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The multifunctional transcription factor Rap1: a regulator of yeast physiology

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TABLE OF CONTENTS

1. ABSTRACT

Transcription is a fundamental process that is tightly regulated by transcription factors to maintain cellular homeostasis. Transcription factors have DNA-binding domains, some of which are sequence specific, and are found throughout the eukaryotic kingdom. Recent studies have revealed the molecular mechanisms by which transcription factors perform their functions. In the budding yeast *Saccharomyces cerevisiae*, Rap1 (ScRap1) can either activate or repress transcription. This bimodal transcriptional activity has led to the widespread study of the mode of action of ScRap1. This review summarizes current knowledge about yeast ScRap1, including its structure, mechanisms of transcription regulation, and biological functions, and the future directions in the field.

2. INTRODUCTION

To survive under variable environmental conditions, organisms adapt themselves according to changes in their surroundings. One of the primary response mechanisms at the cellular level is the adjustment of gene transcription levels (1). To efficiently initiate transcription, the transcription factors and machinery access their binding sites on the genome. However, eukaryotic genomes are tightly packed with the help of histone and non-histone proteins to form a dynamic chromatin. The basic unit of chromatin, the nucleosome, includes two copies of each of the four core histones H2A, H2B, H3, and H4 wrapped by 146 bp of DNA. Modifications in either the DNA or the histone proteins fundamentally affect chromatin structure and function and thus influence transcription (2-4). Transcription of eukaryotic messenger RNA (mRNA) by RNA polymerase II (Pol II) is activated by the binding of trans-activating proteins to enhancer DNA elements, which initiates the recruitment of general transcription factors and Pol II to the cis-linked promoter resulting in the formation of the pre-initiation complex leading to transcription (5). Activation and repression of transcription are primarily caused by gene regulatory proteins (activators and repressors), which act by binding to specific sites on the DNA.

Saccharomyces cerevisiae Rap1 (ScRap1) is a classic example of a transcription regulator that exhibits bimodal function, i.e. acts as both an activator and a repressor. ScRap1 is an abundant nuclear protein encoded by a single-copy essential gene (6-8). ScRap1 was discovered as a positive transcriptional regulator of multiple growth-related genes (9). Later studies revealed that ScRap1 is one of the major double-stranded telomeric repeat-binding proteins in *S. cerevisiae* and has indispensable roles in telomere length regulation (10), sub-telomeric gene silencing, HML and HMR silencing (11), chromatin barrier function (12), and chromosome end protection (13, 14). Moreover, it activates the transcription of a large number of heavily transcribed genes, including those encoding glycolytic

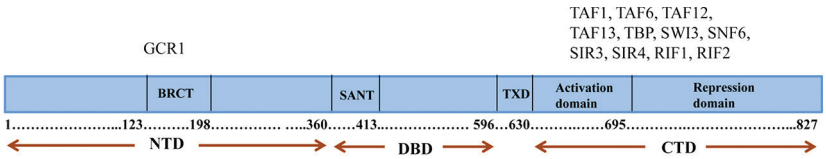

Figure 1. Schematic diagram showing Rap1 functional domains with interacting partners. BRCT (BRCA1 C Terminus), SANT (an *acronym* stands for "Swi3, Ada2, N-Cor, and TFIIIB), TXD (Toxicity domain). The proteins interacting with Rap1 are mentioned in block letters above the schematic domain sketch of Rap1.

enzymes, ribosomal proteins, and several components of the transcriptional machinery (13, 15).

Due to its wide range of targets, ScRap1 is an essential protein required for normal physiological growth and functioning of cells. In the subsequent sections of this review, we will explain the interconnections among the various roles played by this multifunctional protein; we first begin with its structural details.

3. STRUCTURE AND INTERACTING PARTNERS OF ScRap1

The ScRap1 structure has a complex organization with several independent functional domains. The 827-amino acid primary sequence can be subdivided into three regions: a DNA-binding domain (DBD) present in the centre of this protein, the N-terminal and C-terminal domains of approximately similar size as shown in Figure 1.jpg (13). Studies conducted on the DBD of ScRap1 revealed that it is an essential domain of this protein and yeast expressing only the ScRap1 DBD exhibits severe growth defects (16). The non-essential N-terminal domain of ScRap1 is similar to the putative BRCT (BRCA1 C Terminus) domain of higher eukaryotes (17). BRCT domain of ScRap1 corresponds to the region between 123-198 amino acids of this protein and surrounded by non-globular regions composed of non-hydrophobic amino acids (17). The function of the ScRap1 BRCT domain not yet described in the literature, but it has been suggested that it might have role in bending the DNA immediately flanking the ScRap1 recognition site (18). ScRap1 BRCT domain has been shown to physically interact with Gcr1 as revealed through yeast two-hybrid experiments (19). Interestingly, the ScRap1 BRCT domain has been shown to be attain flexible conformation with a small number of secondary structure elements compared to other BRCT domains, therefore, it has been suggested that ScRap1 might exhibit biological functions different from those of other BRCT domains (20). Our recent study has shown the requirement of ScRap1 N-terminal domain in maintenance of cell wall homeostasis (21). We have shown that the N-terminal deletion of ScRap1 leads to hypersensitivity for cell wall-perturbing agents and altered the cell wall structure and composition. We also detected the constitutive phosphorylation of Slt2p (a central kinase of cell wall integrity pathway) in the ScRap1 N-terminal deletion mutant. Altogether, we have shown the novel function of ScRap1 N-terminal domain in the regulation of yeast cell wall integrity (21).

Through a combination of deletion of the N-terminal and C-terminal and internal deletions of ScRap1, the DBD was localized to a central 235-amino acid fragment spanning residues 361–596 (22). The DBD of ScRap1 is composed of two Myb-like motifs, each based on a helix-turn-helix motif (23). Studies have shown that ScRap1 contains two sub-domains that bind DNA in tandem, recognizing a tandem repeat sequence. The two sub-domains are interlinked by a 30 amino acid linker and it has been suggested that the linker might play an important role in determining their relative positions. Moreover, the DNA-binding site of ScRap1 consists of two halves: the 5′ half binds a conserved half-site, the ACACC repeat, and the 3′ half binds to half-sites that exhibits sequence divergence (24, 25). Therefore, ScRap1 is able to recognize different binding sites in the yeast genome. Furthermore it has been shown that the binding of ScRap1 to its binding site leads to distortion of the DNA (26). Another study showed that the ScRap1-DNA complex shows abnormal electrophoretic mobility, and Scanning Tunneling Microscopy (STM) revealed that ScRap1 binding induces a DNA bend >50° (18). Interestingly, the ScRap1 DBD interacts directly with TBP (TATA binding protein); and it has been proposed that this interaction may be the underlying mechanism by which ScRap1 regulates the transcription of its target genes (27). Due to the ability of the ScRap1 DBD to bind to diverse DNA elements, ScRap1 can regulate the transcription of hundreds of genes and is therefore an essential factor for yeast survival.

One of the interesting features of ScRap1 is that its over-expression is toxic to yeast cells (28). The region responsible for this toxicity has been mapped as a 34-amino acid sequence that starts after the DBD and extends towards the C-terminus of ScRap1 and termed as the toxicity domain. Furthermore, it has been also observed that the over-expression of the toxicity domain along with ScRap1 DBD is sufficient to inhibit the growth of yeast cells (28). The underlying growth inhibition mechanism is not well established, but multiple mechanisms have been proposed. One of the most reliable thought is that the over-expression of ScRap1 might lead to squelching or inappropriate Rap1 binding at its low-affinity sites

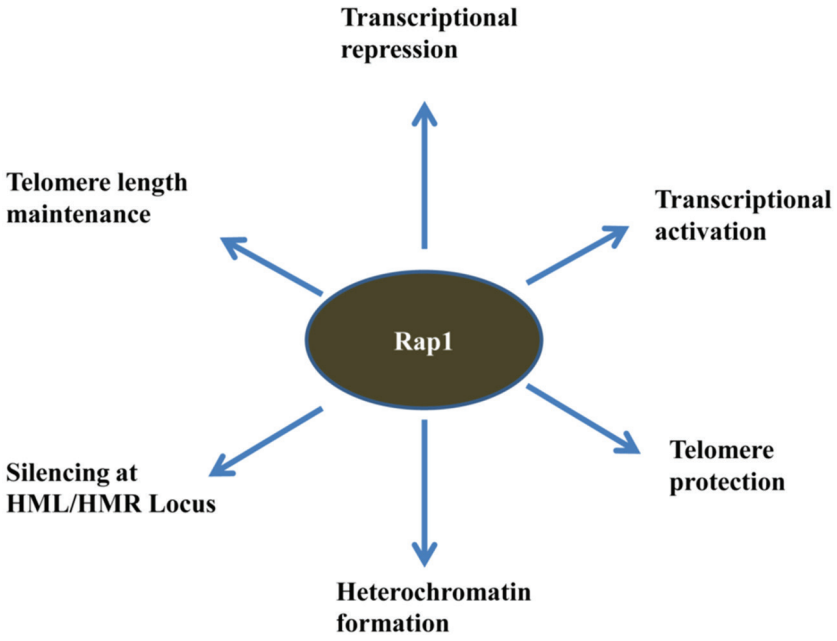

Figure 2. The weblogo was obtained from YeTFaSCo (http://yetfasco.ccbr.utoronto.ca/) showing the multiple sequence alignment of the DNA binding sites of the ScRap1 in budding yeast. The logo shows the conservation of various nucleotides. The *y*-axis represents the bit score. The *x*-axis displays the position of nucleotides in the multiple sequence alignment. The data to generate weblogo was taken from Lieb *et al*, 2001(49).

that could interfere with normal promoter function. It has been also suggested that excess ScRap1 could increase activation of a gene whose product blocks growth or is toxic to cells (29). However, the exact underlying mechanisms for ScRap1-mediated toxicity remain unknown and are a promising target for future work.

The C-terminal domain (CTD) comprises amino acids 630–827 and is the most highly studied of the ScRap1 domains. The crystal structure of the CTD has been solved and indicated an all-helical, containing seven α-helices and one turn of a 3^{10} helix domain with no structural homologues (30). Moreover, functional analysis demonstrated that the CTD of ScRap1 is not essential for vegetative growth although it is the target for most of the protein-protein interactions (16). The CTD is further sub-divided into overlapping activation and repression domains based on the function (16, 31). Transcriptional activation requires only the activation domain that is present between amino acids 630–695. Furthermore, one of the study demonstrated that the activation domain of the CTD fused to a heterologous ScRap1 DBD is sufficient to activate the transcription of downstream reporter genes (32). The repression domain of the CTD is present between amino acids 667-827. Several independent studies concluded that the ScRap1 domains directly interact with several functional partners as shown in Figure 1.jpg. The ScRap1 CTD acts as a center for interactions with proteins such as Rif1, Rif2, Sir3, and Sir4 (33-38). Furthermore, it has been proposed that the binding of Rif or Sir proteins is mutually exclusive (30, 39). The ScRap1 CTD also physically interacts with multi-subunit transcriptional co-regulators, including NuA4/Esa1, SWI/SNF, and TFIID, to control several non-ribosomal genes (28, 40, 41). Together, these evidences suggest that ScRap1 contains distinct domains that can carry out specialized functions.

4. GENOME-WIDE TARGETS OF ScRap1

There is an abundance of published consensus sequences derived either from the analysis of ScRap1-regulated promoters or from direct measurements of ScRap1 binding both *in vitro* and *in vivo* (42). Studies showed that Rap1 doesn't have a strong consensus DNA binding site. However, careful analysis revealed that ScRap1 binds to an extended sequence of 12–14 base pairs with high stability (43, 44). Furthermore, multiple DNA-binding sequence specificities have been observed for Rap1. According to the YeTFaSCo (The Yeast Transcription Factor Specificity Compendium), the DNA binding specificity of ScRap1 was found to be diverse as shown in Figure 2.jpg. Only few conserved nucleotides can be observed from the weblogo, suggesting that Rap1 have the ability to bind with a wide range of DNA sequences. Interestingly, several studies have suggested that ScRap1 binds to most of the UASs of ribosomal protein genes (RPGs) with a strong consensus binding sequence (5′ ACACCCATACATTT 3′) (45). Apart from RPGs, ScRap1 also binds to the *S. cerevisiae* 300-base pair telomeric repeat $(C1–3A)_n$ that produces multiple ScRap1 binding sites (46).

Earlier studies showed that ScRap1 could bind to non-canonical DNA sequences, suggesting that ScRap1 can possibly control the expression of a very large number of yeast genes (47); hence, studies were conducted to determine the genome-wide targets of ScRap1. One study showed considerable alteration of global gene expression due to the loss of ScRap1 binding (48). Another study (49) revealed that in exponentially growing cells ScRap1 targets 294 genomic loci (~5% of total yeast genes). Detailed analysis revealed that ScRap1 binds to the promoters of the most heavily transcribed yeast genes that account for 37% of total yeast mRNA synthesized in rapidly growing cells. Interestingly, it was also observed that ScRap1 binds to the promoters of both active and inactive genes (49). Functional characterization revealed that ScRap1 predominantly binds to 128 RPG promoters and regulates their expression (50). ScRap1 was also found to be the master regulator of the glycolytic pathway because it targets the UASs of most of the genes involved in this pathway including PGK1, ENO1, ENO2, CDC19, PDC1, TDH3, GPD1, and GPD2 (49, 51). Interestingly, another study revealed that binding of ScRap1 to its target sites is also controlled by the carbon sources present in the medium, with expansion of the target set after glucose depletion (52). These experiments led to the identification of 52 ScRap1 targets specific to low-glucose growth conditions (53). Interestingly, another study examined the genomic locations of ScRap1-crosslinked nucleosomes and it was found that approximately 43% of the ScRap1-bound nucleosomes were at the -1 position. Furthermore, it was also suggested that the nucleosomal ScRap1 interactions might be different in telomeric regions compared to promoter regions (54). Altogether, genome wide studies with ScRap1 suggested the indispensible role of this protein in transcriptional turnover of the yeast cells.

5. *IN VIVO* FUNCTIONS OF ScRap1

5.1. ScRap1 acts as an effective transcriptional activator

Transcription is a stepwise process that involves many specialized proteins and protein complexes, all of which must work together to regulate the expression of a given gene. An integral step in this regulatory process is performed by multi-subunit co-activator complexes (55), which have diverse roles in transcriptional control (56, 57). Transcriptional activation and repression are primarily caused by gene regulatory proteins (activators and repressors), which act by binding to specific sites on DNA. Ribosomal protein genes (RPGs) were one of the first targets of ScRap1 discovered, and studies have shown that ScRap1 binds to the regulatory region of RPGs to activate transcription (43, 58). Later studies revealed that binding of ScRap1 to the upstream activator sequences (UAS) of RPGs leads to the formation of nucleosome free regions that stimulates transcription by increasing the local DNA cis-element accessibility for other transcription factor (32, 59, 60). Furthermore, a crystallographic study using cryo-electron microscopy revealed the architecture of ScRap1 nucleoprotein complexes. These structures suggested that that a large ScRap1-dependent DNA loop forms between the activator-binding site and the proximal promoter region leading to the formation of pre-initiation complex (61). Recently, ScRap1 has been shown to interact with several molecules including Ifh1, Flh1, Abf1, Gcr1, and Hmo1 to regulate transcription of RPGs (50, 62-68).

Additionally, ScRap1 genome-wide binding indicated association with the promoters of RNR2 and RNR4 (49). Another study showed that ScRap1 also binds to the promoter of RNR3, and recruitment of ScRap1 to RNR3 is dependent on activation of the DNA damage checkpoint and chromatin remodeling by SWI/SNF (69). Detailed investigation revealed that the CTD of ScRap1 interacts physically with TFIID and SWI/SNF to regulate the expression of RNR3 under genotoxic stress (69). ScRap1 also regulates several glycolytic genes such as PYK, PGK, ENO1, and ADH1 by binding to their UASs and acting as an activator (70, 71), suggesting a mechanism for coordinated expression of several of the glycolytic genes in yeast. Furthermore, ScRap1 also binds to promoters of glucose-inducible genes including SRP1 (72), TPI (73), CAR1 (74), and HIS4 (75) and is required for their activation.

5.2. Role of ScRap1 in transcriptional repression and heterochromatin formation

One of the first suggestions that ScRap1 acts as transcriptional repressor came from studies on the MAT locus (11). ScRap1 is involved in the silencing of the transcription of genes on the HML/HMR locus (76-78) by interacting with Rif and Sir proteins (76-80). Later on it was identified that the minimal domain of ScRap1 (amino acids 667–827) is sufficient to establish transcriptional silencing at the HMR locus (81). It has been suggested that ScRap1 initiates transcriptional silencing at the HML/HMR locus by recruiting Sir3 and Sir4 to DNA. Detailed analysis revealed that the ScRap1 CTD interacts with Sir3, Sir4, and histone H4 to form a transcriptionally silenced heteromeric complex (82, 83). Furthermore, it has been demonstrated that Sir4 is recruited to the telomeric end by ScRap1 and, in turn, it recruits Sir2 and Sir3 to initiate telomeric silencing and heterochromatin formation (26, 84, 85). Several other studies also conclude that the ScRap1 CTD plays an essential role in heterochromatin formation by silencing genes in the telomeric region (86, 87).

5.3. ScRap1 regulates telomere length and structure

In addition to its role in formation of telomeric heterochromatin, ScRap1 regulates telomere length. *S. cerevisiae* telomeres contain 300 base pairs of

double-stranded DNA with the repetitive TG1-3 sequence. *In vitro* studies revealed that ScRap1p binds to sites present in telomeric repeats at an average of about 1 in every 18 base pairs (26). It has been suggested that the binding of additional ScRap1p leads to inhibition of telomerase activity during telomere length elongation. Studies have shown that the removal of the C-terminal domain (CTD) of ScRap1 results into defect in telomere elongation leading to its instability (88). Furthermore, it has been also observed that the interaction of the ScRap1 CTD with the telomere terminal repeat is required for proper telomere length maintenance (89). One of the study showed that the ScRap1 interaction with Rif proteins (Rif1 and Rif2) causes the formation of a protein complex that is capable of regulating telomere length to telomeres (33). It has been also demonstrated that yeast cells probably measures telomere length by counting the bound ScRap1p molecules on the telomere instead of TG1-3 sequences (90-92). Furthermore, it has been shown that extension of short telomeres is enhanced by ScRap1 molecules (93). These observations are supported by several independent studies that show that altered ScRap1 levels can affect telomere length (94-96). Additionally, ScRap1 is involved in the formation of telomere caps. Early studies indicated that ScRap1p was highly concentrated at telomeres (7), and the telomeric repeat was later identified as the predominant DNA-binding site of ScRap1, where it forms a protective telomeric cap structure (26, 96-98). The ability of ScRap1 to bind at long TG arrays suppresses both Mre11 and Cdc13 binding. Interestingly, the binding of multiple ScRap1 molecules to a long telomeric TG tract favors the formation of a t-loop structure similar to that observed in many other higher eukaryotes that physically hides the DSB (double strand break) end from MRX and/or exonucleases, thereby inhibiting initiation of a DNA damage checkpoint (99).

5.4. ScRap1 relocalizes to new genomic targets under different conditions

ScRap1 can expand its transcriptional targets under different physiological conditions. Under normal growth conditions, a significant fraction of ScRap1 (at least 10–15%) localizes to telomeric regions, which can be visualized as prominent foci by fluorescence microscopy (100, 101). ScRap1 tends to relocalize considerably during the G2 phase of the cell cycle (102), and this phenomenon is proposed to be required for normal cell cycle progression. Another study demonstrated that under the low-glucose stress condition, ScRap1 binds to a variety of new target genes (53), suggesting that ScRap1 has highly dynamic transcriptional targets. Similar observations were seen during heat shock; at 25 °C, ScRap1 binds to and activates RPG promoters, but additional ScRap1 accumulates at the same sites upon heat shock, leading to transcriptional repression (103). Thus, these observations suggest that higher ScRap1 occupancy is linked to transcriptional inhibition (104), which is consistent with the finding that multiple ScRap1-binding sites confer transcriptional inhibition. Moreover, one of the study demonstrated that under genotoxic stress conditions, such as in MMS (methyl-methane sulfonate) treatment, ScRap1 is relocalized from telomeric sites to new genomic loci including the RNR3 gene (69). Recruitment of ScRap1 to the RNR3 gene was found to be dependent on activation of the DNA damage checkpoint pathway. Additionally, the ScRap1 CTD physically interacts with SWI/SNF, the chromatin remodeler that causes nucleosome eviction from the RNR3 promoter leading to its activation (69). Thus, ScRap1 regulates the DNA damage response by activating RNR3 expression, which is involved in the formation of the RNR complex and thereby controls the dNTP pools required for DNA repair (105). Additionally, telomere shorting causes ScRap1 redistribution to new genomic loci, as observed through fluorescence microscopy (39, 106). Furthermore, a recent study revealed that ScRap1 localizes upstream of hundreds of new target genes during the senescence process (107), and senescence induces ScRap1 localization to the promoter of core histone genes leading to repression and overall decrease in core histone levels. This decrease in histone levels correlates with a decrease in nucleosome occupancy at the promoters of the genes that are up-regulated during senescence (107). Interestingly, senescence-induced ScRap1 distribution requires Mec1p, a central protein kinase of DNA damage response suggesting the existence of upstream signaling molecules that might regulate ScRap1 distribution and transcriptional activity (107). However, these observations raise the possibility that ScRap1 may have several unknown targets specific to different stress conditions or physiological states that have not been addressed yet and are worth pursuing in the future.

6. HOW ScRap1 CONTROLS TRANSCRIPTION IN YEAST

In spite of recent advances in the field of transcription, the mechanisms governing the bimodal function of ScRap1 *in vivo* remain to be established. Of particular interest is how ScRap1 can efficiently perform both transcriptional activation and repression. One possibility is that post-translational modifications (PTMs) of ScRap1 might regulate its activity. According to the literature, phosphorylation is a common PTM that affects the properties of a wide range of proteins with different functions. Along these lines, one study revealed that phosphorylation influences the binding of ScRap1 to the UAS of the PGK gene (108). Furthermore, potential phosphorylation sites in ScRap1 were determined using the PIP program, and phosphorylation of ScRap1 has been hypothesized to regulate its transcriptional activity (109). Indirect evidence suggests that overexpression of protein kinase A (PKA) leads to an increase in rRNA synthesis, and it has been proposed that PKA phosphorylates ScRap1 leading to

Figure 3. Schematic representation of *in vivo* functions displayed by yeast Rap1.

increases in transcriptional activity, but this hypothesis has yet to be proven (110). Recently, phosphorylation of *Schizosaccharomyces pombe* ScRap1 (spScRap1) during the mitotic (M) phase was shown to be required for faithful chromosome segregation (111). Detailed analysis showed that the spScRap1 is phosphorylated at Ser213, Thr378, Ser422, Ser456, and Ser513 during the M phase *in vivo*, and this causes dissociation of the telomere cap from the nuclear envelope (111). Furthermore, based on sequence similarity, ScRap1 possesses potential phosphorylation sites at Ser288, Ser289, Ser479, Ser658, Ser660, Ser731, and Thr486. However, the conservation of ScRap1 phosphorylation in other species is currently unknown and is worth pursuing in future studies.

Apart from PTMs on ScRap1, direct evidence demonstrates possible mechanisms by which ScRap1 regulates transcriptional activity. The positive role of ScRap1 in transcription might be due to induction of nucleosome disruption upon binding to promoters (112, 113), whereas its negative role may promote nucleosome formation (13, 114). One important clue as to how ScRap1 regulates transcription came from a recent crystallographic study in which cryo-electron microscopy was conducted to determine the architecture of nucleoprotein complexes composed of TFIID, TFIIA, ScRap1, and yeast enhancer-promoter DNA (115). These structures revealed that the central role of ScRap1 is to expose promoters to other factors. ScRap1-dependent formation of a large DNA loop occurs between the activator-binding site and the proximal promoter region that ultimately can lead to pre-initiation complex (PIC) formation (115). Interestingly, another study correlates the binding dynamics of ScRap1 as a crucial factor for determining the fate of downstream genes (116). Experimental evidence suggested that long ScRap1 residence is coupled to transcriptional activation, whereas fast binding turnover was linked to low transcriptional output (116). Another alternative mechanism of ScRap1 transcriptional regulation is the interaction of ScRap1 with a wide variety of proteins, including both coactivators and corepressors. For example, the interaction of ScRap1 with Sir proteins is functionally important for heterochromatin formation and gene silencing at HML/HMR locus (79, 80, 85). Another example of this mode of regulation came from a study on the DNA damage-inducible RNR3 gene, which revealed that ScRap1 utilizes multiple domains to carryout distinct processes in opening the chromatin structure at the RNR3 promoter in response to genotoxic stress. It was observed that the ScRap1 CTD forms physical interactions with SWI/SNF and helps in its recruitment to affect promoter opening and PIC formation (69). Together, these insights represent current hypotheses as to how ScRap1 regulates transcription, but this understanding is incomplete and is an area of intensive research.

7. FUTURE PERSPECTIVES AND CONCLUDING REMARKS

ScRap1 is a well-studied transcription factor in *S. cerevisiae* that performs a diverse range of functions as summarized in Figure 3.jpg, to regulate transcription and yeast physiology. However, the functional role of ScRap1 in the transcriptional regulation remains elusive, mainly because ScRap1 mutations that abolish DNA binding are lethal and overexpression of ScRap1 is toxic (117, 118). Hence, future study should be focused on identifying the direct transcriptional targets of ScRap1 by devising experimental strategies to overcome the issue of lethality. Furthermore, the mechanism underlying the toxicity of ScRap1 overexpression is also not well understood and is worth pursuing in the future. Another exciting and intriguing field is the spatiotemporal regulation of both activation and repression by ScRap1. It has been proposed that a variety of mechanisms must be utilized, but they remains to be elucidated. Recently, the involvement of ScRap1 in the DNA damage response or maintenance of genomic integrity has been elucidated, suggesting that ScRap1 might be involved in other stress pathways, but this hypothesis needs to be validated. Surprisingly, at present, there is limited information about the physiologically relevant signals that might target ScRap1 under different conditions and the pathways by which these signals are transmitted to ScRap1. This will be a promising field for future exploration. We anticipate that continued genetic and biochemical experiments combined with information from genome-wide studies should increasingly converge and contribute to understanding the overall mechanisms by which ScRap1 regulate yeast physiology.

8. CONCLUSIONS

Eukaryotic cells exhibit an incredible number of genetic responses to fluctuating environmental stimuli by fine-tuning the transcriptional state. In *S. cerevisiae*, ScRap1 governs multiple ways to regulate transcription. Due to its wide range of functions, ScRap1 is known to be indispensable for yeast growth and survivability. Recent advances in molecular biology techniques and increasing genome-wide studies will soon reveal the fundamental regulatory mechanisms through which ScRap1 functions in yeast. From its discovery, ScRap1 has been a pioneer candidate for studying yeast transcription, and we believe that future studies on this transcription regulator will reveal a much broader significance. The authors declare that they have no conflict of interests.

9. ACKNOWLEDGEMENTS

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