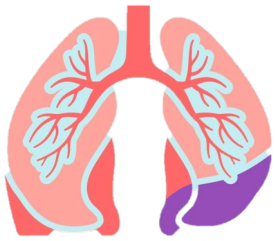
A Case for Cystic Fibrosis

A Quebec Perspective



MODULE 1

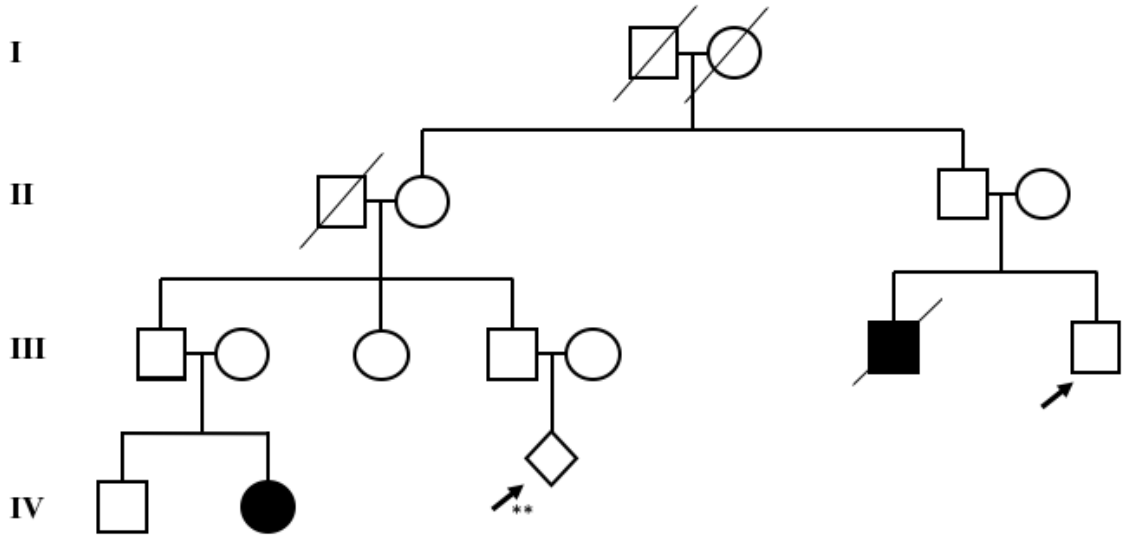
Part I: Mendelian Disorders

Cystic Fibrosis is a common fatal genetic disorder that affects approximately one in every 3,600 children in Canada. It is a disorder that affects the mucous membranes within the body, mainly influencing the airway and digestive tract. The severity of the disease varies based on the individual, but it is often accompanied by persistent lung infections and progressive decline in lung function.

The Morin family lives in Quebec, Canada. Jean-Francois is a native Quebec resident and his partner Claire is from Ontario. Together they have a son, Mathieu (5), and a daughter, Johanne (2). They have started to notice that their daughter, born in November of 2016, has a persistent wheezing cough and isn't growing quite as quickly as her older brother did at her age. Doctors diagnosed her with pneumonia, but after kissing her daughter's forehead and realizing it had a salty taste, Claire decided to get a second opinion. After a genetic screen and a sweat chloride concentration test, the doctors delivered the unfortunate news that their daughter suffers from cystic fibrosis, a disorder to which Jean-Francois recently lost a cousin.

Task:

- Look at the pedigree for the Morin family on the follow page.
- Answer the questions included below.

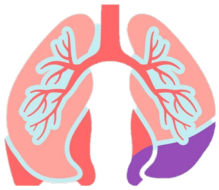


QUESTIONS:

1. When looking at the pedigree, what are the probabilities of individual III-7 (indicated by a black arrow) being a carrier for either mutation, or not carrying a mutation at all?
    a. ¾, 1/4
    b. ½, ¼
    c. 1/3, 2/3
    d. 2/3, 1/3
    e. ¼, 1/2

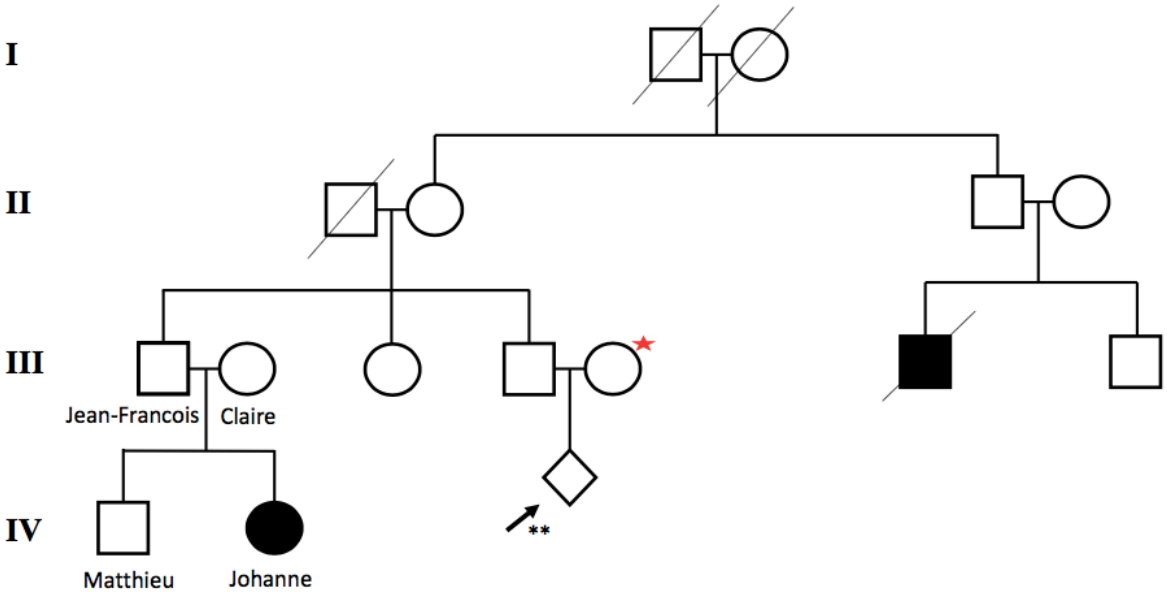
2. Jean-Francois' sister-in-law (born in Quebec) is currently pregnant, and given Johanne's recent diagnosis she and her husband are concerned about the probability that their child (indicated by black arrow and **) may have CF. Given the above pedigree, what is this probability?
    a. ¼
    b. ½
    c. 0
    d. 1/12
    e. 1/6

MODULE 2



Part II: Population Genetics

As the Morin family learned more about their daughter's disorder, they became increasingly aware of the specificity of the Quebec population's genetic basis of CF. The province-wide incidence of CF is higher than the remainder of the country, at one in every 2,500 individuals. In addition, only certain mutations are present, and they are specific to French Canadians. The Morins found a blog based on an article that was written in 2001 which outlined the important differences between the prevalence of CF in the Quebec population and elsewhere. Neither Jean-Francois nor Claire have a background in genetics and must rely on summary information to become informed.



Task:

- Read the section: *The CFTR Locus Reflects Both Inter and Intrapopulation Diversity* in the article Human Genetics: Lessons from Quebec Populations by Charles R. Scriver (2001). Annu. Rev. Genomics Hum. Genet. 2001. 2:69–101. **Note**: Although only one section is required, other aspects of the paper may be helpful for understanding concepts!
- Answer the questions below.

QUESTIONS:

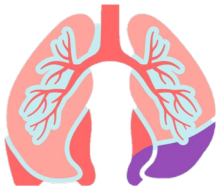
1. While the genetic testing results relieved the minds of Jean-François' brother and sister-in-law, it alarmed his sister, Sophie, especially after reading the article herself. Her new boyfriend, Robert, whom she hopes to marry, comes from the Saguenay-Lac St-Jean region (SLSJ). Using information provided in the article, what is the probability that Sophie (III-3, see arrow on pedigree) and Robert's first child would have CF?

    a. 1/30
    b. ¼
    c. 1/15
    d. 1/6
    e. 1/60

2. Robert, the new boyfriend of Jean-François' sister, Sophie, comes from the Saguenay-Lac St-Jean region (SLSJ). Based on the information in the article, if he were to be a carrier of a CF allele, which are the two most likely alleles that he may be carrying?

    a. 621+1 or A455E
    b. ΔF508 or A455E
    c. ΔF508 or 621+1
    d. ΔF508 or 711+1
    e. ΔF508 or L206W

MODULE 3



Part III: From Genotype to Phenotype

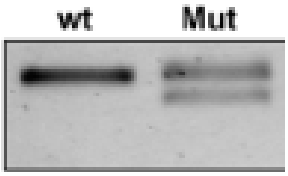
For Cystic Fibrosis, there is a strong correlation between the type of mutation and the phenotype that the person presents with. Even among individuals with the same mutations and genetic basis for CF, there exists a broad range of symptoms and disorder severities. As such, the genetics team at the Montreal Royal Victoria Hospital decided to assess the genetic basis of Johanne's disorder, and which gene(s) is/are specifically altered.

Task:

- Read the following molecular biology information and answer the questions below.

QUESTIONS:

Sequencing of the CFTR gene reveals a single point mutation that converts a G to and A residue at position 2756 of the gene, which is located between exons 16 and 17. They call this allele CFTR 2756GA. The team then carries out a Northern blot analysis to compare expression of the CFTR gene in cells homozygous for the CFTR 2756GA (Mut) to CFTR gene expression in wild type cells. They discover an extra band in the CFTR 2756GA lane compared to wild type.



1. Based on the sequence analysis, what type of mutation is CFTR 2756GA?
    a. Silent
    b. Transition
    c. Transversion
    d. Frameshift
    e. None of the above

2. Propose an explanation for what each band represents and how the 2756GA point mutation might result in an extra band in the CFTR 2756GA mutant lane (max 75 words).

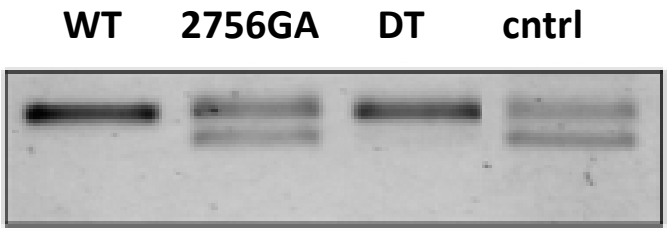
3. The researchers consult a database of CFTR alleles identified in CF patients and find that CFTR 2756GA has been previously found in other patients. Table 1 (below) contains phenotypic information from individuals homozygous for the 2756GA, the F508del allele, and a third allele called 1831TC, a class III mutation that reduces CFTR protein function. Which alleles correspond to columns A, B, and C, respectively?
    a. **2756GA, F508del, 1831TC**
    b. F508del, 2756GA, 1831TC
    c. F508del, 1831TC, 2756GA
    d. 1831TC, 2756GA, F508del
    e. Cannot distinguish from the information provided

Table 1 Phenotypic characteristics of the cystic fibrosis patients

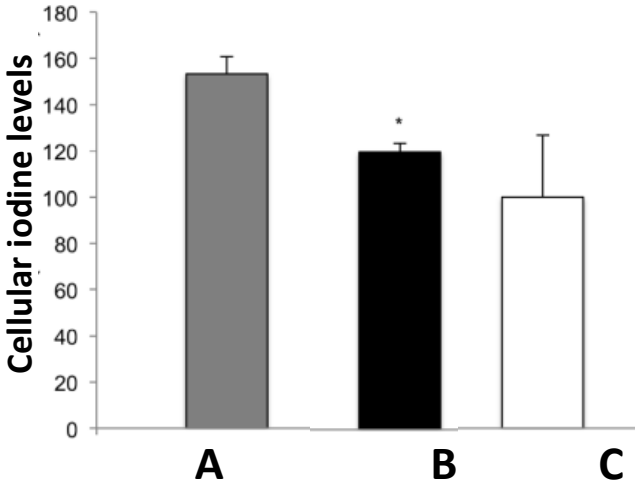
	A		B	C
Sex, (male/female)	5/6	NS	28/17	2/1
Current age, years (mean ± SD)	36.8 ± 7.0	0.0001	23.8 ± 5.3	22.6 ± 3.5
Age at diagnosis, years (mean ± SD)	21.0 ± 8.1	0.0001	3.5 ± 4.5	5.6 ± 1.1
Sweat test, mEq/l (mean ± SD)	103.4 ± 23.3	NS	112.5 ± 20.3	102.3 ± 4.0
FEV1% predicted (mean ± SD)	82.4 ± 31.4	0.004	47.4 ± 23.1	32.8 ± 5.2
FVC % predicted (mean ± SD)	84.2 ± 32.1	0.045	61.4 ± 23.9	53.2 ± 4.7
Oxigen saturation, % (mean ± SD)	96.1 ± 1.5	0.020	93.9 ± 4.6	91.6
BMI (mean ± SD)	22.3 ± 4.3	0.037	19.1 ± 2.7	20.6
Trypsine, μg/l (mean ± SD)	343.8 ± 169.8	0.0001	15.6 ± 20.7	24.5 ± 48.8
Pancreatic insufficiency, *n* (%)	3 (27)	0.0001	43 (93)	3(100)
Pancreatitis, *n* (%)	2 (18)	0.035	0 (0)	0 (0)
Diabetes mellitus, *n* (%)	0	NS	9 (20)	2 (66)
Lung symptoms at diagnosis (%)	82	0.0001	60	—
Bronchiectasis, *n* (%)	11 (100)	NS	45 (100)	3(100)
Bilateral bronchiectasis, *n* (%)	8 (77)	NS	12 (27)	0
Diffuse bronchiectasis, *n* (%)	3 (23)	NS	33 (73)	3(100)
Bronchial colonization, *n* (%)	10 (91)	NS	44 (98)	3(100)
Pseudomonas colonization, *n* (%)	7 (64)	NS	38 (84)	2(66)
Hemoptysis, *n* (%)	11 (100)	0.001	21 (47)	—
Sinusitis, *n* (%)	11 (100)	0.024	30 (66)	3(100)
Admission to hospital (mean ± SD)	1.3 ± 2.7	NS	2.0 ± 4.6	—

FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; BMI, body mass index; NS, not statistically significant.

4. In a cultured cell system, the researchers treat cells containing the 2756GA allele with a compound (DT) that they hypothesize will correct the defect. 2756GA cells that were mock-treated serve as a control (cntrl). The researchers obtained the following Northern blot results.

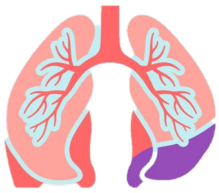


Following up on these Northern blot results, the researchers carried out a functional assay to determine whether treatment with the compound (DT) had a positive effect on the function of the CFTR protein. This assay measures CFTR-mediated transport by loading cells with iodine, stimulating CFTR activity with an additional compound, and then evaluating the function of the CFTR channel by assessing the amount of radiolabeled iodine remaining in the cell. The results are shown in the graph below.



5. Given these data, which samples from the Northern blot analysis above are represented in lanes A, B, and C of the iodine assay, respectively?
   a. Cntrl, DT, 2756GA
   b. DT, cntrl, WT
   c. 2756GA, DT, WT
   d. Cntrl, DT, 2756GA
   e. WT, cntrl, 2756GA

MODULE 4



Part IV: Genetic Screening

Read the article excerpt below (For full article: https://montrealgazette.com/news/local-news/quebec-to-start-screening-newborns-for-cystic-fibrosis**)**

Quebec to start screening newborns for cystic fibrosis

Cystic Fibrosis Canada says this is a "huge win" for the health and well-being of Quebec's children

CATHERINE SOLYOM, MONTREAL GAZETTE Updated: June 8, 2017

The decision to institute neonatal blood screening was based on a study conducted by the Institut national de santé publique du Québec — Quebec's public health institute — which found only one in five children with typical cystic fibrosis was diagnosed before the age of three months, while half were diagnosed in their first year of life.

Another 30 per cent were diagnosed before the age of five, and the remaining 20 per cent were diagnosed sometime before their 18th birthday.

CF Canada said this was a "huge win" for the health and well-being of Quebec's children.

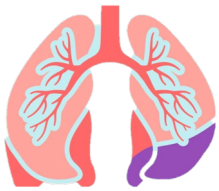
"Since 2006, CF Canada has been calling for the implementation of a newborn screening program in Quebec," said Yannick Brouillette, regional executive director, CF Canada Quebec. "Now that this has been realized, our mission is to continue to support CF families and the 11 Quebec clinics they depend on for essential care. We would like thank Minister Gaétan Barrette, as well as the entire CF community who have worked tirelessly in their efforts to improve the lives of Quebec newborns and their families, and will continue to do so until a cure is found."

Prior to the Spring of 2018, genetic screening for CF was not mandatory for newborns in Quebec. Given Johanne's birth date, she was not included in the screening wave. The Morins are disappointed, but happy to hear that moving forward children will be tested for CF at birth.

Task:

- Write a Tweet style (140 characters) announcement regarding CF screening in Quebec. Simplify what genetic screening does, and why this is beneficial. Remember, the lay public reads Tweets, so no jargon!

MODULE 5



Part V: Bacterial Genetics

The most common pathology amongst CF patients is lung infection. Most patients acquire chronic *Psuedomonas aeruginosa* infections by the time they're teenagers. These bacteria are capable of avoiding host immunity by frequently arising mutator phenotypes. The bacteria with the most beneficial mutations are then able to thrive in the lungs of CF patients, and continue to cause chronic infections.

TASKS:

- Read the attached paper sections.
- Answer two questions. (**Note to instructors**: randomly assign 2 of the 5 questions to students).

QUESTION BANK:

What is the evidence that resistance genes are ancient?

a) They have been found in environments untouched by humans
b) They have sequence homology with organelle DNA
c) They are originated from transposons
d) They are originated from bacterial phage

What is the function of the TetX enzyme in conferring antibiotic resistance?

a) Increasing efflux
b) Hydrolysis of the acyl-enzyme bond in the Beta-lactam ring
c) Inactivates tetracyclines
d) Alteration of the metabolic pathway used by the bacteria

Which of the following methods of horizontal gene transfer does not require physical contact between host and recipient?

a) Transduction
b) Translation
c) Transfer
d) Transformation
e) Conjugation

Which bacteria is able to use the conjugation machinery for HGT into plant cells?

a) *Rhodobacter capsulatus*
b) *Haloferax volcanii*
c) *Agrobacterium*
d) Streptomyces

What is the first thing required for an antibiotic resistance gene to be maintained in a recipient?

a) Selective advantage
b) Ease of transfer
c) Neutral acquisition
d) Does no harm

Microbiology Spectrum
American Society for Microbiology Press

Selection and Transmission of Antibiotic-Resistant Bacteria

DAN I. ANDERSSON and DIARMAID HUGHES
Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

ABSTRACT Ever since antibiotics were introduced into human and veterinary medicine to treat and prevent bacterial infections there has been a steady selection and increase in the frequency of antibiotic resistant bacteria. To be able to reduce the rate of resistance evolution, we need to understand how various biotic and abiotic factors interact to drive the complex processes of resistance emergence and transmission. We describe several of the fundamental factors that underlay resistance evolution, including rates and niches of emergence and persistence of resistant bacteria, time- and space-gradients of various selective agents, and rates and routes of transmission of resistant bacteria between humans, animals and other environments. Furthermore, we discuss the options available to reduce the rate of resistance evolution and/ or transmission and their advantages and disadvantages.

INTRODUCTION AND SCOPE

Antibiotics are compounds that inhibit (bacteriostatic drugs) or kill (bactericidal drugs) bacteria by a specific interaction with a specific target in the bacterial cell, and they are arguably the most important medical intervention introduced by humans. Ever since antibiotics were introduced on large scale in the late 1940s to treat human bacterial infectious diseases, there has been a steady selection and increase in the frequency of antibiotic-resistant bacteria, generating a very problematic situation (1–3). Resistance evolution is a complex process that is driven by the interaction between a number of biotic and abiotic factors. Fundamental factors underlying this dynamic are the rates of emergence and persistence of resistant bacterial clones; time and space gradients of antibiotics and other xenobiotics; and transmission rates within human populations and between humans and various other sources, including animals, the environment, food, etc. Furthermore, with the realization that antibiotic resistance has become a serious medical problem, human attempts to reduce transmission of infectious bacteria in general, and resistant ones in particular, by hygienic measures, vaccination, reduced antibiotic pressures, etc., has also influenced this dynamic.

Here we will describe some of the factors that influence the selection and transmission of resistant bacteria and discuss the options available to prevent these processes and reduce the rate of resistance evolution and/ or transmission. In this description, we will follow the outline shown in Fig. 1, where we track the initial emergence of resistance in environmental bacteria, its transfer into pathogenic bacteria, and the subsequent transmission of these bacteria between different compartments and environments.

WHERE DID RESISTANCE GENES ORIGINATE AND WHY?

The majority of medically relevant antibiotics originate in nature and are synthesized by a variety of species, in particular soil-dwelling bacteria in the genus

Received: 2 February 2017, **Accepted:** 21 February 2017, **Published:** 27 July 2017
Editors: Fernando Baquero, Hospital Ramón y Cajal (IRYCIS), Madrid, Spain; Emilio Bouza, Hospital Ramón y Cajal (IRYCIS), Madrid, Spain; J.A. Gutiérrez-Fuentes, Complutensis University, Madrid, Spain, and Teresa M. Coque, Hospital Ramón y Cajal (IRYCIS), Madrid, Spain
Citation: Andersson DI, Hughes D. 2017. Selection and transmission of antibiotic-resistant bacteria. *Microbiol Spectrum* 5(4):MTBP-0013-2016. doi:10.1128/microbiolspec.MTBP-0013-2016.
Correspondence: Dan Andersson, Dan.Andersson@imbim.uu.se

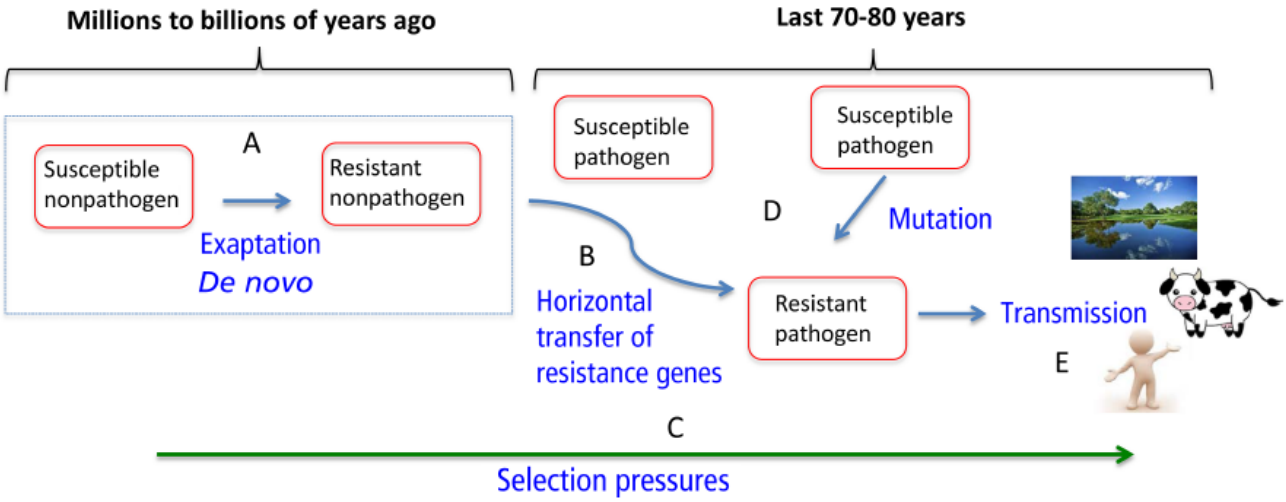


FIGURE 1 Schematic view of the evolution of antibiotic resistance. Key questions in understanding the emergence and transmission include: (A) What are the origins of resistance genes? (B) Where do resistant pathogens emerge? (C) Which are the most significant selective pressures driving resistance evolution? (D) Which are the biological factors that influence rates of resistance development? (E) What are the routes, directions, and magnitudes of flow of pathogens between humans, animals, and the environment?

Streptomyces (4, 5). Both antibiotics and resistance genes are thought to be ancient and far predate the existence of humans. This has been inferred from phylogenetic studies suggesting that class A β-lactamases evolved billions of years ago and were transferred into the Gram-positive bacteria about 800 million years ago and that the progenitors of β-lactamases, like CTX-M's, diverged 200 million to 300 million years ago (6). Further evidence that resistance genes are old is their presence in environments that have been untouched by humans (7–9), including in isolated caves (>4 million years), Beringian permafrost (30,000 years), and Siberian permafrost (15,000 to 40,000 years old).

The benefits of antibiotics for microbial producers is not entirely clear, but the standard explanation is that the producers use them as ecological weapons to inhibit neighboring competitors in the environment (10, 11). However, they might also have a more benevolent function as signals for cell-to-cell communication in microbial communities. A recent study that distinguished between these hypotheses provided strong evidence that antibiotics are weapons but that their expression is influenced by social interactions between competing strains and species (12). It is expected that the biosynthesis and release of antibiotics in various natural environments will expose many bacteria (both the producers and bystanders) to antibiotics and, as a consequence, select for the evolution of resistance mechanisms to protect against self-destruction (in antibiotic producers), to defend against antibiotics produced by other species, and/or to modulate intermicrobe communication.

Another hypothesis is that resistance genes originally performed metabolic functions unrelated to antibiotics and that they had weak secondary promiscuous activities that conferred a low-level resistance that was exapted and further evolved to become bona fide antibiotic resistance functions. For example, aminoglycoside acetylate-modifying enzymes could originally have been involved in sugar metabolism and modification of complex sugars. Similarly, the plasmid-borne, dual-activity fluoroquinolone acetylate-modifying enzyme AAC(6′)-Ib-cr (13–15) belongs to the GNAT (GCN5-related *N*-acetyltransferase) superfamily, with 10,000 known enzymes that perform a variety of coenzyme A-dependent acetylation reactions (16). Considering the low activity of this enzyme on fluoroquinolones (only a fewfold increase in MIC of fluoroquinolones), it is likely that this represents a weak promiscuous activity. Another example is the class A, C, and D (Ser-OH) β-lactamases that have been suggested to originate from penicillin-binding proteins (PBPs) by acquiring the capability to hydrolyze the acyl-enzyme bond between the β-lactam ring and the hydroxyl group of the PBP's active-site serine (17, 18). This notion is supported by experimental data showing that PBPs can evolve weak β-lactamase activity after mutagenesis (19–21). A final example

of where a function might have been coopted is the TetX enzyme, a flavin-dependent monooxygenase that inactivates all known tetracyclines, including tigecycline. This enzyme belongs to the flavoprotein monooxygenase group, whose native metabolic function is in the hydroxylation and degradation of phenolic compounds (22).

Our possibilities to interfere with and slow down the rate by which novel resistance mechanisms evolve in nonpathogenic bacteria (e.g., *Streptomyces*) are at present nonexistent, but this type of knowledge is still useful since it provides the tools that allow us to explore the intrinsic potential of a bacterium to acquire a high-level resistance mechanism to a novel antibiotic by evolving an existing weak promiscuous activity into a more efficient enzyme. For example, by directed *in vitro* evolution of a suspected candidate enzyme or an adaptive evolution experiment with whole cells, one can explore the likelihood and efficiency of such a process (23).

Continues below

Horizontal gene transfer: building the web of life

Shannon M. Soucy[1], Jinling Huang[2] and Johann Peter Gogarten[1,3]

Abstract | Horizontal gene transfer (HGT) is the sharing of genetic material between organisms that are not in a parent–offspring relationship. HGT is a widely recognized mechanism for adaptation in bacteria and archaea. Microbial antibiotic resistance and pathogenicity are often associated with HGT, but the scope of HGT extends far beyond disease-causing organisms. In this Review, we describe how HGT has shaped the web of life using examples of HGT among prokaryotes, between prokaryotes and eukaryotes, and even between multicellular eukaryotes. We discuss replacement and additive HGT, the proposed mechanisms of HGT, selective forces that influence HGT, and the evolutionary impact of HGT on ancestral populations and existing populations such as the human microbiome.

Selfish genetic element
A gene or group of genes that enhance their own transmission and reproductive success without making a positive contribution to the host's fitness.

[1]*Department of Molecular and Cell Biology, University of Connecticut, 91 North Eagleville Road, Storrs, Connecticut 06269–3125, USA.*
[2]*Department of Biology, East Carolina University, Greenville, North Carolina 27858, USA.*
[3]*Institute for Systems Genomics, University of Connecticut, Connecticut 06269–3125, USA.*
Correspondence to J.P.G.
e-mail: gogarten@uconn.edu
doi:10.1038/nrg3962

Horizontal gene transfer (HGT) was first described in microorganisms in the late 1940s[1], and around 20 years later it was speculated to have a role in the adaptation of multicellular eukaryotes — specifically plants[2]. Since then, methods to detect HGT have improved, and these have revealed the surprising extent and relevance of HGT to the variation of viral, prokaryotic and eukaryotic gene content. Many apparent gene duplications, for example, are now known to be the result of HGT, not autochthonous gene duplication, resulting in a 'web of life' rather than in a steadily bifurcating tree[3,4].

For a transferred gene to survive in the recipient lineage for long periods of time, the gene usually needs to provide a selective advantage either to itself (in the case of a selfish genetic element) or to the recipient, and research on HGT initially focused on such genes. However, it is now known that many of the genes that have been identified as transferred through comparative genomics between close relatives have neutral or nearly neutral effects in the recipient in both prokaryotic and eukaryotic organisms[5]. One rule for transferred genes seems to be 'first do no harm' — genes that are successfully integrated into a recipient are often expressed at low levels and encode functions at the periphery of metabolism[6]. These neutral acquisitions, however, can later provide novel combinations of genetic material for selection to act on — in some cases, the transferred material becomes domesticated over time and produces a beneficial phenotype. In other cases, when the imported genes remain neutral and there is no obvious benefit associated with their retention, the genes are likely to be lost over time.

HGT has long been recognized as an important force in the evolution of bacteria and archaea. However, the exchange of genetic information between prokaryotic symbionts and their eukaryotic hosts, and even between eukaryotes, signifies that HGT in eukaryotes occurs more frequently than previously thought[7,8]. Often these transfers involve gene donations to unicellular eukaryotes[9] and are frequently associated with bacterial endosymbionts[10] (known as endosymbiotic gene transfer (EGT) or intracellular gene transfer (IGT)). However, bacterial genes can also be transferred to multicellular eukaryotes[8]. Recent interest in the human microbiome has reinvigorated the search for HGTs from symbionts into the human genome. Although transfers of bacterial genes into the human germ line[11,12] have not be confirmed, evidence is accumulating of HGT from bacteria to human somatic cells[13]. These findings demonstrate the enduring influence of HGT on the evolution of all parts of the web of life, eukaryotes included.

In this Review, we present an overview of how HGT has contributed to innovation throughout the web of life by providing novel combinations of gene sequences for selection to act upon, thus shaping the evolution of species ranging from single-celled microorganisms to multicellular eukaryotes. Advances in the understanding of mechanisms of HGT, methods of identifying HGT events and the growth of genome databases have facilitated these insights.

Mechanisms of HGT

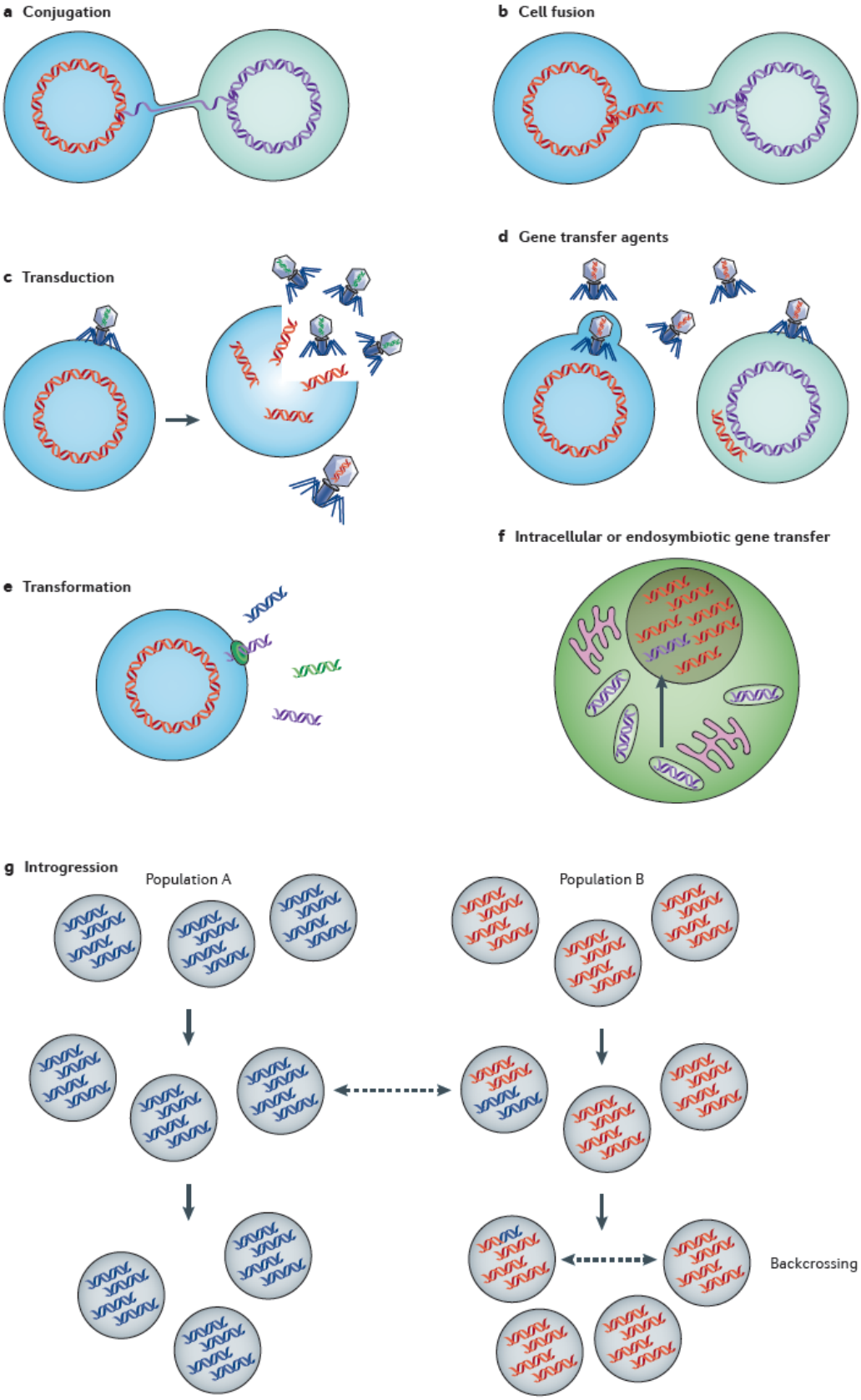
The three most recognized mechanisms of HGT in prokaryotes are conjugation, transformation and

transduction (FIG. 1). Conjugation requires physical contact between a donor and a recipient cell via a conjugation pilus, through which genetic material is transferred. Conjugation is canonically restricted to bacterial cells as the donor and recipient, however, *Agrobacterium* spp. is an exception and uses its conjugation machinery for HGT into plant cells[14,15]. Transformation is the uptake of exogenous DNA from the environment and has been reported in both archaea and bacteria[16,17]. Transduction is the delivery of genetic material through phage predation owing to the integration of exogenous host genetic material into a phage genome, and this phenomenon has been observed in both bacteria and archaea. There are two types of transduction: generalized, in which a random piece of the host DNA is incorporated during cell lysis; and specialized, in which a prophage imprecisely excises itself from a host genome and incorporates some of the flanking host DNAs.

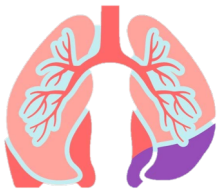
Other mechanisms of gene transfer, such as gene transfer agents (GTAs) and cell fusion, have more recently been described. GTAs are gene delivery systems that are integrated into a host chromosome and are sometimes under host regulatory control. GTAs carry small random pieces of host genome in capsids for delivery to nearby hosts. GTAs are found in both bacteria and archaea. The GTA-encoding genes do not provide an obvious benefit to the host, which donates its DNA to others, nor is the benefit to the GTA-encoding genes obvious, because the GTA does not preferentially transfer the GTA-encoding genes. The question of how these genes remain under selection for function remains enigmatic[18]. One study found that GTAs from *Rhodobacter capsulatus* were able to transfer antibiotic resistance to bacteria from different phyla; however, other studies have shown that not all bacteria, including those with the genes encoding GTAs, are able to receive gene donations via GTAs[18]. GTAs have evolved from prophages that have lost the ability to target their own DNA for packaging[18]. Most GTAs cannot package a long enough segment of DNA to transfer all the genes that are necessary to produce GTAs — that is, in contrast to phages, GTAs cannot transfer all of the genes that encode them to a new host. This is an important distinction from transduction.

Cell fusion has been observed in both Euryarchaeota (*Haloferax* spp.) and Crenarchaeota (*Sulfolobus* spp.)[19,20]. Experimentally, cell fusion has been observed on solid media where *Haloferax volcanii* forms aggregates and cells become physically joined by several small bridges of fused cell membrane[21]. Bidirectional gene transfer that is mediated through cell fusion has also been observed between different *Haloferax* species[22]. The bidirectionality of this method of gene exchange means that it is more similar to sexual reproduction in eukaryotes than it is to conjugation in prokaryotes.

◀ Figure 1 | **Mechanisms of gene transfer.** Each panel represents a method of gene transfer. Conjugation (part **a**) occurs through donor–recipient cell contact, and single-stranded DNA is transferred from the donor cell to the recipient cell. Cell fusion (part **b**) differs from conjugation in that DNA is exchanged bi-directionally after cell contact and bridge formation between two cells. Gene transfer mediated by phage is known as transduction (part **c**). In the case of generalized transduction, any piece of genomic DNA may be loaded into the phage head; a general transducing phage is shown with host DNA (red). Specialized transduction occurs when an activated prophage loads a piece of genomic DNA neighbouring the prophage genome into the phage head together with the phage DNA (not shown). Gene transfer agents (GTAs) (part **d**) are phages that no longer recognize their own DNA and only carry random fragments of host DNA. Like prophage, they reside in the host cell genome. During transformation (part **e**) DNA is taken up from the surrounding environment; in the picture the DNA is depicted as entering the cell in the double stranded form, though many DNA uptake systems degrade one of the strands upon cell entry. Intracellular or endosymbiotic gene transfer (part **f**) occurs when genetic material from an endosymbiont or organelle (such as a chloroplast or mitochondrion) is incorporated into the host genome, this mainly pertains to eukaryotes. Introgression (part **g**) occurs when a hybridization event occurs between two diverging species (orange and blue populations). Backcrosses with one of the parent populations (orange) can lead to only a small piece of the divergent genome (blue) remaining in the recipient.



MODULE 6



Part VI: Vaccine Development and Immunogenetics

The Morins have grown increasingly concerned about their daughters' condition, and the progression of her disease over her lifespan. Although there are many palliative treatments, there is no true cure for CF. They have been researching different treatment and management options, and have stumbled across a clinical trial for a vaccine strategy. This therapy targets the bacterial infections that plague CF patients by priming host response, or exploiting the genomic data derived from bacterium.

Tasks:

- Read the attached paper selection.
- In 3 or 4 sentences, describe what you would use as an active agent to develop a vaccine against Cystic Fibrosis.

HUMAN VACCINES & IMMUNOTHERAPEUTICS
2016, VOL. 12, NO. 3, 751–756
http://dx.doi.org/10.1080/21645515.2015.1102810

Taylor & Francis
Taylor & Francis Group

COMMENTARY

Vaccine strategies against cystic fibrosis pathogens

Vincent Le Moigne[a], Jean-Louis Gaillard[a,b], and Jean-Louis Herrmann[a,b]

[a]INSERM U1173, UFR Des Sciences de la Santé Simone Veil, Université de Versailles-Saint-Quentin, Saint-Quentin en Yvelines, France,; [b]Service de Microbiologie, Groupe Hospitalier et Universitaire Paris Île-de-France Ouest, Assitance Publique Hôpitaux de Paris, (92) Boulogne-Billancourt and Garches, France

ABSTRACT

A great number of cystic fibrosis (CF) pathogens such as *Pseudomonas aeruginosa*, the *Burkholderia cepacia* and the *Mycobacterium abscessus* complex raised difficult therapeutic problems due to their intrinsic multi-resistance to numerous antibiotics. Vaccine strategies represent one of the key weapons against these multi-resistant bacteria in a number of clinical settings like CF. Different strategies are considered in order to develop such vaccines, linked either to priming the host response, or by exploiting genomic data derived from the bacterium. Interestingly, virulence factors synthesized by various pathogens might serve as targets for vaccine development and have been, for example, evaluated in the context of CF.

ARTICLE HISTORY
Received 10 September 2015
Revised 25 November 2015
Accepted 28 September 2015

KEYWORDS
cystic fibrosis; vaccine; *Mycobacterium abscessus*; *Pseudomonas aeruginosa*; *Burkholderia* spp.

Cystic fibrosis and microbial lung infections

Cystic fibrosis (CF) is a disease which arises from a Mendelian defect due to a series of mutations in the *cftr* gene encoding the Cl^- channel.[1] The resulting flaw in this protein is responsible for increasing the viscosity of the mucus, which promotes the accumulation and the attachment of bacteria to mucins. Chronic inflammation[2] and early bacterial infection maintain a vicious circle and are each responsible for the lung damage which ensues. Lung infections in CF patients represent the most frequent but also the more serious manifestations since they are responsible for more than 90% of CF patient deaths.[3] The microorganisms that may infect the respiratory system are bacteria, fungi and viruses. Bacterial colonization occurs very early in the natural history of the disease.[4] The first causative organisms are *Haemophilus influenzae* and *Staphylococcus aureus. S. aureus* is usually the first detected[5] and its prevalence is rising.[6] Affinity of *S. aureus* for CF mucus contributes to persistent colonization and progressive pulmonary damage increasing the potential for further infections to set in, for example *Pseudomonas* spp.[5] *Pseudomonas aeruginosa* colonization arises several months to several years after. Finally, several bacterial complexes are found responsible for severe infections in CF, in addition to be the most difficult to treat: the *Burkholderia cepacia* complex (Bcc) and the *Mycobacterium abscessus* complex, which has emerged recently as a threat in CF patients, and may present with *Mycobacterium avium*, the major non-tuberculous mycobacterium (NTM) present in CF lungs with a significant prevalence.[7,8]

Opportunistic pathogens becoming untreatable weapons in CF patients

P. aeruginosa is the environmental opportunistic pathogen in CF patients. It is the most commonly isolated bacterium that infects individuals with CF, with colonization and chronic infections that may affect up to 80% of adult CF patients.[9] *P. aeruginosa* establishes a chronic endobronchial infection which impacts on morbidity and mortality of CF patients. *P. aeruginosa* is also notable for its resistance to antibiotics, making it therefore a difficult to treat pathogen, which, once acquired, is rarely, if ever, eradicated. In addition, *P. aeruginosa* frequently colonized CF lungs as a biofilm, which reduces the patient's immune response and access by antibiotics.[10] A second opportunistic pathogen, represented as a complex is the *Burkholderia cepacia* complex (Bcc). It is composed of 18 species that are able to cause opportunistic and lethal infections CF patients.[11] The two most clinically relevant species are *Burkholderia cenocepacia* and *Burkholderia multivorans*.[12] These environmental, intracellular and biofilm-forming bacteria are extremely antibiotic resistant organism.[12] Bcc infections are rarely cleared from CF patients once they are colonized, as observed in *P. aeruginosa* infections. The third antibiotic-resistant bacterium found in CF patients with frequency between 3 to 7%[7,13] is *Mycobacterium abscessus*. It is a rapidly growing mycobacterium also existing as a complex: the *Mycobacterium abscessus* complex,[14] with 2 subspecies *M. abscessus abscessus* and *M. abscessus bolletii* respectively. *M. abscessus* is, within the group of rapid-growers, responsible for a broad spectrum of diseases in humans. Lung infections are frequent, with CF patients particularly susceptible,[7,13,15] in addition to muco-cutaneous infections often of nosocomial origin.[16] Recent reports of human-to-human transmission in the context of CF care have been described.[17,18] *M. abscessus* raises very challenging therapeutic issues because of its natural resistance to most available antibiotics.[19,20] Severe, even fatal, infections in CF patients have been

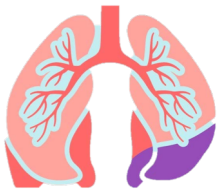
CONTACT Vincent Le Moigne vincent.le-moigne@uvsq.fr Université de Versailles-Saint-Quentin, INSERM U1173, UFR Des Sciences de la Santé Simone Veil, Saint-Quentin en Yvelines, 78180, France.

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/khvi.

described due to therapeutic deadlock.[21] *M. abscessus* infection might represent a contraindication for lung transplantation in several countries,[22] leaving CF patients without therapeutic options.

As such, antibiotic treatment exemplifies a clear challenge now faced with these opportunistic pathogens. We demonstrated for example a significant link between previous intravenous antibiotic courses and the isolation of *M. abscessus* in CF patient lungs, underlining the role of broad-spectrum antimicrobial therapy in the emergence of *M. abscessus* disease.[23] And this is true for the continuous emergence of resistant *P. aeruginosa* or Bcc due to the repeated antibiotic therapeutic regimens given to CF patients.[24] Emergence of multi-resistant bacteria leads to therapeutic impasses with severe and fatal infections.[24]

BONUS ASSIGNMENT



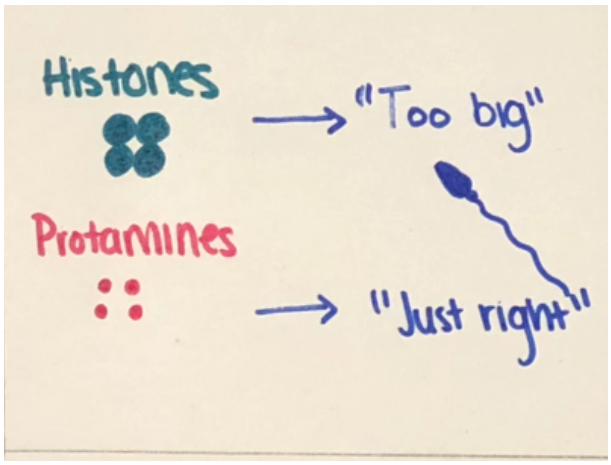
+ 1PT TO OVERALL GRADE

Community engagement by outreach to schools and the public in general is an important role for all scientists to play. For a bonus grade, draw an engaging diagram ("cartoon") of a concept, result, or topic covered in the invited speaker seminar or from the case study this semester. **Your diagram should be aimed at explaining or highlighting science to an 8-year-old child**. In addition to making it simplified (but still accurate), go ahead and be creative - have fun with it!!

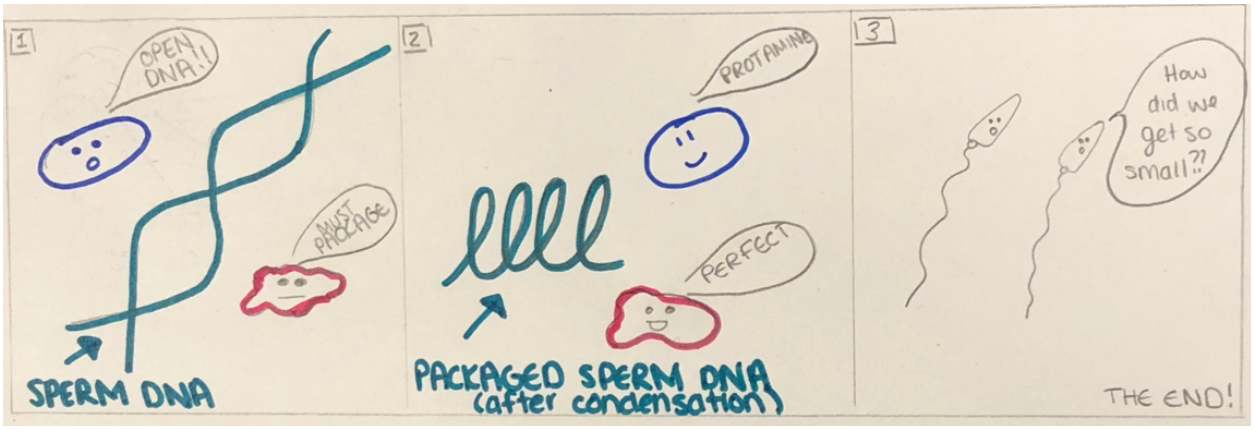
Submission Instructions:

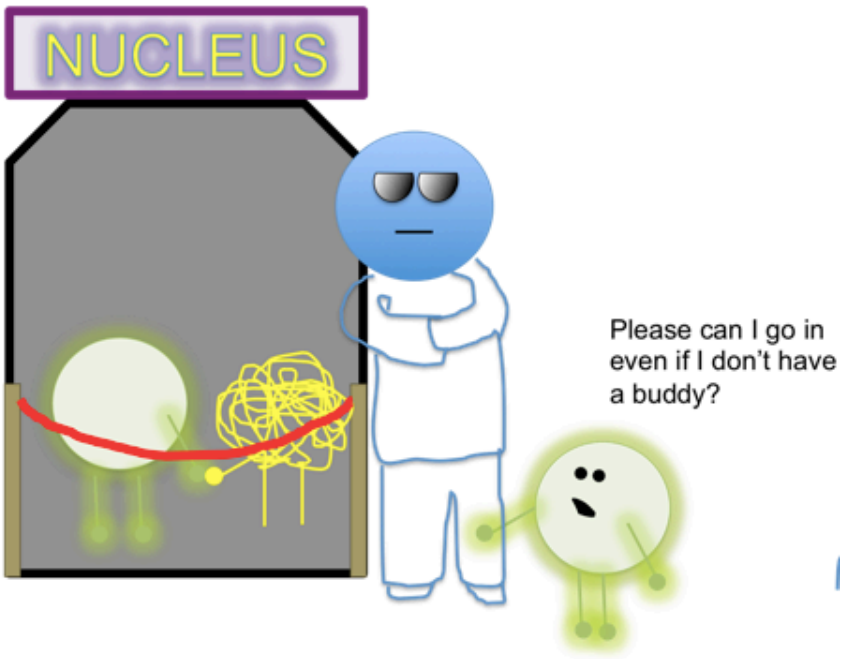
- Include instructions for the students (suggested to submit through the University/College learning platform, such as myCourses or Moodle).

See below for some (non-Genetics) cartoon examples. (You will not be graded on your artistic ability – worry not!)

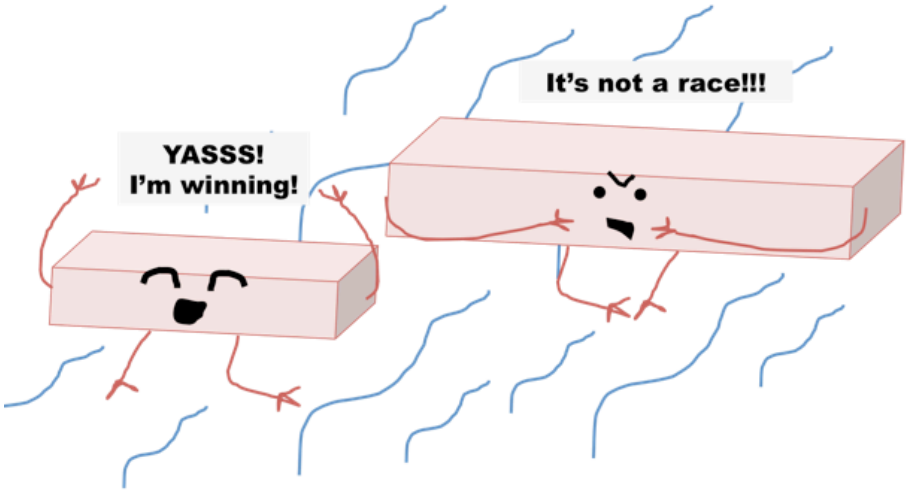

Histones
"Too big"
Protamines
"Just right"


DIVERGENT PLATES
I guess I'm going now...
Mood.


1
OPEN DNA!!
MUST PACKAGE
SPERM DNA
2
PROTAMINE
PERFECT
PACKAGED SPERM DNA (after condensation)
3
How did we get so small??
THE END!



Diffuse GFP: No Protein Fusion, No Entry



Gel Electrophoresis – Everyone's a Winner