Title page

Title:

CELL SYSTEMS BIOLOGY OF TRANSLATION FACTORS AND
PROTEASOME-TARGETED PROTEIN COMPLEXES ASSOCIATED WITH
AGC KINASE SCH9

Minireview

By Alex Sobko *

* Ofakim 8762728, Israel. E-mail: sobkosasha@gmail.com. Formerly at: Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, 76100, Israel; Iogen Corporation, 310 Hunt Club Road East, Ottawa, Ontario, K1V 1C1, Canada. ORCID ID: <https://orcid.org/0000-0003-1873-6414>.

Running title:

CELL SYSTEMS BIOLOGY OF PROTEINS ASSOCIATED WITH AGC
KINASE SCH9

Abstract

Sch9 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. Sch9 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 Sch9, its putative substrates, and other proteins. Sch9 appears to be involved in control of biosynthetic and catabolic pathways. Thus, the analysis of Sch9-associated proteins indicates that this kinase may be involved in regulation of protein synthesis. Sch9 forms a complex with, and, presumably, phosphorylates starvation- and stress-induced protein kinase GCN2, which, in turn, phosphorylates translation initiation factor eIF2 α . Sch9 also interacts with translation factors Arc1, Pab1 and prion-like protein Sup35. Thus, Sch9 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 Sch9 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 homologues of Sch9 are also associated with the proteins involved in translation/protein synthesis and proteasomal degradation.

Key words

Sch9 / AGC kinase / Protein-protein interactions / Shp1-Cdc48-Ufd1 complex / Arc1 / Pab1 / Sup35 / GCN2 / regulation of translation / proteasome / protein degradation / UPS

Text of the article:

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.

Sch9 appears to be the *Saccharomyces cerevisiae* homolog of protein kinases PKB (Sobko, 2006) and S6 kinase (Powers, 2007), (Urban et al., 2007) and is involved in the control of numerous nutrient-sensitive processes, including regulation of cell size, cell cycle progression, and stress resistance. Sch9 has also been implicated in the regulation of replicative (Kaeberlein et al., 2005) and chronological life span (Deprez et al., 2018). The availability of data from global studies of protein-protein interactions now makes it possible to predict and validate functional connections between Sch9, its putative substrates, and other proteins. Sch9 appears to be involved in control of biosynthetic and catabolic pathways. Thus, the analysis of Sch9-associated proteins indicates that this kinase may be involved in regulation of protein synthesis and protein degradation.

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 motif. 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 motif that interacts 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 Sch9. 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 Sch9 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 (Urban et al., 2007): Phosphoserine 711; Phosphothreonine 723; Phosphoserine 726; Phosphothreonine 737; Phosphoserine 758; Phosphoserine 765. Sch9 is also phosphorylated by upstream kinase SNF1 that is also a nutrient sensor. It is presently unknown which motif(s) in Sch9 mediates interactions of the kinase with other proteins.

Control of aging by Sch9 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. Availability of nutrients, sphingolipid metabolites, and exposure to stress factors tune the activity of AGC kinases PDK-like kinases Pkh1 and 2, S6/PKB-like kinase Sch9, SGK-like kinases Ypk1 and 2, and Pkc1. Sch9 receives input from TORC1 complex (Urban et al., 2007), SNF1 and Pkh1/2. Sch9 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 (Sobko, 2006), (Huber et al., 2009), (Deprez et al., 2018). 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 Sch9. 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 (Sobko, 2006).

In the present study we set to further characterize molecular composition of protein signaling complexes, that consist of Sch9 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 Sch9 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 (Krogan et al., 2006), Sch9 entries of BIOGRID and SGD and few other databases were confirmed by data mining of ResNet Yeast database integrated into PathwayStudio software (Ariadne Genomics Inc., now Elsevier). SGD database (<https://www.yeastgenome.org>) lists 883 total interactions for 696 unique genes, 46 physical, 17 combined physical/genetic interactions for Sch9. The BioGrid database (<https://thebiogrid.org>) includes 29 physical interactors; 17 physical/genetic interactors and 650 genetic interactors for Sch9. Based on this data, we assembled comprehensive list of Sch9-associated proteins in *S. cerevisiae*.

Sch9 and control of translation and protein synthesis. Curiously, we found that Sch9 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 (Huber et al., 2009), we noted that Sch9 interacts with protein kinase GCN2 that is activated by either nutrient deprivation or secretion stress. This protein kinase, when activated, phosphorylates translation initiation factor eIF2 α . The nature and functions of GCN2-Sch9 interactions are currently unknown. Sch9 also presumably interacts with and phosphorylates Arc1, Pab1 and prion-like protein Sup35. Arc1 is a component of tRNA synthetase complex. Pab1 functions in association between the 5' cap and 3' mRNA poly(A) tail, and Sup35 functions in the termination of translation. Thus, Sch9 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 Sch9 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 (Anisimova et al., 2020).

Sch9, Ubiquitination and proteasomal protein degradation. The activity of Ubiquitin-Proteasome System (UPS) deteriorates with age. To what extent this activity is regulated by Sch9 signaling is presently unknown. In sch9 null cells, overall level of total ubiquitinated proteins is decreased, pointing to possible role of this protein kinase in Ubiquitination (Qie et al., 2015). Sch9 phosphorylates and activates ubiquitin ligase Cdc34, which was implicated in cell cycle regulation. Sch9-mediated cell cycle progression is triggered by proteolysis of cyclin-dependent protein kinase (CDK) inhibitor Sic1, which is mediated by its Cdc34-controlled Ubiquitination (Cocklin and Goebel 2011). One of the interesting outcomes of the proteome-wide analysis of protein-protein interactions in yeast is the finding that Sch9 associates with Shp1, Cdc48, and

Ufd1, which form a complex responsible for the recognition and targeting of ubiquitinated proteins to the proteasome for degradation (Sobko, 2006). One possibility is that Sch9 itself is ubiquitinated and targeted for degradation by Shp1-Cdc48-Ufd1. An alternative scenario is that Sch9 phosphorylates some of the components of this complex to regulate its activity.

It is unknown and remains to be elucidated, whether mammalian homologues of Sch9 are also associated with the proteins involved in translation/protein synthesis and proteasomal degradation. In my future work, I am interested to study the homologues and functional counterparts of this protein kinase 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.

Conclusions:

- 1) Sch9 forms a complex with, and, presumably, phosphorylates protein kinase GCN2 and translation factors Arc1, Pab1 and prion-like protein Sup35.
- 2) Sch9 associates with Shp1, Cdc48, and Ufd1, which form a complex responsible for the recognition and targeting of ubiquitinated proteins to the proteasome for degradation.
- 3) One possibility is that Sch9 itself is ubiquitinated and targeted for degradation by Shp1-Cdc48-Ufd1. An alternative scenario is that Sch9 phosphorylates some of the components of this complex to regulate its activity.
- 4) Thus, Sch9 may be part of the mechanism that relays availability of nutrients to utilization of glucose and amino acids to the rates of protein synthesis and protein degradation.

The proposed hypothesis states that Sch9 activity controls the rates of protein synthesis (by regulating associated translation factors) and protein degradation (by regulating associated factors linked to Ubiquitin-Proteasome System) and it should be further experimentally validated under physiological conditions and in response to nutrient deprivation, Rapamycin treatment and exposure to specific stress factors.

Acknowledgements

Conflict of interest

The author has no a conflict of interest

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