

November 16, 1998

ID number _____

Biochemistry Cumulative Exam

The Premise:

Today's biology is tomorrow's biochemistry. You should be able to pick up any current issue of Science, Nature, or Cell and find an article of interest, do some reading in the library to research the topic, and be ready to propose biochemical experiments or a whole project that would further our knowledge of that particular topic. In the future, this will be the most critical part of your job as a trained biochemist. Of course, in order to be able to do this, you need to be conversant with contemporary techniques of biochemical analysis and experimentation. This exam will test not only your general knowledge of biochemistry, but your ability to research an unfamiliar topic and propose intelligent experiments to test current ideas in that topic. If you fail this test, then at least you know what you need to work on. Good luck.

Question	Maximum points	Points received
1	7 (<u>must</u> answer)	
2	9	
3	6	
4	9	
5	6	
6	7	
7	5	
8	9	
9	9	
10	5	
11	28	
Total	100	

The topic:

1) Briefly describe what autophagy is. Is it a constitutive or induced process or both? Under what conditions would it be induced? Where in the cell does it take place? (This question is only worth 7 points, but you must be able to answer it. If you cannot do so, then fold up the test and turn it in – you have failed it.)

2) Many of the experiments described in this paper (Mizushima et al, 1998) rely on the analogy with the ubiquitin system. (3 pts each)

a) Describe briefly the ubiquitin system, answering (at least) the following questions: What is ubiquitin? What enzymes operate upon it? How is it activated for its biochemical function?

b) What sort of linkage does ubiquitin make with other molecules? What is an "isopeptide" bond?

c) What happens to the molecules to which ubiquitin is attached? There are several answers to this last question; give at least one.

3) What differences are apparent between the ubiquitin system and the autophagy system? (6 pt)

This paper:

4) Explain the analogy between the ubiquitin system and the system made in this paper. (i.e. Which Apg gene products would be the equivalent of which proteins in the ubiquitin pathway? – ubiquitin, E1, E2, E3, etc...) (9)

5) The authors mention that the vacuolar enzyme aminopeptidase I (API) is not processed from the precursor to the mature form in the *apg* mutants. Describe what is known about how API is transported to the vacuole and this process is unusual. What inferences can be made about the relationship between the defects in the vacuolar import of API and the autophagy process?

6) In Figure 1b, the presence/absence of a ca. 70 kDa band was observed in various *apg* mutants (*apg1*, *apg5*, *apg7*, & *apg10*). If this analysis were continued for all *apg* mutants (and it was – see the paper), what inferences could be drawn about the role of the *APG* gene products in the pathway of autophagy? (I am thinking specifically in terms of their point of action.)

7) The experiments in this paper employ extensively the technique of "epitope tagging." Briefly describe this technique and how it was specifically used in these experiments. (5 pts)

8) The authors hypothesize that there exists a specific covalent linkage between the Apg5 and Apg12 polypeptides. Clearly state this hypothesis. Then list all of the predictions that this hypothesis makes which were tested by experiments reported in this paper. (Hint: I do not mean just the experiments mentioned after the authors statement of their hypothesis, but all experiments that have any bearing on this hypothesis.) In each case, indicate if the predicted result was obtained or not, and mention any criticism that you think the experimental design and interpretation deserve.

9) The Apg12-Apg5 conjugate is purified and subjected to trypsin digestion. The resulting peptides are sequenced. One particular peptide gives strange results when sequenced by Edman degradation. This is a representation of the residues obtained upon the first 10 rounds of degradation. The first 6 rounds produced about equimolar amounts of 2 different residues, while the 7th round gave a very strange peak ("X").

1	2	3	4	5	6	7	8	9	10
F	W	F	H	L	W	V	E	V	C
T	D	D	E	E	I	X	tr	tr	tr

X = Unknown

tr = traces (unidentifiable)

(Note: D = Asp or Asn; E = Glu or Gln)

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AUTOPHAGY PROTEIN Apg5                                ACCESSION 2498143
1  MNDIKQLLWN GELNVLVSID PSFLMKGSPR EIAVLRIRVP RETYLVNYMP LIWNKIKSFL
61 SFDPLTDSEK YFWFEHNKTP IPWNYVPGVL FDCLAGKSAT FTTSFENQVK DVLTFRLRIHL
121 VMGDSLPTTI IPIASSKTQA EKFWFHQWKQ VCFILNGSSK AIMSLSVNEA RKFWGSVITR
181 NFQDFIEISN KISSSRPRHI PLIIQTSRTS GTFRISQPTI SMTGVNPTLK DIEGDILDVK
241 EGINGNDVMV ICQGEIPWH MLLYDLYSKL RSFDGFLYIT LVPIKGGDKA SSEL

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Given the sequence of Apg12 in the paper and the sequence of Apg5 shown above, explain these anomalous results. What is the origin of this peptide? Draw the region of the peptide that caused the X during the peptide sequencing; you may be very schematic about most of the peptide, but I want to see it in molecular detail (i.e. show all bonds, except those to hydrogen) around the critical bond that causes the strange peak.

Future experiments:

(In this section, you are asked to put yourself in the place of a biochemical researcher preparing to perform experiments on the topic described in this paper.)

10) The paper mentions that the yeast APG5 and APG12 genes have animal homologs. What use of this fact could be made in a grant proposal?

11) Come up with 2 new hypotheses regarding the autophagy system in yeast that you have been reading about. For each hypothesis, propose a critical experiment or series of experiments designed to test it. You may use any of the reagents, assays, mutants, etc. that you have encountered during your reading (I would like a reference for anything truly out of the ordinary) You may assume that you would be able to purify any proteins involved, although it would be better if you would detail how you would go about purifying them, and any techniques that would make that job easier. Each hypothesis-experiment pair is worth **14 points**.