[S8-2]

A Novel Mechanism for the Iron-Sparing Response

Simon Labbé* and Alexandre Mercier

Département de Biochimie, Faculté de médecine, Université de Sherbrooke, Sherbrooke, QC, J1H 5N4, Canada

All organisms share a requirement for the regulation of iron metabolism as a function of iron availability. Our studies in *Schizosaccharomyces pombe* have led to the identification of the gene *php4*+ that encodes a negative regulatory subunit of the CCAAT-binding factor, which is induced under conditions of iron starvation. Once synthesized, the Php4 subunit associates with the CCAAT-binding core complex, which is composed of Php2, Php3, and Php5. Once formed, the Php2/3/4/5 transcription complex is required to inactivate a subset of genes encoding iron-using proteins, as well as the *fep1*+ gene, which encodes the iron-responsive transcriptional repressor of iron transport genes. In contrast, when cells undergo a transition from iron-limiting to iron-sufficient conditions, we determined that iron-dependent inactivation of Php4 is controlled at two distinct levels: first, at the transcriptional level by the iron-responsive factor Fep1 and second, at the posttranslational level by a mechanism that remains unclear. In this report, we show that, in cells undergoing a shift from low to sufficient iron concentrations, Php4 accumulates in the cytosol, exhibiting a pancellular pattern. Among four different metal ions tested, iron, manganese, cobalt, and cadmium, only iron fosters the nuclear export of Php4. We determined that the nuclear exit of Php4 requires the presence of a leucine-rich nuclear export signal (NES), 93-LLEQLEML-100, near the middle part of Php4. Furthermore, we found that a reporter GST-GFP fusion protein harboring the Php4 93-NES-100 was exported from the nucleus. Mutagenesis of the NES abolished Php4 translocation to the cytosol. Treatment of cells with leptomycin B, an exportin inhibitor, results in the nuclear accumulation of Php4. Taken together, the results reveal that the negative regulatory subunit Php4 undergoes iron-regulated nuclear-cytoplasmic shuttling, providing a rapid response to changes in the supply of iron.