NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

I Scream for Ice Cream:
Lactase Persistence in Humans

by
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Introduction

It is finals week on college campus and everybody is in the middle of studying for exams, just waiting for summer break. Björn, Chris, Esiankiki, Xiao-Ma, Sanjeet and Linda are studying together in the international students dorm.

Björn: Hey, how are you doing with studying? I'm tired …

Chris: Me too. Let's take a break. I could also use some ice cream after that last final I bombed.

Esiankiki: Did I hear ice-cream? I love ice-cream! Let's go downtown and find a spot. Is everybody coming? Let's go!

Xiao-Ma: Hm, I can't tolerate dairy so I usually don't get ice-cream or other milk products. Sorry guys, I'll stay behind.

Linda: What does that mean? Do you just not like milk? I used to hate milk as a little kid.

Xiao-Ma: No, I actually get really bad stomach cramps, bloating and diarrhea from milk. It's not pretty. My whole family is that way. When we were still living in China, it wasn't a big deal because we don't traditionally eat a lot of milk products there.

Sanjeet: Same for me! I get the same thing, and in India we don't eat a lot of dairy either, so it's not a problem if I eat Indian food. My mom gave me Lactaid® though, so that helps me to eat ice-cream. Xiao-Ma, I can give you some. Let's go!

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Part I – Geographical Variation in Tolerance to Dairy

I.i – Survey of Dairy Intolerance

Björn: Wait, so are you saying that where you're from, people are generally not able to digest milk? At home in Sweden, we eat dairy products like milk and cheese all the time and everyone I know seems to tolerate it well.

Linda: That's so interesting! I'm curious to see how that maps to the world and if what you observed could be generally true. Let's do a survey in the international dorm and find out who is tolerant to dairy and where they are from. I'll put a black dot for a dairy tolerant person in his or her home country and a white dot for a dairy intolerant person. I'll start with Xiao-Ma—one white dot in China!

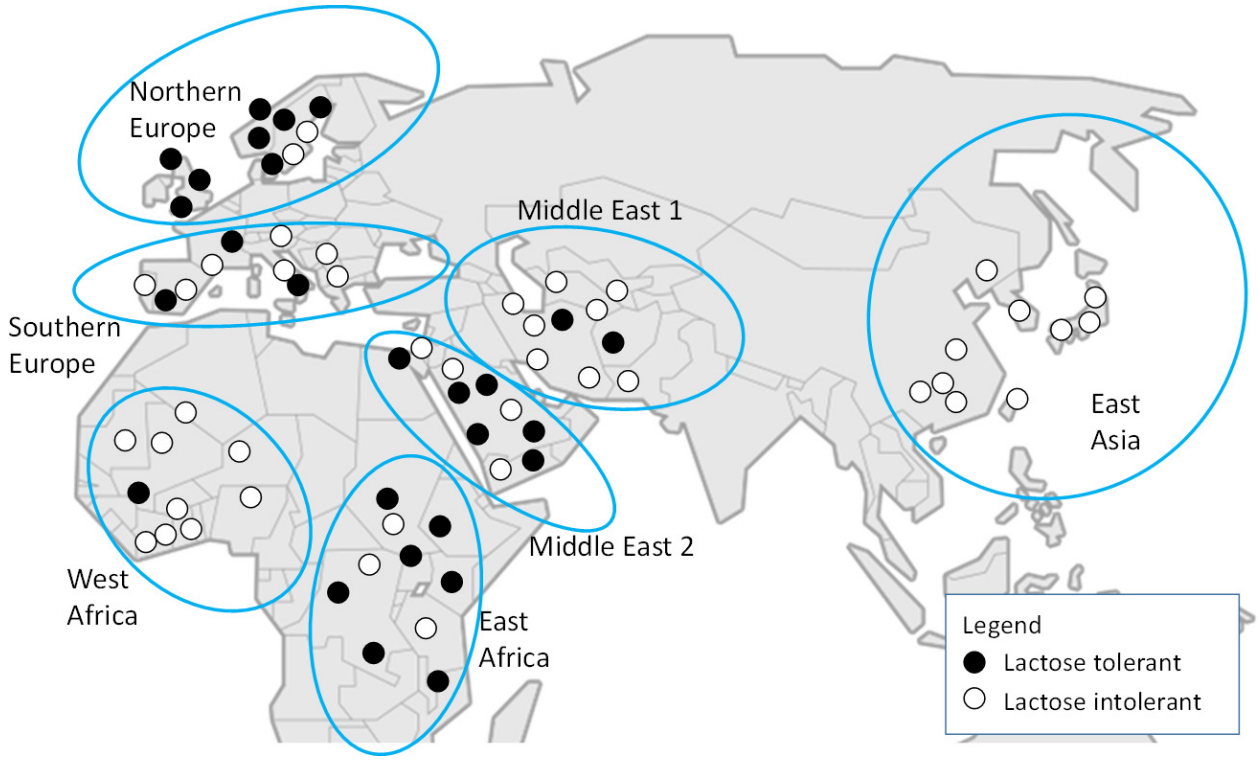


Figure 1. Survey of dairy tolerance. Linda's results on a map of the world

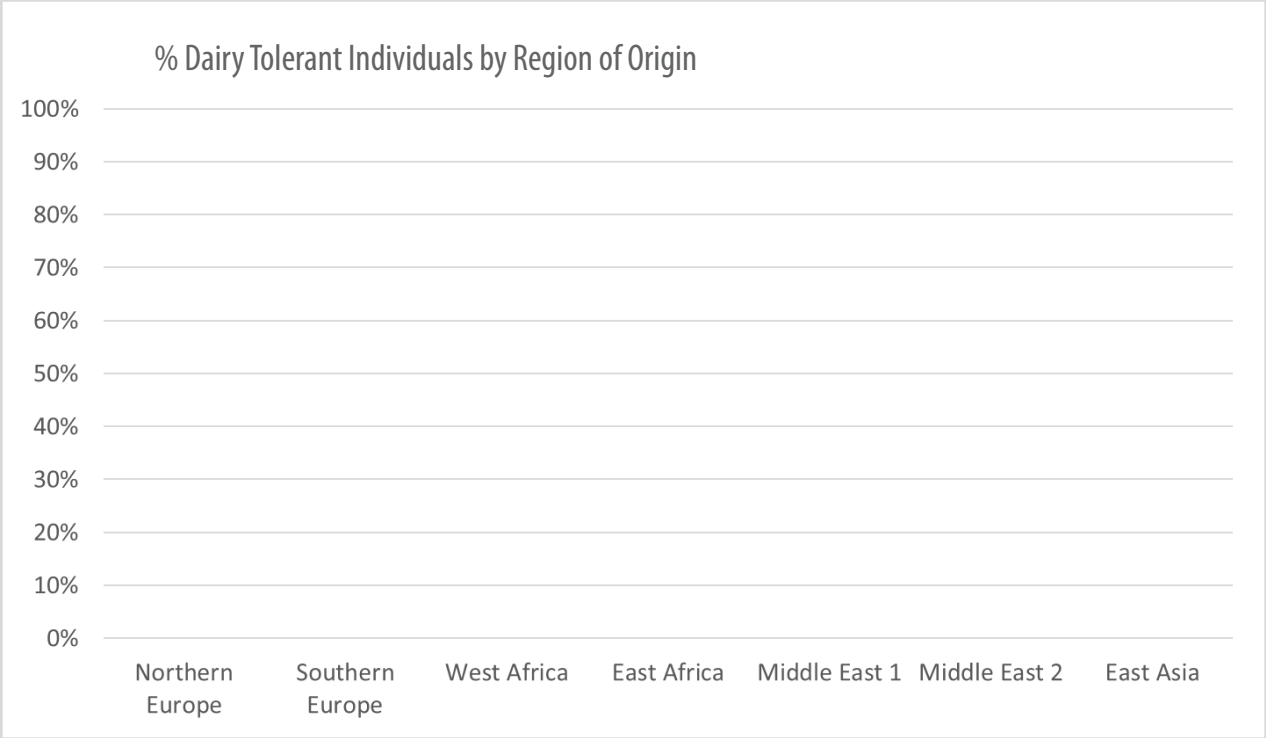
Questions

1. Do you see patterns in the geographic distribution of the black and white dots? Describe the pattern qualitatively.

2. How would you quantify these patterns?

I.ii – Quantification of Dairy Intolerance by Region

3. Count the black and white dots for each circled region and calculate the percentage of dairy tolerant people in each region. Plot your findings in the graph below.



4. What does this data mean in terms of likelihood for a person from a particular geographic area to be dairy tolerant?

Part II — Lactase Catalyzes the Hydrolysis of Lactose

II.i – Enzymes and Sugars

The group of college students is on their way to the ice-cream shop and Sanjeet has offered Xiao-Ma Lactaid so that she could go with the group and also eat ice cream despite her lactose intolerance.

Xiao-Ma: I've never taken Lactaid. What is it?

Sanjeet: My mom told me that you become intolerant to dairy because you don't have the enzyme that can digest lactose, which is the sugar found in milk products. Lactaid is a dietary supplement that contains the lactase enzyme.

Chris: Uh, Sanjeet, remember—I'm a literature major. What's an enzyme? I don't remember what that word really means. Also, is the sugar in the milk different from the other sugar in my food?

Sanjeet: *Finally* my biochem class comes in handy in real life! An enzyme is a protein which catalyzes a reaction in the cell. There are tons of different enzymes in your body. The lactase enzyme catalyzes the degradation of lactose into its subunits. Lactose is a sugar and there are several other sugars; the one that you probably know is sucrose. Sucrose consists of two parts: glucose and fructose. Lactose is a different kind of sugar and consists of glucose and galactose.

You need to digest lactose into the two components because the cells in your intestine can only take up galactose and glucose but not lactose. If lactose stays in the intestine and is not digested into its components, it will be consumed by gut bacteria which produce various gases in the large intestine—that's what leads to the symptoms Xiao-Ma and I experience when we consume dairy.

Here's a picture from my biochem book showing how the lactase enzyme breaks down the milk sugar lactose into its components glucose and galactose:

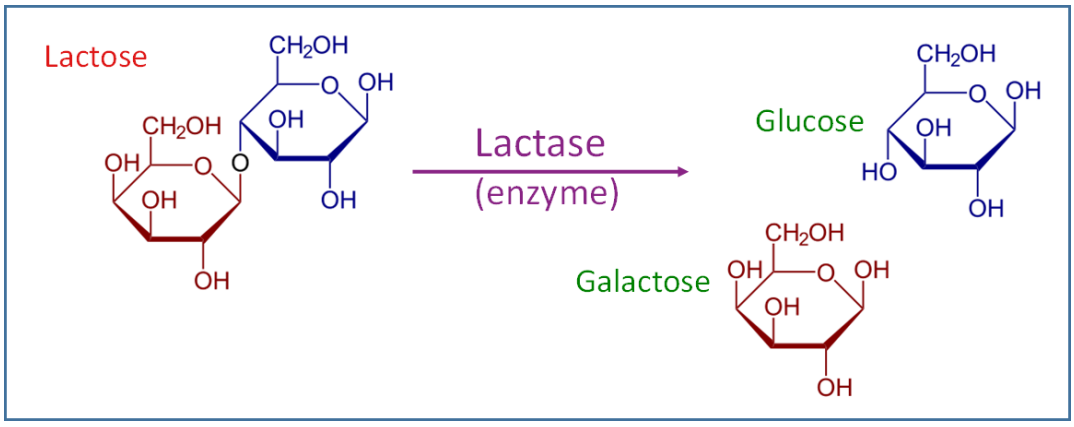


Figure 2. Action of lactase.

It also says in here that you can test if you are intolerant to dairy (so lactose intolerant) by measuring the level of glucose in your blood after drinking milk.

Questions

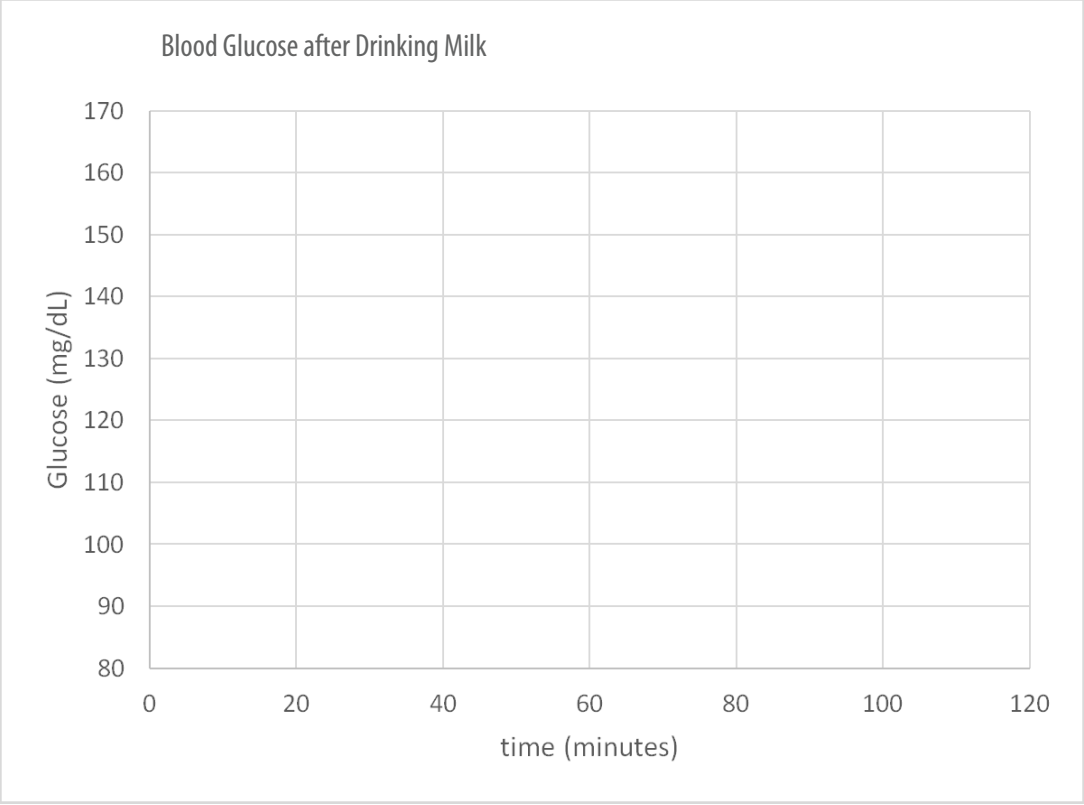
1. What is the difference between a protein and an enzyme? Search the internet if you need more clarification and write down a definition of each term.

2. What is a monosaccharide and what is a disaccharide? Which of these three sugars are mono or disaccharide? Look this up on the internet and label the figure above (Figure 2) with the corresponding terms.

3. How can taking Lactaid help someone who is lactose intolerant to digest lactose?

4. Why would a test for tolerance to lactose measure the levels of blood glucose and not lactose?

5. Would you expect higher or lower glucose levels for dairy tolerant individuals after consuming milk? Why? Draw your predictions for a lactose tolerant and lactose intolerant person into the graph below and explain why.



II.ii – Blood Glucose Test

Esiankiki: Oh, now I remember—I've heard of this test before. My mom's a physician with her own office here in town. I know that my mom is participating in a clinical trial for a new lactose intolerance test that measures blood glucose.

Now I wonder if I'm lactose intolerant. I once had really bad stomach aches when I ate ice cream. Maybe that's why? If we go to her office, we could try to participate in the trial and if we ask nicely, she might give us the data. Wouldn't it be nice to know for sure if you are lactose intolerant?

Xiao-Ma: Yes, I would love that!

Sanjeet: And you know what's interesting—when I was a baby, I didn't have these issues but I was able to digest milk just fine as I was breastfed.

Chris: Wow that's fascinating—so as a baby you probably did produce the lactase enzyme and, if you are right, then your body at some point just stopped making it. Suddenly biology is starting to be more interesting...

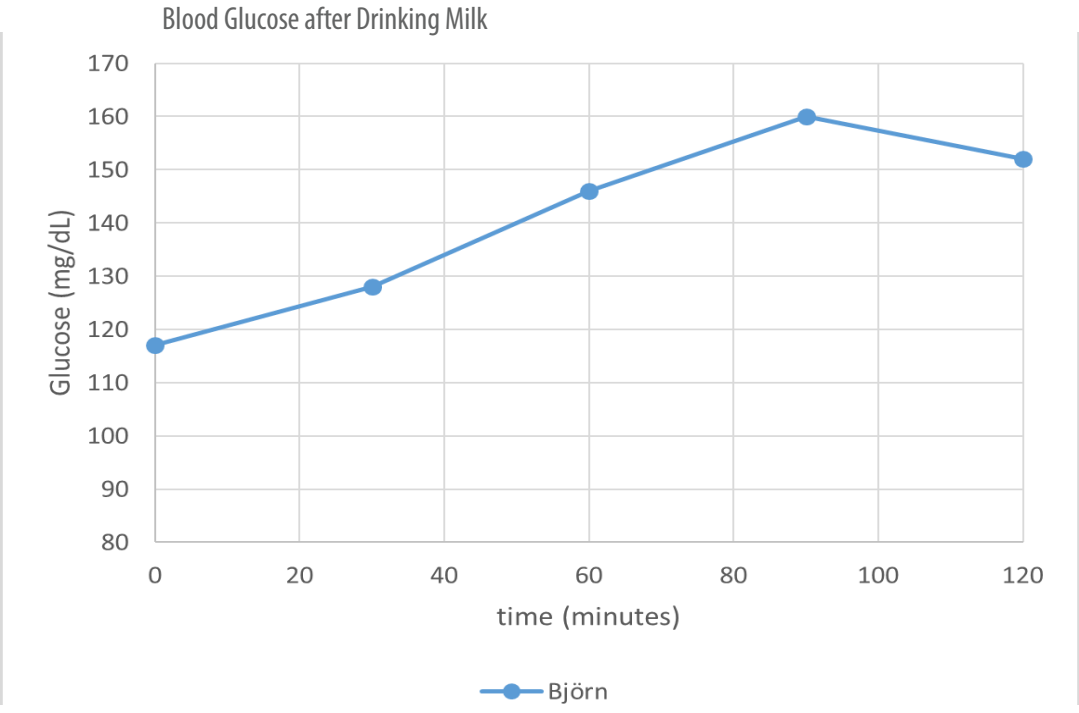
Esiankiki: Well, that would explain why my mom calls people who can eat dairy without problems "lactase persistent." I never really understood what she meant by that. She probably means that the production of lactase enzyme persists in those individuals when they are adults. Let's go and do that blood test; I'm really curious now.

Here are the results that the group of friends obtained from Esiankiki's mother. Each of them had to drink a liquid that contains lactose. At the time the liquid was consumed, a physician assistant took a blood glucose measurement and repeated this every 30 minutes for two hours.

Minutes after drinking milk:	Blood glucose (mg/dL)				
	0	30	60	90	120
Björn	117	128	146	160	152
Chris	97	111	135	154	143
Xiao-Ma	96	99	105	101	98
Esiankiki	108	116	129	141	139
Linda	94	109	128	143	140
Sanjeet	97	96	94	83	88

Questions

6. Plot the results from the five individuals in the graph below. Make sure to include a legend for your graph. The physician assistant has already plotted one individual's data.



7. Can you divide the group of individuals into two groups based on this data? If so, explain according to which observation you would group individuals and who would belong into each group:

   A: _____

   B: _____

8. According to this data and your grouping, who of the groups are probably lactase persistent individuals?

9. Does the data from the blood glucose measurement match what you had heard from the individuals during their conversation on being able to eat ice-cream?

10. How do you think the graph would look like if Sanjeet had taken the test as a baby?

11. A person taking this test is usually asked to fast prior to taking the test. Why do you think that is?

Part III – Regulation of Lactase Gene Expression in Eukaryotes

III.i – Introduction to Gene Regulation and SNPs

Some students are lactose intolerant whereas others are not. This is due to the expression of an enzyme that is required to digest the discaccharide lactose.

Chris: I'm just blown away. I never thought that people could be different on that level. How come we're all generally healthy humans but some of us have a certain enzyme and others don't?

Xiao-Ma: Now I can tell you something I learned in my biology class. Good thing I was just studying that for my finals. Here's an excerpt from my lecture notes:

- Every cell in an organism has the same genetic material and each human has an almost identical genome.
- Individuals differ from each other in regions that are variants of each other.
- The most common genetic variant between individuals are single nucleotide polymorphisms (SNPs), which is a genetic difference of the DNA sequence in just a single base. There are millions of SNPs distinguishing individuals from each other.

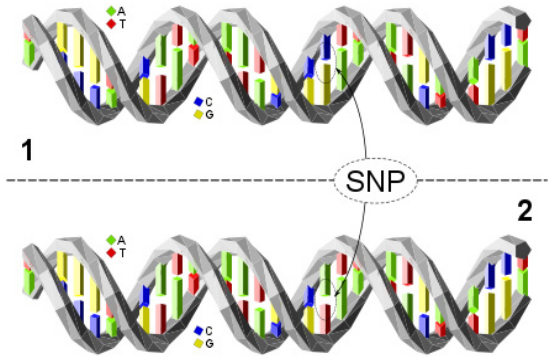


Figure 3. SNP.

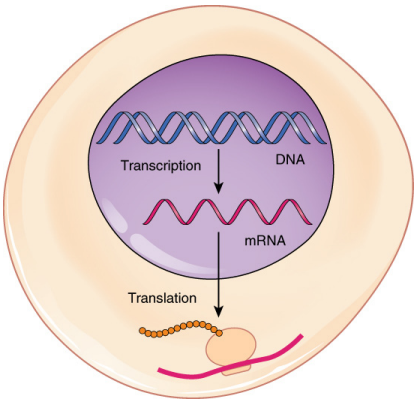


Figure 4. Transcription-translation summary.

-Eukaryotic cells are different from each other by only expressing (=producing, synthesizing, making) proteins from certain genes of the genome. The expression of eukaryotic genes can be regulated at any step along the pathway from DNA to protein .

Questions

1. In which ways can the individual steps of this process be regulated to lead to higher or lower expression of a particular protein? Formulate hypotheses using terms such as: *RNA, protein, stability, splicing, transport, translation,* and *efficiency*.

2. How could SNPs contribute to gene regulation? Formulate hypotheses.

III.ii – Eukaryotic Gene Regulation

Esiankiki: My mom told me that in the case of lactase persistence, it turns out that the persistence is caused by continued expression of the lactase RNA.

Xiao-Ma: That's so interesting! Here are some more notes from my biology class. I wonder how the lactase RNA can be expressed in some adults but not in others?

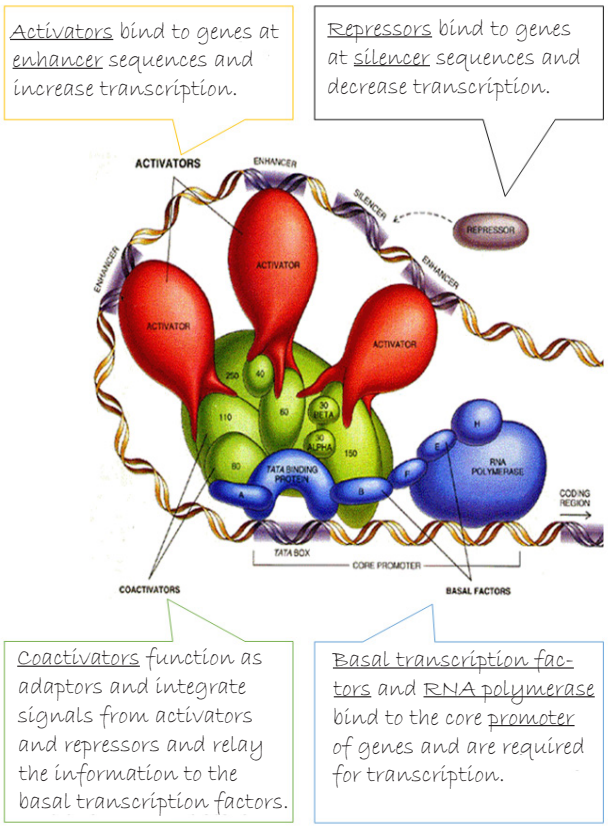


Figure 5. Transcription in eukaryotes.

- The expression of RNA in eukaryotes is initiated by the binding of basal transcription factors to the promoter sequence of a gene. This leads to the recruitment of RNA polymerase to synthesize an RNA transcript of the corresponding gene.
- RNA transcription can be increased or decreased additionally by binding of activator or repressor proteins to enhancer / silencer sequences which can be located far away from the promoter of the gene they are regulating.
- Transcription factors, activators and repressors bind DNA in a sequence specific fashion. This is achieved by chemical interactions (mostly hydrogen bonds) between the amino acids of the protein and the bases of the DNA molecule.

Questions

3. Looking at the diagram of transcription in eukaryotes and using the information provided, how could the expression of lactase RNA be turned on and off by transcription factors, activators and repressors?

4. Would the relative position where a transcription factor or an activator/repressor binds DNA be the same across different individuals? Explain why or why not.

Part III.iii – Regulation of the LCT Gene by Oct1

Linda: I went online and found a paper that had this figure; check it out.

Chris: Let me see that. Wow, now I'm really confused.

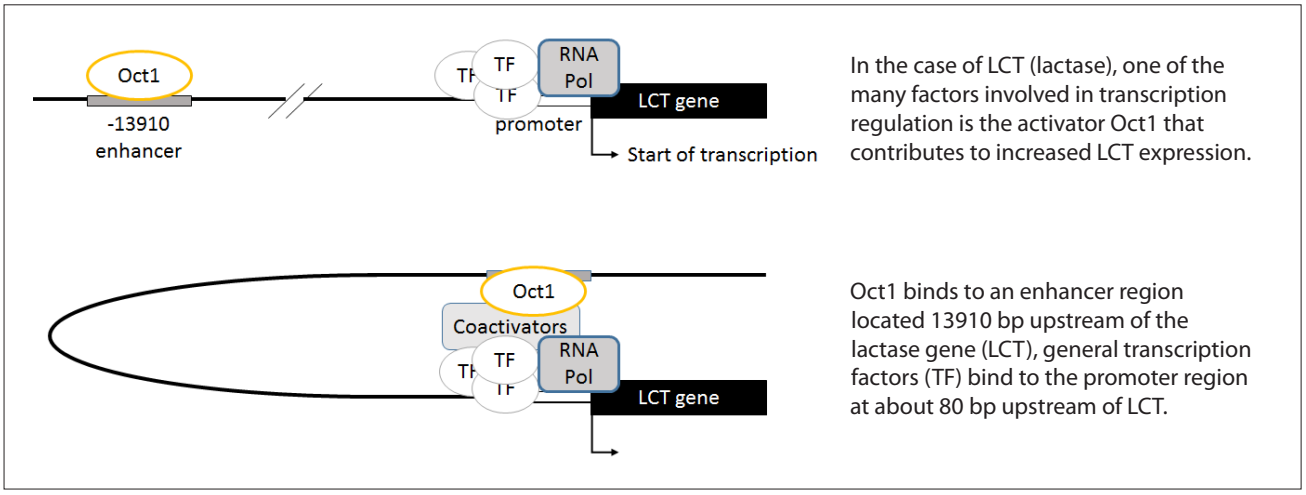


Figure 6. Regulation of the LCT gene by Oct1. Image modified from: Schultheis, P.J. and B.V. Bowlling, 2011, Analysis of a SNP Linked to Lactase Persistence, *BAMBED* 39(2): 133–140.

Questions

5. How does Oct1 recognize the position on the DNA molecule where it should bind?

6. Figure 7 is an illustration of another DNA binding protein. How can the protein recognize specific DNA sequences? Do the bases of the DNA form specific interactions with the protein? Describe how.

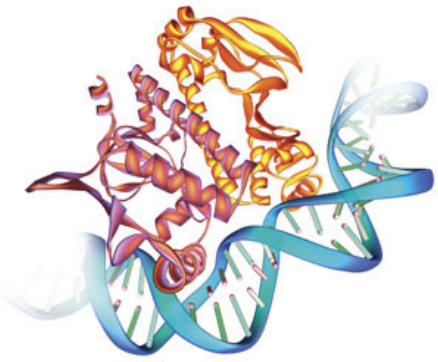


Figure 7. Lactase transcription factor.

7. How could differences between individuals such as SNPs lead to the persistent expression of lactase in some of your friends but not others?

Part III.iv – Influence of SNPs on LCT Expression

Esiankiki: So you just told me about SNPs and now about transcription, but how do they fit together? Can SNPs lead to different levels of lactase in each of us?

Björn: You know, I've been wanting to visit my TA for some questions anyway. I'll ask her what she thinks about this.

In the office of Amy, the TA:

Amy: There are actually a lot of SNPs that are linked to lactase persistence. I'm studying this for my thesis. Look, I was just reading this paper—I think this figure might be helpful for you:

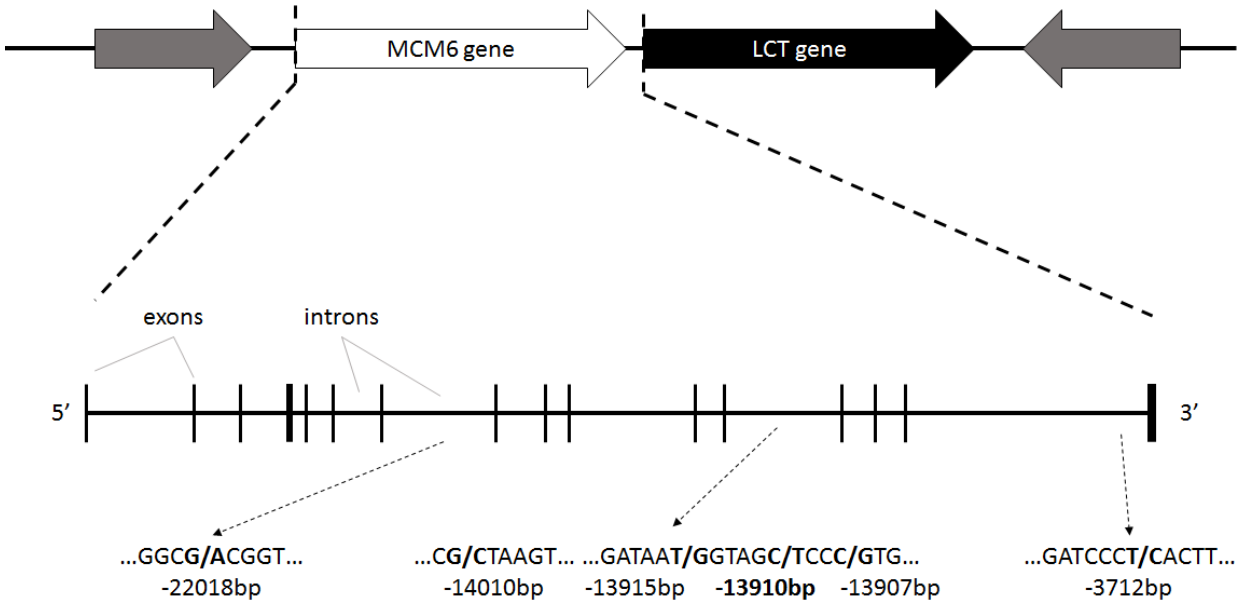


Figure 8. SNPs associated with lactase persistence. *Top panel:* Map of the region of human chromosome 2 containing the human lactase (LCT) and minichromosome maintenance -6 (MCM6) genes. *Middle panel:* Intron-exon organization of the MCM6 gene. *Bottom panel:* SNPs associated with lactase persistence in Europeans and certain African and Saudi pastoralist populations. The location of each SNP relative to the initiation codon of the lactase gene is given in base pairs (bp). Image modified from: Schultheis, P.J. and B.V. Bowlling, 2011, Analysis of a SNP Linked to Lactase Persistence, *BAMBED* 39(2): 133–140.

Björn: Wait a minute; some of the SNPs that are associated with lactase persistence are so far away from the lactase (LCT) gene! And they can even be in the coding region of neighboring genes like MCM6. How can that be?

Amy: Those are most likely enhancer regions that can regulate lactase gene expression from far away by looping to the promoter region.

With his notes from the meeting with the TA, Björn goes back to the dorm and shares what he found out with his group of friends.

Linda: This is just getting more and more interesting. Do you think we could find out what variant we each have and if that correlates to our lactase persistence or not?

Björn: Amy actually offered to do that for us. She is sequencing samples for her thesis and is looking for more volunteers for her study. Let's go and let her sequence our DNA.

Here are the results Amy emailed back to the group. She forgot to include what the results mean.

Individual	Phenotype	Genotype at the enhancer of the Lactase gene (position 13923 – 13902 upstream of the gene)
Björn	Lactose tolerant	Copy 1: TAAGATAATGTAGTCCCTGG
		Copy 2: TAAGATAATGTAGTCCCTGG
Chris	Lactose tolerant	Copy 1: TAAGATAATGTAGTCCCTGG
		Copy 2: TAAGATAATGTAGTCCCTGG
Esiankiki	Lactose tolerant	Copy 1: TAAGATAATGTAGTCCCTGG
		Copy 2: TAAGATAATGTAGCCCCTGG
Xiao-Ma	Lactose intolerant	Copy 1: TAAGATAATGTAGCCCCTGG
		Copy 2: TAAGATAATGTAGCCCCTGG
Linda	Lactose tolerant	Copy 1: TAAGATAATGTAGTCCCTGG
		Copy 2: TAAGATAATGTAGCCCCTGG
Sanjeet	Lactose intolerant	Copy 1: TAAGATAATGTAGCCCCTGG
		Copy 2: TAAGATAATGTAGCCCCTGG

Questions

8. How could a SNP in the -13910 region lead to persistent lactase expression? Formulate a hypothesis and discuss with your team.

9. Looking at the data above, can you correlate a genetic difference between the individuals with tolerance or intolerance to lactose?

10. Does your finding support or disprove the hypothesis that the SNP at 13910bp from LCT is correlated with lactase persistence?

11. Do your findings rule out that other SNPs or other regulatory pathways are involved? Explain.

Part IV – Tracing the Origin of the Lactase Persistence Trait

Sanjeet: I wonder what caused the evolution of something like lactase persistence in humans?

Xiao-Ma: Well, I think that's pretty obvious, right?

Chris: Really? It's not to me. You have to explain that to me.

Xiao-Ma: Well, in my biology class we learned that after a new trait arises from a genetic mutation, it may become prevalent in a population if it is advantageous for individuals to have it.

Chris: That's still over my head.

Xiao-Ma: Well, if it's better for the survival of the individual and the species, then whatever trait gave them the advantage will become more prevalent in the population because those individuals reproduce more successfully.

Figure 9. Cow milking, Egyptioan funerary model (1990-1786 BCE). Photo by Rama, Wikimedia Commons, CC-BY-SA-2.0-FR.

Sanjeet: Wait a minute; are you saying I won't reproduce successfully because I'm lactose intolerant?

Xiao-Ma: Now you're being silly. Let's think about this from more of an evolutionary and historical standpoint.

Questions

1. What role do mutations play in the evolution of new traits such as lactase persistence?

2. What role does the environment play in the evolution of new traits such as lactase persistence?

3. How can a mutation become more prevalent in certain populations?

4. If mutations are random and equally likely in each of these populations, why are there no known mutations in the lactase gene itself in lactose intolerant populations?

5. Why do you think that some populations developed lactase persistence? Using these terms, draw a map of how they might connect to each other. Terms: *environmental factors, diet, dairy farming, changes in DNA, phenotype, genotype, traits, mutation.*

6. *Optional:* How would you calculate the percentage of individuals with a particular trait in a population knowing certain parameters?