Inducing Expression of Red Fluorescent Protein (RFP) or Green Fluorescent Protein (GFP)

Powerpoint adapted from:
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Pierce College, Woodland Hills, CA

What is Inducible Protein Expression?

Inducible protein expression is the ability to ‘turn on’ and ‘turn off’ protein expression in a cell at a specific time.

Why Induce Protein Expression?

Why would we want to have protein expression be inducible?

Wouldn't it be easier if the cells produced the protein all the time?

Can you think of situations where you would want to be able to 'turn on' or 'turn off' protein production at a specific time?

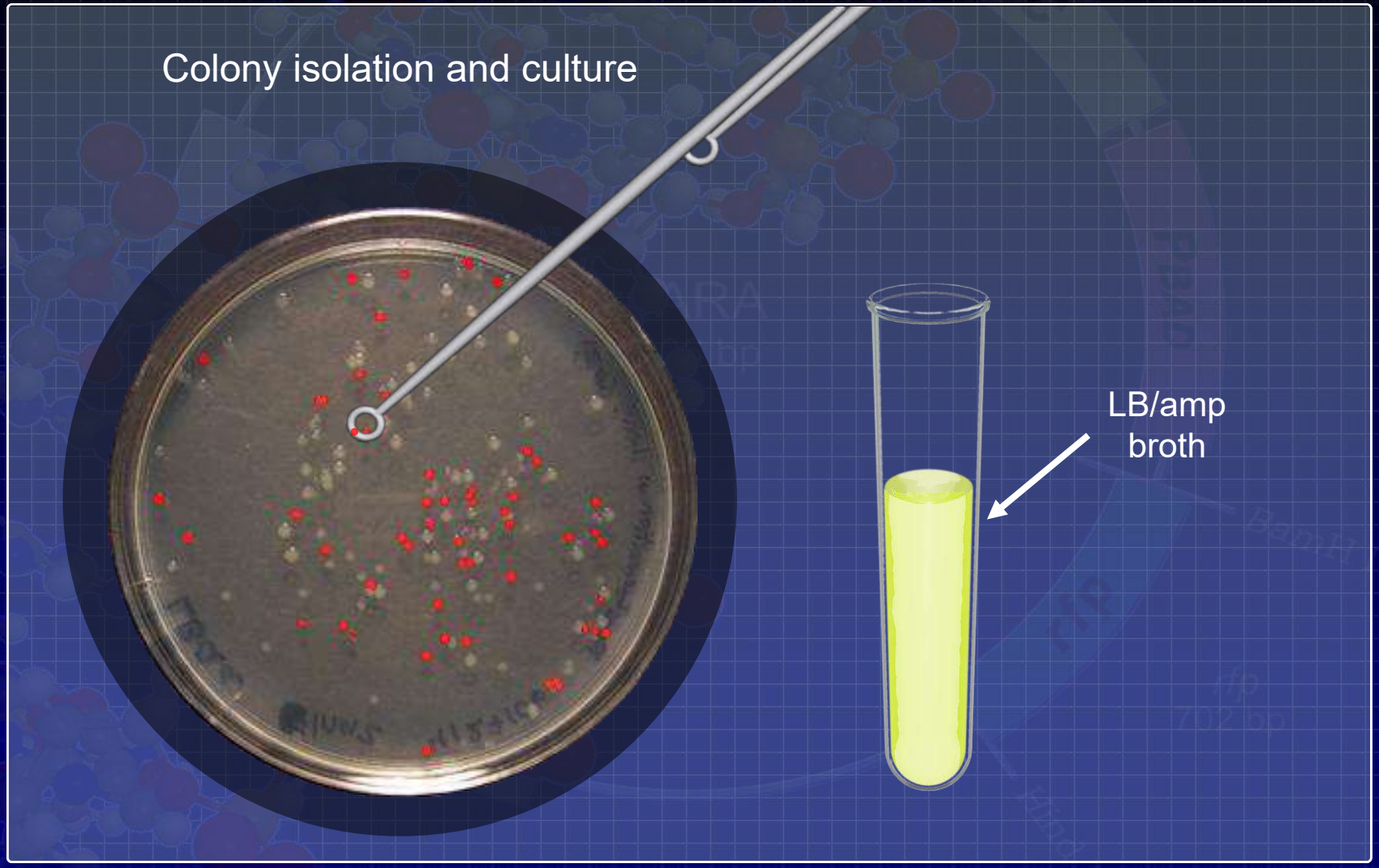
Reasons to Use Inducible Protein Expression?

1. You want to study the effect of the protein in the cell only at certain times.
   Example: You want to study the effect of a protein at a specific stage of cell development or cell growth.

2. The production of the protein is toxic to the cell. You want to turn on protein production only for a short time and then turn it off.

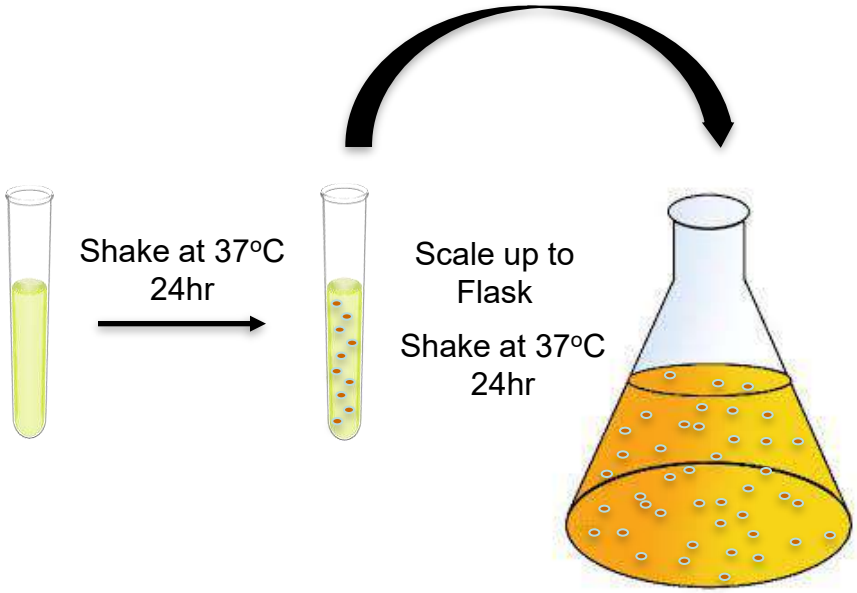
1. The production of the protein impedes cell growth.

How do you make E. *Coli* Express RFP?



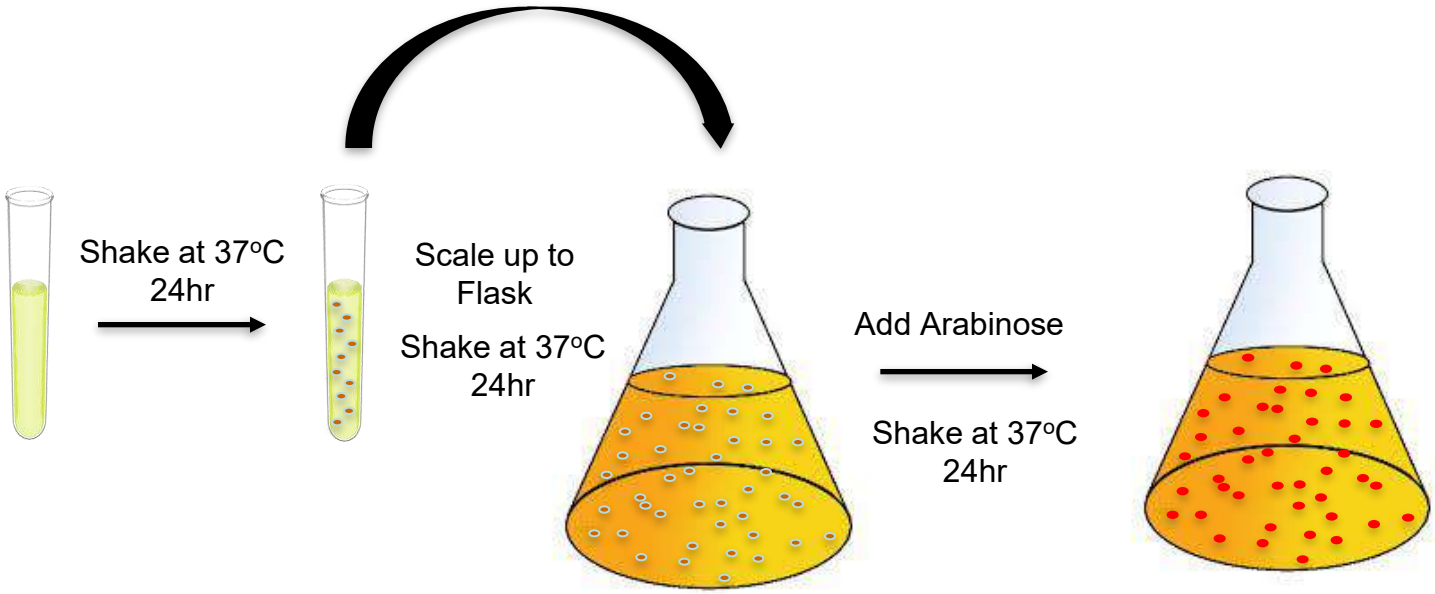
How do you make E. *Coli* Express RFP?

Culture scale up



How do you make E. *Coli* Express RFP?

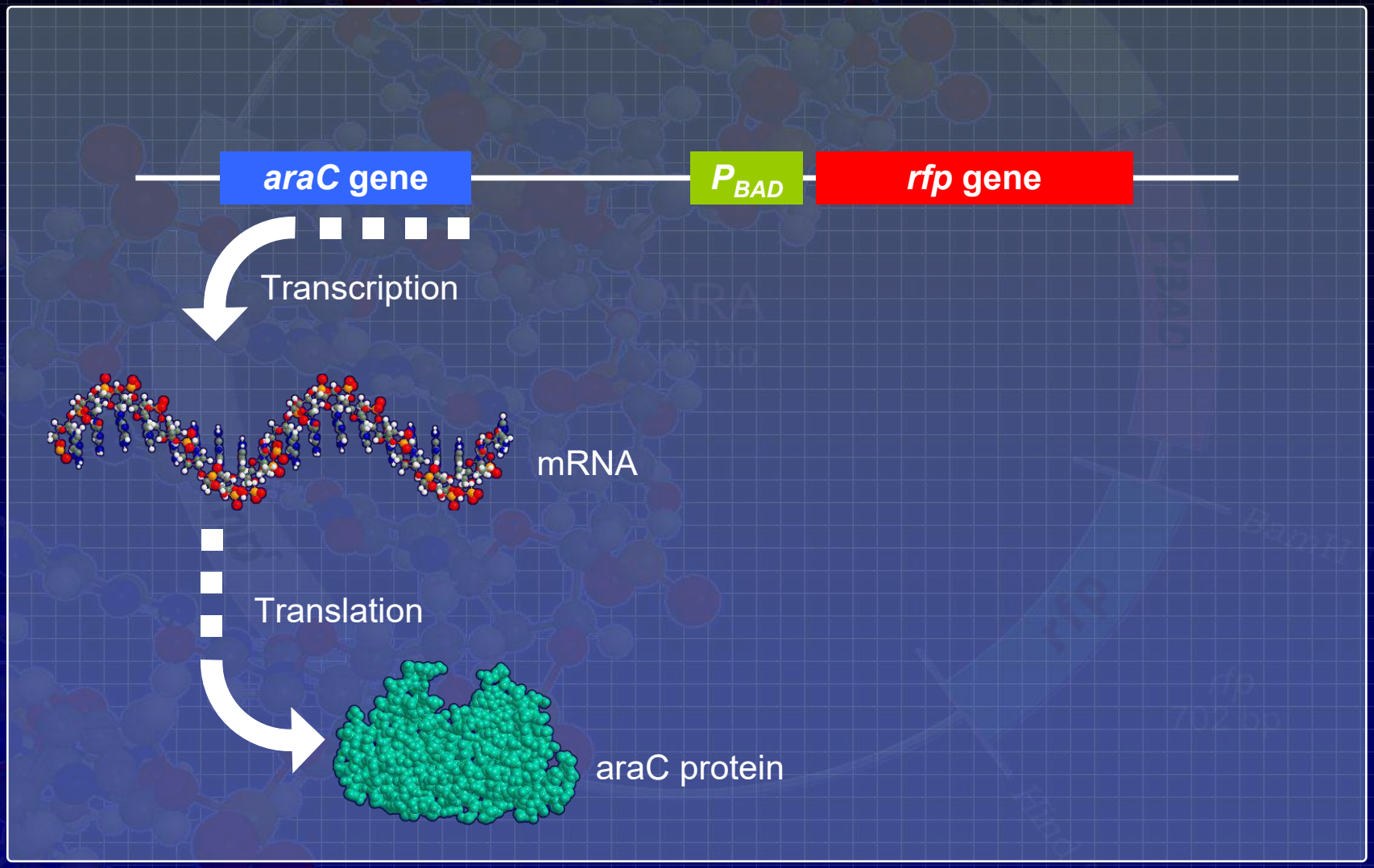
Induction of RFP Expression



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RFP expression

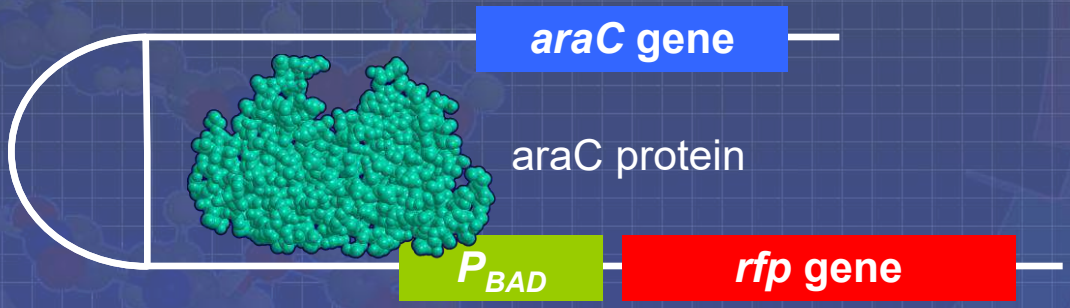


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RFP expression

araC protein prevents RFP transcription by causing a loop to form in the region of the *rfp* gene



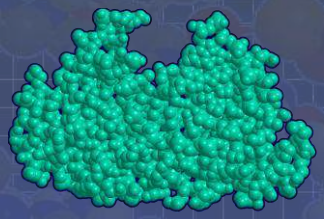
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RFP expression

Arabinose – araC protein complex prevents DNA looping and helps to align RNA polymerase on the promoter site (P_{BAD}).



arabinose



araC protein



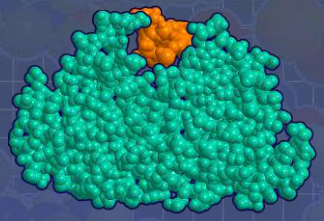
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arabinose



arabinose – araC protein complex



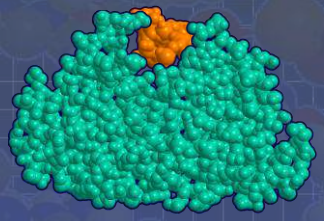
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RFP expression

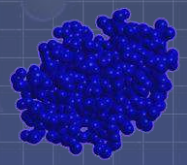
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arabinose



arabinose – araC protein complex



RNA polymerase



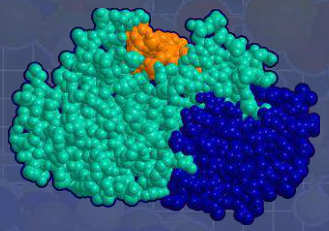
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RFP expression

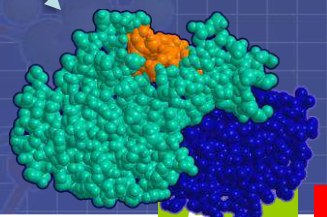
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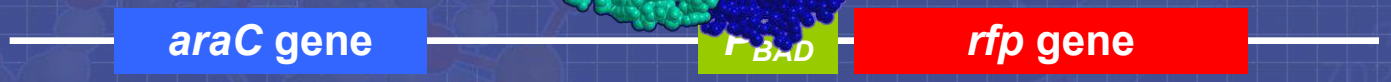


arabinose



arabinose – araC protein complex

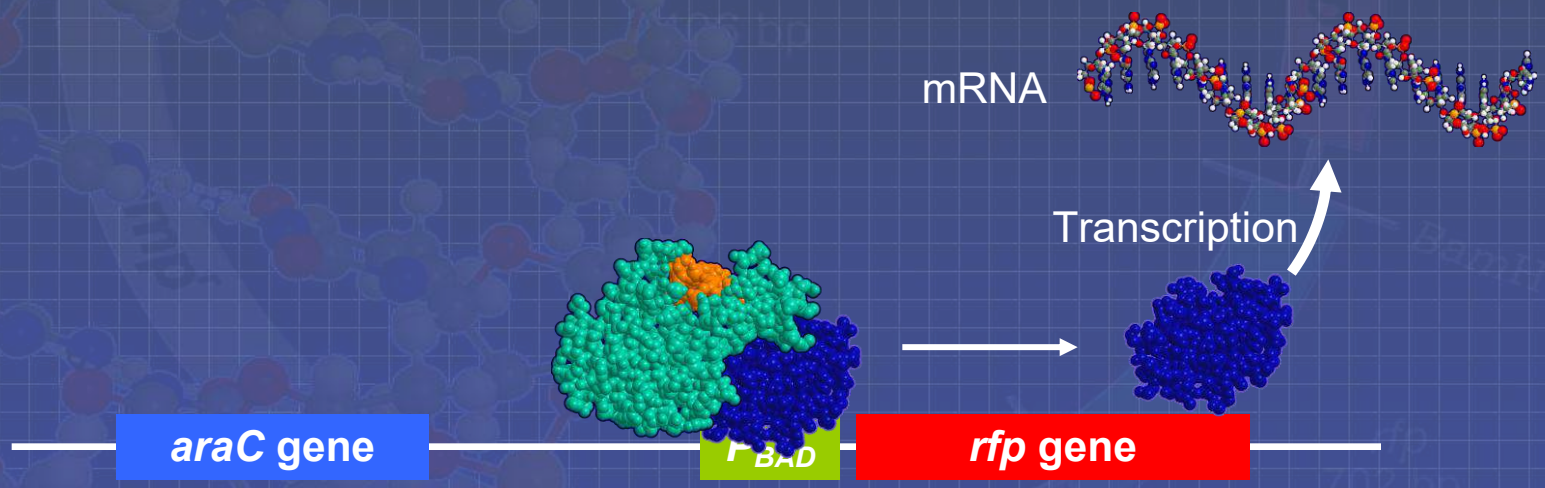




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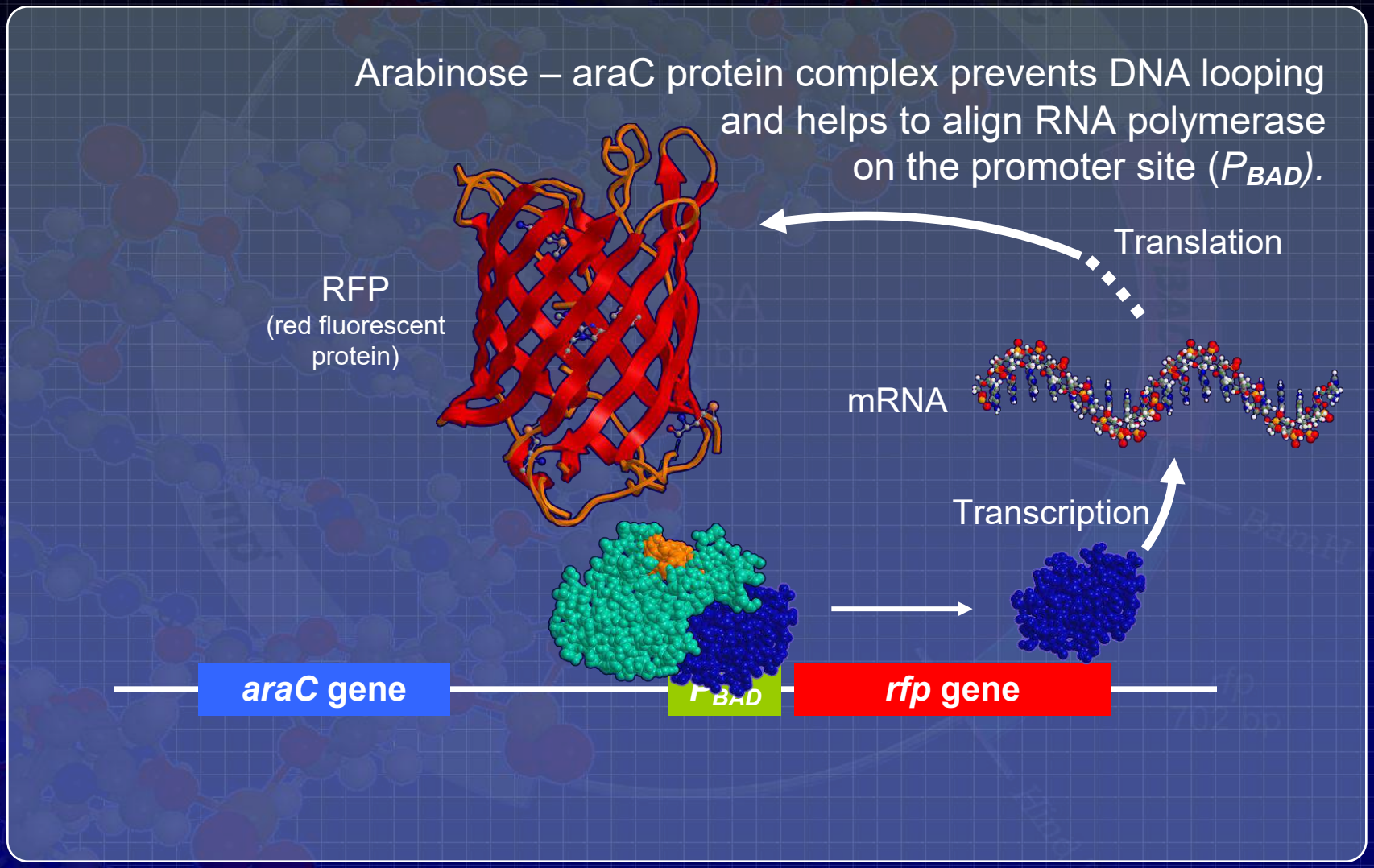
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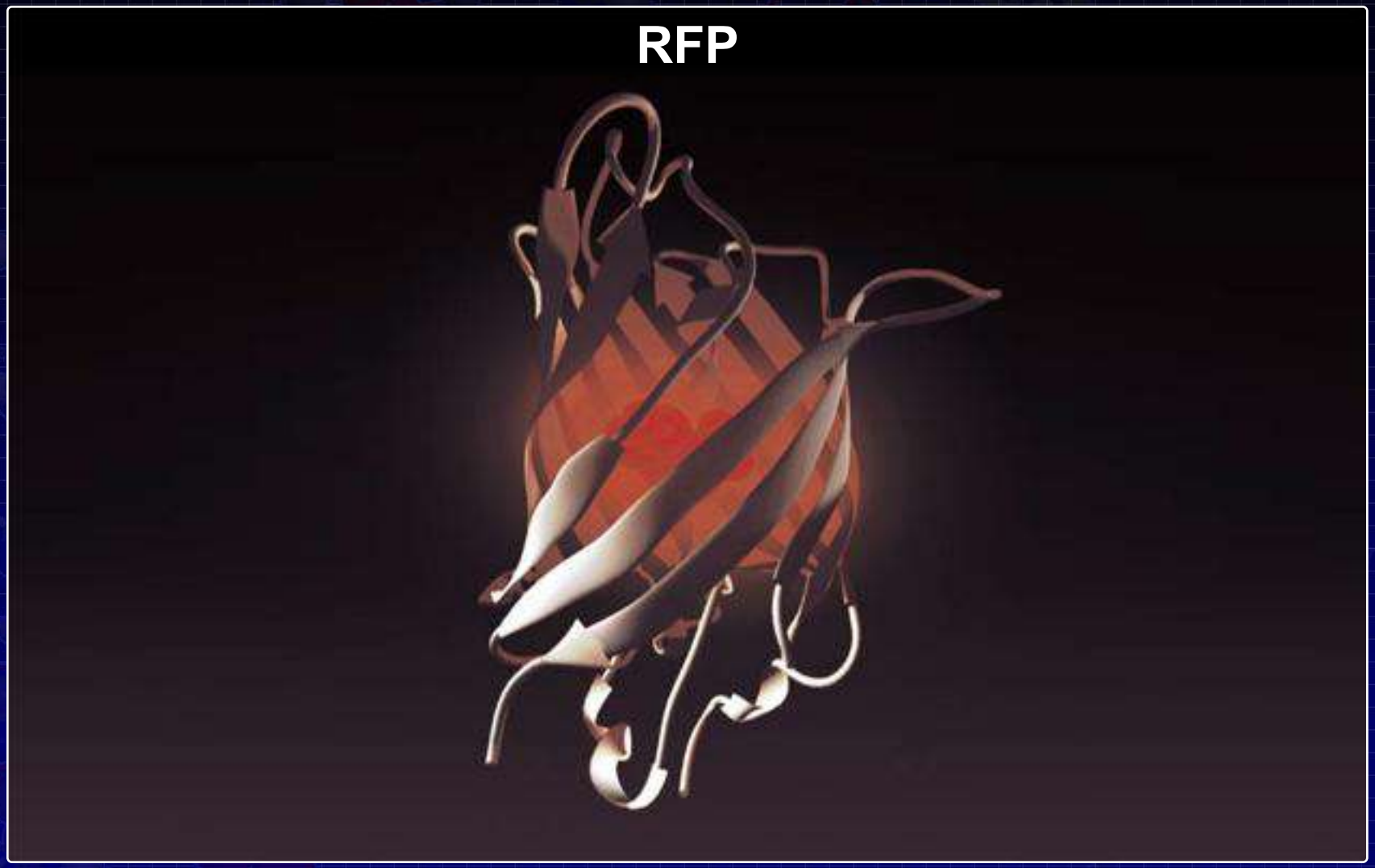
RFP expression



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RFP



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Purification of RFP from an overnight culture

