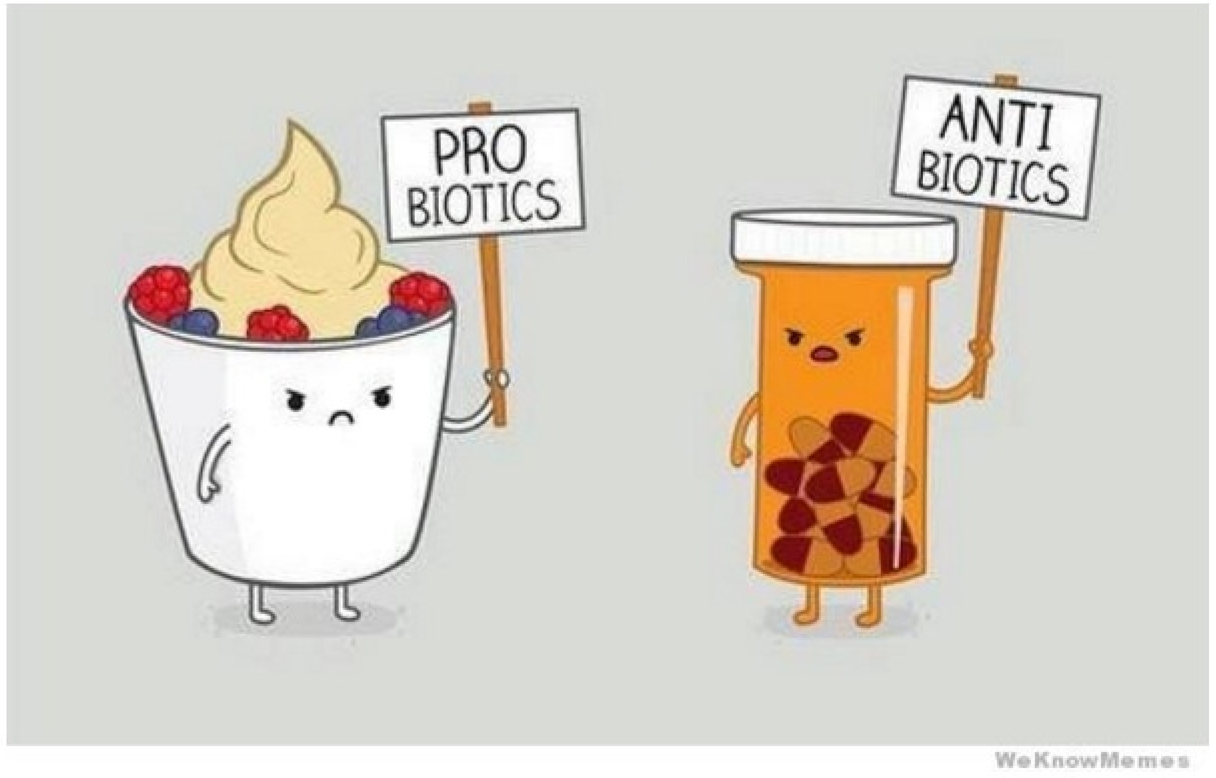
Protein Sequences and Antibotics

- Alexander Fleming discovered Antibotics in 1928
- Small peptide sequences that target and kill bacteria
- Generated naturally by fungi and other bacteria
- Today's questions; Where do they come from? How can one infer their sequence?

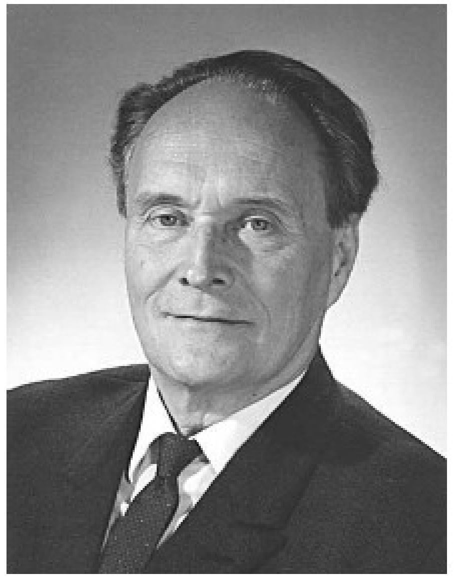


Discovery of Penicillin (1928)

- Upon returning from holiday in 1928 a british biologist and pharmacolgist, Alexander Fleming, discovered a strange pattern of cell death in a stack of *staphylococci* cultures he had on a bench in a corner of his laboratory.
- Fleming noticed that one culture was contaminated with a fungus, and that the colonies of staphylococci immediately surrounding the fungus had been destroyed, whereas other staphylococci colonies farther away were normal, and famously remarked "That's funny".
- He attempted to isolate the agent that killed the bacteria, and even hypothesized that such an agent might be suitable for treating infections known to be caused by *staphylococci*. But, he had little luck.
- It proved hard to collect enough Penicillin to make it viable af a drug throughout the 1930s.
- Eventually, a mouldy cantalope found in Illinois in 1943, would provide a mold capable of generating high quantities of Penicillin and greatly reduce the World War II battlefield infection called *Sepsis*.
- The chemical structure of Penicillin was not determined until 1945 by Dorothy Hodgkin, another british X-ray crystallographer like Rosalind Franklin.

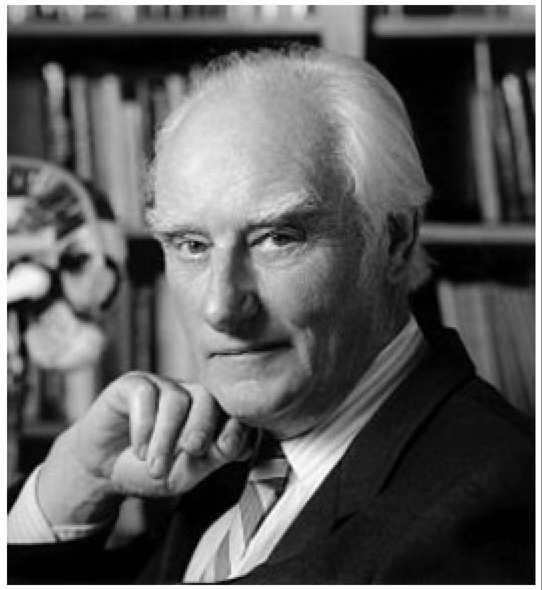
Other Antibotics

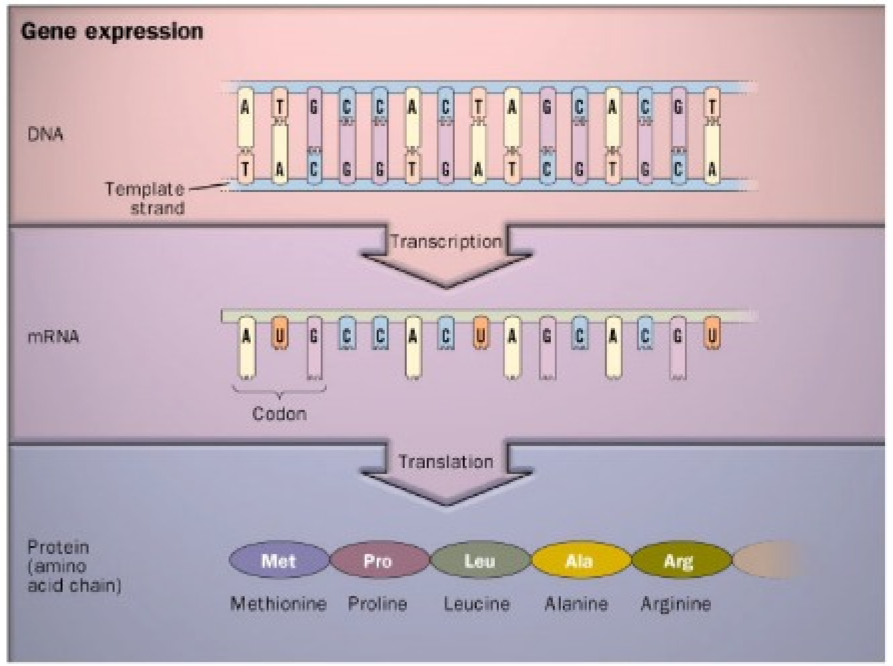
- Meanwhile, a second important antibotic, *Gramicidin S* or *Gramicidin Soviet*, was discovered by Russian microbiologist Georgyi Gause and his wife Maria Brazhnikova in 1942. This antibotic was discovered in another strain of bacteria, *Bacillus brevis*, which had the tendancy to kill *staphylococci* nearby it.
- It was actually easier to mass-produce than Penicillin, and it saved the lives of many russians in the second world war, as well as the lives of its inventors.
- *Gramicidin S* is largly composed of a *cyclodecapeptide*, a protein-like peptide chain composed of 10 amino-acids joined head-to-tail to form a ring, called *Tyrocidine B1*.
- The ***Central Dogma of Molecular Biology*** suggests that templates for such peptide chains are encoded in **DNA**.
- Our first task today is to see if we can find the genomic region where *Gramicidin S* is encoded.



Recall the Central Dogma from Lecture 1

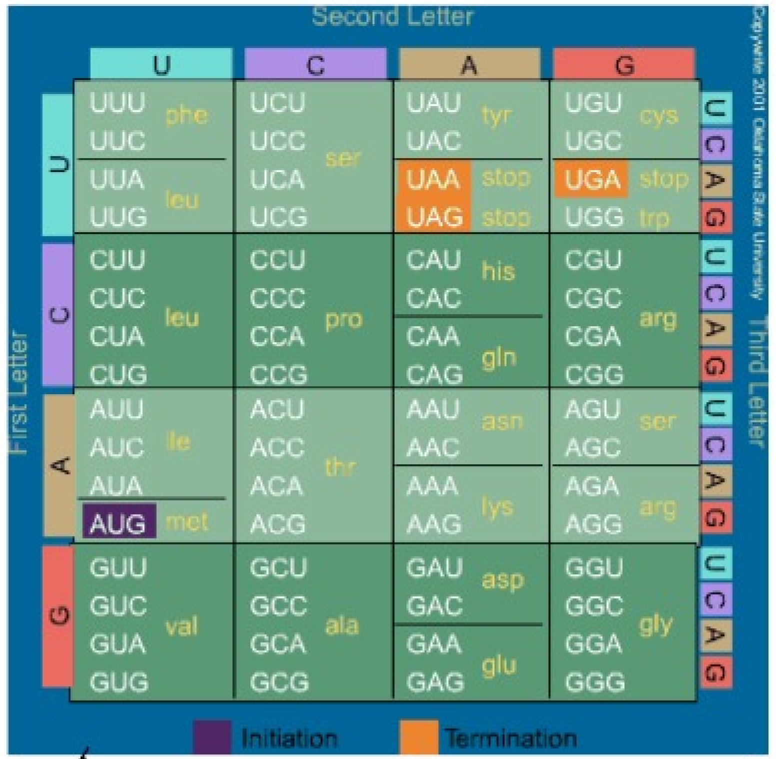
Francis Crick, one of the discoverers of DNA structure, originally coined the term "Central Dogma" in 1958. That choice of words was particularly provocative.


Francis Crick


Gene expression
DNA
A T G C C A C T A G C A C G T
T A C G G T G A T C G T G C A
Template strand
Transcription
mRNA
A U G C C A C U A G C A C G U
Codon
Translation
Protein (amino acid chain)
Met Pro Leu Ala Arg
Methionine Proline Leucine Alanine Arginine

DNA Transcription

- Triplets of nucleotide bases determine one of 20 amino acids in a protien's peptide sequence
- Special STOP codes halt the translation process
- However, the DNA sequence that we see may not be a perfect indicator of the DNA sequence that produced it for two reasons.
    1. Post-transcriptional modifications change the mRNA pattern. For example, in *eucaryotes* sections of code (Itrons) are removed from the transcribed sequence.
    2. Post-translational modifications modify the produced amino acid chain. For example when a protein if circularized
- So our goal is to find a partial pattern in the DNA which might have created *Gramicidin S*
- Recall the codon codings



Second Letter

First Letter	U	C	A	G	Third Letter
U	UUU phe	UCU	UAU tyr	UGU cys	U
	UUC	UCC ser	UAC	UGC	C
	UUA	UCA	UAA stop	UGA stop	A
	UUG leu	UCG	UAG stop	UGG trp	G
C	CUU	CCU	CAU his	CGU	U
	CUC leu	CCC pro	CAC	CGC arg	C
	CUA	CCA	CAA	CGA	A
	CUG	CCG	CAG gln	CGG	G
A	AUU	ACU	AAU asn	AGU ser	U
	AUC Ile	ACC thr	AAC	AGC	C
	AUA	ACA	AAA	AGA arg	A
	AUG met	ACG	AAG lys	AGG	G
G	GUU	GCU	GAU asp	GGU	U
	GUC val	GCC ala	GAC	GGC gly	C
	GUA	GCA	GAA	GGA	A
	GUG	GCG	GAG glu	GGG	G

🟪 Initiation 🟧 Termination

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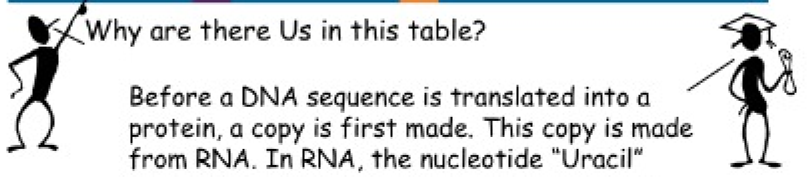
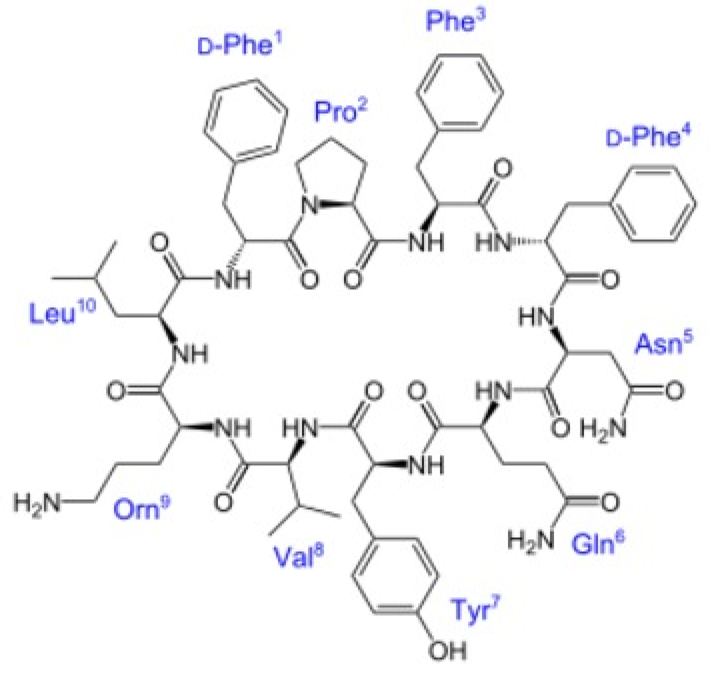


Why are there Us in this table?

Before a DNA sequence is translated into a protein, a copy is first made. This copy is made from RNA. In RNA, the nucleotide "Uracil" replaces "Thymine". Uracil and Thymine are both chemically and structurally very similar.

Tyrocidine B1's Peptide Sequence

Tyrocidine B1 is a small peptide composed of only 10 amino acids.



```
Amino Acid:     Valine      Leucine     Proline Phenylalanine Glutamine
3-letter Abbr:  Val-Lys-Leu-Phe-Pro-Trp-Phe-Asn-Gln-Tyr
1-letter Abbr:   V   K   L   F   P   W   F   N   Q   Y
Amino Acid:          Lysine  Phenylalanine Tryptophan Asparagine   Tyrosine
```

Also note that the Lysine is modified during the circularization into another non-proteinogenic amino acid, *Ornithine.*

A toolkit of Python Dictionarys

```
codon = {
    "AAA": 'K', "AAG": 'K', "AAC": 'N', "AAU": 'N',
    "AGA": 'R', "AGG": 'R', "AGC": 'S', "AGU": 'S',
    "ACA": 'T', "ACG": 'T', "ACC": 'T', "ACU": 'T',
    "AUA": 'I', "AUG": 'M', "AUC": 'I', "AUU": 'I',
    "GAA": 'E', "GAG": 'E', "GAC": 'D', "GAU": 'D',
    "GGA": 'G', "GGG": 'G', "GGC": 'G', "GGU": 'G',
    "GCA": 'A', "GCG": 'A', "GCC": 'A', "GCU": 'A',
    "GUA": 'V', "GUG": 'V', "GUC": 'V', "GUU": 'V',
    "CAA": 'Q', "CAG": 'Q', "CAC": 'H', "CAU": 'H',
    "CGA": 'R', "CGG": 'R', "CGC": 'R', "CGU": 'R',
    "CCA": 'P', "CCG": 'P', "CCC": 'P', "CCU": 'P',
    "CUA": 'L', "CUG": 'L', "CUC": 'L', "CUU": 'L',
    "UAA": '*', "UAG": '*', "UAC": 'Y', "UAU": 'Y',
    "UGA": '*', "UGG": 'W', "UGC": 'C', "UGU": 'C',
    "UCA": 'S', "UCG": 'S', "UCC": 'S', "UCU": 'S',
    "UUA": 'L', "UUG": 'L', "UUC": 'F', "UUU": 'F'
}

AminoAcid = {
    'A': 'Alanine', 'C': 'Cysteine', 'D': 'Aspartic acid', 'E': 'Glutamic acid', 'F': 'Phenylalanine',
    'G': 'Glycine', 'H': 'Histidine', 'I': 'Isoleucine', 'K': 'Lysine', 'L': 'Leucine', 'M': 'Methionine',
    'N': 'Asparagine', 'P': 'Proline', 'Q': 'Glutamine', 'R': 'Arginine', 'S': 'Serine',
    'T': 'Theronine', 'V': 'Valine', 'W': 'Tryptophan', 'Y': 'Tyrosine', '*': 'STOP'
}

AminoAbbrv = {
    'A': 'Ala', 'C': 'Cys', 'D': 'Asp', 'E': 'Glu', 'F': 'Phe', 'G': 'Gly', 'H': 'His', 'I': 'Ile',
    'K': 'Lys', 'L': 'Leu', 'M': 'Met', 'N': 'Asn', 'P': 'Pro', 'Q': 'Gln', 'R': 'Arg', 'S': 'Ser',
    'T': 'Thr', 'V': 'Val', 'W': 'Trp', 'Y': 'Tyr', '*': 'STP'
}
```

Now our Peptide sequence

We'll use the 1-letter Amino Acid abbreviations to represent our sequence, and create a dictionary that provides for every Amino acid the set of codons that can encode it.

```
TyrocidineB1 = "VKLFPWFNQY"
TrimerCodes = {}
for key, code in codon.iteritems():
    TrimerCodes[code] = TrimerCodes.get(code,[]) + [key]
for key in sorted(TrimerCodes.iterkeys()):
    print "%15s (%1s): %s" % (AminoAcid[key], key, TrimerCodes[key])
```

```
           STOP (*): ['UGA', 'UAA', 'UAG']
        Alanine (A): ['GCA', 'GCG', 'GCC', 'GCU']
       Cysteine (C): ['UGU', 'UGC']
  Aspartic acid (D): ['GAU', 'GAC']
  Glutamic acid (E): ['GAG', 'GAA']
  Phenylalanine (F): ['UUU', 'UUC']
        Glycine (G): ['GGU', 'GGG', 'GGA', 'GGC']
      Histidine (H): ['CAC', 'CAU']
     Isoleucine (I): ['AUA', 'AUC', 'AUU']
         Lysine (K): ['AAG', 'AAA']
        Leucine (L): ['CUU', 'CUG', 'CUA', 'CUC', 'UUG', 'UUA']
     Methionine (M): ['AUG']
     Asparagine (N): ['AAC', 'AAU']
        Proline (P): ['CCC', 'CCA', 'CCU', 'CCG']
      Glutamine (Q): ['CAG', 'CAA']
       Arginine (R): ['AGG', 'AGA', 'CGA', 'CGG', 'CGU', 'CGC']
         Serine (S): ['AGC', 'AGU', 'UCU', 'UCG', 'UCC', 'UCA']
      Theronine (T): ['ACC', 'ACA', 'ACU', 'ACG']
         Valine (V): ['GUU', 'GUC', 'GUG', 'GUA']
     Tryptophan (W): ['UGG']
       Tyrosine (Y): ['UAU', 'UAC']
```

How many ways to code Tyrocidine B1?

- Since most Amino Acids have multiple codon encodings, any given sequence of Amino Acids can be codes in mltiple ways.
- Let's figure out exactly how many ways there are to encode our little peptide.

```
codes = 1
for residue in TyrocidineB1:
    print residue, AminoAbbrv[residue], TrimerCodes[residue]
    codes *= len(TrimerCodes[residue])
print "%d possible sequences" % codes
```

```
V Val ['GUU', 'GUC', 'GUG', 'GUA']
K Lys ['AAG', 'AAA']
L Leu ['CUU', 'CUG', 'CUA', 'CUC', 'UUG', 'UUA']
F Phe ['UUU', 'UUC']
P Pro ['CCC', 'CCA', 'CCU', 'CCG']
W Trp ['UGG']
F Phe ['UUU', 'UUC']
N Asn ['AAC', 'AAU']
Q Gln ['CAG', 'CAA']
Y Tyr ['UAU', 'UAC']
6144 possible sequences
```

Where do we start?

- Consider that translation could begin at any point in the genome.
- Genes can be encoded on either strand
- There are 3 possible ***open-read frames*** (mod-3 starting positions) on each strand.
- Meanwhile, our peptide has 10 possible starting positions, since it is circular we can't be sure which codon appears first.
- Let's start with something simple
  - Converting a DNA sequence into a protein sequence.
  - In other words, the computational version of translation

```python
def revComp(dnaSeq):
    return ''.join([{'A':'T','C':'G','G':'C','T':'A'}[base] for base in reversed(dnaSeq)])

def proteinTranslation(dnaSeq):
    rnaSeq = dnaSeq.replace("T", "U")
    protSeq = ''
    for i in xrange(0,len(dnaSeq),3):
        if (i+3 > len(dnaSeq)):
            break
        protSeq += AminoAbbrv[codon[rnaSeq[i:i+3]]]
    return rnaSeq, protSeq
```

Let's test it

```python
# one way to code TyrocidineB1
DNA = 'GTGAAACTTTTTCCTTGGTTTAATCAATAT'
DNAr = revComp(DNA)

print "A short DNA sequence"
print "5'-%s-3'" % DNA
print "3'-%s-5'" % ''.join([{'A':'T','C':'G','G':'C','T':'A'}[base] for base in DNA])
print

print "Read frames in primary sequence"
for frame in xrange(3):
    RNA, Peptides = proteinTranslation(DNA[frame:]+DNA[:frame])
    print "%d  %s" % (frame+1,DNA)
    print "%s   %s" % (frame*" ", RNA)
    print "%s   %s" % (frame*" ", Peptides)
    print

print

print "Read frames in the reverse-complement sequence"
for frame in xrange(3):
    RNA, Peptides = proteinTranslation(DNAr[frame:]+DNAr[:frame])
    print "%d  %s" % (frame+1,DNAr)
    print "%s   %s" % (frame*" ", RNA)
    print "%s   %s" % (frame*" ", Peptides)
    print
```

Let's test it

```
A short DNA sequence
5'-GTGAAACTTTTTCCTTGGTTTAATCAATAT-3'
3'-CACTTTGAAAAAGGAACCAAATTAGTTATA-5'

Read frames in primary sequence
1  GTGAAACTTTTTCCTTGGTTTAATCAATAT
   GUGAAACUUUUUCCUUGGUUUAAUCAAUAU
   ValLysLeuPheProTrpPheAsnGlnTyr

2  GTGAAACTTTTTCCTTGGTTTAATCAATAT
    UGAAACUUUUUCCUUGGUUUAAUCAAUAUG
    STPAsnPhePheLeuGlyLeuIleAsnMet

3  GTGAAACTTTTTCCTTGGTTTAATCAATAT
     GAAACUUUUUCCUUGGUUUAAUCAAUAUGU
     GluThrPheSerLeuValSTPSerIleCys


Read frames in the reverse-complement sequence
1  ATATTGATTAAACCAAGGAAAAAGTTTCAC
   AUAUUGAUUAAACCAAGGAAAAAGUUUCAC
   IleLeuIleLysProArgLysLysPheHis

2  ATATTGATTAAACCAAGGAAAAAGTTTCAC
    UAUUGAUUAAACCAAGGAAAAAGUUUCACA
    TyrSTPLeuAsnGlnGlyLysSerPheThr

3  ATATTGATTAAACCAAGGAAAAAGTTTCAC
     AUUGAUUAAACCAAGGAAAAAGUUUCACAU
     IleAspSTPThrLysGluLysValSerHis
```

Now let's try Something Real

```python
def loadFasta(filename):
    """ Parses a classically formatted and possibly
        compressed FASTA file into a list of headers
        and fragment sequences for each sequence contained"""
    if (filename.endswith(".gz")):
        fp = gzip.open(filename, 'rb')
    else:
        fp = open(filename, 'rb')
    # split at headers
    data = fp.read().split('>')
    fp.close()
    # ignore whatever appears before the 1st header
    data.pop(0)
    headers = []
    sequences = []
    for sequence in data:
        lines = sequence.split('\n')
        headers.append(lines.pop(0))
        # add an extra "+" to make string "1-referenced"
        sequences.append('+' + ''.join(lines))
    return (headers, sequences)

header, seq = loadFasta("data/BacillusBrevis.fa")
for i in xrange(len(header)):
    print header[i]
    print len(seq[i])-1, "bases", seq[i][:30], "...", seq[i][-30:]
    print
```

```
Chromosome dna:chromosome chromosome:GCA_000010165.1:Chromosome:1:6296436:1
6296436 bases +GGGTCTGTGGATATCATTTTATCCACAAA ... AAGGCAAATATCCCATAAAACTATTTCCC
```

Over 6 million bases to consider!

Some simple "Computational" experiments

- Just like a biologist, it is ill-advised to jump into a problem without first getting some sense of what works and what doesn't. Let's first take a look at our data
- One thing you might have noticed about the possible encodings of Tyrocidine B1 is that there is a single *Tryptophan* and only one encoding for this amino acid
- Let's try to anchor our search around that seed and grow from there.
- Where and how many *Tryptophan* encodings

```
def CodonSeqCount(genome, codonSeq):
    N = 0
    start = 0
    while True:
        pos = genome.find(codonSeq, start)
        if (pos < 0):
            break
        N += 1
        start = pos + 1
    return N

genome = seq[0]
tryptophanCode = TrimerCodes['W'][0].replace('U','T')
print "Searching for", tryptophanCode, "Found it", CodonSeqCount(genome,tryptophanCode), "times"
revCompCode = revComp(tryptophanCode)
print "Searching for", revCompCode, "Found it", CodonSeqCount(genome,revCompCode), "times"
```

```
Searching for TGG Found it 106555 times
Searching for CCA Found it 107112 times
```

Is this about what you expected? $\frac{6.3\times10^6}{64} \approx 100,000$. We've narrowed our search some, but there's still alot.

Narrowing things down more

- Rather than searching for every possible string, let's examine the codons around this initial anchor.

```python
def AminoAcidSeqCount(genome, peptideSeq):   # Note: Only checks primary strand
    readframe = []
    anchor = peptideSeq[0]
    for rnaSeq in TrimerCodes[anchor]:
        dnaSeq = rnaSeq.replace('U','T')
        start = 0
        while True:
            pos = genome.find(dnaSeq, start)
            if (pos < 0):
                break
            i = 1
            while (i < len(peptideSeq) and (pos+3*(i+1) <= len(genome))):
                if (codon[genome[pos+3*i:pos+3*(i+1)].replace('T','U')] != peptideSeq[i]):
                    break
                i += 1
            else:
                readframe.append(pos)
            start = pos + 1
        return readframe

peptide = "WFNQY"
for i in xrange(1,len(peptide)+1):
    ORFs = AminoAcidSeqCount(genome,peptide[:i])
    print "Searching for", peptide[:i], "Found it", len(ORFs), "times"
```

```
Searching for W Found it 106555 times
Searching for WF Found it 3922 times
Searching for WFN Found it 111 times
Searching for WFNQ Found it 5 times
Searching for WFNQY Found it 1 times
```

Could we have been Lucky this time?

Let's take a look:

```
for pos in ORFs:
    start = pos
    end = pos + (3*len(peptide))
    print "%9d %s %s" % (start, genome[start:end], proteinTranslation(genome[start:end])[1])
```

```
  2789614 TGGTTCAACCAATAT TrpPheAsnGlnTyr
```

Recall we are looking for a substring of `ValLysLeuPheProTrpPheAsnGlnTyr`, which we found, but to be sure let's look around a little more.

```
for pos in ORFs:
    start = pos - (2*3)
    end = pos + (3*(len(peptide)+2))
    print "%9d %s %s" % (start, genome[start:end], proteinTranslation(genome[start:end])[1])
```

```
  2789608 CATGAATGGTTCAACCAATATTACGGA HisGluTrpPheAsnGlnTyrTyrGly
```

We didn't find what we were expecting.

What Went Wrong?

1. We only searched in one direction from our anchor
2. We didn't search the reverse-complement sequence
3. We didn't consider that the cycle could have been broken somewhere in between "W-F-N-Q-Y"
    - One of the earlier 5, 111, 3922, or 106555 candidates might be the solution
4. The best approach might be to call AminoAcidSeqCount() will all 10 circular permutations of "VKLFPWFNQY" (assuming that we also fix the reverse-complement sequence problem)

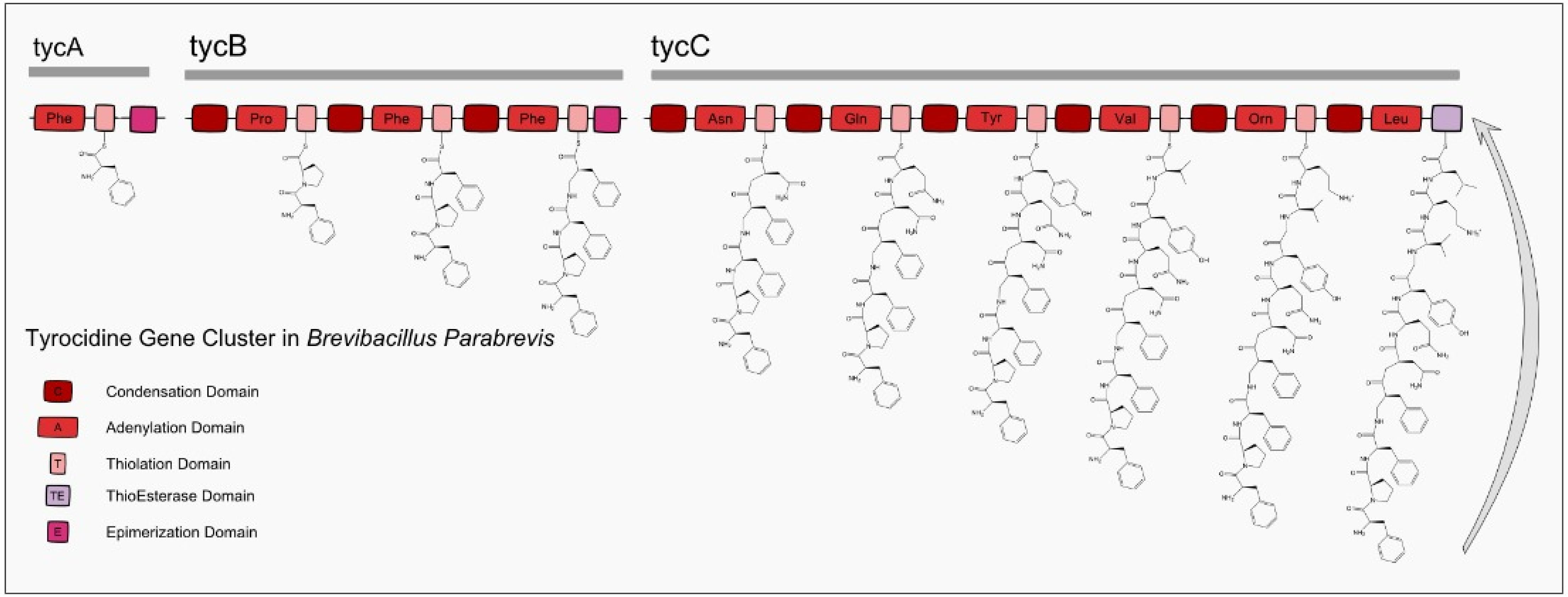
```
[TyrocidineB1[i:]+TyrocidineB1[:i] for i in xrange(len(TyrocidineB1))]
```

```
['VKLFPWFNQY',
 'KLFPWFNQYV',
 'LFPWFNQYVK',
 'FPWFNQYVKL',
 'PWFNQYVKLF',
 'WFNQYVKLFP',
 'FNQYVKLFPW',
 'NQYVKLFPWF',
 'QYVKLFPWFN',
 'YVKLFPWFNQ']
```

But even if you tried alll these fixes, you would still not find the code for Tyrocidine B1 in *Bacillus brevis.*

Proteins that make Petide Chains

Bacteria and fungi use large multifunctional enzymes called *NonRibosomal Peptide Synthetases* (NRPSs) to produce peptide chains that are *not encoded* in DNA. Nonribosomal peptides are synthesized by nonribosomal peptide synthetases, which, unlike the ribosomes, are independent of messenger RNA. Each nonribosomal peptide synthetase can synthesize only one type of peptide. They are often toxins, siderophores, or pigments. Nonribosomal peptide antibiotics, cytostatics, and immunosuppressants are widely used as drugs.



So we are left with a Question

- How do you figure out a peptide's amino acid sequence? It may not appear in the genome.
- Next time we will delve deeper into laboratory methods to start a discussion about peptide sequence inference