

## Ras and Cell Signaling Exercise

### Learning Objectives

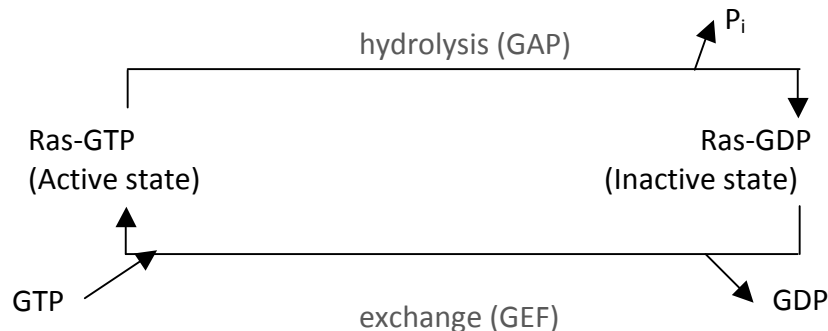
In this exercise, you will use StarBiochem, a protein 3D- viewer, to explore:

- the structure of the Ras protein
- the active and inactive state of Ras and the amino acids that contribute to these two states

### Background

Ras proteins are plasma membrane bound proteins that communicate signals from the outside of the cell to the nucleus. Ras proteins are essential components of various signal transduction pathways that regulate growth, proliferation, differentiation, and apoptosis. The Ras protein family is divided into five major categories based on their sequence and functional similarities: Ras, Rho, Rac, Ran, and Arf.

Ras proteins function as molecular switches by cycling between an active GTP- bound state and an inactive GDP-bound state. The conversion of GTP- to GDP- bound form is mediated by the intrinsic GTPase activity of Ras, which hydrolyzes GTP to GDP, and by the GTPase activating protein (GAP). On its own, Ras protein can hydrolyze GTP, however, this process is too slow for efficient Ras function. Binding of GAP to Ras protein accelerates GTP hydrolysis by Ras. The process of exchange of GDP to GTP is facilitated by a guanine nucleotide exchange factor (GEF).

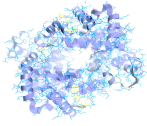


Mutations in Ras genes can result in permanent activation and cause inappropriate signaling even in the absence of activating extracellular signals. Given that activation of Ras signaling results in cell growth and division, dysregulated Ras signaling can ultimately lead to cancer. Mutations in Ras genes are found in 20-25% of all human tumors and in up to 90% of specific tumor types such as leukemias and colon cancer.

### Getting started with StarBiochem

To help you learn how to use the program, a tutorial is available under StarBiochem User Guide.

- To get to StarBiochem, please navigate to <http://web.mit.edu/star/biochem>.
- Click on the **Start** button to launch the application.
- Click **Trust** when a prompt appears asking you if you trust the certificate.
- In the top menu under Samples click on **Select from Samples**, select "H-Ras p21 partial – H. sapiens (5P21)" and click **Open**.



**1 How would you classify this protein: monomeric or oligomeric?**

Answer

**2 Based on the primary sequence of Ras, what is the approximate length of the mature Ras mRNA?**

*Hint: Membrane proteins have an additional amino acid sequence called the signal recognition peptide, comprised of 15-20 amino acid residues, that targets them to the endoplasmic reticulum. The signal recognition peptide sequences is found within the mature mRNA but is cleaved after the protein is inserted into the ER lumen and therefore is not found in the mature protein. Remember that each amino acid is specified by three nucleotide base codon.*

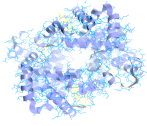
Answer

**3 Identify and draw the other structural component shown in this structure.**

Answer

**4 What form of Ras is shown in this structure: active or inactive? Explain why.**

Answer



**5** We will now analyze the secondary structure of Ras (5p21).

**a) Which of the following options best describes Ras' secondary structure?** Circle your choice.

Answer

- Beta sheets surrounded by helices and coils.
- Coils surrounded by beta sheets and helices.
- Helices surrounded by sheets and coils.

We will now explore one of the secondary structures within Ras.

**b) Is this helix located at the N- or the C-terminus of the protein?** Explain.

Answer

**c) How many amino acid residues does helix 5 have?**

Answer

**d) The C-terminus of Ras is involved in targeting the Ras protein to the plasma membrane.**

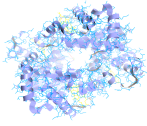
**What should be the nature of the amino acids that make up the C-terminus?**

Answer

**4** Ras is comprised of five major 'G' motifs that bind to GDP/GTP directly. These motifs are: G1 (amino acids #10-17), G2 (amino acid #35), G3 (amino acids #57-60), G4 (amino acids #116-119) and G5 (amino acids #145-147).

**a) Highlight the amino acid residues within the G1 and G2 motifs. To which part of GTP/GDP do these motifs bind to?** Your choices are 'guanine base', 'sugar' or 'phosphates'.

Answer



**b) Which of these two G motifs can bind to both GTP and GDP? Explain your choice.**

Answer

**c) Which of these two G motifs can bind only to GTP? State the most likely mode of interaction between this motif and GTP.** Your choices are 'hydrogen bond', 'ionic bonds', 'peptide bonds', 'hydrophobic interaction' or 'van der Waals forces'. **Draw this interaction and indicate the residues involved.** Indicate the alpha-carbon atom and the side-chains of the amino acid residues involved.

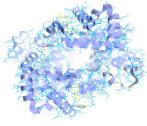
Answer

**d) Highlight the amino acid residues within the G3, G4 and G5 motifs. Which motif(s) interact with the guanine base of GTP/GDP?**

Answer

**e) Identify the amino acids within the G3, G4 and G5 motifs that interact with the guanine base of GTP/GDP by hydrogen bonding. Draw this interaction(s) and indicate the residues involved.** Indicate the alpha-carbon atom and the side-chains of the amino acid residues involved.

Answer



Name \_\_\_\_\_

StarBiochem

**g) Amino acid #30 interacts with the sugar component of GTP/GDP. *What is the most likely mode of interaction between these two?* Your choices are 'hydrogen bond', 'ionic bonds', 'peptide bonds', 'hydrophobic interaction' or 'van der Waals forces'. *Draw this interaction and indicate the residues involved.* Indicate the alpha-carbon atom and the side-chains of the amino acid residues involved.**

Answer

**h) The interaction of Ras with GTP/GDP also involves  $Mg^{++}$  ions. *Identify the part of GTP/GDP that interacts with  $Mg^{++}$  ions.* Your choices are 'guanine base', 'sugar' or 'phosphates'. *Draw this interaction.***

Answer

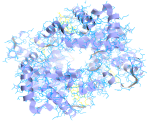
**j) *State the mode of interaction between  $Mg^{++}$  ions and GTP/GDP.* Your choices are 'hydrogen bond', 'ionic bonds', 'peptide bonds', 'hydrophobic interaction' or 'van der Waals forces'.**

Answer

**5** Mutations in the genes that encode Ras protein family members are very common in all human tumors. Therefore it is reasonable to speculate that a pharmacological approach that curtails Ras activity may represent a possible method to inhibit certain cancer types.

Here are three individual amino acid substitutions that may affect Ras activity.

- Substitution 1: amino acid residue #17 substituted by asparagine.
- Substitution 2: amino acid residue #119 substituted by asparagine.
- Substitution 3: amino acid residue #61 substituted by lysine.



For each of these amino acid substitutions:

**a) Identify the original amino acid present at these locations.**

Answer

**b) State which of these substitutions may result in a Ras protein that is active at all times.**

Explain your choice.

Answer

**c) State which of these substitutions may result in a Ras protein that is inactive at all times.**

Explain your choice.

Answer

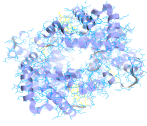
### Keywords:

Ras proteins, GTP, GDP, GEF, GTPase, cancer, tumors, differentiation, apoptosis, nucleotides, sugars, and endoplasmic reticulum.

### Thought questions

**1** Change in the activity of Ras can influence various cellular processes such as proliferation, differentiation and cell death. Propose a mechanism to explain how a single protein can influence diverse cellular processes.

**2** Mutations in Ras protein are commonly observed in most cancers and are regarded as gain of function. Would you classify these mutations as dominant or recessive? Explain.



## PROTEIN STRUCTURE BASICS

Each protein has the following three levels of protein structure:

### Primary structure

Lists the amino acids that make up a protein's sequence, but does not describe its shape.

### Secondary structure

Describes regions of local folding that form a specific shape, like a helix, a sheet, or a coil.

### Tertiary structure

Describes the entire folded shape of a whole protein chain.

In addition, some proteins interact with themselves or with other proteins to form larger protein structures. How these proteins interact and fold to form a larger protein complex is termed

### Quaternary structure.

## CHEMICAL STRUCTURES OF THE AMINO ACIDS

The 20 amino acids share a common backbone and are distinguished by different 'R' groups, highlighted in various colors below.

