To investigate the role of the ORF47 protein kinase of varicella-zoster virus (VZV), we constructed VZV recombinants with targeted mutations in conserved motifs of ORF47 and a truncated ORF47 and characterized these mutants for replication, phosphorylation, and protein-protein interactions in vitro and for infectivity in human skin xenografts in the SCID-hu mouse model in vivo. Previous experiments showed that ROka47S, a null mutant that makes no ORF47 protein, did not replicate in skin in vivo (J. F. Moffat, L. Zerboni, M. H. Sommer, T. C. Heineman, J. I. Cohen, H. Kaneshima, and A. M. Arvin, Proc. Natl. Acad. Sci. USA 95:11969-11974, 1998). The construction of VZV recombinants with targeted ORF47 mutations made it possible to assess the effects on VZV infection of human skin xenografts of selectively abolishing ORF47 protein kinase activity. ORF47 mutations that resulted in a C-terminal truncation or disrupted the DYS kinase motif eliminated ORF47 kinase activity and were associated with extensive nuclear retention of ORF47 and IE62 proteins in vitro. Disrupting ORF47 kinase function also resulted in a marked decrease in VZV replication and cutaneous lesion formation in skin xenografts in vivo. However, infectivity in vivo was not blocked completely as long as the capacity of ORF47 protein to bind IE62 protein was preserved, a function that we identified and mapped to the N-terminal domain of ORF47 protein. These experiments indicate that ORF47 kinase activity is of critical importance for VZV infection and cell-cell spread in human skin in vivo but suggest that it is the formation of complexes between ORF47 and IE62 proteins, both VZV tegument components, that constitutes the essential contribution of ORF47 protein to VZV replication in vivo.

Received 3 December 2002/Accepted 25 February 2003
trafficking to the Golgi for virion assembly (11). The efficient replication of the ROKa47S mutant in cell cultures suggests that ORF47 protein-mediated phosphorylation of the IE62 protein and gE and ORF47-IE62 protein binding are not essential for VZV infectivity in vitro. In the absence of ORF47 kinase, CKII phosphorylates gE sufficiently to permit VZV cell-cell spread (11). In contrast, cell-cell spread and virion synthesis cannot occur in intact host tissues in vivo in the absence of ORF47 protein (21).

Having established that the ORF47 protein is essential in vivo (21), we investigated whether its kinase activity accounts for the essential role of ORF47 as a virulence determinant. To address this question, we constructed VZV recombinants with ORF47 mutations that were designed to disrupt kinase activity. Recombinant viruses with mutations that altered conserved kinase motifs or resulted in a C-terminal truncation of ORF47 protein were characterized for phosphorylating activity, protein complex formation, and intracellular localization of ORF47 and IE62 proteins in vitro and for infectivity in human skin in the SCID-hu mouse model.

The ORF47 protein kinase mutations were designed by analysis of the ORF47 sequence to identify predicted kinase domains. By sequence comparison, the ORF47 protein appears to contain the 11 kinase subdomains typical for all members of the large serine/threonine kinase family and the sequences that distinguish it from tyrosine kinases (5, 6). The central core of the catalytic subdomains starts with subdomain VI, which corresponds to amino acid residue 240 in the ORF47 protein. Within the central core subdomains of kinases, conserved motifs (containing invariant amino acids) are essential for catalytic functioning. In subdomain VII, for example, the highly conserved DFG motif is necessary for ATP binding. The invariant aspartate in this motif is believed to orient the γ phosphate of ATP for substrate transfer by chelating the activating Mg2+ ions. Point mutations at this site have been shown to abolish kinase activity in v-fps, a viral tyrosine kinase (22). In ORF47 protein, the corresponding putative ATP-binding site reads DYS, with phenylalanine and glycine being very conservative changes from tyrosine and serine, respectively. The APE motif is another highly conserved sequence in subdomain VIII. This site, which corresponds to PPE in the kinase domain (5, 6). Mutational analysis has shown that all three amino acids in the APE motif are essential for kinase function in v-src (2). Exchange of a conserved amino acid, lysine 169, in the ORF47 protein, which is upstream of the central core kinase domains in subdomain II, reduced ORF47 autophosphorylation in vitro by almost 50% (12).

In the present experiments, ORF47 mutations that resulted in a C-terminal truncation or disrupted the DYS kinase motif abolished ORF47 kinase activity and were associated with extensive nuclear retention of ORF47 and IE62 proteins in vitro. Disrupting ORF47 kinase function resulted in a marked decrease in VZV replication and cutaneous lesion formation in skin xenografts in vivo. However, infectivity was not blocked completely as long as binding of the ORF47 protein and the IE62 protein, which was mapped to the N-terminal domain of the ORF47 protein, was preserved. These experiments indicate that ORF47 kinase activity is of critical importance for VZV infection and cell-cell spread in human skin in vivo, but it is the interaction between the two VZV tegument proteins, the ORF47 protein and the IE62 protein, that defines the essential contribution of the ORF47 protein to VZV replication in vivo.

MATERIALS AND METHODS

Construction of mutations in ORF47. A modification of a previously described cosmid transfection system divided the largest of the original cosmids, pPm2c, into two overlapping cosmids, pAI171 and pAvr102, facilitating mutations of ORF47 (17, 26a). The first step in making VZV ORF47 mutant viruses was to introduce targeted mutations into ORF47 in the cosmid pAvr102, which was the fourth of five overlapping VZV DNA fragments cloned into SuperCos1 cosmid vectors (Stratagene, La Jolla, Calif.) (10, 26a). A SalI-Accl fragment (12 kb) was excised from pAvr102 and cloned into pNEd19. A 2.5-kb SalI-ScaI fragment was subcloned and used as a template for PCR to introduce the mutations. Three mutations were made, including a truncation of ORF47, designated ORF47AC, which terminated ORF47 at the start of the sequence for the kinase domain in the 3′ half of ORF47, directly behind a unique SalI site at nucleotide 799; an ORF47 D-N point mutation at nucleotide 847 that replaced aspartic acid with asparagine in the conserved DYS kinase motif; and an ORF47 P-S point mutation at nucleotide 997 which replaced the second conserved proline in the PPE motif with serine. To make ORF47AC, the sense primer included the SalI restriction sequence preceded by eight random nucleotides, a Flag Tag sequence, a stop sequence with stop codons in all three frames, an introduced PciI restriction site, and 36 bases of the ORF47 sequence directly following the SalI site (5′-GGCCCGCCACGTCGACAGTTAACAAGGCAGGACG 

The replication kinetics and peak titers of rOka, rOka47S, and rOka47AC viruses were determined by plaque focus assay (7, 19).
FIG. 1. Schematic illustration of the construction of ORF47 mutants. (Panel 1) VZV genome, showing the localization of the ORF47 gene. (Panel 2) Five-cosmid system used to produce mutant viruses. ORF47 is located in pAvr102. (Panel 3) Schematic depiction of ORF47 indicating the coding sequence of the kinase domain in the 3’ half of the gene and the conserved kinase motifs that were targets for point mutations (shown in amino acid single-letter code). (Panel 4) A 2.5-kb SalI-SacI fragment subcloned into pNEB193 (via a 12-kb SalI-AscI fragment cloned into pNEB193; step not shown) containing the 3’ half of ORF47 and downstream sequences. This plasmid was used to produce the ORF47 mutations as follows: (a) insertion of STOP codons at the beginning of the kinase domain code, yielding the ORF47ΔC construct; (b) point mutation in the DYS motif, yielding the ORF47ΔN construct; and (c) point mutation in the PPE motif, yielding the ORF47ΔP-S construct. All mutants were constructed via PCR using the SalI restriction site as a sense and mutagenesis primer and a SalI site located 250 bp downstream of the SalI fragment cloned into pNEB193 (via a 12-kb SalI-SacI fragment subcloned into pNEB193; step not shown) containing the 3’ half of ORF47 and downstream sequences. This plasmid was used to produce the ORF47 mutations as follows: (a) insertion of STOP codons at the beginning of the kinase domain code, yielding the ORF47ΔC construct; (b) point mutation in the DYS motif, yielding the ORF47ΔN construct; and (c) point mutation in the PPE motif, yielding the ORF47ΔP-S construct. All mutants were constructed via PCR using the SalI restriction site as a sense and mutagenesis primer and a SalI site located 250 bp downstream of the SalI site and very close to the PPE motif as a location for the antisense primer (a and b) or an AscI site located 250 bp downstream of the SalI site and very close to the PPE motif as a location for the antisense and mutagenesis primer (c). All mutants were reinserted into pAvr102 via the SalI-AscI construct.
The rOka and rOka47P-S viruses replicated to close to $10^4$ PFU per implant at day 14 after inoculation; rOka47P-S titers remained equivalent to those of rOka at days 21 and 28 (Fig. 3A). In contrast, growth of rOka47ΔC and rOka47D-N was decreased and was delayed significantly. On day 14, infectious virus was recovered from only 3 of 10 implants inoculated with rOka47ΔC; the mean titer for positive implants was $10^3$ PFU per implant; and 1 of 10 implants inoculated with rOka47D-N yielded infectious virus on day 14, with a mean titer of $5 \times 10^2$ PFU in this implant. On days 21 and 28, rOka47ΔC and rOka47D-N titers remained approximately 10% or less of those in skin xenografts inoculated with rOka (Fig. 3A). Over all time points, infectious virus was recovered from 82% (23/28) of rOka-infected implants and 83% (25/30) of rOka47P-S-infected implants. Conversely, viral growth was detected in only 32% (10/31) of implants inoculated with rOka47ΔC and 22% (7/32) of those inoculated with rOka47D-N (Fig. 3B). The expected ORF47 sequence was confirmed for all mutants recovered from skin implants (data not shown).

**Immunohistologic analyses of rOka47 mutants in human skin xenografts.** Infection with rOka and rOka47P-S resulted in cutaneous lesions by day 14, which appeared in the epidermis and progressed through the dermis-epidermis junction (basement membrane) (Fig. 4). In contrast, rOka47ΔC infection progressed much more slowly, involving only the epidermal layer at day 14; VZV-infected cells were not detected at day 14 after inoculation of rOka47D-N. By day 21, rOka and rOka47P-S had produced extensive lesions at the epidermal surface, disrupted the basement membrane extensively, and infected multiple hair follicles located on the dermal side of the dermis-epidermis junction. In contrast, rOka47ΔC and rOka47D-N generated small lesions, infection did not penetrate the basement membrane, and hair follicles were not infected. By day 28, all of these cytopathologic differences were even more pronounced; the rOka and rOka47P-S viruses had spread throughout the epidermal layer and into many hair follicles, and large sections of the basement membrane had disappeared. In contrast, rOka47ΔC and rOka47D-N infection remained highly localized without penetrating the basement membrane. Infection with rOka47ΔC had some disruptive effect on the cells of the basement membrane, which were enlarged and less well ordered, but no virus-infected cells were detected in the dermis. rOka47D-N was detected in a single hair follicle on the dermal side of the basement membrane (Fig. 4).

Since CKII can complement ORF47 kinase function, we examined whether the impaired replication of rOka47ΔC and rOka47D-N in skin xenografts was related to a difference in CKII expression in skin versus cells grown in cultures in vitro. When CKII was assessed by Western blot analysis of uninfected skin xenografts, uninfected melanoma cells, and human lung fibroblasts (HEL and MRC-5 cells), CKII detection was equivalent in lysates of skin cells and cells grown in cultures (Fig. 5).

**Immunoprecipitation and ORF47 kinase experiments.** Based upon the observed differences in the pathogenicity of rOka47 mutants in vivo, further investigations of ORF47 kinase function and ORF47 protein binding to IE62 protein were done in melanoma cells infected with the rOka47ΔC,
rOka47D-N, and rOka47P-S mutants in vitro. A series of immunoprecipitation and kinase assays demonstrated that the binding of ORF47 and IE62 proteins was mediated by the N-terminal region of the ORF47 protein, whereas phosphorylation depended upon the kinase domain located in the C-terminal region and, specifically, on the DYS motif at residues 282 to 284.

Melanoma cells were infected with ROka47S, rOka, rOka47ΔC, rOka47D-N, or rOka47P-S, cell lysates were prepared, and ORF47 protein and associated proteins were precipitated with ORF47 antiserum. Complex formation between ORF47 and IE62 proteins was detected by Western blotting in cells infected with rOka, rOka47ΔC, rOka47D-N, and rOka47P-S (Fig. 6a). No complex formation occurred in cells infected with ROka47S, a recombinant virus that expresses only the first 165 amino acids of ORF47 protein due to the insertion of a STOP codon (8); ROka47S (kindly provided by Jeffrey Cohen, National Institutes of Health) was used as a negative control. Expression of the full-length ORF47 kinases and the ΔC-truncated protein was documented as a 30-kDa band (Fig. 6b), and testing for IE62 protein expression confirmed equivalent infection levels in all lysates (Fig. 6c). ORF47 protein binding to IE62 protein was preserved in both point mutants and the truncation mutant but was not observed with the ROka47S control.

To investigate whether mutant ORF47 proteins phosphorylate IE62 protein and autophosphorylate, melanoma cells were infected with rOka, rOka47ΔC, rOka47D-N, and rOka47P-S; cell lysates were then immunoprecipitated with ORF47 antiserum and evaluated by in vitro kinase assays. The intact ORF47 protein from rOka-infected cells and the ORF47P-S protein phosphorylated IE62 protein and autophosphorylated in vitro, whereas the ORF47ΔC and ORF47D-N mutant proteins did neither (Fig. 7A). As the second step in these experiments, the filter was probed with IE62 antiserum, again confirming preservation of IE62 protein binding for all of the three mutant forms of ORF47 protein (Fig. 7B).

To document that this functional analysis of ORF47 protein domains was valid and relevant to VZV pathogenesis in vivo, the ORF47 kinase assay was also performed using rOka, rOka47ΔC, rOka47D-N, and rOka47P-S viruses that were recovered 28 days after infection of skin xenografts. These experiments showed that the mutant ORF47 kinases ORF47ΔC and ORF47D-N did not phosphorylate IE62 protein or autophosphorylate, whereas intact ORF47 protein and the ORF47P-S point mutant phosphorylated IE62 and ORF47 proteins (Fig. 7C). This result indicates that the slow-growing rOka47ΔC and rOka47D-N viruses did not acquire any kinase gain-of-function mutation during replication in skin in vivo, which might have
restored the capacity of their respective ORF47 proteins to phosphorylate IE62 or to autophosphorylate. ORF47 protein binding to IE62 protein was again demonstrated for all mutants by probing with IE62 monoclonal antibody (Fig. 7D).

Localization of ORF47 and IE62 proteins in infected cells in vitro. To further evaluate the effects of ORF47 mutations, the intracellular localization of ORF47 protein and IE62 protein was examined at 20, 30, and 42 h after infection of melanoma cells with rOka and rOka47 mutants. As shown in representative examples (Fig. 8), ORF47 protein localized to the cytoplasm and was expressed quite extensively on plasma membranes in cells infected with rOka but was detected in only a few nuclei within syncytia formed by rOka-infected cells. In contrast, ORF47 protein was detected in the nuclei of most

![Image](https://example.com/image1.png)

FIG. 5. Expression of CKII in cell cultures and SCID-hu skin xenografts. Lane 1, CKII α and β chain (Upstate Biotechnology, Inc.) (+); lane 2, melanoma cells (MeWo); lane 3, MRC-5 cells; lane 4, HEL cells; lane 5, skin xenograft cells. Lane 1 was used as positive control. All other lanes showed similar levels of CKII expression.

![Image](https://example.com/image2.png)

FIG. 6. Immunoprecipitation of IE62 protein with ORF47 antiserum from melanoma cells infected with rOka or rOka ORF47 mutants. (a) Melanoma cells were infected with rOka47S (47S), rOka, rOka47ΔC (ΔC), rOka47D-N (D-N), or rOka47P-S (P-S). Infected cell lysates were incubated with ORF47 antiserum (+) or preimmune serum (−) and subjected to SDS-PAGE. A Western blot filter was probed with IE62 antiserum. A band slightly above the 172.6-kDa marker (which corresponds to the 175-kDa weight of IE62) was detected in lanes rOka, ΔC, D-N, and P-S (containing the ORF47 antiserum) but not in the lanes containing the rOka47S virus (kindly provided by J. Cohen, National Institutes of Health). (b and c) Western blot of total lysates. As a control, 20-μl portions of the infected cell lysate described above were directly subjected to SDS-PAGE. The filters were probed with ORF47 antiserum (b) or IE62 antiserum (c).
cells infected with rOka47ΔC or rOka47ΔD-N as well as in the cytoplasm, indicating nuclear import and retention of ORF47 protein when kinase domains were disrupted. Like intact ORF47 protein, ORF47P-S protein was not detected in nuclei of cells infected with rOka47P-S. These patterns of ORF47 protein distribution were observed at all time points in cells infected with rOka and the rOka47ΔC, rOka47ΔD-N, and rOka47P-S mutants.

The intracellular distribution of IE62 protein was also altered dramatically in cells infected with rOka47ΔC and rOka47ΔD-N, with IE62 protein being almost exclusively nuclear at all time points. In contrast, IE62 protein was present in cytoplasm as well as nuclei at 20, 30, and 42 h after infection with rOka and rOka47P-S (Fig. 8). IE62 protein is also cytoplasmic at these times in cells infected with the rOka47S null mutant (data not shown) (13). Thus, preserving the binding of ORF47 and IE62 proteins while abolishing ORF47 kinase activity was associated with an aberrant nuclear retention of ORF47 and IE62 proteins. The changes in cellular localization of these critical VZV proteins were not associated with altered production of infectious virus in cells infected with rOka47ΔC or rOka47ΔD-N in vitro (Fig. 2), whereas viral replication was significantly reduced in differentiated human skin cells in vivo (Fig. 3).

**DISCUSSION**

The construction of VZV recombinants with targeted mutations in the ORF47 gene and evaluation of these rOka47 mutants in vitro and in human skin xenografts in vivo indicate that the ORF47 protein has two important and distinct functions, which are kinase activity and IE62 protein binding. Experiments with the ROKa47S stop codon mutant demonstrated that ORF47 protein is essential for skin infection (21). Since kinase activity was its defined function, the requirement of ORF47 protein for replication in vivo was attributed to its phosphorylation of viral and (perhaps) cellular protein substrates. We report now that rOka47 mutants with disrupted ORF47 kinase function retain the capacity to replicate in human skin, albeit with a markedly delayed and diminished growth phenotype. Since the ORF47 protein made by rOka47ΔC (which is truncated at 266 amino acids) binds IE62 protein, we suggest that ORF47 protein complex formation with IE62 protein, and perhaps with other proteins, is an essential function. This prediction is consistent with a model of VZV tegument formation in which ORF47 protein binds to IE62 protein (and perhaps to other tegument proteins encoded by ORFs 4, 9, 10, and 63) (reviewed in reference 35). Based on this model, we suggest that the N terminus of the ORF47 protein recruits the IE62 protein to the intracellular site of virion assembly and/or binds to the IE62 protein as a structural component of the tegument. If ORF47 protein were only acting as a kinase, abolishing its capacity to phosphorylate IE62 protein should have made its formation of complexes with IE62 protein irrelevant. In contrast, preserving these interactions was associated with some VZV replication in skin, whereas no infection occurred in the absence of ORF47 protein in vivo. Thus, we conclude that ORF47 protein has a function, mapped to its N terminus and independent of its kinase activity, which is necessary and sufficient for viral growth in vivo, and we suggest that this function involves binding to IE62 protein. Further, defining these two independent contributions of ORF47 protein to VZV pathogenesis required evaluation of ORF47 mutants in vivo in the SCID-hu model, because ORF47 protein is completely dispensable in vitro.

While ORF47 protein kinase activity was not essential in vivo, blocking kinase function severely restricted VZV infectivity in skin xenografts. Constructing VZV recombinant viruses with ORF47 mutations confirmed that kinase activity maps to the C terminus, as indicated in transient expression experiments, and established that the predicted kinase motif, DYS, is necessary for autophosphorylation and phosphorylation of IE62 protein. Most importantly, rOka47ΔD-N experiments demonstrated that the DYS motif is the essential kinase domain required for the characteristic replication of VZV in differentiated human skin cells within the intact tissue microenvironment in vivo. The conserved DFG motif (DYS in ORF47 protein) is invariant in virtually all kinases and is considered necessary for phosphate transfer from ATP to the substrate; ORF47 kinase activity was completely abolished by the D-N substitution. Mutation of the conserved motif, APE (PPE in ORF47 protein), to construct rOka47P-S had no effect on ORF47 kinase function or on VZV infectivity in vivo. Because the role of subdomain VIII appears to be to ensure the stability of protein folding, single residue changes, even within
the conserved central motif, may not necessarily generate a protein impaired in kinase activity (5, 6). Our experiments suggest that the proline-serine exchange did not alter the tertiary structure of ORF47 protein significantly. rOka47ΔC expressed a truncated ORF47, which might have retained some kinase function because sequence analysis predicts that the ORF47 kinase domain starts at amino acid 132 (http://smart.embl-heidelberg.de/) (16, 32). However, the core of the kinase domain, containing the putative ATP binding site, resides in the C terminus, and truncated ORF47 protein did not mediate phosphorylation. Of particular interest, whereas CKII compensates for ORF47 kinase activity in vitro (11), little infectious virus was recovered from skin xenografts infected with rOka47ΔC or rOka47D-N, despite the presence of CKII.

The comparative analysis of the consequences of disrupting ORF47 kinase activity on VZV replication in vitro and in skin xenografts in vivo provides new insights about how the ORF47 protein contributes to VZV pathogenesis. Our experiments with rOka47ΔC and rOka47D-N mutants support the accumulating evidence that cell-cell spread of VZV depends upon cell fusion and syncytia formation but does not require complete virion assembly in vitro (11). Conversely, experiments with these mutants in vivo indicate that cell-cell spread in intact human skin is highly dependent upon ORF47 kinase function. Further, based upon the failure to recover any infectious virus from skin xenografts infected with the ROka47S null mutant and upon the recovery of some infectious virus when the capacity of ORF47 protein to bind to IE62 protein was preserved, we suggest that complete virion assembly is required for VZV infection of human skin in vivo.

Our mutational analysis using VZV recombinants confirmed that ORF47 protein phosphorylates IE62 protein, which has been considered to reduce IE62 protein-mediated transactivation in the nucleus at early and late time points and enhance its accumulation at the cytoplasmic site of tegument formation. Experiments with the ROka47S stop codon mutant have shown no difference (13) or some delay in the translocation of IE62 protein from the nucleus to the cytoplasm (11), suggesting that the second VZV serine/threonine kinase (encoded by ORF66) or CKII substitutes to phosphorylate IE62 protein (11, 13). In our experiments, replication was not affected by the persistence of a predominantly nuclear localization of both IE62 and ORF47 proteins in cell cultures infected with rOka47ΔC or rOka47D-N. Nuclear expression of ORF47 protein was rare in rOka-infected syncyta but was quite pronounced in rOka47ΔC- and rOka47D-N-infected cells. The limited transport of IE62 protein from the nucleus to the cytoplasm in cells infected with rOka47ΔC or rOka47D-N may be secondary to increased transfer of nonphosphorylated ORF47 protein into the nucleus, where it binds but does not phosphorylate IE62 protein. Nevertheless, infectious virus yields were not reduced, indicating that only minimal cytoplasmic localization of IE62 and ORF47 proteins, presumed to be mediated by ORF66 kinase or CKII phosphorylation, is necessary for VZV replication in vitro. In contrast, inoculation of skin xenografts with rOka47ΔC or rOka47D-N suggests that such complementation provides very limited support for VZV replication in vivo, probably by allowing some cytoplasmic translocation of IE62 and ORF47 proteins and virion tegument assembly which does not occur in skin in the absence of ORF47 protein. The documentation that rOka47ΔC or rOka47D-N viruses recovered from skin xenografts remained “kinase dead” and did not phosphorylate IE62 protein supports the interpretation that the contribution of ORF47 protein to virion formation was the critical factor in permitting some replication in vivo.

The immediate phosphorylation of other virion tegument proteins, including IE62 protein, after viral entry to facilitate tegument disassembly is another possible function of ORF47 protein. HSV tegument disassembly has been shown to be regulated by phosphorylation of component proteins (24). Even if VZ virions could be assembled despite the blocking of ORF47 kinase activity, phosphorylation by ORF47 protein could be important for tegument disassembly. Cell-cell spread and replication of rOka47ΔC and rOka47D-N would not be affected in vitro, assuming that complete virion assembly is not required. However, slower tegument disassembly when ORF47 protein is made but its kinase activity is abolished could contribute to the delayed and restricted replication of rOka47ΔC and rOka47D-N mutants in differentiated skin cells in vivo.

In addition to its interactions with IE62 protein, both as a phosphorylation substrate and as a tegument component, disruption of ORF47 kinase function may have altered the intracellular trafficking of gE, which would be predicted to impair VZV replication in vivo. Kenyon et al. showed that ORF47 protein targets serines in the acidic cluster of gE for phosphorylation and causes gE recycling from plasma membranes to the trans-Golgi network (TGN), whereas CKII preferentially phosphorylates threonines and enhances gE association with plasma membranes (11). Trafficking of gE to the TGN, the putative site of virion assembly, was reduced substantially in cells infected with the ROka47S mutant (11), but increased trafficking of gE to plasma membranes enhanced syncytium formation and cell-cell spread, and infectious virus yields were not affected in vitro. Nevertheless, some low-affinity phosphorylation of serines in the gE acid cluster was mediated by CKII in ROka47S-infected cells and some gE localization to the TGN was observed (11). Given the presence of CKII in skin cells, these observations are consistent with the limited replication of rOka47ΔC and rOka47D-N in vivo, despite the blocking of ORF47 kinase function.

The VZV ORF47 protein is representative of homologous gene products that are conserved in all of the herpesviruses (3, 31). This evaluation of VZV recombinants with targeted mutations in ORF47 protein for effects on VZV replication in human skin in vivo in the SCID-hu model of VZV pathogenesis suggests that ORF47 protein and related gene products are likely to be important as both virion components and viral kinases.

FIG. 8. Confocal analysis of melanoma cells infected with rOka and rOka ORF47 mutants for expression of ORF47 and IE62 proteins. Melanoma cells were infected with rOka, rOka47ΔC (ΔC), rOka47D-N (D-N), or rOka47P-S (P-S) for 30 h. ORF47 kinase was detected with a secondary fluorescein isothiocyanate-conjugated antibody (green), and IE62 was detected with a secondary Texas Red-conjugated antibody (red). Images of each row were merged. Colocalizations of ORF47 kinase and IE62 appear as yellow.