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Cell Systems Biology of Translation Factors and Proteasome-Targeted Protein Complexes Associated with AGC Kinase Sch 9

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Abstract

Sch-9 appears to be the *Saccharomyces cerevisiae* homolog of protein kinase B and S6 kinase and is involved in the control of numerous nutrient-sensitive processes, including regulation of cell size, cell cycle progression, and stress resistance. *Sch-9* has also been implicated in the regulation of replicative and chronological life span. The availability of data from global studies of protein-protein interactions now makes it possible to predict and validate functional connections between *Sch-9*, its putative substrates, and other proteins. *Sch-9* appears to be involved in control of biosynthetic and catabolic pathways. Thus, the analysis of *Sch-9*-associated proteins indicates that this kinase may be involved in regulation of protein synthesis. *Sch-9* forms a complex with, and, presumably, phosphorylates starvation- and stress-induced protein kinase GCN2, which, in turn, phosphorylates translation initiation factor eIF2-alpha. *Sch-9* also interacts with translation factors Arc1, Pab1 and prion-like protein Sup-35. Thus, *Sch-9* may be part of the mechanism that relays availability of nutrients to utilization of glucose and to the rates of protein synthesis. One of the interesting outcomes of the proteome-wide analysis of protein-protein interactions in yeast is the finding that *Sch-9* associates with Shp1, Cdc48, and Ufd1, which form a complex responsible for the recognition and targeting of ubiquitinated proteins to the proteasome for degradation. It is unknown and remains to be elucidated, whether mammalian paralogues of *Sch-9* are also associated with the proteins involved in translation/protein synthesis and proteasomal degradation.

Keywords: *Sch-9* • AGC Kinase • Protein-protein interactions • Shp1-Cdc48-Ufd1 Complex • Arc1 • Pab1 • Sup-35 • GCN2 • Regulation of translation • Proteasome • Protein degradation • UPS

Introduction

The AGC kinases constitute a large family of serine/threonine protein kinases consisting of 60 members, including the cAMP- and cGMP-dependent protein kinases (PKA and PKG), the protein kinase C family (PKC), PKB/Akt, ribosomal protein S6 kinases, and the 3-phosphoinositide-dependent protein kinase (PDK1). They regulate many critical processes including metabolism and cell proliferation. *Sch-9* appears to be the *Saccharomyces cerevisiae* homolog of protein kinases PKB and S6 kinase and is involved in the control of numerous nutrient-sensitive processes, including regulation of cell size, cell cycle progression, and stress resistance [1-3]. *Sch-9* has also been implicated in the regulation of replicative and chronological life span [4,5]. The availability of data from global studies of protein-protein interactions now makes it possible to predict and validate functional connections between *Sch-9*, its putative substrates, and other proteins. *Sch-9* appears to be involved in control of biosynthetic and catabolic pathways. Thus, the analysis of *Sch-9*-associated proteins indicates that this kinase may be involved in regulation of protein synthesis and protein degradation.

Literature Review

Domain organization of AGC family kinases

Typical domain organization shared by the members of this group includes (1) N-terminal membrane-targeting, lipid binding domains (C1, C2 or PH), (2) catalytic kinase domain, and (3) C-terminal regulatory hydrophobic (H) motif.

AGC Kinase docking motifs

Members of this family contain a hydrophobic surface in the N-terminal lobe of their catalytic domain, called the PDK1 Interacting Fragment (PIF) pocket, and a non-catalytic C-terminal tail containing different motifs, including the AGC Kinase docking motifs that interact with the PIF pocket. Both these regions are conserved in Eukaryotic AGC kinases, except for PDK1, which lacks the C-tail. The AGC Kinase docking motif mediates intramolecular interactions to the PIF pocket, serving as a cis-activating module, but can also act as a PDK1 docking site that trans-activates PDK1, which in turn will phosphorylate the docked AGC kinase.

Domain organization of *Sch-9*

C2 domain includes amino acid residues 166-378; protein kinase domain – residues 412-671; AGC kinase C-terminal domain – residues 672-748. Within C-terminal domain (residues 733-738), AGC Kinase docking motif is situated. On the basis of sequence comparisons, it is expected that the C2 domain is responsible for targeting *Sch-9* to vacuolar or vesicular membranes. Threonine 570 within the kinase domain is phosphorylated by PKH1 or PKH2 activated by sphingolipid phytosphingosine (PHS). Multiple serine and threonine residues within AGC-kinase C-terminal domain are phosphorylated by TORC1 complex: Phosphoserine 711; Phosphothreonine 723; Phosphoserine 726; Phosphothreonine 737; Phosphoserine 758; Phosphoserine 765 [3]. *Sch-9* is also phosphorylated by upstream kinase SNF1 that is also a nutrient sensor. It is presently unknown which motif (s) in *Sch-9* mediates interactions of the kinase with other proteins.

Control of aging by *Sch-9* and other AGC family kinases

We proposed the model, showing the network of signaling components that control stress responses and aging in *S. cerevisiae*, regulated by AGC kinases [1]. Availability of nutrients, sphingolipid metabolites, and exposure to stress factors tune the activity of AGC kinases PDK-like kinases Pkh1 and

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2, S6/PKB-like kinase *Sch-9*, SGK-like kinases Ypk1 and 2, and Pkc1. *Sch-9* receives input from TORC1 complex, SNF1 and Pkh1/2. *Sch-9* is involved in the coordination of several transcriptional responses, including induction of antioxidants, heat shock proteins, ribosomal protein genes, and ribosomal biogenesis, and genes that promote progression through the G1 phase of the cell cycle [6]. These coordinated responses affect metabolic rates and biomass accumulation, and ultimately set the limits of chronological and replicative aging.

Genetic interactions and protein-protein interactions that involve *Sch-9*

The availability of data from global studies of protein-protein interactions and PTMs of protein kinases and other signaling proteins now makes it possible to predict and validate functional connections between the kinases, their putative substrates, modulators and other proteins (Supplementary Tables 1-6) [1]. In the present study we set to further characterize molecular composition of protein signaling complexes that consist of *Sch-9* and associated proteins. We also used the data of publicly and privately available databases and high-throughput proteomics studies to characterize protein-protein interactions that take part in signaling via this multiprotein complex. We set to identify putative *Sch-9* phosphorylation substrates and regulatory proteins and attempt to understand their biological functions, such as protein synthesis (regulation of translation) and proteasomal protein degradation.

The data of published studies of protein-protein interactions, *Sch-9* entries of BIOGRID and SGD and few other databases were confirmed by data mining of ResNet Yeast database integrated into Pathway Studio software (Ariadne Genomics Inc., now Elsevier) [7]. SGD database (https://www.yeastgenome.org) lists 883 total interactions for 696 unique genes, 46 physical, 17 combined physical/genetic interactions for *Sch-9*. The BioGrid database (https://thebiogrid.org) includes 29 physical interactors; 17 physical/genetic interactors and 650 genetic interactors for *Sch-9*. Based on this data, we assembled comprehensive list of *Sch-9*-associated proteins in *S. cerevisiae*.

Sch-9 and control of translation and protein synthesis

Curiously, we found that *Sch-9* forms a complex with, and, presumably, phosphorylates proteins that function in translation and protein synthesis. In addition to previously reported ribosomal and ribosomal biogenesis proteins, we noted that *Sch-9* interacts with protein kinase GCN2 that is activated by either nutrient deprivation or secretion stress [6]. This protein kinase, when activated, phosphorylates translation initiation factor eIF2-alpha. It is known that *Sch-9* might control phosphorylation and activity of eIF2-alpha [3]. The nature and functions of GCN2-*Sch-9* interactions are currently unknown. *Sch-9* also presumably interacts with and phosphorylates Arc1, Pab1 and prion-like protein Sup-35. Arc1 is a component of tRNA synthetase complex. Pab1 functions in association between the 5′ cap and 3′ mRNA poly (A) tail, and Sup-35 functions in the termination of translation. Thus, *Sch-9* may be part of the mechanism that relays availability of nutrients to utilization of glucose and to the rates of protein synthesis.

The connection between the control of aging by *Sch-9* and the rates of translation and protein synthesis are of special interest, given recent findings in other systems that protein synthesis and ribosome biogenesis machinery regulated by the mTOR pathway are deteriorated with age [8]. It is unknown and remains to be elucidated, whether mammalian paralogues of *Sch-9* are also associated with the proteins involved in translation/protein synthesis.

In mammalian cells, S6K1 was also shown to control healthy mammalian life-span, as the knock-out female mice have a phenotype which mimics exposure to caloric restriction and increased longevity [9]. It regulates initiation and elongation of translation [10]. S6K associates with the eukaryotic initiation factor 3 (eIF3) translation initiation complexes, which serves as a scaffold [11]. Cell stimulation triggers TOR activation, recruitment to S6K-eIF3 complex and phosphorylation of S6K1, leading to S6K1 dissociation, activation, and subsequent phosphorylation of its translational targets, including eucaryotic translation initiation factor 4B (eIF4B), ribosomal protein S6 and elongation factor 2 kinase (EEF2K) [11]. S6 kinase phosphorylates and activates several substrates in the preinitiation complex, including the EIF2B complex and the cap-binding complex component EIF4B. It also interacts with EIF3B and EIF3C. S6 kinase controls translation initiation by phosphorylating a negative regulator of EIF4A, PDCD4, targeting it for ubiquitination and subsequent proteolysis [12]. S6 kinase promotes initiation of the pioneer round of protein synthesis by phosphorylating RNA binding protein POLDIP3/SKAR [13]. In response to IGF1, it also activates translation elongation by phosphorylating EEF2K, which leads to its inhibition and thus activation of EEF2 (for the summary of S6 kinase regulation of translation factors, see S6K1/2 entries in GeneCards database).

Other AGC-family kinases do not appear to interact with the eukaryotic initiation factor 3 (eIF3) complexes in unstimulated or stimulated cells. Therefore, we don't presently know whether S6 kinase homologue *Sch-9* interacts with yeast homologues of eIF3 subunits and whether it phosphorylates homologues of eIF4B, S6 and other translation factors.

Sch-9, Ubiquitination and proteasomal protein degradation

The activity of Ubiquitin-Proteasome System (UPS) deteriorates with age. To what extent this activity is regulated by *Sch-9* signaling is presently unknown. In *Sch-9* null cells, overall level of total ubiquitinated proteins is significantly decreased, pointing to possible role of this protein kinase in Ubiquitination. This effect was shown to be not due to the enhanced proteasomal degradation. The decrease in *Sch-9* protein level during the transition from log to stationary phase, when overall protein ubiquitination level went down also suggests the involvement of *Sch-9* in the regulation of protein ubiquitination [14]. Sch9 phosphorylates and activates E2 ubiquitin-conjugating enzyme Cdc34, which was implicated in cell cycle regulation. *Sch-9*-mediated cell cycle progression is triggered by proteolysis of cyclin-dependent protein kinase (CDK) inhibitor Sic1, which is mediated by its Cdc34-controlled Ubiquitination [15].

One of the interesting outcomes of the proteome-wide analysis of protein-protein interactions in yeast is the finding that *Sch-9* associates with Shp1, Cdc48, and Ufd1, which form a complex responsible for the recognition and targeting of ubiquitinated proteins to the proteasome for degradation. One possibility is that *Sch-9* itself is ubiquitinated, as it is the case with S6 kinase 1/2 (see S6K1/2 entries in PhosphoSite-Plus® database) and targeted for degradation by Shp1-Cdc48-Ufd1. It will be important to search among *Sch-9*-associated proteins to identify putative E3 ubiquitin ligase (s), such as RING finger and the components of SCF complexes that might catalyze *Sch-9* ubiquitination. To test this hypothesis further, we might need to co-immunoprecipitate *Sch-9* with poly-ubiquitin chains (such as Lysine-48-linked ubiquitin that is known to be targeted to proteasome) and validate direct *Sch-9* ubiquitination by IP-mass-spectrometry approach. If *Sch-9* ubiquitination will be established by *in vivo* and *in vitro* assays, we can further apply such assays in order to map exact ubiquitin attachments sites within *Sch-9* protein sequence by systematic lysine-to-arginine substitution of candidate attachment sites by side-directed mutagenesis in *Sch-9* expression constructs and in this way to determine whether ubiquitination is eliminated. Ultimate detection of such PTMs in *Sch-9* protein by mass-spectrometry might be also very instrumental. If established, *Sch-9* ubiquitination will raise the question of coupling between *Sch-9* modifications and its activity as protein kinase.

An alternative plausible scenario is that *Sch-9* phosphorylates some of the components of Shp1-Cdc48-Ufd1 complex to regulate its activity. To test this hypothesis, one needs to demonstrate that at least some of these proteins serve as phosphorylation substrates of *Sch-9* protein kinase activity. It is possible to examine and compare phosphorylation status of these proteins in wild-type and *Sch-9* null cells and also subject purified proteins to *in vitro Sch-9* phosphorylation assays. Whether phosphorylation status of Shp1-Cdc48-Ufd1 complex might affect recruitment of ubiquitinated substrate proteins to proteasome is also an open question. In my future work, I am interested to study the paralogues and functional counterparts of *Sch-9* in human genome/proteome and to identify the functional DNA sequence variants of these proteins and their roles in normal cell biology, cancer and aging-associated diseases.

Discussion and Conclusion

AGC kinase *Sch-9*, master regulator of longevity and stress resistance,

appears to be involved in control of biosynthetic and catabolic pathways. Even though large body of evidence has been accumulated regarding *Sch-9*-associated proteins, very little is known about *Sch-9* substrates and regulatory proteins. *Sch-9* forms a complex with, and, presumably, phosphorylates proteins that function in control of protein synthesis, such as nutrient- and stress-sensitive protein kinase GCN2 and translation factors Arc1, Pab1 and prion-like protein Sup-35. *Sch-9* associates with Shp1, Cdc48, and Ufd1, which form a complex responsible for the recognition and targeting of ubiquitinated proteins to the proteasome for degradation. Future studies will show whether *Sch-9* is itself ubiquitinated and targeted for degradation by Shp1-Cdc48-Ufd1. An alternative scenario is that *Sch-9* phosphorylates some of the components of this complex to regulate its activity. Thus, *Sch-9* may be part of the mechanism that relays availability of nutrients and exposure to stress to the rates of protein synthesis and protein degradation. The proposed hypothesis states that *Sch-9* activity controls the rates of protein synthesis (by regulating associated translation factors) and protein degradation (by regulating associated factors linked to UPS) and it should be further experimentally validated in wild-type and *Sch-9* null cells under physiological conditions and in response to nutrient deprivation, Rapamycin treatment and exposure to specific stress factors.

Conflict of Interest

The author has no a conflict of interest.

Author Contributions

Alex Sobko, PhD. is fully responsible for conception, design, acquisition, analysis and interpretation of data; drafting the manuscript, revising it critically for important intellectual content; gave final approval of the version to be published. He is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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