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

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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.

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.,

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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 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.

Table 1
Viruses that replicate in yeast

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

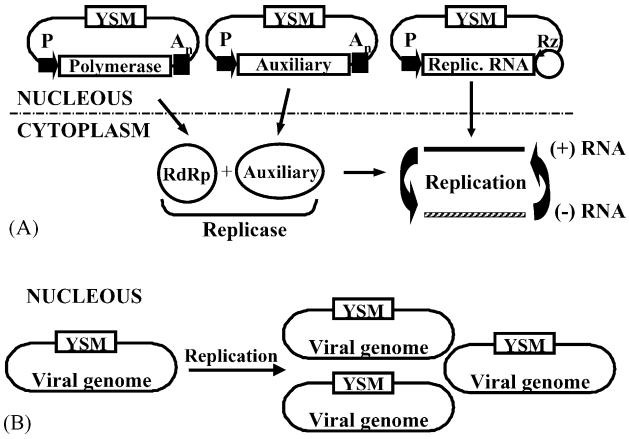

Fig. 1. (A) Schematic illustration of common features of positive-strand RNA virus replication in yeast. The viral RNA-dependent RNA polymerase (RdRp) and the auxiliary protein (if required) are expressed from mRNA transcripts derived from two yeast plasmids by using inducible *GAL*1 or constitutive *ADH*1 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 *GAL*1 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.

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-