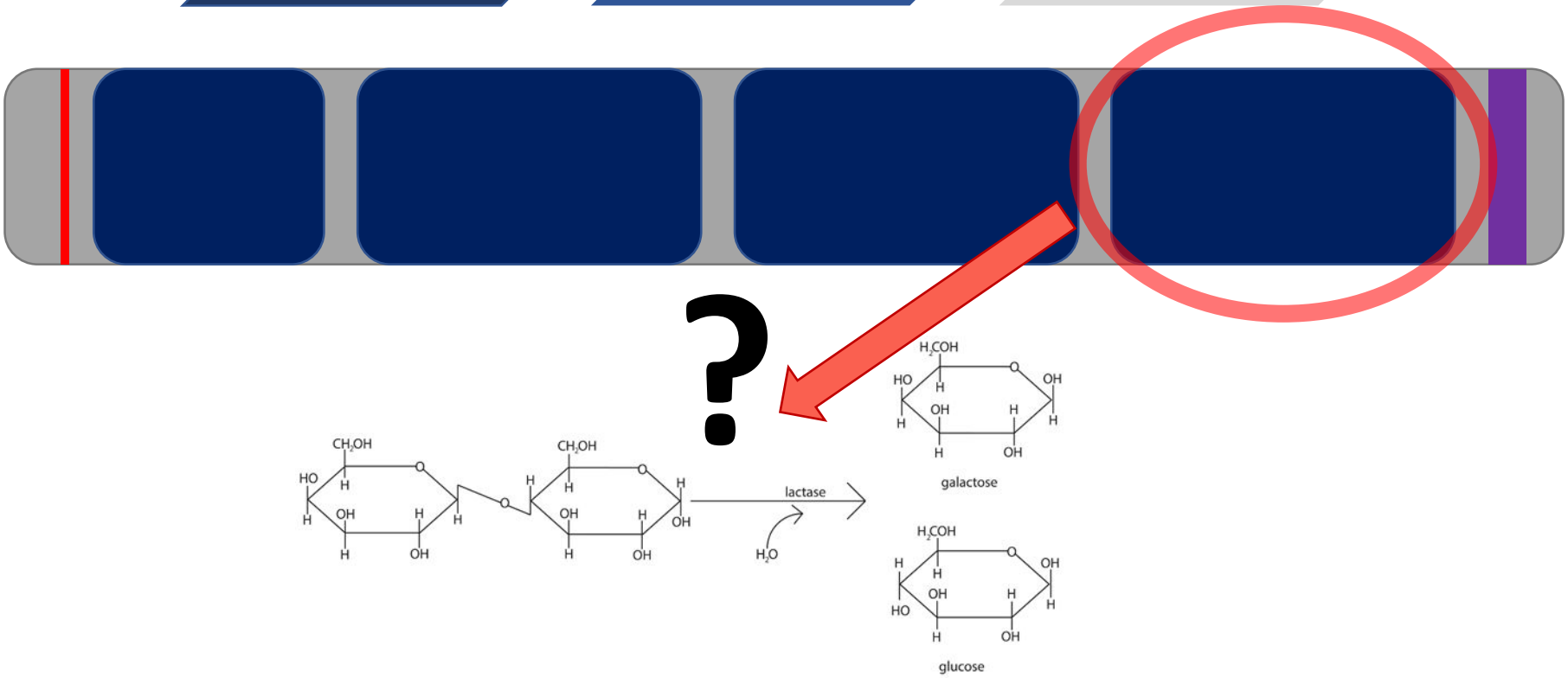
What → **Why** → **Hypothesis**



Approach: Use amino acid sequence to build phylogenetic trees and observe conserved regions

Aim 1: Understand evolutionary history of LCT and conservation of R1587 and the C-terminus

What → **Why** → Hypothesis



Rationale: Evolutionary history can elucidate the importance of R1587 in functional lactase among milk consuming organisms

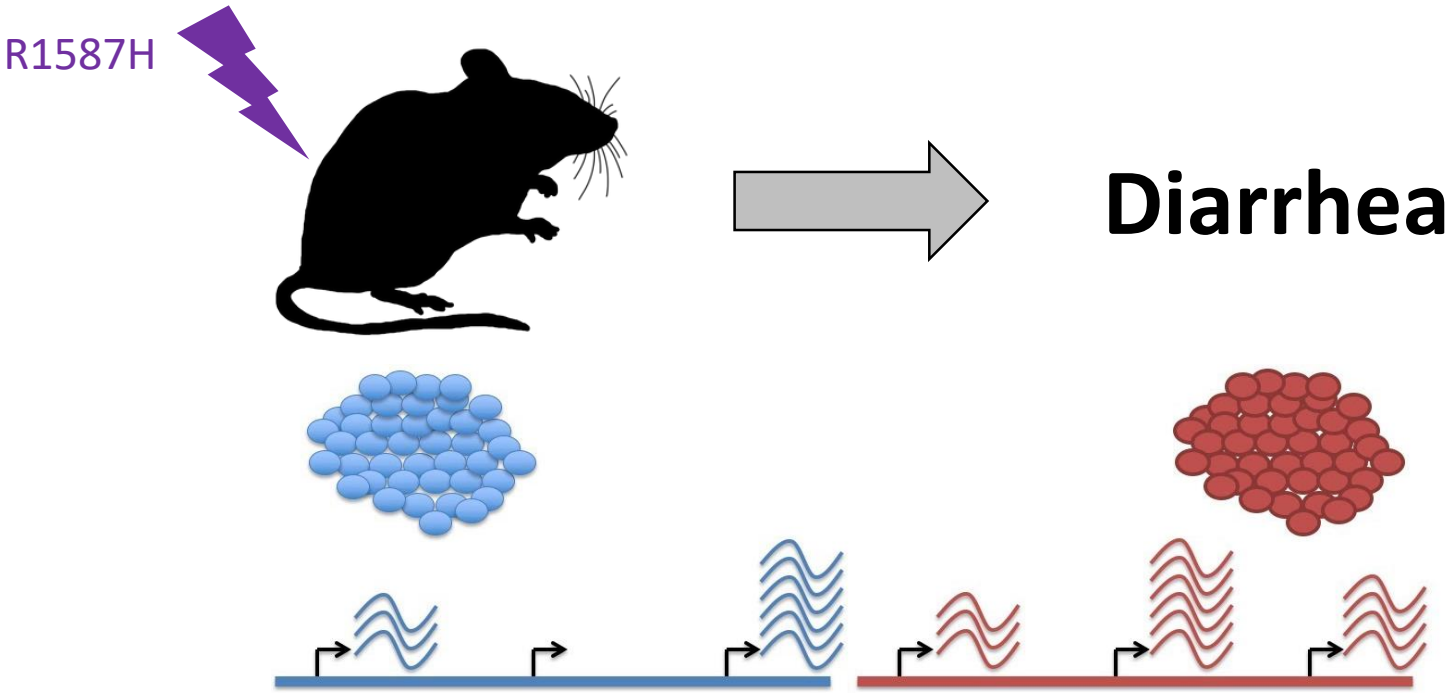
Aim 1: Understand evolutionary history of LCT and conservation of R1587 and the C-terminus

What → **Why** → **Hypothesis**

Hypothesis: R1587 will be conserved among milk consuming organisms

Aim 2: Determine differentially expressed genes and their functions in R1587H and WT mice

What → Why → Hypothesis



Approach: Create mutant mouse line and confirm CLD phenotype, then perform RNA-seq to identify gene expression

Aim 2: Determine differentially expressed genes and their functions in R1587H and WT mice

What → **Why** → **Hypothesis**

GENEONTOLOGY
Unifying Biology

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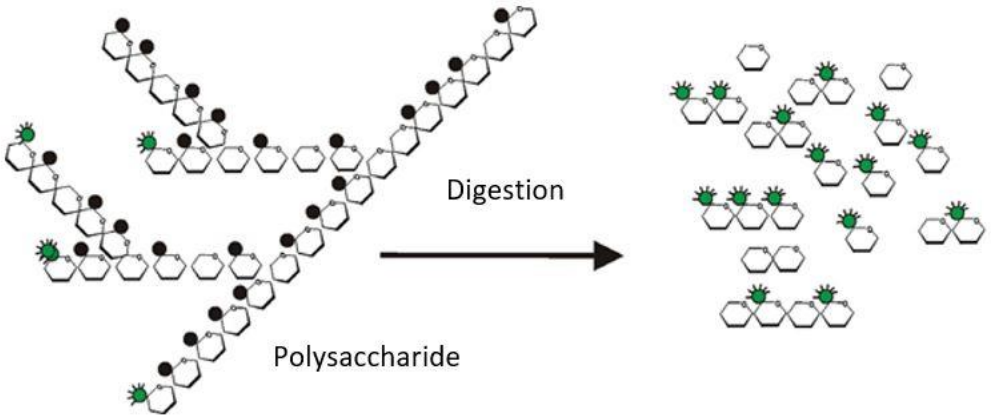
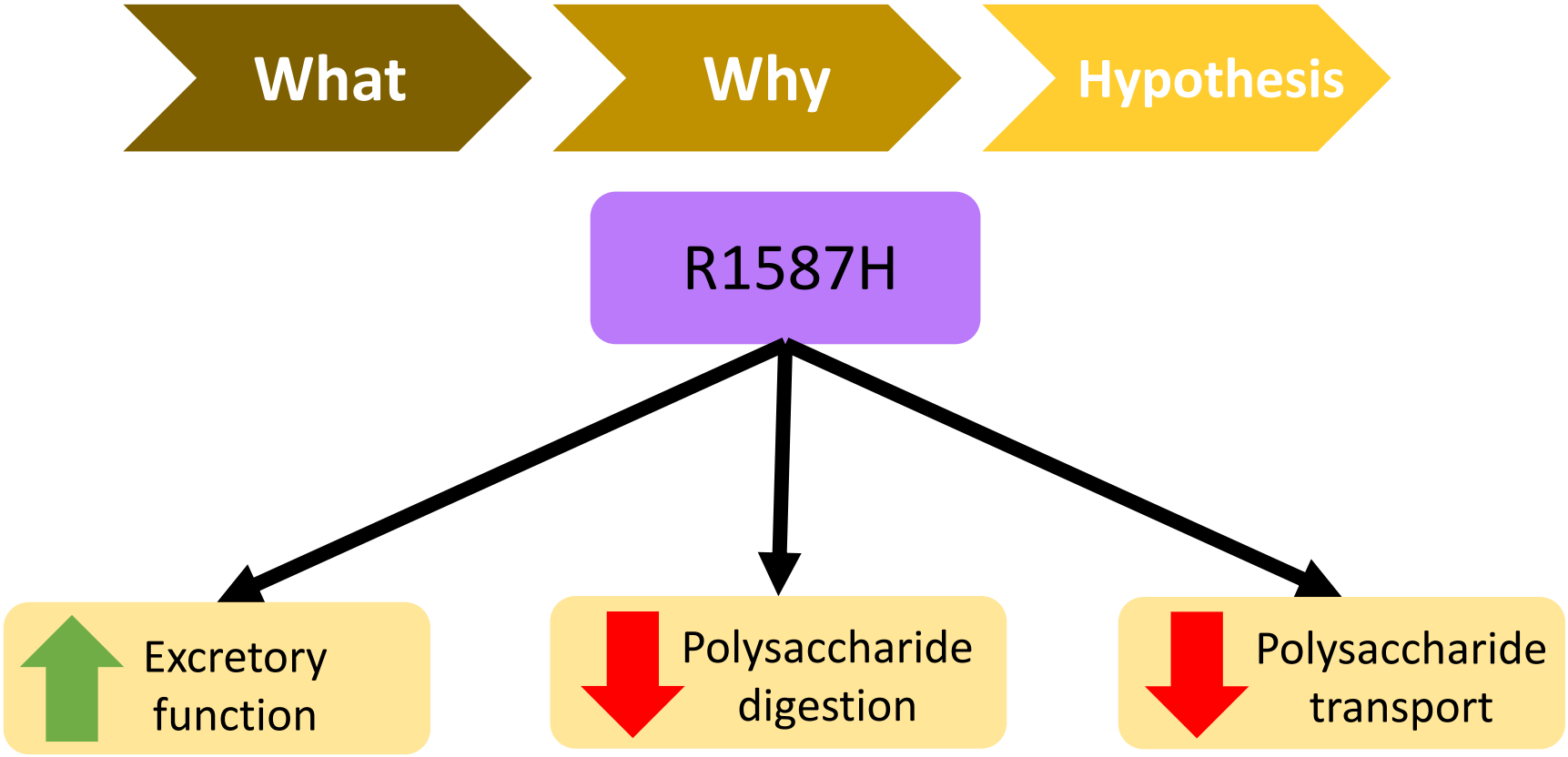


Rationale: Determining differentially expressed genes will elucidate interrupted biological processes

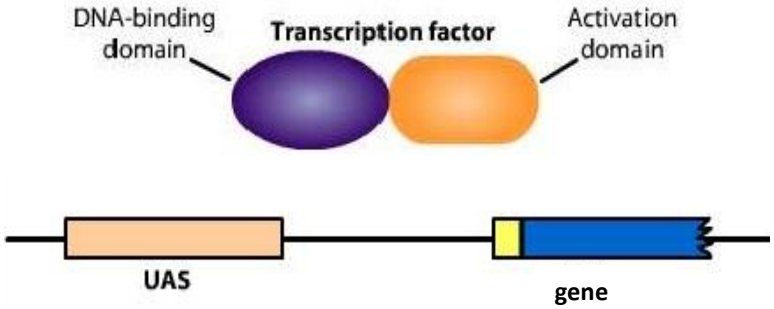
Aim 2: Determine differentially expressed genes and their functions in R1587H and WT mice

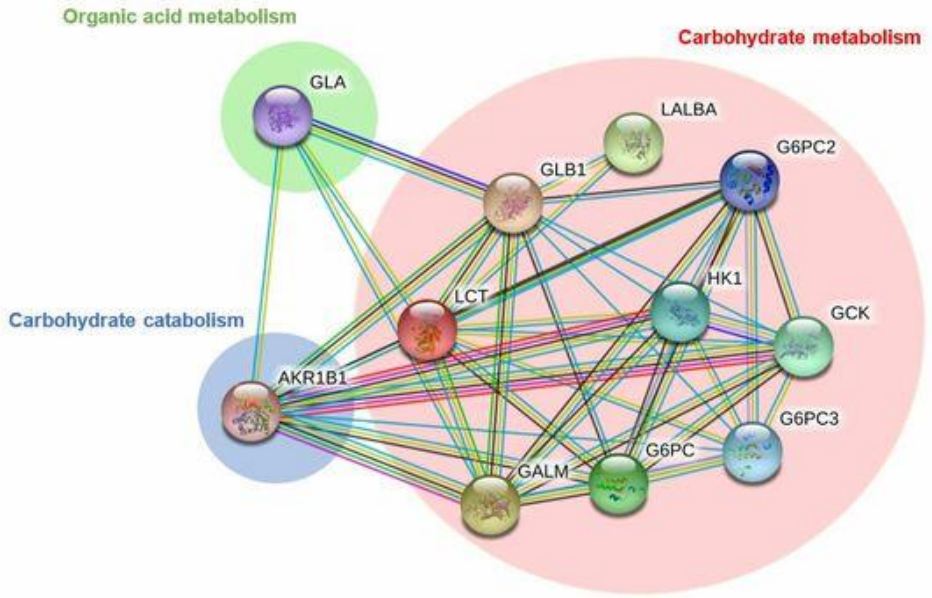


Hypothesis: R1587H mice will have upregulated genes related to excretory function downregulated genes in polysaccharide digestion/transport

Aim 3: Experimentally determine protein-protein interactions of WT and R1587H LCT

What → **Why** → **Hypothesis**

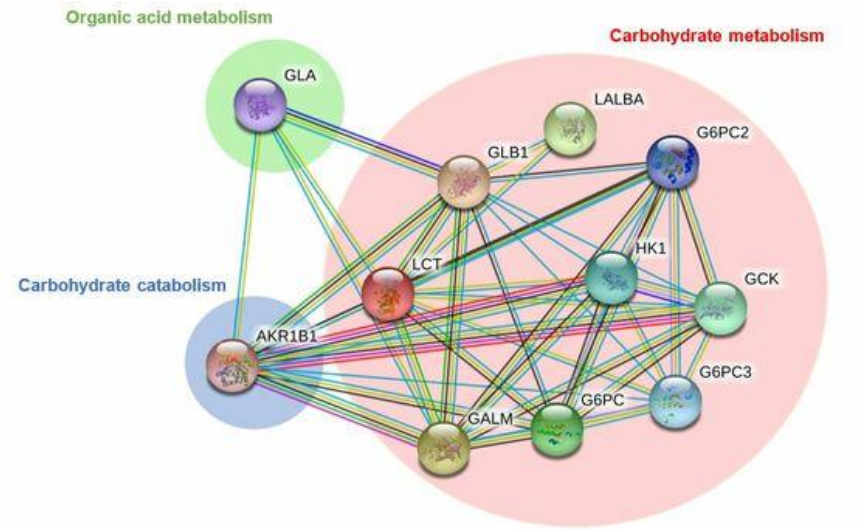


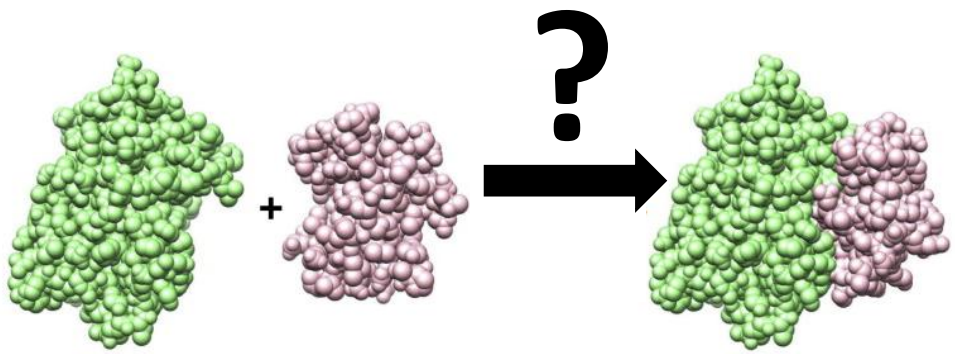


Approach: Use mammalian two-hybrid system to identify protein-protein interactions and sort according to GO terms

Aim 3: Experimentally determine protein-protein interactions of WT and R1587H LCT

What → **Why** → Hypothesis

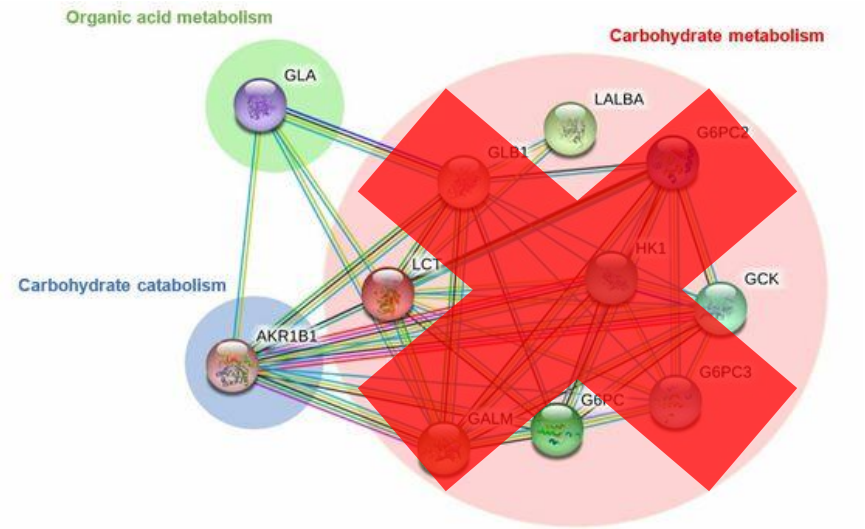


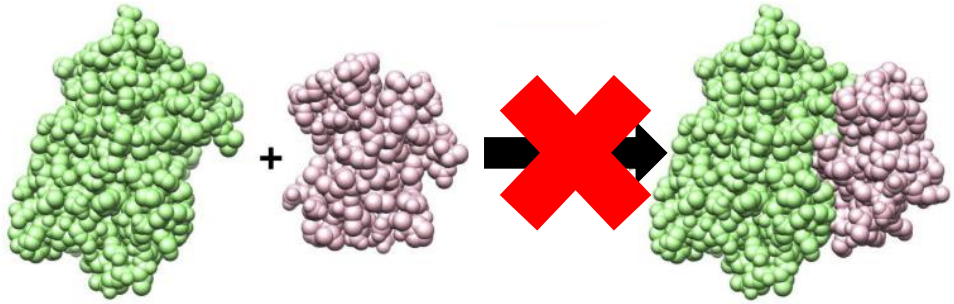


Rationale: Create first experimentally determined interaction network for LCT and determine which protein-protein interactions inhibited by R1587H

Aim 3: Experimentally determine protein-protein interactions of WT and R1587H LCT

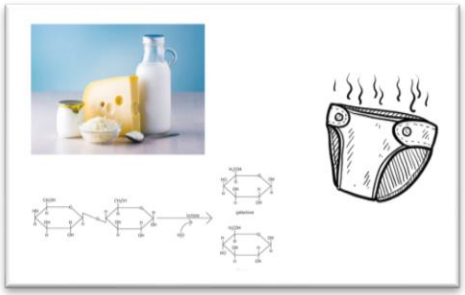
What → **Why** → **Hypothesis**



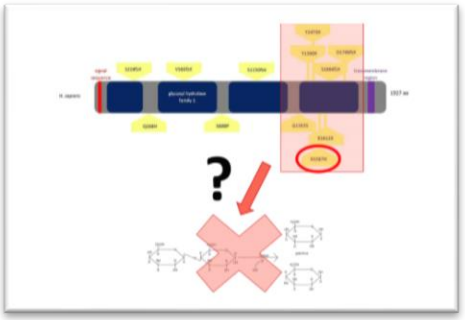


Hypothesis: Interaction network will involve carbohydrate metabolism proteins and R1587H will interrupt many of these interactions

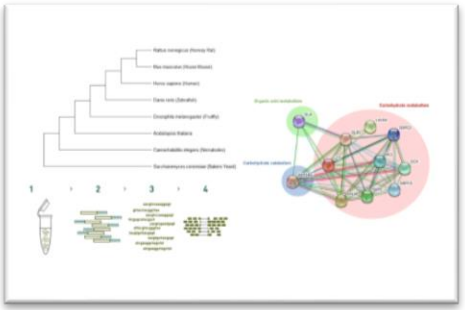
Summary



CLD is a disease caused by lack of lactase (LCT) functional enzyme resulting in inability to digest lactose leading to diarrhea



It is unknown how missense mutations in the C-terminal glycosyl-hydrolase family domain lead to CLD



Phylogenomic, transcriptomic, and proteomic techniques will be used to study the functional contribution of R1587H to LCT

Future Directions

Develop treatment for CLD

Questions?

References

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***all images hyperlinked**