Simon Labbé's work on iron and copper homeostasis

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Author contributions: Labbé S solely contributed to this manuscript.

Supported by The Canadian Institutes for Health Research (MOP-36450 to Labbé S), Natural Sciences and Engineering Research Council of Canada (MOP-238238-2010 to Labbé S), and the Fonds de la Recherche en Santé du Québec (Senior Investigator Scholarship to Labbé S)

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Received: May 8, 2010 Revised: May 18, 2010

Accepted: May 25, 2010

Published online: May 26, 2010

Figure 1 Simon Labbé, PhD, Professor, Department of Biochemistry, Faculty of Medicine, Université de Sherbrooke, Canada.

Abstract

Iron and copper have a wealth of functions in biological systems, which makes them essential micronutrients for all living organisms. Defects in iron and copper homeostasis are directly responsible for diseases, and have been linked to impaired development, metabolic syndromes and fungal virulence. Consequently, it is crucial to gain a comprehensive understanding of the molecular bases of iron- and copper-dependent proteins in living systems. Simon Labbé maintains parallel programs on iron and copper homeostasis using the fission yeast *Schizosaccharomyces pombe* (*Schiz. pombe*) as a model system. The study of fission yeast transition-metal metabolism has been successful, not only in discerning the genes and pathways functioning in *Schiz. pombe*, but also the genes and pathways that are active in mammalian systems and for other fungi.

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Key words: Iron and copper transport systems; Metalloregulatory sensors; Iron- and copper-responsive

genes; Metal trafficking pathways; Metalloenzymes; Fungi as model systems

Peer reviewer: Gaetano Cairo, PhD, Professor, Department Human Morphology and Biomedical Sciences-Città Studi-Via Mangiagalli 31, Milano, 20133, Italy

Labbé S. Simon Labbé's work on iron and copper homeostasis. *World J Biol Chem* 2010; 1(5): 196-200 Available from: URL: <http://www.wjgnet.com/1949-8454/full/v1/i5/196.htm> DOI: <http://dx.doi.org/10.4331/wjbc.v1.i5.196>

INTRODUCTION AND EDUCATIONAL EXPERIENCE

Dr. Simon Labbé is a Professor of Biochemistry in the Faculty of Medicine and Health Sciences at the Université de Sherbrooke, Canada (Figure 1). He received his bachelor's degree in Microbiology from the Université Laval (Québec, Canada) in 1987. He pursued graduate work at Université Laval and received his Masters and Ph.D. in microbiology and molecular/cellular biology, respectively. Subsequently, Dr. Labbé trained as a postdoctoral fellow in biological chemistry with Professor Thiele DJ at the University of Michigan (Ann Arbor, USA). He was sup-

Figure 2 A proposed transcription model for an “iron economy”. Under low iron supply conditions, Fep1 cannot bind to DNA and *php4⁺* is expressed. Once biosynthesized, Php4 interacts with the Php2/Php3/Php5 heterotrimer to mediate repression of genes that encode iron-storage (Pcl1) and iron-containing (Sdh4 and Isa1) proteins, as well as the *feh1⁺* gene. Conversely, under iron-replete conditions, Fep1 binds DNA and, with the aid of Tup11 and Tup12, turns off *php4⁺* gene expression. This inactivation of *php4⁺* enables genes involved in storage and utilization of iron to be expressed via the Php2/Php3/Php5 heterotrimeric CCAAT binding factor.

ported by Fellowship awards, including the prestigious Centennial Fellowship from the Medical Research Council of Canada. Dr. Labbé has been an invited speaker at international meetings, and is a peer reviewer for scientific journals, as well as career and research grant committees. As an independent investigator, he has received the New Investigator Award from the Canadian Institutes of Health Research, as well as the Junior II and Senior Investigator Awards from the Fonds de la Recherche en Santé du Québec.

ACADEMIC STRATEGY AND GOALS

Over recent years, Dr. Labbé's research group has developed a productive yeast model [*Schizosaccharomyces pombe* (*Schiz. pombe*)] for investigating both iron and copper metabolism at the molecular level. Because *Schiz. pombe* provides an excellent and useful model that permits the drawing of parallels with other eukaryotic living systems, his studies have led to significant advances in understanding how cells regulate the acquisition of iron and copper. Dr. Labbé's research also utilizes the model organism *Saccharomyces cerevisiae* (*S. cerevisiae*) as an intermediate step to advance our understanding of metal ion signaling and trafficking in eukaryotic cells. By combining the best features of both species of yeast, *Schiz. pombe* and *S. cerevisiae*,

he expects to elucidate mechanisms and genes common to all eukaryotes. Thus far, Dr. Labbé's research has contributed to the identification of molecular defects in iron and copper transport, as well as uncovering different strategies that yeasts have acquired to take up iron and copper from their environment and/or hosts.

ACADEMIC ACHIEVEMENTS

The following contributions highlight Dr. Labbé's activities in the field of iron and copper homeostasis.

Discovery of Fep1, an iron sensor that represses iron transport gene expression

Dr. Labbé and his team took advantage of the *Schiz. pombe* model to identify the GATA-type transcription factor Fep1^[1-3]. The identification of Fep1 was significant because it revealed that iron-regulated gene expression in fungi other than *S. cerevisiae* is mediated by a group of non-canonical GATA-type transcription factors. These studies also demonstrated, for the first time, a central role for Fep1 in coordinating regulation of genes that encode components of the reductive and non-reductive iron transport systems^[4]. Using a genetic approach to find GATA-factor-associated proteins, his research group identified the co-repressors Tup11 and Tup12 as physi-

Figure 3 A model (steps 1-4) for copper homeostasis in *Schizosaccharomyces pombe* (*Schiz. pombe*). Step 1: Copper is reduced from Cu^{2+} to Cu^+ by a putative cell surface reductase. Following reduction, copper is transported across the plasma membrane by a heteroprotein complex formed by the Ctr4 and Ctr5 proteins; Step 2: Three copper chaperones, namely, Atx1, Pccs and Cox17, deliver copper to specific intracellular localizations. Atx1 shuttles copper from the cytosol to post-Golgi vesicles by docking specifically with the Ccc2 copper-transporting P-type ATPase. Once loaded, Ccc2 pumps copper into the lumen of the Golgi, which provides copper to the ferroxidase Fio1 as part of its maturation (with Fip1). Atx1 can function as a copper chaperone for a molecule other than Ccc2, which participates in the delivery of copper to Cao1. In the mitochondria, copper delivered by Cox17 is incorporated into cytochrome c oxidase, with the aid of Sco1 and Cox11. Pccs delivers copper to SOD1 in the cytosol; Step 3: When the pool of cytoplasmic copper is insufficient, Ctr6 is involved in copper efflux from the vacuole, which provides copper to cytosolic copper-dependent enzymes. Step 4: In response to copper deficiency, Cuf1 is localized in the nucleus where it activates the transport of copper by upregulating expression of the *ctr4+*, *ctr5+* and *ctr6+* genes. Conversely, in cells that undergo a shift from low to sufficient copper concentrations, Cuf1 translocates from the nucleus to the cytoplasm. The functional link between copper transport and iron uptake is explained by the observation that the Fip1 iron permease must couple with Fio1, which is a copper-dependent enzyme, to move through the secretory pathway and assemble as a functional iron-transport complex at the plasma membrane. Therefore, the inability to deliver copper to the interior of the cell, or to traffic copper appropriately to the lumen of the secretory compartment where it binds to Fio1, which leads to an iron-starvation defect.

ological partners of Fep1^[5]. This is significant because these results describe the first example of a physical interaction between a co-repressor and an iron-sensing factor that control the expression of iron uptake genes^[6,7].

Discovery of the iron-responsive regulatory subunit Php4 of the CCAAT-binding complex

Dr. Labbé's laboratory has shown that, under high iron conditions, transcriptional activation of genes for iron utilization results from a transcriptional activator complex composed of Php2, Php3 and Php5. In contrast, under low iron conditions, his group has determined that Php4 associates with the Php2/Php3/Php5 heterotrimer and inhibits target gene expression^[8]. The outcome of these findings is considerable because it establishes a new mechanism for downregulation of iron metabolic genes during iron deficiency (Figure 2). Using microarrays, many new Php4 target genes have been identified^[9]. These results are important because future studies that address the function of these genes should provide new insights into how cells adapt to conditions of iron deficiency. Although the mechanism by which Php4 is inactivated by iron remains poorly understood, Dr. Labbé's

group recently has found that the glutaredoxin Grx4 and the exportin Crm1 are involved in this process^[10]. Taken together, these findings have been highlighted in influential reviews^[11,12].

One of the first demonstrations that vacuoles communicate with the plasma membrane to ensure that sufficient copper is acquired for effective functioning of copper-dependent enzymes

Dr. Labbé and coworkers have published a seminal paper on the monitoring of copper uptake and the role of the vacuolar membrane copper transporter Ctr6^[13]. Using a cDNA library, they have isolated *ctr6+*, a novel Ctr family member (Figure 3). With sustained expression of Ctr6, cells were hypersensitive to copper, but ⁶⁴Cu uptake was reduced by approximately 70%. Consistent with this reduction in copper uptake, transcript levels of the plasma membrane copper transporters Ctr4 and Ctr5 were also reduced^[14]. On the basis of these results, we have proposed a model of vacuole to plasma membrane communication. As Ctr6 mobilizes vacuolar copper, the copper-sensing transcription factor Cuf1 senses a larger pool of labile copper, which in turn downregulates the Cuf1-

dependent transcription of Ctr4 and Ctr5, which results in a reduction of copper uptake. As a sign of recognition, the work has been discussed in leading reviews^[15,16].

Pccs copper chaperone functions in dual pathways to deliver and sequester copper, while Atx1 carries copper to Cao1

The copper chaperone for superoxide dismutase 1 (SOD1), termed CCS, functions in the delivery of copper to SOD1^[17]. In *Schiz. pombe*, a functional homolog of CCS is named Pccs (Figure 3). It harbors an extra domain at the C terminus. Dr. Labbé and his colleagues have shown that the first three domains of Pccs are required to incorporate copper into SOD1 in the presence of low copper levels, whereas the fourth domain of Pccs functions to sequester metals under conditions of elevated copper concentrations^[18]. These results describe the first example of a metalloprotein that functions as a copper chaperone when copper levels are low and as a detoxifier when copper levels are high. Dr. Labbé's team has demonstrated that the metallochaperone Atx1 directly activates the copper amine oxidase Cao1^[19]. Although it has been shown that Atx1 specifically delivers copper to the copper ATPase Ccc2 in the secretory pathway^[20,21], Dr. Labbé's studies have described the first example of the ability of Atx1 to act as a copper carrier for a molecule other than Ccc2^[22].

Identification of the nutritional copper transcription factor Cuf1: its function and regulation

To gain insight into important structural and functional features of copper-sensing transcription factors, Dr. Labbé has cloned a gene that encodes a putative copper metalloreulatory protein (Cuf1) from *Schiz. pombe*^[23]. Cuf1 is composed of functional modules that resemble two distinct types of copper metalloreulatory factors, which share similar DNA sequence binding specificity as the toxic copper sensor Ace1, while playing an essential role in the activation of the copper transporter gene expression in *Schiz. pombe*, as does the Mac1 protein in *S. cerevisiae*^[24,25]. Dr. Labbé's group has found that Cuf1 localizes to the nucleus in copper-deficient cells but is retained in the cytoplasm when cells are grown under copper-replete conditions^[26]. Furthermore, his group has discovered that, in cells that undergo a shift from low to sufficient copper concentrations, Cuf1 translocates from the nucleus to the cytoplasm^[27]. The exportation of Cuf1 to the cytoplasm is likely to serve as a cellular defense mechanism to ensure that no copper transport gene expression takes place in excess copper conditions.

CONCLUSION

Identification of the genes and proteins involved in the regulation of iron and copper homeostasis, and elucidation of their primary functions, is a crucial step for attaining a more detailed, integrated understanding of iron and copper metabolism. Due to the facilitation of analysis

of protein function from yeast genetics, *Schiz. pombe* is an excellent model organism for studying fundamental processes in eukaryotic cells, especially those of metal ion homeostasis. Overall, Dr. Labbé's research has provided a better understanding of the key mechanistic aspects of iron and copper homeostasis that includes iron/copper-mediated cellular sensing, assembly, trafficking and compartmentalization.

ACKNOWLEDGMENTS

I am grateful to the past and present members of my laboratory for their contributions to our studies. I also wish to express my gratitude to several investigators worldwide for their collaborations.

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S- Editor Cheng JX **L- Editor** Lutze M **E- Editor** Zheng XM