494f Determination of Metabolically Distinct Cellular Physiologies Using Metabolic Rate Screening

R. Robert Balcarcel

With the aim of understanding the role of biological building blocks within cells, experiments and analyses in computational and functional genomics study changes in transcription and translation profiles obtained using large-scale techniques. Of great importance in designing such work is knowledge that, in addition to changes at the molecular level, there are indeed changes in cellular physiology. Having such knowledge should help guide experiment design and increase the relevance of results.

Metabolic rate screening (MRS) is a new cell culture methodology for obtaining data sets for average rates of biochemical reactions of mammalian cells. It is based on the premise that changes in metabolic rates ought to portray changes in building blocks and regulatory mechanisms, they being cumulative outcomes of the underlying biomolecular interactions. In this talk, two MRS assays will be described in the perspective of defining metabolically distinct cellular physiologies. The first assay, a 96-well plate assay for sublethal metabolic activity (SMA), provides average glucose uptake and lactate production rate, as well as relative viable cell concentration, during incubations lasting 6- to 24-h. The second assay, a 24-well plate metabolic flux analysis assay (MFA assay), provides average rates for glucose uptake, lactate production, and CO2 production during 6-h incubations, as well as ten other calculated metabolic variables using a model. Both assays provide precise data sets (errors on rates within 1% to 10%), are fairly inexpensive ($25 to $100 in supplies per well-plate experiment), and can be done with ease using manually operated multi-pipettors.

Use of these assays to define metabolically distinct cellular physiologies will be demonstrated in regard to defining concentrations of various small molecules to use in various cell culture research projects. Examples include effect of CTZ on hexokinase detachment (and glucose uptake and cell death), effect of rapamycin on transcription and translation (and ultimately cell death and specific productivity), study of detoxification pathways for methylglyoxal (and consequence for cell death), and study of lactate *consumption* pathways, all in hybridoma cell culture. Despite the molecules, their molecular targets, and the purpose of studies being quite dissimilar and not all being “metabolic inhibitor/activators”, MRS was beneficially used to define metabolically distinct conditions of use in designing further experiments. Projects in computational and functional genomics should benefit from first-hand knowledge from MRS-style assays that distinguish metabolic consequences resulting from changes in transcription and translation profiles.