The markers used by researchers for studying e. coli

Health & Medicine

“ Green Fluorescent Protein Functions as a Reporter for Protein Localization in Escherichia coli”

In order to study and analyze a wide variety of cellular characteristics, scientists have developed many markers to indicate the presence or absence of certain cellular components and activities. Because of our well-established knowledge of its overall structure and function, as well as its relative simplicity, Escherichia coli (henceforth E. coli) becomes a model microorganism through which to study the activity of these reporters specifically in bacteria. Many classic markers like alkaline phosphatase (encoded by phoA) and β-lactamase (encoded by bla) function best only in the periplasm, while others, like β-galactosidase, are enzymatically active only in the cytoplasm and are subsequently inactivated in the periplasm. Because of its unique activity, β-galactosidase in particular has led to the discovery of many genes encoding significant components of E. coli which aid in bringing newly manufactured proteins inside the cell to the surface for secretion, known as the protein export machinery. However, sometimes indicators like these can be overproduced by the cell, in turn jamming this machinery and causing cell toxicity known as overproduction lethality. Mutations that counteract overproduction lethality have been used to uncover significant features of the signal sequences of exported proteins, revealing the utility of markers like β-galactosidase. However, limitations exist when using β-galactosidase, making it likely impossible to isolate all mutants of particular interest. An alternative reporter that is active only in the cytoplasm yet can be efficiently transported across the cytoplasmic membrane would be more ideal, such as green fluorescent protein (GFP) – its

ability to aid in monitoring gene expression and assess viability is well-established, it is active in E. coli, and it is significantly smaller than β-galactosidase. Perhaps most significant, however, is that its three-dimensional structure is similar to that of bacterial porin proteins, making it likely that GFP would be exported from the cytoplasm if fused to the appropriate signaling sequences. Combined with its ease of detection by UV light, GFP provides numerous advantages worth investigating.

To study GFP as a reporter to locate proteins in bacteria, a protein called maltose-binding protein (MBP) was selected, with a modified GFP fused to it as an indicator. This is created by fusing the MBP gene, malE, to the modified GFP gene, GFPuv. MBP was selected primarily because it is known to be exported from the cell. Additionally, it serves as a useful affinity tag for purification of the hybrid protein. GFPuv was used in place of GFP largely because it fluoresces up to 18 times brighter under UV excitation; moreover, it is soluble in E. coli, and is therefore expressed very efficiently. GFP, on the other hand, may sometimes be unpredictably expressed as a nonfluorescent protein, making it a much less reliable reporter of protein location.

To make the MBP-GFP hybrid proteins, four different plasmids were created: two with the full plasmid sequence (pMGP2 and pMGC2), and two with part of the plasmid deleted (pMGP22 and pMGC22). pMGP2 and pMGP22 contained the signal sequence for MBP, and pMGC2 and pMGC22 did not. A signal sequence attached to a protein tells the cell that the protein will eventually be secreted; if no signal sequence is present, the protein will remain in the cytoplasm. By creating plasmids both with and without a signal sequence,

researchers were able to test whether GFP could function outside of the cytoplasm, as this functionality was unknown.

To create pMGP2 which encoded protein SS418, the GFPuv gene (henceforth gfp) and the vector pMalp2 were both cut with the restriction enzyme HindIII-ScaI, and were then ligated with DNA ligase to create a modified plasmid. To create the corresponding deletion derivative, pMGP22, the pMGP2 plasmid was cut at a different restriction site by the restriction enzyme BglII-BamHI to excise part of the new plasmid, and was then rejoined by DNA ligase. This plasmid encoded protein SS128. The same process was used to create pMGC2 (full plasmid) encoding protein SSΔ418 and pMGC22 (deletion derivative of pMGC2) encoding protein SSΔ128, using pMalc2 as a target vector instead.

As predicted, protein SSΔ418 was active and fluoresced. However, no fluorescence was observed of protein SS418, indicating the export of this protein somehow inactivated GFP. Later it was determined this was due to improper folding of GFP in the periplasmic space. The various MBP-GFP hybrid proteins were localized by separating the samples into cytoplasmic, periplasmic, and membrane samples, and then running them on a Western blot with antibodies specific to either MBP or GFP. A Western blot is used to identify the presence of certain proteins in a sample by blocking the sample with fluorescent antibodies specific to the protein of interest. Through this procedure, it was determined that protein SSΔ418 remained in the cytoplasm, as well as protein SSΔ128. Conversely, the proteins encoded by

pMGP2 and pMGP22 were located in the periplasmic space, indicating that these hybrid proteins can indeed be exported by the cell.

In E. coli that are defective in protein export, known as sec mutants, it was predicted that if the hybrid protein was expressed in the bacteria, and it is in fact exported by the normal sec-dependent pathway, fluorescence would occur. This is exactly what was observed: protein SSΔ418 but also protein SS418 caused fluorescence in the colonies, clearly indicating that the hybrid protein resulting from pMGP2 was not exported. The two shortened proteins encoded by pMGP22 and pMGC22 also showed fluorescence in the sec mutants, but only when the proteins were expressed without the MBP signal sequence (SSΔ128) or were expressed in a mutant that caused the protein to be located in the membrane space, and not the periplasmic space.

Interestingly, when expression of the full-length protein SS418 was induced, normal cell growth resulted with no overproduction lethality like that caused by β-galactosidase gene fusions. Conversely, induced expression of the shortened hybrid protein SS128 did cause overproduction lethality. Researchers did further tests by fusing gfp gene with phoA in one study and with bla in another, observing fluorescence in both cases only when the protein was localized inside the cytoplasm with no cell toxicity. These results suggest that GFP is not toxic to cells, as well as the possibility that folding of MBP in the periplasmic space does not completely account for the lack of GFP fluorescence outside the cytoplasm in the case of malE-gfp fusions.

Because of the results described above, researchers confidently used fluorescence itself to isolate export-defective mutants. In a successful

attempt to do so, researchers randomly mutagenized SS418 proteins to create abnormal proteins that would fail to be exported. Indeed, fluorescent colonies were detected, indicating failed export. Even when replacing a fragment of a normal pMGP2 plasmid with a mutant fragment containing malE, fluorescence occurred, suggesting that the fluorescence seen is in fact due to a malE signal sequence mutation. From this series of experiments, it can be reasonably concluded that the periplasmic MBP-GFP hybrid protein is exported by the sec-dependent pathway and requires a signal sequence for this export.

Though the series of experiments performed were overall successful, researchers found that not every GFP hybrid protein may be appropriate for analysis of protein export – some may cause overproduction lethality likely due to some cause other than jamming of protein export machinery, as was the case with protein SS128. Additionally, it was suggested that using a machine that sorts fluorescent cells from nonfluorescent cells could heighten the sensitivity of detection, increasing the probability of discovering new export-defective mutants. Overall, the results observed of the various MBP-GFP hybrid proteins indicate that GFP may be very useful in studying protein localization in bacteria.