Here's a longer, more complex (and wordier) version of the same question (used for a take-home Final Exam!). Work your way through the data and questions and see if you come away with a similar understanding of plasma membrane recycling.

6. (45 pts) Cells and their organelles are dynamic entities. In particular, cellular membranes seem constantly in motion, carrying out their various functions *and* being newly synthesized, degraded, recycled or transformed. The existence of these dynamic phenomena is well-document, but we do not yet completely understand how the various processes are regulated.

For example, under some circumstances a portion of the plasma membrane (PM) may be endocytosed and become associated with lysosomal vesicles. In another context, labeled PM becomes associated with different, non-lysosomal organelles following endocytosis. (See data from the previous question, reproduced in more detail in i. And ii. Below.) Data from more recent research, carried out in a laboratory near the one that produced the data in i. And ii., are presented in iii. below. You may assume all three data sets were obtained under similar conditions unless described differently.

I. Many cells that secrete hormones are themselves under hormonal control. One such cell, the pituitary gonadotroph, secretes two polypeptide hormones (LH and FSH) when stimulated by GnRH (Gonadotropin-releasing Hormone). To investigate the regulation of LH and FSH secretion, a biologically active, radioactively iodinated (^{125}I) analogue of GnRH was injected into rats. At varying periods of time post injection, pituitary glands were fixed and sectioned, and the sections were then examined by EM-autoradiography. Photographic grains resulting from radioactive decay were counted and their location in the sections noted. These data are presented in the table below, as the **percentage of total grains per cell**.

	Time Post Injection, min			
Location	**5**	**15**	**30**	**60**
1. Plasma Membrane	60%	15%	20%	5%
2. AP'ase-positive vesicles	20	30	60	80
3. GalTran'ase-positive vesicles	2	2	2	2
4. rER	3	3	2	2
5. Nucleus	1	1	1	1
6. Secretory Vesicles	5	8	9	8

Note: "AP'as- positive" indicates the presence of acid phosphatase;
"GalTran'ase-positive" indicates the presence of galactosyl transferase.

II. In another set of experiments using a culture of cancerous bone-marrow cells (myeloma), an iodination procedure was used to label plasma membranes directly, using ^{125}I. The fate of the radioactive label was then followed with EM-autoradiography. Chemical analysis of the iodinated species revealed 15% of the label was covalently bound to membrane lipid and 85% to IMP. (Separate experiments confirmed the labeling process did not affect the viability of

these cells or their ability to secrete IgG.) Cells fixed 5 and 60 min after labeling showed the following distribution of grains.

Location	Time Post Labeling, min	
	5	60
1. Plasma Membrane	85%	55%
2. AP'ase-positive vesicles	2	2
3. GalTran'ase-positive vesicles	2	25
4. rER	1	1
5. Nucleus	1	1
6. Secretory Vesicles	2	10

III. The internalization and intracellular transport of the Fc receptor (FcR was examined in cultures of mouse macrophages using ^{125}I-labeled antibodies against the receptor. Following a brief pulse for labeling the receptors, labeled antibodies remaining at the surface were removed with a brief wash at pH 4.0, cells were incubated for varying periods of time and the intracellular fates of the label was monitored by cell fractionation and radioisotopic assay. The data in Table I was obtained using antibody fragments (F'ab) with only one receptor binding domain; those in Table II made use of intact IgG (with two receptor binding domains).

Table I

Location	Time Post Washing, min			
	5	15	30	60
1. Plasma Membrane	10%	40%	50%	60%
2. Lysosomes	8	9	7	7
3. Trans-Golgi vesicles	3	4	3	5
4. Cis-Golgi vesicles	4	5	5	4
5. rER	1	1	1	1
6. Nucleus	2	1	2	1
7. Secretory Vesicles	62	40	32	22

Table II

Location	Time Post Washing, min			
	5	15	30	60
1. Plasma Membrane	12%	8%	7%	7%
2. Lysosomes	60	60	50	50
3. Trans-Golgi vesicles	5	10	12	11
4. Cis-Golgi vesicles	4	5	5	4
5. rER	1	1	1	1
6. Nucleus	1	2	1	1
7. Secretory Vesicles	18	17	25	28

A. (25 pts) Discuss critically and as completely as possible what these data indicate concerning the regulation of plasma membrane flow/turnover during brief periods in the lives of cells. Illustrate your discussion with well-labeled diagrams and attend closely to the following questions:

i. what components in each experiment are being labeled and traced?
ii. what appear to be the fate of these components?
iii. how do these results compare with other endocytotic experiments you have examined: e.g., LDL uptake by fibroblasts?
iv. are the date equivocal – that is, open to different interpretations? How so?
v. what additional experiment could be performed to remove ambiguity or to test your hypothesis concerning plasma membrane turnover?

B. (10 pts) The data in all experiments represent *relative* amounts of label in each fraction at the indicated time points. Considering both parts of Experiment III, would you expect the *total* amount of label within the cell to vary among the time periods. Why or why not?

C. (10 pts) Given your answer in C. above, hypothesize how the insertion of newly synthesized F_C R into the plasma membrane might occur and how the insertion might be regulated.