16 january 2012

Exam for Masters II in Systems and Synthetic Biology

The 10 questions in this exam are based on Marlière et al, 2011 “Chemical Evolution of a Bacterium's Genome” *Angew Chem Int Ed Engl* PMID: 21710668. Each question is worth 10 pts.

1. This paper reports the evolution of *E. coli* to use 5-chlorouracil in place of thymine. DNA consists of 4 nucleobases: adenine, cytosine, guanine, and thymine. Among these 4 bases, why did they decide thymine was the best candidate for replacement?

7.5 pts: Thymine is the **only nucleobase specific to DNA** (ie not present in RNA). Thus, replacement of thymidine would not affect RNA-based processes.

2.5 pts: Thymine synthesis can be **disrupted by mutation of a single gene**, *thyA*.

2. This study used *E. coli* strain THY1 for 5-chlorouracil transliteration. How is strain THY1 different from wild-type *E. coli* K12? How did these genetic changes help with the replacement of thymine with 5-chlorouracil?

5pts to include all 4 genetic changes:
1. Deletion of ***thyA*** *(*thymidylate synthase)
dUMP + 5,10-methylenetetrahydrofolate <=> dTMP + 7,8-dihydrofolate monoglutamate.

2. Deletion of ***udp*** (uridine phosphorylase)
phosphate + uridine <=> alpha-D-ribose-1-phosphate + uracil

3. Deletion of ***deoCABD***:
deoA (thymidine phosphorylase)
thymidine + phosphate <=> deoxyribose-1-phosphate + thymine

4. Insertion of a plasmid-born copy of ***ntd*** gene from *Lactobacillus leichmannii*
thymine (or 5-chlorouracil) + deoxyuridine <=> thymidine (or 5-chlorodeoxyuridine). Addition of *ntd* allows growth in the absence of *udp* and *deoCABD*.

5 pts to describe that these mutations make THY1 require exogenous thymine.
Disruption of *thyA* prevents the synthesis of thymine nucleotides. Deletion of *udp* and *deoCABD* prevents the salvage pathway to make dTMP from thymine. THY1 thus can grow only if given **exogenous thymine for growth**.

3. The selection of 5-chlorouracil containing mutants was accomplished using a continuous culture device containing two identical growth chambers connected with tubing to allow transfer between chambers. Why was this culture device constructed with these two identical chambers?

Cells were transferred to the second chamber to allow **sterilization (**5 pts**)** of the first chamber with NaOH. Cleaning of the chamber **prevented biofilm formation** (5 pts).

4. THY1 was adapted to growth on 5-chlorouracil to produce strains CLU2 and CLU4. These two strains were then re-adapted to growth on thymine to make strains THY2 and THY4. What did the observation that THY2 and THY4 could still grow on 5-chlorouracil without re-adaption show?

THY2 and THY4 could still grow on 5-chlorouracil without adaptation, showing that the ability to grow on 5-chlorouracil was **heritable and encoded in the genome** (10 pts) of these strains.

5. A previous study adapted a hamster cell line to bromo-deoxyuridine, but did not measure the amount of incorporation of this alternative base into the DNA. What two methods did Marlière et al use to directly measure the amount of incorporation of 5-chlorouracil into DNA?

First, the composition of hydrolyzed DNA in THY1, CLU2, and CLU4 was measured by **HPLC fractionation** (5 pts).

Second, **mass spectrometry** was used to confirm the presence of chloro-2'-deoxyuridine in the DNA of strains CLU2, CLU4, and CLU5 (5 pts).

6. Both cultures of THY1 adapted to 5-chlorouracil in the same amount of time (Fig 3A vs Fig 3B). How did these two cultures differ? What does this difference suggest about how the adaptation process occurred?

The two cultures differ in terms of their **generation times** (7.5 pts); the culture in Fig3A was growing twice as fast as in Fig3B. Both cultures adapted in the same amount of time. Thus, **adaptation occurred independent of the number of generations** (2.5 pts).

7. After adaptation to 5-chlorouracil, strains CLU2 and CLU4 still contained about 10% thymine. What could account for this 10% residual thymine in strains CLU2 and CLU4?

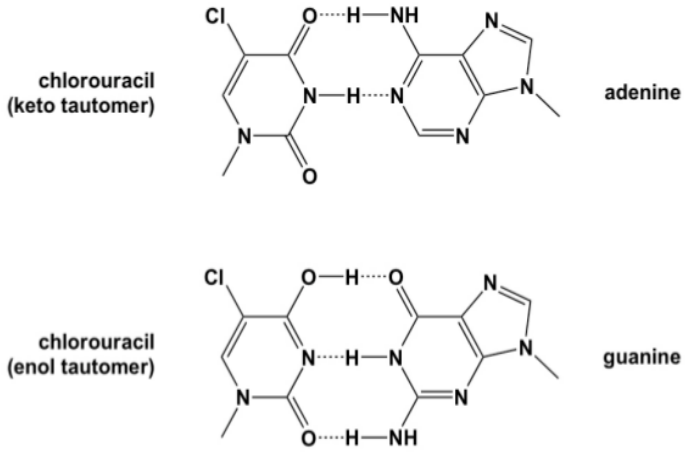
The remaining thymidine might come from from **recycling pathways**. For example, position 54 of tRNAs is a uridine that is posttranscriptionally converted to ribothymidine, which could

provide a metabolic source of thymine (10 pts to mention recycling of thymidine or give a specific example).

8. What additional mutation did they make to produce strain CLU5, which had only 1.5% thymidine in the DNA?

They deleted ***trmA*** (10 pts)**,** which encodes the SAM-dependent U54 tRNA methyltransferase.

9. They sequenced the genomes of THY1, THY2, and THY4. The most common mutation in THY2 was 1023 A:T to G:C transitions. How might this diagram explain why this type of mutation was so common?



The authors propose that in addition to pairing with adenine (top), **5-chlorouracil forms an anionic enol tautomer that mispairs with guanine** (bottom). This mispairing resulted in the numerous transitions observed in THY2. (10 pts).

10. What technical or environmental applications could a chemically modified organism like 5-chlorouracil-dependent strains have?

A strain with a chemically-modified genetic code **would not exchange DNA with natural strains**, which would prevent propagation of engineered DNA in the environment. Also, a strain that uses an alternative genetic code might be resistant to viruses, making it **economically advantageous in industrial settings**. Other logical answers also acceptable (10 pts).