Abstract

Inositol pyrophosphates are energy-rich signalling molecules which contain one or more diphosphate or pyrophosphate moieties. As the energy released upon hydrolysis of their pyrophosphate moiety is nearly equivalent to the energy of hydrolysis of ATP, these molecules are considered as “high-energy” phosphate donors. The most abundant inositol pyrophosphates that occur in all eukaryotes from yeast to mammals are 5-IP$_7$ and IP$_8$. In the budding yeast, *Saccharomyces cerevisiae*, synthesis of inositol pyrophosphates starts with cleavage of PI(4,5)P$_2$ by Plc1 which results in release of IP$_3$ into the cytoplasm. Sequential phosphorylation of IP$_3$ by Ipk2 and Ipk1 results in the synthesis of IP$_6$, which is further phosphorylated by Kcs1 to form 5-IP$_7$. Vip1 further phosphorylates 5-IP$_7$ and synthesizes IP$_8$. Another form of IP$_7$ i.e. 1-IP$_7$ is synthesized by Vip1 from IP$_6$, which in turn is converted to IP$_8$ by Kcs1. The pyrophosphate group on these molecules is removed by the enzyme Ddp1 in *S. cerevisiae*, to convert IP$_8$ and both forms of IP$_7$ to IP$_6$. The physiological concentration of 5-IP$_7$ ranges from 0.5 to 5 µM in yeast. IP$_7$ and IP$_8$ regulate multiple cellular pathways by two molecular mechanisms; (a) by directly binding to proteins and modulating their function, and (b) protein pyrophosphorylation, in which a β phosphate is transferred from inositol pyrophosphates to pre-phosphorylated serine residues that are surrounded by acidic amino acids. *S. cerevisiae* strains that lack inositol pyrophosphates (*kcs1Δ*) display defects in growth, telomere length maintenance, vesicular trafficking, stress response, energy metabolism, and phosphate homeostasis. Inositol pyrophosphates are termed as ‘energy sensors’ or ‘metabolic messengers’, as they couple cellular signalling pathways with the energy status of the cell. One such signalling pathway is ribosome biogenesis which shows a swift response to the energy status of the cell as it consumes most of the cell’s energy.

Ribosome biogenesis is a complex process that involves 35S rRNA synthesis by RNA Pol I, which is processed to give rise to mature 25S, 18S and 5.8S rRNA. Mature 18S rRNA is then assembled into the 40S ribosome subunit and the mature 25S and 5.8S rRNA assemble into the 60S subunit along with 5S rRNA that is synthesized by RNA Pol III. Approximately 5500 nucleotides of rRNA and 79 ribosome proteins (r-proteins) are assembled into one ribosome unit. An actively growing cell synthesizes approximately 2000 ribosomes per minute, and therefore the energy demand for this
process is high compared to other cellular processes. In general, actively dividing yeast require a high amount of protein synthesis, and consistent with this a reduction in the rate of protein synthesis slows down yeast growth.

Previous studies noted that yeast lacking 5-IP$_7$ displayed slow growth and that proteins involved in yeast ribosome biogenesis such as Nsr1 and Srp40 were pyrophosphorylated by 5-IP$_7$. These observations prompted us to study the role of inositol pyrophosphates in ribosome biogenesis. In this study we observed that yeast lacking IP$_7$ displayed increased sensitivity to different translation inhibitors and a decreased rate of protein synthesis compared to wild type yeast. kcs1Δ yeast exhibited compromised levels of polysomes, monosomes, 40S and 60S ribosome subunits, indicating that yeast lacking IP$_7$ were defective in ribosome biogenesis. These observations persuaded us to investigate the first step of ribosome biogenesis i.e. rDNA transcription, which revealed a decrease in rRNA synthesis in kcs1Δ yeast. Though there is impaired rRNA transcription in yeast lacking the inositol pyrophosphate 5-IP$_7$, recruitment of RNA Pol I on rDNA is not compromised, suggesting a defect in RNA Pol I mediated elongation.

RNA Pol I is a 14 subunit complex and we examined the protein sequences of all subunits for acidic serine motifs which are known to be potential IP$_7$ target sites. Five proteins were found to contain acidic serine regions, out of which three subunits- A34.5, A43 and A190, were pyrophosphorylated by IP$_7$. Mutation analyses of these proteins helped localise the sites of pyrophosphorylation. Functional analyses of these sites suggested that IP$_7$-mediated pyrophosphorylation of these three subunits together may impact RNA Pol I function, as loss of pyrophosphorylation on individual subunits did not have any effect. Ribosome biogenesis is an indicator of cellular energy levels as it utilizes 80% of a cell’s energy. IP$_7$ is considered as a “metabolic signalling molecule” and we speculate that in kcs1Δ yeast, loss of IP$_7$ may lead to impaired co-ordination between cellular energy and ribosome biogenesis.

This work has been published recently as Thota, S. G. et al., Inositol pyrophosphates regulate RNA polymerase I-mediated rRNA transcription in Saccharomyces cerevisiae (2015), Biochem. J, 466:105-14.
Given below is the chapter-wise organization of this thesis.

**Chapter 1** summarizes the literature related to this study and is organized into three sections describing the metabolism and physiological roles of inositol pyrophosphates, synthesis of ribosomes in yeast, and the rationale for this study.

**Chapter 2** provides a description of experimental materials and methods used in this study.

**Chapter 3** describes the results obtained from biochemical experiments to characterise the process of ribosome biogenesis in yeast lacking inositol pyrophosphates. We studied different stages involved in the ribosome biogenesis pathway to home in on the precise step that is regulated by inositol pyrophosphates.

**Chapter 4** gives a detailed account of identification of pyrophosphorylatable proteins involved in rRNA synthesis. This chapter also includes the demonstration of the location of pyrophosphorylation sites, followed by mutation analyses to establish the functional relevance of these sites.

**Chapter 5** provides a detailed analyses of the results obtained, summarises the findings of the current study, and presents future prospects offered by this study.