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A Baculovirus Blocks Insect Molting by Producing Ecdysteroid UDP-Glucosyl Transferase

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The predicted amino acid sequence of a newly identified gene of the insect baculovirus *Autographa californica* nuclear polyhedrosis virus was similar to several uridine 5′-diphosphate (UDP)–glucuronosyl transferases and at least one UDP-glucosyl transferase. Genetic and biochemical studies confirmed that this gene encodes an ecdysteroid UDP-glucosyl transferase (egt). This enzyme catalyzes the transfer of glucose from UDP-glucose to ecdysteroids, which are insect molting hormones. Expression of the *egt* gene allowed the virus to interfere with normal insect development so that molting was blocked in infected larvae of fall armyworm (*Spodoptera frugiperda*).

BACULOVIRUSES CONSTITUTE A LARGE group of DNA-containing viruses that infect only invertebrate hosts. These viruses, many of which infect pest lepidopteran species, are of particular interest because of their potential as biological control agents (*1*). Studies of *Autographa californica* nuclear polyhedrosis virus (AcMNPV), the model system for baculovirus research, are revealing the molecular mechanisms by which the virus implements its replication strategy. Most of the genes identified to date are involved in the interaction of the virus with its host cell, but little is known of the molecular aspects of infection at the organismal level. We now report that AcMNPV has a gene that allows the virus to manipulate the hormonal regulation of development of its larval host.

The region of the AcMNPV genome containing the *egt* gene, spanning 8.4 to 9.6 map units, first came to our attention as a hypermutable region in serially propagated viruses (*2*). The sequence of this region revealed an open reading frame that could encode a 57-kD polypeptide of 506 amino acids (Fig. 1). The *egt* product shares 21 to 22% amino acid sequence identity with several mammalian UDP-glucuronosyl transferases, also shown in Fig. 1. In mammals, the UDP-glucuronosyl transferases catalyze the transfer of glucuronic acid to a wide variety of exogenous and endogenous lipophilic substrates (*3*). This conjugation reaction is of critical importance in the detoxification and safe elimination of a multitude of drugs and carcinogens. In addition, the normal metabolism and disposal of various endogenous compounds, such as bilirubin and steroid hormones, proceed through their conjugation with glucuronic acid. Available evidence on insect systems indicates that sugar conjugation reactions of this type involve glucose rather than glucuronic acid transfer (*4*). No sequences of UDP-glucosyl transferase genes were available in GenBank at the time of our search, but the sequence of a UDP-glucosyl transferase gene from *Zea mays* (maize) was subsequently reported (*5*). The COOH-terminal portion of this protein also displays homology to egt and to mammalian UDP-glucuronosyl transferases (Fig. 1).

Mammalian UDP-glucuronosyl transferases are known to be membrane-bound, and the amino acid sequences of these proteins

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Table 1. Substrate specificity of *egt* gene product. Substrates were incubated in the presence of medium derived from appropriately infected cells and 0.05 μCi UDP–[U-^{14}C]glucose (312.5 mCi/mmol) for 1 hour at 37°C. All substrates were used at a concentration of 1 m*M*. Other conditions were as described in the legend to Fig. 2. Amounts of glucose transferred were calculated after scintillation counting of the appropriate regions of the chromatography plates. A hyphen indicates that less than 1% of the glucose was transferred.

Substrate	wt (pmol glucose transferred)	vEGTZ (pmol glucose transferred)
p-Aminobenzoic acid	–	–
Bilirubin	–	–
Chloramphenicol	–	–
Cholesterol	–	–
Diethylstilbestrol	–	–
Ecdysone	155.7	–
β-Estradiol	–	–
20-Hydroxyecdysone	87.8	–
8-Hydroxyquinoline	–	–
Makisterone A	89.6	–
(–) Menthol	–	–
Methylumbelliferol	–	–
α-Naphthol	–	–
p-Nitrophenol	–	–
Phenolphthalein	–	–
Testosterone	–	–
α-Tetralol	–	–

contain both a putative signal sequence at the NH_2-terminus and a halt-transfer sequence at the COOH-terminus (*6–8*). Although the predicted amino acid sequence of *egt* contains a putative signal sequence, the protein is approximately 30 amino acids shorter than the mammalian enzymes at the COOH-terminus (Fig. 1) and lacks the residues that constitute the halt-transfer sequence. These facts suggested the possibility that the egt protein is secreted.

The predicted amino acid sequence of the AcMNPV egt protein suggested to us that the enzyme might conjugate and eliminate some compound in the insect hemolymph. As in mammals, a wide variety of both exogenous and endogenous substrates are prone to conjugation in insect systems (*4*). Recently, an ecdysteroid-glucose conjugate was reported (*9*). Therefore, several ecdysteroids were included among the compounds tested as potential substrates for egt-mediated conjugation (Fig. 2).

Spodoptera frugiperda (fall armyworm) cells, which serve as hosts for AcMNPV, were infected with wild-type (wt) AcMNPV L-1 (*10*) or the recombinant virus vEGTZ. This mutant virus differs from wt AcMNPV only in the disruption of the *egt* gene by the insertion of the *Escherichia coli* β-galactosidase gene (Fig. 1). Twelve hours after infection, lysates or media were incubated in the presence of [^{3}H]ecdysone and either UDP-glucose (Fig. 2) or UDP-glucuronic acid. Samples were then analyzed for ecdysone derivatives with altered polarities by thin-layer chromatography. A novel ecdysone derivative (EG) was observed only when the lysate or extracellular medium of wt AcMNPV-infected cells was used (Fig. 2A). When UDP–[U-^{14}C]glucose was included in the reaction mix, it was possible to detect both ^{3}H and ^{14}C in the product (Fig. 2B), confirming that the conjugation reaction involves the transfer of glucose from UDP-glucose to ecdysone. The ecdysteroid UDP-glucosyl transferase activity was found predominantly in the extracellular medium. No ecdysteroid-conjugating activity was produced by mock-infected or vEGTZ-infected cells.

Although several ecdysteroids could function as substrates for this enzyme, none of the other compounds tested were conjugated to a significant extent (Table 1). The ecdysteroids ecdysone, 20-hydroxyecdysone, and makisterone A were conjugated only in the presence of medium from wt-infected cells when UDP-glucose was included in the reaction mix. They were not conjugated when UDP-glucuronic acid was used (*11*) or when they were incubated in the presence of medium from vEGTZ (Table 1) or mock-infected cells (*11*). Thus, we conclude that the product of the *egt* gene is an ecdysteroid UDP-glucosyl transferase that is secreted into the extracellular medium by wt virus–infected cells.

Hemolymph titers of ecdysteroids fluctuate in a cyclic fashion to regulate both larval-larval and larval-pupal molts (*12*). Since glucose conjugation is suspected to act as an

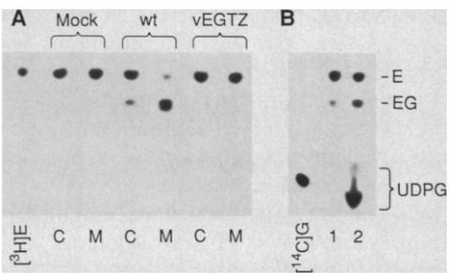

Fig. 2. AcMNPV-specific ecdysone UDP-glucosyl transferase activity. (**A**) *Spodoptera frugiperda* cells were infected with wt AcMNPV (wt) or vEGTZ at a multiplicity of infection of 20. Twelve hours later, the cells (C) and overlying medium (M) were harvested separately; the cells were lysed in tissue culture fluid by several strokes of a Dounce homogenizer. Mock-infected cell cultures were treated in parallel. The enzymatic assay was modified from Bansal and Gessner (*16*). The standard incubation mixture included the following: cell lysate containing 10 μg of total protein or medium from the equivalent number of cells; 10 m*M* $MgCl_2$; 10 m*M* tris maleate, *p*H 7.4; 1 m*M* unlabeled UDP-glucose (Sigma Chemical); 100 μ*M* unlabeled ecdysone (Sigma Chemical); and 0.25 μCi [^{3}H]ecdysone (DuPont Biotechnology Systems). The final reaction volume was 50 μl. Reactions were carried out for 5 min at 37°C, then stopped by the addition of two volumes of ethanol. Products were evaporated and resuspended in 60% ethanol. (**B**) Cell lysates from wt-infected cells were assayed as described above except that the concentration of UDP-glucose was reduced to 3.16 μ*M* and the reaction was allowed to proceed for 30 min. The UDP-glucose used was either unlabeled (lane 1) or UDP–[U-^{14}C]glucose (0.05 μCi; DuPont Biotechnology Systems) (lane 2). [^{3}H]Ecdysone was included in both reactions. Products were separated by thin-layer chromatography on silica-gel plates (Merck) as described (*16*). [^{3}H]Ecdysone ([^{3}H]E) and UDP-[U-^{14}C]glucose ([^{14}C]G) were also subjected to chromatography. Autoradiographs of the silica-gel plates are presented. Scintillation counting confirmed the presence of both ^{3}H and ^{14}C in the ecdysone-glucose conjugate in (B), lane 2. The positions of unconjugated (E) and conjugated (EG) ecdysone, as well as UDP-glucose (UDPG), are indicated.

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E  MTILCWLALLS-----TLTAVNAANILAVFPTPAYSHHIVYKVYIEALAEKCHNVTV
H  Msm....ALL......fss.s.gkvL-V.PT-.fSH.m..K..ld.L.qr.HeVTV
M  M......ALL.....f.sVk.gkvL-V.P.-.fSH.m..Ki.ld.L.qr.HeVTV
R  M......ALf.....f.s.h.gkvL-V.P.-.fSH.m..Ki.ld.L.qr.HeVTV
                                          ↓
E  VKP-KLFAYSTKTYCGNITEI-NADMSVE-----QYKKLVANSAMFRKRGVVSDTDT
H  l.s...isf..ns.....Ev....lt.....-...KqLV...A-...kd......s
M  lrP...y....K...G...E...t.vS.d.......K.v...t.-...Rd.......
R  lKP...F....K...d...EI.st.iS.d.......K.L...t.-...Rd.......

E  VTAANYLGLIEMFKDQF-DNINVRNLIANNQ--TFDLVVVEAFADYALVFGHLYDPA
H  ........f.dilr....D.vs.kkLm.k.Q...FDvVl.dAl..fg.llaeL...p
M  l.......f.d.F.....D.vs.keLmtk.Q...FDvll.dpiA..g.liaeL.q.p
R  i.......f...y.....D.vs.kqLmtk.Q...FDvl..dpiA..g.liaeL.h.p

E  PVI--QIAPGYGLAENFDTVGAVAR-HPVHHPNIW-RSNFDDTEANVMTEMRLYKEF
H  .V...rfsPGYai..h.g.l...p...PV..sel..q..F.e...Nmi-.v-LY.EF
M  .l...rfsPGY.i..s.g.....p...PV..s.l..q..F.e...Nmi-.M-LY.dF
R  .l....fsPG..L..sig.....p...PV..s.l..k..F.D...Nmi-.M-LY.dF

E  --KIL-ANMSNALLKQQFGPNTPTIEKLRNKVQLLLLNLHPIFDNNRPVPPSVQYLG
H  ..qIf..k..d.f..e.lG..T-Tl.....K.di.Li.....Fq..hPl.PnVefv-
M  ..qmf..k..dsf..e.lG..T-Tl.....q.em.Li..n..le..hP..PnVdYv-
R  ....L..k..dtf..e.lG..T-Tvd...sKVei.Li.....l...hP..PnVdYi-

E  GGIHLVKSAPLTKLSPVINAQMNKSKSGTIYVSFGSSIDTKSFANEFLYMLINTFKT
H  GGlH...a.PL.K....f-.Q.s..ng..vf-SlGS.v-.-n.seE...vi.sal..
M  GGlH...a.PL.K....f-.Q.s...g..vf-SlGS.v-.-n.teE....i..al..
R  GGlH...a.PL.K....f-.Q.s...g..vf-SlGS.v-.-n.teE....i..al..
Z                    285  qp..G..YVSFGt....rp...El...L.ds...
                                            ^

E  LDNYTILWKIDDEVVKNITLPANVITQNWFNQRAVLRHKKMAAFITQGGLQSSDEAL
H  i.-..vLWrfDgn....l.L.t.l--.kWi.Q..lL.H.K..AFIThGG.ng...Ai
M  i.-..vLWKfDg.....l...t.V--.kWl.Q..lL.H.K..AFhThGG.ng..EAi
R  i.-..vLWKfDg.....l...t.V--.kWl.Q..lL.H.K..AFvThGG.ng..EAi
Z  L.....W.l....l...a..g..l...W..Q.AVLRH..vgAFvThaG..S..EgL
   ^^      ^

E  EAGIPMVCLPMMGDQFYHAHKLQQLGVARALDTVTVSSDQLLVAINDVLFNAPTYKK
H  .p.IPMV.vPl.aDQ..n...mk..G.A.sLD..TmSS.dLL.Alk.Vi-N.P.YK.
M  ..GIPMi.iPl.GeQ..n...m...G.A.ALn..TmS..dvL.AleeVi-..P.YKK
R  ..GIPMi.iPl.GDQ..n...m...G.A.sLn..TmS..dfL.AleeVi-d.P.YKK
Z  .sGvPM.C.P..GDQ..nAr.v.hvG.G.Afe.-amtS..v..AveelL......rr
                           ^                              ^
                                ↓
E  HMAELYALINHDKATFPPLDKAIKFTERVIRYRHDISRQLYSLKTTAANVPYSNYYM
H  n...L-s.IhHDqp-..PLDrA-.F....v-.RH..akhL...---A.dl.---.f.
M  n...L-s.IhHDqp-..PLDrA-.F....v-.RH..akhL.pL---g.Nl.---.f.
R  nv..L-s.IhHDqp-..PLDrA-.F....I-.RH..akhL.pL---g.NlP---.Y.
Z  ..AEL.ALv.e..g......K.frF.E.V.R.*
     ^           ^

E  YKSVFSIVMNHLTHF*
H  Y.S-l.v....La.............-..............*
M  Y.S-l.vi...Ls.............................*
R  Y.S-l.vi...LT.F............................*
```

Fig. 1. Similarity of *egt* gene product to several UDP-glucuronosyl transferases and a UDP-glucosyl transferase. The predicted amino acid sequence of *egt* (E) (*13*) was compared to human (H) (*6*), mouse (M) (*7*), and rat (R) (*8*) UDP-glucuronosyl transferases by the use of the FASTP algorithm (*14*) as implemented by International Biotechnologies, Inc. The homology of a plant UDP-glucosyl transferase (Z) (*5*) to the above proteins is also displayed. Uppercase letters denote exact matches; lowercase letters correspond to substitutions that occur frequently among related proteins (*15*). Substitutions that occur infrequently are indicated by a dot. Gaps in a sequence are designated by hyphens; a caret marks where an amino acid has been deleted from the sequence. The amino acid sequence between the vertical arrows was replaced by the β-galactosidase gene in vEGTZ.

ecdysteroid inactivation mechanism (*9*), we tested whether AcMNPV infection, through the expression of *egt*, would disrupt the normal developmental process of the host insect. Sixteen newly ecdysed fourth instar *S. frugiperda* larvae were infected by injection either with wt AcMNPV or vEGTZ and monitored daily for any perturbations in their development. One cohort of 16 larvae was injected with tissue culture fluid as a negative control. All larvae injected with tissue culture fluid molted to fifth instar as expected. Only 1 of 16 larvae infected with wt virus made this transition. In contrast, all larvae infected with the mutant vEGTZ underwent a fourth-to-fifth instar molt. Thus, it is clear that *egt* expression by wt AcMNPV specifically inhibits host molting.

Both infected groups of larvae subsequently succumbed to the viral infection, showing that disruption of *egt* did not prevent vEGTZ from completing its infectious cycle. Using newly ecdysed fifth instar larvae, we have observed that no wt-infected larvae showed any signs of pupation, whereas the majority of vEGTZ-infected insects displayed several behavioral modifications (feeding cessation, wandering, and spinning) characteristic of an impending larval-pupal molt. However, all virus-infected animals died before pupation.

The data show that the baculovirus AcMNPV specifically interrupts the normal development of its insect host by producing an ecdysteroid UDP-glucosyl transferase, which is possibly secreted into the hemolymph by infected cells. Our observations provide the first genetic and developmental evidence in support of the hypothesis (*9*) that glucose conjugation by UDP-glucosyl transferases is a mechanism for ecdysteroid inactivation. AcMNPV *egt* is the first identified gene encoding a glucosyl transferase capable of conjugating ecdysteroids. The identification of an insect virus gene encoding such an enzyme should greatly facilitate the study of equivalent insect genes.

It is probable that other baculoviruses and other insect viruses also interfere with the development of their hosts by altering hormonal regulation. A more detailed analysis of the regulation of AcMNPV *egt* expression in the insect, as well as a closer study of the hormonal changes brought about by viral infection, will lead to a deeper understanding of the significance of this activity to the viral life cycle. In particular, such studies should reveal the evolutionary advantage gained by viruses expressing an ecdysteroid UDP-glucosyl transferase.

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T Cells Against a Bacterial Heat Shock Protein Recognize Stressed Macrophages

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Heat shock proteins are evolutionarily highly conserved polypeptides that are produced under a variety of stress conditions to preserve cellular functions. A major antigen of tubercle bacilli of 65 kilodaltons is a heat shock protein that has significant sequence similarity and cross-reactivity with antigens of various other microbes. Monoclonal antibodies against this common bacterial heat shock protein were used to identify a molecule of similar size in murine macrophages. Macrophages subjected to various stress stimuli including interferon-γ activation and viral infection were recognized by class I–restricted CD8 T cells raised against the bacterial heat shock protein. These data suggest that heat shock proteins are processed in stressed host cells and that epitopes shared by heat shock proteins of bacterial and host origin are presented in the context of class I molecules.

EXPOSURE OF CELLS TO VARIOUS stress conditions leads to the synthesis of a family of polypeptides termed heat shock proteins (hsp's) (*1*). After immunization with tubercle or leprosy bacilli, a major fraction of T cells and antibodies is directed against the 65-kD hsp, a newly discovered member of the hsp family (*2–4*). This hsp is a homolog of the GroEL hsp of 60 kD in *Escherichia coli* and shares some degree of homology with the *E. coli* DnaK hsp of 70 kD, which is a homolog of the mycobacterial 71-kD hsp (*2*). Pathogenic mycobacteria preferentially live and replicate inside macrophages, and it can be assumed that the stress imposed by activated macrophages induces abundant hsp synthesis in their intracellular parasites, which will then result in a strong immune response to this antigen. T cells with specificity for the 65-kD hsp are not only demonstrable in patients but also in many healthy individuals, and a great variety of microbes are known to have similar hsp's (*2, 3*). Indeed, hsp's have been highly conserved in evolution, and in some cases similar molecules are shared by prokaryotic and eukaryotic cells (*1*). Our study reveals that a homolog of the mycobacterial 65-kD hsp is present in murine macrophages. This molecule is recognized by T cells raised against the mycobacterial 65-kD hsp and could serve as a target for protective and autoreactive immune responses.

Lysates of bone marrow–derived macro-

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