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A model for long-term infection of bovine papillomavirus type 1 in *Saccharomyces cerevisiae*

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Summary. – We have previously reported that bovine papillomavirus type 1 (BPV1) can replicate its genome and produces infectious virus-like particles in short-term BPV1 virion-infected *Sacharomyces cerevisiae* (Zhao and Frazer, 2002). Here, we report viral RNA transcription and L1 capsid protein expression in long-term BPV1 virion-infected *S. cerevisiae* culture. Northern blot hybridization showed that viral RNA was detected in long-term BPV1-infected *S. cerevisiae* cultures (82–108 days). The levels of the viral RNA transcription varied significantly over the long time period, which showed active transcription at an early stage (Day 3 to Day 16), weak transcription at a middle stage (Day 23 to Day 45) and stable transcription at the late stage of culture (Day 55 to Day 82/85/95). Three major BPV1 transcripts of 4.3, 2.6 and 1.8 Kb were identified, with 4.3 Kb a minor transcript and the 1.8 Kb the most prominent transcript compared with the 2.6 Kb species. Immunoblotting showed that L1 capsid protein was expressed, with its variable amounts corresponding to the levels of RNA transcription over the time period. ^{35}S-methionine/cysteine labeling and immunoprecipitation proved that the detected L1 protein was newly synthesized in BPV1-infected *S. cerevisiae* cultures. 33.3–54.2% of the cell colonies expressed L1 protein. Thus, the *S. cerevisiae* system, as a promising model, may be used not only for the study of virus like particle formation of BPV1 *in vitro,* but also for further functional analysis of individual viral genes in BPV1 life cycle.

Keywords: BPV1; viral RNA transcription**;** expression of L1 capsid protein; virion-infected Saccharomyces cerevisiae

Introduction

Papillomaviruses are a family of small double-stranded circular DNA viruses with more than 200 genotypes (Frazer *et al*., 2011a; Stanley, 2007; Zur Hausen, 2009). Human papillomaviruses (HPVs) have been demonstrated to be responsible for causing several human cancers and genital warts (Moody *et al*., 2010). The persistent infection with high-risk human papillomavirus (hr-HPV) is closely related to the pathogenesis of cervical cancer and many other cancers. HPVs account for more than 30% of all infection-associated cancers in humans (Zur Hausen, 2009). Preventive vaccines including 2-valent, 4-valent and 9-valent vaccines against HPV-associated cervical cancer are now available, which prevent an estimated 92% of the cancers attributable to HPV types (Frazer *et al*., 2011b; Huh *et al*., 2017; Zhang *et al*., 2021). Considering that the preventive vaccines are unable to wipe out the existing HPV viruses in already infected people, scientists have been focusing on developing therapeutic HPV vaccines that have reached the clinical trial phase in cervical cancers and diseases. Many therapeutic HPV vaccines tested

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Abbreviations: BPV(s) = bovine papillomavirus(es); D3, D23, D34 ... = Day 3, 23, 34 ...; HPV(s) = human papillomavirus(es)

in clinical trials show the potential use of the vaccines as safe and effective pharmacological tools (Zhang *et al*., 2021; Zottnick *et al*., 2020). Development of the potential therapeutic vaccines for HPV infection is a very exciting area of HPV research. However, a number of features of HPV biology prevent the development of the therapeutic vaccines. First, our understanding of the mechanism, by which spontaneous clearing of warts is generated, and the immune response to HPV infection is still very poor. Second, there is no ready source of live virus that might be exploited for use as a live attenuated vaccine, such as in case of poliovirus (Bodily *et al*., 2010). Thus, it is very difficult to eliminate HPV virus existence in host cells to facilitate the treatment of HPV-associated cervical cancer. While most of the other viruses spend a portion of their life cycle in the systemic circulation, where they are vulnerable to neutralizing antibodies, HPVs remain exclusively in the epithelium, thus antibodies must transverse the basement membrane and reach the other layers of the skin or mucosa to be effective in preventing infection (Bodily *et al*., 2010; Psyrri *et al*., 2008). Although the developed raft culture systems allow replication and virus production for high-risk HPV types 16, 18 and 31 (Flores *et al*., 1999; Frattini *et al*., 1996; Meyers *et al*., 1997), such systems have not shown to promote vegetative replication of low-risk HPVs. Furthermore, while a few cell lines containing the DNA of high-risk HPVs, such as types 16, 18 and 31, in an episomal state are available for biological studies, no cell lines containing benign cutaneous HPVs have been reported. Thus, an efficient *in vitro* system is still needed to facilitate the studies of HPV biology and therapeutic vaccines.

Papillomaviruses have also been identified in most domestic animals such as bovine papillomaviruses (BPVs), which are the most typical animal PVs (Modolo *et al*., 2017). BPVs are a cosmopolitan virus, worldwide distributed, independently of the level of expertise on livestock exploration (He *et al*., 2016). BPVs have 15 types, including BPV1, BPV-2, and BPV13, which induce fibropapilloma (Munday *et al*., 2015; Thomson *et al*., 2015). Since 1970, BPV1 has been used as a prototype papillomavirus to study PV biology and oncology (Araldi *et al*., 2017; Borzacchiello *et al*., 2008; Borzacchiello *et al*., 2006; Koller *et al*., 1972). It has also been used to study DNA replication, viral transcription and latency. Transcription of BPV1 viral RNAs has been previously reported in BPV1-transformed cells and in warts (Amtmann *et al*., 1982). The studies using BPV have greatly improved our understanding of HPV-associated oncogenic process and carcinogenesis (Munday *et al*., 2015). We and others have previously developed an *in vitro S. cerevisiae* (budding yeast) system, which is permissive for replication of BPV1 and different types of HPVs (Angeletti *et al*., 2002; Zhao *et al*., 2002a,b) We discovered that *S. cerevisiae* can be infected with BPV1 virions, leading not only to the replication of BPV1 genomic DNA, but also to the production of infectious virus-like particles (VLPs) (Zhao *et al*., 2002a, b). According to a published review, the use of *S. cerevisiae* for replication of both plant and animal viruses was a breakthrough (Rubino *et al*., 2005). Since the establishment of the *S. cerevisiae* system for replication of HPVs and BPV1, many studies have been carried out to study HPV biology and to express L1 capsid protein as a vaccine (Angeletti, 2005; Chattopadhyay *et al*., 2005; Kim *et al*., 2007; Pittayakhajonwut *et al*., 2008). Kim *et al.* reported that elements of papillomavirus DNA could substitute for the function of the autonomously replicating sequence (ARS) (Erdmann *et al*., 2000) and centromere (CEN) elements that are both normally required for the stable replication of extra chromosomal DNAs in yeast (Kim *et al*., 2005). However, these studies could not find that RNA transcription and L1 protein expression were detectable in *S. cerevisiae* although multiple HPV genomes and BPV1 replicated stably in this system for a long term, up to 75 h (Rogers *et al*., 2008). Considering that the infectious virus-like particles were produced in BPV1 virions-infected *S. cerevisiae*, we believe that BPV1 RNA should be transcribed and L1 capsid protein is expressed to generate VLPs. Thus, we focused on investigating how BPV1 viral RNA was transcribed and L1 protein was expressed in BPV1 virions-infected *S. cerevisiae* cultures. Here, we report that the transcription of BPV1 RNA with three transcripts was distinguishably detected and the L1 capsid protein was persistently expressed in long-term BPV1 virions-infected *S. cerevisiae* cultures.

Materials and Methods

BPV1 virion preparation. BPV1 virions used for *S. cerevisiae* protoplast infection were prepared from bovine papillomas as described previously (Zhao *et al*., 2002a,b). Virions in suspension were dialyzed against 0.15 M phosphate-buffered saline (PBS) (pH 7.4) for 30 min and then used for infecting *S. cerevisiae* protoplasts.

Cultures of BPV1-infected S. cerevisiae *cells and sample collection*. BPV1-infected *S. cerevisiae* cells were grown *in vitro* for a long term, as previously described (Zhao and Frazer, 2002a,b) with some modifications. Briefly, *S. cerevisiae* protoplasts (10 ml; 5 x 10^7cells/ml) infected with 0.6 μg of BPV1 were grown in *S. cerevisiae* medium containing 0.8 M sorbitol and 0.2 M glucose on a shaker with gentle agitation at 28°C in the dark for a long time course (about 3 months). *S. cerevisiae* protoplasts without infection of BPV1 virions were cultured under the same conditions. Ten to twelve time points were designed to collect *S. cerevisiae* cells for analysis of viral RNA transcription and L1 capsid protein expression. At each time point, 5 ml of *S. cer-*

evisiae cells were collected and allocated for RNA and protein preparation, with 5 ml of fresh medium without sorbitol added for continuous culture.

RNA preparation and Northern blot hybridization. Total RNA was extracted from BPV1-infected *S. cerevisiae* cultures using a NucleoSpin RNAII Kit (Mackery-Nagel). Following DNase I treatment, 30 µg RNA samples were electrophoresed in 1.2% denatured agarose gels and blotted onto a Nylon N+ membrane (Amersham). The Northern blots were probed with a ^{32}P-labeled BPV1 DNA probe. To visualize internal controls, the Northern blots were stripped and re-probed with a ^{32}P-labeled *S. cerevisiae* actin gene probe.

Protein preparation and Western blot analysis. Both BPV1-infected and uninfected *S. cerevisiae* cultures were lysed in lysis buffer and sonicated for 40 s. Fifty-microgram total protein samples were separated by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and blotted onto polyvinylidene difluoride (PVDF) membrane. The blots were probed by monoclonal antibody against BPV1 L1 protein (Zhao and Frazer, 2002b). Blots were then probed with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG; Sigma) and visualized using a chemiluminescence system (Amersham).

Synthesis of L1 protein in BPV1-infected S. cerevisiae. Synthesis of BPV1 L1 capsid protein was studied by labeling of [^{35}S]-Cys/Met at two time points during the time course. Five ml of *S. cerevisiae* cell suspension with or without infection of BPV1 at Day 3 (D3) and Day 23 (D23) were pelleted. The pelleted *S. cerevisiae* cells were incubated in 4 ml of Cys/Met -free medium supplemented with 20 µCi of L-[^{35}S] Cys/Met (370 kBq) for 24 h. The Cys/Met-labeled *S. cerevisiae* cells were collected for preparing protein samples by lysis and sonication. The protein samples were incubated with 0.3 µl of L1-specific MAb for 2 h and then with protein G-Sepharose (Sigma) beads at 4°C overnight. Beads were washed with radio immuno-precipitation assay buffer (RIPA; 100 mM Tris, pH 7.5; 150 mM NaCl; 5 mM $CaCl_2$; 0.1% Triton X-100) five times and with sterile water once and then boiled in Laemmli buffer; next, supernatant was applied to an SDS 10% polyacrylamide gel. Gels were dried and exposed to a film at -70°C for 48 h.

Expression of L1 protein in individual BPV1-infected S. cerevisiae cell colonies. Twenty microliters of BPV1-infected *S. cerevisiae* cell suspension at two time points (Day 11 and Day 23) in 85-days BPV1-infected *S. cerevisiae* culture were plated on two yeast plates to grow colonies overnight. Twenty-four cell colonies were collected at each time point. A single cell colony was amplified in 1 ml of yeast medium in a 10 ml culture tube for two days on a shaker with gentle agitation at 28°C in the dark. Then the individual colony cultures were pelleted and washed with PBS at pH 7.4. The colony pellets were suspended in 1 ml lysis buffer containing 2 mM of phenylmethylfluorane sulphate (PMSF), leupeptin and pepstatin A and homogenized in a Dounce homogenizer with a tight-fitting pestle for 10 min. The released cell lysates were collected by centrifugation at 12,000 rpm at 4°C for 15 min, and suspended in 100 µl of 1x Laemmli buffer and sonicated for 40 s. Forty microliters of the protein samples were separated by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and blotted onto polyvinylidene difluoride (PVDF) membrane. The blots were probed by monoclonal antibody against BPV1 L1 protein as described above (Zhao *et al.*, 2002a,b).

Results

BPV1 RNA transcription in 5-days BPV1-infected S. cerevisiae culture

Published studies from other and our laboratories have demonstrated that *S. cerevisiae* is a good model for study of DNA replication of different types of HPVs and BPV1 (Zhao *et al.*, 2002a, b). We also detected L1 and L2 capsid proteins in the fractions of CsCl gradients prepared from the short-term BPV1-infected *S. cerevisiae* culture (Zhao *et al.*, 2002a,b). However, it is unclear how the BPV1 RNA is transcribed in BPV1-infected *S. cerevisiae* cells. Thus, we determined first BPV1 RNA transcription in a 5-days BPV1-infected *S. cerevisiae* culture (Fig. 1a). Five milliliters of *S. cerevisiae* culture infected with 100 ng of BPV1 virus

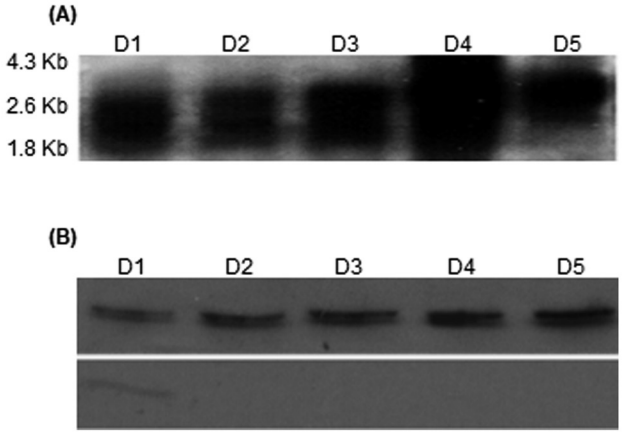

Fig. 1

Viral RNA transcription and L1 protein expression in 5-days BPV1-infected *S. cerevisiae* culture

(a) Viral RNA transcription was detected by Northern blot analysis. The BPV1 viral DNA was transcriptionally active for the period of 5 days. **(b)** L1 capsid protein expression in *S. cerevisiae* culture with infection of BPV1 virions was detected by immunoblotting analysis. The signals of the L1 protein were slightly increased during the 5-days period correspondingly to the viral RNA transcription, while the L1 signal in medium was only detected on the first day. *Upper panel*: BPV1-infected *S. cerevisiae* cultures. *Lower panel:* Medium only with infection of BPV1 virions was used as control.

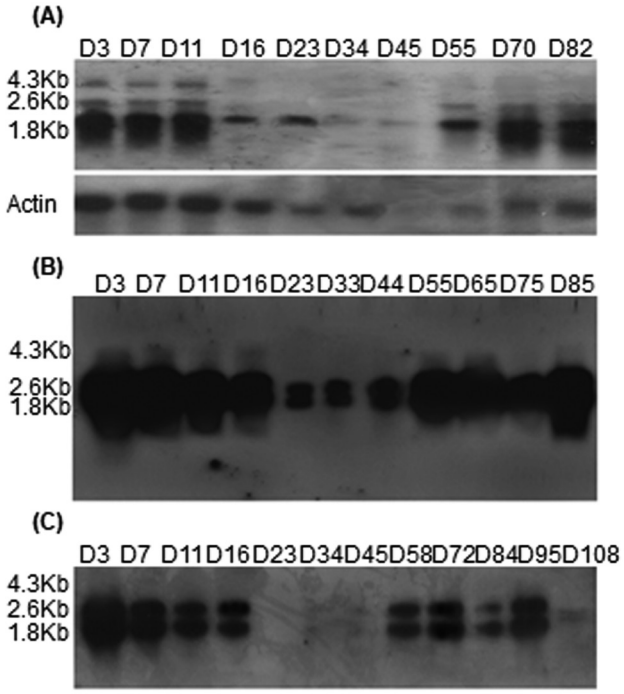

Fig. 2

BPV-1 RNA transcription in three long-term BPV1-infected *S. cerevisiae* cell cultures

(a) Viral RNA transcription detected up to 82 days. Upper panel shows the RNA blot hybridized with ^{32}P-labeled BPV-1 DNA probe. Lower Panel shows the RNA blot hybridized with ^{32}P-labeled *S. cerevisiae* actin gene probe. **(b)** Viral RNA transcription in virions-infected *S. cerevisiae* up to 85 days. **(c)** Viral RNA transcription in virions-infected *S. cerevisiae* up to 108 days.

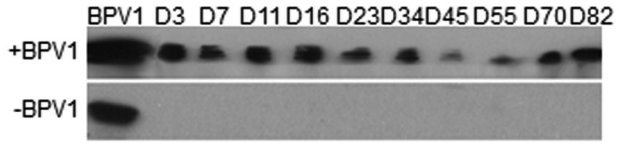

Fig. 3

Expression of L1 protein in one long-term BPV1-infected *S. cerevisiae* cell culture (82 days)

Upper panel shows L1 immunoblotting assay in *S. cerevisiae* cells with infection of BPV1 virions; lower panel shows L1 immunoblotting assay in *S. cerevisiae* cells without infection of BPV1 virions.

were split every day, with one third used for preparing RNA, one third for protein preparation and the other one third for continuing culture with adding two thirds (3.33 ml) fresh medium. Northern blot hybridization showed clearly that the BPV1 viral DNA was transcriptionally active for the period of 5 days although multiple BPV1 transcripts were not well separated (Fig. 1a). We then carried out the time course analysis of L1 protein expressed in the 5-days BPV1-infected *S. cerevisiae* by immunoblotting assay (Fig. 1b). Results showed that signals of the L1 protein were slightly increased during the 5-days period correspondingly to the viral RNA transcription, while the L1 signal in medium was only detected on the first day (Fig. 1b).

BPV1 mRNA transcription in long-term BPV1-infected S. cerevisiae cultures

We next determined whether BPV1 RNA was actively transcribed in a long-term BPV1-infected *S. cerevisiae* culture (82 days). Northern blot visualization showed that BPV1 RNA was transcribed in BPV1-infected *S. cerevisiae* cells for the long time period of 82 days (Fig. 2a). Three BPV1 transcripts with different sizes (4.3, 2.6 and 1.8 Kb) were distinguishably detected (Fig. 2a), with the 4.3 Kb species being a minor transcript mainly observed at the early stage of the BPV1-infected *S. cerevisiae* culture (Fig. 2a). Compared with the 2.6 Kb species, the 1.8 Kb band was the most prominent transcript species (Fig. 2a). Over the time period from D3 to D82, the levels of BPV1 RNA were substantially altered. The lowest levels of BPV1 RNA were observed at three time points (D23, D34, and D45) at the middle stage (Fig. 2a), correspondingly, the levels of the host gene actin at these three time points were the lowest (Fig. 2a). We then investigated the reproducibility of the RNA transcription in two long-term BPV1-infected *S. cerevisiae* cultures (85 and 108 days) (Fig. 2b,c). The weak transcription of BPV1 RNA also occurred at the three time points D23, D33/34 and D44/45 (Fig. 2b,c), indicating that the transcriptional activity at both early and late stages was significantly stronger than that at the middle stage. The weak RNA transcription was observed associating with unhealthy growth of the BPV1-infected *S. cerevisiae* cells (Data not shown), suggesting that active growth of the BPV1-infected *S. cerevisiae* cells might facilitate transcription of the viral RNA. Although three BPV1 transcripts: 4.3, 2.6 and 1.8 Kb species could be detected, the 4.3 Kb transcript was scarce (Fig. 2b,c). The results of the Northern blot hybridization experiments provide strong evidence that BPV1 viral RNA transcription continued in BPV1-infected *S. cerevisiae* cells for a long time period.

L1 capsid protein expression in long-term BPV1-infected S. cerevisiae culture

We previously reported that L1 capsid protein could be detected in short-term BPV1-infected *S. cerevisiae* culture (Zhao *et al.*, 2002a,b). Here, lysates of *S. cerevisiae*

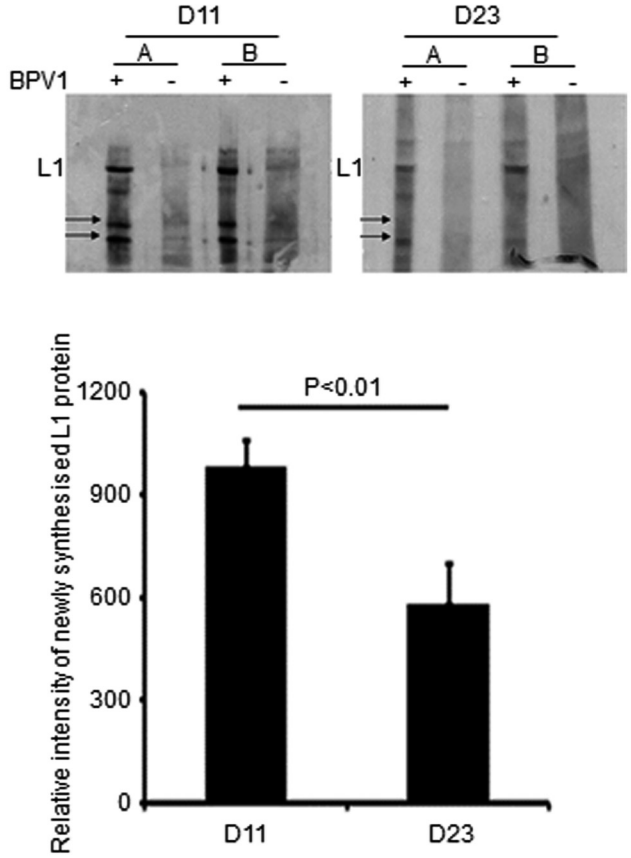

Fig. 4

Detection of newly synthesized L1 capsid protein at two time points in a long-term culture of BPV1-infected *S. cerevisiae* by [^{35}S]-Cys/Met labeling and immunoprecipitation

Upper panel: [^{35}S]-Cys/Met labeling for detection of newly synthesised L1 capsid protein. The [^{35}S]-Cys/Met labeling experiments were carried out in duplicates **(A)** and **(B)** at each time point. *Lower panel:* Relative intensity analysis of the newly synthesised L1 capsid protein at two time points. Histogram and error bars shown are representative of mean and SD of the duplicates (P <0.01).

cells infected with BPV1 virions in one long-term culture (82 days) were subjected to Western blot analysis using monoclonal anti-L1 antibody. The results clearly showed that the L1 protein was expressed over the time course (Fig. 3). As observed for the mRNA transcription in Fig. 2, the signals of the L1 protein were altered from one time point to another over the culture period (Fig. 4), suggesting that expression of L1 protein as an end product was mediated by the mRNA transcription in the long-term BPV1-infected *S. cerevisiae* cultures.

To prove that the changes of the L1 protein expression over the time course were due to the newly synthesized L1 protein, we labeled BPV1-infected *S. cerevisiae* cultures with ^{35}S-methionine/cysteine at D9 and D21, respectively. The labeling of the BPV1-infected cell cultures with ^{35}S-methionine/cysteine lasted for two days to provide sufficient radioactivity to be incorporated into the newly synthesized L1 protein. The lysates prepared from the BPV1-infected and ^{35}S-methionine/cysteine-labeled *S. cerevisiae* cultures at D11 and D23 were immunoprecipitated with specific L1 monoclonal antibody (Fig. 5, upper panel). The BPV1 L1 protein of a molecular mass of 55 kDa was clearly detected in BPV1-infected cultures (Fig. 5, upper panel). Other labeled proteins smaller than L1 protein were also precipitated. No band corresponding to L1 protein was precipitated from *S. cerevisiae* cultures without infection of BPV1 virions (Fig. 5, upper panel). Relative intensity of the L1 protein in D11 BPV1-infected *S. cerevisiae* culture was significantly higher than that in D23 BPV1-infected *S. cerevisiae* culture (Fig. 5, lower panel). Incorporation of ^{35}S-methionine/ cysteine into the L1 protein confirmed that the L1 protein detected in BPV1-infected *S. cerevisiae* culture over the time period was newly synthesized.

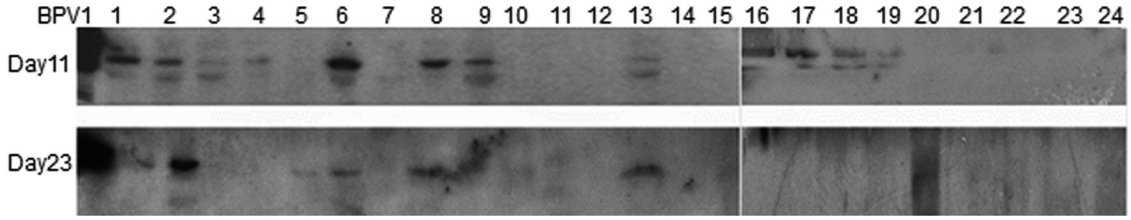

Fig. 5

Expression of L1 capsid protein in individual cell colonies at two time points (Day 11 and Day 23) in a long-term BPV1-infected *S. cerevisiae* cell culture was examined by immunoblotting.

The lysates prepared from the BPV1-infected and ^{35}S-methionine/cysteine-labeled *S. cerevisiae* cultures at D11 and D23 were immunoprecipitated with specific L1 monoclonal antibody (upper panel). The BPV1 L1 protein of a molecular mass of 55 kDa was clearly detected in BPV1-infected cultures (upper panel). Other labeled proteins smaller than L1 protein were also precipitated. No band corresponding to L1 protein was precipitated from *S. cerevisiae* cultures without infection of BPV1 virions (upper panel). Relative intensity of the L1 protein in D11 BPV1-infected *S. cerevisiae* culture was significantly higher than that in D23 BPV1-infected *S. cerevisiae* culture (lower panel).

L1 capsid protein expression in long-term BPV1-infected S. cerevisiae cultures

Previously, we observed that a total of 30 to 40% of BPV1-infected *S. cerevisiae* cells showed L1 expression by immunofluorescence microscopy (Zhao *et al*., 2002b). Here, we plated the infected cells on solid plates to grow individual cell colonies overnight at two time points (D11 and D23). Twenty-four individual cell colonies were amplified for determining the expression of L1 protein (Fig. 5). Immunoblotting assay revealed that 13 out of 24 colonies (54.2%) were positive for L1 protein at Day 11, significantly higher than that 8 out 24 colonies (33.3%) at Day 23 (Fig. 5). The results keep in agreement with the results of viral RNA transcription and L1 protein expression over the time course experiments (Fig. 2, 3 and 4).

Discussion

BPV1 has served as the prototype papillomavirus for the study of DNA replication, viral transcription and latency. Transcription of BPV1 viral RNAs has been previously reported in BPV1-transformed cells and in warts induced by BPV1 (Amtmann *et al*., 1982; Engel *et al*., 1983; Freese *et al*., 1982; Heilman *et al*., 1982). In this paper, we report that BPV1 RNA transcription occurred in long-term BPV1-infected *S. cerevisiae* cultures up to 108 days. The active viral RNA transcription at the early stage (up to 16 days) in the long-term BPV1-infected *S. cerevisiae* cultures can be ascribed to the active replication of the BPV1 DNA. At this stage, the replicating molecules had a high degree of superspiralization in at least part of the replicating genome due to the intensive ongoing elongation of the synthesized DNA strand (Henno *et al*., 2017). The weak transcription of the BPV1 RNA at the middle stage of culture (Day 23–45) was obviously the result of the low BPV1 DNA replication, which could produce dramatic biochemical and structural changes leading to yeast cell damage. As a result, the virion-infected yeast cells grew slowly and unhealthy at this stage. At the late stage (45–108 days), a stable RNA transcription corresponding to DNA replication was probably due to the viral latency, likely indicating that the BPV1 virus was going to lie dormant within yeast cells, which is a typical characteristic shared by papillomaviruses (Araldi *et al*., 2017).

In our study, we showed that three major RNA transcripts (4.3, 2.6 and 1.8 Kb) were present in BPV1-infected *S. cerevisiae* cultures, and the amounts of the three transcripts varied greatly over the time course. Furthermore, 4.3 and 2.6 Kb transcript levels were very low or scarce in BPV1-infected *S. cerevisiae* cultures at the middle of the studied time interval. The variation and scarcity of the detected viral transcripts might reflect the weak replication of the viral DNA, the unhealthy growth status of the BPV1-infected *S. cerevisiae* cells and the change in the viral life cycle. In this study, we did not characterize the individual viral RNA species expressed in BPV1-infected *S. cerevisiae* cultures. According to the published studies, the structural RNAs of BPV4, BPV1 and CRPV show a similar organization despite differences in their molecular weight (Engel *et al*., 1983; Nasseri *et al*., 1984; Phelps *et al*., 1985). Transcripts of 2.8, 1.7 and 2.6 Kb, respectively, span the L1 open reading frame (ORF), whereas a larger transcript, of 3.8, 4.2, and 4.8 Kb respectively, covers both the L2 and L1 ORFs (Smith *et al*., 1986). One study has indicated that HPV-58 genome could replicate its DNA extrachromosomally and transcribe both early and late genes in HPV-58-transformed *S. cerevisiae* (Li *et al*., 2010). Both HPV-58 short and long L1 mRNAs can efficiently translate their L1 proteins in a yeast cell-free lysate system (Wang *et al*., 2010). Thus, the L1 protein detected in the present study and L1 and L2 proteins reported in our previous study (Zhao *et al*., 2002b) may be expressed from either the smaller or the larger transcript. Furthermore, Li *et al.* (2010) observed that HPV-58 genome lost its mitotic stability and the levels of E6 and E7 transcripts were upregulated *in S. cerevisiae* when E2 gene was mutated. Thus, it is necessary to investigate whether and how both early and late genes are transcribed in BPV1-infected *S. cerevisiae*. The results of such studies will help us understand how the trans genes (E1 and E2) contribute to continuous BPV1 genome replication and RNA transcription in the *S. cerevisiae* system. In conclusion, we report here that BPV1 genome transcribed continuously viral RNA leading to the expression of L1 capsid protein in BPV1 virion-infected *S. cerevisiae* for over a long time period (over 100 days). Three major transcripts at 4.3, 2.6 and 1.8 Kb were detected in BPV1-infected *S. cerevisiae* cultures. The amounts of the detected transcripts significantly varied over the time period. Expression of L1 protein changed correspondingly to the viral RNA transcription over the same time period. As reported previously (Zhao *et al*., 2002a,b), this system may be a promising model used not only for the propagation of free virus particles of BPV1 *in vitro* but also for further genetic analysis of the BPV1 life cycle.

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