Principles of Modern Biology SCI 1210 Fall 2012
Prof. Jean Huang
Dr. Sadie Aznavoorian-Cheshire

PTC Phenotype and Genotype:
Pt. 1: Taste testing and Collection/Isolation of Human Genomic DNA

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Overview:

In Part 1, each student will do a taste test of paper strips coated with phenylthiocarbamide (PTC), the related chemical thiourea, and benzoate; some of you will be able to taste these chemicals, and others will not. You will also carry out genomic DNA isolation from your own buccal cells collected today (see preliminary protocol). This DNA will be the template for a PCR reaction (11/7/12) in which you will amplify a specific gene involved in the tasting of PTC (and possibly the other chemicals). Finally (11/14/12), the amplified PCR products will be purified and sent out for sequencing.

Our hypothesis is that those who can taste PTC will have an 'active' version of this gene, and those who cannot will have a mutated- and inactive-version. However, questions still remain so we are actually contributing to on-going studies.

PTC Overview

Introduction

More than 70 years ago a chemist working at DuPont made a startling accidental discovery concerning a chemical called phenylthiocarbamide[1] or PTC.

"Dr. A. L. Fox had occasion to prepare a quantity of phenyl-thio-carbamide… as he was placing this compound in a bottle some of it was dispersed into the air as dust. Thereupon another occupant of the laboratory complained of the bitter taste of the dust. This surprised Fox, who being much closer to the scene of operations had of course inhaled more of the dust, but had perceived no taste. He was so positive that the stuff was tasteless that he went so far as to taste some of the crystals directly, finding them as tasteless as chalk. Nevertheless the other chemist was convinced the substance was bitter and was confirmed in this impression when he in turn tasted the crystals and found them to be intensely bitter. Naturally a lively argument arose. In an attempt to settle it, the two chemists called in various other laboratory workers, friends and other people with whom they could establish contact. Some people declared the substance was tasteless and some again found it bitter."

As cited in Annals of Human Biology (2001) 28:111-142

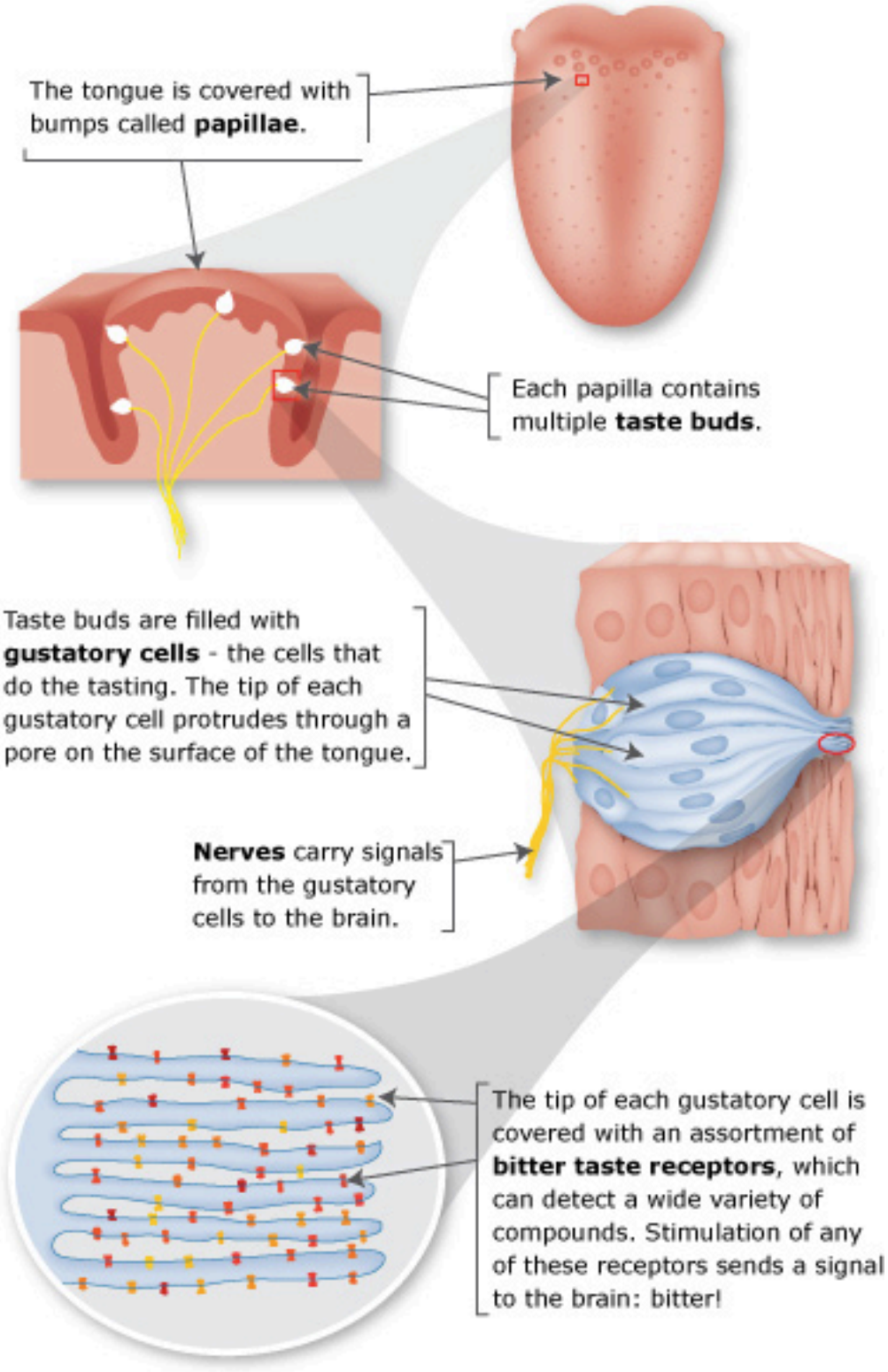
In the years since this discovery, the ability to taste PTC has become one of the most studied traits in human genetics, second only to the ABO blood group

[1] Phenylthiocarbamide is also known as phenylthiourea.

system. In almost all populations throughout the world there exist both tasters and non-tasters. In America it is estimated that about 30% of the population are PTC non-tasters, while 70% are tasters of PTC. In 2002, scientist reported the identification of a gene (on chromosome 7) that determines the ability to taste the compound PTC.

The PTC gene encodes a protein that is part of a bitter taste receptor complex found in the taste buds on the tongue (see illustration on page 3). Everyone has two copies of the PTC gene, but there are three single nucleotide changes (polymorphisms, or 'SNPs') that are present in some versions of the gene. These different versions produce slightly different proteins. It turns out that throughout the world there are five different alleles, and one type confers a severe deficit in the ability to taste the bitterness of PTC (Kim et al., 2003).

We will be devoting several lab sessions to the analysis of your PTC gene alleles, and their correlation with your tasting phenotypes (and food preferences). Once the sequence results are in, we will spend one additional lab period analyzing your sequencing data and observing the correlation between your tasting phenotype and your PTC haplotype.



http://learn.genetics.utah.edu/content/begin/traits/ptc/

(**Laboratory adapted from Princeton Univ. MOL 101 – Fall 2004)**

Protocol: Determination of PTC (and related chemical) tasting phenotype

Phenotype determination:

In addition to PTC, there will be 2 other related chemicals to taste-test: thiourea (also known as thiocarbamide) and sodium benzoate (widely used as a food preservative). These are each embedded onto color-coded test paper strips, in matchbox-like packets. There is also a packet of "control" paper strips, bringing the total to 4 (green tape has been placed over the identity of the chemical on each matchbox, so that the taste-test will be blind initially).

1. **Wearing gloves remove a test paper from one of the matchbox packets (it doesn't matter which one you begin with).**

2. **Place the test paper on your tongue. Close your mouth on the paper and get a good impression of the taste.**

3. **Remove and discard the paper, then record the taste you perceived using the following categories:**
   No taste
   Bitter
   Salty
   Soapy
   Sweet
   Nauseating

4. **Take a drink of water to clear any taste in your mouth.**

5. **Wearing gloves, repeat this procedure for each of the 4 test papers, recording your results in your book for each colored strip. It would be best to record your impressions immediately after tasting each paper, when your memory is freshest. (The color-code will be broken by the end of this lab). In addition to recording the type of taste you perceived (if any), please also note the relatively intensity of the taste, or any additional adjectives you can think of to describe it. i.e.,**

Jacqueline, a PTC taster in an early study of PTC sensitivity (around 1939). From S. Wooding, 2006 (see references).

Make a good attempt to read the research paper (to be posted soon) that describes the characterization of the PTC gene (Kim et al., 2003). The paper is quite technical but you should be able to decipher the main points. Also, the additional papers should be at least skimmed through to get the main points. We will discuss the biochemistry of the PTC protein (and its variants) in subsequent labs.

Genomic DNA isolation

Overview of DNA isolation:

In this laboratory each student will isolate his/her own genomic DNA sample from cells gathered from inside the mouth, using a sterile swab (Whatman "Omniswab"). Cells gathered from this area are termed buccal cells. We will use a commercial kit produced by Qiagen, Inc. ("QIAamp DNA Mini Kit, catalog # 51304), which allows a quick preparation to be made that is sufficiently pure for our purposes. This kit employs silica-gel membrane technology in the form of a spin column to isolate genomic DNA from small quantities of human cells/tissues. In brief, the cells are collected on the swab, then washed into a tube with PBS. Cell membranes are ruptured (with SDS-containing lysis buffer, 'AL'), cellular proteins are digested (with 'Proteinase K'), and the DNA-containing lysate is bound to the silica column by centrifugation. Under these conditions, DNA is selectively bound to the silica membrane filter while the non-nucleic acid cell debris is washed through (with wash buffers 'AW1' and 'AW2'). Finally, pure DNA is released (eluted) from the column into a salt-free elution buffer ('AE'). DNA up to 50 kb in length (predominantly 20-30 kb) and suitable for direct use for polymerase chain reactions (PCR) is isolated. The yield of DNA is typically 0.5-3.5 µg for one buccal swab.

DNA binds in a reversible manner to silica in the presence of high concentrations of chaotropic ("chaos-causing") salts, which are a component of the lysis buffer "AL". While the mechanism of binding is not entirely understood, it is postulated that dehydration of the DNA phosphodiester backbone (and the silica surface) under these conditions allows the exposed phosphate residues to adsorb to the silica, possibly mediated by positively charged Na+ ions forming a salt bridge between the negatively charged silica and the negatively charged DNA backbone. This interaction is stable under wash conditions that remove other biopolymers (RNA, carbohydrates, etc.). Upon rehydration of the DNA with aqueous, low-salt (or salt-free) buffers, such as Tris-EDTA (designated "AE" by Qiagen) or dH_2O, the purified 'ready-to-use' DNA elutes from the silica matrix into a clean tube.

Protocol: Isolation of Human genomic DNA

Note: Be sure to wear gloves for this procedure. Some of the chemicals used in this procedure are irritants, so if you choose not to wear safety goggles, take appropriate precautions and always work carefully! Change gloves as needed (Biohazard waste containers, w/red bags). For each student sample:

1. **Add 600 µl Phosphate Buffered Saline (PBS) to the dried buccal swab sample in the 2 ml tube.**

2. **Add 20 µl of Proteinase K solution directly into the PBS in your sample tube, followed by 600 µl of Buffer AL (lysis buffer). Mix immediately by vortexing at high speed for 15 sec. In order to ensure efficient lysis, it is essential that the sample and Buffer AL are mixed immediately and thoroughly. *DO NOT ADD PROTEINASE K DIRECTLY TO BUFFER AL. (Its enzymatic activity would be destroyed.)**

3. **Incubate at 56^{o}C for 10 min (water bath). After incubation, briefly microfuge the tube containing the reaction mixture to remove drops from inside the lid.**

4. **Add 600 µl 96-100% ethanol ("ETOH") to the sample, and mix again by vortexing. Briefly centrifuge the microfuge tube containing the reaction mixture to remove drops from inside the lid.**

Remove your QIAamp Spin Column from its package and label the top with your initials. The spin column is packaged inside of a 2 ml collection tube; get 4 additional collection tubes and have them handy. At this point you have a volume of sample that is too large for the spin column, so the sample must be loaded onto the column in two steps:

5. **Carefully apply 700 µl of the mixture from step 4 to the spin column without wetting the rim, and without poking the silica membrane with your pipette tip; close the cap, and centrifuge at 8000 rpm for 1 min. Transfer the QIAamp Spin Column into a clean 2 ml collection tube, and discard the tube containing the filtrate (i.e. the one that you just centrifuged the 700 µl into).**

Note: Close each spin column in order to avoid aerosol formation during centrifugation. Use the microfuge rotor lids.

6. **Repeat step 5 by applying up to 700 µl of the remaining mixture to the spin column. Spin again as above, then transfer spin column to**

a new 2 ml collection tube, and discard the tube containing the filtrate.

7. **Open the QIAamp Spin Column and add 500 µl Buffer AW1 (wash buffer #1) without wetting the rim. Close the cap and centrifuge 8000 rpm for 1 min. Place the QIAamp Spin Column into a clean 2 ml collection tube and discard the collection tube containing the filtrate.**

8. **Open the QIAamp Spin Column and add 500 µl Buffer AW2 (wash buffer #2) without wetting the rim. Close the cap and centrifuge at full speed (~13,000 rpm) for 3 min. Place the QIAamp Spin Column into a clean 2 ml collection tube and discard the collection tube containing the filtrate.**

9. **Centrifuge the "empty" spin column at 14,000 rpm for 1 min. (This additional centrifugation step removes any residual wash buffer AW2 from the silica membrane, which would have contaminated the DNA and interfered with subsequent PCR reactions.) You may see a tiny amount of liquid at the bottom of the collection tube after this step.**

10. **Place the QIAamp Spin Column into a clean, labeled eppendorf tube (labeled with your initials and date), and discard the collection tube containing the filtrate. (The eppendorf tube will have to remain uncapped for the duration of the microfuge spin.)**

11. **Open the QIAamp Spin Column and add 150 µl of Elution Buffer 'AE' (10 mM Tris-Cl; 0.5 mM EDTA, pH 9.0) directly to the center of the silica membrane. Incubate at room temperature for at least 1 min., and then centrifuge at 8000 rpm for 1 min. After the spin has ended, discard the spin column, and SAVE the labeled eppendorf tube with your eluted DNA.**

The DNA samples in buffer AE will be used for the next step of the project (although there will be plenty of DNA left over). In the meantime, they will be stored at -20^{o}C.

For more information, go to Qiagen's website (Qiagen.com)

There are a number of publications that can be found by navigating the "Support" tab. For a PDF file of the handbook that comes with this kit, go to Support: Handbooks and Protocols: Genomic DNA Stabilization and Purification: From Clinical Samples: QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit
These additional 2 should also be very helpful:

Support: Other Qiagen Publications: Brochures and Applications Guides: Nucleic Acid Purification: Genomic DNA Purification

Support: Technical Guidelines: Qiagen Purification Technologies: “Anion-exchange, silica-gel-membrane, and magnetic particle technologies”