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Short communication

An Activity for Cell Biology Course to Implement Active Learning

Surya Banerjee*

Department of Biological Sciences, Arkansas Tech University, USA

***Corresponding author:** Surya Banerjee, Biological Sciences Department, Arkansas Tech University, Russellville, USA.

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Abstract

Practices involving active learning engage students in doing activities that develop their understanding and higher order thinking in a lecture or laboratory course. The activities promote students' skill due to their dynamic participation in problem solving process through application of scientific principles, research techniques and tools. This article introduces a design of a class activity ideal for a Cell Biology course taught virtually or in-person. The activity includes training to retrieve sequence of a protein of interest from a database, feeding the sequence in freely available online bioinformatic tools that predict protein localization followed by a set of analytical questions to assess the structure and function of the protein. Practice of this inquiry-driven activity in a classroom can be extremely valuable for its practical use in research with novel proteins.

Keywords: Cell biology; Active learning; Protein localization; DeepLoc-1.0; SignalP-5.0; TargetP-2.0

Introduction

Study of a protein includes description of its structure, function, and localization. Proteins are synthesized by free ribosomes in cytoplasm or by bound ribosomes in RER (rough endoplasmic reticulum) from mRNAs during translation in eukaryotes. Subsequently, proteins localize within specific subcellular structure or extracellular space where they work. One or more amino acid signal sequences in a protein precisely determine in which cellular compartment and through what mechanism the protein will be synthesized, modified, sorted, and trafficked to reach its destination. For example, cytosolic proteins imported to nucleus (e.g., transcription factors, nuclear enzymes, histone proteins etc.) contain nuclear localizing signal (NLS); nuclear proteins exported to cytoplasm (e.g., ribosomal proteins) contain nuclear export signal (NES); "proteins synthesized in RER contain signal peptide (SP) (e.g., RER resident proteins such as chaperons, transposons, plasma membrane proteins with transmembrane domain(s) such as receptors, and secreted proteins such as hormones and enzymes in which SP is cleaved etc.)" contain signal peptide (SP) and/ or KDEL signal (e.g., RER resident soluble proteins in which SP is cleaved); and cytosolic proteins imported to mitochondria or chloroplast (e.g., enzymes that work in mitochondria and plastid respectively) contain different signal sequences for matrix, outer and inner membrane bound proteins of the respective organelles. Additionally, a set of possible distinct functions can be predicted for a protein depending on its localization [1]. Therefore, learning the use of bioinformatic tools that predict protein localization could be handy while characterizing novel proteins as shown in recent research articles [2].

Active learning engages students in learning by doing activities which enhance their learning experience [3]. Inquiry-driven class activities in which students analyze a case study or a primary research article using guided instructions and questions [4] often rely on the students' ability to combine and apply the learning outcomes from previously taught topics in a course [5]. Such practices improve understanding of a topic as they get involved in critical thinking [3]. Thus, an activity to find protein localization was designed and implemented for the first time in Spring 2021 iteration in an upper division undergraduate Cell Biology course. The activity utilizes databases to retrieve eukaryotic protein sequence, and subsequently submitting the sequence in

highly accurate and freely available bioinformatic tools that uses algorithms to predict subcellular protein localization depending on its amino acid sequence in the primary structure [6]. It will be appropriate to run the activity after covering the concepts of central dogma, protein structure and function, protein synthesis and protein sorting, trafficking, and localization in eukaryotes in the course. Students then will be able to appreciate the implication of the activity and to answer the follow-up analytical questions that connect the outcome of the activity with the concepts of protein sorting, trafficking, and function.

Methods

A protein of interest is selected. The FASTA file of the protein sequence is retrieved and copied from databases such as NCBI or fly base (flybase.org). The sequence is then submitted in the "DeepLoc-1.0: Eukaryotic protein subcellular localization predictor" and the outcome is recorded. The tool scores the likelihood of eukaryotic protein localization in 10 different compartments including nucleus, cytoplasm, cell membrane, extracellular space, RER etc., and predicts the final subcellular localization that has the highest score. The outcome also predicts the pathway of protein transport with more than 70 percent accuracy [6]. If it is predicted as a secreted protein, then the sequence is submitted in "SignalP-5.0 Server" to predict the cleavage site in the protein [6]. If it is predicted as a cytoplasmic protein the sequence is submitted to "TargetP-2.0 Server" which can further predict whether it is likely to be a mitochondrial or plastid protein [6]. An example of the workflow for this activity is added in the supplementary file 1.

Follow-up analytical questions

- What is the first amino acid in the protein and why?
- What is the predicted localization of the protein? If it is a secreted protein, then where is it cleaved? If it is a cytoplasmic protein, does it enter mitochondria or chloroplast? If so, which part of the organelle is likely to reside.
- Where the protein is likely to be synthesized, in Cytoplasm or RER? If it is synthesized in RER, how the protein is likely to be modified and transported through endomembrane system?
- Which specific signal sequence(s) the protein is likely to contain?
- Predict few functions of the protein depending on its subcellular localization.

Conclusion

Effective practice-related activity such as the one described in this article using online tools for teaching biological concepts can enable others to quickly design and implement new activities in virtual and in-person courses [7]. This activity can be complementary to another activity that explores structural and functional conserved domains in a protein as identified by bioinformatic tools such as NCBI Conserved Domain Database and together can provide better insight of the structure, function and localization of a novel protein.

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Conflict of Interest

Author has no conflict of interest.

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