Regulation of icosahedral virion capsid size by the *in vivo* activity of a cloned gene product

(cosmid/morphogenesis/satellite virus P4/scaffolding protein/self-assembly)

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ABSTRACT Determination of icosahedral virion capsid size can be directly studied during helper-dependent lytic development of satellite P4 because the assembly pathway specified by the P2 helper virus is altered to yield smaller-sized capsids. Size determination (*sid*) mutations identify a P4-encoded function regulating this process. To determine whether the *sid* gene product is necessary and sufficient to redirect the assembly pathway, we (i) cloned the *sid* structural gene in a plasmid vector (pMA30) under the control of an inducible promoter and (ii) constructed a packaging substrate (pMA1), a P4 genome-sized plasmid containing only that region of P4, the *cos* site, necessary for encapsidation. Superinfection by P2 of a host carrying pMA30 under induced conditions resulted in a shift from large to small capsid production. P2 superinfection of a host carrying the *cos* plasmid pMA1 plus pMA30 under induced conditions yielded pMA1-transducing particles of P4 capsid size. These cloning-based analyses directly demonstrate that *sid* protein is the only P4 gene product required for small-capsid size determination. In the absence of the P2 *O* gene product no capsids of any size are assembled during solo infection by P2. Nevertheless, P2 *Oam* mutant superinfection of a host carrying pMA1 and pMA30 under induced conditions yielded small P4-sized transducing particles. We therefore propose that (i) the *sid* gene product competes with the *O* gene product to determine the assembly of small vs. large capsid sizes and (ii) both gene products probably function as temporary scaffolding proteins.

Regular polyhedra composed of identical subunits were proposed by Crick and Watson (1) as the basic capsid structure of simple spherical viruses. To account for both the 5- and 6-fold clustering of such subunits necessary to the formation of icosahedral shells, Casper and Klug (2) conceived of a related model involving conformational flexibility of the identical subunit proteins based on the "quasi-equivalence" of their capsid shell environments. Such flexibility proved to be not an intrinsic property but to result from specific interactions with virally encoded scaffolding proteins (3–5). Thus, rather than the simple "self-assembly" of identical subunits, *in vivo* construction of an icosahedral virion shell typically involves a pathway of ordered steps that provide the perturbations necessary to induce the quasi-equivalence of the capsid subunits. A striking example of this phenomenon occurs during the lytic development of satellite virus P4. Here, the assembly pathway normally followed by a helper virus-encoded major subunit capsid protein is perturbed by the activity of a P4-encoded gene product (gp). The result of this interference is a distinct class of smaller-sized functional icosahedral capsids appropriate for encapsidation of the P4 genome (6). This report focuses on the gps that shift the

helper virus-encoded capsid subunits into alternate assembly pathways.

Although originally described as a satellite virus (7), P4 *virI* is a naturally occurring phasmid (phage plasmid hybrid) because it is capable of plasmid, lysogenic, and lytic modes of propagation. During lytic development, P4 requires the 17 morphogenic gps of a coinfecting or lysogenic helper virus such as coliphage P2 (8). P4 appears to redirect the assembly process specified by the helper-encoded morphogenic gps because the resultant icosahedral capsids are one-third the volume of those found assembled by P2 in the absence of P4 (9). These small-sized capsids, while appropriate for encapsidation of the 11.4-kilobase (kb) P4 genome, are incapable of completely encapsidating the larger 33-kb P2 genome (6).

Size determination (*sid*) mutants of P4 are unable to shift the P2-specified capsid assembly pathway into the small-capsid assembly mode (10). P4 *sidI* infection of a P2 lysogen yields large P2-sized capsids, into which are packaged dimeric or trimeric copies of the P4 *sidI* genome (10). The precise role of the *sid* gp has been difficult to identify because of a perplexing labyrinth of reciprocal regulatory interactions involved in the P4–P2 interaction. In particular, the *sid* gene is located at the proximal end of a polycistronic operon that is composed of genes *sid*, δ , and *psu* (11). Transcriptional polarity of amber mutations in the *sid* gene results in a complex pleiotropic phenotype due to the failure to express the downstream genes δ and *psu*, the gps of which normally act in cis and in trans to regulate expression of P4 and P2 (12). To circumvent these complex regulatory interactions involving the *sid*– δ –*psu* operon and its encoded gps, we cloned the *sid* gene in a plasmid vector under the inducible control of the *araBAD* promoter (13). Consequently, the effect of the *sid* gp on the capsid assembly pathway specified by the helper phage could be characterized in the absence of all other P4-encoded functions.

MATERIALS AND METHODS

Bacterial Strains. *Escherichia coli* indicator strains C1055 *nal*^r (14) and C1a (15) were used for P2; C1792 (P2 *lg cc*) (16) was used for P4; and JM107 was the host for pGBT518 (17). David Kurnit (University of Michigan) provided strains C3150, a C1a Δ (*srl-recA*) 306 strain; C3152, a P2 *lg cc* lysogen of C3150, and DK1, a MC1061 Δ (*srl-recA*) 306 strain (18).

Phages and Plasmids. P2 *virI* is an immunity-sensitive clear-plaque mutant (19); P2 *lg cc* is a large-plaque mutant (20). P4 *virI* is a spontaneously occurring immunity-insensitive clear-plaque mutant (7). The *virI* mutation also

Abbreviations: gp, gene product; PFU, plaque-forming unit(s); *sid*, size determination.

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FIG. 1. Schematic diagram displaying the strategy for construction of the *sid* expressor plasmid pMA30, a plasmid carrying a pACYC184 origin of replication and the P4 *sid* structural gene cloned under inducible control of the *araBAD* operon promoter. The *sid* gene was isolated on a 1337-bp *XmnI*–*HindIII* fragment of pP4 *virI* (11, 26) and subcloned into the deletion mutagenesis Altergene M13 derivative vector pGBT518 (Gold Biotechnology, Saint Louis) to generate pMA131. Single-stranded (ss) DNA was isolated after infection with helper phage M13KO7. This ssDNA served as a template for deletion of the *sid* promoter (P_{sid}) based on oligonucleotide-directed site-specific *in vitro* deletion mutagenesis. For this purpose a complementary 33-mer oligonucleotide (gift of Derek Woods, Ortho Diagnostics) of the sequence 5'-GATGATTAATTGT CAAGTCGACTGACGAGGAGC-3' was prepared to hybridize, as

allows for maintenance of P4 in the plasmid state (21). pP4 (plasmid P4) *virI* *EcoRI* AD:Km^r (kanamycin resistant) and pP4 *virI* *sidI* *EcoRI* AD:Km^r are derivatives of pP4 *virI* and pP4 *virI* *sidI*, respectively, constructed by replacement of the P4 *EcoRI* restriction fragments B and C with a Km^r gene; all such derivatives can be propagated either as plasmids or helper-dependent phages (22).

Preparation of Infected Cell Lysates. Overnight bacterial cultures were diluted 1:100 in LB medium [+/-1% (wt/vol) L-arabinose]. Appropriate antibiotics were added, and the cells were grown at 37°C to midlogarithmic phase. Calcium chloride was added to a final concentration of 5 mM to promote phage adsorption. Phage were added at a multiplicity of infection of 10 and allowed to adsorb at room temperature for 15 min. The infected cells were then pelleted at $10,000 \times g$ for 10 min at 4°C and resuspended in fresh medium. This step was repeated twice to remove unadsorbed phage, after which the cells were resuspended in 5 ml of LB medium plus appropriate antibiotic and allowed to grow in a shaking water bath at 37°C until lysis.

Construction of Cosmid pMA1, a Packagable P4-Sized DNA Substrate That Expresses No P4 *gps*. pMA1 was constructed by cloning a 430-base-pair (bp) *EcoRI*–*ClaI* fragment carrying the *cos* site of pP4 *virI* between the unique *EcoRI*–*ClaI* sites of pBR322 (23, 24). To expand the size of this P4 *cos*–pBR322 hybrid to that of the P4 genome, a 6203-bp *BamHI* fragment from adenovirus type 2 (25) was cloned into the unique *BamHI* site of the P4 *cos*–pBR322 hybrid plasmid.

Construction of the *Sid* Expressor Plasmid pMA30. See legend to Fig. 1.

Electron Microscopy. Preparation and analyses were as described (10, 30).

RESULTS

***cos* Plasmid pMA1 Is a Viable DNA Substrate for Packaging into Small and Large Capsids of P4- and P2-Size, Respectively.** P4 infection of an *E. coli* host lysogenic for helper P2 results in a burst of small P4-sized progeny phage (6, 9). If the *cos* plasmid pMA1, a genome of P4-size containing no P4 sequences except the *cos* site, can serve as a substrate for packaging into small P4-sized capsids, then P4 superinfection of a host lysogenic for P2 and also harboring pMA1 should result in a mixed burst of P4-sized virions that have encapsidated either a copy of the P4 genome or pMA1. Because P4 can form plaques on a P2 lysogenic host and pMA1 can confer

follows, to the pMA131 ss template: the 5' terminus (5'-GATGATTAAT-3') with the truncated promoter for *lacZ* at one end and the 3' terminus (5'-TGACGAGGAGC-3') to regions immediately downstream of P_{sid} . The strategy was to create a 460-base loop-out containing P_{sid} to be targeted for deletion. The central portion of the oligonucleotide, which would be looping in during hybridization to template pMA131, contained the sequence 5'-TGTCAAGTCGAC-3' so as to reconstitute the -35 region of the truncated *lacZ* promoter and to provide an engineered *SalI* restriction site to allow subcloning of the *sid* structural gene from the resultant deletion plasmids. Deletion mutants of pMA131 were identified by blue-white screening of colonies (27). From one of these constructs, pMA132, the 1.5-kb *SalI*–*EcoRI* fragment encoding the *sid* structural gene, was subcloned into the multicloning site of the expression vector pING1 (13), to yield pMA135. This placed the *sid* structural gene under the inducible control of the promoter for the arabinose *BAD* operon (P_{araBAD}) of *Salmonella typhimurium* carried by pING1. To make pMA135 compatible with cosmid pMA1, the *EcoRI*–*AvaI* fragment of pMA135 carrying a pBR322 origin of replication was exchanged for the 3.1-kb *EcoRI*–*AvaI* fragment of pACYC184 carrying a p15A origin of replication (28, 29); the resulting plasmid was designated pMA30. Abbreviations: P_{sid} , promoter for the *sid*–*psu* operon; *cat*, chloramphenicol acetyltransferase gene conferring chloramphenicol resistance; δ' , truncated fragment of the δ gene.

ampicillin resistance, the encapsidated genomes can be differentiated. After infection with P4 *vir1*, the P2 lysogenic host C3152 carrying pMA1 produced a lysate containing 1.6×10^9 P4 plaque-forming units (PFU)/ml and 3.8×10^6 pMA1 Amp^r transducing particles per ml.

We also expected that pMA1 could be packaged into P2-sized capsids because (i) it carried the *cos* site sequence required for encapsidation of both the P2 and P4 genome (31) and (ii) we had previously observed that large P2-sized capsids encapsidated dimers and trimers of the genome P4 *vir1 sid1* (10). Lysates prepared after P2 superinfection of *E. coli* strain C3150 harboring pMA1 yielded 1.4×10^9 P2 PFU/ml and 9×10^3 pMA1 Amp^r transducing particles per ml. Thus, pMA1 could be encapsidated and transduced by small-sized P4 capsids with ≈ 400 -fold greater efficiency than by large-sized P2 capsids.

Complementation of pP4 *vir1 sid1 EcoRI AD:Km^r* by pMA30. P2 infection into host cells carrying P4 *vir1* in the plasmid state results in lytic induction of P4 (21, 32). The resulting lysate includes both large helper P2-sized and small P4-sized progeny virions that can be separated based on their densities. Likewise, P2 superinfection of a host carrying the *sid* mutant P4 *vir1 sid1* in the plasmid state yields a progeny burst of both P2 PFU and P4 *sid1* PFU. However, >99% of the P4 PFU consist of large P2-sized virions carrying two or three copies of the P4 *vir1 sid1* genome (P. Lozano, D. Kurnit, and R.G., unpublished results). After CsCl equilibrium density-gradient centrifugation, P4 *sid1* trimeric and dimeric PFUs can be resolved at slightly below P2 density and at an intermediate density between that normally found for P2 and P4 PFUs, respectively (6, 10).

A functional *sid* gp expressed from pMA30 (Fig. 1) should complement the P4 *sid1* mutant for the small-capsid size determining function. We therefore analyzed density-gradient profiles of the lysate progeny resulting from P2 *vir1* superinfections of *E. coli* strain DK1 carrying (i) pP4 *vir1 sid1 EcoRI AD:Km^r* and the negative control pACYC184 (Fig. 2A) and (ii) pP4 *vir1 sid1 EcoRI AD:Km^r* and pMA30 under induced conditions for expression of *sid* (Fig. 2B). Expression of *sid* from pMA30 resulted in a 200-fold increase in the burst of P4 *sid1* progeny particles, from 5×10^8 (Fig. 2A) to 10^{11} PFU/ml (Fig. 2B). Concomitantly, a new PFU band appeared at P4 *wt* density (Fig. 2B). There was a 200-fold increase in progeny phage banding at this P4 *wt* density compared with the P4 PFUs banding at P2 density and intermediate density (comparing bands within Fig. 2B). These latter two non-P4 density peaks correspond to trimers and dimers of P4, respectively, that are encapsidated within large P2-sized capsids. Capsid sizes were confirmed by EM. These results show that the *sid* gp as expressed from pMA30 is a biologically functional gp able to complement pP4 *vir1 sid1*.

Sid Redirects P2 Morphogenic gps to Make Viable Small-Sized Capsids. If *sid* is sufficient for small-capsid size determination, then its expression from pMA30 should switch P2-specified morphogenesis from large-to-small capsid pathways. If the resultant small capsids are functional, then they should encapsidate the P4-sized cosmid pMA1, thereby producing infectious particles that can confer Amp^r to indicator strains. Induction of *sid* protein from pMA30 should also reduce the burst of P2 because the larger genome of P2 cannot be completely encapsidated within a small P4-sized capsid.

The experimental observations conformed to all of these predictions. The density-gradient profiles of lysates resulting from P2 *vir1* superinfection of strain DK1 harboring pMA30 and pMA1 are shown for noninducing conditions (Fig. 3A) and inducing conditions (Fig. 3B). Induction of *sid* resulted in a 500-fold decrease in helper P2 PFUs (compare Fig. 3A and B) and a 200-fold increase in the burst of Amp^r pMA1 transducing particles. Further, a new Amp^r transducing par-

FIG. 2. CsCl equilibrium density-gradient centrifugation profiles of lysates resulting from P2 *vir1* superinfection of *E. coli* strain DK1 carrying plasmids: pACYC184 and pP4 *vir1 sid1 EcoRI AD:Km^r* (A) and pMA30 and pP4 *vir1 sid1 EcoRI AD:Km^r* (B). Equilibrium density centrifugation of the lysates was according to Shore *et al.* (10). Every fraction was titrated for P2 PFU and P4 PFU. P2 ρ and P4 ρ denote fractions, based on density, at which wild-type P2 and P4 PFUs normally band.

title peak appeared that banded at P4 density and contained ≈ 200 -fold more progeny than was detected at P2 density (Fig. 3B). EM was used to confirm capsid sizes. Thus, the *sid* protein is necessary and sufficient for redirection of the capsid pathway of helper phage P2 and the subsequent assembly of functionally active small P4-sized capsids.

Complementation of P2 *Oam279* by Sid Protein Yields Small Capsids. Only the genes *N* and *O* of phage P2 are required for the assembly of large P2-sized precursor capsid structures during solo infection by P2 (9, 33). Although a processed form of the *N* gp accounts for the major subunit of both the P2- and P4-sized capsid, the *O* gp is not found as a structural component within either type of capsid (33, 34). The final form of the *N* protein as found in the P2 capsid results from a multistep cleavage process that is strictly dependent on the activity of the *O* gp (9, 33). This relationship accounts for the finding that no capsid structures are assembled during solo infection by the P2 mutant *Oam279* (33). Because only the *O* gp of P2 is required for the assembly of large capsids composed of *N* protein cleavage subunits, we assessed whether *sid* could complement an *O* gene mutation of P2 and thereby restore the capsid assembly process.

P2 *Oam279* superinfection of strain DK1 harboring pMA30 and pMA1 under conditions of induction of *sid* expression resulted in a 2000-fold increase in the burst of Amp^r transducing particles containing pMA1 compared to that found for the noninduced condition (1.2×10^6 particles/ml vs. 6×10^2

FIG. 3. CsCl equilibrium density-gradient centrifugation profiles of lysates resulting from P2 *vir1* superinfection of *E. coli* strain DK1 carrying the compatible *sid* expressor plasmid pMA30 and the DNA packaging substrate cosmid pMA1 under conditions of noninduction of *sid* from pMA30 (A) and conditions of induction of *sid* expression from pMA30 (B). See legend to Fig. 2.

particles/ml). This increase in lysate titer correlated quantitatively with the appearance of small P4-sized capsid phage particles as visualized by EM. No burst of P2 was detected in the induced or the uninduced lysates. Thus, the P4 *sid* protein can complement for the absence of the otherwise essential P2 *O* gp. Moreover, the resulting capsids are of small P4-size, suggesting that it is the *O* gp of P2 that directs the assembly of the larger P2-sized capsids.

DISCUSSION

Scaffolding-type proteins, as a class, are essential in determining the singular functional size of the capsid assembled by icosahedral viruses such as P22 (35), λ (36), and T4 (37). In each case, the scaffolding proteins directly interact with major capsid subunits to form a temporary core structure essential for correct capsid size determination. In this respect, the P2–P4 interaction is unusual because “functional” icosahedral capsids of two different sizes can be polymerized from the same subunits. Nonetheless, because the mature capsids of both P2 and P4 PFUs are composed of identical major and minor subunits encoded by the P2 helper (34), the P2–P4 interaction likewise directly demonstrates that all of the information needed for correct assembly of a complex icosahedral capsid is not necessarily contained in the polymerized subunits.

Although the mechanism of large-sized capsid determination occurring during solo infection by P2 remains obscure, there are mutations of P4, designated as *sid*, that identify a P4-encoded function essential for small-sized capsid determination (10, 30). In the current study, we investigated (i) whether the *sid* protein is the only P4-encoded factor required

to shift the large-capsid assembly pathway specified by the P2 virus helper and (ii) whether the expression of the *sid* gene from a cloning vector could be used in complementation analyses to identify the P2-encoded function that determines large-capsid size.

Inducible Expression of the *Sid* Gene from a Cloning Vector. We initially evaluated the ability of the *sid* gp, as expressed from the construct pMA30, to complement the P4 *sid1* mutant. P2 superinfection of *E. coli* carrying pP4 *vir1 sid1* AD:Km^r plus pMA30 under conditions for *sid* expression resulted in a 200-fold increase in P4 *sid1* progeny burst size over that observed in the absence of *sid* expression. Equilibrium density-gradient profiles resolved a new P4 *vir1 sid1* AD:Km^r PFU peak at P4 density, consisting of small P4 capsid size virions; this peak was found only after expression of *sid* from pMA30 (Fig. 2B vs. A). There were 10⁶-fold more P4 *vir1 sid1* AD:Km^r PFU banding at this density than were found in the absence of *sid* expression. Thus, the *sid* gp as expressed from pMA30 can redirect the P2-specified assembly pathway. However, in these experiments an additional gp expressed by the P4 *sid* mutant could also have been involved in this process.

Only the *Sid* gp Is Required to Redirect the Capsid Assembly Pathway Specified by Helper Phage P2. To assay further the *sid* activity expressed from pMA30 (Fig. 1), we constructed a compatible, self-replicating, P4 genome-sized artificial DNA packaging substrate, pMA1, which carried the P4 *cos* site required for encapsidation plus a β -lactamase gene serving as an Amp^r selection marker. Based on its encapsidation and transduction, this cosmid was used to test the *in vivo* functionality of the small capsids assembled after expression of *sid* from pMA30 in the absence of the P4 genome.

After P2 superinfection of a host carrying pMA30 and pMA1, the burst of pMA1 transducing particles produced under conditions of induction of *sid* from pMA30 was 200-fold greater than under noninducing conditions. Further, equilibrium density-gradient analysis revealed that the increased burst included a new pMA1 Amp^r transducing peak, composed of small-capsid sized virions of P4 density (Fig. 3B). There were 5000-fold more pMA1 transducing units banding at this density than found in the absence of *sid* expression from pMA30 (Fig. 3A). Concomitant with this shift in the pMA1 banding pattern from P2 to P4 density was a 500-fold drop in P2 titer. This indicates interference with lytic development of the helper due to a shift in the assembly pathways, as first described by Diana *et al.* (6). We also observed a dosage-dependent effect of the *sid* protein on capsid size determination (data not shown; D. Kurnit and R.G., unpublished results). Taken together, these findings directly demonstrate that the switch from large-to-small capsid assembly pathways during the P2–P4 interaction is mediated by the activity of a single P4-encoded gp, *sid*.

P2 *N* Gene or *N* gp Is a Likely Target for *Sid* Activity. *Sid* responsiveness (*sir*) mutations, which render P2 resistant to small-capsid sized determination, map to the *N* gene of P2 (12); consequently, we examined the effect of the *sid* protein expressed from pMA30 on the size of the capsids assembled by the mutant P2 *sir2*. After P2 *vir1 sir2* superinfection of strain DK1 carrying pMA30 plus pMA1, the equilibrium density-gradient profiles of the P2 *vir1 sir2* PFU and pMA1 particles were identical regardless of whether or not *sid* expression was induced (data not shown). Thus, in contrast to the results obtained with P2 *vir1* (Fig. 3), *sid* expression caused essentially no interference with the large-capsid size assembly pathway specified by the P2 *sir* mutant. This result suggests that the P2 *N* gene or *N* gp is the most likely target for *sid* activity in redirecting the P2-specified capsid assembly pathway.

Scaffolding Proteins of Satellite Phage P4 and Its P2 Helper.

Is *sid* a scaffolding-like protein, analogous to those that determine icosahedral capsid size during the assembly of other viruses? The most compelling evidence is our observation that the *sid* protein is both necessary and sufficient to "redirect" the P2-specified morphogenic pathway. We have also observed a direct correlation between increased amounts of *sid* gp and increased numbers of small-sized capsids assembled [D. Kurnit and R.G., unpublished results; ref. 6]; such a stoichiometric relationship is likewise indicative of scaffolding-like activity. Further, the finding that *sir* mutations of the major capsid subunit render the helper resistant to *sid* activity strongly suggests that the *sid* protein forms the alternate scaffold necessary to assemble P4-sized capsids composed of these helper-encoded subunits.

From these observations, we hypothesized that there is an analogous P2-specified protein, *lrg* (large-capsid size determination), that directs the assembly of the larger P2-sized capsid, and, consequently, that among the morphogenic mutants of P2, only a P2 *lrg* gene mutant would be complemented by *sid* protein, yielding capsids of small size. Previous observations suggest that the *O* gp of P2 might have such a role. Aside from the major capsid structural subunit, which is a cleavage product of the P2 *N* gp, only the *O* gp is required for the assembly of precursor P2 capsid structures (9, 33). Nevertheless, like the essential capsid size determination proteins of viruses P4 (10, 34), P22 (35), T4 (37, 38), and λ (36, 39), the P2-encoded *O* protein is not found as a structural subunit of the mature capsid (34).

To test this hypothesis, we used the nonpolar amber mutant P2 *Oam279* (33) to superinfect strain DK1 carrying both pMA1 and pMA30. Compared with noninduced conditions, the expression of *sid* under induced conditions resulted in a 2000-fold increase in pMA1 transducing units and a corresponding increase in phage particles with P4-sized capsids. These results suggest that the *sid* gp expressed from pMA30 complemented for the absence of the P2 *O* gp and that the activities of *O* versus *sid* protein determine the expression of large- vs. small-sized capsids, respectively.

If the *O* gp is the hypothesized *lrg* factor, then this protein should not be required by P4 during its lytic development with a coinfecting or lysogenic helper. Such a premise appears to conflict with the finding of Six (8) that a P2 lysogenic helper with an *O* gene mutation did not support the lytic propagation of P4. Because this report is not in accord with the current results as to *sid* complementation of *O* to yield small capsids, an alternative and more complex model would be necessary to account for this finding. This model would involve the *O* gp as the scaffold protein for both P2- and P4-sized capsids and a secondary interaction of *sid* with the helper-encoded major capsid protein subunit to bring about subunit conformational flexibility necessary to the assembly of small-sized capsids. We do not favor the latter model because it does not account for all of the results described in this report. Rather, this apparent dichotomy (8) can more likely be attributed to the complex pleiotropic phenotypes of the involved mutations and the multiply reciprocal regulatory interactions between P2 and P4 (12). It is precisely for this reason that we analyzed capsid assembly using our simplified system for the inducible expression of *sid* from a cloning vector.

These interactions will be clarified further by cloning and inducibly expressing the *O* gene in the presence of the *sid*-expressor plasmid pMA30. Likewise, expression of the cloned P2 *N* gene in the presence of *sid* should clarify whether the *sid*, like the *O*, protein mediates the cleavage of the *N* gp, but by an alternate pathway that results in the assembly of small-sized capsids.

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