Rom1p and Rom2p are GDP/GTP exchange proteins (GEPs) for the Rho1p small GTP binding protein in *Saccharomyces cerevisiae*

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The RHO1 gene encodes a homolog of the mammalian RhoA small GTP binding protein in the yeast Saccharomyces cerevisiae. Rho1p is localized at the growth site and is required for bud formation. Multicopy suppressors of a temperature-sensitive, dominant negative mutant allele of RHO1, RHO1(G22S, D125N), were isolated and named ROM (RHO1 multicopy suppressor). Rom1p and Rom2p were found to contain a DH (Dbl homologous) domain and a PH (pleckstrin homologous) domain, both of which are conserved among the GDP/GTP exchange proteins (GEPs) for the Rho family small GTP binding proteins. Disruption of ROM2 resulted in a temperature-sensitive growth phenotype, whereas disruption of both ROM1 and *ROM2* resulted in lethality. The phenotypes of $\Delta rom 1 \Delta$ *rom2* cells were similar to those of $\Delta rho1$ cells, including growth arrest with a small bud and cell lysis. Moreover, the temperature-sensitive growth phenotype of $\Delta rom2$ was suppressed by overexpression of RHO1 or RHO2, but not of CDC42. The glutathione-S-transferase (GST) fusion protein containing the DH domain of Rom2p showed the lipid-modified Rho1p-specific GDP/GTP exchange activity which was sensitive to Rho GDP dissociation inhibitor. These results indicate that Rom1p and Rom2p are GEPs that activate Rho1p in S.cerevisiae.

Keywords: GDP/GTP exchange protein/Rho/S.cerevisiae

Introduction

The Rho family belongs to the small GTP binding protein superfamily and consists of the Rho, Rac and Cdc42 subfamilies (Hall, 1994; Takai *et al.*, 1995). The Rho subfamily is composed of three highly homologous members, RhoA, -B and -C. Evidence is accumulating that, through reorganization of the actin cytoskeleton, Rho regulates various cell functions, such as maintenance of cell morphology, formation of stress fibers and focal adhesions, cell motility, membrane ruffling, cytokinesis, cell aggregation and smooth muscle contraction. However, it remains to be clarified how Rho regulates reorganization of actin filaments.

Rho has two interconvertible forms: GDP-bound inactive and GTP-bound active forms (Hall, 1994; Takai et al., 1995). The GDP-bound form is converted to the GTP-bound form by the GDP/GTP exchange reaction which is stimulated by GDP/GTP exchange proteins (GEPs) and is inhibited by GDP dissociation inhibitors (GDIs). The GTP-bound form is converted to the GDPbound form by the GTPase reaction which is regulated by GTPase activating proteins (GAPs). Two GDIs, including Rho GDI (Fukumoto et al., 1990; Ueda et al., 1990) and Ly/D4 GDI (Lelias et al., 1993; Scherle et al., 1993), and four GEPs, including Smg GDS (Yamamoto et al., 1990; Kaibuchi et al., 1991), Dbl (Hart et al., 1991; Yaku et al., 1994), Ost (Horii et al., 1994) and Lbc (Toksoz and Williams, 1994) have so far been reported to be active on Rho. These GEPs, except Smg GDS, share two conserved domains: one is the DH (Dbl homologous) domain, which is supposed to possess the GDP/GTP exchange activity for Rho (Hart et al., 1994), and the other is the PH (pleckstrin homologous) domain, which is found in many signaling and cytoskeletal proteins and is suggested to be responsible for localization to the plasma or organellar membranes (Musacchio et al., 1993; Gibson et al., 1994). Rho is found in both cytosol and membrane fractions. Rho in the cytosol fraction was shown to be complexed with Rho GDI, indicating that Rho in this fraction is in the inactive state (Takai et al., 1995). Therefore, it is likely that Rho complexed with Rho GDI in the cytosol is released from the inhibitory action of Rho GDI and is activated by its GEPs at the plasma membrane. However, the GEPs for Rho themselves have been shown not to be sufficient to activate Rho complexed with Rho GDI (Kikuchi et al., 1992; Yaku et al., 1994). It remains to be clarified how Rho is activated.

The budding yeast Saccharomyces cerevisiae, possessing the Rho family members, including RHO1, RHO2 (Madaule et al., 1987), RHO3, RHO4 (Matsui and Toh-E, 1992) and CDC42 (Adams et al., 1990; Johnson and Pringle, 1990), offers an attractive system to solve the problems described above, since a powerful molecular genetical approach is applicable. Cells of this yeast grow by budding for cell division, and the actin cytoskeleton plays a pivotal role in the budding process (Drubin, 1991). RHO1 is a homolog of the mammalian RhoA gene and has been shown to be an essential gene in S.cerevisiae (Madaule *et al.*, 1987). We have shown recently that cells of *rho1* mutants stop growing with small budded cells under restrictive conditions (Yamochi et al., 1994). Moreover, immunofluorescence microscopic studies indicate that Rholp is localized at the growth site where cortical actin patches are localized, including the presumptive

budding site, the bud tip and the cytokinesis site (Yamochi *et al.*, 1994). These results suggest that *RHO1* is involved in the process of bud formation and that Rho1p also regulates reorganization of the actin cytoskeleton as in mammalian cells. We have shown recently that Rho1p performs its functions through at least Pkc1p, which is a homolog of mammalian protein kinase C, as a downstream target of Rho1p (Nonaka *et al.*, 1995). Concerning the upstream regulators of *RHO1*, recently we have purified a homolog of Rho GDI in yeast, cloned its gene and named it *RD11* (Masuda *et al.*, 1994). However, the gene for a GEP for *RHO1* has not yet been cloned in yeast.

Accumulating evidence indicates that there is a small GTP binding protein cascade operating in the budding process in *S.cerevisiae* (Chant and Stowers, 1995). In the budding process, Rsr1p/Bud1p, a homolog of mammalian Rap1, selects the site for budding, Cdc42p initiates the budding site assembly, and then Rho1p promotes the bud formation. Therefore, in this cascade, it is presumed that the GTP-bound form of Rsr1p/Bud1p activates Cdc42p and then the GTP-bound form of Cdc42p activates Rho1p. In accordance with this model, it has been shown recently that the GTP-bound form of Rsr1p/Bud1p binds directly to Cdc24p, which is a GEP for Cdc42p (Zheng *et al.*, 1995a). However, the linkage between Cdc42p and Rho1p has not yet been established at a molecular level. To this end, the gene for a GEP for Rho1p should be cloned.

In the present work, we have cloned two homologous genes, *ROM1* and *ROM2*, as multicopy suppressors of a temperature-sensitive dominant negative mutation of *RHO1*. Genetic and biochemical studies indicate that Rom1p and Rom2p are Rho1p-specific GEPs in *S.cerevisiae*.

Results

Isolation of a temperature-sensitive dominant negative mutation of RHO1, RHO1(G22S, D125N)

It has been reported that an amino acid substitution of glycine at position 22 of Ras2p results in a dominant negative phenotype (Powers et al., 1989). This glycine is located in the amino acid sequence, GXXXXGK, which is highly conserved among small GTP binding proteins of the Ras superfamily, including Rho1p. By analogy, it was presumed that a corresponding mutation of RHO1 would also result in a dominant negative phenotype. An analogous mutation was introduced into RHO1 by PCR mutagenesis, and the resultant RHO1(G22S) gene was cloned into pRS316 (URA3, CEN6). Since transformation of a wild-type yeast strain OHNY1 with pRS316-RHO1(G22S) gave no transformants, RHO1(G22S) seemed to behave as a dominant lethal mutation (data not shown). To make this RHO1(G22S) mutant gene amenable to genetic analyses, a temperature-sensitive mutation of RHO1(G22S) was searched for. Since a temperaturesensitive RHO1(G22S) protein is active at a lower temperature, a wild-type strain carrying the temperature-sensitive RHO1(G22S) gene would show a cold-sensitive growth phenotype. Such a mutation, RHO1(G22S, D125N), was isolated by the hydroxylamine mutagenesis method as described in Materials and methods. It is noteworthy that the aspartic acid at amino acid position 125 in Rho1p corresponds to GNKXD, which is a guanine base binding site conserved among small GTP binding proteins of the Ras superfamily. Cells of OHNY1 carrying pRS316-RHO1(G22S, D125N) arrested with tiny or small budded cells (60–70% of ~300 cells counted) at 25°C. This phenotype is similar to that of *rho1* mutants, indicating that *RHO1*(G22S, D125N) behaved as a dominant negative mutation at 25°C.

Isolation of ROM genes, multicopy suppressors of RHO1(G22S, D125N)

To isolate genes functionally relevant to RHO1, we isolated multicopy suppressors of RHO1(G22S, D125N). OHNY1 carrying pRS316-RHO1(G22S, D125N) and pRS315-RHO1(G22S, D125N) were transformed with yeast genomic DNA libraries constructed in episomal vectors, YEp13 (LEU2) and YEp24 (URA3), respectively. Transformants were selected on SD -leu -ura medium at 25°C for 4-10 days. Of 180 000 transformants screened, ~2000 transformants formed colonies at 25°C with various growing rates. Since many transformants which grew as quickly as wild-type cells had been found to possess a rearrangement or a mutation in the RHO1(G22S, D125N) gene itself, we selected transformants with slower growing rates, and these transformants were analyzed further. Of ~200 transformants tested, 105 transformants showed the library plasmid-dependent growth phenotype at 25°C. Library plasmids were recovered from these transformants through Escherichia coli transformation, and 94 clones were found to confer the ability to grow at 25°C on OHNY1 carrying pRS316-RHO1(G22S, D125N) or pRS315-RHO1(G22S, D125N). Restriction enzyme mapping and Southern blot analyses revealed that 50 and five clones encoded RHO1 and RHO2, respectively. One clone encoded PKC1, which is a yeast homolog of protein kinase C and is one of the downstream targets of Rho1p (Nonaka et al., 1995). The other 38 clones comprised five genes that turned out to be new or poorly characterized and were designated ROM1, ROM3, ROM4, ROM5 and ROM6 (RHO1 multicopy suppressor). ROM1 consisted of one clone, YEp24-ROM1, and was characterized further in this study.

YSCL8039.3 is homologous to ROM1 and functions as a ROM gene

The deletion mapping indicated that ROM1 was located in the 3.8 kb SalI-ClaI fragment (Figure 1). DNA sequencing of the fragment revealed that the ROM1 coding region was truncated at its N-terminal region. Thus, the insert DNA encoded the truncated Rom1p, Δ N-Rom1p, of 1013 amino acid residues. Searches for sequence homology to ΔN-Rom1p using BLAST and FASTA programs and the GenBank database revealed that a predicted protein, encoded by YSCL8039.3 (GenBank accession number U19103) on chromosome XII of S.cerevisiae, was significantly homologous to ΔN -Rom1p. A 5.3 kb DNA fragment, containing the 660 bp upstream region, 4071 bp open reading frame (ORF) and 550 bp downstream region of YSCL8039.3, was amplified by PCR and was cloned into YEp352. The resulting plasmid suppressed the coldsensitive growth phenotype of OHNY1 carrying pRS315-RHO1(G22S, D125N) (Figure 2). Thus, YSCL8039.3 was named ROM2 and was characterized further with ROM1.



Fig. 1. Restriction enzyme map and deletion mapping of *ROM1*. (A) Restriction enzyme map of the insert DNA. The thick line represents the 12 kb original genomic DNA fragment cloned into YEP24-ROM1, whereas the thin lines represent portions of the vector YEp24. *S*, *Sall*; *B*, *Bgl*[1; *K*, *Kpn*]; *BG*[1, *Bsr*G1; *P*, *Pvu*[1; *C*, *Clal*. The *Clal* site is not unique in the insert DNA. The extent and direction of the *ROM1* ORF truncated at its N-terminal region (ΔN -*ROM1*) are shown by an arrow. (B) Deletion mapping of *ROM1*. Various deletion fragments of the insert DNA were cloned into YEp24 or YEp352. The constructed plasmids were transformed into OHNY1 carrying pRS315-RHO1(G22S, D125N) to examine their ability to suppress the coldsensitive growth phenotype. a, YEp24-ROM1 Δ BglII; b, YEp352-ROM1[ClaI– PvuII]; e, YEp352-ROM1[SalI–PvuII]; d, YEp352-ROM1[ClaI– PvuII]; e, YEp352-ROM1[SalI–ClaI].



Fig. 2. Suppression of the cold-sensitive growth phenotype of the *RHO1*(G22S, D125N) mutant by overexpression of *ROM1* or *ROM2*. OHNY1 carrying pRS315-RHO1(G22S, D125N) was transformed with YEp24-RHO1 (RHO1), YEp24 (vector), YEp352-ROM1[ClaI–SalI] (ROM1) or YEp352-ROM2 (ROM2). The resultant transformants were streaked onto the SD-ura-leu plates and then incubated at 35°C (left) or at 25°C (right) for 2 days.

ΔN -Rom1p and Rom2p possess the DH and PH domains

Rom2p is a protein of 1356 amino acid residues with a predicted M_r of 153 kDa. The predicted amino acid sequences of ΔN -Rom1p and Rom2p were aligned optimally with 42% amino acid sequence identity (Figure 3). It should be noted that Rom2p is rich in serine (12.7%), resulting in many potential sites for phosphorylation by protein kinases. More importantly, ΔN -Rom1p and Rom2p possess a region of ~200 amino acid residues with significant sequence homology to the DH domain (Figures 3 and 4A). This DH domain is found to be present in many GEPs for the Ras superfamily members, including Cdc24p, Dbl, Ect2, Vav, mSos-1, Ras-GRF, Bcr (Boguski and McCormick, 1993), Abr (Heisterkamp et al., 1993), Tiam1 (Habets et al., 1994), Ost (Horii et al., 1994), Lbc (Toksoz and Williams, 1994), Lfc (Whitehead et al., 1995) and Tim (Chan et al., 1994). Of these, Cdc24p (Zheng et al., 1994), Dbl (Hart et al., 1991, 1994; Yaku et al., 1994), Tiam1 (Michiels et al., 1995), Ost (Horii et al., 1994) and Lbc (Zheng et al., 1995b) have been shown to possess the GDP/GTP exchange activity for the Rho family members. The conservation of the DH domain varies from

Rom2p	1	SCOLUDNETE	NTONUNVLLS	MSETNVDSLG	DRNDIYSQIF	GVERRPDSFA AIDDSDIPNN	TFDSDSHGDI EMKGKSSNYI
Rom2p	111	LSQQTNIKEV	PDTQSLSSAD	NTPVSSPKKA	RDATSSHPIV	HAKSMSHIYS	TSNSASRQAK
Rom2p Rom2p	171	HYNDHPLPPM	SPRNEVYQKN FSRPSSKDLH	KSTTAFVPKR EOHOHHOHIO	KPSLPQLALA HNNINNHNNN	GLKKQSSFST NTNNNGAHYO	GSASTTPTQA VGSSNSNYPO
Tomp	232	lator byor or	101100010000			-	
N-Rom1p	1	SN *	* *	.HSSPSLLSF	S.KNSGSHMG	DPNQLSTPPT	PKSAGHTMEL *
Rom2p	281	HSHSISSRSM	SLNSSTLKNI	ASSFQSKTSN	SRKATQKYDI	TSNPFSDPHH	HHHHHHSSNS
N-Romip	49	HSSFNGKHSS	SSTSSLFALE	SLKPQNR	RSSNSSNHSS	QYRRHTNQHQ	RHHSRSKSSP
Rom2p	341	HSSLNNVHGS	GNSSSVMGSS	SNIGLGLKTR	VSS-TSLALK	RYTSVSGTSL	SSPRRSSMTP
N-Romip	106	VSVTEISM	IKGTPLVYPA	LLSLIAIKFK	QTIQLGTHKK	MGLLYRDSFT	GKQAIDTLCL
Rom2p	400	LSASRPVMSA	SSKKPQVYPA	LLSRVATKFK	SSIQLGEHKK	DGLVYRDAFT	GQQAVDVICA
N-Romlp	164	IIGSLDRNLG	MLIGKSLEAQ	KLFHDVLYDH	GVRDSVLEIY	ELSSESIF	MAH
Rom2p	460	IIRTSDRNLA	LLFGRSLDAQ	KLFHDVVYEH	RLRDSPHEVY	EFTDNSRFTG	TGSTNAHDPL
N-Romlp	215	QSQSST	SIA	NTFSSSSSSV	NS.LRTKT		I
Rom2p	520	* * MLLPNSSSFN	* SGNHSYPNSG	MVPSSSTSSL	NSDQATLTGS	RLHMSSSLSQ	QKNPAAIHNV
AN-Romip	243	NGVFVPLTHC	YSSTCSLEKL	CYSISCPNRL	QQQANLHLKL	GGGLKRNISL	ALDKEDDERI
Rom2p	580	**** * * NGVFTLLAEC	** ** * YSPTCTRDAL	CYSISCPRRL	*** * ** EOOARLNLKP	NGGLKRNISM	ALDDDDEEKP
AN-Romin	303	SWINSVPKSV	WESLSKOOIK	ROBALYELFT	TERREVESLE	IIRDTTNKKL	LETNIIPSDV
Dom?m	640	*** ** *	** * * * **		*** ******	* ****** *	
Romap	040	SWISSVSKED	WENDERKEIK				
∆N-Romlp	363	* ** *****	YINEIYSVNR	EFLKALAORO	SLSPICPGIA	SIFLOYLPFF	* ** ***
Rom2p	700	RENFIKEVTA	HINDIYSVNR	RFLKALTDRQ	RSSPVVRGIG	DIVLRFIPFF	EPFVSYVASR
∆N-Romlp	423	PYAKYLIETQ	RSVNPNFARF	DDEVSNSSLR	HGIDSFLSQG	VSRPGRYSLL	* ** **
∆N-Romlp Rom2p	423 760	PYAKYLIETQ PYAKYLIETQ	RSVNPNFARF RSVNPYFARF	DDEVSNSSLR	HGIDSFLSQG HGIDSFLSQG	VSRPGRYSLL	VREIINFSDP
∆N-Romlp Rom2p ∆N-Romlp	423 760 483	PYAKYLIETQ PYAKYLIETQ VTDRDDLQML	RSVNPNFARF RSVNPYFARF MKVQDLLKDL	DDEVSNSSLR	HGIDSFLSQG HGIDSFLSQG AQDRYDVKVL	VSRPGRYSLL VSRPGRYMLL KQKILFKNEY	VREIINFSDP
∆N-Romlp Rom2p ∆N-Romlp Rom2p	423 760 483 820	PYAKYLIETQ PYAKYLIETQ VTDRDDLQML ERDKSDYEDL	RSVNPNFARF RSVNPYFARF MKVQDLLKDL * * * * SKAMDALRDF	DDEVSNSSLR DDDHMSSSLR MKRIDRASGA	HGIDSFLSQG HGIDSFLSQG AQDRYDVKVL **** *** * AQDRHDVKLL	VSRPGRYSLL VSRPGRYMLL KQKILFKNEY	VREIINFSDP * ** ** VREINKSTDP VNLGLNNEKR ****** * VNLGLNDERR
ΔN-Romlp Rom2p ΔN-Romlp Rom2p ΔN-Romlp	423 760 483 820 543	PYARYLIETQ PYARYLIETQ VTDRDDLQML EKDKSDYEDL KIKHEGLLSR	RSVNPNFARF ***** RSVNPYFARF MKVQDLLKDL * * * * SKAMDALRDF KDVNKTDASF	DDEVSNSSLR ** **** DDDHMSSSLR MKRIDRASGA ***** **** MKRIDQASGA SGDIOFYLLD	HGIDSFLSQG ********** HGIDSFLSQG AQDRYDVKVL **** *** * AQDRHDVKLL 	VSRPGRYSLL VSRPGRYMLL KQKILFKNEY KQKILFKNEY VNKWHOHTVF	VKEIIMSTDP VKEIMKSTDP VNLGLNNEKR VNLGLNDERR
ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p	423 760 483 820 543 880	PYARYLIETQ PYARYLIETQ VTDRDDLQML KIKHEGLLSR KIKHEGILSR	RSVNPNFARF RSVNPYFARF MKVQDLLRDL SKAMDALRDF KDVNKTDASF KELSKSDGTV	DDDHNSSSLR DDDHNSSSLR MKRIDRASGA SGDIOFYLLD	HGIDSFLSQG HGIDSFLSQG AQDRYDVKVL **** *** AQDRHDVKLL NMLLFLKSKA	VSRPGRYSLL VSRPGRYMLL KQKILFKNEY KQKILFKNEY VNKWHOHTVF	VKEIIMSTDP
ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p ΔN-Rom1p	423 760 483 820 543 880 603	PYAKYLIETQ PYAKYLIETQ VTOKODLQML EKOKSDYEDL KIKHEGLLSR KIKHEGILSR ICPAEDMPPI	RSVNPNFARF RSVNPYFARF MKVQDLLKDL SKAMDALRDF KDVNKTDASF KELSKSDGTV KRYVTENPNC	DDDWSSSLR DDDWSSSLR MKRIDQASGA SGDIOFYLLD VGDIOFYLLD SAGVLLPOYO	HGIDSFLSQG AQDRYDVKVL AQDRHDVKVL NMLLFLKSKA NMLLFLKSKA TSNPKNAIVF	VSRPCRYSLL VSRPCRYMLL KQKILFKNEY KQKILFKNEY VNKWHOHTVF AYYGTKOOYO	VKEINKSTOP VNLGLNNEKR VNLGLNDERB ORPIPLPLLF ORPIPLPLLF 2 VTLYAPQPAG
ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p	423 760 483 820 543 880 603 940	PYAKYLIETQ PYAKYLIETQ VTDKDDLQML EKDKSDYEDL KIKHEGLLSR KIKHEGLLSR ICPAEDMPPI ACPGEDMPAL	RSVNPNTARF RSVNPYTARF MKVQDLLKDL SKAMDALRDF KDVNKTDASF KELSKSDGTV KRYVTENPNC RKYIGDHPDC	DDDWSSSLR DDDMSSSLR MRRIDRASGA SGDIOFYLLD SAGVLLPYUD SAGVLLPYUD	HGIDSFLSQG AQDRYDVKVL AQDRHDVKVL NMLLFLKSKA NMLLFLKSKA TSNPKNAIVF	VSRPCRYSLL VSRPCRYMLL KQKILFKNEY KQKILFKNEY VNKWHOHTVF VNKWHOHKVF AYYGKOOYO LYYGAKORYO	VKEINKSTOP VNLGLNDERB VNLGLNDERB ORPIPLPLLF ORPIPLPLLF VTLYAPOPAG
ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p ΔN-Rom1p	423 760 483 820 543 880 603 940 663	PYAKYLIETQ PYAKYLIETQ PYAKYLIETQ PYAKYLIETQ EKDKSDYEDL KIKHEGLLSR KIKHEGILSR KIKHEGILSR ICPAEDMPPI ACPGEDMPAL LQTLIEKVKQ	RSVNPYTARF RSVNPYTARF MKVQDLLKDL SKAMDALRDF KDVNKTDASF KELSKSDGTV KRYVTENPNC RKYJGDHPDC EQKRLLDETK	DDEWSNSSLR DDDMSSSSLR MRRIDRASGA SGDIOFYLLD VGDIOFYLLD SAGVLLPOYO SGTVIOPEYN HITFKQMVGQ	HGIDSFLSQG AQDRYDVKVL AQDRHDVKVL NMLLFLKSKA NMLLFLKSKA TSNPKNAIVF TSNPKNAIVF	VSRPGRYSLL VSRPGRYMLL KQKILFKNEY VNKWHOHTVF VNKWHOHTVF AYYGTKOOYO LYYGAKORYO VNDVLICSAG	VKEINKSTOP VNLGLNNEKR VNLGLNDERR ORPIPLPLLF ORPIPLPLLF 2 VTLYAPQPAG 2 VTLYAPQPAG 5 KILLVATNMG
ΔN-Romlp Rom2p ΔN-Romlp Rom2p ΔN-Romlp Rom2p ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p	423 760 483 820 543 880 603 940 663 1000	PYAKYLIETQ PYAKYLIETQ VTDKDDLQML EKDKSDYEDL KIKHEGLLSR KIKHEGLLSR KIKHEGLLSR ICPAEDMPPI ACPGEDMPAL LQTLIEKVKQ LQTLIEKIKQ	RSVNPYTARF RSVNPYTARF MKVQDLLKDL SKAMDALRDF KDVNKTDASF KELSKSDGTV KRYVTENPNC RKYIGDHPDC EQKRLLDETK GQAALISKTE	DDDMMSSSLR DDDMMSSSLR MKRIDRASGA KKRIDRASGA SGDIOFYLLD SAGVLLPOYO SGTVIOPEYN HITFKQMVGQ MFNVTKMSDR	HGIDSFLSQG HGIDSFLSQG AQDRYDVKVL AQDRIDVKLL NMLLFLKSKA NMLLFLKAKA TSNPKNAIVF TSNPKNAIVF FFHSYINTNC FFJYINK	VSRPGRYSLI VSRPGRYMLL KQKILFKNEY KQKILFKNEY VNKWHOHTVF VNKWHOHKVF AYYGTKOOYO LYYGAKORYO VNDVLICSAG	VKEINKSTDP VNLGLNNEKR VNLGLNDERR ORPIPLPLLF VTLYAPOPAG VTLYAPOPAG KILLVATNMG RKLLIATNSG
ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p ΔN-Rom1p Rom2p ΔN-Rom1p	423 760 483 820 543 880 603 940 663 1000 723	PYAKYLIETQ PYAKYLIETQ VTOKODLQML KIKHEGLISR KIKHEGLISR CPGEDMPAL LQTLIEKVKQ LQTLIEKVKQ LQTLIEKIKQ LFVLNYATSI	RSVMPYTARF RSVMPYTARF MRVQDLL#DL SRAMDALRDF KELSKSDGTV KRYVTENPNC RKYIGDHPDC EQKRLLDETK GQAAIISTE N	DDDMMSSSLR DDDMMSSSLR MKRIDAASGA SGDIOFYLLD SAGVLLPOY SGTVIOPEYN HITFKQMVGQ MFNVTKMSDR .QKPVHLLH	GIDSFISQG HGIDSFISQG AQDRYDVKVL AQDRBDVKLL NMLLFLKSKA NMLLFLKSKA TSNPKNAITF TSNPKNAITF FFNJNTNC FFFDYTNK KISISQISVL	VSRPGRYSLI VSRPGRYMLI KQKILFKNEY KQKILFKNEY VNKWHOHKVF AYYGTKOOYO LYYGAKORYO VNDVLICSAG INSVTSCDGG EEYNVMILLI	VKEINKSTOP VALGLANEKR VALGLANEKR VALGLANEKR VALGLANERR ORPIPLPLLF VTLYAPQPAG VTLYAPQPAG VTLYAPQPAG VTLYAPQPAG KLLUATNAG RKLLIATNSG
AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p	423 760 483 820 543 880 603 940 663 1000 723 1057	PYAKYLIETQ PYAKYLIETQ VTORDDLQML EKOKSDYEDL KIKHEGLLSR ICPAEDMPPI ACPGEDMPAL LQTLLEKVKQ LQTLLEKKKQ LFVLNYATSI LYMSNIKRQQ	RSVBPNARF RSVMPYTARF MKVQDLLKDL SKAMDALRDF KDUNKTDASF KELSKSDGTV KRVYTENPAC RKVIGDHPDC EQKRLLDETK GQAAIISKTE N NKOHRHKSSA	DDDMMSSSLR DDDMMSSSLR MKRIDAAGA SGDIOFYLLD SAGVLLPOYO SGTVIOPEYN HITFKQMVGQ MFNVTKMSDR .QKPVHLLH FFSTFIQLVQ	GIDSFISQG AQDRYDVKVL AQDRADVKLL NMLLFLKSKA ISNPKNAIVF ISNPKNAIVF FFDYTNK KISISQISVL	VSRPCRYSLL VSRPCRYMLL KQKILFKNEY KOKILFKNEY VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKULCSAG INSVTSCDGG EEYNVMILLI	VKEIIAFSDP VKEIAKSTDP VNLGLNNEKR VNLGLNDERR ORPIPLPLLF ORPIPLPLLF VTLYAPOPAG VTLYAPOPAG VTLYAAQYAG KILLVATNMG KKLLIATNSG DKKLYSCPLD
AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip	423 760 483 820 543 880 603 940 663 1000 723 1057 772	PYAKYLIETO PYAKYLIETO VTORDDLOML EKOKSDYEDL KIKHEGLLSR KIKHEGLLSR LICPAEDMPPI LOTLLEKVKO LOTLLEKVKO LOTLLEKKKQ LFVLNYATSI LYMSNIKRQQ VIDDAENADF	RSVBPNARF RSVMPYTARF MKVQDLLKDL SKAMDALRDF KDUNKTDASF KELSKSDGTV KRYVTENPNC RKYIGDHPDC EQKRLLDETK GQAAIISKTE N NKOHRHKSSA LFRKNSKVLF	DDDMMSSSLR DDDMMSSSLR MKRIDAASGA SGDIOFYLLD SAGVLLPOYO SGTVIOPFYLL HITFKOMVGQ MFNVTKMSDR .QKPVHLLH FFSTFIQLVQ KYVAMFKDGF	GIDSFISQG GIDSFISQG AQDRYDVKVL AQDRDVKLL NMLLFLKSKA NMLLFLKSKA TSNPKNAIVF FFNFNNAIVF FFFSUNTNC FFFDYTNK KISISQISVL RNNITQIAVL CNGKRIIMIA	VSRPCRYSLL VSRPCRYMLL KQKILFKNEY KOKILFKNEY VNKWHOHKVF AYYGTKOOYO LYYGKKOOYO VNDVLICSAG EEYNVMILLI EEFKSILLI HHFLHTVK.I	VKEIIAF5DF VKEIAKSTDP VNLGLNNEKR VNLGLNDERR ORPIPLPLLF ORPIPLPLLF VTLYAAQYAG KILLVAINMG KKLLVAINMG DKKLYGCPLD DKKLYGCPLD DKKLYGCLS LUVNPLIFDF
AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p	423 760 483 820 543 880 603 940 663 1000 723 1057 772	PYAKYLIETQ PYAKYLIETQ VTDRDDLQML EKDKSDYDDL KIKHEGILSR ICPAEDMPPI ACPGEDMPAL LQTLIEKVKQ LQTLIEKVKQ LQTLIEKKKQ LFVLNYATSI LYMSNIKRQQ VIDDAENADF	RSVBDNAAF RSVNDYTAR MKVQDLLKDL SKAMDALROF KDUNKTDASF KELSKSDGTV KRYVTENPNC RKYIGDHPDC EQKRLLDETK GQAAIISKTE N NKDHRHKSSA LFRKNSKVLF FFKKHHELL	DDDMMSSSLR DDDMMSSSLR MRRIDDASGA SGDIOFYLLD VGDIOFYLLD SAGVLIPOYO SGTVIOPEYN HITFKOMVGQ MFNVTKMSDR .QKPVHLLH FFSTFIQLVQ KYVAMFKDGF	GIDSFISQG GIDSFISQG AQDRYDVKVL AQDRDVKLL NMLLFLKSKA NMLLFLKSKA NMLLFLKSKA TSNPKNAIFF FFHSVINTNC FFFDYTNK KISISQISVL RNNITQIAVL CNGKRIIMIA CNGKRIIVIA	VSRPCRYSLL VSRPCRYMLL KQKILFKNEY KOKILFKNEY VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF IYYGKKORYO VNVLICSAG HYYGKKORYO VNVLICSAG EEYNVMILLI EEFKSILLI HHFLHTVK.I HSSSISIXYF	VKEILMFSDP VKEIMKSTDP VNLGLNNEKR VNLGLNDERR ORPIPLPLLF ORPIPLPLLF VTLYAAQYAG VTLYAAQYAG KILLVAINMG KILLVAINMG DKKLYSCPLD LKKLYSCPLD LLVNPLIFDF
AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p AN-Romip Rom2p	423 760 483 820 543 880 603 940 663 1000 723 1057 772 1117 831	PYAKYLIETQ PYAKYLIETQ VTDRDDLQML EKDKSDYEDL KIKHEGILSR KIKHEGILSR LICPAEDMPPI ACPCEDMPAL LQTLLEKVKQ LQTLLEKVKQ LQTLLEKKKQ LFVLNYATSI LYMSNIKRQQ VIDDAENADF LIEAEGNGTS N SONEKK	RSVRPNTARF RSVRPYTARF MKVQDLLKDL SKAWDALROF KDUNKTDASF KELSKSDGTV KRUVTENPNC RKVJGDRPDC EQKRLLDETK GQAATISKTE N NKDHRHKSSA LFRKNSKVLF FFKKHHKELI	DDDMMSSSLR DDDMMSSSLR MRRIDRASGA SGDIOFYLLD VGDIOFYLLD SGTVIOPEYN HITFKQMVGQ MFNVTKMSDR .QKPVHLLH FFSTPIQLVQ KYVAMFKDGF NHVSFFAEGD	HGIDSFISQG HGIDSFISQG AQDRIDVKUL NMLLFLKSKA NMLLFLKSKA TSNPKNAITF FFHSVINTNC FFFDYTNK KISISQISVL KISISQISVL CNGKRIIMIA CNGKRIIMIA	VSRPCRYSLL VSRPCRYMLL KQKILFKNEY KQKILFKNEY VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNVULCSAG INSVTSCDGG EEVNVMILL EEFKSIILLI HHFLHTVK.I HSSSHSITYF KNIKILAUPPF	VKEIINF5DF VKEINKSTDF VNLGLNNEKR VNLGLNDERB ORPIPLPLLF ORPIPLPLLF VTLYAAQYAG VTLYAAQYAG KLLVATNNG KLLVATNNG DKKLYSCPLD LKKLYSCPLD LLVNPLIFDF EHEHPLLAEK
AN-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip	423 760 483 820 543 880 603 940 663 1000 723 1057 772 1117 831	PYAKYLIETQ PYAKYLIETQ VTDRDDLQML EKDKSDYEDL KIKHEGILSR KIKHEGILSR ICPAEDMPPI ACPCEDMPAL LQTLLEKVKQ LQTLLEKVKQ LQTLLEKVKQ LQTLLEKKKQ LYMSNIKRQQ VIDDAENADF LIEAEGNGTS NSGNFKK	RSVRPNTARF RSVRPYTARF MKVQDLLKDL SKAWDALROF KDUNKTDASF KELSKSDGTV KRUVTENPNC RKYUGDHPDC EQKRLLDETK GQAATISKTE N NKDHRHKSSA LFRKNKVLF FFKKHHKELI NLKAGLVDFS SLEWETTD	DDDMMSSSLR DDDMMSSSLR MRRIDRASGA SGDIOFYLLD VGDIOFYLLD SGTVIOPEYN HITFKQMVGQ MFNVTKMSDR .QKPVHLLH FFSTPIQLVQ KYVAMFKDGF NHVSFFAEGD VDSEPLSFSF	HGIDSFISQG HGIDSFISQG AQDRIDVKUL NMLLFLKSKA NMLLFLKSKA TSNPKNAITF FFHSYINTNC FFDYTNK KISISQISVL RNNITQIAVL CNGKRIIMIA CNGKRIIMIA	VSRPCRYSLL VSRPCRYMLL KQKILFKNEY KQKILFKNEY VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNVWHOLTXSAC VNDVLICSAC INSVTSCDGG EEVNVMILL EEFKSIILLI HHFLHTVK.1 HSSSHSIKYF KNIKILNVFF	VKEILMFSDP VKEILMFSDP VNLGLNNEKR VNLGLNDERB ORPIPLPLLF ORPIPLPLLF VTLYAPQPAG VTLYAPQPAG VTLYAPQPAG VTLYAPQPAG KLLVATNMG KLLVATNMG DKKLYSCPLD LVNPLIFDF EHEHPLLAEK VSLSONAH
AN-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip	423 760 483 820 543 880 603 940 663 1000 723 1057 772 1117 831 1177	PYAKYLIETO PYAKYLIETO VTORDDLOML EKDKSDYDDL KIKHEGILSR KIKHEGILSR ICPAEDMPPI ACPCEDMPAL LQTLIEKVKQ LQTLLEKIKQ LQTLLEKIKQ LFVLNYATSI LYMSNIKRQQ VIDDAENADF LIEAEGNGTS NSGNFKK	RSVRPNTARF RSVRPYTARF MKVQDLLKDL SKAWDALROF KDUNKTDASF KELSKSDGTV KRUVTENPNC RKYJGDHPDC EQKRLLDETK GQAATISKTE N NKDHRHKSSA LFRKNSKVLF FFKKHHKELI NLKAGLVDFS SLKKKITEVI	DDDMMSSSLR DDDMMSSSLR MTRIDRASGA SGDIOFYLLD VGDIOFYLLD SGTVIOPEYN HITFKQMVGQ MFNVTKMSDR .QKPVHLLH FFSTPIQLVQ KYVAMFKDGF NHVSFFAEGD VDSEPLSFSF	GIDSFISQG GIDSFISQG AQDRIDVKUL AQDRIDVKUL NMLLFLKSKA NMLLFLKSKA NMLLFLKSKA TSNPKNAITF FFHSYINTNC FFDYTNK KISISQISVL CNGKRIIMIA CNGKRIIMIA CNGKRIIMIA LENKICIGCK	VSRPCRYSLL VSRPCRYMLL KQKILFKNEY KQKILFKNEY VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNVULCSAG UNVULCSAG INSVTSCDGG EEVNVMILL EEFKSIILLI HHFLHTVK.1 HSSSHSIKYF KNIKILNVFF KGFQI	VKEILMFSDP VKEIMKSTDP VNLGLNNEKR VNLGLNDERB VNLGLNDERB VNLGLNDERB VTLYAPQPAG VTLYAPQYAG
AN-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip	423 760 483 820 543 880 603 940 663 1000 723 1057 772 1117 831 1177 838	PYAKYLIETO PYAKYLIETO VTDRDDLQML EKDKSDYEDL KIKHEGILSR ICPAEDMPPI ACPGEDMPAL LQTLIEKVKQ LQTLIEVKXQ LQTLIEVKXQ LQTLIEVKXQ LQTLIEVKXQ LQTLIEXVX LQTLIEVKXQ LQTLIEXVX LQTXXXXX LQTXXXXXXXXXXXXXXXXXXXXXXXXXX	RSVBENTARF RSVMPYTARF MKVQOLLKDL SKANDALROF KDUNKTDASF KELSKSDGTV KRVYEENKC GQAAIISKTE NKURHKSA LFRKNKVLF FFKKHHELL NLKAGLVDFS SLKKKITEVI LANMYKETFK	DDDMMSSSLR DDDMMSSSLR MTRIDRASGA SGDIOFYLLD VGDIOFYLLD SGTVIOPEYN HITFKQMVGQ MFNVTKMSDR .QKPVHLLH FFSTPIQLVQ KYVAMFKDGF NHVSFFAEGD VDSEPLSFSF FDSEPVSISF	HGIDSFISQG HGIDSFISQG AQDRIDVKUL AQDRIDVKUL NMLLFLKSKA NMLLFLKSKA ISNPKNAIFF FFHSYININC FFDYINK KISISQISUL RNNITQIAVL CNGKRIIMIA CNGKRIIVIA LENKICIGCK TFACFPELCF	VSNPCRYSLL VSRPCRYMLL KQKILFKNEY KQKILFKNEY VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNVLICSAG HYGTKOYG VNDVLICSAG HYGTKOYG HYGTKOYG NDVLICSAG HYGTKOYG NDVLICSAG HYGTKOYG NDVLICSAG HYGTKOYG NDVLICSAG HYGTKOYG HYGTKOYG HYGTKOYG NDVLICSAG HYGTKOYG HYGTKO	VKEILMFSDP VKEILMFSDP VNLGLNNEKE VNLGLNDERB VNLGLNDERB VVLYAPQPAG VTLYAPQPAG VTLYAPQPAG VTLYAPQPAG VTLYAPQPAG VTLYAPQPAG VTLYAPQPAG KLLVATNMG KLLVATNMG DKKLYSCPLS LVNPLIFDF EHEHFLLAEK VCDKNGFKMR .VSISQNAH
AN-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p	423 760 483 820 543 880 603 940 663 1000 723 1007 722 1117 831 1177 888 1229	PYAKYLIETO PYAKYLIETO VTDRDDLQML EKDKSDYEDL KIKHEGILSR ICPAEDMPPI ACPGEDMPAL LQTLIEKVKQ LQTLIEVKXQ LQTLIEVKXQ LQTLIEVKXQ LQTLIEVKXQ LQTLIEKVXQ LQTLIEVKXQ LQTLIEVXXQ LQTLIEVXXQ LQTLIEVXXQ LQTLIEVXXQ LQTLIEVXXQ LQTLIEVXXQ LQTLIEVXXQ LQTLIEVXXQ LQTLIEVXXQ LQTLIEVXXQ LQTLIEVXXQ LQTLIEVXXQ LQTLIEXXXQ	RSVHENNARF RSVNEYTARF MKVQOLLKDL SKANDALROF KDUNKTDASF KELSKSDGTV KRVYCENNC EQKRLLDETK GQAAIISKTE N NKDHRHKSSA LFRKNKKVLF FFKKHHEELI NLKAGLVDFS SLKKKITEVI LANMYKETFK	DDDMMSSSLR DDDMMSSSLR MCRIDQASGA SGDIOFYLLD VGDIOFYLLD SGTVIOPEYN HITFKQMVGQ MFNVTKMSDR .QKPVHLLH FFSTPIQLVQ KYVAMFKDGF NHVSFFAEGD VDSEPLSFSF FDSEPVSISF FDSEPVSISF FDSEPVSISF	HGIDSFISQG HGIDSFISQG AQDRIDVKUL AQDRIDVKUL NMLLFLKSKA NMLLFLKSKA NMLLFLKSKA TSNPKNAITF FFDYINK KISIQISVL RNNITQIAVL CNGKRIIMIA CNGKRIIVTA LENKICIGCK TFACFPELCF FLCYTEFAF	VSRPCRYSLL VSRPCRYMLL KQKILFKNEY KQKILFKNEY VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNVKWHOHTVF VNVKWHOHTVF NVKWHOHTVF VNVCGARCA KUTCH KILL KUTCH KU	VKEILMFSDP VKEILMFSDP VNLGLNNEKE VNLGLNDERB VNLGLNDERB VNLGLNDERB VTLYAPQPAG VTLYAPQPAG VTLYAPQPAG VTLYAPQPAG VTLYAPQPAG VTLYAPQPAG KLLVATNMG KLLV
AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip	423 760 483 820 543 880 603 940 663 1000 723 1007 722 1117 831 1177 838 1229 948	PYAKYLIETO PYAKYLIETO VTDRDDLQML EKDKSDYEDL KIKHEGILSR KIKHEGILSR ICPAEDMPPI ACPGEDMPAL LQTLLEKVKQ LQTLLEKVKQ LQTLLEKVKQ LQTLLEKVKQ LQTLLEKVKQ LQTLLEKVKQ LQTLLEKVKQ LQTLLEKVKQ ELINLEKJKQ VIDDAENADF LIEAEGNGTS NSGNFKK MGSGSGN.KK ELLNLHDDKV ESLLDPADTS PEQFACSYPY	RSVRPNTARF RSVRPYTARF MKVQOLLKDL SKANDALROF KDUNKTDASF KELSKSDGTV KRVYCENPKC EQKRLLDETK GQAAIISKTE N NKDHRHKSSA LFRKNKVLF FFKKHHEELI NLKAGLVDFS SLKKKITEVI LANMYKETFK LEFALROTLK IVAINSNFIE	DDDMMSSSLR DDDMMSSSLR MCRIDQASGA SGDIOFYLLD VGDIOFYLLD SGTVIOPEYN HITFKQMVGQ MFNVTKMSDR .QKPVHLLH FFSTPIQLVQ KYVAMFKDGF NHVSFFAEGD VDSEPLSFSF FDSEPVSISF FDSEPVSISF FDSEPVSISF FMAINRVGRM IRHIENGELV	HGIDSFISQG HGIDSFISQG AQDRIDVKUL AQDRIDVKUL NMLLFLKSKA NMLLFLKSKA ISNPKNAIFF FFHSYINTNC FFDYINK KISISQISUL RNNITQIAVL CNGKRIIMIA CNGKRIIVTA LENKICIGCK TFACFPELCF FLCVIENKIRM	VSRPCRYSLL VSRPCRYMLL KQKILFKNEY KQKILFKNEY VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF VNVKHOHTVF VNVKHOHTVF VNVKHOHTVF KNVCLSAG KUNV	VKEILMFSDP VKEILMFSDP VKEILMFSTP VNLGLNNEKE VNLGLNDERB ORPIPLPLLE ORPIPLPLLE VTLYAAQYAG VTLYAAQYAG KLLVATNMG KLLVATN
AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p	423 760 483 820 543 880 603 940 663 1000 723 1057 772 1117 831 1177 838 1229 948 1229	PYAKYLIETO PYAKYLIETO VTDRDDLQML EKDKSDYEDL KIKHEGILSR ICPAEDMPPI ICPAEDMPPI LOTLLEKVKQ LOTLLEKVKQ LOTLLEKVKQ LOTLLEKVKQ LIDAENADF LIEAEGNGTS NSGNFKK SGSGSN.KK ELLNLHDDKV ESLLDPATTS PEQFACSYPY POKFAIWYPY	RSVHENRARF RSVHENRARF KELSKSDGTV KELSKSDGTV KELSKSDGTV KRVVEENKG GQAAIISKTE N NKDHRHKSSA LFRKNKVLF FFKKHHELI NLKAGLVDFS SLKKKITEVI LANMYKETFK LEFALROTLK IVAINSNFIE ILAFDSNFIE	DDDMMSSSLR DDDMMSSSLR MCRIDQASGA SGDIOFYLLD VGDIOFYLLD SGTVIOPEYNL SGTVIOPEYN HITFKQMVGQ MFNVTKMSDR .QKPVHLLH FFSTPIQLVQ KYVAMFKDGF NHVSFFAEGD VDSEPLSFSF USSEPLSFSF SDSEPVSISF FMAIYRVGMM IRHIENGELV IRHIENGELV	HGIDSFISQG HGIDSFISQG AQDRIDVKUL AQDRHDVKLL NMLLFLKSKA ISNPKNAIFF FFDYINK KISIQISVL CNGKRIIMIA CNGKRIIVIA LENKICIGCK IFACFPELCF FLCYTEFAF RCVLGNKIRM	VSRPCRYSLL VSRPCRYMLL KQKILFKNEY KQKILFKNEY VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF AYYGTROOYO VNDVLICSAG INSVTSCDGG EEYNVILLI EEFKSIILLI HHFLHTVK.I HSSSNSIKYF KNIKILNVFF KGFQI FLNKQGKREF FVNNQGKREF LKSYAKKILY LQTSTQEILX	VKEILMFSDP VKEIMKSTDP VNLGLNNEKE VNLGLNDERB ORPIPLPLLF ORPIPLPLLF VTLYAAQYAG VTLYAAQYAG KLLVATNM
AN-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AN-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip Rom2p AM-Romip	423 760 483 820 543 880 603 940 663 1000 723 1057 772 1117 831 1177 888 1229 948 1229 948	PYAKYLIETO PYAKYLIETO VTDRDDLQML EKDKSDYEDL KIKHEGILSR KIKHEGILSR ICPAEDMPPI ICPAEDMPPI LQTLIEKVKQ LQTLIEKVKQ LQTLIEKVKQ LQTLIEKVKQ VIDDAENADF LIEAEGNGTS NSGNFKK NGSGSGN.KK ELLNLHDDKV ESLLOPATS PEQFACSYPY FOKFAIWYPY IGLLMF	RSVHENRARF RSVHENRARF KELSKSDGTV KELSKSDGTV KELSKSDGTV KRVVEENKG RKYIGDHPDC EQKRLLDETK GQAAIISKTE N NKDHRHKSSA LFRKNKKVLF FFKKHHELI NLKAGLVDFS SLKKKITEVI LANMYKETFK LEFALROTLK IVAINSNFIE ILAFDSNFIE	DDDMMSSSLR DDDMMSSSLR MCRIDQASGA SGDIOFYLLD VGDIOFYLLD SGTVIOPEYNL SGTVIOPEYN HITFKQMVGQ MFNVTKMSDR .QKPVHLLH FFSTPIQLVQ KYVAMFKDGF NHVSFFAEGD VDSEPLSFSF VDSEPLSFSF SEPVSISF SMAIYRVGNM IRHIENGELV IRHIENGELV	HGIDSFISQG HGIDSFISQG AQDRIDVKUL AQDRIDVKUL NMLLFLKSKA ISNPKNAITF FFDYINK KISISQISVL RNNITQIAVL CNGKRIIMIA CNGKRIIVIA LENKICIGCK IFACFPELCF FLCYIEFAF RCVLGNKIRM	VSRPCRYSLL VSRPCRYMLL KQKILFKNEY KQKILFKNEY VNKWHOHTVF VNKWHOHTVF VNKWHOHTVF AYYGTROOYO VNDVLICSAG UYYGAKORYO VNDVLICSAG INSVTSCHOOYO VNDVLICSAG EEYNVLILI EEFKSIILLI HHFLHTVK.I HSSSNSIKYF KNIKILNVPF KGFQI FLNKQGKREE FVNNQGWRKH LKSYAKKILY	VKEIINF3DP VKEINKSTDP VNLGLNNEKE VNLGLNDERB ORPIPLPLLF ORPIPLPLLF VTLYAAQYAG VTLYAAQYAG KLLVATNM

Fig. 3. Amino acid sequences of the ΔN -Rom1p and Rom2p. The predicted amino acid sequences of ΔN -Rom1p and Rom2p are shown in single-letter code. Two sequences are aligned optimally on the basis of residue identity indicated with an asterisk (*). The DH domains are shown in bold letters and the PH domains are underlined.

20 to 30% identity (40–60% similarity) among these proteins and Δ N-Rom1p/Rom2p, and is 72% identity (86% similarity) between Δ N-Rom1p and Rom2p. Like most of these GEPs, Δ N-Rom1p and Rom2p possess a PH domain C-terminally adjacent to the DH domain (Figures 3 and 4B), which is found in many cytoskeletal and signaling proteins (Musacchio *et al.*, 1993; Gibson *et al.*, 1994).

Disruption of both ROM1 and ROM2 results in lethality

ROM1 and *ROM2* were disrupted with *TRP1* and *HIS3*, respectively, by one-step gene replacement. The *ROM1/* $\Delta rom1::TRP1$ diploid strain, DIOY10B, and the *ROM2/* $\Delta rom2::HIS3$ diploid strain, DIOY20B, were sporulated and subjected to tetrad analysis at 24°C. With regard to

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AN-Rom1p	323	ROPATYRIET	TERREVESLE	TIRCTEMERT	LETNITERD	UPTNEUKUV
Rom2p	660	ROEATYEVYI	TEKNEVKSLE	ITROTEMET.	AFTNIISAD	TRENETRUM
Lbc	75	ROEVIYELMO	TEFHHVRTLK	IMSGVYSOGM	MA.DLL	FEOOMVERT.
Lfc	237	KODVIYELIO	TELHHVRTLK	IMTRLERTGM	LE.ELO.	MEREVVOGL
DE1	496	KNHVLNELIQ	TERVYVRELY	TVLLGYRAEM	DNPEMEDLMP	PLURNKKDIL
Cdc24p	161	YVKIIKEFVA	TERKYVHDLE	IL.DKYROOL	LDSNL	. ITSEELYML
Tiaml	1041	LRKVICELLE	TERTYVKDLN	CLMERYLKPL	QKETF	.LTQDELDVL
Vav	195	RCCCLREIQQ	TEEKYTDTLG	SIQQHEMKPL	QRF	.LKPQDMETI
		<u>F 0 e0 0</u>	F11	<u>+</u> р		+ 9 0 0
$\Delta N-Rom 1p$	371	FAYINEIYSV	NRE.FLKALA	QRQSLSPI	CP	GIADIFL.QY
Rom2p	708	FAHINDIYSV	NRR.FLKALT	DRQRSSPV	VR	GI G DIVL.RÉ
Lbc	119	PCLDELISI	HSO.FFORIL	ERKKESLVDK	SEKNFLIK	RIGDVLVNQF
LIC	281	FPCVDELSD1	HTR. FLNQLL	ERRRQALCPG	STRNFVIH	RLGDLLISQF
	240	FGNMAEIYEF	HNDIFLSSLE	NCAHAPE		RVGPCFL.ER
Caczep	1005	POLICIAIDE	OVE PLISLE	INAL. VEPS	KQ	RIGALFM.HS
Tiami	1082	FGNLIEMVEF	OVE FLATLE	DGVR.LVPD	LEKLEKVDQF	KKVLFSLGGS
vav	231	EVNILLEDESV	HIH.FLKELK	DALAGPG	A:	TLYQVFI.KY
		f	v 0.0 +	a k 1	<i>f f</i>	
AN-Romin	409	LPFFD	PLLSYIASBP	YA. KYLTETO	RSVNPNPARF	DDEVSNSST
Rom2p	746	IPEFE	PEVSYVASRP	YA. KYLIETO	RSVNPYFARE	DDDMMSSSI
Lbc	166	SGENAERLKK	TYGKFCGOHN	OSVNYFKDLY	AKDKR . FOAF	VKKKMSSSVV
Lfc	328	SGSNAEQMRK	TYSEFCSRHT	KALKLYKELY	ARDKR . FOOF	IRKMTRSAVL
Dbl	582	KDDFQ	MYAKYCQNKP	RSETIWRKYS	EC AF	FOECORKLK.
Cdc24p	242	KHF F K	LYEPWSIGQN	ANIEFLSSTL	HKMR	VDESQRFIIN
Tiaml	1132	FL.YYADR F K	LYSAFCASHT	KVP K V L VKAK	TDTAFKAF	LDAQNPRQQ.
Vav	274	KERFL	VYGRYCSQVE	SASKHLDQVA	TARED.VQMK	LEECSQRANN
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		<u>r (s()</u>	gR_+Y	lLot+eîo) š		<u>L ald +</u>
AN-Romip	452	.RHGIDSFLS	QGVS R PGR Y S	LLVREIIHFS	DPVTDKDDLQ	MILMKVQDLLK
Rom2p	/89	.RHGIDSFLS	QGVSRPGRYM	LLVKEIMKST	DPEKDKSDYE	DLSKAMDALR
LDC	215	KREGIPECIE	LVTORITKYP	VLFQRILQCT	KDNEVEQE	DLAQSLSLVK
DEI	420	UNIDIDIDIVI	KDUORITKYO	VLINRILONS	HGVEELYQ	DLASALGLVK
Cdc24p	281	NKIELOSELY	KEVORI COVD	TUNELLARG	RDCEGSA	ELENALDAML
Tiaml	1178	HSSTLESYLI	KP TOPVIEVP	LIPPIFALT	DAESEEU V	HT DVA TETMN
Vav	318	GRETIBDULM	VPMORVLEYH	LLORIVKHT	ODATEKE	NT.PLAT.DAMP
					201101010111	
		dee ed	e 🔮			
$\Delta N-Rom 1p$	501	DLMKRIDRAS	GAAQDRYDV	519		
Rom2p	838	DFMKRIDQAS	GAAQDRHDV	856		
Lbc	262	BULL OF LUC OF L	ACVERNUDI	281		
	203	DVIGAVDSKV	ROIERRYRE			
Lfc	425	ELLSNVDQDV	HELEKEARL	443		
Lfc Dbl	425 667	DUIGAVDSKV ELLSNVDQDV DLLKSVNDSM	HELEKEARL HQIAINGYI	443 685		
Lfc Dbl Cdc24p	425 667 328	DUIGAVDSKV ELLSNVDQDV DLLKSVNDSM NIARSINENQ	HELEKEARL HQIAINGYI RRTENHQVV	443 685 346		
Lfc Dbl Cdc24p Tiaml	425 667 328 1226	DUIGAUDSKU ELLSNUDQDU DLLKSUNDSM NIARSINENQ KVASHINEMQ	HELEKEARL HQIAINGYI RRTENHQVV KIHEEFGAV	443 685 346 1244		
Lfc Dbl Cdc24p Tiam1 Vav	425 667 328 1226 365	DVIGAVDSKV ELLSNVDQDV DLLKSVNDSM NIARSINENQ KVASHINEMQ DLAQCVNEVK	HELEKEARL HQIAINGYI RRTENHQVV KIHEEFGAV RDNETLRQI	443 685 346 1244 383		
Lfc Dbl Cdc24p Tiam1 Vav	425 667 328 1226 365	DVIGAVDSKV ELLSNVDQDV DLLKSVNDSM NIARSINENQ KVASHINEMQ DLAQCVNEVK	HELEKEARL HQIAINGYI RRTENHQVV KIHEEFGAV RDNETLRQI	443 685 346 1244 383		
Lfc Dbl Cdc24p Tiaml Vav	263 425 667 328 1226 365	DVIGAVDSKV ELLSNVDQDV DLLKSVNDSM NIARSINENQ KVASHINEMQ DLAQCVNEVK	HELEKEARL HQIAINGYI RRTENHQVV KIHEEFGAV RDNETLRQI	443 685 346 1244 383		
Lfc Dbl Cdc24p Tiaml Vav	263 425 667 328 1226 365	DUIGAUDSKU ELLSNVDQDV DLLKSVNDSM NIARSINENQ KVASHINEMQ DLAQCVNEVK	HELEKEARL HQIAINGYI RRTENHQVV KIHEEFGAV RDNETLRQI	443 685 346 1244 383		
Lfc Dbl Cdc24p Tiaml Vav	263 425 667 328 1226 365	DVIGAVDSKV ELLSNVDQDV DLLKSVNDSM NIARSINENQ KVASHINEMQ DLAQCVNEVK ++k +-	g l +k	443 685 346 1244 383	<u>- 69:4 -</u>	<u></u>
Lfc Dbl Cdc24p Tiaml Vav B	263 425 667 328 1226 365	DVIGAVDSKV ELLSNVDQDV DLLKSVNDSM NIARSINENQ KVASHINEMQ DLAQCVNEVK ++k +- NNEKRKIKHE	G 1 +k GLLSRKDVNK	443 685 346 1244 383	<u>- ¢¢;¢ -</u> SgDiQFY LL	<u>. 41 føk k</u> NMLI FIKSR A
Lfc Dbl Cdc24p Tianl Vav B AM-Rom1p Rom2p	263 425 667 328 1226 365 538 875	DVIGAUSKV ELLSNVDQDV DLLKSVDSM NIARSINENQ KVASHINEMQ DLAQCVNEVK ++k +- NNEKRKIKHE NDERRKIHE	G 1 +k GLLSRKEUSK	443 685 346 1244 383 TDASF SDGTV	- \$910 - SGDIQFYLLD VGDIQFYLLD	<u>- 01 fok k</u> N MLLPIRSRA N MLLPIRARA
Lfc Db1 Cdc24p Tian1 Vav B AN-Rom1p Rom2p Lbc	203 425 667 328 1226 365 538 875 306	DULIGAUSKU DLLKSVNDSM NIARSINENQ KVASHINEMQ DLAQCVNEVK *** + NNEKRKIKHE NDERRKIKHE DLKRKKLVRD	GIL+k GLLSRKDVNK GILSRKELSK GSVFLKNAA.	443 685 346 1244 383 TDASF SDGTV GR	- 0010- SGDIQFYLLD VGDIQFYLLD LKEVQAVLLT	<u>ei fok k</u> Nmllpikska Nmllpikska Dilveikosko
Lfc Dbl Cdc24p Tiaml Vav B AN-Rom1p Rom2p Lbc Lfc	203 425 667 328 1226 365 538 875 306 468 704	DULIGAUSKU DLLKSVNDQDV DLLKSVNDSM NIARSINENQ DLAQCVNEVK *** NNERKKINHE DLRKKKLINE DLRKKKLINE LGRKCAKLINE	C 1 +k C 1 +k C 1 +k C 1 +k C 1 +k C 1 SRRDVNK C 1 SRRDVNK C 1 SRRDVNK C 1 SRRDVNK C 1 SVF1 KNAA. C 2 VF1 KNAA.	443 685 346 1244 383 TDASF SDGTV GR	- 9010 - SGDIQFYILD VGDIQFYILD LKEVQAVILT FKDVLLILT FKDVLLITY	<u>el fok k</u> NMLIFIKSKA MILIFIKSKA DILVFIQEKD DVLVFIQEKD DVLVFIQEKD
Lfc Dbl Cdc24p Tianl Vav B AM-Rom1p Rom2p Lbc Lfc Dbl	203 425 667 328 1226 365 538 875 306 468 704 355	DVIGAVDSKV DLLKSVNDQDV DLLKSVNDSM NIARSINENQ DLAQCVNEVK MIRKKIKHE NDERRKIKHE DLRRKLIHE IGHKKGAIKM MWKQPDIEKE	GL1+k GLLSRKDVNK GLLSRKDVNK GSVFLKNAA. GCLLWKTAT. KDLARFK	443 685 346 1244 383 TDASF SDGTV GR	- 4010 - SGDIQFYLLD VGDIQFYLLD LKEVQAVLLT FKDVLLLLMT PMQRHLFLYE EDEFEVYE	<u>el fok k</u> NMLLFIRSRA NMLLFIRSRA DILVFLQEKD VILVFLQEKD KALVFCKRR.
Lfc Dbl Cdc24p Tiaml Vav B AN-Romlp Rom2p Lbc Lfc Dbl Cdc24p Plec	203 425 667 328 1226 365 538 875 306 468 704 355 1	DVIGAUSKU ELLSNVDQDV DLLKSVNDSM NIARSINENQ VLAGCVNEVK DLAGCVNEVK MDERRKIKHE DLKRKLVRE ELLRRKLIHE ELLRRKLIHE ELLRRKLIHE MEYKRIKE	G 1 +k G 1 +k G 1 +k G 1 st k G 1 st k St k St k St k St k St k St k St k S	443 685 346 1244 383 TDASF SDGTV GR GR GR FNT	- 4010 - SGDIQFYLLD VGDIQFYLLD LKEVQAVLLT FKDVLLLMT PMQRHLFLYE EREFEVYLFE	<u>91 fok k</u> NMLIFIKSRA NMLIFIKSRA DILVFLGERD DVLVFLGERD DVLVFLGERK KIIVICKR. KIIIFSEV.
Lfc Dbl Cdc24p Tiam1 Vav B AM-Rom1p Rom2p Lbc Lfc Dbl Cdc24p Plec	263 425 667 328 1226 365 538 875 306 468 704 355 1	DVIGAUSKU ELISNVDQDV DLIKSVNDSM NIARSINENQ KVASHINEMQ DLAQCVNEVK DLAQCVNEVK DLAQCKIKHE DLKRKKLHE IGHKKGATKM MEYKRIKE MEPKRIRE	G 1 +k GLLSRKDVNK GLLSRKDVNK GLLSRKDVNK GLLSRKLSK GVELKNAA. GCLLYRKNAA. GCLLYRKNAA. GCLLYRKVAFK.	443 685 346 1244 383 TDASF SDGTV GR GR GR	- SGDIQFYILD VGDIQFYILD LKEVQAVILT FKDVLLLIMT PMCRHLFLY EREFEVYIFE WKPMWVVILE	<u>. 01 fok k</u> NMLLPIARKA NMLLPIARKA DULVTIGERD DULVTIGERD KAIVTORR. KIIILFSEV. DGIEFYRKK.
Lfc Dbl Cdc24p Tiaml Vav B AN-Rom1p Rom2p Lbc Lfc Dbl Cdc24p Plac	203 425 667 328 1226 365 305 306 468 704 355 1	DVIGAUSKU ELISNVDQDV DLIKSVNDSM NIARSINENQ DLAQCVNEVK NDERRKIKHE DLAQCKIKHE DLKRKKIKHE DLKRKKIKHE ELIKRKKIKHE IGHKKGAIKM NWKQYRISKF MEPKRIRE	G L +k GLLSRKEUNK GLLSRKEUNK GSVFLKRAL GSVFLKNA GSVFLKNA GSVFLKNA GSVFLKNA GSVFLKNA GSVFLKNA GSVFLKNA GSVFLKNA GSVFLKNA GSVFLKNA GSVFLKNA GSVFLKNA GSVFLKNA GSVFLKNA GSVFLKNA GSVFLKA GSVFLKA GSV	443 685 346 1244 383 TDASF SDGTV GR GR FNT ISTINSSEP FNT	- SGLQ- SGDIQFYLLD VGDIQFYLLD LKEVQAVLLT FKDVLLLLMT PMQRHLFLY ERFFEVYLFE WKPMWVVLLE	01 for k NMLLFLKSRA NMLLFLKSRA DILVFLGERD DULVFLGERD KAIVFCKRR. KIILFSEV. DGIEFYKKK. ± f i
Lfc Dbl Cdc24p Tiaml Vav B AM-Rom1p Rom2p Lbc Lfc Dbl Cdc24p Plec AM-Rom1p	263 425 667 328 328 328 328 328 328 328 328 326 468 355 1 583	LLSNVDQDV DLLKSVNDQDV NIARSINENQ KVASHINEMQ DLAQCVNEVK DLAQCVNEVK NDERRKINHE DLKRKKIVRD ELLRRKIIHE IGHKKGATHE MEPKRIRE VNKWHQHIVF	C 1 +k C 1 +k CLLSREVNK CLLSREVNK CSVFLKNAA. CCLLWKIAT. CELLYFDKVF CYLVKKGSV. QR	443 685 346 1244 383 SDGTV SDG	- 0010 - SGDIQFYILD SGDIQFYILD LKEVQAVILT FKDVLLLIMT FKDVLLLMT PMRHLFLYE ERFEVVIFE WKPMWVVILE PAEDMPPI	<u>el fok k</u> NMLLFLÆSRA NMLLFLÆSRA DULVFLGERD DULVFLGERD DULVFLGERD KLIVFCRR. KIILLFSEV. DGIEFYÆKK. + <u>f i</u> KRVVTENPNC
Lfc Dbl Cdc24p Tianl Vav B AM-Rom1p Rom2p Lbc Lfc Dbl Cdc24p Plec	263 425 667 328 1226 365 306 468 704 355 1 583 920	DVIGAUSKU ELISNVDQDV DLIKSVNDSM NIARSINENQ KVASHINEMQ DLAQCVNEVK NNEKRKIKHE DLRRKIKHE DLKRKIKHE DLKRKKINE DLKRKKINK MEYKRIRE WEYKRIRE VNKWHQHIVF VNKWHQHIVF	GILSRKEDNK GILSRKEDNK GILSRKEDNK GILSRKEDNK GSVFLKNAT. KDLARFK GELLYFDKVF GYLVKKGSVQR	443 685 346 1244 383 TDASF SDGTV GR GR GR ISTINSSEP FNT PIPLPLLFIC PIPLPLLFAC	- \$910 - SGDIQFYILD VGDIQFYILD LKEVQAVLIT PMORHLETY ERFFEVYLFE WKPMWVVILE PAEDMPPI PGEDMPAL	•1 fok k NMLIPIKSKA NMLIPIKSKA DILVFIGEKD KAIVICKRR. KIILFSEV. DGIEFYKKK. ± f 1 KRVTENPNC RKVIGHPPC
Lfc Dbl Cdc24p Tim1 Vav B AM-Rom1p Rom2p Lbc Lbc Dbl Cdc24p Plec AM-Rom1p Rom2p Lbc	243 425 667 328 1226 365 306 468 704 355 1 583 920 347	DULADUSKU ELLSNDQDV DLLSVNDBM KVASHINENQ DLAQCVNEVK NNEKKKIKHE DLKRKKIKHE DLKRKKIKHE DLKRKKIKHE DLKRKKIKHE NNKGYRISKF MEPKRIRE	GLISKEVSKU GLISKEVSKU GLISKEVSK GULSKEVSK GVLIKKOVSK GULSKELSK GVLIKKOVSK GVLIKKOVSK GVLIKKOVSK GLISKELSK GVLIKKOVSK GVLISKEVSK GVLISKEVSK GVLISKEVSK GVLISKEVSK GVLISKEVSK GVLISKEVSK GVLIKKOVSK GVLIKKOVSK	443 685 346 1244 383 TDASF SDGTV SDGTGR GR GR GR ISTINSSEPT FNT PIPLPLLFIC PIPLPLFIC PIPLPLFIC	- 00:0- SEGIGFYLLD VEDIGFYLLD LKEVQAVLLT FNOVLLIMP PMERVELE PAEDMPPI PGEDMPAL	<u>ei fek k</u> NMLIFIKSRA NMLIFIKSRA DILVFIGERD DILVFIGERD DILVFIGERD KRIVICKR. KIIIESEV. DGIEFYKKK. KRYICENENC RKYIGDHEDC RKYIGDHEDC GIFILISMONT
Lfc Dbl Cdc24p Timl Vav B AM-Romlp Rom2p Libc Cdc24p Plec AM-Romlp Rom2p Libc Lfc Dbl Cdc24p Plec	203 425 667 328 1226 365 306 468 704 355 1 583 920 347 509	DILAQUSKU DLLSVNDDM NLASSINENG KVASHINENG DLAQCVNEVK NDERKKIKHE NDERKKIKHE DLKRKLIKE LLRKKLIKE IGHKKGATKM NMKGYRISK MEPKRIRE VNKHGHVF VNKHGHKVF QKYIFT	ADIANYAS HAIKARAH HOTAINGYI RATENHQYI KIHEFGAV GLISAKUVAK GLISAKUVAK GLISAKUVAK GLISAKUVAK GUSYIKNAA. GLISAKUVAK GULYAKKOSV. 	443 685 346 1244 383 S037	- 0016 - SGDIOFYLLD VGDIOFYLLD HEWOAVLLL FROUTLLIMT PMORHLFLYE EREFEYLLE PAEDMPPI POEDMPAL EVAHEK	<pre> fok k NMLIFIKSKA NMLIFIKSKA NMLIFIKSKA ULVTLOEKD ULVTLOEKD ULVTLOEKD ULVTLOEKK KIILFSV. DGIEFYKKK. f i KRVTENNC RKYLGOHPOC GLFLSMGMT GMFLSSG </pre>
Lfc Dbl Cdc24p Timl Vav B M-Romlp Rom2p Lbc Lfc Dbl Cdc24p Plec	203 425 667 328 1226 365 306 468 704 355 1 583 920 347 509 740	DULAGVOSKU ELLSNVDDU DLLSVNDDM NLASINEN ULASVNDSM NNEKKKIKHE DLAKKKIKHE DLKKKKIKHE DLKKKKIKHE DLKKKGATKM NWKGYGISKF WIKHOHTVF VNKHOHTVF VNKHOHTVF QXIFA	AJIANYAL HAIKKARI HOIAINOYI RATEMAUV KIHEFGAV RDNETLRQI GLISKEVSK GUJIKKAA GULSKELSK GUJIKKAA GELLYPBKY GILSKESS QR QR 	443 685 346 1244 383 SDGTV SDGTV SDGTV SDGTV PTPPLFLFIC PTPPLFIC STURSSEP VISUKKIVR VVSLVKKIVR VVSLVKKIVR CVMEVPEGIT	- 00:0- SGDIGFYLLD VGDIGFYLLD LEWQAVLLL PRORHLEIVE EREFEVYLFE WKPMVVLLE PAEDMPPI PAEDMPPI DIANGAK EVANBEK DIANGAK	01 for k NMLIPIKARA DILVFIQERD DULVFIQERD DULVFIQERD KIVIGER, KIVIESEV. SGIPJYEKK. t f 1 KRVVTENPRC GUFLISMGAT GMFLISSG RKVELYGEK
Lfc Dbl Cdc24p Timl Vav B ΔM-Romlp Rom2p Lbc Dbl Cdc24p Plec	203 425 667 328 1226 365 306 468 704 355 1 583 920 509 740 347 509 740	ULAYDOSKU DLLSVNDDM NLASSINEN KVASHINENQ DLAQCVNEVK NDERKKIKHE DLKKKKIVRD LLKKKKIVRD LLKKKKIVRD LLKKKKIVRD VNKHORVFY VNKHORVFY QKYIFA QKYIFA VYSSGUS VSSGUS VSSGUS	ALININAL HAIKAAL MIINAYI KINHOYI KINHEFGAV KINEFGAV GLISRKUVKK GLISRKUVKK GLISRKUVKK GLISRKUVKKGV. GLISRKUVKGV. GLIVFKVGV. QR 	443 685 336 1244 383 SOGTV SOGTV SOGTV SOGTV SOGTV ISTINSSEP ILI PIEPELEAC VISEKKIVP VISEKKIVP VISEKKIVP RIMENDALI	- 0010 SGDIOFYLLD VGDIOFYLLD LKEVQAVLLL FROULLLMT FROULLLMT PORDHELVE BREFEVYLFE WKPMWVULLE PAEDMPPI DIANGAK UIANGAK UIANGAK	<pre></pre>
Lfc Dbl Cdc24p Tiaml Vav B AM-Romlp Rom2p Lbc Dbl Cdc24p Plec AM-Romlp Rom2p Lbc Lfc Dbl Cdc24p Plec	203 425 667 328 1226 365 306 468 704 355 1 583 920 347 509 740 347 509 740 483 40	DULAGUDSKU DLLSVNDDGW NLASSINENG NLASSINENG DLAQCVNEVK DLAQCVNEVK DLAQCVNEVK DLARKIKHE DLSKRKKIVRD LLSKRKIKHE JLSKRKKUVRD LLSKRKKUVRD UKKWHOHTVF VNKWHOHTVF	ADIANYAL HAIKARI HOIAINOYI RATENHQYI KIHEFGAV RINHTLRGI GLLSRKDVNK GLLSRKDVNK GLLSRKDVNK GLLSRKDVKK GLLSRKDVKK GVLVKKGSV. OR CLLYFDKY GYLVKKGSV. OR N.SLDKST SPKFKA MAEFKLDLGG SDNSFKG	443 685 346 1244 383 SDGTV SDGTV SDGTV SDGTV PIPLPLE SDGTV SDGTV SDGTV SDGTV SDGTV VISUREV STARTAN	- delte - SGDJGFYLLD VGDJGFYLLD HKEVQAVLLL FKDVLLLIMT FKDVLLLIMT FKDVLLLIMT FKEFEYLLE RAEFEYLLE RAEFEYLLE RAEFMFV- ULANQAK EVYKGN SPCQDFGKRM	<pre></pre>
Lfc Dbl Cdc24p Tiami Vav B AM-Romip Rom2p Libc Dbl Cdc24p Plec AM-Romip Rom2p Libc Dbl Cdc24p Plec	203 425 667 328 1226 365 306 468 875 1 583 920 347 509 509 509 509 509 509 483 40	DULAGUDSKU ELLSNVDDU DLLSVVDDM NLASINEN ULASINEN DLACVNEVK NNEKKKIKHE DLKRKKIKHE DLKRKKIKHE IGHKKGATKM NNKGYRISKF MEPKRIFE VNKHOHTVF VNKHOHTVF CKYIFA QKYIFA QKYIFA	2 1 +k HGTAINNEL HGTAINNEYI RATIMHQVI KIHEFGAV RDNETLRQI GLISKELSK GVFIKNAA GGLISKELSK GVFIKNAA GGLISKELSK GVFIKNAA GGLISKELSK GVLIKKGSV QR 	443 685 336 1244 383 5057V 5057V 5057V 5057V 1571N558P 71PLP1LF1C 7157N558P 71PLP1LF1C 715NK1V9 715KK1V9 715KK1V9 715KK1V9 715KK1V9 715KK1V9 715KK1V9 715KK1V9 715KK1V9 715KK1V9 715KK1V9 715KK1V9 715KK1V9 715KK1V9 715 715 715 715 715 715 715 715 715 715	- 61.0 - SGDIQFYLLD VGDIQFYLLD LKEVQAVLLL PROBALFLY PAEDMPPI PAEDMPPAEDMPPI PAEDMPP.	<u>ei fok k</u> NMLIFILKSRA NMLIFILKSRA DILVTLOEKD DULVTLOEKD DULVTLOEKD KRIVICKR. KIILFSEV. DGIEFYKKK. KRIVTENPNC RKVIGHEDC RKVIGHEDC RKVIGHEDC GULISSG. GULISSG. RKVEINVGSK RSINITWESI FVFKITTKO
Lfc Dbl Cdc24p Timl Vav B ΔN-Romlp Rom2p Libc Lfc Dbl Cdc24p Flec ΔN-Romlp Dbl Cdc24p Flec	203 425 667 328 875 365 538 875 306 468 704 355 1 583 347 509 740 483 347 509 740 483 40 623	DILAOUSKU DLLSVNDDM NLASINEN KVASHINEN DLAQCVNEVK NDERKKIKHE NDERKKIKHE DLKRKLIKE LLRKKLIKE LLRKKLIKE LLRKKLIKE MEPKRIRE VNKHEGHKVF QKYIFT QKYIFT QKYIFT SAG.VLLEOV	ADIANYAL HOIANYAL KIRKARU CILSKENYAK	443 665 346 1244 383 SOJ SOJ SOJ GR SOJ GR SOJ GR SOJ FNT PIPEPLEFAC VISLKKLIVR VVSQKLIVR VVSQKLIVR MIPLEFAC VVSQKLIVR MIPLEFAC FARMMENDAL	- 0016 - SGD1QFYLLD VGD1QFYLLD HEVQAVLLL FROULLIMT PAEDMPP.I PAEDMPP.I PAEDMPP.I PAEDMPP.S DIANQAK EVVKGON SPCQDFOKRM OVILYAPOPA	<pre></pre>
Lfc Dbl Cdc24p Timl Vav B AM-Romlp Rom2p Dbl Cdc24p Plec AM-Romlp Rom2p Dbl Cdc24p Plec	203 425 667 328 1226 365 538 875 365 468 704 355 1 583 920 347 509 740 483 40 623 960	DULAVUSKU ELLSNVDDU DLLSVVDSM NLASINEN NNASINEN DLAQCVNEVK NNEKKIKHE DLKAKKIKHE DLKKKGATKM NMKGYTSKF VNKHHOHUF VNKHHOHUF VNKHHOHUF SAG.VLLPQY SGT.VLPPY SGT.VLPPY	ADIANYAL HAIKKARI HOIAINOYI RATENHQYI KIHEFGAV KIHEFGAV GLISRKDVNK GILSRKEISK GVTIKNAA GULYRKNAA GULYRKKAN SLIJKSS DAYFSYSFKH ANEFKAIV ANEFKAIV	443 685 336 1244 383 883 883 883 883 883 883 883 883 8	SGDIGFYLD SGDIGFYLD HEWQANLIL FROULLLMT FROULLLMT FROULLLMT PAEDMPP.I PAEDMPP.I EVAHEDK DIANGAK EVAGDN SPCDFORM QVILYAPQPA	OL FOR K NMLLFLERRA NMLLFLERRA NMLLFLERRA NMLLFLERRA KIVICKR. KIVICKR. KIVICKR. KIVICKR. KIVICKR. KIVICKR. KIVICKR. KIVICKR. KIVICKR. KIVICKR. KIVICKR. GOLLSMGMT GWTLSSG. KVFCKIVCKR. SLNIWESI FVFKITTKQ G 663 G 1000
Lfc Dbl Cdc24p Timl Vav B ΔM-Romlp Rom2p Lbc Dbl Cdc24p Plac ΔM-Romlp Rom2p Lbc Cdc24p Plac	2032 425 667 328 1226 365 538 875 306 468 704 483 355 1 583 920 347 5740 483 40 623 960 387	DILAOUSKU DLLSUNDOW DLLSUNDSM NYASINEM DLAOUNDSM NYASINEMO DLAOUNDSK NYASINEMO DLAOUNDSK NYASINEMO DLAOUNDSK NYASINEMO DLAOUNDSK NYASINE NYASIN NYASINE NYASINE NYASINE NYASINE NYASINE NYASINE NYASINE NYASINE NYASINE NYASINE NYASINE NYASINE NYASINE NYASINE NYASIN'I NYASIN'I NY ANY I NY I	ALINING HOLIKCARL HOTAINOYI RATENHQUY KIHEFGAV RDNUTLRQI GLISRKUVNK GLISRKUVNK GLISRKUVNK GLISRKUVNK GLISRKUVNK GLISRKUVNK GSVFLKNAA SUDIST GSLIYFDKV GYLVKKGSV. OR OR OR SLDYFSYSHKA ABEYKLING ASD FF GSLIYFDKV TSSFKRAT NSSFKERNAIT	443 685 336 1244 383 SOGTV SOGTV ISTINSSEP GR 		<pre>bl fok k NMLLFLKSRA NMLLFLKSRA NMLLFLKSRA NMLLFLKSRA KILLFSEV. DGIEFYKKK. + f i KRVYTENNC RKYIGDHPDC GMFLISSG GMFLISSG GMFLISSG GMFLISSG GMFLISSG G 663 G 1000 N 424</pre>
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Fig. 4. Alignments of the DH and PH domains in Δ N-Rom1p, Rom2p and other proteins. Amino acid sequences were aligned by the Edit Align program (Hirosawa et al., 1993) and further optimized by eye. Identical amino acids present in more than half of the proteins are shown in bold. Dots (.) indicate gaps introduced to maintain the alignment. A large insertion (79 amino acid residues) in the PH domain of Cdc24p has been removed and the missing number is given in blankets (<>). The amino acid residues and symbols in the consensus lines represent recurring features; upper case (identical in all proteins), lower case (identical in more than half of the proteins), +/- (cationic/anionic in more than half of the proteins) and ϕ (hydrophobic in all proteins). Lines under the consensus lines represent the conserved alignment blocks. (A) The DH domains of ΔN-Rom1p, Rom2p, Lbc, Lfc, Dbl, Cdc24p, Tiam1 and Vav are aligned. (B) The PH domains of Δ N-Rom1p, Rom2p, Lbc, Lfc, Dbl, Cdc24p and pleckstrin (Plec, the first PH domain of two PH domains) are aligned.

the disruption of ROM1, 11 complete tetrads were recovered, all of them showed a 2 Trp⁺:2 Trp⁻ segregation pattern, and all of the Trp⁺ segregants grew at 14 or 35°C as wild-type cells did, indicating that ROM1 is not an essential gene. In contrast, the disruption of ROM2 resulted



Fig. 5. Growth phenotypes of $\Delta rom 1$, $\Delta rom 2$ and $\Delta rom 1 \Delta rom 2$ mutant cells. (A) Tetrad analysis of DIOY70B (*\Deltarom1::TRP1/ROM1 ROM2/* $\Delta rom2$::HIS3). Of 23 tetrads dissected, 10 tetrads are shown. Bigger segregants showed either Trp⁻ His⁻ (ROM1 ROM2) or Trp⁺ His (*\(\Deltarom1::TRP1 ROM2*) phenotype, whereas smaller segregants showed a Trp⁻ His⁺ (ROM1 Δrom2::HIS3) phenotype. No Trp⁺ His⁻ (Δrom1::TRP1 Δrom2::HIS3) segregant was recovered. (B) Growth phenotypes of $\Delta rom1$. $\Delta rom2$ and $\Delta rom1\Delta rom2$ mutants under various growth conditions. Strains, IOS00a (ROM1 ROM2, WT), IOS10a (Δrom1::TRP1 ROM2, Δrom1), IOS20a (ROM1 Δrom2::HIS3, Δrom2) and IOY73a (Arom1::TRP1 Arom2::HIS3 pRS316-PGALI-ROM2, $\Delta rom 1 \Delta rom 2$), were streaked onto various plate media and the plates were incubated at 33°C for 2 days or at 20°C for 4 days. IOY73a carried pRS316-PGAL1-ROM2 which expressed ROM2 in the presence of galactose in the medium. Residual growth of IOY73a seems to be due to a time lag for the repression of the GAL1 promoter.

in a temperature-sensitive growth phenotype. Nine complete tetrads were recovered and all of them showed a 2 $His^+:2 His^-$ segregation pattern. All of the His⁺ segregants grew more slowly than the His⁻ segregants at 20°C and did not grow at 33°C as described below.

To characterize the phenotypes of the $\Delta rom 1 \Delta rom 2$ double disruption mutant, a haploid strain, IOY10a ($\Delta rom 1::TRP1$), was mated with a haploid strain, IOY20a ($\Delta rom 2::HIS3$), and the resultant diploid strain, DIOY70B, was sporulated and subjected to tetrad analysis at 24°C (Figure 5A). In 22 tetrads out of a total of 23 tetrads dissected, 17, one and four tetrads showed tetratype, parental ditype and non-parental ditype segregation patterns, respectively, for the His and Trp phenotypes. Genotypes of inviable spore clones could be inferred from



Fig. 6. Growth arrest of the $\Delta rom 1 \Delta rom 2$ mutant cells with a small bud. Cells of diploid strains, OHNY3 (WT) and DIOY73A ($\Delta rom 1 \Delta rom 2$), were inoculated into YPGalAU or YPDAU medium and were cultured at 30°C for 12 h. Cells were fixed and double-stained with rhodamine-phalloidin for actin and with DAPI for DNA, then were subjected to microscopic observation. All fields were photographed at the same magnification.

those of viable spore clones. No His⁺ Trp⁺ segregant was recovered, suggesting that the $\Delta rom 1 \Delta rom 2$ double mutant is inviable. To examine whether ROM1 and ROM2 are required for vegetative cell growth, a conditional $\Delta rom l$ - $\Delta rom2$ mutant was constructed. The DIOY70B strain was transformed with pRS316-P_{GAL1}-ROM2 which expresses *ROM2* under the control of the *GAL1* promoter (P_{GAL1}). The resultant transformants were grown, sporulated and dissected on YPGalAU medium. From one ascus showing a tetratype segregation pattern for the His and Trp phenotypes, four strains, IOS00a (ROM1 ROM2), IOS10a $(\Delta rom1::TRP1 ROM2)$, IOS20a (ROM1 $\Delta rom2::HIS3$) and IOY73a (*Arom1::TRP1 Arom2::HIS3* pRS316-PGAL1-ROM2), were isolated. Growth phenotypes of these four strains were examined under various growth conditions. As shown in Figure 5B, the $\Delta roml$ cells grew as wildtype cells did under all growth conditions examined, whereas the $\Delta rom2$ cells did not grow at 33°C. This temperature-sensitive growth phenotype of the $\Delta rom2$ mutant was partially suppressed in the presence of 1 M sorbitol in the medium in the same way as that of a rhol mutant was suppressed (Yamochi et al., 1994). The $\Delta rom 1 \Delta rom 2$ cells did not grow at 20 or 33°C even in the presence of 1 M sorbitol in the medium, as was the case for the $\Delta rho1$ mutant, unless ROM2 was expressed under the control of the GAL1 promoter. Thus, we con-



Fig. 7. Cell lysis phenotype of the $\Delta rom 1\Delta rom2$ mutant. Cells of strains IOS00 α (*ROM1 ROM2*), IOS10 α ($\Delta rom1::TRP1 ROM2$), IOS20 α (*ROM1 \Delta rom2::HIS3* $) and IOY73<math>\alpha$ ($\Delta rom1::TRP1 \Delta rom2::HIS3$ pRS316-P_{GAL1}-ROM2) were streaked onto a YPDAU plate. This plate was incubated at 24°C for 15 h and then incubated further at 37°C for 3 h. The patches were stained with a chromogenic substrate, BCIP, of alkaline phosphatase at 37°C for 15 h. (a) IOS00 α ; (b) IOS10 α ; (c) IOS20 α ; (d) IOY73 α .

cluded that disruption of *ROM1* and *ROM2* in combination is lethal, and that *ROM1* and *ROM2* are functionally redundant.

The $\Delta rom 1 \Delta rom 2$ mutant shows phenotypes similar to those of the $\Delta rho1$ mutant

Since ROM1 and ROM2 are multicopy suppressors of RHO1(G22S, D125N) and possess the DH domain, ROM1 and ROM2 may encode GEPs for Rholp. If this is the case, the $\Delta rom 1 \Delta rom 2$ mutant would show phenotypes similar to those of the $\Delta rhol$ mutant. We have shown recently that Rholp-depleted cells are arrested with a small bud (i.e. smaller than about one-third of its mother cell in diameter) (Yamochi et al., 1994). To investigate this point, cells of a diploid strain, DIOY73A (Δrom1::TRP1/ Δrom1::TRP1 Δrom2::HIS3/Δrom2::HIS3 pRS316-PGALI-ROM2), were grown under ROM2-expressing (YPGalAU) or non-expressing (YPDAU) conditions, along with cells of a wild-type strain OHNY3 as control. As shown in Figure 6, under the ROM2-non-expressing conditions, DIOY73A cells were arrested with a small bud or an abnormally elongated and abnormally shaped bud. Such cells accumulated from 50 to 80% in the DIOY73A culture in YPDAU medium, but not in that in YPGalAU medium or in the OHNY3 culture in either medium (>500 cells were counted for each culture). Arrested cells of DIOY73A possessed a single nucleus and, in spite of these morphological defects, showed almost normal localization of cortical actin patches and actin cables as did $\Delta rhol$ cells (Yamochi et al., 1994). IOY20 α ($\Delta rom 2$::HIS3) cells were also arrested with a small bud at 37°C (data not shown).

Another phenotype specific to the $\Delta rho1$ mutant is the cell lysis phenotype: the $\Delta rho1$ mutant cells release the intracellular materials into the medium without apparent cell lysis under the restrictive conditions (Yamochi *et al.*, 1994). This phenotype can be assayed by staining cells for alkaline phosphatase activity (which is normally localized at vacuoles in wild-type cells) released into the medium. As shown in Figure 7, cells of the $\Delta rom1\Delta rom2$ mutant or the $\Delta rom2$ mutant released alkaline phosphatase as a result of cell lysis under the restrictive conditions. These results indicate that the $\Delta rom1\Delta rom2$ mutant shows

phenotypes very similar to those of the $\Delta rhol$ mutant, suggesting that Rom1p/Rom2p and Rho1p function in the same signal transduction pathway.

The temperature sensitivity of the $\Delta rom2$ mutant is suppressed by overexpression of RHO1

If ROM1 and ROM2 function as upstream regulators of *RHO1*, it could be expected that overexpression of *RHO1* would suppress the temperature-sensitive growth phenotype of the $\Delta rom2$ mutant. A strain, IOY20 α ($\Delta rom2$:: HIS3), was transformed with a multicopy plasmid bearing ROM1, ROM2, CDC42, RHO1, RHO2, RHO3 or RHO4 or with a vector as a control. As shown in Figure 8, the temperature sensitivity of the $\Delta rom2$ mutant was suppressed by either RHO1 or RHO2 and was weakly suppressed by either RHO3 or RHO4, but not by CDC42. Since RHO2 is a non-essential homolog of RHO1, these results suggest that a major function of Rom2p is to activate Rho1p. The temperature sensitivity of the $\Delta rom2$ mutant was also suppressed by ROM1 (Figure 8), being consistent with the result that $\Delta rom1$ and $\Delta rom2$ were synthetically lethal.



Fig. 8. Suppression of the temperature-sensitive growth phenotype of the $\Delta rom2$ mutant by overexpression of *RHO1*. IOY20 α (*ROM1* $\Delta rom2::HIS3$) was transformed with YEp352-ROM1[SaII–ClaI] (ROM1), YEp352-ROM2 (ROM2), YEp24 (vector), YEp13M4-CDC42 (CDC42), YEp24-RHO1 (RHO1), YEp24-RHO2 (RHO2), pYO324-RHO3 (RHO3) and pYO324-RHO4 (RHO4). Two independent transformants obtained from each transformation were streaked onto YPDAU plates which were incubated at 20°C for 4 days or at 30°C for 2 days.

Rom2p interacts with a dominant negative form of Rho1p in the two-hybrid system

The genetic and structural analyses of ROM1 and ROM2 described above suggested that Rom1p and Rom2p are GEPs for Rho1p. This possibility was examined by the two-hybrid method (Fields and Song, 1989). A dominant active mutation in Ras, Ras(Q61L), has been shown to keep Ras in the GTP-bound form (Bourne et al., 1991), whereas two dominant negative mutations in Ras, Ras(T17N) and Ras(G15A), have been suggested to keep Ras in either the GDP-bound or nucleotide-free form (Chen et al., 1994). Of these dominant negative mutants of Ras, only Ras(G15A) has been shown to form a stable complex with Cdc25 [a GEP for Ras (Martegani et al., 1992)], even in the presence of GDP or GTP (Chen et al., 1994). Since Cdc25 has been shown to form a stable complex only with the nucleotide-free form of wild-type Ras (Lai et al., 1993), Ras(G15A) seems to be in the nucleotide-free form even in the presence of GDP or GTP. Recently, a nucleotide-free form of Cdc42 also has been shown to form a stable complex with Dbl, which is a GEP for Cdc42 (Hart et al., 1994). A mutation corresponding to G15A, Q61L or T17N was introduced into RHO1 or CDC42, and the resultant mutant alleles were fused to the LexA DNA binding domain (DBD_{1exA}) of a two-hybrid vector pBTM116. A DNA fragment encoding the fulllength Rom2p was fused with the transcriptional activating domain of Gal4p (AD_{GAL}) and the resultant plasmid was transformed into yeast strains which carried the LexA fusion genes. The activity of β -galactosidase, which is a reporter enzyme of the two-hybrid system, was measured in each transformant. As described in Table I, AD_{GAL}-ROM2 interacted with DBD_{LexA}-RHO1(G22A) to an extent similar to that of the interaction of DBD_{LexA}-Ras(G12V) with VP16-Raf, which is a target of Ras (Vojtek et al., 1993). AD_{GAL}-ROM2DH (Rom2p amino acid position from 623 to 917), which contains the Rom2p DH domain, also interacted with DBD_{LexA}-RHO1(G22A), indicating that Rom2p interacts with Rho1p through the DH domain (data not shown). However, AD_{GAL}-ROM2 did not interact with DBD_{LexA}-RHO1, DBD_{LexA}-DBD_{LexA}-RHO1(T24N), DBD_{LexA}-RHO1(Q68L), CDC42(G15A) or DBD_{LexA}-CDC42(T17N). These results suggest that Rom2p is a GEP for Rho1p, but not for Cdc42p.

The DH domain of Rom2p shows the Rho1p-specific GDP/GTP exchange activity

In the next experiment, we examined whether Rom2p actually shows the GDP/GTP exchange activity for Rho1p

Fable I. Two-hybrid interactions between Rom2p and Rho1p				
DNA binding domain fusions	Transcriptional activating domain fusions	Colony color	Units of β -galactosidase ^a	
RHO1(G22A)	ROM2	blue	67 ± 9	
RHO1(G22A)	vector	white	<1	
RHO1	ROM2	white	<1	
RHO1(068L)	ROM2	white	<1	
RHO1(T24N)	ROM2	white	<1	
CDC42(G15A)	ROM2	white	<1	
CDC42(T17N)	ROM2	white	<1	
Ras(G12V)	Raf	blue	55 ±6	

^aThe values are average \pm SE for three transformants.



Fig. 9. GEP activity of GST-Rom2DH. The GEP activity to stimulate the dissociation of $[{}^{3}H]$ GDP from or the binding of $[{}^{35}S]$ -GTP γS to the lipid-modified form of Rho1p was assayed. (A and B) Dissociation of $[{}^{3}H]$ GDP. (C and D) Binding of $[{}^{35}S]$ GTP γS . (A and C) Time course (4 pmol of GST-Rom2DH or GST); (B and D) Dose-response (5 min). (\bullet) GST-Rom2DH; (\bigcirc) GST.

in vitro. A fusion protein, GST-Rom2DH, which is glutathione-S-transferase (GST) fused with amino acid positions 623-917 of Rom2p was expressed in E.coli and purified. This GST-Rom2DH possessed the DH domain and the first one-third of the PH domain of Rom2p. As shown in Figure 9A and B, this GST-Rom2DH stimulated the dissociation of GDP from the lipid-modified form of Rholp in a dose- and time-dependent manner. Moreover, GST-Rom2DH stimulated the binding of $[^{35}S]GTP\gamma S$ to the lipid-modified form of Rho1p (Figure 9C and D). These results indicate that GST-Rom2DH is a GEP for Rho1p. The substrate specificity of GST-Rom2DH was examined further. As shown in Figure 10, GST-Rom2DH stimulated the binding of [35S]GTPyS to the lipid-modified form of RhoA as well as to the lipid-modified form of Rholp. This result is consistent with our finding that the expression of RhoA suppressed the rho1 mutation (Yamochi et al., 1994). GST-Rom2DH did not stimulate the binding of [35S]GTPYS to the lipid-modified form of Rac1, Ki-Ras or Rab3A, indicating that Rom2p is a Rholp- or RhoA-specific GEP.

We have shown previously that the post-translational lipid modifications of small GTP binding proteins are required for the GDP/GTP exchange activities of Smg GDS (Mizuno *et al.*, 1991) and Dbl (Yaku *et al.*, 1994). Consistent with these results, GST–Rom2DH did not stimulate the binding of [35 S]GTP γ S to the non-lipid-modified form of Rho1p (Figure 10). GST–Rom2DH also did not stimulate the binding of [35 S]GTP γ S to the non-lipid-modified form of Rho1p purified from *E.coli* (data not shown).

We have shown also that Rho GDI inhibits the GDP/ GTP exchange reaction stimulated by Smg GDS (Kikuchi *et al.*, 1992) or Dbl (Yaku *et al.*, 1994). Recently, we have



Fig. 10. Substrate specificity of GST–Rom2DH. The binding of $[^{35}S]$ GTP γ S to various small GTP binding proteins was assayed for 5 min in the presence of 4 pmol of GST–Rom2DH.



Fig. 11. Inhibition of the GEP activity of GST-Rom2DH by GST-Rdi1. The binding of $[^{35}S]$ GTP γ S to the lipid-modified form of Rho1p was assayed for 5 min in the presence of 4 pmol of GST-Rom2DH or GST and various amounts of GST-Rdi1. (\bullet) GST-Rom2DH; (\bigcirc) GST.

cloned a gene, *RDI1*, which is a yeast homolog of mammalian Rho GDI and have shown that the recombinant GST-Rdi1 is active on the lipid-modified form of Rho1p (Masuda *et al.*, 1994). As shown in Figure 11, GST-Rdi1 inhibited the GST-Rom2DH-stimulated binding of $[^{35}S]$ GTP γ S to the lipid-modified form of Rho1p. Therefore, the functional relationship between GEP and GDI seems to be conserved for Rho1p in *S.cerevisiae*.

Discussion

In this work we have isolated a temperature-sensitive dominant negative mutation of RHO1, RHO1(G22S, D125N), and have demonstrated that this type of mutation is valuable in the isolation of genes functionally relevant to RHO1. We have isolated six new genes (the ROM genes) as multicopy suppressors of RHO1(G22S, D125N) and have demonstrated that two of them, ROM1 and ROM2, encode Rho1p-specific GEPs. These ROM genes could not be isolated as multicopy suppressors of a temperature-sensitive recessive mutation of RHO1

(H.Nonaka *et al.*, unpublished results). Therefore, this approach is an alternative method for the isolation of genes functionally relevant to *RHO1* and may be applicable to the study of other small GTP binding proteins.

The $\Delta rom 1 \Delta rom 2$ mutant was lethal and showed phenotypes very similar to those of the $\Delta rhol$ mutant, including arrest with a small bud and cell lysis. The temperature sensitivity of the $\Delta rom2$ mutant was suppressed by overexpression of RHO1, but not of CDC42. Moreover, the full-length Rom2p interacted with a dominant negative form of Rholp, Rholp(G22A), but not with the corresponding mutant of Cdc42p, Cdc42p(G15A), in the twohybrid system. These results clearly indicate that ROM1 and ROM2 function as upstream regulators of RHO1. Consistent with the genetic results, Rom2p showed the Rholp-specific GDP/GTP exchange activity in vitro. Although we have not yet tested whether Rom1p also shows the GDP/GTP exchange activity for Rho1p, the amino acid sequence similarity and functional redundancy of ROM1 and ROM2 suggest that Rom1p also possesses the Rholp-specific GDP/GTP exchange activity.

The GDP/GTP exchange activity of Rom2p resides in the amino acid region 623-917 of Rom2p (Rom2DH), which contains the DH domain. This result is consistent with the finding that the GDP/GTP exchange activity of Dbl also resides in the DH domain-containing region (Hart et al., 1994). The PH domain is located just downstream of the DH domain in Rom2p, and GST-Rom2DH lacks the C-terminal two-thirds of the PH domain. Therefore, the PH domain is not required for the GDP/GTP exchange activity of Rom2p. The PH domains have been shown to bind to phosphatidylinositol derivatives (Harlan et al., 1994) or to the $\beta\gamma$ subunits of a heterotrimeric G protein (Touhara et al., 1994), and their functions are implicated in the translocation of the PH domain-containing proteins to the plasma or organellar membranes. Consistent with this model, it has been shown recently that the PH domain of Lfc is required for its activity in transforming NIH 3T3 cells and can be replaced functionally by an isoprenylation site which recruits this protein to the plasma membrane (Whitehead et al., 1995). Since the GTP-bound form of Rholp seems to be localized in the plasma membrane area of the growth site (Yamochi et al., 1994), it is probable that Rom2p is also localized at the same site in order to activate Rho1p. The role of the PH domain in the intracellular localization of Rom2p currently is under investigation.

The functions of the regions of Rom1p or Rom2p other than the DH and PH domains are currently unknown. The amino acid sequences of these regions do not show significant homology to any known protein. Recently, Cdc24p has been shown to bind to Bem1p, which is also involved in the bud site assembly, through the C-terminal 75 amino acid region of Cdc24p (Peterson et al., 1994). In mammalian systems, it has been reported that the N-terminal truncation is responsible for the transforming activity of the DH domain-containing proteins, including Dbl (Ron et al., 1989), Vav (Katzav et al., 1991) and Ost (Horii et al., 1994), suggesting that the N-terminal regions of these proteins negatively regulate the transforming activity of these proteins. Thus, these regions of Rom1p and Rom2p may play some role in the regulation of activity of the DH domain.

We have demonstrated recently that there is a homolog of Rho GDI, RDI1, in S.cerevisiae, suggesting that there is a functional interaction between Rdilp and Rom1p/ Rom2p. It has been demonstrated in mammalian cells that Rho GDI is complexed with Rho in the cytosol (Kuroda et al., 1992; Regazzi et al., 1992). Moreover, Rdi1p has also been found to be complexed with Rho1p in the yeast cytosol (Koch et al., in preparation). Based on these results, we have proposed that Rho complexed with Rho GDI is in the inactive state and that Rho should be released from Rho GDI for its activation (Takai et al., 1995). It is most probable that the Dbl family members are involved in the activation process of Rho. However, no Dbl family members have circumvented the inhibitory activity of Rho GDI so far. In the present study, Rom2p also did not circumvent the inhibitory activity of Rdilp. Therefore, there should be another factor which enables Rom1p/ Rom2p to interact with and activate Rho1p complexed with Rdilp. Recently, it has been reported that such a factor is present in the membrane fraction in neutrophils (Bokoch et al., 1994). Isolation of this factor would be important in order to clarify the molecular mechanisms of activation of Rholp and we are currently trying to isolate this factor genetically.

The GTP-bound form of Rsr1p/Bud1p has been shown to interact directly with Cdc24p (Zheng *et al.*, 1995a). This result is consistent with the physiological roles of these proteins: Rsr1p/Bud1p chooses the site for budding which is initiated by the activation of Cdc42p by Cdc24p. Since Rho1p seems to function after the establishment of bud site assembly that is regulated by Cdc42p, it is possible that Cdc42p might activate Rom1p/Rom2p directly or indirectly. Molecular cloning of *ROM1/ROM2* has enabled us to examine the regulatory mechanisms of Rom1p/ Rom2p in the small GTP binding protein cascade regulating the budding process in *S.cerevisiae*.

Materials and methods

Strains, media and yeast transformations

Yeast strains used in this study are listed in Table II. An *E.coli* strain DH5 α was used for construction and propagation of plasmids. Yeast strains were grown on YPDAU medium that contained 2% glucose, 2% Bacto-peptone (Difco Laboratories, Detroit, MI), 1% Bacto-yeast extract (Difco), 0.04% adenine sulfate and 0.02% uracil. YPGalAU medium contained 3% galactose, 0.2% sucrose, 2% Bacto-peptone, 1% Bacto-yeast extract, 0.04% adenine sulfate and 0.02% uracil. Yeast transformations were performed by the lithium acetate method (Gietz *et al.*, 1992). Transformants were selected on SD medium that contained 2% glucose and 0.7% yeast nitrogen base without amino acids (Difco). The SD medium was supplemented with amino acids when required. Standard yeast genetic manipulations were performed as described (Sherman *et al.*, 1986).

Plasmid construction and other molecular biological techniques

Standard molecular biological techniques were used for the construction of plasmids, PCR and DNA sequencing (Sambrook *et al.*, 1989). Plasmids used in this study are listed in Table III. PCRs were performed using GeneAmp PCR System 2400 (Perkin Elmer) and DNA sequences were determined using the ALFred DNA sequencer (Pharmacia Biotech, Inc.). Point mutations were introduced by the PCR mutagenesis methods (Higuchi, 1989).

Isolation of a temperature-sensitive dominant negative mutation, RHO1(G22S, D125N)

pRS316-RHO1(G22S) was constructed by PCR mutagenesis and was found not to give any transformant when introduced into wild-type cells.

Table II. Yeast strains used in this study

Strains	Genotype
OHNYI	MATa ura3 leu2 trp1 his3 ade2
OHNY3	MATa/MATa ura3/ura3 leu2/leu2 trp1/trp1 his3/his3 ade2/ade2
IOY10a	MATa ura3 leu2 trp1 his3 ade2 Δrom1::TRP1
ΙΟΥ20α	MATα ura3 leu2 trp1 his3 ade2 Δrom2::HIS3
IOS00a	MATa ura3 leu2 trp1 his3 ade2
IOS10a	MATα ura3 leu2 trp1 his3 ade2 Δrom1::TRP1
IOS20a	MATa ura3 leu2 trp1 his3 ade2 ∆rom2::HIS3
IOY73a	MATa ura3 leu2 trp1 his3 ade2 \Deltarom1::TRP1 \Deltarom2::HIS3 pRS316-P _{GAL1} -ROM2
DIOY10B	MATa/MATα ura3/ura3 leu2/leu2 trp1/trp1 his3/his3 ade2/ade2 ROM1/Δrom1::TRP1 ROM2/ROM2
DIOY20B	MATa/MATo. ura3/ura3 leu2/leu2 trp1/trp1 his3/his3 ade2/ade2 ROM1/ROM1 ROM2/∆rom2::HIS3
DIOY70B	MATa/MATα ura3/ura3 leu2/leu2 trp1/trp1 his3/his3 ade2/ade2 Δrom1::TRP1/ROM1 ROM2/Δrom2::HIS3
DIOY73A	MATa/MATa ura3/ura3 leu2/leu2 trp1/trp1 his3/his3 ade2/ade2 \strom1::TRP1/\strom1::TRP1 \strom2::HIS3/\strom2::HIS3 pRS316-
	P _{GALI} -ROM2
L40	MATa trp1 leu2 his3 ade2 LYS2::lexA-HIS3 URA3::lexA-lacZ

Strains used in this study are isogenic except L40.

This plasmid was mutagenized in vitro with hydroxylamine at 55°C for 24 h as described (Rose and Fink, 1987), and the resultant mutagenized DNA was used to transform a wild-type haploid strain, OHNY1. Transformants were grown on SD-ura medium at 35°C for 3 days and were replica-plated onto SD -ura medium that subsequently was incubated at 20°C for 5 days. Of 200 transformants screened, five transformants did not grow at 20°C. Plasmids were recovered from these cold-sensitive clones and were transformed back into OHNY1. All clones conferred the cold-sensitive growth phenotype on OHNY1. One of these clones was selected and the 2.2 kb SmaI-SacI RHO1(G22S) DNA fragment was cleaved out and cloned into the Smal-Sacl site of pRS315 (LEU2, CEN6). The resultant plasmid again conferred the cold-sensitive growth phenotype on OHNY1, indicating that the temperature-sensitive mutation occurred in the RHO1(G22S) gene, but not in the vector. The nucleotide sequence of the mutant RHOI(G22S) gene was determined, and one point mutation, $G \rightarrow A$, was found at nucleotide position 373 of the RHO1 ORF, consistent with the specificity of base changes by hydroxylamine mutagenesis. This mutation caused amino acid substitution from Asp to Asn at amino acid 125 of Rho1p. OHNY1 carrying the mutant plasmid, pRS316-RHO1(G22S, D125N) or pRS315-RHO1(G22S, D125N) was used for isolation of multicopy suppressors.

Determination of the nucleotide sequence of ROM1

The 3.8 kb Sall-ClaI DNA fragment containing the ROM1 gene was cloned into YEp352 to construct YEp352-ROM1[Sall-ClaI]. Nested deletions were made from both ends of the ROM1 insert as described (Henikoff, 1984) and the DNA sequence of ROM1 DNA portions of these deletion plasmids was determined. The ROM1 DNA sequence reported in this work is based on the determination of DNA sequences of both strands of the ROM1 DNA.

Disruption of ROM1 and ROM2

To disrupt ROM1, pUC19- Δ rom1::TRP1 was cut with Sal1 and EcoR1and the digested DNA was introduced into a diploid strain OHNY3. To disrupt ROM2, pUC19- Δ rom2::HIS3 was cut with *No1* and the digested DNA was introduced into a diploid strain OHNY3. The genomic DNA was isolated from each transformant and the proper disruption of ROM1or ROM2 was verified by PCR (data not shown). A diploid strain in which one ROM1 allele was disrupted was named DIOY10B, whereas a diploid strain in which one ROM2 allele was disrupted was named DIOY20B. These strains were used for subsequent experiments.

Two-hybrid method

A strain L40 was transformed with derivatives of pBTM116 bearing wild-type or mutant forms of *RHO1* or *CDC42* fused to the LexA DNA binding domain (DBD_{LexA}). In these pBTM116 fusion plasmids, the carboxy-terminal lipid modification sites (CVLL in Rho1p and CTIL in Cdc42p) were deleted. A resultant transformant was transformed with a vector pACTII-HK or pACTII-HK-ROM2 in which *ROM2* was fused with the *GAL4* transcriptional activation domain (AD_{GAD}). Western blot analysis using a monoclonal antibody raised against the hemagglutinin epitope confirmed that cells carrying pACTII-HK-ROM2 expressed a protein of ~165 kDa, the expected size of the protein of Rom2p fused with AD_{GAD}. For quantitative assay, cells of each transformant were cultured in SD-trp-leu medium and the β -galactosidase activity was

measured according to the ONPG assay method described (Guarente, 1983). For qualitative assay, cells of each transformant were placed on the nitrocellulose filter and stained with 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside for β -galactosidase activity as described (Vojtek *et al.*, 1993).

Materials and chemicals for biochemical assays

The lipid-modified and non-lipid-modified forms of Rho1p were purified from the membrane and cytosol fractions of *Spodoptera frugiperda* cells, respectively, which were infected with the baculovirus carrying the *RHO1* gene as described (Matsuura *et al.*, 1987; Mizuno *et al.*, 1991). The lipid-modified forms of RhoA, Rac1 and Ki-Ras were purified similarly from the membrane fraction of the insect cells (Mizuno *et al.*, 1991; Ando *et al.*, 1992). The lipid-modified form of Rab3A was purified from bovine brain membranes as described (Kikuchi *et al.*, 1988). Recombinant yeast Rho GDI (Rdi1p) and Rom2DH were purified from overexpressing *E.coli* DH5\alpha as GST fusion proteins using a glutathione– Sepharose 4B column (Pharmacia P-L Biochemicals Inc.) as described (Kikuchi *et al.*, 1992). The BA-85 (0.45 µm pore size) nitrocellulose filter was purchased from Schleicher & Schuell.

Assay for the GEP activity of Rom2p

The GEP activity of GST-Rom2DH was assayed by measuring the dissociation of [³H]GDP from or the binding of [³⁵S]GTP₇S to each small GTP binding protein (3 pmol). The dissociation of [³H]GDP or the binding of [³⁵S]GTP₇S was assayed at 25°C by the filtration method using nitrocellulose filters by measuring the radioactivity of [³H]GDP or [³⁵S]GTP₇S bound to each small GTP binding protein after incubation with GST-Rom2DH in a 100 µl reaction mixture as described (Yamamoto *et al.*, 1990).

Cytological techniques

Actin and DNA were stained with rhodamine-phalloidin (Molecular Probes, Inc., Eugene, OR) and 4',6'-diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma Chemical Co.), respectively, as described (Yamochi *et al.*, 1994). Stained cells were observed and photographed on Neopan Super Presto film (Fuji Photo film, Tokyo, Japan) using a Zeiss Axiophoto microscope (Carl Zeiss, Oberkochen, Germany). Cell lysis was examined by assaying the alkaline phosphatase activity using a chromogenic substrate 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) (Sigma Chemical Co., St Louis, MO) as described (Paravicini *et al.*, 1992).

Accession numbers

The DDBJ/EMBL/GenBank accession number for the sequence reported in this paper is D83171.

Acknowledgements

We thank Hideyuki Ogawa and Yoshikazu Ohya for providing yeast genomic libraries constructed in YEp24 and YEp13, respectively. We also thank Yasushi Matsui for providing pYO324-RHO3 and pYO324-RHO4. The two-hybrid vector plasmid, pACTII, was kindly provided by Stephen J.Elledge. This investigation was supported by grants-in-aid

Table III. Plasmids used in this study

Characteristics
LEU2. CEN6 (Sikorski and Hieter, 1989)
URA3, CEN6 (Sikorski and Hieter, 1989)
<i>RHO1</i> , <i>URA3</i> , CEN6; made by inserting the 2.1 kb <i>Bsu361–Bg/II RHO1</i> genomic DNA fragment containing the 1.1 kb upstream and 414 bp downstream regions into pRS316
<i>RHO1</i> (G22S), <i>URA3</i> , CEN6; a derivative of pRS316-RHO1 in which the codon for amino acid position 22 of Rho1p, GGT (Gly), was mutated to TCA (Ser) by PCR mutagenesis
<i>RHO1</i> (G22S, D125N), <i>URA3</i> , CEN6; a derivative of pRS316-RHO1(G22S) in which the codon for amino acid position 125 of Rho1p, GAT (Asp), was mutated to AAT (Asn) by hydroxylamine
<i>RHO1</i> (G22S, D125N). <i>LEU2</i> , CEN6; made by inserting the 2.2 kb <i>SmaI-Sac1 RHO1</i> (G22S, D125N) DNA fragment from pRS316-RHO1(G22S, D125N) into the <i>SmaI-Sac1</i> site of pRS315
$URA3, 2 \mu m$ (Hill <i>et al.</i> , 1986)
RHO1, URA3, 2 µm; isolated from a genomic library in YEp24 (Carlson and Botstein, 1982)
RHO2, URA3, 2 µm; isolated from a genomic library in YEp24 (Carlson and Botstein, 1982)
ΔN - <i>ROM1</i> , <i>URA3</i> , 2 µm; isolated from a genomic library in YEp24 (a gift of H.Ogawa) as a clone containing the 12 kb genomic DNA fragment of <i>ROM1</i>
ΔN -ROMI, URA3, 2 µm; made by deleting the 0.9 kb KpnI fragment from YEp24-ROMI
URA3, 2 μ m; made by deleting the 4.0 kb Bg/II fragment from YEp24-ROM1
ΔN- <i>ROM1</i> , URA3, 2 μm; made by inserting the 7.5 kb SalI–PvuII fragment from YEp24-ROM1 into the SalI–SmaI site of YEp352
URA3, 2 µm; made by deleting the 3.8 kb Clal-Sall fragment from YEp352-ROMI[Sall-PvuII]
ΔN- <i>ROM1</i> . URA3. 2 μm; made by deleting the 3.6 kb <i>ClaI-KpnI</i> (<i>KpnI</i> site in the multicloning site of YEp352) fragment from Yep352-ROM1[SaII-PvuII]
ROM2, URA3, 2 µm; made by inserting the BamHI-Sall 5.2 kb ROM2 PCR fragment containing 670 bp
upstream and 450 bp downstream regions into the <i>Bam</i> HI- <i>Sal</i> I site of YEp352. The 5.2 kb <i>ROM2</i> DNA was amplified by PCR using an upstream primer 5'-GGCGGATCC <u>GATGAGCACTCTGTCGGCGGG</u> and a downstream primer 5'-GGCGTCGAC <u>ATGCGGAAAATACACAGTACC</u> . The underlined sequences
are pointons of the $ROM2$ ORF $P_{abc} = ROM2$ ($RO3$ CEN6; mode by inserting the 4.1 kb RawHI Sural DNA fragment containing the
<i>ROM2</i> ORF from pACTII-HK-ROM2 into the <i>Bam</i> HI- <i>Sma</i> I site of pRS316-P _{GAL1} to place <i>ROM2</i> downtream of the promote from pact of <i>CAL1</i> .
CDC42 <i>LEU</i> 2 um isolated from a genomic library in YEn13M4 (Yamochi <i>et al.</i> unpublished)
RHO3 TRP1 2 µm; made by inserting the 1.8 k RHO3 fragment into the Knel-Xhol site of pYO324
RHO4 TRP1 2 µm; made by inserting the 2.0 kb RHO4 fragment into the Korl site of pYO324
$DBD_{1} \rightarrow TRP / 2$ µm; (Bartel et al., 1993)
BBD_{Level} - BHQ1, $TRP(-2 \ \mu m; (Nonaka et al., 1995)$
DBDL $_{av}$ -RHO1(O68L), TRP1, 2 µm: (Nonaka et al., 1995)
DBD _{LexA} -RHO1(G22A), <i>TRP1</i> , 2 μ m; made by inserting <i>RHO1</i> (G22A) into pBTM116 as described (Nonaka <i>et al.</i> , 1995)
DBD_{Lova} -RHO((T24N), TRP1, 2 µm; (Nonaka et al., 1995)
DBD _{LevA} -CDC42, <i>TRP1</i> , 2 μ m; made by inserting the 580 bp <i>Eco</i> RI- <i>Pst</i> I PCR fragment containing the <i>CDC42</i> ORF into the <i>Eco</i> RI- <i>Pst</i> I site of pBTM116
DBD _{LexA} -CDC42(G15A). <i>TRP1</i> , 2 µm; a derivative of pBTM116-CDC42. A codon for amino acid position 15 of Cdc42p, GGG (Gly) was mutated to GCG (Ala) by PCR mutagenesis
DBD _{LevA} -CDC42(T17N). <i>TRP1</i> , 2 μ m; a derivative of pBTM116-CDC42. A codon for amino acid position 17 of Cdc42n ACG (Thr) was mutated to AAC (Asn) by PCR mutagenesis
AD_{GAD} , LEU2, 2 µm; a derivative of pACTII. The sequence of the multicloning site of pACTII. 5'-
CATATOGCCATGG <u>AGGCCCCCGGGGATCCGAATCGAAGC</u> TCGAGAGATCT-3' was changed to 5'-
recults) The changed courses are underlined
$\Delta D_{0,m}$, ROM2 <i>FI</i> /2 2 µm; made by inserting the 4.1 kb <i>Ram</i> HI-Smal PCR fragment containing the
ROM2 ORF into the BamHI-Smal site of pACTIL-HK. The 4.1 kb ROM2 DNA was amplified by PCR
using an upstream primer 5'-GCGGGATCCATGAGCGAAACCAACGTTG-ACAGC and a downstream
primer 5'-GCGCCCGGGTTAACCCCAGAAATCTAACGACGC. The underlined sequences are portions
of the <i>ROM</i> 2 ORF
GST-ROM2DH; made by inserting the 884 bp HaeIII-HaeIII DNA fragment of ROM2 containing the
DH domain (amino acid positions 623–917 of Rom2p) into the Smal site of pGEX2T
ΔN-ROM1: made by inserting the 3.8 kb Sall-EcoRI DNA fragment from YEp352-ROM1[Sall-Cla1]
encoding the ΔN -Rom1p into the Sal1–EcoR1 site of pUC19
a derivative of pUC19-ROM1; made by replacing the 2.1 kb $AfII-Bg/II$ internal fragment of $ROM1$ corresponding to amino acid positions 287–996 of ΔN -Rom1p with the 0.8 kb $TRP1$ fragment
made by inserting a (NotI-EcoRV-NotI) linker into the PvuII-PvuII site of pUC19
made by inserting the 2.4 kb XhoI(filled in)-BamHI(filled in) PCR fragment corresponding to amino acid
positions 358-1175 of Rom2p into the EcoRV site of pUC19-(NotI-EcoRV-NotI). The 2.4 kb ROM2
DNA was amplified by PCR using an upstream primer 5'-
GGCCTCGAGGGGAAGCTCGTCTAATATTGGGTTGG and a downstream primer 5'-
GGCGGATCC <u>CGGCCAATAATGGATGTTCATGCTCG</u> . The underlined sequences are portions of the
KUM2 UKF
a derivative of pUC19-ROM2; made by replacing the 0.8 kb <i>LcoRV</i> – <i>LcoRV</i> internal fragment of <i>ROM2</i> corresponding to amino acid positions 713–988 of Rom2p with the 1.8 kb <i>HIS3</i> fragment

for Scientific Research and for Cancer Research from the Ministry of Education, Science, Sports and Culture, Japan (1995), by grants-in-aid for Abnormalities in Hormone Receptor Mechanisms and for Aging and Health from the Ministry of Health and Welfare, Japan (1995) and by a grant from the Uehara Memorial Foundation (1995).

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Received on November 23, 1995; revised on January 5, 1996