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Purification and analysis of human *muc* reporter proteins

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Exocrine mucous glycoproteins are a family of multifunctional heavily glycosylated, anionic macromolecules that typically possess a high serine/threonine content. The size and complexity of these glycoprotein molecules, while physiologically beneficial, prohibits direct analysis of small-to-moderate changes within the side-chain oligosaccharides. These polydispersed molecules have a protein core encoded by muc-genes, which possess multiple repeats within their sequences. To assist in developing a method to study these complex molecules, a reporter-DNA construct, for eventual eukaryotic expression, consisting of an IgK secretory leader sequence, two polyHis regions, two HSV and one myc antigenic sites was synthesized. This construct was then utilized by incorporating two separate muc repeat-sequence units; one consisting of muc-2/muc-2 and one possessing muc-2/muc-4 (i.e., amuc2c and amuc24c, respectively). DNA plasmids pET28-amuc2c and pET28-amuc24c were transformed into the bacterial strain Ecoli BL21DE3. Expressed proteins from transformants were isolated and purified, and then analyzed by MALDI-TOF MS. Mass+H+ (avg) of 16306.2 Da and 17062.8 Da for amuc2c and amuc24c, respectively, were observed. MS and MS/MS analysis of the tryptic digests of these expressed proteins also confirmed their respective sequences. To test the efficacy of possible coexpression of fluorescent protein transfection markers with the muc-constructs in eukaryotic cells, preliminary transfections of fluorescent DNA plasmids pEGFPc1 (green, cytoplasmic), pDSRed2-N1 (red, secretory-IgK), pEYFP-Golgi (yellow) and pECFP-Golgi (cyan) into MATLyLu cells (rat prostate cancer) are being performed. The successful conclusion of these ongoing studies will result in the expression of a small, glycated, and secreted muc-protein from transfected human intestional and respiratory cells in vitro that also are producing muc-related macromolecules. These posttranslationally modified amuc2c and amuc24c reporter proteins can then be analyzed, in detail, by contemporary methods. They will be employed as tools to help provide an insight into the changes that occur in the posttranslational modifications of macromolecular glycoproteins in human disease, such as cystic fibrosis.