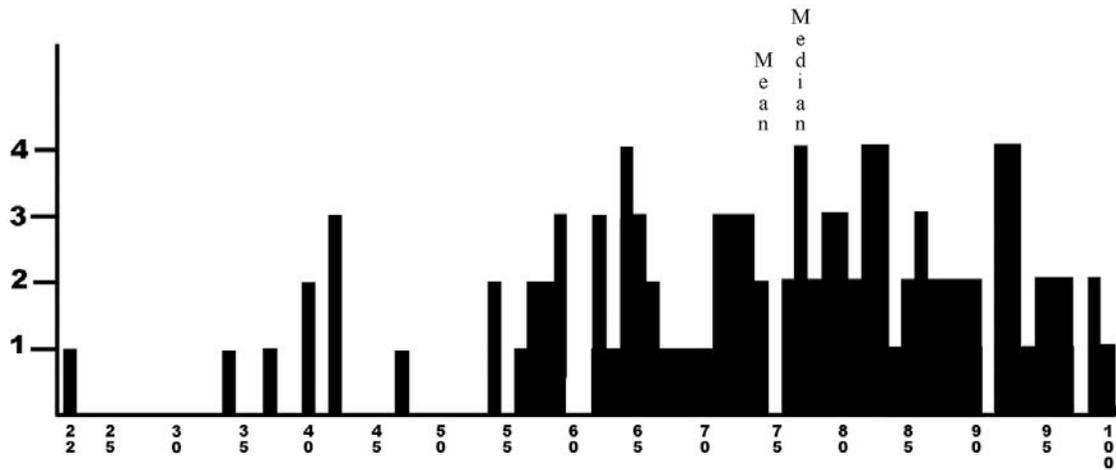


Midterm 1

Average score: 74.4

Median score: 77



Name: _____

NAME: _____

TA (circle one) Jody Westbrook or Jessica Piel
Section (circle one) Tue Wed Thur

MCB 141 – First Midterm Feb. 21, 2008

Only answer 4 of these 5 problems. You MUST put an “X” below on this page to indicate the question that you are NOT answering. Only 4 out of the 5 questions will be graded.

Write your name on each page of the exam. Write your answers with a pen. If you need extra space, continue on the back of the exam pages, but clearly indicate this on the front page of the question.

Problem 1 (25 points) _____/25

Problem 2 (25 points) _____/25

Problem 3 (25 points) _____/25

Problem 4 (25 points) _____/25

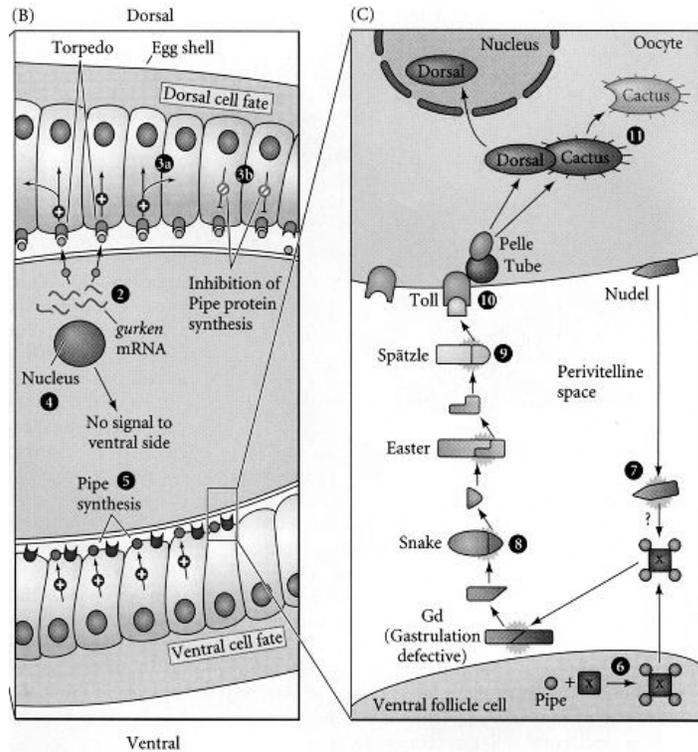
Problem 5 (25 points) _____/25

TOTAL (100 points) _____/100

Name: _____

Problem 1)

Here is a schematic of the dorsal-ventral patterning pathway in *Drosophila*:



Describe the phenotype (normal, dorsalized, or ventralized) of the following embryos that come from mothers with the following genotypes:

a. 1 point) Homozygous mutant for complete lack of function for Toll.

Dorsalized

b. 1 point) Homozygous mutant for complete lack of function for cactus.

Ventralized

c. 1 point) Homozygous mutant for complete lack of function for easter.

Dorsalized

d. 1 point) Homozygous mutant for complete lack of function for torpedo.

Ventralized

Name: _____

e, 2 points) Homozygous mutant for complete lack of function for torpedo AND easter.
Explain in 1-3 sentences.

Dorsalized. Easter is downstream of torpedo. Torpedo activity normally inhibits pipe function dorsally, but even with pipe function on everywhere, the cascade is still stopped by lack of easter, and the embryo is dorsalized.

f, 2 points) Homozygous mutant for complete lack of function for Toll AND easter.
Explain in 1-3 sentences.

Dorsalized. Both mutations cause dorsalization. Toll cannot activate the degradation of cactus regardless of what has happened to easter.

g, 2 points) Homozygous mutant for complete lack of function for cactus AND easter.
Explain in 1-3 sentences.

Ventralized. Cactus is downstream of easter. If cactus is not functional, Dorsal will enter the nucleus regardless of the state of easter.

h, 1 point) You obtain some embryos that have just been laid by a female homozygous for a complete lack of function mutation in the snake gene. What is the phenotype you expect for these embryos?

Dorsalized.

Name: _____

i, 3 points) You inject these same eggs described in (h) above (within the first few minutes of egg laying) with some synthetic full-length snake protein. Your injection is into the perivitelline space that completely surrounds the embryo. What is the phenotype you expect for these embryos? Explain your answer in 1-3 sentences.

Normal (or wildtype). The synthetic snake protein will only be cleaved and activated on the ventral side (where activated Gd is present)

j, 3 points) You inject these same eggs described in (h) above (within the first few minutes of egg laying) with some synthetic snake protein that you have pre-cleaved to yield the active form of the snake protein. Your injection is into the perivitelline space that completely surrounds the embryo. What is the phenotype you expect for these embryos? Explain your answer in 1-3 sentences.

Ventralized. Since activated snake is everywhere, it will set off the signaling cascade all around the embryo, leading to a ventralized phenotype.

Name: _____

k, 4 points) You inject these same eggs described in (h) above (within the first few minutes of egg laying) with some synthetic easter protein that you have pre-cleaved to yield the active form of the easter protein. Your injection is into the perivitelline space that completely surrounds the embryo. What is the phenotype you expect for these embryos? Explain your answer in 1-3 sentences.

Ventralized. Easter is downstream of snake, and will thus bypass the lack of snake. However, since activated easter is now all around the embryo, the embryo will be ventralized.

l, 4 points) You inject these same eggs described in (h) above (within the first few minutes of egg laying) with some synthetic Gd (gastrulation defective) protein that you have pre-cleaved to yield the active form of the Gd protein. Your injection is into the perivitelline space that completely surrounds the embryo. What is the phenotype you expect for these embryos? Explain your answer in 1-3 sentences.

Dorsalized. Gd is upstream of snake, so activated Gd will make no difference since snake is still absent, stopping the cascade and resulting in a dorsalized phenotype.

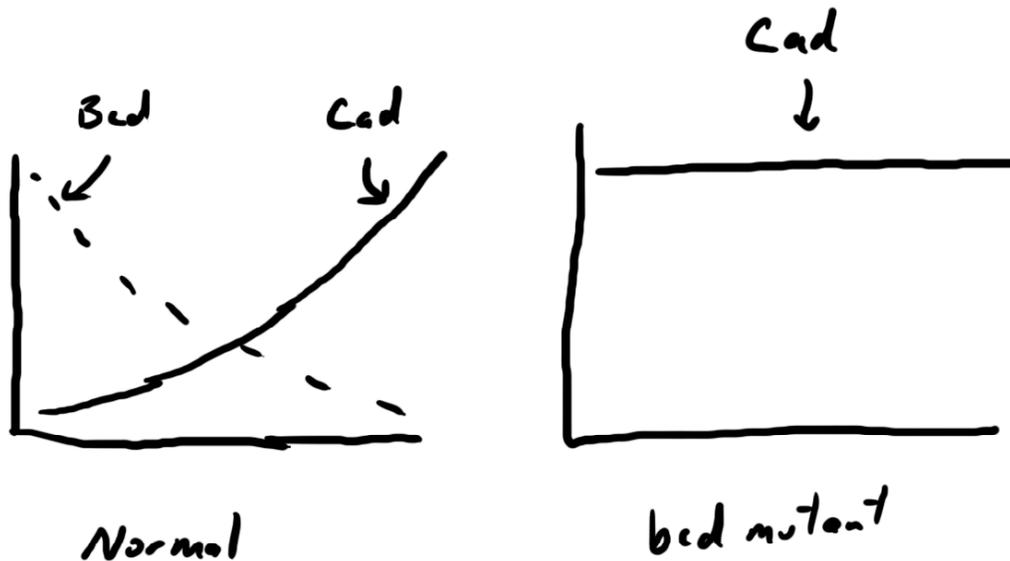
Name: _____

Problem 2)

You isolate embryos laid by a female *Drosophila* that lacks both copies of the bicoid gene.

a. 6 points) Describe (in 1-3 sentences or with a clearly labeled diagram) the spatial distribution of caudal protein at the syncytial blastoderm stage that is made from the corresponding maternal caudal mRNA in these mutant embryos (laid by female homozygous mutant for bicoid lack of function mutation). Explain in an additional 1-3 sentences how and why this distribution is different from that seen in normal embryos.

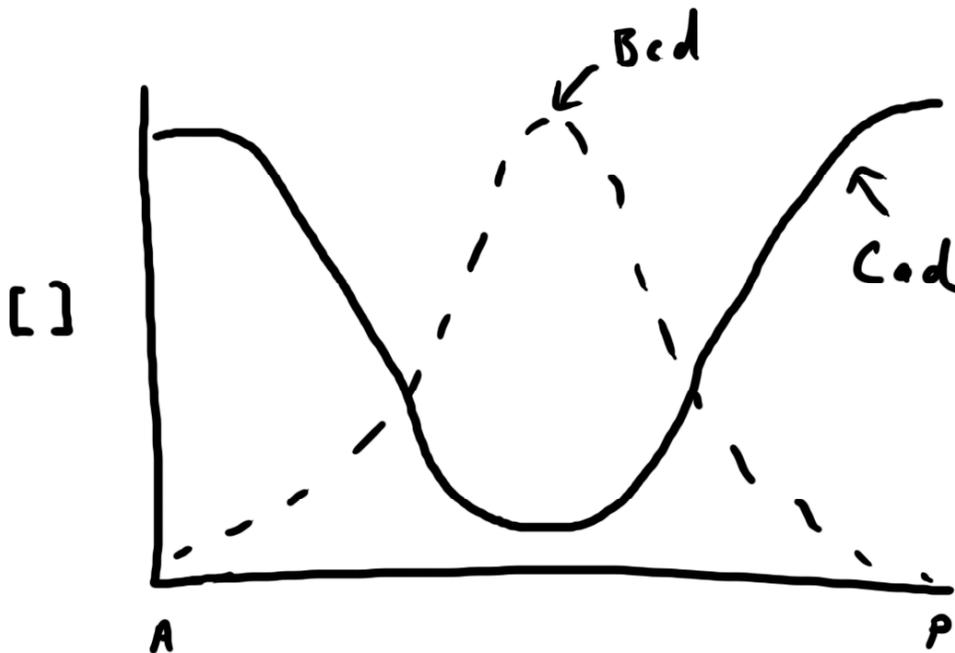
In the bicoid mutants, caudal protein is distributed uniformly throughout the embryo, just as is the caudal mRNA. In a wild type embryo, caudal protein forms a concentration gradient that is highest at the posterior end of the embryo and then decreases anteriorly. This is because the bicoid protein, which is in an anterior to posterior gradient, represses the translation of the uniformly distributed caudal mRNA.



Name: _____

Into some freshly laid mutant embryos (laid by females homozygous mutant for a bicoid lack of function mutation) you inject synthetic bicoid mRNA into the middle of the egg. b, 9 points) Describe (in 1-3 sentences or with a clearly labeled diagram), the spatial distribution of caudal protein at the syncytial blastoderm stage that is made from the corresponding maternal caudal mRNA in these embryos. Explain how this distribution is achieved in an additional 1-3 sentences.

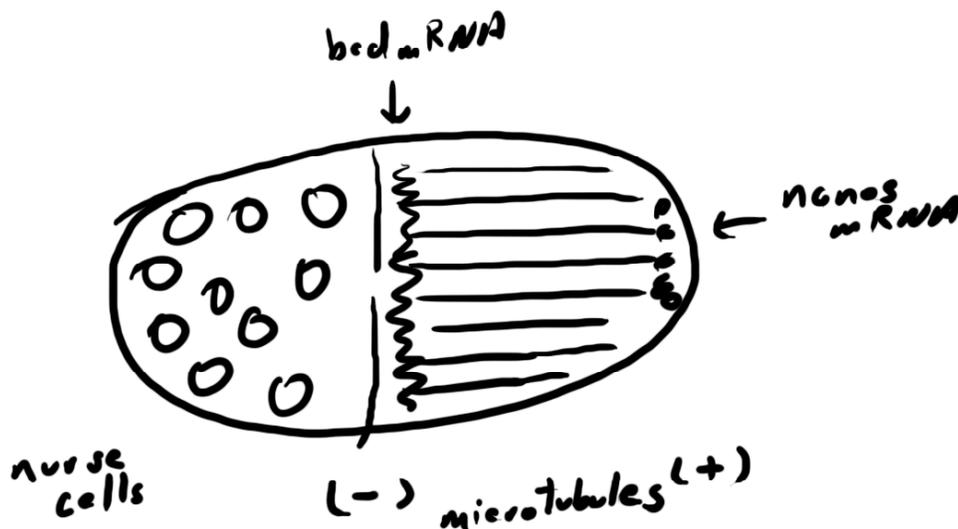
Caudal protein concentration will be highest at the anterior and posterior ends of the embryo, and will be lowest in the middle. Caudal mRNA translation will be inhibited by Bicoid protein, and since you injected bicoid mRNA into the middle of the embryo, Bicoid protein will be in a gradient highest in the middle of the embryo, and getting lower towards each end.



Name: _____

c. 10 points) Describe in 3-6 sentences (plus any diagrams you wish to use) the mechanisms that cause bicoid and nanos mRNAs to be localized during *Drosophila* oogenesis [note: its okay if you don't specifically remember which end is (+) versus (-)].

In the oocyte, the microtubules are organized (polarized) so that the (-) ends are at what will become the anterior end of the embryo and the (+) ends are at what will become the posterior end. Bicoid mRNA associates with motor proteins that move to the (-) end of the microtubules and nano associates with motor proteins that move to the (+) end. The mRNAs are thus localized to opposite sides of the oocyte, with bicoid at what will become the anterior and nanos at what will become the posterior end of the embryo.



[Note: some of you provided the more detailed information that was in the textbook. This was fine as long as you made mention of the polarization of the cytoskeleton and the importance of directed motors in the overall process]

Name: _____

Problem 3)

a, 7 points) Describe in 3-6 sentences how the cuticle patterns of mutant embryos can be used to distinguish between gap, pair-rule, segment polarity, and homeotic mutants.

In gap mutants, several contiguous segments are missing. In pair-rule mutants, deletions are seen in a two segment periodicity. In segment polarity mutants, all the segments are present but pattern within each segment is abnormal (deletions with duplications sometimes). In homeotic mutants, all the segments are present but segment identities are altered [Note: some points were deducted if you said that transformations were always posterior segments taking on anterior identity, as the reverse definitely occurs].

Name: _____

b. (10 points) Describe how the information provided by Krüppel, giant, bicoid, and hunchback is used to create the expression pattern seen for *eve* stripe 2. Use diagrams as needed.

The *eve* stripe 2 enhancer is activated by bicoid and hunchback and repressed by Krüppel and giant. The resulting stripe of *eve* expression will occur between the expression domains of giant and Krüppel, where hunchback and bicoid concentrations are still sufficiently high (see diagram below).



Name: _____

c, 8 points) Both wingless and engrailed are segment polarity genes and in a wild-type embryo they are expressed in adjacent cells within each segment. In an embryo homozygous for the complete loss of the wingless gene, engrailed mRNA and protein expression begins normally (end of the blastoderm stage through the onset of gastrulation), but then fades away. Explain in 3-6 sentences why engrailed expression starts out normally in these wingless mutants, but then fades away.

Engrailed expression is initially regulated by pair-rule genes. However, pair-rule gene regulation of engrailed stops after gastrulation, and engrailed expression is maintained by a positive feedback loop with the adjacent row of cells that express wingless. Thus when wingless is missing, pair-rule gene expression will still ensure that engrailed expression starts normally, but the absence of the feedback from wingless will cause engrailed expression to fade later.

Name: _____

Problem 4)

a, 7 points) In *Drosophila*, Ubx expression inhibits the formation of appendages in the first abdominal segment, but Ubx expression also makes the appendages of the third thoracic segment different from the appendages of the second thoracic segment. At first these two functions seem contradictory. Explain in 5-8 sentences how it is that Ubx can play both roles.

Explanation comes from temporal differences in the spatial distribution of Ubx. Early in development Ubx is expressed in the abdomen, but not the thorax. At this time Ubx inhibits Dll expression in A1 – a sign that it is repressing appendage primordia from forming in A1. Later, Ubx expression begins in the T3 segment. However, at this point Dll has become autoregulatory, and Ubx is no longer capable of inhibiting appendage formation in T3. Ubx expression in T3 does, however, cause these appendages (halteres for example) to be different from the ones that form in T2.

Name: _____

You discover a new crustacean species in which there are 8 thoracic segments and 6 abdominal segments. All eight thoracic segments have identical large walking legs. Both Ubx and abd-A in this species are uniformly expressed (at the mRNA and protein levels) at all stages in all thoracic segments and all abdominal segments. All six abdominal segments are identical and have identical small feathery legs that are used to hold developing eggs. Abd-B is uniformly expressed at all stages in all six abdominal segments and is not expressed in the thoracic segments.

b, 4 points) Predict the phenotype, in terms of leg patterns, that you would expect if you had a mutant of your new crustacean species that lacked all Abd-B expression. Explain your answer in 1-3 sentences.

It would have all large legs (14 pairs of large legs and no pairs of feathery legs) because all of the abdominal segments would take on the identity of T8. This would occur because Abd-B would no longer be present in the abdominal segments to repress Ubx and Abd-A activity in these segments. Segments now lacking Abd-B expression would take on the identity of segments anterior to them.

c, 4 points) Predict the phenotype, in terms of leg patterns, that you would expect if Abd-B were to be uniformly expressed at all stages throughout the entire thorax and abdomen of your new crustacean species. Explain your answer in 1-3 sentences.

It would have all feathery legs (14 pairs of feathery legs and no large legs). This is because Abd-B would repress Ubx and Abd-A function in all of the segments, and following posterior prevalence, cause all of the thorax segments to take on the identity of abdominal segments.

Name: _____

d, 8 points) You isolate a new mutant allele of Ubx in Drosophila. It creates a dominant phenotype in which the wings of the adult fly are transformed into halteres (there are no embryonic defects). You find that this mutation does not alter Ubx expression (at either the mRNA or protein level) during embryogenesis, but during late larval and pupal development it causes Ubx mRNA and protein to be expressed more anteriorly than normal – that is to say that you observe Ubx expression in what should be the wing disks of T2. Explain in 1-3 sentences how this mutation might be acting to change Ubx expression. In an additional 1-3 sentences, explain why this is a dominant instead of a recessive allele of Ubx.

This is a regulatory mutation in Ubx, altering an enhancer region, that causes Ubx to be misexpressed in the T2 wing disks. Ubx expression is normally repressed in T2, but this mutation causes the repression to be relieved. This is a dominant phenotype since it involves inappropriate additional expression, which would occur with even just one copy of the mutant allele.

[Note: some of you answered that the altered expression of Ubx might be due to changes in the expression of a repressor (hunchback for example), but since the question stated that this was an allele of Ubx, this cannot be correct as the mutation has to be in the Ubx gene itself and not in an upstream regulator.]

Name: _____

e, 2 points) You examine the mRNA expression pattern of the pair-rule gene even-skipped in a blastoderm stage embryo that is homozygous mutant for a complete loss of function allele of Ubx. In 1-2 sentences, describe the pattern that you would see.

Even-skipped should be expressed in its normal pattern of seven stripes (since pair-rule genes are not regulated by Hox genes).

Name: _____

Problem 5)

While exploring in the Amazon, you discover two new species of beetles that you name species A and species B. Both species have 8 abdominal segments. In species A, the first two abdominal segments are blue, and the remaining six are red. In species B, the first four abdominal segments are blue, and the remaining four are red.

You carry out a genetic screen in species A, and discover a mutant with all blue abdominal segments. Further analysis of the mutant leads you to discover gene Q, which is normally expressed (both mRNA and protein) in the last six abdominal segments of species A. If gene Q is eliminated, the abdomen of species A becomes completely blue. If you express gene Q in all the abdominal segments of species A, the animals now have abdomens in which all eight segments are red. You also discover that species B has a gene that encodes a protein identical to that encoded by gene Q in species A, but in species B, this gene Q is normally expressed (both mRNA and protein) in just the last four abdominal segments.

You isolate the regulatory region for gene Q from species A. When combined with a lacZ reporter gene, this regulatory region from species A gives the expression of lacZ in the last six abdominal segments when placed into species A. You also isolate the regulatory region for gene Q from species B. When combined with a lacZ reporter gene, this regulatory region from species B gives the expression of lacZ in the last four abdominal segments when placed into species B.

You hypothesize that the differences in expression of gene Q between species A and B are responsible for the coloration differences between the two species. You now wish to test whether the differences in expression are due to differences in the enhancers of gene Q between species A and B (cis regulatory changes), or if the differences are due to differences in genes that regulate gene Q transcription (trans regulatory changes).

Name: _____

a, 6 points) In 4-6 sentences, explain the experiment you would carry out to test the two possibilities (cis versus trans).

To test this, you would take the lacZ reporter with the A enhancer for Q ($Q_{A-Enh}:lacZ$) and place it in species B. Also, take the lacZ reporter with the B enhancer for Q ($Q_{B-enh}:lacZ$) and place it in species A. Then, monitor the expression pattern of lacZ in the two transgenic organisms.

Name: _____

b, 6 points) Describe in 2-4 sentences the outcome of your experiment if the answer is that “cis” changes are responsible.

Expression follows the source of the enhancer, and not that of the host. The B organism carrying the $Q_{A-Enh}:$ lacZ reporter gene would expression lacZ in the last 6 abdominal segments, characteristic of the Q expression pattern in species A. Also, the A organism carrying the $Q_{B-Enh}:$ lacZ reporter construct would show lacZ expression in the last four abdominal segments, characteristic of the Q expression pattern in species B.

c, 6 points) Describe in 2-4 sentences the outcome of your experiment if the answer is that “trans” changes are responsible.

Expression matches that of the host, and not that of the source of the enhancer. The B organism carrying the Q_{A-Enh} lacZ reporter construct would show lacZ expression in the last 4 abdominal segments, characteristic of the Q expression pattern in species B. The A organism carrying the Q_{B-Enh} lacZ reporter construct would show lacZ expression in the last 6 abdominal segments, characteristic of the Q expression pattern in species A.

[Note: some of you switched the complete genes between species, and talked about how the actual color of the segments would change. This is fine, but you lost some points if your answer in part (a) used lacZ constructs as this would not change the actual color of the segments and could only be seen by detecting the lacZ mRNA or protein product.]

Name: _____

d, 7 points) Imagine that you find that “trans” changes are responsible for the difference between the two species. In species A, you discover genes X and Y. Gene X encodes a protein X that is expressed in all segments except the last six abdominal segments of species A. Gene Y encodes a protein Y that is found everywhere in the animal. You find that the X protein directly binds to the enhancer of gene Q and represses the transcription of gene Q, while the Y protein binds to the enhancer of gene Q and activates transcription. Predict the pattern you might expect to see for gene Q (CORRECTION announced during the test - this should be gene X, not gene Q) in species B and how this would account for the “trans” result you obtained.

X would be expressed in the first four abdominal segments of species B (or could say everywhere except the final four abdominal segments; Y would presumably still be everywhere). This accounts for the trans results because there is no difference between the cis regulatory elements of gene Q in species A and B. The different Q expression patterns (and lacZ patterns) result instead from the different expression domains of the X protein (which repressed Q expression) between these two species.

Problem 6) 0 POINTS

Explain the sentence

“Time flies like an arrow, fruit flies like a banana”

No explanation needed, the humor of Groucho Marx is timeless