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An improved strategy for tandem affinity purification-tagging of *Schizosaccharomyces pombe* genes

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Abstract

Tandem affinity purification (TAP) is a method that allows rapid purification of native protein complexes. We developed an improved technique to fuse the fission yeast genes with a TAP tag. Our technique is based on tagging constructs that contain regions homologous to the target gene cloned into vectors carrying a TAP tag. We used this technique to design strategies for TAP-tagging of predicted *Schizosaccharomyces pombe* genes (http://mendel.imp.ac.at/Pombe_tagging/). To validate the approach, we purified the proteins, which associated with two evolutionarily conserved proteins Swi5 and Sfr1 as well as three protein kinases Ksg1, Orb6 and Sid1.

Keywords

MS; *Schizosaccharomyces pombe*; Tandem affinity purification; Technology

Many biological processes are carried out by multiprotein complexes. A crucial step toward understanding of cellular biology is mapping networks of physical protein–protein interactions.

This can be achieved by purification of individual protein complexes followed by identification of purified proteins by MS. Tandem affinity purification (TAP) has been developed as a tool that allows rapid purification of TAP-tagged proteins together with associated proteins [1]. It has been successfully applied to purify protein complexes from various organisms (reviewed in [2-4]). Different strategies for tagging of proteins have been described [5-8]. Ideally, a gene at its normal locus is substituted with an allele encoding epitope-tagged version of the protein, thus ensuring expression of the tagged protein at physiological levels. PCR-based strategy using long oligonucleotides, which contain typically about 80 bp regions of homology to the target gene, is frequently used for tagging of genes in the fission yeast *Schizosaccharomyces pombe* [9]. However, longer regions of homology are required to ensure the high efficiency of gene targeting [5, 10]. Here, we

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describe a novel strategy for tagging fission yeast genes using long regions of homology to the target gene.

Our technique is based on tagging constructs that contain long regions homologous to the target gene cloned into vectors carrying a dominant drug-resistance marker (Supporting Information Fig. S1) [11, 12]. PCR primers are used to amplify regions upstream and downstream of the stop codon of the target gene. These homology regions are 200–800 bp long. Restriction sites in the primers allow cloning of the homology regions into the pTAPKan1 vector carrying the TAP tag and the *KanMX* gene conferring resistance to geneticin (G418) (Supporting Information Fig. S2). The vector-containing homology regions is amplified in *E. coli* and after linearization by a restriction enzyme transformed into yeast. Homologous recombination is used to replace the target gene with an allele encoding TAP-tagged version. Finally, a successful tagging is verified in yeast transformants by colony PCR.

Although the epitope tagging is an easy way to detect and purify proteins, the epitope tag can reduce the function of the tagged protein. To reduce the negative impact of the TAP tag on the tagged protein, we introduced a flexible linker between the TAP tag and the open reading frame of a given gene. The linker potentially improves folding and function of both tagged protein and the tag itself [13]. We found that only three out of five tagged genes were functional in the absence of the linker (Supporting Information Table S1 and Fig. S3).

Based on our tagging technique, we designed a strategy for cloning tagging constructs for 4901 predicted fission yeast genes, which is available in a form of a searchable web-accessible database http://mendel.imp.ac.at/Pombe_tagging/. Apart from the in-silico derived cloning strategies, this database provides supporting information, such as gel-previews with predicted PCR and ligation products as well as information on both coding and intergenic regions with links to the *S. pombe* GeneDB database [14]. We failed to design oligonucleotides required to amplify homology regions of 123 genes due to unfavorable sequence composition upstream of the stop codon. Other strategies (e.g. *N*-terminal tagging) should be used for tagging of these genes.

To validate our approach, we TAP-tagged two evolutionarily conserved proteins Swi5 and Sfr1 as well as three essential protein kinases Ksg1, Orb6 and Sid1. We obtained high efficiency of homologous integration for all five genes. The efficiency was higher compared with tagging strategy using long oligonucleotides containing 80 bp regions of homology to genomic target regions (93% correct integration with pTAPKan1 strategy compared with 30% with the long oligonucleotides strategy, in 100 colonies tested) (Supporting Information Table S1). Swi5 is involved in two related processes, the mating-type switching and homologous recombination [15]. Genetic epistasis analysis suggested that there are two different Swi5-containing protein complexes in the fission yeast *S. pombe*. A protein complex containing Swi5 and Swi2 specifically promotes mating-type switching, whereas another complex containing Swi5 and Sfr1 is involved in Rhp51-dependent homologous recombinational repair [16]. Rhp51 recombinase initiates a reciprocal DNA-strand exchange reaction between two homologous duplex DNA molecules by forming a helical nucleoprotein filament on single-stranded DNA. Accessory proteins referred to as mediators are required for the full activity of the Rhp51 recombinase. Recent studies showed that Swi5–Sfr1 complex is a mediator that stabilizes and activates the Rhp51 filaments [17-19]. On the other hand, Swi5–Swi2 complex is required for efficient mating-type switching, which occurs during the cell cycle by gene conversion. Mating-type switching is initiated by formation of the double-strand break at the *mat1* locus, which recruits DNA repair machinery that facilitates the search for homologous donor sequences. The molecular function of Swi5–Swi2 complex in this process is largely unknown [20].

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We used TAP to isolate proteins associated with Swi5-TAP, Sfr1-TAP Ksg1-TAP, Orb6-TAP and Sid1-TAP from cycling *S. pombe* cells. Purified proteins were separated by SDS-PAGE gels and silver stained (Supporting Information Fig. S4). In parallel, purified proteins were subjected to analysis by MS. Table 1 shows proteins specifically copurifying with Swi5-TAP and Sfr1-TAP. Sfr1-TAP associated with high levels of Swi5, while Swi5-TAP associated with high levels of both Sfr1 and Swi2. Swi5 was previously shown to co-immunoprecipitate with Swi2 and Sfr1, however only when Swi2 and Sfr1 were overexpressed from a strong promoter [16]. Here, we show that Swi5-TAP physically interacts with Swi2 and Sfr1 when expressed from their own promoters. Our observation that Swi2 associates with Swi5-TAP, but not with Sfr1-TAP provides the first biochemical evidence that there are two different Swi5-containing protein complexes, namely Swi5-Swi2 and Swi5-Sfr1.

In addition to the previously identified interactors, we identified novel proteins co-purifying with Swi5-TAP and Sfr1-TAP. Notably, Swi5-TAP associated with Sir2 protein (Table 1). Sir2 proteins (sirtuins) have NAD(+)-dependent protein deacetylase activity and target for deacetylation histones as well as multiple non-histone proteins [21]. Interestingly, Sir2 and Swi5 share similar biological functions. Both Sir2 and Swi5 are required for proper function of mating-type loci and efficient DNA repair. It is intriguing to speculate that the protein deacetylase activity of the Sir2 is required for the Swi5 function. Sfr1-TAP associated with an uncharacterized protein, which belongs to the XPG/RAD2 family of structure-specific nucleases. Members of the XPG/RAD2 family not only play a central role in nucleotide excision repair, but also fulfill other biological roles [22]. Additional studies will be needed to clarify a possible role of the SPAC139.01c in Swi5-Sfr1-mediated DNA repair.

Previous studies showed that Swi5 interacts with Rhp51 and Swi6 proteins. A small amount of Rhp51 was co-immunoprecipitated by the anti-Swi5 antibody and yeast two-hybrid analysis suggested that Swi5 interacts with Swi6 *via* Swi2 [16]. However, we were not able to detect Swi5–Rhp51 or Swi5–Swi6 interactions, suggesting that these interactions are transient or easily disrupted or that the interactions may be substoichiometric.

Interestingly, we found that both Swi5 and Sfr1 proteins were phosphorylated. Swi5 was phoshorylated on serine 72 and Sfr1 contained three phosphorylated serine residues (serines 26, 109 and 165) (Table 2 and Supporting Information Fig. S5). Further studies are required to analyze the functional relevance of Swi5 and Sfr1 phosphorylation and to identify the relevant protein kinases.

Swi5 and Sfr1 are also involved in meiotic recombination. Swi5 acts in a branched pathway of joint molecule formation to repair meiotic DNA breaks and it is required for the mating-type bias of gene conversion [23-26]. It will be interesting to purify proteins interacting with Swi5 and Sfr1 during meiosis and identify possible differences in the composition of protein complexes between mitosis and meiosis.

In summary, we developed an improved strategy for tagging proteins. In addition to its high efficiency of homologous integration, our strategy has the added advantage that tagging plasmids are created. These plasmids can be easily used to tag proteins in strains with various genetic backgrounds. Our protocol is also suitable for high-throughput approaches and it should help to speed up the functional analysis of the fission yeast genes not only on the level of single genes, but also in systematic approaches. Our strategy of cloning homology regions into a vector is not limited to tagging. We have successfully used this strategy to knock out genes in a high-throughput screen [12, 27]. Apart from the fission yeast, this technique should also be applicable to other species where homologous recombination can be used to target genes. We used this strategy to identify proteins co-

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purifying with two evolutionarily conserved proteins Swi5 and Sfr1. We recapitulated previously characterized protein–protein interactions and importantly, we discovered novel proteins co-purifying with Swi5 and Sfr1. Our analysis is consistent with the notion that there are two different Swi5-containing protein complexes, namely Swi5-Swi2 and Swi5-Sfr1. Further studies are required to explore the roles of Swi5 and Sfr1-interacting proteins in DNA repair and mating-type switching.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviation

TAP tandem affinity purification

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Table 1

List of proteins identified by MS co-purifying with *S. pombe* Swi5-TAP and Sfr1-TAP. Only proteins identified with more than two peptides are included[a)]

Purification	Accession number	Co-purifying proteins	Unique peptides	Assigned spectra	Sequence coverage [%]
Swi5-TAP	gi|19112460	Sfr1	17	245	63
	gi|19112245	Swi5	5	168	60
	gi|68000325	Swi2	19	30	32
	gi|68012661	Sir2, histone deacetylase.	5	5	13
Sfr1-TAP	gi|19112460	Sfr1	18	353	78
	gi|19112245	Swi5	6	215	68
	gi|162312386	SPAC139.01c, XPG/RAD2 nuclease family protein (predicted)	4	4	6

[a)]Proteins found in other unrelated purifications are omitted from this table; the full list of proteins is provided in Supporting Information Table S2 and the gel image is shown in Supporting Information Fig. S4.

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Table 2

Phospho-peptides detected by MS

Protein	Peptide	$[M+H]^+$ (Da)	Delta M (Da)	z
Swi5-TAP purification				
Sfr1	R.LFKS#PISNCLNPK.S	1597.8	0.7	2
Sfr1	K.S#PISNCLNPK.S	1209.6	0.1	2
Sfr1	K.VSLS#ESDLR.D	1085.5	0.2	2
Sfr1	K.NILLKPFKS#PLR.Q	1505.8	−0.4	2
Swi5	K.CTS#VELFDR.F	1206.5	0.7	2
Sfr1-TAP purification				
Sfr1	K.VSLS#ESDLR.D	1085.5	0.6	2

Phosphorylated residues are labeled by #.