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Review

Quantitative imaging of subcellular metabolism with stable isotopes and multi-isotope imaging mass spectrometry

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ABSTRACT

Multi-isotope imaging mass spectrometry (MIMS) is the quantitative imaging of stable isotope labels in cells with a new type of secondary ion mass spectrometer (NanoSIMS). The power of the methodology is attributable to (i) the immense advantage of using non-toxic stable isotope labels, (ii) high resolution imaging that approaches the resolution of usual transmission electron microscopy and (iii) the precise quantification of label down to 1 part-per-million and spanning several orders of magnitude. Here we review the basic elements of MIMS and describe new applications of MIMS to the quantitative study of metabolic processes including protein and nucleic acid synthesis in model organisms ranging from microbes to humans.

Contents

1. Introduction

Radiolabeled nucleotides [1] and halogenated nucleotide analogs [2] have been extensively used to label and track nucleic acids during chromosome replication and cell division. These approaches, however, are associated with pitfalls related to reagent toxicity [3,4] and the fidelity of techniques used for label measurement. Indeed, numerous active controversies in cell biology can be traced to limitations inherent to the use of label detection methods such as autoradiography or immunofluorescence to answer questions of cell turnover and fate.

One such area of active debate is whether some stem cells non-randomly segregate chromosomes during mitosis [5,6]. The resultant preservation of DNA template strands may influence the genetic stability of the stem cell lineage or impact daughter cell fate by the asymmetric inheritance of epigenetic gene-regulatory elements. The mere fact that the debate over the existence of so-called "immortal DNA template strands" is unresolved after nearly four decades of investigation [7] illustrates the limitations of the methods used to test the hypothesis.

Here we review a new approach to quantitatively study intracellular metabolic processes, including DNA synthesis, a methodology that we call multi-isotope imaging mass spectrometry (MIMS) [8]. MIMS is a synergy of two methods pioneered in the first half of the

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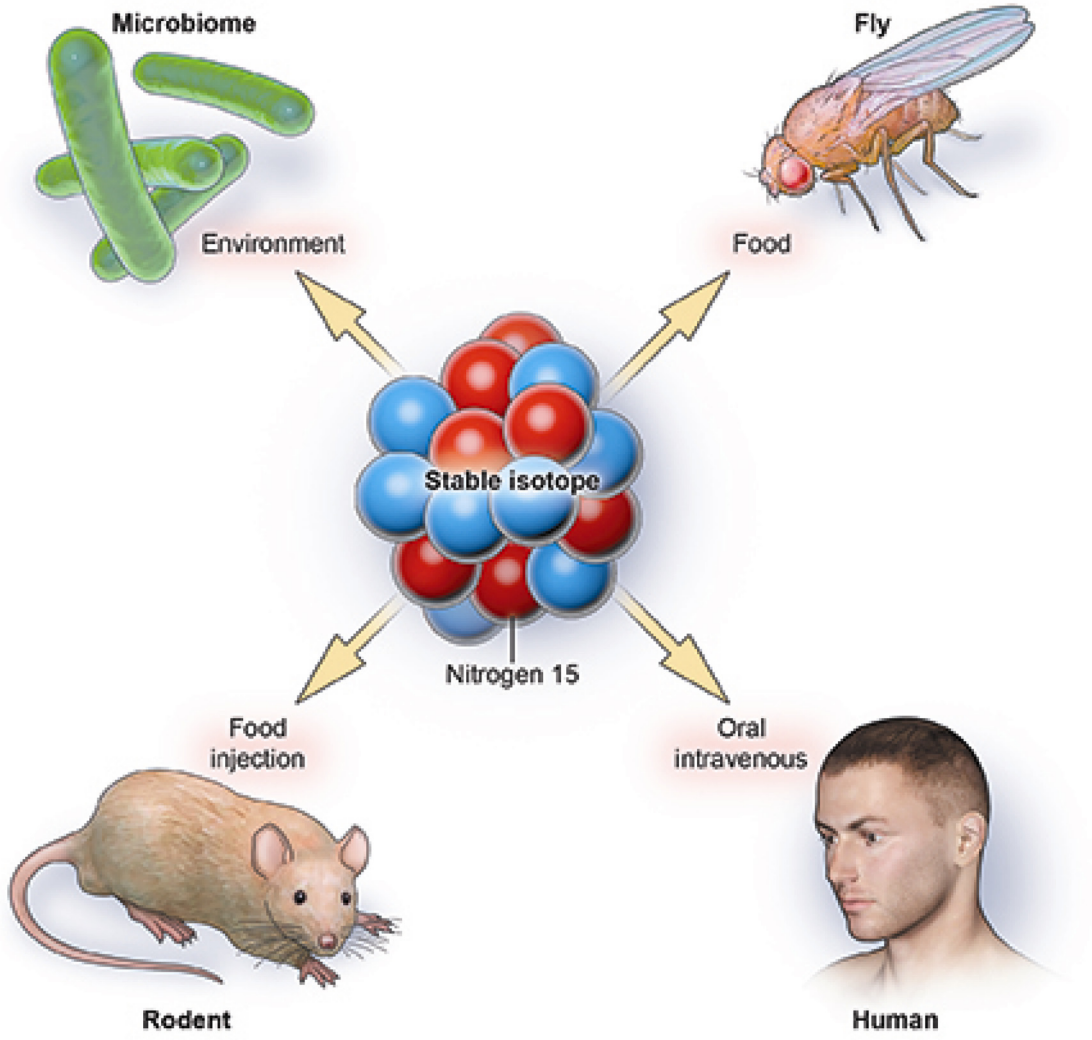

Fig. 1. Stable isotopes as metabolic tracers. An immense advantage of stable isotope tracers is that they do not decay and are natural in the environment. When delivered in enriched form as a component of tracer molecules, they seamlessly incorporate into the metabolic pathway under study, and they are detectable with mass spectrometers, including NanoSIMS. The innocuous nature of stable isotopes has led to their widespread use as tracers in organisms ranging from microbes to humans. Since Urey [18] discovered deuterium and Schoenheimer [9–12] pioneered the use of stable isotopes as tracers to study metabolism, they have been increasely used in human studies.

last century: the vision of using stable isotope tracers (Figs. 1 and 2) to quantitatively study metabolism as shown by Schoenheimer [9–12] and the extension by Leblond [13] to use autoradiography with electron microscopy to visualize radiotracers intracellularly. With MIMS, stable isotope tracers can be quantitatively imaged and measured in domains at least 10-fold smaller than a micron cubed. Here we summarize the important components of MIMS, including (i) stable isotopes and their advantages as metabolic tracers, (ii) a new-generation secondary ion mass spectrometer (Fig. 3) that emerged after crucial advances in ion optics, and (iii) an evolving computational interface that enables efficient data analysis [8]. We conclude by illustrating the power of the methodology, with particular emphasis on the study of immortal strands and the immense potential for human translation.

2. Stable isotope tracers

Stable isotopes are ideal metabolic tracers, because they are easily detectable and topographically mappable with high precision, they seamlessly integrate in the biochemical and physiologic processes of cells and organisms, and there is extensive precedent of safety in model organisms and humans (Fig. 2). Although radiolabels, fluorescent compounds, and halogenated analogs have been invaluable tools for biological studies, they each have the potential for toxicity and direct influence on the pathway under study. The toxicity of radiolabels includes the induction of DNA damage and modification of cell cycle activity [14]. The covalent attachment of a reporter, such as a fluorescent protein or halogen atoms, may alter the structure and biochemical properties of the parent molecule, resulting in cytotoxicity or cell cycle alterations [4,15–17].

In contrast to radioisotopes, stable isotopes do not decay and simply are isotopic variants that differ in atomic mass due to an alternative number of neutrons [18,19]. Many elements have more than one stable isotope form, but the term “stable isotope” often denotes the heavier, less abundant variants. Stable isotopes exist in animate and inanimate matter in constant ratios; therefore, incorporation of stable isotope tracers in a domain of interest is detectable by a measurable change in the isotope ratio. Schoenheimer pointed to their existence in equivalent ratios in both animate and inanimate matter as evidence that living organisms must not distinguish the few molecules containing the heavier variant from those containing the lighter variant [11].