ACTA ABP BIOCHIMICA POLONICA

Vol. 60, No 2/2013
233–238
on-line at: www.actabp.pl

Regular paper

Cohesin Irr1/Scc3 is likely to influence transcription in *Saccharomyces cerevisiae via* interaction with Mediator complex

Agata Cena, Marek Skoneczny, Anna Chełstowska, Piotr Kowalec, Renata Natorff and Anna Kurlandzka✉

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

The evolutionarily conserved proteins forming sister chromatid cohesion complex are also involved in the regulation of gene transcription. The participation of SA2p (mammalian ortholog of yeast Irr1p, associated with the core of the complex) in the regulation of transcription is already described. Here we analyzed microarray profiles of gene expression of a *Saccharomyces cerevisiae irr1-1/IRR1* heterozygous diploid strain. We report that expression of 33 genes is affected by the presence of the mutated Irr1-1p and identify those genes. This supports the suggested role of Irr1p in the regulation of transcription. We also indicate that Irr1p may interact with elements of transcriptional coactivator Mediator.

Key words: sister chromatid cohesion, transcription, *Saccharomyces cerevisiae*

Received: 28 February, 2013; revised: 02 April, 2013; accepted: 18 April, 2013; available on-line: 31 May, 2013

INTRODUCTION

Sister chromatid cohesion complex has initially been characterized as a set of four evolutionarily conserved proteins essential for chromosome segregation (Guacci *et al.,* 1997; Michaelis *et al.,* 1997). It comprises three core subunits called cohesins: Mcd1/Scc1, Smc1 and Smc3, and an accesory protein Irr1/Scc3 (yeast nomenclature). For recruiting cohesin to the chromosome two proteins Scc2 and Scc4 (also evolutionarily conserved) are necessary (Ciosk *et al*., 2000; Tomonaga *et al*., 2000). The first years of cohesion research focused on its function in chromosome segregation and chromosome condensation, where cohesin has been proposed to facilitate chromatin loop formation (Guacci et al., 1997, Novak et al., 2008).

However, it has also been noticed that mutations in genes encoding cohesins Smc1 and Smc3 of *S. cerevisiae* cause a loss of function of the boundary element surrounding the HMR silent-mating-type loci (Donze *et al*., 1999), which indicated a role of these proteins in transcription regulation. Further characterization of *Drosophila* Nipped B protein, a homolog of yeast Scc2, also indicated its role in regulation of gene expression (Rolins *et al*., 1999, Dorsett, 2009). Similarly, a closer examination of SA2p, a mammalian homolog of yeast Irr1p, indicated that it may act as a transcriptional co-activator by interacting with transcription factors (Lara-Pezzi *et al*., 2004). Subsequently, it has been evidenced that cohesin regulates transcription *via* multiple mechanisms (for review: Dorsett, 2011). Cohesin facilitates looping out of DNA and communication between transcriptional enhancers and gene promoters, at sites that bind CCCTC-binding factor (CTCF) (Kagey *et al*., 2010; Cien *et al*., 2011; Hadjur *et al*., 2009; Hou *et al*., 2010). There is also evidence for CTCF-independent cohesin recruitment to various genomic regions, which suggests another mechanism of gene regulation (Kagey *et al*., 2010, Schmidt *et al*., 2010, Zeng *et al*., 2009). In *Drosophila* and in mouse embryonic stem cells cohesin represses many genes, acting in concert with the Polycomb group (PcG) repressor proteins, especially these which control growth and development (Dorsett, 2011, Kagey *et al*., 2010, Schaaf *et al*., 2009).

Genome-wide mapping showed that cohesin binds to the chromosome at discrete loci both in yeast and vertebrate cells, although the exact mechanisms of cohesin recruitment in yeast and humans may differ (Glynn *et al.,* 2004; Lengronne *et al.,* 2004; Parelho *et al.,* 2008; Rubio *et al.,* 2008; Wendt *et al.,* 2008). In yeast, most cohesin-binding sites correspond to locations where genes are transcribed in a convergent orientation, and are likely to be linked with transcript termination (Glynn *et al.* 2004; Lengronne *et al.* 2004, Gullerova & Proudfoot 2008). During yeast meiosis chromosomal binding of cohesin is sufficient for target-gene activation. The protein Scc2 activates the expression of the gene *REC8* (encoding meiotic equivalent of Mcd1p) by recruiting cohesin to an upstream region in a position-dependent manner (Lin *et al*., 2011A, Lin *et al*., 2011B). Surprisingly, conditional inactivation of the most thoroughly characterized cohesin Mcd1 was sufficient to significantly alter transcriptional profiles of many genes which had highly related functions (Skibbens *et al.* 2010).

Understanding cohesin function in transcription regulation is of clinical importance since mutations in cohesion pathways cause developmental abnormalities such as Cornelia de Lange Syndrome, Roberts Syndrome/ SC-Phocomelia, and Rothman-Thompson Syndrome (Skibbens 2009, Gartenberg 2009, Liu *et al*., 2009, Dorsett, 2007). Subtle mutations in cohesin components that apparently do not affect the chromosomal cohesion can alter the transcriptome and have been associated with diseases (Liu *et al*., 2009).

Accumulating evidence on the participation of SA2p (a mammalian ortholog of the yeast Irr1p) in the regulation of transcription (Wendt *et al*., 2008; Parelho *et al*., 2008; Degner *et al*., 2008) prompted us to verify the role of Irr1p in this process in yeast. We used a well-characterized *irr1-1/IRR1* yeast heterozygous diploid strain. The mutation *irr1-1* is lethal in the haploid but the dip-

✉e-mail: ania218@ibb.waw.pl

Abbreviations: HU, hydroxyurea; MMS, methyl methane sulfonate

loid *irr1-1/IRR1* bears two types of the Irr1 protein: one wild-type and one with the F658G substitution. Such strain mimics, to some extent, a mammalian somatic cell with a cohesin defect (Cena *et al.*, 2007; 2008, 2012). We analyzed microarray profiles of RNA expression of the *irr1-1/IRR1* strain and report here the identity of those genes whose regulation was affected by the presence of the mutated Irr1-1p. Moreover, by performing two-hybrid study, we identified a putative indirect link between Irr1p cohesin and elements of the Mediator, a key regulator of eukaryotic transcription, connecting activators and repressors bound to regulatory DNA elements with RNA polymerase II (Boube *et al.*, 2002, Kornberg, 2005, Conaway *et al.*, 2005). This finding should be helpful in characterizing the gene regulatory networks involving cohesin.

MATERIALS AND METHODS

Strains, media and general methods. Yeast strains used in the present study were isogenic with the strain W303. *Escherichia coli* XL1-Blue MRF0 (Stratagene) was used for molecular manipulations. Yeast culture media were prepared as described (Rose *et al.*, 1990). YPD contained 1% Bacto-yeast extract, 2% Bacto-peptone and 2% (all w/v) glucose. SD contained 0.67% yeast nitrogen base without amino acids (Difco) and 2% glucose.

Microarrays. For microarray analyses yeast cells were cultivated in YPD complete medium to a density of 5×10^6 cells/ml. The resulting cultures were harvested by centrifugation and RNA extracted from the pellets using hot acid phenol procedure. RNA quality was assessed by 260/280 and 260/230 OD ratios. Fluorescently labeled cDNA probes were prepared using Superscript II RT (Fermentas) reverse transcriptase and Cyanine-3- or Cyanine-5-dCTP (Perkin Elmer). Two-color hybridizations were run in quadruplicates with dye swap between duplicates of the same variant. Labeled samples were hybridized to yeast oligonucleotide microarrays (Transcriptome Platform, Ecole Normale Superieure, Paris), spotted in duplicate with Operon v 2.2 60-mer oligo set representing 6,388 ORFs and controls. Axon GenePix 4000B scanner and GenePix software (Molecular Devices) were used for scanning and feature extraction. Statistical evaluation employing Student's *t*-test was performed with Acuity software (Molecular Devices). A *p* value lower than 0.05 was accepted as significant. Upregulation of particular gene expression level in either variant was inferred from a positive $\log_2$ ratio value.

Determination of mRNA levels and RT-PCR. Total RNA was isolated from yeast cells using the RNeasy Mini Kit (QIAGEN, Germany). Reverse transcription (RT) was performed in duplicate using the QuantiTect Reverse Transcription Kit (QIAGEN), according to the manufacturer's recommendations. qPCR amplification was performed using a LightCycler 1.5 and LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics GmbH, Germany) according to the manufacturer's instructions. The Pfaffl model (Pfaffl, 2001) and the relative expression software tool (REST-384 ©) (Pfaffl *et al.*, 2002) were used to estimate the changes in relative mRNA levels. Data normalization was carried out against the 35S rRNA transcript. The sequences of all primers and qPCR amplification parameters are available upon request.

Two-hybrid analysis. Two independent two-hybrid studies were carried out. In the first assay Swc4-24 bait protein was fused to the Gal4p DNA binding domain on pGBKT7 plasmid (Clontech). The protein Swc4-24 consists of 269 amino acids and is devoid of 24 C-terminal amino acids (Micialkiewicz & Chelstowska, 2008). The procedure was done in our laboratory, according to protocols described by Vojtek *et al.* (1997), by sequential transformation using the genomic library of Fromont-Racine *et al.*, (1997). Direct two-hybrid analyses were done with cotransformed haploid cells. The host strain for the two-hybrid studies was PJ69-4α (James *et al.*, 1996). In the second assay, performed by Dualsystems Biotech AG (Switzerland), the bait LexA-Swc4-24p was used, and the whole procedure was carried out according to the company protocols.

RESULTS AND DICSUSSION

Previous work from our laboratory showed that the heterozygous diploid *irr1-1/IRR1* exhibits irregularities in mitotic and meiotic divisions, chromosome segregation errors, disturbances in segregation of nuclei, and in cytokinesis. However, the chromosome segregation errors of mitotically dividing *irr1-1/IRR1* cells, which did not lead to cell lethality, were increased only to 9.4% compared to 0.2% in the control *IRR1/IRR1* strain. On the other hand, a majority of mutant cells displayed phenotypic defects in cell wall stability and increased HU (hydroxyurea) and MMS (methyl methane sulfonate) sensitivity (Cena *et al.*, 2007, 2008). Such a spectrum of defects suggested that the growth defects of the mutant could result from changes of transcription, manifested in all cells, rather than from errors in chromosome segregation. Transcription could be affected due to the pool of mutated Irr1-1p present in the diploid *irr1-1/IRR1* at a level similar to the wild-type Irr1p.

To verify this assumption we compared transcriptomes of *IRR1/IRR1* and *irr1-1/IRR1* strains by ge-

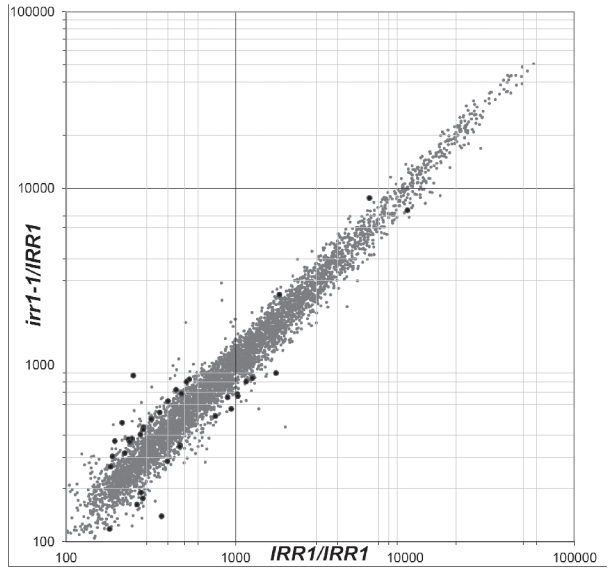

Figure 1. Scatter plot summarizing the transcriptome data obtained in this study.
Each point on the plot represents the expression data for a single gene in *irr1-1/IRR1* heterodiploid compared to *IRR1/IRR1* wild-type. Data were Lowess normalized and averaged across all microarray experiments. Smaller gray dots denote genes for which significant fluorescence values were obtained in at least two out of six microarray experiments. Larger black dots correspond to genes with Log Ratio of expression >0.5 or <-0.5 and *p*-value <0.05. X axis: fluorescence intensity for *IRR1/IRR1* wild-type, Y axis: fluorescence intensity for *irr1-1/IRR1* heterodiploid.

Table 1. Thirty-three genes showing altered expression in response to the presence of mutated Irr1-1p cohesin.
Bold — gene/ORF up regulated, regular — down regulated.

Common name	ORF	Chromosome	LogR	Fold change
AAD6	YFL056C	VI	–0.7	1.6
AMS1	**YGL156W**	VII	1.0	2.0
BNA4	YBL098W	II	–0.7	1.6
CMK2	**YOL016C**	XV	0.6	1.5
CRC1	**YOR100C**	XV	0.8	1.7
DCS2	**YOR173W**	XV	0.7	1.6
DEF1	YKL054C	XI	–0.7	1.6
ENP1	YBR247C	II	–0.5	1.4
GLC3	**YEL011W**	V	1.2	2.3
HAP4	YKL109W	XI	–0.7	1.6
HXK1	**YFR053C**	VI	1.9	3.7
MAE1	YKL029C	XI	–0.6	1.5
MAK5	YBR142W	II	–0.6	1.5
MEC1	YBR136W	II	–0.6	1.5
MSI1	YBR195C	II	–0.5	1.4
NRD1	YNL251C	XIV	–0.6	1.5
PEP12	**YOR036W**	XV	0.5	1.4
PHO3	YBR092C	II	–0.5	1.4
PYK2	**YOR347C**	XV	0.7	1.6
PWP2	YCR057C	III	–0.6	1.5
RKM3	YBR030W	II	–0.6	1.5
RTC3	**YHR087W**	VIII	2.3	4.9
SPT7	YBR081C	II	–0.5	1.4
SPS18	YNL204C	XIV	–0.8	1.7
STF2	**YGR008C**	VII	0.9	1.9
SWC3	YAL011W	I	–0.5	1.4
YBP1	YBR216C	II	–0.5	1.4
	YBR063C	II	–1.0	2.0
	YBR074W	II	–0.5	1.4
	YGR079W	VII	–0.6	1.5
	YGR149W	VII	0.5	1.4
	YKL151C	XI	0.7	1.6
	YNL134C	XIV	–0.9	1.9

nome-wide microarray expression analysis. An overall view on the gene expression changes in the *irr1-1/IRR1* strain is given in Fig. 1. We limited our analyses to those genes whose expression level in the mutant was significantly ($p < 0.05$) increased or decreased 1.4-fold or more, relative to wild-type cells. This was represented by microarray signal Log Ratio (LogR) $|\log_2 R| > 0.5$. As a result we identified with high confidence 33 loci exhibiting highly reproducible and significant changes common to all data sets, which are summarized in Table 1. To validate microarray data we verified a random sample of five genes by realtime quantitative RT-PCR. We analysed the level of transcription of *RTC3*, *HXK1*, *DEF1*, *YER079W* and *YBR063C*, and normalized the data to 35S ribosomal RNA (rRNA), a housekeeping gene. The qRT-PCR results (not shown) were in agreement with the microarray results.

Among the 33 genes identified by microarray analysis 11 are localized on chromosome II. Ten of them, upregulated at the 1.4–2.0 fold level, are spread along the right arm and are not clustered [*RKM3* (*YBR030W*), *YBR074W*, *MEC1* (*YBR136W*), *MAK5* (*YBR142W*), *YBR063C*, *SPT7* (*YBR081C*), *PHO3* (*YBR092C*), *MSI1* (*YBR195C*), *YBP1* (*YBR216C*), *ENP1* (*YBR247C*)]. Thus, the changes of their transcription rather did not result from a coordinated induction of adjacent loci. Moreover, we did not find binding sites for a common transcription factor in their promoters (http://rsat.ulb.ac.be/rsat/). However, the fact that they are localized on the same arm of one chromosome suggests that changes of transcription could result from a rearrangement of the chromosome. Gene ontology analysis does not show any direct relations among the proteins encoded by these genes. They are involved in RNA processing, transcription (directly or through interactors), chromatin assembly, and protein modifications. The transcription of one gene (*BNA4*) localized on the left arm is downregulated. The five affected genes localized on chromosome XV [*PEP12* (*YOR036W*), *DCS2* (*YOR173W*), *CMK2* (*YOL016C*), *CRC1* (*YOR100C*), *PYK2* (*YOR347C*)] also seem unrelated in function. As above, we did not find binding sites for a common transcription factor in their promoters. They are not clustered, although they are all upregulated at a similar level (1.4–1.6 fold). The protein encoded by *PEP12* is involved in vesicular transport. Cmk2p is a calmodulin-dependent protein kinase which may play a role in stress response, Crc1p is a mitochondrial inner membrane carnitine transporter, and *PYK2* encodes pyruvate kinase. What is interesting, a regulatory protein Dcs2 was identified among factors which allow cells to tolerate the adverse effects of aneuploidy (Torres *et al.*, 2010).

Six other genes localized on various chromosomes encode proteins involved, at least to some extent, in carbohydrate metabolism (*RTC3, GLC3*, *HXK1*, *AMS1*, *PYK2* and *MAE1*). Changes in transcription levels of *RTC3, GLC3, HXK1* and *AMS1* may, to some extent, be related to the aberrated cell-wall phenotypes of *irr1-1/IRR1* strain. These genes, the transcription of which is induced, are localized on various chromosomes. Transcriptomic studies indicated the relevance of *RTC3*-encoded protein to transcription control during the response to high sugar and stress conditions, or a role in the stationary phase (Gasch *et al.*, 2000, Jimenez-Marti *et al.*, 2011). However, the protein has also been implicated in the cell wall integrity pathway, although this function has not been studied in detail (Garcia *et al.*, 2009). *GLC3* encodes 1,4-α-glucan branching enzyme involved in the synthesis of glycogen, a major intracellular reserve polymer formed upon limitation of carbon, nitrogen, phosphorus or sulfur. The branching is important for glycogen function because both its synthesis and degradation occur from the nonreducing ends of the α-1,4 chains (Roach *et al.*, 2001). The link of the *GLC3*-encoded protein to the cell wall integrity may also be explained by a general function of glycogen in the basal matrix where it binds cell wall β-1,3-glucans and holds other carbohydrate components covalently linked to the cell wall (Arvin-

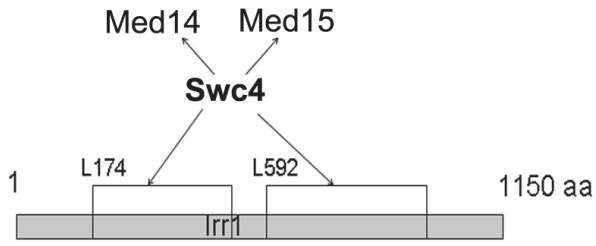

Figure 2. Swc4p, an element of NuA4-HAT, interacts with the Irr1/Scc3 cohesin and two elements of the Mediator complex. The interaction between Swc4p and Irr1p has been detected in the yeast two-hybrid system. Two different fragments of the Irr1p, one starting from L174 and the second starting from L592, were identified. Independently, interactions of Swc4p with Med14p and Med15p subunits of the Mediator were found.

dekar & Patil 2002). A change in the *HXK1* transcript level may constitute another link to the cell wall phenotypes. Hexokinase Hxk1p, together with the isoenzyme Hxk2p and glucokinase Glk1p, catalyzes phosphorylation of glucose. A targeted proteomics approach clustered Hxk2 in the glycolytic branch, Glk1 — with tricarboxylic acid cycle proteins, and Hxk1 — with the 1,4- α-glucan branching enzyme Glc3p, discussed above (Costenoble *et al*., 2011). Both Glk1 and Hxk1 have been speculated to be involved in glycogen storage rather than in the regular glycolytic path involving Hxk2 (Ihmels *et al*., 2004). Moreover, *HXK1* was also identified among genes induced by cell wall perturbation and it was upregulated by calcofluor white, a cell-wall damaging agent (Boorsma *et al*., 2004). *AMS1* encodes vacuolar alpha mannosidase involved in free oligosaccharide (fOS) degradation. The biological relevance of this process is poorly understood. It has been shown that in budding yeast almost all fOSs are formed from misfolded glycoproteins. However, an up-regulation of Ams1 activity was apparent for cells treated with cell-wall-perturbing agents, which may constitute a possible link between the catabolism of fOSs and cell wall stress (Hirayama & Suzuki, 2011).

Another group comprised genes whose transcription was significantly decreased in the *irr1-1/IRR1* strain. These are four genes involved in the regulation of transcription or chromatin organization *HAP4*, *SPT7*, *MSI1* and *SWC3*. The decrease of the level of *HAP4* (exerts an effect on respiratory gene expression) may reflect minute differences in the metabolic status between the mutant and control strain. Genes *SPT7* and *MSI1* are both localized on the right arm of chromosome II and are separated by a distance of about 13 000 bp. The similar level of down-regulation of both genes may be an example of the spreading of changes in chromatin structure to neighboring domains elicited by cohesin, postulated by Skibbens *et al.* (2010).

The protein encoded by *SWC3* is a component of the SWR1 complex, which exchanges histone variant H2AZ (Htz1p) for chromatin-bound histone H2A (Bao & Shen, 2011). This complex shares some subunits with NuA4 complex — a histone acetyltransferase (NuA4-HAT), which plays fundamental roles in regulating gene expression (Brown *et al*., 2000; Narlikar *et al*., 2002). It is believed that the NuA4-HAT and SWR1 complexes may, to some extent, have overlaping functions (Lin *et al*., 2008, Lu *et al*., 2009). Swc3p is not a subunit of NuA4-HAT, but it was identified as an interactor of a few proteins assigned to this complex.

While working on the putative role of Irr1p in transcription, we simultaneously carried out a two-hybrid assay to look for new physical interactions of Swc4p of the NuA4-HAT complex (Krogan *et al*., 2003). In this assay we identified Irr1p as one such interactor. Two Swc4p-interacting fragments of Irr1p comprised 319 and 379 aminoacids, respectively, the first starting from leucine 174, the second from leucine 592. Moreover, the same screen idenified Swc4p interaction with two components of a multisubunit transcriptional coactivator complex — Mediator. Mediator interacts directly with a number of transcription factors to facilitate RNA polymerase II recruitment to target genes. The subunit Med14/Rgr1 (*YLR071c*) was identified three times, and the subunit Med15/Gal11 (*YOL051w*) — two times (summarized in Fig. 2).

It has been found that in murine embryonic stem cells Mediator and the cohesin complex physically and functionally connect the enhancers and core promoters of active genes (Kagey *et al*., 2010). Elements identified in that screen comprised cohesins Smc1, Smc3, Nipbl (a homolog of fungal Scc2) and SA2/Stag2 (one of two mitotic mammalian equivalents of Irr1p) and subunits Med1, Med12 and Med15-17 of Mediator. Subsequent findings confirmed the interactions between Mediator and cohesins in the regulation of gene expression (Dorsett, 2011, Prenzel *et al*., 2012). Our data reported here are insufficient to conclude that in yeast Swc4p mediates an interaction of Irr1p with Mediator, since we did not show that Swc4p can interact with its partners simultaneously. However, it seems plausible that, similarly to higher eukaryotes, Irr1p may play a role in regulating transcription *via* Mediator.

In conclusion, the data presented in this report suggest that the phenotypes of the heterozygous diploid *irr1-1/IRR1* unrelated to chromosome segregation defect could result from changes in expression of individual genes. The aberrated cell-wall phenotypes could be related to changes of expression of *RTC3, GLC3, HXK1* and *AMS1*. The increased HU and MMS sensitivity could result from decreased levels of transcription of four genes involved in the regulation of transcription itself or in chromatin organization: *HAP4*, *SPT7*, *MSI1* and *SWC3*. These observations support the suggested role of Irr1 protein in the regulation of transcription. Moreover, for the first time in yeast we indicate for a link between the cohesin and elements of Mediator. These data may contribute to better understanding of the role of Irr1p in transcription.

Acknowledgements

We thank Drs: Arkadiusz Miciałkiewicz, Marzena Sieńko and Anna Wójcicka for help in performing and discussing experiments.

This work was supported in part by the Ministry of Science and Higher Education, grants 2 P04C01130 and N N303 456838.

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