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Gene-protein assignments within the yeast *Yarrowia lipolytica* dsRNA viral genome

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Summary. Some strains of the yeast *Yarrowia lipolytica* possess virus-like particles (VLPs) which encapsidate a double-stranded RNA (dsRNA) genome designated L_y. We report here that these VLPs have two associated polypeptides of molecular weights 83 kd (VL_y-P1) and 77 kd (VL_y-P2). Denatured L_y-dsRNA was used to program a cell-free rabbit reticulocyte translation system, resulting in the appearance of four major products, viz. L_y-P1 (83 kd); L_y-P2 (77 kd); L_y-P3 (74 kd) and L_y-P4 (68 kd). The in vivo viral-associated protein VL_y-P1 co-migrated on SDS-polyacrylamide gels with the in vitro product L_y-P1 and, similarly, VL_y-P2 co-migrated with L_y-P2. Peptide mapping data confirm the identity of the in vivo products (VL_y-P1 and VL_y-P2) and their in vitro counterparts. The conclusion made is that VL_y-P1 and VL_y-P2 are almost identical primary translation products of the L_y genome, derived from a single or multiple species of L_y-dsRNA. RNA blot hybridizations using L_{1A} M_1 and separately, L_{2A} M_2 probes prepared from appropriate K1 and K2 *Saccharomyces cerevisiae* killer strains, failed to show any detectable homology to L_y-dsRNA, substantiating the uniqueness of the L_y genome with respect to the K1 and K2 *S. cerevisiae* dsRNA killer systems.

Key words: Double-stranded RNA (dsRNA) – *Yarrowia lipolytica* – *Saccharomyces cerevisiae* – Virus-like particles

Introduction

Killer phenotypes in fungi have been identified not only in strains of *S. cerevisiae* (Makower and Bevan 1963), but also in a wide variety of other genera, including *Ustilago, Torulopsis, Debaromyces, Hansenula, Kluyveromyces, Candida, Pichia,* and *Cryptococcus* (Puhalla 1968; Hankin and Puhalla 1971; Maule and Thomas 1973; Bussey and Skipper 1975; Philliskrik and Young 1975; Stumm et al. 1977; Kandel and Stern 1979). In *S. cerevisiae,* killer strains may have one of three killer (K^+) and resistance (R^+) specificities, $K_1^+R_1^+$, $K_2^+R_2^+$ or $K_3^+R_3^+$ (see Table 1 for nomenclature). Type K1 Killers were originally recognized in laboratory strains of *S. cerevisiae* (Makower and Bevan 1963; Bevan et al. 1973). Type K2 Killers were subsequently found among wine yeasts (Naumov and Naumova 1973) as contaminants of a two-stage continuous beer fermentation (Maule and Thomas 1973, Rogers and Bevan 1978) and in the National Collection of Yeast Cultures (Young and Yagiu 1978). Type K3 Killers are defined by a sole strain of *S. capensis,* 761 (Young and Yagiu 1978), which is now classified as *S. cerevisiae* 761.

Among pathogenic yeasts, K1 Killers were first observed in *Candida* by Mitchell (1974) and K2 Killers were seen in *Torulopsis* by Bussey and Skipper (1975). Middelbeek et al. (1980) reported sensitivity among 116 of 142 strains of *Candida* and *Torulopsis,* especially to toxins produced by strains of *Hansenula* and *Pichia kluyveri.*

Recently, strains of the dimorphic, hydrocarbon-utilizing yeast *Yarrowia lipolytica* have been shown to possess VLPs with a species of dsRNA of the same approximate size as L-dsRNA genomes of *S. cerevisiae* (Jewers et al. 1982, 1983; Groves et al. 1983; Treton et al. 1985). It has been suggested that *Y. lipolytica* also produce a killer toxin (Groves et al. 1983), although this has not yet been confirmed.

In this paper we present a characterization of the in vivo and in vitro translation products of L-dsRNA isolated from *Y. lipolytica* (L_y-dsRNA), and a comparison

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of dsRNA and protein homologies between L_y-dsRNA and different species of L-dsRNA from *S. cerevisiae* (L_{1A}, L_{1BC} and L_2). We demonstrate that L_y-dsRNA of *Y. lipolytica* encodes its own capsid polypeptide. The major in vivo VLP protein (VL_y-P1), is identical by peptide mapping to the major in vitro translation product (L_y-P1) of L_y-dsRNA, and essentially identical to a second, less abundant virus-associated protein VL_y-P2. VL_y-P1 and VL_y-P2 are distinct from the capsid polypeptides produced by either L_{1A}- or L_{1BC}-dsRNA of *S. cerevisiae*. Furthermore, L_y-dsRNA is shown to share very little or no sequence homology with either L_{1A}, L_{1BC} or L_2-dsRNAs, thus establishing that *Y. lipolytica* VLP's constitute a distinctly different dsRNA viral system than the K1 or K2 killer systems in *S. cerevisiae*.

Materials and methods

Yeast strains and media. The yeast strains used in this work, their genotypes, dsRNA content, and sources are listed in Table 1. Cells were grown in yeast extract bacto-peptone (YEPD) medium (Rogers and Bevan 1978).

Preparation and analysis of VLPs. Virus particles were prepared, purified and analyzed on SDS-polyacrylamide gels as described in EL-Sherbeini et al. (1984).

Preparation, fractionation and analysis of RNAs. Total cellular nucleic acids were prepared from *Y. lipolytica* and *S. cerevisiae* by standard H_2O-saturated phenol extraction (El-Sherbeini et al. 1984). Single stranded RNA (ssRNA) was eliminated from the total nucleic acid preparations by lithium chloride precipitation (Barlow et al. 1963) and dsRNA isolated from the LiCl soluble fraction by chromatography on Whatman CF11 cellulose (Franklin 1966). The double-strandedness of the purified dsRNAs was ascertained on the basis of their sensitivity to RNAse III but not to pancreatic RNAse at elevated salt concentration (Bevan et al. 1973; Vodkin and Fink 1973). Treated and untreated RNAs were electrophoresed in 1% agarose gels and visualized by staining with ethidium bromide. RNAs were further analyzed by nitrocellulose blot hybridization of samples fractionated by formaldehyde gel electrophoresis (Thomas 1980; Bostian et al. 1983b). dsRNAs were denatured by treatment for 5 min at 65 °C in 50% formamide – 2.2 M formaldehyde – 20 mM morpholine propane sulfonic acid (MOPS), pH 7.5, before fractionation by formaldehyde gel electrophoresis (Bostian et al. 1983b). To prepare radiolabelled hybridization probes, purified dsRNAs were hydrolyzed at 95 °C for 90 min in 0.05 Tris, pH 9.5 – 1 mM EDTA and then labelled with T4 polynucleotide kinase and γ-^{32}P-ATP as previously described (El-Sherbeini et al. 1984).

Protein characterization. Peptide maps were obtained by limited proteolysis with *Staphylococcus aureus* V8 protease using a modification (El-Sherbeini et al. 1984) of the method of Cleveland et al. (1977). In this instance, protein bands were localized in preparative gels without Coomassie staining, by use of marker proteins fluorometrically labeled with fluorescamine (4-phenylspiro[furan-2(3H), 1'-phthalon]-3-3'-dione. Labeling conditions are described in Vondekerckhove and Van Montagu (1973). The standard proteins used migrated at 92 kd (phosphorylase A) and 67 kd (bovine serum albumin), a range bracketing the size of all the VLP proteins of interest.

Bands of purified VLP proteins and translation products of denatured dsRNAs were excised from 10% SDS polyacrylamide gels, the gel pieces placed in 4 cm wide sample wells of a second SDS-polyacrylamide gel, the protein stacked into the spacer gel, and hydrolyzed for 30 min before continuing electrophoresis.

Cell-free protein synthesis. Total cellular RNAs and dsRNAs (native and denatured) were translated in a reticulocyte cell-free translation system (Pelham and Jackson 1976) with ^{35}S-methionine to label the products as described in previously published procedures (Bostian et al. 1984). Double-stranded RNAs were prepared for translation by heating to 100 °C for 1 min in sealed capillary tubes, and then rapidly freezing on dry ice. Samples were thawed individually and immediately added to otherwise complete translation reaction mixtures. Translation products were analyzed by electrophoresis and autoradiography as in Bostian et al. (1983a).

Table 1. Strains

Designation	Genotype and phenotype	dsRNA	Source
S. cerevisiae			
K7.S1	a *arg*9 (S)[a]	L_{1A}	Authors
K7	a *arg*9 (K)[b]	$L_{1A}M_1$	Authors
MES7.11A	*spo*11 *ura*3 *ade*1 *his*1 *leu*2 *lys*7 *met*3 *trp*5 (S)	L_{1BC}	Authors
K12-1	a *ade2-5*	$L_{1A}L_{1BC}M_1$	Authors
K23.A	a *ade2* (K)	$L_{1A}L_{1BC}M_1$	Authors
K396-11A	a *spo*11 *ura*3 *ade*1 *his*1 *lys*7 *met*3 *trp*5 (S)	$L_{1A}L_{1BC}$	S. Esposito
Y110	wt (K)	$L_{2A}L_{2B}M_2$	Authors
482	wt (N)[c]	$L_{2A}L_{2B}M_2$	Authors
Y. lipolytica			
CBS 6124.2	wt	L_y	Central Bureau vor Schimmelculture, Delft, The Netherlands

[a] S, Sensitive; [b] K, Killer; [c] N, Neutral