Check Met!

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We are made up of billions of cells. These cells need to talk to each other and sense their environment for various cues such as available nutrients, toxins, mechanical pressure, fluctuations in temperature. Different sensing mechanisms operate for each cue, and ensure that cells coordinate their growth and survival functions according to their immediate environment. How a cell senses its environment and utilises the available nutrients is a fascinating area of research. It is important to study nutrient sensing because failure in sensing mechanisms have implications in several disorders. I joined my postdoc laboratory (Dr Sunil Laxman's group at inStem, Bangalore) with the determination to work on how cells *sense* nutrients. As the laboratory was new, I had complete freedom to choose any topic: the thought may scare some of you, but it sure is a delight for researchers!

While I was learning the basic tools in the field using baker's yeast (*Saccharomyces cerevisiae*) as a model organism, I was thinking about interesting topics that I could pursue. A few years ago, two observations were made related to a nutrient called methionine (Met). Met belongs to a class of molecules called amino acids. There are 20 natural amino acids present in all living organisms. These amino acids can be strung together in different sequences to form proteins (poly-amino acid-chains, much like how Lego blocks are strung together to form chains and toys!), and can be used for generating energy or other molecules. More importantly, amino acids can even act as

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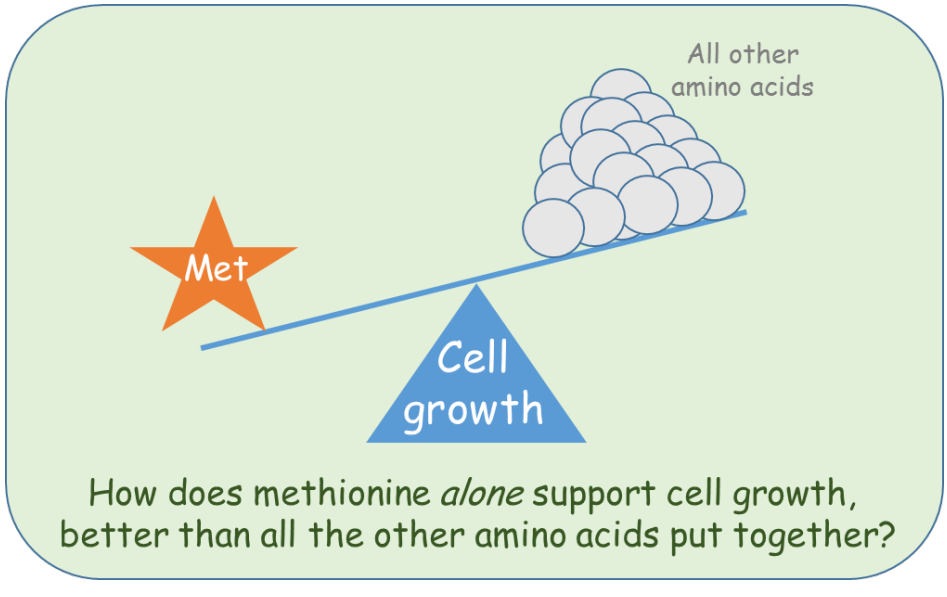
signaling molecules as a part of nutrient sensing mechanisms, an angle which we were interested in the laboratory.

The first observation dealt with Met availability and showed that the presence of this molecule determines the output of a particular nutrient sensing pathway. The second observation was related to overall growth wherein it was shown that, under amino acid limitation, Met *alone* could boost cell growth as good as providing the rest of the amino acids put together. Just as a starting point, I was interested in finding out the mechanism related to the first observation. Although we had good reasons to follow this observation, we were caught up in the detail and were finding it hard to see the big picture.

One fine day, while having a regular discussion, my mentor and I realised that it made more sense to look at the *global picture* of methionine-mediated cellular growth (the second observation) and investigate what was happening in response to Met, rather than trying to find its role in one particular cellular process. *You cannot see the forest for the trees!* From the nutrient sensing point, it was an outstanding puzzle: it is a well-known fact that cells need all the amino acids for making proteins, other important molecules and in general for growth. *Then, how does a single amino acid (Met) facilitate growth of cells, and that too, during overall amino acid limitation!?*What happens globally? And why *only Met*? In short, we were interested in knowing how methionine can single handedly alter the fate of a cell.



Now, one could do the global analysis by looking at many outputs such as levels of proteins, metabolite pools (small molecules such as amino acids that are required for growth), expression of genes (the ultimate effectors of nutrient sensing machineries, which determine which proteins and metabolites are produced) or likewise outputs. Just when we were thinking of which global analysis to take forward, results from one of my experiments showed that a protein called Gcn4,increases

specifically in the presence of Met. It is known that Gcn4 is a global regulator and *controls* expression of genes involved in synthesis of amino acids. This gave us a strong reason to follow the *gene expression* angle for our global analysis, in the presence and absence of Met. Cells grown in the presence of all other amino acids were also included to find out Met-specific effects and cells devoid of Gcn4 were included to find out Gcn4-dependent processes.

We looked at the expression profile of individual genes across the stated conditions in wild type (a fully functional, natural isolate of yeast)and Gcn4-devoid cells. Our analysis showed many interesting features of Met-mediated cell growth. First, when we compared the expression profile of wild type cells grown a) without amino acids, b) with Met only, and c) with all amino acids except Met, we could clearly see that presence of Metelicits a structured, hierarchical response. We identified three key nodes of cellular processes that were strongly Met-dependent. These nodes culminate to form *precursors* for biosynthesis of amino acids (other than Met) and nucleotides, another class of molecules required for cell growth. We observed that the expression of genes involved in amino acid and nucleotide biosynthesis wasalso upregulated in the presence of Met, suggesting that the response is structured and ultimately leads to accumulation of molecules required for overall growth. It was surprising to see that a single amino acid could do so much!

Next, the gene expression profiles between wild type and Gcn4-devoid cells in the stated conditions indicated that Gcn4 plays a crucial role in mediating the Met response. Most of the genes involved in synthesis of amino acids and nucleotides were strongly dependent on Gcn4. We also found that there are some processes (such as protein synthesis) which are only Met-dependent and Gcn4 does not play any role in them. The overall analysis suggested an interesting model where Met, with the help of Gcn4, increases biosynthesis of other amino acids and nucleotides, and thereby induces cellular proliferation!

To test the validity of this model more directly, we performed more experiments using mass spectrometry and other relevant biochemical tools. Athorough analysis revealed that Met availability increases *new* synthesis (yes, we can distinguish the newly formed metabolites from the old metabolites using isotope-labeling experiments; details in future articles) of amino acids and nucleotides in a Gcn4-dependent manner. The new synthesis of these molecules allows cell growth in the adverse conditions of amino acid limitation. Overall, we find that Met acts as a strong *growth cue* and Gcn4 acts as a *coordinator*.

Of course, as applicable for any other study, we need to do more experiments to comprehend the complete system and above analysis lead us to a more interesting set of questions such as: How does Met increase the levels of Gcn4? How do other global regulators “talk” with Gcn4?Also, the core question, i.e. *why* methionine alone? We have done some experiments that suggest the mechanism by which Met controls Gcn4 levels and are planning more to find the answers to the above questions.