Laishram Rajendrakumar Singh
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Proteostasis and Chaperone Surveillance

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Preface

Human diseases have been the matter of gravest concern ever since the conception of scientific research. Whether it is basic or applied research, all intend to cater one single curiosity of man, and that is attainment of illness-free healthy life. Indeed, we are in an era where many of the earlier deadly considered disorders no longer exist, but still we have to cover a very long distance in order to find cure for all. However, in this search we have realized that it is the basic biomolecules that are of esteem importance and has a great deal of involvement in disease development. Protein is one such biomolecule of keen interest for researchers as it is responsible for the ultimate biological function. It is the fine balance of proteostasis inside the cell that is responsible for smooth functioning of various cellular activities, and any lapse in this molecular balance can lead to a fatal disorder. For instance, loss of function of a protein either due to impaired protein synthesis or improper protein folding is known to cause various disorders such as cystic fibrosis, phenylketonuria, etc., and on the other hand, gain of function can generate a cohort of aggregated species leading to various incurable disorders like Parkinson's and Alzheimer's disease. To ensure proper management of proteostasis, including protein synthesis, its proper folding, and transportation to the correct target region, cell has evolved an effective surveillance system. Innumerable research carried out to date has clearly indicated the molecular and chemical chaperones to be an essential part of this surveillance system. They not just ensure proper folding and targeting of the protein to respective organelles but also remove the improperly folded protein and various aggregates of proteins by targeting them toward the cellular debris clearance system, including proteasome, lysosome, and cell-mediated autophagy. Moreover, there are various reports which show failure in chaperone surveillance to be the reason behind the aggressive disease progression. Therefore, in the hope of finding the novel ways of curing proteopathies by altering these modulators and sentinels of protein homeostasis, it is important to understand the role of chaperone surveillance in maintaining the fine molecular balance of cellular proteostasis. This edited volume is made with an intention to help a curious beginner as well as an expert who sought the knowledge regarding protein homeostasis, the diseases that could develop due to imbalance of this homeostasis, and complete erudition of the current assessment of molecular and chemical chaperone surveillance.

The edited book entitled *Proteostasis and Chaperone Surveillance* comprises 9 different chapters arranged in three different sections that enable the

book to cover and highlight major aspects of proteostasis and chaperone functions. Section I is introduced to give a complete picture of maintaining proteome or protein stability in the cells. The section allows one to understand that there are different approaches of maintaining protein stability via structural allostery (Chap. 1) that we explained with a suitable transcription factor adaptor protein, protein posttranslational modifications (Chap. 2), and small molecule chaperones (Chap. 3) that we discuss with several small molecules and enzyme systems. Section II basically deals with the consequences brought about due to the failure of the proteostatic system. Each of the different chapters under (Chaps. 5, 6, 7, and 8) this section describes the different proteo-pathologic conditions making the section covered with almost all proteopathic mechanisms. Section III is dedicated about the chaperonic machineries that cells have, to cope up with the changing need of the proteostatic system. This section gives a detail information of the almost all of the treatment strategies of diseases caused due to failure of the proteostatic system. Chapter 9 under this section also covers the pharmaceutical strategy of inhibiting proteostatic failure by using small molecule compounds.

Chapters contributed in this book have been published keeping intact authors' justifications. Necessary editorial changes were made wherever required, and authors have been requested to revise the manuscripts multiple times to address the editorial issues. We tried our best to gather information on different aspects of this volume; however, there is a possibility of some errors still creeping in the book for which we seek reader's indulgence and feedback. We owe our sincere thanks to the authors for their valuable contribution. We are also very thankful to Springer India Limited and their staff members who were directly or indirectly associated with this project for their constant help, valuable suggestions, and efforts in bringing out the timely publication of this volume.

Delhi, India
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Dr. Laishram R. Singh is an assistant professor in the University of Delhi. He obtained his master's degree from Jamia Millia Islamia, New Delhi. After receiving a doctoral degree in protein biophysics from Jamia Millia Islamia, New Delhi, Dr. Singh continued his postdoctoral research at Fox Chase Cancer Center, Philadelphia (FCCC). During his doctoral study, he was engaged in investigating how small molecule compounds affect native protein structure, stability, and enzymatic catalysis. At FCCC his main research interest encompasses understanding the proteostasis and modulators of mutant proteins including mutants of p53, cystathionine beta synthase, and methyl tetrahydrofolate reductase. Currently, Dr. Singh (at Delhi University) is working how the dysregulated proteostasis, which is the common hallmark of many neurodegenerative and metabolic disorders, could be reversed. Dr. Singh is a well-known enzymologist and protein biochemist. He has published more than 37 publications in many esteemed journals in the field of proteostatic regulation by small molecules and various heat shock proteins. Dr. Singh is also a recipient of Elizabeth Knight Peterson Fellowship sponsored by FCCC, Philadelphia.

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Part I

Maintaining Proteostasis

Structural Allostery and Protein–Protein Interactions of Sin3

1

Tauheed Hasan and Daman Saluja

Contents

Abstract

Sin3, a global transcription regulator, acts as a molecular scaffold for complex assembly and also as a molecular adapter bridging HDAC (histone deacetylase complex) with an astonishingly large and diverse group of DNA-binding transcription factors and chromatin-binding proteins. Most of the protein-protein interactions are achieved through six conserved domains of Sin3 that include four paired amphipathic helices (PAH 1–4), one histone deacetylase interaction domain (HID) and one highly conserved region (HCR). These PAH domains, though, are structurally homologous to one another having different amino acid sequences which result in differential protein-protein interactions. The PAH domains also show conformational flexibility under different physiological conditions such as pH and temperature and conformational heterogeneity upon interaction with different proteins. Interaction with large groups of transcription factors and co-repressor complexes is also possible due to the fact that Sin3 shows structural modification such as phosphorylation, myristoylation, ubiquitination, and SUMOylation as well as structural allostery

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upon interaction with proteins. Till now, only N-terminal region of Sin3 has been characterized, which makes it more important to characterize C-terminal region of Sin3 so as to find and understand its interacting partners and their role in Sin3-mediated gene regulation. Future studies should also be directed towards understanding the regulation of Sin3 protein under different physiological conditions and modulation of its biological activity.

Keywords

Sin3 complex • Protein-protein interaction • Allosteric regulation • Post-translation modification • Co-repressor • Ubiquitination

1.1 Introduction

Sin3, a global transcription regulator, helps to regulate many biological functions including nucleosome remodelling, DNA methylation, cell proliferation and apoptosis. Sin3 does not bind to DNA but is a scaffold protein that helps the transcription of various genes by interacting with different transcription factors, forming Sin3 complex (Grzenda et al. 2009). The core complex of Sin3 consists of eight components in humans: Sin3, SAP18, SAP30, HDAC1, HDAC2, RbAp46, RbAp48 and SDS3 (McDonel et al. 2009). Presence of different sub-complexes has also been reported in different organisms; these sub-complexes contain additional components besides the components present in a core complex. Sin3 interacts with numerous transcriptional factors through its six distinct yet conserved domains that include the four imperfect repeats of paired amphipathic helices (PAH 1–4), histone deacetylase interaction domain (HID) and highly conserved region (HCR) (Grzenda et al. 2009). These PAH domains, though distinct in sequence, are structurally homologous to one another and recognize unique sets of proteins. NMR and X-ray crystallographic studies of individual PAH domains as well as PAH domains complexed with DNA-binding domains and co-repressor complex revealed that each PAH domain folds in a distinct way to interact with the proteins. There are a number of other factors which are responsible for making PAH domains an ideal region of Sin3 for interacting with large numbers of transcription factors. Presence of different splice variants and isoforms of Sin3 in humans and various other organisms further increases the flexibility of Sin3 protein to recruit a large set of proteins (Ayer et al. 1995). Interaction of Sin3 with the proteins indirectly via co-repressor complex adds another level of complexity to Sin3 functions. Post-translation modifications of Sin3 such as phosphorylation, myristoylation, ubiquitination and SUMOylation help in maintaining protein-protein stability and proper regulation of genes. Structural variation in domains of Sin3 and allosteric regulation of Sin3 further increase the flexibility and conformational heterogeneity of Sin3 protein, making it an ideal regulator of gene expression.

1.2 Sin3 Complex

Sin3 is a large protein which is thought to function as a molecular scaffold due to presence of several protein-protein interaction domains. It helps in the assembly of co-repressor complex and as a molecular adapter to bridge components of the complex with DNA-bound repressors (Sheeba et al. 2007; Silverstein and Ekwall 2005). Chromatographic data of components of Sin3 complex suggested that there are different Sin3 complexes and these are variable in their structural components. Certain proteins are found to be conserved between them and thus referred to as "core complex" while some have additional proteins. The mammalian Sin3 complex analogous to Sin3/RPD3 complex of yeast comprises of at least seven polypeptides besides Sin3, including HDACs HDAC1 and HDAC2, two histone-binding proteins RbAp46 and RbAp48 (Rb-associated polypeptides) and three Sin3-associated polypeptides SAP18, SAP30 and SAP45/Sds3. Human family with sequence similarity 60 member A (FAM60A) protein, a new member of Sin3 core complex, has been discovered recently (Smith et al. 2012). Other protein components might vary between different sub-complexes. $p33^{ING1b}$ is one such example as it is thought to associate with only certain species of

Sin3/HDAC complex (Campos et al. 2004). Similarly, Sin3A, an isoform of Sin3 present in mammals, contains five extra polypeptides besides components of core complex which includes three SAP polypeptides SAP25, SAP130, SAP180 and one RBP1 (Rb-binding protein 1) protein (Xie et al. 2011). In the years following the elucidation of the core complex, a number of other associated proteins were uncovered, including BRMS1, CpG methylated binding protein (MeCP2) and ING1/2 (Grzenda et al. 2009; Nan et al. 1998; Zhang et al. 2014). The ability to support different compositions within the complex may be yet another way to expand the functional flexibility of Sin3 complex.

In the core complex, Sin3 is the platform for protein interaction, most importantly the enzymatic activity of HDAC1 and HDAC2. RbAp46 and RbAp48 are able to bind histone H4 and H2A and might thus function in stabilizing the interaction between co-repressor complex and chromatin. HDAC1 and HDAC2 bind to conserved HID region on Sin3 and provide the enzymatic activity to the complex. RbAp46 and RbAp48 that bind to nucleosomal histones are involved in chromatin modelling such as histone acetylation, nucleosome remodelling and nucleosomal assembly (Spencer and Davie 1999). Sin3-associated proteins (SAP) provide structural support and stabilize the complex. Brief functions of different components of Sin3 complex present in different organisms are listed in Table 1.1.

1.3 Structural Overview

The basic structure of Sin3 is evolutionary conserved from yeast to mammals. It contains four paired α-helices known as PAH (paired amphipathic helix) domains separated by less conserved amino acids forming the spacer region. There are also two other conserved protein interaction domains, HID (histone interacting domain) located between PAH3 and PAH4 regions and HCR (highly conserved region) situated C-terminally to PAH4 (Fig. 1.1). The PAH domains are meant for protein-protein interaction; HID interacts with HDACs and many other components of Sin3/HDAC complex and HCR, recently identified as another protein interacting domain (Grzenda et al. 2009). The current evidence advocates Sin3 as a modular protein where PAH1-3 are earmarked for interactions with different transcription factors while the HID and PAH4 domains primarily serve scaffolding function by interacting with other components/subunits of the repressor complex. PAH1-3 domains form pre-folded binding modules on full-length Sin3, like a beads-on-a-string model (Le Guezennec et al. 2006). Unlike the other PAH domains, PAH4 most likely does not fold as a four-helix bundle but instead adapts a distinct fold (van Ingen et al. 2006). Out of the four Sin3 PAH domains, the tertiary structures of only mSin3A and mSin3B PAH1 and PAH2 domains have been determined by NMR and X-ray crystallography (Kumar et al. 2011; Nomura et al. 2005; Sahu et al. 2008). The fundamental structures of both PAH1 and PAH2 are similar; however, the helices formed in PAH1 are shorter than that of PAH2 (Sahu et al. 2008). The Sin3A PAH2 domain homodimerizes and exists in unfolded state, but Sin3B PAH2 domain is monomeric and is fully folded while PAH1 exists in homodimeric form in both isoforms. Thus, PAH2 domain shows conformational heterogeneity in two isoforms of Sin3 (Kumar et al. 2011). Structures of PAH1, PAH2 and PAH3 complex with SID domain of interacting partners have also been determined by NMR and X-ray crystallography (Sahu et al. 2008; Swanson et al. 2004). PAH2 domains of mSin3A and mSin3B hold a wedged four-helix bundle structure associated with the Sin3-interacting domain (SID) of Mad1, a transcription factor involved in cell proliferation and differentiation in mammalian cells. PAH2 domain complex with the Mad1 SID adopts an amphipathic α- helix whereas PAH1 holds a rather globular four-helix bundle structure with a semi-ordered C-terminal tail on interaction with the NRSF/REST repressor domain (Nomura et al. 2005). Solution structure of PAH3 and SAP30 complex showed that PAH3 forms a canonical hydrophobic cleft and a discrete surface formed largely by the PAH3 α2, α3 and α3′ helices. Sin3 interaction domain (SID) of SAP30 binds to PAH3 via a tripartite structural motif, including a C-terminal helix that targets the

Table 1.1 Components present in Sin3 core complex in different organisms

Component of Sin3 complex	Functions	References
Sin3	Act as a molecular scaffold to provide a platform for the assembly of numerous transcription factors and co-repressor complex and also as a molecular adapter bridging components of the complex with DNA-bound repressors	Binda et al. (2006)
HDAC1/2 (Histone deacetylase complex)	Constitute the major catalytic subunits of Sin3/HDAC complexes and provide enzymatic activity resulting in deacetylation of both histones H3 and H4	He et al. (2009)
RbAp46/48 (Retinoblastoma-associated proteins)	Rb-associated proteins can interact with histone H4 and H2A and thus are predicted to help stabilize the interaction between the Sin3/HDAC complex and histone H4 and target the Sin3/HDAC to nucleosome and thus stabilize the interaction of core-complex with chromatin	Grzenda et al. (2009)
FAM60A	Newest member of Sin3 core complex. Interact with HDAC1/2 and stabilize the core complex. It also helps in repressor of TGF-beta signalling and cell migration	Smith et al. (2012)
SAP18	Ubiquitously expressed in all mouse tissues tested and interacts directly with both mammalian Sin3 and HDAC1. Besides stabilization function of the Sin3 core complex it also has been discovered to interact with GAGA factor in drosophila and Chick hairy 1 in mammal to carry out transcription regulation	Sheeba et al. (2007)
SAP25	Besides stabilizing Sin3 core complex, it is also involved in transcription repression mediated by Sin3A in mammals by interacting with different transcription factors	Shiio et al. (2006)
SAP30	Proteins of the SAP30 family (SAP30 proteins) have a functional nucleolar localization signal and they are able to target Sin3A to the nucleolus in mammals, also interact with transcription factor and co-repressor to bring transcription regulation. Interacts directly with Rb-associated polypeptides and with HDAC1and help in stabilization of core complex	Viiri et al. (2009)
SAP45/SDS3	Important for the integrity and catalytic activity of the Sin3/HDAC core complex	Grzenda et al. (2009)
SAP130	Besides stabilization of Sin3A core complex, it can also interact with mSin3A- and HDAC-independent co-repressors and transcription factors	Fleischer et al. (2003)
SAP180	Involved in stabilizing the mSin3A complex on DNA and in mediating interactions between mSin3A and HDAC-independent co-repressors	Fleischer et al. (2003)
RPB1	Repressor for the key tumor suppressor gene Rb; functions in senescence and development and cell cycle. Interacts directly with SAP30 thus helps in stabilizing SAP30 assembly in Sin3 complex	Xie et al. (2011)
$p33^{ING1b}$	Like RBP1, it also directly interacts with SAP30 and helps in stabilization SAP30 on Sin3 core complex. Interactions between p33ING1b and Sin3A are also required for the antiproliferative function of cell	Kuzmichev et al. (2002)
BRMS1	Suppresses metastasis of multiple human and murine cancer cells without inhibiting tumorigenicity. Directly bind to SAP30 of Sin3A core complex in mammals, helps in maintaining of the core complex	Meehan et al. (2004)

(continued)

Table 1.1 (continued)

Component of Sin3 complex	Functions	References
ING1/ING2	Directly binds to SAP30 of Sin3 core complex in mammals, helps in maintaining of the core complex. Together with p33ING1b function to repress cell proliferation and import of proteins in nucleus	Grzenda et al. (2009)
MeCP2	Methy-CpG-binding protein involved in the long-term repression of genes during mammalian development in Sin3A. Interact directly with HDAC1 of Sin3 core complex helps in stabilizing the interaction between HDAC1 and HID domain	Nan et al. (1998)

Fig. 1.1 The yeast Sin3 protein: Contains 1,536 amino acids and has six regions that are highly conserved throughout evolution. The PAH (paired amphipathic helix) domains appear to be protein-interaction domains, separated by spacer. The HID (Histone interacting domain) region between PAH3 and PAH4 interacts with HDACs and many of the other core components of the Sin3/ HDAC complex. The HCR (Highly conserved region) was recently identified as another protein-interaction domain that resides on C-terminal region of Sin3 protein

canonical PAH hydrophobic cleft while two other helices and an N-terminal extension target a discrete surface of the PAH3 domain formed by α helices (α2, α3 and α3′) (Xie et al. 2011).

In spite of substantial structural homology and similarity between PAH1 and PAH2 domains of Sin3 in various organisms, the two domains recognize different sequence motifs, thereby enabling differential target specificity. However, PAH3 domains share relatively low levels of sequence identity with PAH1 and PAH2 domains (25 and 16 %, respectively), yet these domains are structurally homologous to one another (Xie et al. 2011). Recent studies from our lab have shown that the three PAH domains, although showing homology in the tertiary structure, respond differentially to the environmental signals (pH and temperature). We found that PAH2 and PAH3 domains of Sin3B largely exist in unfolded state and are thermodynamically unstable in nuclear pH condition with respect to physiological pH while the structural identity of PAH1 remains unaltered at both the pH values (Tauheed Hasan et al. 2015). Based on these studies, we assume that there exists a flexibility and conformational heterogeneity in structure of Sin3 which provides additional new surfaces for protein-protein interactions.

1.4 Isoforms of Sin3

The basic protein structure of Sin3 is highly conserved among eukaryotes from yeast to mammals and has a varied number of isoforms (Fig. 1.2). *Saccharomyces cerevisiae* has only one isoform of Sin3 while *Schizosaccharomyces pombe* has three isoforms Pst1, Pst2 and Pst3, each encoded by a separate gene. Study on three paralogues of fission yeast (*Sch. Pombe*) revealed that these isoforms have originated due to gene duplication. Pst1 is most closely related to Sin3 of budding yeast (*S. cerevisiae*), suggesting that gene duplication occurred early in the evolution process. Pst2 appears to have arisen from Pst1 gene duplication during evolution of fission yeast. Pst3 seems to have arisen from duplication very early in evolution (Benedik et al. 1999). In Drosophila, three isoforms of Sin3 (Sin3 187, Sin3 190 and Sin3 220) are reported, which are produced by alternate splicing, having different C-terminals. Sin3 220 is expressed in proliferating cells