

MCB 130 Final Questions, Spring 2002

1. Ribosome assembly begins in the nucleolus, a specialized compartment in the nucleus. Answer the following questions about this process.

(5pts) a. Ribosomal protein subunits have an NLS (nuclear localization sequence). True or False? Explain your answer briefly.

True. Ribosomal proteins must first be imported into the nucleus where they assemble into ribosomes which are then exported to the cytoplasm.

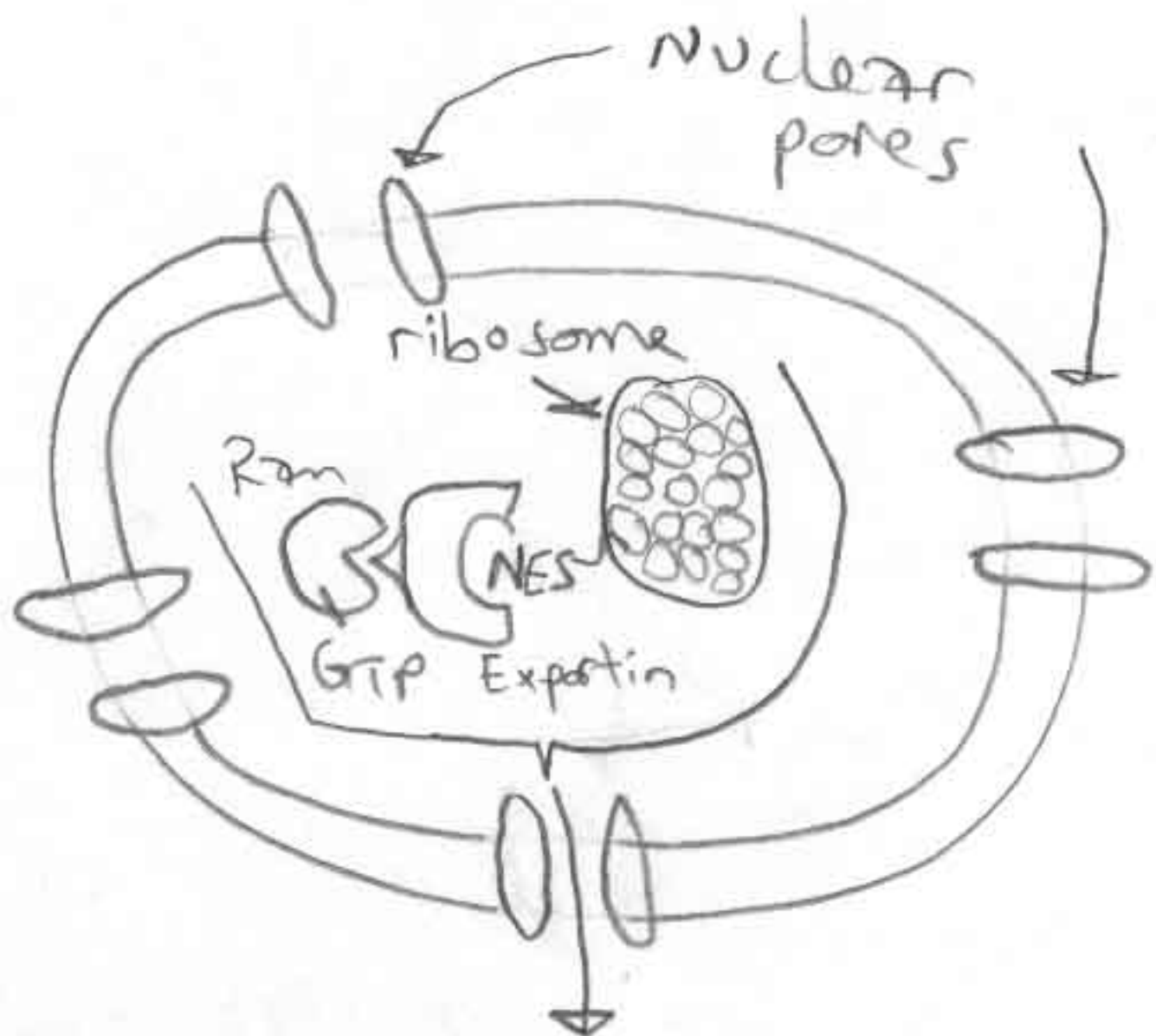
(5pts) b. Ribosomes assembled in the nucleolus have one or more NES (nuclear export signal). True or False? Explain your answer briefly.

True. The assembled ribosome must have at least one NES to allow the structure to be exported to the cytoplasm.

(5pts) c. Assembled ribosomes diffuse back and forth through the nuclear pore complex. True or False? Explain.

False. Ribosomes are much larger than the approx. 60KD size of a protein that is the upper size limit for diffusion through the nuclear pore.

(10pts) d. Provide a list of the nuclear transport factors required to get assembled ribosomes into the cytoplasm. Include a diagram of the process and indicate how each factor participates in the process.



assembled ribosome (or ribosome subcomplex) exposes NES which binds to exportin in the presence of Ran-GTP complex is transported through pore where it decomposes in the cytosol when Ran-GAP stimulates GTP hydrolysis by Ran.

2. Membrane fusion requires SNARE proteins that bridge adjoining membranes. One theory is that SNARE proteins are necessary and sufficient to catalyze fusion as long as one participating membrane has a v-(vesicle) SNARE and the other has a t-(target) SNARE.

(5 pts) a. Provide a brief explanation of the meaning of the two criteria, necessary and sufficient, in the context of a protein, such as a SNARE, which is essential for a cell process such as secretion.

necessary would mean, for example, that mutation of SNARE genes would produce cell deficient in a particular membrane fusion step.

sufficient would mean, for example, that SNARE proteins by themselves would be enough to catalyze membrane fusion. Thus, liposomes reconstituted with a v-SNARE in one and a t-SNARE in the other, when mixed together, would fuse their membranes to make larger, mixed liposomes.

(10 pts) b. Describe an experiment to show that SNARE proteins are necessary for membrane fusion.

isolate a SNARE mutation and show that a particular step in vesicle traffic is blocked at the fusion step

(10 pts) c. Describe an experiment to show that SNARE proteins are sufficient to catalyze membrane fusion.

reconstitute vesicles with a ^{pure} v-SNARE in one population of vesicles and a t-SNARE in another population. Mix the two together and observe fusion by content mixing or by an increase in vesicle size when the membranes mix.