

Secretion Homework Key

1. Can the Sec pathway be used to export essentially any protein? That is, if you fused a Sec-leader sequence to any peptides coding sequence, could you get that protein exported into the periplasm? Why or why not? Can you cite evidence from the text to support your answer?

No. The Sec system cannot push every protein through the channel. The book cites early studies when budding bacterial geneticists tried to do this by fusing genes encoding huge cytoplasmic proteins such as LacZ with secreted proteins. They jammed up the system and killed the bacteria. In fact, they used spontaneous mutants that failed to transport the hybrid proteins to identify components of the secretion system. (It looks like some of you didn't read this. Some got confused by the successful use of PhoA fusions to probe secretion, but PhoA is a naturally exported protein.)

2. Where does the energy come from for translocation with the SecB/SecA system versus the SRP system?

SecB/SecA – primarily ATP; SRP – the energy of protein synthesis/elongation (which is GTP)

3. Which gram-negative secretion systems do not directly rely on the Sec system to get the secreted protein outside of the cytoplasm?

Type 1 – ABC Type 3 – Injection Type 4- Injection
Type 6 – probably not (did anyone actually look at the linked comment on type VI secretion?)

4. What motif is common to all of the proteins that secrete proteins through the outer membrane?

Beta-Barrel. Please note that only some of the proteins will have disulfide bonds.

5. Which one of the terminal secretion systems would be most useful to researchers studying mammalian cell biology (not necessarily protein secretion)? Why?

Type 3 (or Type 4) because they inject proteins into host cells. Type 4 can also inject DNA, which is another interesting option.

6. If you wanted to design a plasmid vector to enable people get their favorite protein secreted by E. coli, even though it wasn't normally a secreted protein, which of the 6 systems would you choose? Why? Keep in mind that the standard laboratory strain of E. coli K-12 does not possess any of these 6 systems.

Type 5 Autotransporter – All of the other systems involve multicomponent apparatuses which would have to be included on your vector. However, the autotransporter system requires only a single gene/protein to do the job. Some groups gave already begun engineering type 5 systems for their use. I'll also accept Type 1.

7. How does the fate of proteins secreted by the sortase system of gram-positive bacteria differ from that of proteins secreted by gram-negative bacteria?

They are covalently attached to peptidoglycan or other cell wall components. The gram-negative secretion systems don't attached the proteins to anything.