Molecular Methodologies in Nutrition Research

Steven D. Clarke and Sooja K. Kim
Nutritional Sciences and the Institute for Molecular and Cellular Biology, The University of Texas, Austin, TX 78712

In the early stages of the evolution of life forms, cellular growth and evolutionary success required that the developing organism respond to a myriad of environmental factors. In particular, an organism had to be able to fulfill its nutrient needs as well as to sense periods of nutrient deficiency or excess and then turn on pathways of synthesis or storage. Because of this need to sense changes in nutrient environment, early cell life forms developed switches that regulated the transcription of genes encoding proteins involved in such metabolic functions as sugar synthesis and amino acid transport. This regulation of gene expression and metabolism was, in essence, a primitive hormonal signaling system used for the survival and growth of the organism.

As single cell organisms evolved into complex life forms, nutrients continued to act as environmental signals to regulate gene expression and metabolism. These dietary signals and their effects on gene expression could be both beneficial and detrimental in the etiology of such nutritionally related pathophysiologies as diabetes, cancer and heart disease.

We are now beginning to understand how nutrients govern gene expression and the abundance of pivotal metabolic and structural proteins. We now know that dietary constituents can govern the expression of proteins at a number of transcriptional and posttranscriptional points. For example, fatty acids, retinoic acid and cholecalciferol have specific nuclear receptors. Following ligand activation, the receptors interact with specific DNA recognition sequences within a particular gene and either up or down regulate the gene's rate of transcription (Alroy et al. 1995, Mangelsdorf and Evans 1995, Schoonjans et al. 1996). Recent data also suggest that carbohydrates and polyunsaturated fatty acids may govern the cytosolic content of specific transcripts by regulating the processing events involved in mRNA maturation (Girard et al. 1997). Another example is ferritin mRNA translation and transferrin receptor mRNA stability, which have now been shown to be regulated in response to changes in cytosolic iron status (Mascotti et al. 1995). Similarly, selenium governs the abundance of glutathione peroxidase protein by regulating the stability of glutathione peroxidase mRNA (Weiss and Sunde 1997).

Besides enabling researchers to elucidate the way nutrients govern the expression of proteins, the tools of molecular biology (e.g., over-expression and knock-outs) enable researchers to identify the relative importance of specific proteins in nutrient metabolism, growth and disease development (Hotamisligil et al. 1996).

Clearly, the Nutritional and Metabolic Sciences Initial Review Group (NMSIRG) of the Center for Scientific Review at the National Institutes of Health, which reviews applications addressing the role of dietary constituents as regulators of gene expression and metabolism, needs reviewers familiar with a wide array of molecular techniques to identify proteins pivotal to metabolism and growth and development. Recognizing the need, especially in the Nutrition and Metabolic Study Sections, for reviewers with broad expertise in molecular techniques, the NMSIRG sponsored a workshop to provide an overview of the utility, strengths, and weaknesses of certain techniques commonly used in the study of gene expression for nutritional and metabolic sciences.

As this workshop evolved, three areas of molecular research were most often used by investigators applying molecular techniques to nutritional and metabolic questions: (1) measures of mRNA abundance; (2) identification and characterization of nuclear transcription factors and their DNA recognition sequences; and (3) use of transgenic animals to identify proteins involved in the etiology of nutritionally related pathophysiological processes.

The utility, strengths, and weaknesses of these methods were evaluated in the following three studies. First, Dr. Karen Reue reviewed a number of approaches used for the quantitation of transcript abundance. Especially noteworthy in this review was the focus on the power or weakness of reverse transcriptase PCR methodology for quantifying mRNA abundance. Dr. Vincent Yang next provided a well reasoned overview of nuclear transcription factor structures as well as approaches to mapping their interaction with DNA response elements. Finally, Dr. David Hui described using homologous recombination in embryonic stem cells to "knock-out" the expression of a specific gene. Dr. Hui demonstrated the utility of the knock-out procedure for defining protein function and illustrated how knock-out mice could become unique animal models for developing nutritional and drug therapy approaches to pathophysiology.
Because a very broad research spectrum is reviewed by the NMS IRG, requiring broad differences in member expertise, the workshop was very useful for the review panel in evaluating the research grant applications assigned to the NMS IRG.

LITERATURE CITED


