Description for the general public

Phosphoglycerate mutase, abbreviated as PGAM, is a protein involved in glycolysis – one of the basic metabolic processes in the cell. Its cellular function was establish very early in the evolution of life. PGAM has changed very little since then and is strikingly similar in all living organisms – PGAM homologue form E. Coli bacteria has around 50% of sequence identity with human PGAM. Glycolysis and the function of proteins involved were described in the 30s and 40s, and for years it seemed as if there remains nothing new to be discovered about PGAM. It came as a big surprise when scientists found PGAM presence in nucleoli – substructures of cell nucleus responsible for synthesis of ribosomal RNA. Available results suggest that nucleolar PGAM is connected with frequency of cellular division. Cellular division is a process requiring extremely precise control. Both too frequent and too infrequent divisions are causes or symptoms of various diseases. Uncontrolled rapid division of cells is the main cause of cancer, while premature cessation of divisions, also called cellular senescence, is responsible for some of the symptoms of e.g. type II diabetes, chronic hepatitis C, tobacco-related emphysema and aging. The more scientists know about molecular mechanisms controlling a cellular process, the more possibilities for human intervention exist. Explanation of PGAM function in control of cellular divisions can help to design new, innovative therapies for the pathological states mentioned above. However, PGAM, as all parts of the cell, does not work alone. So in order to understand its role in nucleolus we have to identify its binding partners. Preliminary studies have resulted in a list of probable candidates, however, as all scientific results, it has to be confirmed using alternative methods. In this project we propose using affinity chromatography. PGAM will be chemically bound to gel beads immobilized inside a glass column. The gel with PGAM will be flushed several times with nucleolar extract causing PGAM-binding proteins to remain in the gel. Next, the gel will be rinsed with a solution causing release of PGAM-bound proteins. The result will be a mixture of nucleolar partners of PGAM. Proteins in the mixture will be identified using mass spectrometry or MS. In this method proteins are broken down into smaller electrically charged fragments which masses are precisely measured. Each protein breaks into a characteristic set of fragments producing so called “molecular fingerprint”. Comparison of sample fingerprint with a database produces a set of hits indicating which proteins are likely to be present in the sample. Protein identification will be confirmed using a method called Western Blot in which proteins bound to a membrane are detected using specific antibodies. Binding of identified partners to PGAM will be confirmed using microscale thermophoresis – MST, which utilizes differences in the speed of thermal movements of complexes and their free constituents caused by heating a part of the sample with a laser. This method allows us not only to confirm that a complex is forming but also compute the strength of binding. Interactions confirmed in previous experiments will be demonstrated in samples taken from cell cultures. To this end, we will use a method called FRET in which two different kinds of antibodies, each bound to a different fluorescent dye, are used. One kind of antibodies will be specific for PGAM and the other for its binding partner. The fluorescent dyes will form a pair which can transfer energy from one dye to the other, provided they are close enough. If PGAM and its binding partner are close enough in the cell to interact, the energy transfer will be present and excitation of one dye will cause fluorescent emission of the other. Altogether, experiments proposed here are able to determine binding partners of PGAM with good confidence. This will form a necessary basis for future investigation into the role of PGAM in nucleoli and its involvement in cellular division control.