FACULTY: Ben Cheyette (6 points)

Question #1 (6 points):

The reasons why scientists, as individuals or collectively, favor one theory over another are not always obvious – but are worth thinking a bit about. Vincent B. Wigglesworth was on the right track in 1940 when he proposed that bristle-forming plaques in the larvae of his favorite blood-sucking bug have an inhibitory influence on neighboring cells that prevents them from also becoming bristles. But he appears to have strayed off course when he proposed as a mechanism that these plaques draw a positive "essential element" for bristle formation from the zone of tissue surrounding them, and that the limiting amounts of this essential element thereby prohibits too many bristles from forming in close proximity to each other.

1a. Given his results, can you think of a reason why he chose this mechanism over other possibilities? Are any of his observations more consistent with this model than with what we now call lateral inhibition/specification? Or do you think he might have favored his model for some other reason (*e.g.* his non-scientific experience, cultural background, anthropomorphic thinking, limited knowledge about something we now understand better, etc)?

1b. If he had thought of it, is there an experiment he could have done in his own day to distinguish between a diffusible positive signaling factor *versus* a non-diffusible inhibitory signaling mechanism?

1c. How might a system of lateral inhibition/specification cooperate with a system based on a diffusible positive factor? What are some possible developmental, evolutionary, or other advantages of combining the two? Using the experimental techniques you have at your disposal today, can you think of a way to test whether two such mechanisms cooperate - in any system where most scientists today believe that lateral specification is chiefly at work?

FACULTY: Eric Huang (12 points)

Nucleus of Virchow, located in the anterior thalamus, is a highly evolutionarily conserved structure in the vertebrate brains that controls intelligence (*hypothetical, do not look it up on Pubmed or Wiki*). You are in a race to identify a neurotrophic factor that supports survival, dendritic growth and synapse formation of neurons located in this nucleus. To your advantage, you have generated a knock-in mouse line, *NucVir-DsRed*, in which the reporter DsRed is robustly expressed in the cell body and neuronal processes of all mature neurons in the Nucleus of Virchow. Using biochemical approaches, you have also isolated a candidate factor called VPNF (*V*ery *P*romising *N*eurotrophic *F*actor) that seems to promote survival of neurons in the Nucleus of Virchow.

Question #1 (4 points): In addition to the survival assay, propose two assays to prove that VPNF is indeed a neurotrophic factor for neurons in the Nucleus of Virchow. At least one of your experiments must use mouse genetics approach to demonstrate the *in vivo* properties of VPNF.

Question #2 (4 points): Sequence analyses show that VPNF protein has a "pro" domain (containing 50 amino acids) that requires to be cleaved to generate the mature and biologically active form of VPNF. Interestingly, Human Genetics analyses show that Val-to-Met polymorphism on amino acid #48 is associated with mental retardation.

(2a) Propose a hypothesis to explain how the Val-to-Met polymorphism might affect the biological activity of VPNF.

(2b) Propose at least two experiments to test your hypothesis.

Question #3 (4 points): Your talented graduate student developed a highly sensitive in situ probe that specifically labels *VPNF* mRNA. She found that *VPNF* mRNA were highly abundant in the dendrites of neurons in the Nucleus of Virchow, and was eager to propose this as her PhD Candidacy Exam. What would be her hypothesis and how to test it? She may propose either *in vitro* or *in vivo* approaches, but keep in mind that her assays need to focus on the biological functions of VPNF.

FACULTY: Erik Ullian (12 points)

Question 1 (6 points)

You have discovered a novel muscle protein that you think interacts with MUSK and its co-receptor. You think this protein might be secreted and could explain the pre-patterning of muscle nAChRs seen before axons innervate the muscle. What experiments could you do to test if this protein is required for pre-patterning? How can you show a direct interaction with MUSK?

Question 2 (6 points)

Two observations in studies of synaptic competition at synapses innervated by two axons at the NMJ are: 1) if action potentials and presumably presynaptic release is impaired at one axon, the impaired axon will "lose" and retract from the muscle synapse; leading to the idea that there is an activity dependent synaptic competition that drives competition. And 2) if an axon innervates multiple muscles and is competing at one of those synapses with an axon that only innervates that single muscle, the axon with only one synapse will always "win"; leading to the idea that there may be limited resources that determine the outcome of competition. Do these two observations suggest different mechanisms driving competition or could they be the same? With any tools and methods you can think of, how would you test this?