1. A stomach cell line growing in nutrient broth has a mutation in the gene encoding a particular type of tRNA. Assuming the mutation is not lethal to the cells, explain why the amount of SIRT3 mRNA in this cell line is identical to the amount produced by a normal stomach (NS) cell line but the amount of SIRT3 protein is less than in the NS cells.

   (a) There could be many reasons, however only two are likely. One, the stomach cell in the nutrient broth has just a point-shift or deletion mutation. Two, the stomach cell is cancerous, however are ineffective due to a reason unknown. In either case, the amount of SIRT3 produced will always be different, likely less than a functional cancerous cell.
   0/1 - Looking for an explanation that tRNAs are involved in translation, not transcription.

2. Identify a control group for the analysis shown in Figure 3. Justify analyzing SIRT3 protein level in four different cancer cell lines, as shown in Figure 1. Based on Figures 1 and 3, describe the relationship between SIRT3 expression and cytoplasmic ATP levels. Calculate the percent change in cytoplasmic ATP levels by SC+RNA cells compared with SC+plasmid cells.

   (a) The control group presented in Figure 3 would be the NS, or Normal Stomach cells. In Figure 1, they show 5 bars, one for the control group, NS, and 4 for the different cancer patient levels. The justification for having 4 different bars of levels lies in the bias of only having one average. Having just one average bar, let alone just one patient, would present false and misleading data. There seems to be a heavy correlation between having stomach cancer cells, SIRT3 protein levels, and high cytoplasmic ATP levels. As seen in Figure 1, the patients with the stomach cancer cells presented higher than normal SIRT3 protein levels. And then in Figure 3, the stomach cells present higher than normal cytoplasmic ATP levels. This indicates a fairly strong correlation between the SIRT3 protein and cytoplasmic ATP levels.
   3/4 - Response included an acceptable control group, a justification for more than one cell line (small sample size). Explained the coorelation between SIRT3 protein levels and cytoplasmic ATP levels. Did not, however, explain the percent change in cytoplasmic ATP levels by SC+RNA cells compared with SC+plasmid cells. The answer is -59%.

3. Researchers analyzing the SIRT3 gene sequence engineer a SIRT3 gene that is identical to the normal gene except that a single nucleotide has been deleted from the 5‘ end of the second exon of the gene; the deletion does not affect splicing. The researchers introduce this gene into normal stomach cells to determine how the primary AND tertiary structures of the protein expressed from the engineered SIRT3 gene will compare with the protein expressed from the normal SIRT3 gene in these cells. Predict the results of this experiment. The researchers claim that the data in Figure 3 provide evidence that the stomach cancer cells perform fermentation to a greater extent than do normal stomach cells. Provide reasoning to justify the claim. Based on the information provided, predict the effect on the ratio of NADH/NAD+ in the cells treated with the compound that blocks the electron transport chain compared with similar cells not treated with the compound.

   (a) The results from the experiment are likely going to show the structures differing. This is due to the fact that a nucleotide was deleted, not replaced. And while that doesn't affect splicing, it will affect how the protein folds both its primary and tertiary structures. The researchers also claimed that Figure 3 provides data for cancer cells performing fermentation to a greater extent than normal cells. This is true, as in both the SC-1 and SC+Plasmid bars, we see an increase in lactic acid production — the byproduct of fermentation.
   1/3 Did not explain NADH/NAD+ relation to fermentation, or detailed other stuff. Mostly the last part of the question.

4. Based on the data provided, explain how testing for SIRT3 gene expression in samples of stomach cells might be used by physicians to predict which individuals have an increased risk of developing stomach cancer. Human stomach cells have plasma membrane receptors for the binding of certain

extracellular signaling molecules such as growth factors. Explain why the irreversible binding of chemicals that mimic the growth factors will most likely change the normal cells into cancer cells.

(a) Testing for SIRT3 could be used to indicate an increased risk of stomach cancer via testing for the increased levels. Figure 1 supports this by showing that stomach cancer cells lead to increased levels of the SIRT3 protein. The physicians would likely get a blood, gastric, or bile sample, looking for increased SIRT3 protein levels. If presented, the patient would likely be recommended for a more thorough cancer screening. If a chemical that mimics a growth factor or some other type of ligand were to bind to the plasma-membrane receptor, the cells would likely become cancerous. This is thanks to the process being irreversible. In other words, the ligand will *never* detach from the receptor. This would in turn lead to 'cancerous' activity, such as never ending production, inefficiency, and/or inflated consumption of glucose.
2/2 - Explained how a higher level of SIRT3 proteins could lead to a reccomendation for stomach cancer, and how

Figure 1: 1

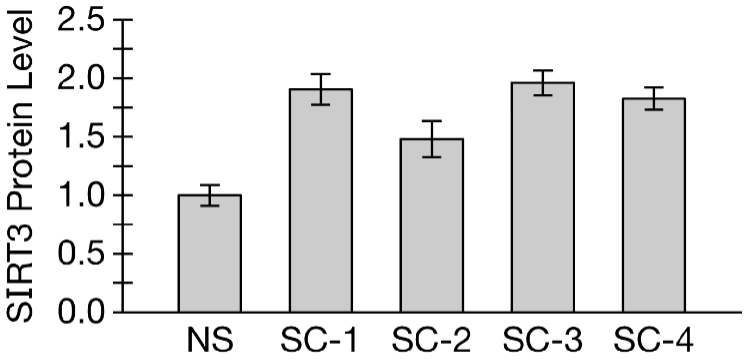

Figure 1. SIRT3 protein levels in normal stomach cells (NSNS) and stomach cancer cell lines from four different patients (SC-1SC-1, SC-2, SC-3, SC-4, shown relative to the level in normal stomach cells (NS). Error bars represent ±SEx¯.

Figure 2: 2

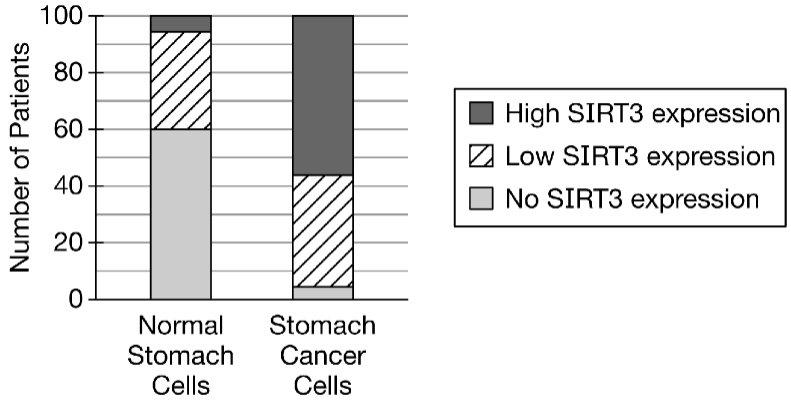

Figure 2. The number of patients, from 100 tested, whose stomach cancer cells or normal stomach cells adjacent to the cancer cells express high, low, or no SIRT3 protein.

Figure 3: 3

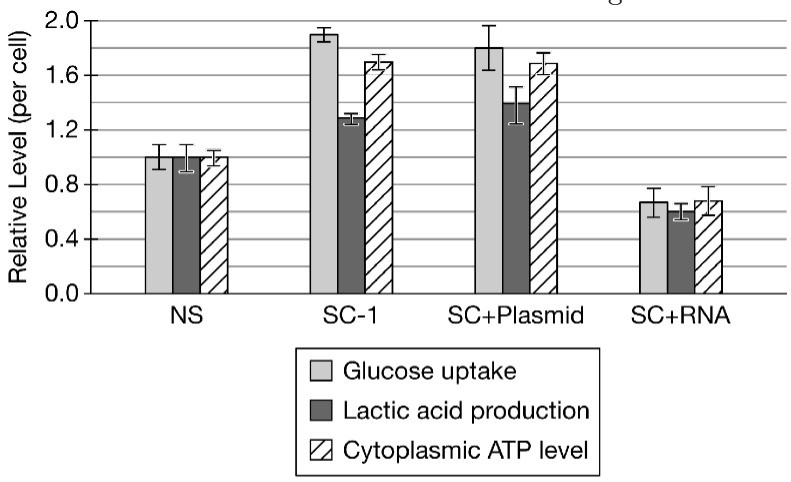

Figure 3. Glucose uptake, lactic acid production, and cytoplasmic ATP levels in stomach cells. NS, normal stomach cells; SC-1, stomach cancer cells; SC+ plasmid, SC-1 cells with added plasmid only; SC+RNA, SC-1 cells with added plasmid encoding small RNA complementary to a portion of SIRT3. Error bars represent ±SEx¯.