Learning Group 5 (Task 1)

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Title	A functional state transition in the fate of EGFR trafficking.
Introduction/ Description	Following its stimulation with EGF, the EGFR is internalized through either clathrin-mediated endocytosis or non-clathrin endocytosis. However, the intracellular sorting mechanisms of EGFR are dependent on specific molecular events, and may result in EGFR recycling or degradation.
Question	Which of the following most accurately describes the fate of EGFR following its endocytosis under normal physiological conditions?

Choice 1	Following internalization downstream of stimulation with a low concentration of EGF, EGFR is exposed to a lower pH at the early endosome. Due to the acidity of the early endosome, Cbl ubiquitinates EGFR, which becomes irreversibly targeted to the lysosome via intralumenal vesicles, where it is degraded by proteases.
Choice 2	The fate of EGFR is entirely determined by the clathrin-dependent nature of its internalization: clathrin-mediated endocytosis results in EGFR recycling, whilst non-clathrin endocytosis results in lysosomal degradation.
Choice 3	Following either clathrin-mediated or non-clathrin endocytosis, the EGFR may be recycled to the plasma membrane, or degraded in the lysosome. These processes are driven by limited versus extensive EGFR ubiquitination, respectively. The ability to achieve a high activity state of the E3 ligase Cbl and a stable Cbl-EGFR interaction is crucial for EGFR degradation.
Choice 4	In low concentrations of EGF, internalization of EGFR occurs by clathrin-mediated and clathrin-independent endocytosis, which may result in receptor recycling or receptor degradation depending on the action of ubiquitin-modifying enzymes.

Feedback sheet – Please **label** the feedback to the choices as “CORRECT” or “INCORRECT”. Provide detailed feedback to explain why the choice was correct or incorrect.

Feedback Choice 1	INCORRECT. Whilst the pH at the EE is lower than the cytosol, the change in pH has no effect on the E3 ligase Cbl, since it is cytosolic. Cbl becomes active due to phosphorylation by EGFR, and its activity is independent of endosomal pH. Furthermore, Cbl is primarily located at the plasma membrane (see choice 3).
Feedback Choice 2	PARTIALLY CORRECT. The key fate determinant of recycling vs degredation is the Ub state of the EGFR receptor. Ub EGFR is recognized by the ESCRT complex factor Hrs, which leads to internalization of the receptor into ILV in LE and subsequent transport to lysosomes. It is true that non-clathrin endocytosis and clathrin-mediated endocytosis usually lead to degradation and recycling of EGFR, respectively, and this is linked to the likelihood of Ub. The fate decision can be reversed after internalization, due to EGFR (de-)ubiquitination, which is controlled by DUBs. Indeed, the action of DUBs, which removes ubiquitin chains from EGFR would lead to EGFR recycling downstream of non-clathrin endocytosis, since ESCRT can no longer bind the polyubiquitinated receptor and sequester it into ILVs.
Feedback Choice 3	CORRECT. EGFR recycling may occur only when it is not extensively ubiquitinated. This may correspond to limited ubiquitination following CME, or to removal of ubiquitin chains by DUBs, if Cbl has already ubiquitinated EGFR. In conditions of high EGF, non-clathrin endocytosis (occurring primarily at lipid rafts) can take place. Due to a lipid modification on Cbl, Cbl preferentially resides on the cytosolic face of lipid rafts. Thus, EGFR becomes more ubiquitinated at the plasma membrane during non-clathrin endocytosis compared to clathrin-mediated endocytosis. After non-clathrin internalization, interactions at the early endosome between ubiquitinated-EGFR and ESCRT lead to sorting of EGFR into intralumenal vesicles (ILVs), which are destined for lysosomal degradation. However, through the action of DUBs, EGFR de-ubiquitination may prevent interactions with ESCRT at the early endosome, leading to EGFR recycling. This demonstrates the control of EGFR fate through molecular events at the PM and EE, that are driven by EGFR ubiquitination.
Feedback Choice 4	INCORRECT. This answer would be correct if describing the effect of high EGF. Indeed, at low concentrations of EGF (such as those in plasma, serum and saliva), EGFR is internalized in a clathrin-dependent manor, whilst at high concentrations of EGF, both clathrin- and non-clathrin endocytosis occur. In non-clathrin

	endocytosis, the likelihood of EGFR ubiquitination at the plasma membrane is increased due to the increased likelihood of encountering Cbl. The action of Cbl and other ubiquitin-modifying enzymes defines EGFR sorting through determining the ubiquitination-depended recognition and sequestration of EGFR into ILVs by ESCRT.