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## *Literature Highlights*

# A SIDEWAYS GLANCE

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When I was a child, in the clear nights my father used to teach me the name of the stars and how to recognise the most important constellations, drawing imaginary lines from one bright point to another in the dark sky of the northern hemisphere. He also told me that with a naked eye we can see only a tiny little part of them, however to me they were so many and so magnificent they kept me staring at the night sky for endless hours.

Still, there was a group of stars always escaping my focus, sliding into fuzziness as soon as I was trying to look straight at them. My father told me I should look out of the corner of my eye and, breathless, I was finally able to clearly see the Pleiades!!

This little space of Genes & Nutrition is dedicated to commentaries intended to open new perspectives and hypotheses. I hope, with this first short commentary on 3 recently published articles, focusing on different molecular aspects of nutrition, to encourage our readers to look at results with "a sideways glance" and to consider them under a different perspective. Further contributions from those who enjoy staring at the stars is more thanwelcome….

**Ubiquitous yet still elusive?**

Metallothioneins (MT) belong to a family of small, heavy metal binding proteins (Hamer, 1986). Genes encoding MTs are strongly and efficiently regulated by different heavy metals. Thanks to this feature, but not only because of that, MT genes became a paradigm for a large number of studies on metal-dependent transcriptional activation and have been extensively studied. In spite of the large body of literature produced over the past twenty and more years, three recently published papers demonstrate that the story is not yet complete, and that MT genes can still surprise us.

Walter Schaffner and co-workers (Egli et al., 2006) recently reported the first total KO of all MT genes in higher eukaryotes. They successfully generated a D. melanogaster quadruple knockout for all four MT genes (Mtn A, B, C and D) present in this species. Molecular genetics characterization of the mutant flies enabled the authors to unravel and highlight some important functions of MTs, and to describe a novel mechanism regulating their expression. D. melanogaster is an attractive model for studies on eukaryotic transgenic organisms and their development: larvae can be grown on petri dishes containing food with defined composition (in this particular case different metal concentrations) and the phenotype checked within few days. Mutant flies were viable and fertile when raised on a standard laboratory diet, but they were very sensitive to copper, cadmium and, to a lesser extent, to zinc loads during early development. Adult MT mutants displayed significantly shortened life span with mild copper overload, the males being affected earlier than females.

The authors report an important difference between MT-KO mutants and mutants for the metal-dependent transcription factor (MTF-1), which is the main regulator of MT gene expression in response to metal ions. Flies lacking MTF-1 were more sensitive to zinc, silver and mercury overload than flies lacking MTs. This observation led the authors to suggest that induction of other genes regulated by MTF-1 was responsible for this sensitivity. Using reporter gene constructs and a shortened version of the MT promoter, containing only four tandem metal response elements (MRE), the authors demonstrate that MREs are sufficient to mediate tissue-specific expression of MT during development. Moreover, tissue-specificity of MT expression was dependent on metal distribution in the tissue rather than the opposite. The experimental evidence comes from MTF-1-dependent expression of an MtnB-reporter gene construct, that was higher in MT mutant flies, as compared to wild type flies, after induction with copper, cadmium, silver, zinc and mercury. Since no differences were found in total metal content between wild type and mutant flies, this surprising result led to the conclusion that a negative feedback mechanism operates in MT gene regulation and that once metals are bound to MT, they are not able to activate MTF-1 anymore.

In a quite distinct field of research, Robert Tjian and collaborators provided additional results addressing the fine-tuning of regulation of MT expression (Marr et al., 2006). They used D. melanogaster MT genes as an expedient model to investigate the role of two regulatory co-activator complexes: TFIID and Mediator (MED). Their results demonstrate that MTF-1 binding to the MT promoter depends on its previous binding to TFIID, and that transcription starts only when MED subunits are added to the complex. Using RNA interference and quantitative PCR analysis of the Mtn A, B and D genes, whose transcription is induced by MTF-1, they reported a differential requirement of each promoter for specific MED subunits. For instance, MED13 is not essential for Mtn A induction, while it is important for both Mtn B and

Mtn D activation. These results highlight the importance of the context of the core promoter for appropriate expression levels of MT genes, which must be taken into account for a deep understanding of the regulation of expression of these metal-binding proteins.

However, "the wise man" says that transcription of a gene, like the end of a caterpillar, is not the end of the story but rather the beginning of a butterfly's life. According to this tale, in the third paper whose reading I would like to suggest, John Hesketh and co-workers add another piece to the jig-saw puzzle of MT expression, clarifying the role of MT mRNA structure in targeting newly translated MT protein (Mickleburgh et al., 2006). This work stems from previous observations by the same Authors, which indicated that MT-1 mRNA is associated with cytoskeletal-bound polysomes in the perinuclear space (Mahon et al., 1997). Such localization can therefore facilitate import into the nucleus, where MTs have been suggested to play an important role in protecting DNA from oxidative damage. Hesketh and collaborators further demonstrate with different experimental approaches that a stem/loop secondary structure in the 3'-untraslated region of MT-1 mRNA engaged in translation binds to eukaryotic elongation factor 1α (eEF1α). Following eEF1α binding, MT-1 mRNA is anchored to the cytoskeleton in the perinuclear cytoplasm, suggesting a multifunctional role for translation factors, which might play key roles in mRNA localization as well as in translational regulation.

Taken together these papers are pointing at the importance of the molecular, cellular physiological context in modulation of MT gene expression in response to metals (and possibly other stresses). This point of view is of great interest, considering the highly conserved structure of these proteins and their widespread distribution in eukaryotes, from yeast to humans. To further emphasize this point, I would like to recall a "vintage" paper published about 19 years ago by Hurst, Schats and Matts (good wine gets better while ageing) (Hurst et al., 1987). They had investigated and reported the effect of heavy metal ions on protein synthesis in a cell-free system, clearly demonstrating that metals are able to inhibit protein synthesis through phosphorylation of the eukaryotic Initiation Factor 1α (eIF1α), thus blocking protein chain initiation. Furthermore, the extent of inhibition of PS paralleled the relative biological toxicity of each metal ion tested. It is certainly intriguing to speculate whether this could be another checkpoint for control of MT expression....

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