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Vectors for expression of proteins with single or combinatorial fluorescent protein and tandem affinity purification tags in *Dictyostelium*

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Abstract

We constructed a series of expression vectors for purification of native proteins and protein complexes in *Dictyostelium*. Protein purification is achieved by either a C-terminal or N-terminal fusion of the protein of choice to the tandem affinity purification (TAP) tag. The TAP tag consists of a protein A tag and a calmodulin binding peptide (CBP) and has been successfully used for purification of native protein complexes from yeast and animal cells. Protein expression is driven by the constitutive actin 15 promoter and the vectors optionally carry additional green- or yellow fluorescent protein (GFP or YFP) tags for fusion at either a C- or N-terminal location. Tandem affinity purification of native *Dictyostelium* protein complexes was tested by using pArc-34, one of the members of the well characterized *Dictyostelium* Arp2/3 complex, as bait. After denaturation and SDS–PAGE separation of the pArc-34 associated proteins all members of the Arp2/3 complex could be identified.

Keywords: Tandem affinity purification; Expression vector; Yellow fluorescent protein; *Dictyostelium discoideum*; Native protein complexes

Dictyostelium discoideum is a genetically tractable model system for studying remodeling of the actin cytoskeleton during cell locomotion, cell division, phagocytosis and vesicle trafficking and for elucidation of signaling pathways that control chemotaxis and development. Several metazoan-type cytoskeletal components were identified for the first time in *Dictyostelium* [1–3], and the process of pleckstrin homology domain mediated recruitment that plays such crucial roles in leukocyte chemotaxis and phagocytosis was also first demonstrated in the Dictyostelids [4]. The *Dictyostelium* genome is now completely sequenced and each gene is therefore available for functional analysis by gene disruption, overexpression and mutagenesis [5]. Genetic approaches to create tagged mutations, such as REMI (restriction enzyme mediated integration), have identified numerous genes with crucial roles in developmental or cellular processes [6]. However in only a few cases has it been possible to use genetic approaches, such as screens for suppressor mutations, to determine epistatic relationships between genes [7]. Or, in other words, to determine in what order their cognate proteins act on each other in a pathway. In other genetically tractable systems, such as the fruit fly and the nematode, epistatic relationships are usually determined by crossing in loss- and gain-of-function mutants of genes in the pathway. The poor accessibility of the Dictyostelid sexual stage, the macrocyst, has prohibited this approach.

Other genetic screens, such as the yeast two-hybrid system or the split-ubiquitin method are being successfully used to identify interacting proteins in a pathway [8,9]. However, these methods depend on successful heterologous expression of the proteins in yeast, which is not possible for many proteins. The TAP tag was developed about seven years ago to purify native protein complexes from yeast [10,11] and was used for systematic identification of protein complexes in yeast [12–15] and metazoans [16,17]. For C-terminal tagging, the TAP tag contains in tandem, a

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Table 1
Oligonucleotide primers used in this work

Name	DNA sequence
MCS1	5′-GATCTAAAAAATGGGATCCAAGCTTACTAGTTCTAGAGAATTC ATCGATTAAC-3′
MCS2	5′-TCGAGTTAATCGATGAATTCTCTAGAACTAGTAAGCTTGGATC CCATTTTTTA-3′
NTAP1	5′-GCAGGATCCGCAGGCCTTGCGCAACACG-3′
NTAP2	5′-CCGTAAGCTTATCGTCATCATCAAGTGCC-3′
CTAP1	5′-GCATCGATGAAAAGAGAAGATGGAAAAAGAATTTCATAGCCG-3′
CTAP2	5′-GCTCTCGAGTTAGGTTGACTTCCCCGCGGAATTCG-3′
tapYFP1	5′-GGTCAAGCTTGTGAGCAAGGGCGAGGAGCTG-3′
tapYFP2	5′-GCACTAGTCTTGTACAGCTCGTCCATGCCG-3′
tapYFP3	5′-GTCGAATTCGTGAGCAAGGGCGAGGAGCTG-3′
tapYFP4	5′-GCCATCGATCTTGTACAGCTCGTCCATGCCG-3′
p34Arc1	5′-GCGGGATCCTTATTATTAGAAACACACAATCG-3′
p34Arc2	5′-GTCTCTAGAATTTTGTTTAAAGAATTTACCAGTGATTG-3′

Table 2
Plasmid vectors that were used or prepared in this work.

Vector name	Backbone	Tag position	Accession #
EXP4(+)	pAT153	None	EF028663
EXP5(+)	EXP4(+)	None	EF028664
pDV-NYFP	EXP5(+)	N-terminal YFP	EF028665
pDV-NTAP	EXP5(+)	N-terminal TAP	EF028666
pDV-CGFP	EXP5(+)	C-terminal YFP	EF028667
pDV-CYFP	EXP5(+)	C-terminal GFP	EF028668
pDV-CTAP	EXP5(+)	C-terminal TAP	EF028669
pDV-NTAP-NYFP	EXP5(+)	N-term. YFP and TAP	EF028670
pDV-NYFP-CTAP	EXP5(+)	N-term. YFP, C-term. TAP	EF028671
pDV-CGFP-CTAP	EXP5(+)	C-term. GFP, C-term. TAP	EF028672
pDV-CYFP-CTAP	EXP5(+)	C-term. YFP, C-term. TAP	EF028673
pDV-NTAP-CGFP	EXP5(+)	N-term. TAP, C-term. GFP	EF028674
pDV-NTAP-CYFP	EXP5(+)	N-term. TAP, C-term. YFP	EF028675

calmodulin binding peptide tag, a TEV protease cleavage site and a protein A tag. For N-terminal tagging the order of these units is reversed. We have constructed a series of vectors for C- and N-terminal tagging of proteins expressed in *Dictyostelium*. Protein expression is driven by the strong constitutive actin15 promoter. Additional cloning sites were introduced to allow replacement of this promoter by an inducible promoter. A third green- or yellow fluorescent protein tag was introduced for C- or N-terminal expression in some of the vectors for rapid assessment of the levels of protein expression and the cellular location of the expressed proteins.

Materials and methods

Construction of plasmid vectors

Gene fragments were amplified by polymerase chain reaction (PCR)[2] with a 4:1 mixture of the Taq and Pfu DNA polymerases (Promega, Madison, WI). All oligonucleotide primers used in this study are listed in Table 1. The integrating *Dictyostelium* vector EXP4(+) [18] was used as starting material. An *Xba*I and a *Hin*dIII site upstream of the actin15 promoter were first successively destroyed by filling in the overhanging sites after digestion with the Klenow fragment of polymerase I, followed by religation, resulting in vector EXP4(-XH). This also generated a unique *Nhe*1 site replacing the *Hin*dIII site.

A long multiple cloning site (MCS) was generated by deleting the existing polylinker by *Bgl*II/*Xho*I digestion of the vector and by ligating a duplex of primers MCS1 and MCS2 that carries compatible sticky ends into the *Bgl*II and *Xho*I sites. The resulting vector, EXP5(+) was used as the backbone for all subsequent constructs containing TAP and/or EGFP/EYFP fragments (Table 2).

The N-terminal TAP fragment (NTAP) was amplified from the plasmid pBS1761 [11] using primers NTAP1 and NTAP2 and ligated into the *Bam*HI and *Hin*dIII sites of EXP5(+), yielding pDV-NTAP. The C-terminal TAP fragment (CTAP) was amplified from plasmid pBS1479 [10] using primers CTAP1 and CTAP2 and ligated into the *Cla*I and *Xho*I sites of EXP5(+), yielding vector pDV-CTAP. Enhanced yellow- and green fluorescent protein genes (YFP) and (GFP) were amplified from the vectors pEYFP-N1 and pEGFP-C3 (Clontech, Mountain View, CA), respectively. For N-terminal location of YFP, primers tapYFP1 and tapYFP2 were used and insertion occurred in the *Hin*dIII and *Spe*I sites of EXP5(+), yielding pDV-NYFP. For C-terminal location of YFP or GFP, primers tapYFP3 and tapYFP4

[2] *Abbreviation used:* PCR, polymerase chain reaction; TAP, tandem affinity purification; CBP, calmodulin binding peptide; GFP, green fluorescent protein; YFP, yellow fluorescent protein; CTAP, C-terminal TAP fragment; NTAP, N-terminal TAP fragment; MCS, multiple cloning site.