



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Saccharomyces cerevisiae: A useful model host to study fundamental biology of viral replication

Isabel Alves-Rodrigues^a, Rui Pedro Galão^a, Andreas Meyerhans^b, Juana Díez^{a,*}

^a Department of Experimental and Health Sciences, Universitat Pompeu Fabra, 08003 Barcelona, Spain

^b Department of Virology, University of the Saarland, Homburg, Germany

Received 19 August 2005; received in revised form 16 November 2005; accepted 21 November 2005

Available online 15 May 2006

Abstract

Understanding the fundamental steps of virus life cycles including virus–host interactions is essential for the design of effective antiviral strategies. Such understanding has been deferred by the complexity of higher eukaryotic host organisms. To circumvent experimental difficulties associated with this, systems were developed to replicate viruses in the yeast *Saccharomyces cerevisiae*. The systems include viruses with RNA and DNA genomes that infect plants, animals and humans. By using the powerful methodologies available for yeast genetic analysis, fundamental processes occurring during virus replication have been brought to light. Here, we review the different viruses able to direct replication and gene expression in yeast and discuss their main contributions in the understanding of virus biology.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Bromovirus; Tombusvirus; Nodavirus; Papillomavirus; Viral replication; Yeast

1. Introduction

Viruses continue to threaten humans and husbandry. Well known examples are the chronic viral infections caused by HIV, *Hepatitis B* and *Hepatitis C viruses*, and the emergence of novel viral agents like the *severe acute respiratory syndrome coronavirus (SARS)*. This, together with the insufficient therapy options of today, has increased markedly the demand for new antiviral strategies. In this respect, a detailed understanding of the biology of a pathogenic virus including its interactions with host proteins at a molecular level is most helpful (Magden et al., 2005). As the cell biology and genetics of higher eukaryotes are highly complex, researchers have turned to the use of yeast as a simpler system to propagate viruses.

The yeast *Saccharomyces cerevisiae* is a simple eukaryotic organism with just approximately 6000 genes. The complete sequence is known since 1996. More than 60% of the genes have an assigned function, while more than 40% share conserved sequences with at least one known or predicted human gene (Lander et al., 2001; Venter et al., 2001; www.yeastgenome.org).

Due to the high conservation of fundamental biochemical pathways, yeast has been used as a model to unravel biological processes in higher eukaryotes (Mager and Winderickx, 2005). The studies of mRNA translation and mRNA degradation are just two examples to name (Coller and Parker, 2004; Donahue, 2000; Schwartz and Parker, 2000). These were possible because yeast is easy to grow in culture and to manipulate genetically. An additional experimentally valuable tool is a yeast library in which each non-essential gene is deleted. The collection is commercially available and covers around 85% of all yeast genes (Winzeler et al., 1999). It was used for multiple studies including genome-wide screenings for human disease genes and host factors that support virus replication (Kushner et al., 2003; Panavas et al., 2005b; Steinmetz et al., 2002).

In virus research, *S. cerevisiae* is a very fruitful organism. From the view of public health, the use of yeast to produce vaccines is notable. For example, the recombinantly expressed hepatitis B surface antigen has become a safe and efficient prophylactic vaccine worldwide (Valenzuela et al., 1982). In addition, yeast has been used for drug discovery including the cellular pathways involved (Hughes, 2002; Lum et al., 2004). In basic research, yeast has assisted the elucidation of the function of individual proteins from important pathogenic viruses such as HIV, *Hepatitis C virus*, and *Epstein-Barr virus* (Blanco et al.,

* Corresponding author. Tel.: +34 93 542 2887; fax: +34 93 542 2802.
E-mail address: juana.diez@upf.edu (J. Díez).

2003; DeMarini et al., 2003; Kapoor et al., 2005). Furthermore, studies of yeast viruses have made important contribution to the dissection of the life cycle of RNA viruses and the host factors involved (Wickner, 2001).

The first higher eukaryotic virus reported to replicate in yeast was *Brome mosaic virus*, a positive-strand RNA (+)RNA virus that infects plants (Janda and Ahlquist, 1993). Since then, a growing list of viruses have been reported to undergo replication in yeast. These include RNA and DNA viruses that infect plants, insects, mammals and humans (Table 1) (Angeletti et al., 2002; Panavas and Nagy, 2003; Pantaleo et al., 2003; Price et al., 1996, 2005; Raghavan et al., 2004; Zhao and Frazer, 2002a). This wide range of viruses emphasizes the general applicability of the yeast system. In this review, we will describe each of the yeast/virus systems developed and point out their main findings and contributions.

2. Replication of viruses with RNA genomes in yeast

All RNA viruses that have been described to replicate in yeast have (+)RNA genomes. These include three plant viruses, *Brome mosaic virus* (BMV), *Carnation Italian ringspot virus* (CIRV) and *Tomato bushy stunt virus* (TBSV), and two animal viruses, *Flock House virus* (FHV) and *Nodamura virus* (NoV) (Table 1). The (+)RNA group of viruses encompasses over one third of all virus genera and include important human pathogens such as the *Hepatitis C virus* and the *severe acute respiratory syndrome coronavirus* (SARS). All (+)RNA viruses share fundamental features in their replication process, (i) the genomic RNA serves as mRNA and as template for replication, (ii) replication complexes are associated with intracellular membranes, and (iii) host factors are required for viral replication (Ahlquist et al., 2003; Andino et al., 1999; Salonen et al., 2005). Details of these features have been clarified from studies in yeast, i.e. how the template is selected for replication and how the replication complex is formed in association with cellular membranes. Furthermore, the use of traditional yeast genetics and genome-wide screening approaches have resulted in the groundbreaking

Table 1
Viruses that replicate in yeast

Family	Virus	Genome	Natural host
RNA viruses			
Bromoviridae	<i>Brome mosaic virus</i>	(+)RNA	Plants
Tombusviridae	<i>Carnation Italian ringspot virus</i>	(+)RNA	Plants
	<i>Tomato bushy stunt virus</i>	(+)RNA	Plants
Nodaviridae	<i>Flock House virus</i>	(+)RNA	Animals
	<i>Nodamura virus</i>	(+)RNA	Animals
DNA viruses			
Papillomaviridae	<i>Human papillomavirus</i>	dsDNA circular	Humans
	<i>Bovine papillomavirus</i>	dsDNA circular	Animals
Geminiviridae	<i>Mung bean yellow mosaic India virus</i>	ssDNA circular	Plants

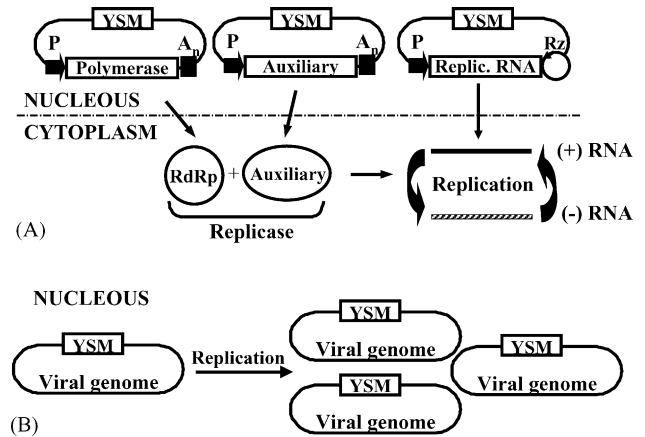


Fig. 1. (A) Schematic illustration of common features of positive-strand RNA virus replication in yeast. The viral RNA-dependent RNA polymerase (RdRp) and the auxiliary protein (if required) are expressed from mRNA transcripts derived from two yeast plasmids by using inducible *GAL1* or constitutive *ADH1* yeast promoters. The expression of the two mRNA transcripts will generate the viral replicase. The replicative RNA is normally transcribed from a plasmid by using the inducible *GAL1* promoter and contains a ribozyme sequence to generate authentic 3' ends. In some of the systems, the replicative RNA can also include a reporter gene, which expression is dependent on viral RNA replication. All yeast plasmids carry a selectable marker to allow stable expression. (B) Schematic illustration of common features of DNA virus replication in yeast. The full length circular DNA genomes are linked in cis to a selectable yeast marker gene. Alternatively, for BPV-1, the incubation of yeast protoplast with virions is enough to obtain viral replication and production of infectious BPV-1 particles. P, yeasts promoter; A_n, poly(A) signal; Rz, self-cleaving ribozyme; YSM, yeast selectable marker.

identification of multiple host factors required for viral RNA replication and recombination. These studies represent a big step forward in the understanding of virus–host interactions and could provide new targets for antiviral drug development.

Experimentally, the (+)RNA virus systems in yeast have some common characteristics (Fig. 1A). The viral RNA-dependent RNA polymerase (RdRp) as well as an auxiliary viral replication protein, if required, are expressed from yeast plasmids by using yeast promoters. Next, a replication-competent RNA is introduced into yeast cells either by transfection or by in vivo transcription from a yeast plasmid. In both cases it is necessary that the 5' and 3' ends of the RNA are authentic because they harbour important replication signals. This can be achieved by designing within the yeast plasmid an appropriate transcription start and by using a ribozyme sequence to generate the exact 3' end. Replication is then measured by detecting replication intermediates or by the expression of a reporter gene, which depends on viral RNA replication. It is important to note that in all cases the viral replicases are able to function in trans on the replicative RNAs. The yeast systems developed reproduce the known features of virus replication in their corresponding natural hosts. Below each system is presented in detail.

2.1. *Brome mosaic virus*

BMV was the first virus described to efficiently replicate and encapsidate its RNA genome in *S. cerevisiae* (Janda and Ahlquist, 1993; Krol et al., 1999). BMV is a well-studied mem-

ber of the alphavirus-like superfamily that includes animal and plant (+)strand RNA viruses. The genome of BMV consists of three 5' capped RNAs which have a tRNA-like structure at their 3' ends (Ahlquist, 1992). RNA1 and RNA2 encode the essential RNA replication factors 1a and 2a, respectively. The 1a protein contains a C-terminal helicase-like domain and an N-terminal capping domain required for viral RNA capping in vivo (Ahola and Ahlquist, 1999; Garriga et al., 2004). 2a is the viral polymerase and contains a central RdRp domain. RNA3 encodes a cell-to-cell movement protein that enables the spread of infection between cells in the natural host. In addition, it encodes the capsid protein through a subgenomic RNA that is generated from an internal promoter in the negative-strand RNA3 replication intermediate.

The replication of BMV in yeast has been analyzed by following RNA3 or RNA3 derivatives. Yeast expressing 1a and 2a replicase proteins are sufficient to support RNA 3 replication and subgenomic RNA synthesis (Janda and Ahlquist, 1993). RNA3 has been introduced into yeast (i) by transfection of in vitro transcripts, (ii) by in vivo transcription from a yeast plasmid, or (iii) by in vivo transcription from a cDNA-based RNA3 launching cassette integrated into a yeast chromosome (Ishikawa et al., 1997a,b; Janda and Ahlquist, 1993). Most valuable for screening purposes has been the construction of RNA3 derivatives in which the coat gene was substituted with a reporter gene. Because its expression requires 1a-, 2a-directed negative-strand RNA3 and subgenomic mRNA synthesis, the reporter gene provides a screenable marker for viral replication (Ishikawa et al., 1997a,b; Kushner et al., 2003).

Important aspects of the process of template selection and replication complex formation have been discovered through the study of BMV replication in yeast. Biochemical analysis and microscopy studies have shown that 1a induces the formation of vesicles in the membranes of the endoplasmic reticulum (Schwartz et al., 2002). 1a protein specifically recruits both the BMV RNA and the 2a polymerase to these vesicles that serve as compartments for RNA replication (Chen et al., 2001; Chen and Ahlquist, 2000; Janda and Ahlquist, 1998). Interestingly their assembly, structure and function have similarities to the replicative cores of retroviruses and virions of double strand-RNA viruses (Schwartz et al., 2002). The domains of 1a and 2a, and the BMV RNA cis-signals involved in these processes have been thoroughly characterized (Ahola et al., 2000; Baumstark and Ahlquist, 2001; Chen et al., 2001; Chen and Ahlquist, 2000; den Boon et al., 2001; Sullivan and Ahlquist, 1999). Together, the obtained results explain many features of the replication of (+)RNA genomes and could help to shed light in parallel processes of other virus groups.

Traditional yeast mutagenic analysis has been used to identify and characterize host genes that function in controlling BMV translation (Noueiry et al., 2000, 2003), selecting BMV RNAs as replication templates (Diez et al., 2000), activating the viral RNA replication complex through chaperones (Tomita et al., 2003) and maintaining a lipid composition required for membrane-associated RNA replication (Lee et al., 2001; Lee and Ahlquist, 2003). A more global and systematic approach to identify host factors affecting viral RNA replication was the use

of a diploid yeast deletion library for a genome-wide screening (Kushner et al., 2003). Each of the single gene deletion strains was transformed with plasmids expressing BMV 1a, 2a, and a BMV RNA3 replication template with a luciferase reporter gene replacing the coat gene. Hence, expression of luciferase was used as a screenable marker for BMV RNA replication. By this approach, approximately 4500 yeast deletion strains (~80% of all yeast genes) were tested twice in two independent assays. Nearly 100 genes were identified whose absence either inhibited or stimulated BMV RNA replication and gene expression. They include (i) genes involved in RNA function or turnover, (ii) genes involved in membrane or lipid membrane synthesis and modification pathways, (iii) genes whose connection to BMV RNA replication is less evident and whose function, for example, in protein turnover, glutathione synthesis, ubiquitin metabolism, tubulin folding or nitrogen utilization, and (iv) genes of as yet unknown function.

Among the identified genes that facilitate BMV RNA replication, four of them (*LSM1*, *LSM6*, *PAT1* and *DHH1*) have been shown by traditional yeast genetic studies to play a key role in the regulated transition of the BMV RNA from the cellular translation machinery to the site of replication (Diez et al., 2000; Mas et al., 2006; Noueiry et al., 2003). The fact that these genes were identified by two independent methods validates the genome-wide screening approach. Among the identified genes that inhibit BMV RNA replication, it is worth noting the four *SKI* genes (*SKI2*, -3, -7, and -8). These genes function in exosome-mediated 3' to 5' mRNA degradation and inhibit the accumulation of non-polyadenylated mRNAs, including those of the yeast dsRNA virus L-A (Araki et al., 2001). Thus, these genes not only participate in the control of model virus/yeast systems but also play a role in natural yeast replicons.

Overall, the genome-wide screening has been extremely valuable to identify multiple host factors that affect BMV RNA replication and, most importantly, has brought to light the involvement of previously unconsidered cellular pathways. A detailed analysis, however, has still to be performed to unravel the underlying mechanisms. Nonetheless, despite the impressive achievements one should keep in mind that the used genome-wide screening approach has one limitation. Only non-essential yeast genes can be analyzed because the essential ones cannot be deleted without affecting yeast viability. To analyze essential yeast genes concerning their role in virus replication, other approaches need to be applied.

2.2. Carnation Italian ringspot virus and Tomato bushy stunt virus

CIRV and TBSV are members of the family *Tombusviridae*. Their genomes consist of a single, uncapped and non-polyadenylated (+)strand RNA which contains five open reading frames (ORFs) (White and Nagy, 2004). ORF1 and ORF2 are directly translated from the genomic RNA to generate the two proteins essential for viral replication, the auxiliary protein and the viral RdRp, respectively. The viral polymerase is translated by a read-through mechanism ignoring a stop codon located at the end of ORF1. Translation of ORFs 3 to 5 requires the synthe-

sis of subgenomic RNAs. These ORFs code for the coat protein and two additional proteins which have roles in virus movement and phenotypic changes in infected plants.

During toombusvirus infections, defective interfering RNAs (DI RNAs) are generated spontaneously during the replication of the viral genomes (White and Morris, 1999; White and Nagy, 2004). DI RNAs are deletion derivatives that contain non-contiguous regions of the viral RNA. Since they do not code for any protein, their replication depends on the replication machinery of the parental toombusvirus. This trans-replication process is very effective, in fact, DI RNAs are often replicated more efficiently than the viral genome itself. These features have made DI RNAs ideal replicons for studying toombusvirus replication in yeast.

The expression of the viral replicase proteins in yeast is sufficient for DI RNA replication of TBSV and CIRV viruses (Panavas and Nagy, 2003; Pantaleo et al., 2003). Both yeast systems were simultaneously developed and use similar experimental approaches. The DI RNAs were in vivo transcribed from a yeast plasmid. The auxiliary and the polymerase ORFs were separately cloned and expressed from two additional plasmids. This was necessary because the read-through mechanism that generates the toombusvirus RNA polymerase does not work efficiently in yeast. Transformation of yeast with the three plasmids resulted in such a robust replication that the TBSV-derived DI RNA accumulated to ribosomal RNA levels as it does in plants (Panaviene et al., 2004; White and Nagy, 2004). The following findings derived from toombusvirus studies in yeast. As in plants, CIRV replicates in association with intracellular membranes of mitochondria whereas TBSV replicates at peroxisomal membranes (Panavas et al., 2005b; Rubino et al., 2000). The necessary molecular interactions between the auxiliary protein and the viral polymerase together with their association with the membranes have been characterized in detail (Panavas et al., 2005a; Pantaleo et al., 2004; Rajendran and Nagy, 2004; Weber-Lofti et al., 2002). In addition, four cis-acting RNA elements that are required for replication were also defined. The one localized within the RdRp ORF is specifically recognized by the auxiliary protein. This protein–RNA interaction then results in the recruitment of the viral RNA to the replication complex (Pogany et al., 2005). The other three cis-acting viral RNA elements stimulate the assembly of the toombusvirus replicase and could be important to ensure fidelity of template selection during replication (Panaviene et al., 2005).

To identify host factors required for replication of TBSV in yeast, a genome-wide screen was carried out with the same yeast deletion library that was previously used for BMV (Kushner et al., 2003; Panavas et al., 2005b). Each strain of the collection was simultaneously transformed with two plasmids expressing the toombusvirus auxiliary and the RdRp proteins, respectively, and a third plasmid that directed the transcription of the DI-72 RNA replicon. Gene deletion effects were then detected by direct visualization of the TBSV DI RNA in ethidium-bromide-stained agarose gels. The screening experiment identified 96 host genes whose absence inhibited or stimulated TBSV replication, a number similar to the one from the BMV screening. As with BMV, the identified genes are involved in diverse cellular pathways

including nucleic acid, protein and lipid metabolism, protein targeting and transport, and general and stress metabolism. Surprisingly, only four genes were found to be common in both studies, three of them function in the ubiquitin pathway (*DOA4*, *LGE1*, *STW14*) and the fourth is a transcription regulator (*CDC50*). This number increases to fourteen when host genes with similar functions are considered. These genes are mainly involved in protein biosynthesis, protein metabolism and DNA remodeling. Interestingly, the TBSV-influencing host genes that were involved in protein targeting, membrane association, vesicle-mediated transport and lipid metabolism were not found in the BMV screening. This may relate to the fact that BMV and TBSV replicate in association with cellular membranes of different intracellular compartments. As a consequence, the transport of the replicase components and the assembly of the replication complex may be guided by different host factors.

TBSV DI RNAs not only replicate in yeast but also undergo recombination (Panavas and Nagy, 2003). Since host factors may also influence this important mechanism of virus evolution, an analogous genome-wide screening experiment was performed to identify such factors (Serviene et al., 2005). As a read-out, TBSV DI RNAs were separated by agarose gels and characterized by Northern blotting. Recombinants were identified by length variation and further analyzed by sequencing. Five genes that suppress RNA recombination, four genes that increase RNA recombination and two that changed the profile of recombinants were identified. Four of the recombination suppressing genes are involved in mRNA degradation. The known functions of the other genes suggest that intracellular transport of viral or host proteins to the site of recombination and the lipid content of the replication compartment could have an effect on RNA recombination efficiency. While much of the mechanistic details have yet to be determined, these exciting findings open new avenues in the study of virus evolution.

2.3. Flock House virus and Nodamura virus

FHV and NoV are members of the family *Nodaviridae* that primarily infect insects. Only NoV infects and also kills mammals such as suckling mice and hamsters. The nodavirus genome is composed of two 5' capped and 3' non-polyadenylated (+)strand RNAs (Ball and Johnson, 1999). RNA1 encodes for protein A, the viral RdRp. This polymerase is the only viral protein required for RNA replication. RNA2 encodes the virion capsid protein precursor. In addition, a small subgenomic RNA is produced from the 3' end of RNA1 during replication, which encodes for protein B1 and B2 of mostly unknown function. B2 has been connected with suppression of RNA interference (Li et al., 2002).

Today, FHV and NoV are the only higher eukaryotic RNA viruses that are able to replicate their entire genomes in *S. cerevisiae* (Price et al., 1996, 2005). Transfection of yeast with RNA isolated from FHV or NoV virions resulted in full viral RNA replication including the production of subgenomic RNA and, for FHV, the assembly of infectious virions. To facilitate the studies of the viral and host functions involved in nodavirus replication, yeast DNA plasmids were constructed to express RNA1

(Price et al., 2000, 2005). After induced transcription, a robust replication is observed, comparable to the one in nodavirus-infected animal cells. For some studies it was useful to separate the replication template activity of RNA1 from its polymerase coding function (Lindenbach et al., 2002). This was achieved by generating FHV RNA1 derivatives that cannot express protein A. The RNA1 derivatives replicate when protein A is co-expressed in trans from a separate plasmid. These constructs were very valuable to define cis-acting elements in RNA1 that control RNA2 replication, as well as subgenomic RNA production (Lindenbach et al., 2002). Also they were used to show that protein A targets and anchors FHV RNA replication complexes to outer mitochondrial membranes through an N-proximal mitochondrial localization signal and transmembrane domain (Miller and Ahlquist, 2002). Interestingly, when the N-proximal mitochondrial localization signal of protein A is replaced by an endoplasmic reticulum localization signal, protein A and FHV replication complexes are targeted to the endoplasmic reticulum and efficient replication takes place (Miller et al., 2003). These results suggest that at least for FHV no specific intracellular membranes are required for the formation and function of the viral RNA replication complex. For future studies, a more sophisticated system has been developed that allows visualization of FHV and NoV replication via reporter gene expression or selection by nutritional markers (Price et al., 2002, 2005). Studies to gain a more detailed understanding in the nodavirus life cycle are in progress.

3. Replication of viruses with DNA genomes in yeast

The developed yeast systems that replicate DNA viruses have only been exploited for replication studies in a limited way. Until today, three DNA viruses with circular genomes are described to replicate in *S. cerevisiae*. These include *Human papillomavirus* (HPV) and *Bovine papillomavirus* (BPV), two viruses from the family *Papillomaviridae*, and *Mungbean yellow mosaic India virus* (MYMIV), a plant virus from the family *Geminiviridae* (Table 1).

The replication of most DNA viruses largely depends on the cellular DNA replication machinery. Between yeast and human cells, many components of this machinery are highly conserved. Accordingly, in the three yeast systems, the simple introduction of viral DNA, or viral DNA linked in cis to a yeast selectable marker, resulted in full DNA viral replication and the maintenance of the viral genome as an episome (Fig. 1B). The characteristics of each system are discussed below.

3.1. *Human papillomavirus* and *Bovine papillomavirus*

Papillomaviruses (PVs) are a group of small, double-stranded, oncogenic DNA viruses that replicate their genomes as nuclear plasmids in mammalian epithelial cells (Howley and Lowy, 2001). A subset of HPV genotypes such as HPV-16, HPV-18 and HPV-31 are associated with anogenital cancers, including cervical carcinoma, the second most prevalent cancer in women worldwide (Parkin et al., 2005). The lack of an efficient conven-

tional in vitro cell culture system has hampered the study of the PV life cycle.

The PV genomes encode for an average of eight proteins that are classified as either early (E) or late (L), depending on the time of expression during infection. From these, only proteins E1 and E2 function in PV replication (Howley and Lowy, 2001). E1 is a DNA helicase that recruits cellular DNA polymerase- α to the E1-dependent origin of replication (E1 ori). However, recent data have shown that there is also an E1-independent mode of replication (Kim and Lambert, 2002). E2, a transcriptional activator, assists the binding of E1 to the E1 ori. In addition, it also contributes to the stable inheritance of PV DNA to daughter cells during mitosis by attaching the viral genomes to host chromosomes (Ilves et al., 1999; Lehman and Botchan, 1998; Skiadopoulou and McBride, 1998).

Full length genomes of HPV-11, HPV-16, HPV-18, HPV-31, and BPV-1 have been linked in cis to a selectable yeast marker gene and shown to replicate stably as episomes in *S. cerevisiae* (Angeletti et al., 2002). Interestingly, none of the viral proteins, including E1 and E2, were required for the replication of the viral episomes. Moreover, the autonomous replication sequence (ARS) and centromeric maintenance (CEN) elements that are normally required for the replication and maintenance of extrachromosomal DNA in yeast were not present in the viral genome (Angeletti et al., 2002). Recent work has identified the cis-acting elements in the HPV-16 genome that mediate the ARS- and CEN-independent replication mechanism (Kim et al., 2005). Further studies should help to understand PV genome replication and stable inheritance to daughter cells in more detail.

Remarkably, a replication of BPV-1 genomes was also observed after exposing *S. cerevisiae* protoplasts to natural purified virions (Zhao and Frazer, 2002a). Thus, protoplasts were infected and fully supported viral replication. Moreover, the infected protoplasts also supported the production of infectious BPV-1 particles (Zhao and Frazer, 2002b). While the exact entry mechanism may not be physiologically relevant, this experiment is the first demonstration of a complete multiplication cycle of a higher eukaryotic virus in yeast.

3.2. *Indian mung bean yellow mosaic virus*

A large variety of plant species are susceptible to infection by geminiviruses. This group of DNA viruses is characterized by twin virion particles each of which encapsidates one single-stranded circular DNA (Gutierrez, 2000). For IMYMV the two DNA molecules are named DNA-A and DNA-B. DNA-A codes for six proteins that function in viral DNA replication, transcription regulation and encapsidation. DNA-B encodes two movement proteins that are dispensable for replication.

In the IMYMV/yeast system, two copies of DNA-A were cloned into a yeast plasmid deficient in ARS-related activity but containing a CEN element and a selectable marker (Raghavan et al., 2004). The generated plasmid expressed the DNA-A viral genes and was efficiently replicated by viral proteins and the viral replication origin, as in plants. This study clarified the role of the previously uncharacterized viral protein AC5 as an important

contributor to virus replication and opened up many possibilities for future geminivirus research.

4. Conclusion

The yeast *S. cerevisiae* has been proven to be a versatile organism for the study of viruses. While an impressive progress has been made to unravel many of the fundamental steps of replication, we are just standing at a beginning. Until today, all higher eukaryotic RNA viruses that have been described to replicate in yeast have relatively simple (+)RNA genomes with, for example, few viral proteins, no IRES elements and no need for proteolytic cleavage of precursor proteins. This situation might be rapidly changing. Recently, internal initiation of translation mediated by the IRES of the *Hepatitis C virus* has been demonstrated in *S. cerevisiae* (Rosenfeld and Racaniello, 2005). In addition, human proteins that are essential for virus replication but lack a functional counterpart in yeast may be expressed in yeast and thereby allow the development of more complex yeast/virus systems. This approach would be analogous to the so-called “humanized yeast systems” for studying mechanisms of human diseases like Huntington’s and Parkinson’s disease (Mager and Winderickx, 2005). Moreover, major advances in proteomics, metabolomics and genome-wide screening approaches in yeast are noteworthy. For example, the recently established yeast strains with increased membrane permeability and deleted major flux pumps will facilitate the entry of chemical compounds and thus the screening for antivirals in the yeast/virus systems. Taken together, the yeast *S. cerevisiae* will continue to provide interesting insights into the complex world of virus–host interactions and keep us excitedly waiting for further pioneering findings.

Acknowledgements

The work is supported by grants from the Spanish Ministerio de Ciencia y Tecnología (BFU2004-00654) and the Deutsche Forschungsgemeinschaft. I.A.-R. is supported by Fundação para a Ciência e Tecnologia (SFRH/BD/9630/2002) through the Gabba programme from the University of Porto, Portugal.

References

- Ahlquist, P., 1992. Bromovirus RNA replication and transcription. *Curr. Opin. Genet. Dev.* 2, 71–76.
- Ahlquist, P., Noueiry, A.O., Lee, W.M., Kushner, D.B., Dye, B.T., 2003. Host factors in positive-strand RNA virus genome replication. *J. Virol.* 77, 8181–8186.
- Ahola, T., Ahlquist, P., 1999. Putative RNA capping activities encoded by brome mosaic virus: methylation and covalent binding of guanylate by replicase protein 1a. *J. Virol.* 73, 10061–10069.
- Ahola, T., den Boon, J.A., Ahlquist, P., 2000. Helicase and capping enzyme active site mutations in brome mosaic virus protein 1a cause defects in template recruitment, negative-strand RNA synthesis, and viral RNA capping. *J. Virol.* 74, 8803–8811.
- Andino, R., Boddeker, N., Silvera, D., Gamarnik, A.V., 1999. Intracellular determinants of picornavirus replication. *Trends Microbiol.* 7, 76–82.
- Angeletti, P.C., Kim, K., Fernandes, F.J., Lambert, P.F., 2002. Stable replication of papillomavirus genomes in *Saccharomyces cerevisiae*. *J. Virol.* 76, 3350–3358.
- Araki, Y., Takahashi, S., Kobayashi, T., Kajihio, H., Hoshino, S., Katada, T., 2001. Ski7p G protein interacts with the exosome and the Ski complex for 3'-to-5' mRNA decay in yeast. *EMBO J.* 20, 4684–4693.
- Ball, L.A., Johnson, K.L., 1999. Reverse genetics of nodaviruses. *Adv. Virus Res.* 53, 229–244.
- Baumstark, T., Ahlquist, P., 2001. The brome mosaic virus RNA3 intergenic replication enhancer folds to mimic a tRNA TpsiC-stem loop and is modified in vivo. *RNA* 7, 1652–1670.
- Blanco, R., Carrasco, L., Ventoso, I., 2003. Cell killing by HIV-1 protease. *J. Biol. Chem.* 278, 1086–1093.
- Chen, J., Ahlquist, P., 2000. Brome mosaic virus polymerase-like protein 2a is directed to the endoplasmic reticulum by helicase-like viral protein 1a. *J. Virol.* 74, 4310–4318.
- Chen, J., Noueiry, A., Ahlquist, P., 2001. Brome mosaic virus Protein 1a recruits viral RNA2 to RNA replication through a 5' proximal RNA2 signal. *J. Virol.* 75, 3207–3219.
- Coller, J., Parker, R., 2004. Eukaryotic mRNA decapping 3. *Annu. Rev. Biochem.* 73, 861–890.
- DeMarini, D.J., Johnston, V.K., Konduri, M., Gutshall, L.L., Sarisky, R.T., 2003. Intracellular hepatitis C virus RNA-dependent RNA polymerase activity. *J. Virol. Methods* 113, 65–68.
- den Boon, J.A., Chen, J., Ahlquist, P., 2001. Identification of sequences in Brome mosaic virus replicase protein 1a that mediate association with endoplasmic reticulum membranes. *J. Virol.* 75, 12370–12381.
- Diez, J., Ishikawa, M., Kaido, M., Ahlquist, P., 2000. Identification and characterization of a host protein required for efficient template selection in viral RNA replication. *Proc. Natl. Acad. Sci. U.S.A.* 97, 3913–3918.
- Donahue, T.F., 2000. Genetic approaches to translation initiation in *Saccharomyces cerevisiae*. In: Sonenberg, N., Hershey, J.W.B., Mathews, M.B. (Eds.), *Translational Control of Gene Expression*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 487–502.
- Garriga, D., Diez, J., Oliva, B., 2004. Modeling the helicase domain of Brome mosaic virus 1a replicase. *J. Mol. Model. (Online)* 10, 382–392.
- Gutierrez, C., 2000. Geminiviruses and the plant cell cycle. *Plant Mol. Biol.* 43, 763–772.
- Howley, P.M., Lowy, D.R., 2001. Papillomaviruses and their replication. In: Knipe, D.M., Howley, P.M. (Eds.), *Fields Virology*. Lippincott Williams & Wilkins, Philadelphia, pp. 2197–2229.
- Hughes, T.R., 2002. Yeast and drug discovery. *Funct. Integr. Genomics* 2, 199–211.
- Ilves, I., Kivi, S., Ustav, M., 1999. Long-term episomal maintenance of bovine papillomavirus type 1 plasmids is determined by attachment to host chromosomes, which is mediated by the viral E2 protein and its binding sites. *J. Virol.* 73, 4404–4412.
- Ishikawa, M., Diez, J., Restrepo-Hartwig, M., Ahlquist, P., 1997a. Yeast mutations in multiple complementation groups inhibit brome mosaic virus RNA replication and transcription and perturb regulated expression of the viral polymerase-like gene. *Proc. Natl. Acad. Sci. U.S.A.* 94, 13810–13815.
- Ishikawa, M., Janda, M., Krol, M.A., Ahlquist, P., 1997b. In vivo DNA expression of functional brome mosaic virus RNA replicons in *Saccharomyces cerevisiae*. *J. Virol.* 71, 7781–7790.
- Janda, M., Ahlquist, P., 1993. RNA-dependent replication, transcription, and persistence of brome mosaic virus RNA replicons in *S. cerevisiae*. *Cell* 72, 961–970.
- Janda, M., Ahlquist, P., 1998. Brome mosaic virus RNA replication protein 1a dramatically increases in vivo stability but not translation of viral genomic RNA3. *Proc. Natl. Acad. Sci. U.S.A.* 95, 2227–2232.
- Kapoor, P., Lavoie, B.D., Frappier, L., 2005. EBP2 plays a key role in Epstein-Barr virus mitotic segregation and is regulated by aurora family kinases. *Mol. Cell Biol.* 25, 4934–4945.
- Kim, K., Angeletti, P.C., Hassebroek, E.C., Lambert, P.F., 2005. Identification of cis-acting elements that mediate the replication and maintenance of human papillomavirus type 16 genomes in *Saccharomyces cerevisiae*. *J. Virol.* 79, 5933–5942.
- Kim, K., Lambert, P.F., 2002. E1 protein of bovine papillomavirus 1 is not required for the maintenance of viral plasmid DNA replication. *Virology* 293, 10–14.

- Krol, M.A., Olson, N.H., Tate, J., Johnson, J.E., Baker, T.S., Ahlquist, P., 1999. RNA-controlled polymorphism in the in vivo assembly of 180-subunit and 120-subunit virions from a single capsid protein. *Proc. Natl. Acad. Sci. U.S.A.* 96, 13650–13655.
- Kushner, D.B., Lindenbach, B.D., Grdzlishvili, V.Z., Noueiry, A.O., Paul, S.M., Ahlquist, P., 2003. Systematic, genome-wide identification of host genes affecting replication of a positive-strand RNA virus. *Proc. Natl. Acad. Sci. U.S.A.* 100, 15764–15769.
- Lander, E.S., Linton, L.M., Birren, B., Nussbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J., Kann, L., Lehoczky, J., Levine, R., McEwan, P., McKernan, K., Meldrum, J., Mesirov, J.P., Miranda, C., Morris, W., Naylor, J., Raymond, C., Rosetti, M., Santos, R., Sheridan, A., Sougnez, C., Stange-Thomann, N., Stojanovic, N., Subramanian, A., Wyman, D., Rogers, J., Sulston, J., Ainscough, R., Beck, S., Bentley, D., Burton, J., Clee, C., Carter, N., Coulson, A., Deadman, R., Deloukas, P., Dunham, A., Dunham, I., Durbin, R., French, L., Grafham, D., Gregory, S., Hubbard, T., Humphray, S., Hunt, A., Jones, M., Lloyd, C., McMurray, A., Matthews, L., Mercer, S., Milne, S., Mullikin, J.C., Mungall, A., Plumb, R., Ross, M., Showkeen, R., Sims, S., Waterston, R.H., Wilson, R.K., Hillier, L.W., McPherson, J.D., Marra, M.A., Mardis, E.R., Fulton, L.A., Chinwalla, A.T., Pepin, K.H., Gish, W.R., Chissole, S.L., Wendl, M.C., Delehaunty, K.D., Miner, T.L., Delehaunty, A., Kramer, J.B., Cook, L.L., Fulton, R.S., Johnson, D.L., Minx, P.J., Clifton, S.W., Hawkins, T., Richardson, E., Predki, P., Richardson, P., Wenning, S., Slezak, T., Doggett, N., Cheng, J.F., Olsen, A., Lucas, S., Elkin, C., Uberbacher, E., Frazier, M., Gibbs, R.A., Muzny, D.M., Scherer, S.E., Bouck, J.B., Sodergren, E.J., Worley, K.C., Rives, C.M., Gorrell, J.H., Metzker, M.L., Naylor, S.L., Kucherlapati, R.S., Nelson, D.L., Weinstock, G.M., Sakaki, Y., Fujiyama, A., Hattori, M., Yada, T., Toyoda, A., Itoh, T., Kawagoe, C., Watanabe, H., Totoki, Y., Taylor, T., Weissenbach, J., Heilig, R., Saurin, W., Artiguenave, F., Brottier, P., Bruls, T., Pelletier, E., Robert, C., Wincker, P., Smith, D.R., Doucette-Stamm, L., Rubenfield, M., Weinstock, K., Lee, H.M., Dubois, J., Rosenthal, A., Platzer, M., Nyakatura, G., Taudien, S., Rump, A., Yang, H., Yu, J., Wang, J., Huang, G., Gu, J., Hood, L., Rowen, L., Madan, A., Qin, S., Davis, R.W., Federspiel, N.A., Abola, A.P., Proctor, M.J., Myers, R.M., Schmutz, J., Dickson, M., Grimwood, J., Cox, D.R., Olson, M.V., Kaul, R., Raymond, C., Shimizu, N., Kawasaki, K., Minoshima, S., Evans, G.A., Athanasiou, M., Schultz, R., Roe, B.A., Chen, F., Pan, H., Ramsay, J., Lehrach, H., Reinhardt, R., McCombie, W.R., de la, B.M., Dedhia, N., Blocker, H., Hornischer, K., Nordtsiek, G., Agarwala, R., Aravind, L., Bailey, A., Bateman, A., Batzoglou, S., Birney, E., Bork, P., Brown, D.G., Burge, C.B., Cerutti, L., Chen, H.C., Church, D., Clamp, M., Copley, R.R., Doerks, T., Eddy, S.R., Eichler, E.E., Furey, T.S., Galagan, J., Gilbert, J.G., Harmon, C., Hayashizaki, Y., Haussler, D., Hermjakob, H., Hokamp, K., Jang, W., Johnson, L.S., Jones, T.A., Kasif, S., Kasprzyk, A., Kennedy, S., Kent, W.J., Kitts, P., Koonin, E.V., Korf, I., Kulp, D., Lancet, D., Lowe, T.M., McLysaght, A., Mikkelsen, T., Moran, J.V., Mulder, N., Pollara, V.J., Ponting, C.P., Schuler, G., Schultz, J., Slater, G., Smit, A.F., Stupka, E., Szustakowski, J., Thierry-Mieg, D., Thierry-Mieg, J., Wagner, L., Wallis, J., Wheeler, R., Williams, A., Wolf, Y.I., Wolfe, K.H., Yang, S.P., Yeh, R.F., Collins, F., Guyer, M.S., Peterson, J., Felsenfeld, A., Wetterstrand, K.A., Patrino, A., Morgan, M.J., de, J.P., Catanese, J.J., Osoegawa, K., Shizuya, H., Choi, S., Chen, Y.J., 2001. Initial sequencing and analysis of the human genome. *Nature* 409, 860–921.
- Lee, W.M., Ahlquist, P., 2003. Membrane synthesis, specific lipid requirements, and localized lipid composition changes associated with a positive-strand RNA virus RNA replication protein. *J. Virol.* 77, 12819–12828.
- Lee, W.M., Ishikawa, M., Ahlquist, P., 2001. Mutation of host delta9 fatty acid desaturase inhibits brome mosaic virus RNA replication between template recognition and RNA synthesis. *J. Virol.* 75, 2097–2106.
- Lehman, C.W., Botchan, M.R., 1998. Segregation of viral plasmids depends on tethering to chromosomes and is regulated by phosphorylation. *Proc. Natl. Acad. Sci. U.S.A.* 95, 4338–4343.
- Li, H., Li, W.X., Ding, S.W., 2002. Induction and suppression of RNA silencing by an animal virus. *Science* 296, 1319–1321.
- Lindenbach, B.D., Sgro, J.Y., Ahlquist, P., 2002. Long-distance base pairing in flock house virus RNA1 regulates subgenomic RNA3 synthesis and RNA2 replication. *J. Virol.* 76, 3905–3919.
- Lum, P.Y., Armour, C.D., Stepaniants, S.B., Cavet, G., Wolf, M.K., Butler, J.S., Hinshaw, J.C., Garnier, P., Prestwich, G.D., Leonardson, A., Garrett-Engle, P., Rush, C.M., Bard, M., Schimmack, G., Phillips, J.W., Roberts, C.J., Shoemaker, D.D., 2004. Discovering modes of action for therapeutic compounds using a genome-wide screen of yeast heterozygotes. *Cell* 116, 121–137.
- Magden, J., Kaariainen, L., Ahola, T., 2005. Inhibitors of virus replication: recent developments and prospects. *Appl. Microbiol. Biotechnol.* 66, 612–621.
- Mager, W.H., Winderickx, J., 2005. Yeast as a model for medical and medicinal research 1. *Trends Pharmacol. Sci.* 26, 265–273.
- Mas, A., Alves-Rodríguez, I., Noueiry, A., Ahlquist, P., Díez, J., 2006. Host deadenylation-dependent mRNA decapping factors are required for a key step in brome mosaic virus RNA replication. *J. Virol.* 80, 246–251.
- Miller, D.J., Ahlquist, P., 2002. Flock house virus RNA polymerase is a transmembrane protein with amino-terminal sequences sufficient for mitochondrial localization and membrane insertion. *J. Virol.* 76, 9856–9867.
- Miller, D.J., Schwartz, M.D., Dye, B.T., Ahlquist, P., 2003. Engineered retargeting of viral RNA replication complexes to an alternative intracellular membrane. *J. Virol.* 77, 12193–12202.
- Noueiry, A.O., Chen, J., Ahlquist, P., 2000. A mutant allele of essential, general translation initiation factor DED1 selectively inhibits translation of a viral mRNA. *Proc. Natl. Acad. Sci. U.S.A.* 97, 12985–12990.
- Noueiry, A.O., Díez, J., Falk, S.P., Chen, J., Ahlquist, P., 2003. Yeast Lsm1p-7p/Pat1p deadenylation-dependent mRNA-decapping factors are required for brome mosaic virus genomic RNA translation. *Mol. Cell Biol.* 23, 4094–4106.
- Panavas, T., Hawkins, C.M., Panaviene, Z., Nagy, P.D., 2005a. The role of the p33:p33/p92 interaction domain in RNA replication and intracellular localization of p33 and p92 proteins of Cucumber necrosis tobravirus 1. *Virology* 338, 81–95.
- Panavas, T., Nagy, P.D., 2003. Yeast as a model host to study replication and recombination of defective interfering RNA of Tomato bushy stunt virus. *Virology* 314, 315–325.
- Panavas, T., Serviene, E., Brasher, J., Nagy, P.D., 2005b. Yeast genome-wide screen reveals dissimilar sets of host genes affecting replication of RNA viruses. *Proc. Natl. Acad. Sci. U.S.A.* 102, 7326–7331.
- Panaviene, Z., Panavas, T., Nagy, P.D., 2005. Role of an internal and two 3'-terminal RNA elements in assembly of tobravirus replicase. *J. Virol.* 79, 10608–10618.
- Panaviene, Z., Panavas, T., Serva, S., Nagy, P.D., 2004. Purification of the cucumber necrosis virus replicase from yeast cells: role of coexpressed viral RNA in stimulation of replicase activity. *J. Virol.* 78, 8254–8263.
- Pantaleo, V., Rubino, L., Russo, M., 2004. The p36 and p95 replicase proteins of Carnation Italian ringspot virus cooperate in stabilizing defective interfering RNA. *J. Gen. Virol.* 85, 2429–2433.
- Pantaleo, V., Rubino, L., Russo, M., 2003. Replication of Carnation Italian ringspot virus defective interfering RNA in *Saccharomyces cerevisiae*. *J. Virol.* 77, 2116–2123.
- Parkin, D.M., Bray, F., Ferlay, J., Pisani, P., 2005. Global cancer statistics, 2002 1. *CA Cancer J. Clin.* 55, 74–108.
- Pogany, J., White, K.A., Nagy, P.D., 2005. Specific binding of tobravirus replication protein p33 to an internal replication element in the viral RNA is essential for replication. *J. Virol.* 79, 4859–4869.
- Price, B.D., Ahlquist, P., Ball, L.A., 2002. DNA-directed expression of an animal virus RNA for replication-dependent colony formation in *Saccharomyces cerevisiae*. *J. Virol.* 76, 1610–1616.
- Price, B.D., Eckerle, L.D., Ball, L.A., Johnson, K.L., 2005. Nodamura virus RNA replication in *Saccharomyces cerevisiae*: heterologous gene expression allows replication-dependent colony formation. *J. Virol.* 79, 495–502.
- Price, B.D., Roeder, M., Ahlquist, P., 2000. DNA-Directed expression of functional flock house virus RNA1 derivatives in *Saccharomyces cerevisiae*, heterologous gene expression, and selective effects on subgenomic mRNA synthesis. *J. Virol.* 74, 11724–11733.

- Price, B.D., Rueckert, R.R., Ahlquist, P., 1996. Complete replication of an animal virus and maintenance of expression vectors derived from it in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. 93, 9465–9470.
- Raghavan, V., Malik, P.S., Choudhury, N.R., Mukherjee, S.K., 2004. The DNA-A component of a plant geminivirus (Indian mung bean yellow mosaic virus) replicates in budding yeast cells. J. Virol. 78, 2405–2413.
- Rajendran, K.S., Nagy, P.D., 2004. Interaction between the replicase proteins of Tomato bushy stunt virus in vitro and in vivo. Virology 326, 250–261.
- Rosenfeld, A.B., Racaniello, V.R., 2005. Hepatitis C virus internal ribosome entry site-dependent translation in *Saccharomyces cerevisiae* is independent of polypyrimidine tract-binding protein, poly(rC)-binding protein 2, and La protein 1. J. Virol. 79, 10126–10137.
- Rubino, L., Di, F.A., Russo, M., 2000. Expression of a plant virus non-structural protein in *Saccharomyces cerevisiae* causes membrane proliferation and altered mitochondrial morphology 1. J. Gen. Virol. 81, 279–286.
- Salonen, A., Ahola, T., Kaariainen, L., 2005. Viral RNA replication in association with cellular membranes. Curr. Top. Microbiol. Immunol. 285, 139–173.
- Schwartz, D.C., Parker, R., 2000. Interaction of mRNA translation and mRNA degradation in *Saccharomyces cerevisiae*. In: Sonenberg, N., Hershey, J.W.B., Mathews, M.B. (Eds.), Translational Control of Gene Expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 807–825.
- Schwartz, M., Chen, J., Janda, M., Sullivan, M., Den, B.J., Ahlquist, P., 2002. A positive-strand RNA virus replication complex parallels form and function of retrovirus capsids. Mol. Cell 9, 505–514.
- Serviene, E., Shapka, N., Cheng, C.P., Panavas, T., Phuangrat, B., Baker, J., Nagy, P.D., 2005. Genome-wide screen identifies host genes affecting viral RNA recombination. Proc. Natl. Acad. Sci. U.S.A. 102, 10545–10550.
- Skidopoulos, M.H., McBride, A.A., 1998. Bovine papillomavirus type 1 genomes and the E2 transactivator protein are closely associated with mitotic chromatin. J. Virol. 72, 2079–2088.
- Steinmetz, L.M., Scharfe, C., Deutschbauer, A.M., Mokranjac, D., Herman, Z.S., Jones, T., Chu, A.M., Giaever, G., Prokisch, H., Oefner, P.J., Davis, R.W., 2002. Systematic screen for human disease genes in yeast. Nat. Genet. 31, 400–404.
- Sullivan, M.L., Ahlquist, P., 1999. A brome mosaic virus intergenic RNA3 replication signal functions with viral replication protein 1a to dramatically stabilize RNA in vivo. J. Virol. 73, 2622–2632.
- Tomita, Y., Mizuno, T., Diez, J., Naito, S., Ahlquist, P., Ishikawa, M., 2003. Mutation of host DnaJ homolog inhibits brome mosaic virus negative-strand RNA synthesis. J. Virol. 77, 2990–2997.
- Valenzuela, P., Medina, A., Rutter, W.J., Ammerer, G., Hall, B.D., 1982. Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. Nature 298, 347–350.
- Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., Evans, C.A., Holt, R.A., Gocayne, J.D., Amanatides, P., Ballew, R.M., Huson, D.H., Wortman, J.R., Zhang, Q., Kodira, C.D., Zheng, X.H., Chen, L., Skupski, M., Subramanian, G., Thomas, P.D., Zhang, J., Gabor Miklos, G.L., Nelson, C., Broder, S., Clark, A.G., Nadeau, J., McKusick, V.A., Zinder, N., Levine, A.J., Roberts, R.J., Simon, M., Slayman, C., Hunkapiller, M., Bolanos, R., Delcher, A., Dew, I., Fasulo, D., Flanigan, M., Florea, L., Halpern, A., Hannenhalli, S., Kravitz, S., Levy, S., Mobarry, C., Reinert, K., Remington, K., Burt Threideh, J., Beasley, E., Biddick, K., Bonazzi, V., Brandon, R., Cargill, M., Chandramouliswaran, I., Charlab, R., Chaturvedi, K., Deng, Z., Di, F.V., Dunn, P., Eilbeck, K., Evangelista, C., Gabrielian, A.E., Gan, W., Ge, W., Gong, F., Gu, Z., Guan, P., Heiman, T.J., Higgins, M.E., Ji, R.R., Ke, Z., Ketchum, K.A., Lai, Z., Lei, Y., Li, Z., Li, J., Liang, Y., Lin, X., Lu, F., Merkulov, G.V., Milshina, N., Moore, H.M., Naik, A.K., Narayan, V.A., Neelam, B., Nusskern, D., Rusch, D.B., Salzberg, S., Shao, W., Shue, B., Sun, J., Wang, Z., Wang, A., Wang, X., Wang, J., Wei, M., Wides, R., Xiao, C., Yan, C., Yao, A., Ye, J., Zhan, M., Zhang, W., Zhang, H., Zhao, Q., Zheng, L., Zhong, F., Zhong, W., Zhu, S., Zhao, S., Gilbert, D., Baumhueter, S., Spier, G., Carter, C., Cravchik, A., Woodage, T., Ali, F., An, H., Awe, A., Baldwin, D., Baden, H., Barnstead, M., Barrow, I., Beeson, K., Busam, D., Carver, A., Center, A., Cheng, M.L., Curry, L., Danaher, S., Davenport, L., Desilets, R., Dietz, S., Dodson, K., Doup, L., Ferreira, S., Garg, N., Gluecksmann, A., Hart, B., Haynes, J., Haynes, C., Heiner, C., Hladun, S., Hostin, D., Houck, J., Howland, T., Ibegwam, C., Johnson, J., Kalush, F., Kline, L., Koduru, S., Love, A., Mann, F., May, D., McCawley, S., McIntosh, T., McMullen, I., Moy, M., Moy, L., Murphy, B., Nelson, K., Pfannkoch, C., Pratts, E., Puri, V., Qureshi, H., Reardon, M., Rodriguez, R., Rogers, Y.H., Romblad, D., Ruhfel, B., Scott, R., Sitter, C., Smallwood, M., Stewart, E., Strong, R., Suh, E., Thomas, R., Tint, N.N., Tse, S., Vech, C., Wang, G., Wetter, J., Williams, S., Williams, M., Windsor, S., Winn-Deen, E., Wolfe, K., Zaveri, J., Zaveri, K., Abril, J.F., Guigo, R., Campbell, M.J., Sjolander, K.V., Karlak, B., Kejariwal, A., Mi, H., Lazareva, B., Hatton, T., Narechania, A., Diemer, K., Muruganujan, A., Guo, N., Sato, S., Bafna, V., Istrail, S., Lippert, R., Schwartz, R., Walenz, B., Yooseph, S., Allen, D., Basu, A., Baxendale, J., Blick, L., Caminha, M., Carnes-Stine, J., Caulk, P., Chiang, Y.H., Coyne, M., Dahlke, C., Mays, A., Dombroski, M., Donnelly, M., Ely, D., Esparham, S., Fosler, C., Gire, H., Glanowski, S., Glasser, K., Glodek, A., Gorokhov, M., Graham, K., Gropman, B., Harris, M., Heil, J., Henderson, S., Hoover, J., Jennings, D., Jordan, C., Jordan, J., Kasha, J., Kagan, L., Kraft, C., Levitsky, A., Lewis, M., Liu, X., Lopez, J., Ma, D., Majoros, W., McDaniel, J., Murphy, S., Newman, M., Nguyen, T., Nguyen, N., Nodell, M., 2001. The sequence of the human genome. Science 291, 1304–1351.
- Weber-Lofti, F., Dietrich, A., Russo, M., Rubino, L., 2002. Mitochondrial targeting and membrane anchoring of a viral replicase in plant and yeast cells. J. Virol. 76, 10485–10496.
- White, K.A., Morris, T.J., 1999. Defective and defective interfering RNAs of monopartite plus-strand RNA plant viruses. Curr. Top. Microbiol. Immunol. 239, 1–17.
- White, K.A., Nagy, P.D., 2004. Advances in the molecular biology of tombusviruses: gene expression, genome replication, and recombination. Prog. Nucleic Acid Res. Mol. Biol. 78, 187–226.
- Wickner, R.B., 2001. Viruses of Yeasts, Fungi and Parasitic Microorganisms. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology. Lippincott Williams & Wilkins, Philadelphia, pp. 629–658.
- Winzeler, E.A., Shoemaker, D.D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J.D., Bussey, H., Chu, A.M., Connelly, C., Davis, K., Dietrich, F., Dow, S.W., El, B.M., Foury, F., Friend, S.H., Gentalen, E., Giaever, G., Hegemann, J.H., Jones, T., Laub, M., Liao, H., Liebundguth, N., Lockhart, D.J., Lucau-Danila, A., Lussier, M., M'Rabet, N., Menard, P., Mittmann, M., Pai, C., Rebischung, C., Revuelta, J.L., Riles, L., Roberts, C.J., Ross-Macdonald, P., Scherens, B., Snyder, M., Sookhai-Mahadeo, S., Storms, R.K., Veronneau, S., Voet, M., Volckaert, G., Ward, T.R., Wysocki, R., Yen, G.S., Yu, K., Zimmermann, K., Philippsen, P., Johnston, M., Davis, R.W., 1999. Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science 285, 901–906.
- Zhao, K.N., Frazer, I.H., 2002b. *Saccharomyces cerevisiae* is permissive for replication of bovine papillomavirus type 1. J. Virol. 76, 12265–12273.
- Zhao, K.N., Frazer, I.H., 2002a. Replication of bovine papillomavirus type 1 (BPV-1) DNA in *Saccharomyces cerevisiae* following infection with BPV-1 virions. J. Virol. 76, 3359–3364.