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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phages (BMMs) were separated by gel electrophoresis, blotted, and stained with the murine monoclonal antibody (MAb) IA10 raised against *Mycobacterium bovis* and specific for an epitope located between amino acids 172 and 224 of the bacterial 65-kD hsp (*5*). A 68- to 70-kD band, slightly larger than the *M. bovis* 65-kD hsp, was revealed by one-dimensional separation (Fig. 1A), and two spots of slightly different size and distinct isoelectric points (*p*I) were identified after two-dimensional separation (Fig. 1B). Conversely, the MAb N27F3-4 against constitutive and inducible members of the human 70-kD hsp family (*6*) cross-reacted with the *M. bovis* recombinant (r-65-kD) hsp (*7*) and with spot b in murine BMMs (Fig. 1C). Thus, molecules similar to the bacterial 65-kD hsp were constitutively present in BMMs.

We examined the possibility that T cells against the bacterial 65-kD hsp recognize activated BMMs. Cytolytic T lymphocytes (CTLs) with specificity for the bacterial 65-kD hsp were activated by culturing spleen cells from naïve C57B1/6 mice with tryptic fragments of this molecule. Most of these cells were $CD8^+CD4^-$ and almost all expressed the CD3 molecule and the α/β T cell receptor (*8, 9*). The cytolytic activity of these CTLs was determined in a conventional ^{51}Cr release assay with unstimulated BMMs alone, unstimulated BMMs labeled with peptides, or IFN-γ–activated BMMs alone as targets. Unstimulated BMMs, in the absence of peptides, showed only marginal lysis, whereas unstimulated BMMs presenting one or more peptides were lysed to a significant degree (Fig. 2, A and B). IFN-γ–activated BMMs, in the absence of exogenous peptides, were lysed equally well. Both T cell–derived and recombinant IFN-γ were active. These CTLs were devoid of natural killer activity as assessed with YAC cells as targets (*10*). BMMs were infected with murine cytomegalovirus (CMV) and used as targets for CTLs with specificity for the bacterial 65-kD hsp. Viral infection rendered these macrophages susceptible to cytolysis (Fig. 2B). For control purposes, CTLs were generated against tryptic fragments of ovalbumin. In confirmation of work by others, these CTL-lysed BMMs primed with ovalbumin fragments but not BMMs primed with fragments of the 65-kD hsp or BMMs stimulated with recombinant IFN-γ (r-IFN-γ) (Fig. 2D). Conversely, CTLs directed against the 65-kD hsp did not lyse BMMs labeled with ovalbumin peptides (Fig. 2C). Thus, lysis of stressed BMMs was a specific function of CTLs directed against one or more epitopes of the 65-kD hsp. BMMs infected with *M. bovis* were also lysed by CTLs directed against the 65-kD hsp (*10*). This experiment, however, does not show whether the relevant epitope was of bacterial or host origin.

CTLs with specificity for the 65-kD hsp were tested in the presence of MAbs directed against CD4 or CD8. Lysis was blocked by the MAb against CD8, but not by the MAb against CD4 (Fig. 3, A to C). Similarly, CTL activity was abrogated by treatment with complement plus the MAb against CD8, whereas treatment with complement plus MAb against CD4 was ineffective (*10*). The genetic restriction of CTLs from C57B1/6 (bbb), B10.MBR (bkq), or B10.A(2R) (kkb) mice was determined by raising CTLs against trypsinized 65-kD hsp. CTLs from B10.A(2R) mice, but not those from B10.MBR mice, were capable of lysing peptide-labeled and IFN-γ–stimulated BMMs from C57B1/6 mice (Fig. 3, D to F).

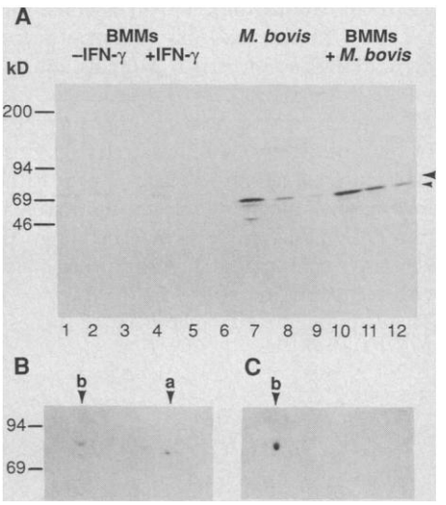

Fig. 1. Identification of 65-kD hsp cross-reactive proteins in murine BMMs. Protein immunoblot after (A) one- or (B and C) two-dimensional gel electrophoresis. BMMs from C57B1/6 mice were cultured in complete Iscove's modified Dulbecco's medium as described (*21*). For BMMs stimulation, 1000 U ml^{-1} r-IFN-γ were added to the cultures 2 days before use. BMMs (2×10^7) were lysed in 200 μl of lysis buffer [0.05*M* tris (*p*H 7.5), 1 μ*M* leupeptin, 1 μ*M* pepstatin, 100 μ*M* EDTA, 200 μ*M* phenylmethylsulfonyl fluoride, and 0.5% Triton X-100]. (**A**) Lysates were run under nonreducing conditions in a discontinuous SDS–polyacrylamide gel electrophoresis (PAGE) system with a 5 to 20% linear gradient, electroblotted, and stained with MAb IA10. Lysates of unstimulated BMMs (lanes 1 to 3) and lysates of r-IFN-γ–stimulated BMMs (lanes 4 to 6) were titrated by loading 15, 5, or 2.5 μl of lysate per lane. Unstimulated BMMs infected with viable *M. bovis* at a 1:1 ratio for 2 days (lanes 10 to 12), and lysates of *M. bovis* containing 2×10^7 bacteria in 200 μl (lanes 7 to 9) were titrated by loading 15, 5, or 2.5 μl of lysate per lane. Arrows indicate the position of the two different immunoreactive bands: the 65-kD antigen of *M. bovis* (small arrow) and the slightly larger cross-reactive antigen of BMMs (large arrow). (**B** and **C**) Lysates (30 μl) of r-IFN-γ–stimulated BMMs were separated by isoelectric focusing and on a discontinuous SDS-PAGE system (*22*). In (B), MAb IA10 detected two distinct spots (a and b) with apparent *p*I of 5.2 and 5.7, respectively, and slightly different molecular mass (68- to 70-kD for a and 73- to 75-kD for b). In (C), MAb N27F3-4 detected spot b only.

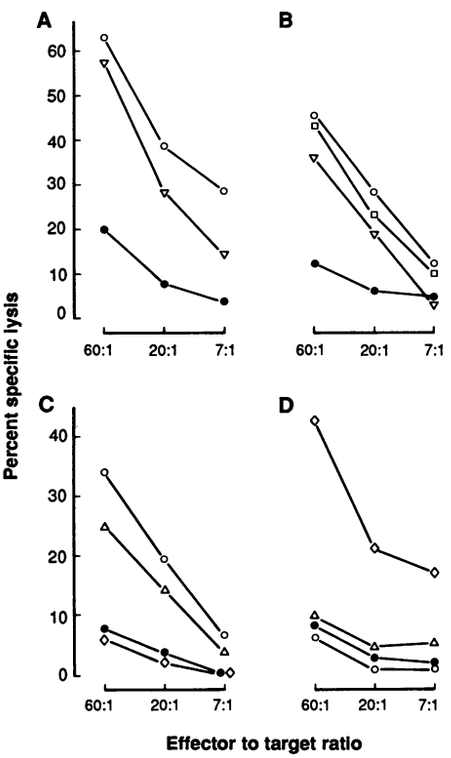

Fig. 2. Specific lysis by 65-kD hsp–specific CTL of peptide-primed BMMs, IFN-γ–activated BMMs, or CMV-infected BMMs. CTLs against the 65-kD hsp (**A**, **B**, and **C**), or ovalbumin (**D**) were tested on BMMs as targets. ●, Unstimulated BMMs; ○, BMMs primed with 65-kD hsp; ▽, BMMs activated with IFN-γ containing T cell factors; △, r-IFN-γ–activated BMMs; □, CMV-infected BMMs; and ◇, BMMs primed with ovalbumin. Spleen cells from C57B1/6 mice (6×10^6 ml^{-1}) were cultured with 100 μg ml^{-1} of tryptic fragments of the 65-kD hsp or ovalbumin (*23*). On day 6, cytolytic activity of the T cells was assessed with BMMs as targets (*21*) Tryptic peptides (10 μg per well) were added immediately before the cytolytic assay. The r-IFN-γ (2000 U ml^{-1}) or supernatant from concanavalin A–activated T cells from mice infected with *Listeria monocytogenes* (25%) were used for activation of BMMs. Two days before assay, BMMs were infected with CMV at 1.6×10^6 ml^{-1}. BMMs were labeled with ^{51}Cr by standard procedures, and 2×10^3 BMMs were cultured with effector cells for 4 hours in V-bottomed microtiter plates (Nunc) at effector to target ratios indicated in the figure and percent specific lysis of target cells was calculated as described (*21*). Symbols represent mean values of at least three determinations; the SD in all experiments is <10%; and each experiment was reproduced at least twice with similar results.

Similarly, CTLs from C57B1/6 mice lysed peptide-labeled and IFN-γ–stimulated BMMs from B10.A(2R) mice, but not those from B10.MBR mice (*10*). Thus, the relevant CTLs were $CD8^+$ and class I (H-2D)–restricted (*8, 9*).

The hsp's are a family of highly conserved polypeptides that were originally identified after exposure of cells to various stress conditions, including increased temperature, lymphokine activation, virus infection, and attack by reactive oxygen metabolites (*1, 11*). Later studies indicated an essential role for hsp's in the assembly and transmembrane transport of certain proteins and led to their designation as "chaperonins" (*12*). More recent studies showed that members of the 70-kD hsp family are released from cultured rat embryo cells and appear to be involved in antigen processing and presentation (*13*). Finally, two genes encoding 70-kD hsp were mapped within the major histocompatibility complex (MHC) (*14*). These findings relate members of the hsp family to antigen presentation. In agreement with these observations, we assume that the cross-reactive molecules in BMMs are hsp's. Indeed, a human 70-kD hsp (*15*) and the bacterial 65-kD hsp (5) have significant sequence similarity [30% similarity at gap-weight 10 (*16*)], and a human homolog of the bacterial 65-kD hsp with marked sequence similarity [66% similarity at gap-weight 10 (*16*)] was identified (*17*). Four segments of 12 or more amino acids are identical or almost identical in the human and bacterial 65-kD hsp's, and three of these segments are enclosed by trypsin cleavage sites. These peptides, therefore, represent candidate epitopes for cross-reactive CTLs.

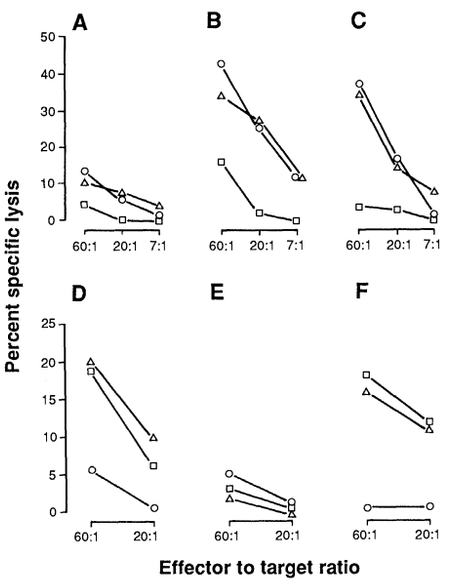

Fig. 3. Determination of CTL phenotype and genetic restriction. CTL against peptides of the 65-kD hsp were tested on unstimulated BMMs (**A**), BMMs primed with peptides of the 65-kD hsp (**B**), or on IFN-γ–stimulated BMMs (**C**) in the presence of antibody to CD4 MAb or antibody to CD8 MAb. ○, No MAb; △, anti-CD4 MAb; □, anti-CD8 MAb. CTL from C57B1/6 (**D**), B10.MBR (**E**), or B10.A(2R) (**F**) mice were tested on BMMs from C57B1/6 mice. ○, Unstimulated BMMs; □, BMMs primed with peptides of the 65-kD hsp; △, r-IFN-γ–stimulated BMMs. CTLs were raised against tryptic fragments of the 65-kD hsp. For antibody blocking, effector cells were incubated with MAb 191 (anti-CD4) or MAb 169 (anti-CD8) at 4°C for 30 min immediately before the CTL assay as described (*21*). Symbols represent mean values of at least three determinations; the SD in all experiments is <10%; and each experiment was reproduced at least twice with similar results.

In the ovalbumin system, T cells against tryptic fragments do not recognize epitopes generated through the cytoplasmic "class I processing pathway" (*18*). In our study, T cells raised against tryptic fragments of the bacterial 65-kD hsp recognized epitopes arising from cytoplasmic class I processing. Although the proteases involved in cytoplasmic class I processing are elusive, their sites of cleavage must be defined by the physicochemical structure of their substrate. We therefore have to assume that trypsin and cytoplasmic proteases show some overlap in the 65-kD hsp system but not in the ovalbumin system.

T cells with specificity for the mycobacterial 65-kD hsp have been identified in patients and in healthy individuals (*3*), and homologs of this hsp are present in different bacteria (*2*). This indicates that T cells reactive with the 65-kD hsp are induced by various microbes. Our data show that even T lymphocytes that cross-react with stressed host cells can be activated. Hence, these autoreactive T cells are present and must have evaded clonal deletion (*19*). These T cells were capable of recognizing host cells harboring bacteria and also those infected with viruses. Thus, T cells with specificity for the bacterial 65-kD hsp—by recognizing a specific but cross-reactive epitope—could contribute to a first line of defense against a wide variety of infections caused by viral and bacterial agents.

Furthermore, T cells with reactivity to the 65-kD hsp could be relevant to autoimmunity. Expression of host-derived epitopes may not suffice for primary T and B cell activation, which may depend on priming with exogenous peptides of bacterial origin. Because in our study unstimulated macrophages were not lysed, they should be relatively protected from T cell attack although they already harbor the cross-reactive molecule inside. Under stress, however, the molecule is processed and presented in the context of MHC class I molecules and could then serve as a target for T cells against shared epitopes of the bacterial 65-kD hsp. Human and experimental animal studies suggest that the mycobacterial 65-kD hsp is involved in rheumatoid arthritis (*20*). A variety of other microbial species have been implicated in this type of autoimmunity as well. Because hsp's are highly conserved, it is conceivable that the same protein can be introduced into the host by various microbes. Hence, hsp's may have a double-sided role in the host response to infectious agents.

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22. Isoelectric focusing gels contained 4% (v/v) of *p*H 5 to 7 and 1% (v/v) of *p*H 3 to 10 carrier ampholyte [Servalyt (Serva)], 9*M* urea, and 2% Triton X-100 (*16*). After incubation in 2% SDS, 10% glycerin, and 60 m*M* tris (*p*H 6.8) for 15 min, gels were run on a discontinuous SDS-PAGE system with a linear gradient of 5 to 20%. Proteins were electroblotted onto nitrocellulose, membranes were blocked for 1 hour (0.05*M* tris (*p*H 7.5), 0.5% casein, and 0.5% Tween 20), incubated for 12 hours at 4°C with MAb IA10, washed in blocking buffer, and incubated for 1 hour with alkaline phosphatase–conjugated goat antibody to mouse immunoglobulin G (Promega, Biotec Madison). Blots were developed with 98 μ*M* nitrobluetetrazolium, 92 μ*M* 5-bromo-4-chloro-3-indolyl phosphate in 1*M* diethanolamine, and 0.5 m*M* $MgCl_2$, *p*H 9.8.
23. The method used for the generation of tryptic peptides was as follows. The r-65-kD hsp of *M. bovis* was isolated from *E. coli* M1103 (*4*) containing the full-length gene of 65-kD hsp in the expression vector pPLc 236. The soluble constituents of bacteria were fractionated by ammonium sulfate precipitation. The precipitate that formed between 20% and 55% saturation of ammonium sulfate was bound on the anion exchanger Q-Sepharose Fast Flow (Pharmacia) in 50 m*M* tris, *p*H 8.0, and the 65-kD hsp was eluted with a linear gradient of 0 to 0.3*M* NaCl. For trypsin digestion, the 65-kD hsp was treated with 8*M* urea, and after dialyzing against 0.1*M* ammonium bicarbonate, up to 100 mg of protein was cleaved overnight at room temperature with 25 U of immobilized trypsin [*N*-tosyl-L-phenylalanine chloromethyl ketone–treated, attached to beaded agarose, (Sigma)]. Ovalbumin was denaturated and trypsinized as described (*18*).
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Primary Structure of the β Subunit of the DHP-Sensitive Calcium Channel from Skeletal Muscle

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Complementary DNAs for the β subunit of the dihydropyridine-sensitive calcium channel of rabbit skeletal muscle were isolated on the basis of peptide sequences derived from the purified protein. The deduced primary structure is without homology to other known protein sequences and is consistent with the β subunit being a peripheral membrane protein associated with the cytoplasmic aspect of the sarcolemma. The protein contains sites that might be expected to be preferentially phosphorylated by protein kinase C and guanosine 3′,5′-monophosphate–dependent protein kinase. A messenger RNA for this protein appears to be expressed in brain.

THE DIHYDROPYRIDINE (DHP)-SENSITIVE Ca^{2+} channel seems to play an important role in excitation-contraction coupling, and when purified from rabbit skeletal muscle, it contains three main subunits with molecular masses of 165,000 (α_1), 55,000 (β), and 32,000 daltons (γ) (*1–9*). Both the α_1 and the β subunits are substrates for protein kinases in vitro, and phosphorylation of these subunits may serve to regulate the in vivo function of the Ca^{2+} channel (*1–5*). A disulfide-linked dimer of 130,000- (α_2) and 28,000-dalton (δ) polypeptides has been observed in preparations of the channel either in variable (*6*) or constant (*7*) amounts with respect to the other subunits. The subunits have been reconstituted and form functional Ca^{2+} channels that are modulated by phosphorylation (*10*) and by monospecific antibodies for the α_1, β, and γ subunits (*11*). The α_1 polypeptide is the principal transmembrane subunit of the channel and forms the ion-conducting pore (*12*). This polypeptide binds DHPs, phenylalkylamines, and benzothiazepines (*1, 2, 6–9*) and is readily phosphorylated in vitro at Ser^{687} by adenosine 3′,5′-monophosphate (cAMP)–dependent protein kinase (*4*). Microinjection of an expression plasmid carrying the α_1-subunit cDNA restores a DHP-sensitive Ca^{2+} current and excitation-contraction coupling in dysgenic muscle (*13*). It is not known whether these functions require the presence of the other channel subunits. The α_2 polypeptide has been cloned (*14*) and is present in dysgenic muscle cells (*15*), which may also contain the β and γ subunits.

We now report the primary structure of the β subunit from rabbit skeletal muscle as deduced from cloned cDNA and the tissue distribution of the corresponding mRNA. A total of eight peptides, six of which were unique, were isolated from two separate proteolytic digests of the isolated β subunit (*16*). In one case the purified channel containing the β subunit was phosphorylated by cAMP-dependent protein kinase before separation of the subunits. Peptide 1 contained 32% of the recovered radioactivity (recovered radioactivity was 74% of the radioactivity initially present in the β subunit). The remaining radioactivity was associated with several peptides. Four oligodeoxyribonucleotides, each containing 14 nucleotides (nt), which were complementary to all possible cDNA sequences encoding the amino acid sequence Asp-Met-Met-Gln-Lys (excluding the third nucleotide residue of the Lys codon) in peptide 2, were synthesized. These oligomers were used as specific primers for reverse transcription of the β-subunit mRNA. An equimolar mixture of 64 synthetic 14-nt oligodeoxyribonucleotides, which were complementary to all the possible cDNA sequences corresponding to the amino acid sequence Gly-Tyr-Glu-Val-Thr (excluding the third nucleotide residue of the Thr codon) in peptide 2, was used to probe 2×10^5 transformants. Two clones, pCaCHβ1-I and pCaCHβ10-I, which both contained the coding region for part of peptide 2, were isolated. In addition, clone pCaCHβ1-I contained the cDNA encoding peptides 1, 3, 4, and 7. This clone was used as a probe for cloning larger cDNA sequences (Fig. 1A).

The 1835-nt cDNA sequence obtained contains an open reading frame encoding a sequence of 524 amino acids (Fig. 1B). The calculated molecular mass of 57,868 daltons is similar to that estimated by SDS–polyacrylamide gel electrophoresis (55 kD). The cDNA contains all sequenced peptides. The NH_2-terminus of the isolated β subunit was blocked and could not be sequenced. We selected the first methionine (amino acid 1) rather than the second methionine (amino acid 7) as the translation initiation site be-

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