Maximizing Production of Estrogen Receptor β with the Baculovirus Expression System

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ABSTRACT

*Steroid hormone/nuclear receptor expression in cultured insect cell lines is routinely driven by a baculovirus vector. An advantage of the baculovirus production of these receptors is that large amounts of functional receptors are obtained for subsequent in vitro studies. Most laboratories produce nuclear receptors in *Spodoptera frugiperda* (Sf)9 cells. However, no one has determined whether this cell line is optimal for the production of any nuclear receptor. We compared the time course and level of estrogen receptor β (ER β) production from a baculovirus in two *S. frugiperda* cell lines, IPLB-SF21AE (Sf21) and Sf9, and two *Trichoplusia ni* cell lines, Tn368 and BTI-TN5b1-4 (High FiveTM). Cells were harvested at various times (0.5–5 days) after infection. ER β expression and activity was determined by specific [³H]estradiol (E₂) binding, Western blot analysis, and estrogen response element (ERE) binding in vitro. The highest functional, bioactive ER β expression both at the earliest time after infection and in the amount of ER β produced/cell was with the Sf21 cell line. Baculovirus expressed ER β -bound EREs with high affinity in a DNA sequence-dependent manner. We conclude that Sf21 cells are the best-suited cells for ER β production.*

INTRODUCTION

The estrogen receptor (ER), which functions both as a signal transducer and transcription factor that modulates the expression of target genes, is the progenitor for all steroid receptors (42) and is a member of the nuclear receptor superfamily that currently includes 48 known receptors plus two pseudogenes (30). In mammals, ER is encoded by two genes, α and β (26). ER α and ER β function as dimers (28). To study the functional properties of ER α and ER β , large quantities of appropriately post-transcriptionally processed proteins are needed for in vitro assays.

Early studies demonstrated that steroid receptors overproduced in yeast (31) or *E. coli* (37) were generally ineffective for in vitro analyses compared to receptors extracted from animal tissues because of insolubility, proteolysis, inappropriate or missing post-translational modification, and interfering proteins (41). The reasons for the failure of these systems included codon usage, solubility, stability of the protein in the absence of the chaperonin complexes with which steroid receptors interact, the absence of appropriate post-translational modifications, and variations in proteolytic activity (41). The baculovirus expression vector system (BEVS) has been widely adopted for the expression of steroid and nuclear receptors and many other intracellular, secreted, and membrane proteins because it provides a reproducible method for the production of large amounts of recombinant proteins (33). The most widely used virus for BEVS is the *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV). Al-

though a wide variety of cells are susceptible to AcMNPV infection, *Spodoptera frugiperda* (Sf) cells Sf21 or their subclone Sf9 (Invitrogen, Carlsbad, CA, USA) are generally used for the production of nuclear receptors.

Insect cells have been demonstrated to perform many post-translational modifications that confer correct folding, biological activity, and antigenicity on expressed proteins (25,32,33). These modifications include the phosphorylation, glycosylation, myristylation, and palmitoylation, signal peptide processing, post-translational cleavage, and disulfide bond formation (25). The first report of the baculovirus expression of hER α with BEVS (9) used the human estrogen receptor (HEO) clone (18) that was later found to have a single nucleotide change that resulted in a glycine-to-valine amino acid substitution (Gly-400 to Val-400) that decreased E₂ binding affinity and destabilized the protein (43). Subsequently, the corrected HEGO clone; that is, with Gly-400 (43), was used to generate a baculovirus for ER α expression in Sf9 cells (2–4,10,34). Other investigators have expressed hER α in Sf9 cells, either as a wild-type or fusion protein with GST or FLAG[®] (6,7,47,48). Mouse ER α (16), chicken ER (8), and mouse ER β (11,12) have also been expressed in Sf9 cells, and mouse ER α has been expressed in Sf21 cells (1). Researchers have yet to examine whether insect cell lines differ in the level of ER or other nuclear receptor produced.

To produce the most pure functional receptor while simultaneously conserving costly reagents, we compared the production and functional characteristics of recombinant rat ER β driven by a

recombinant baculovirus in four commonly used insect cell lines: Sf9, Sf21, Tn368, and BTI-TN5b1-4 (High Five™; Invitrogen). We found that ERβ expression is highest in Sf21 cells compared to the most commonly used Sf9 cells. The procedure for the infection, harvesting, and characterization of recombinant ERβ should be applicable to all nuclear receptors as well as other transcription factors.

MATERIALS AND METHODS

Plasmids and Strains

The cDNA for rat ERβ in pCMV5 was generously provided by Dr. J.-A. Gustaffson (27). There were no unique restriction sites near the beginning of the ERβ open reading frame, so the cDNA sequence was modified by PCR with a primer designed to yield a product that better conforms to base biases near the translation starts in the AcMNPV genome open reading frames (5) and to remove upstream noncoding regions. The PCR product was inserted into the PCR 2.1 plasmid (Invitrogen), which enabled the introduction of an *EcoRI* restriction site near the translation start site. The upstream region and first part of the open reading frame were sequenced and then subcloned from the PCR 2.1 plasmid, from the plasmid *EcoRI* site to the first *HindIII* site in the open reading frame, into the minimal baculovirus transfer plasmid, pBAC-1 (Novagen, Madison, WI, USA), which uses the polyhedrin promoter. The final immediate upstream sequence was 5'-AATTCGGCTTACATG-3'. The transfer plasmid was completed by subcloning the remainder of the open reading frame (*HindIII*-*HindIII*) from pCMV5-rat ERβ. pBAC-1-ERβ was co-transfected into IPLB-Sf21AE cells with BacVector®-3000 viral DNA (Novagen) in six-well plates with the Eufectin™ lipid transfection reagent from the Novagen BacVector-3000 transfection kit. The Sf21 cells were overlaid with agarose, and the initial cloning was done directly from the transfected cell monolayer. Clones were purified by repeated plaque purification in *Trichoplusia ni* (Tn)368 cells cultured in 24-well plates (49).

Baculovirus Titer Determination

ERβ baculovirus titer was determined by plaque assay (51) in 24-well plates. Tn368 cells were used because they demonstrate easily recognizable morphological changes in response to infection. In brief, after the optimal density was determined, Tn368 cells were plated in 1 mL TNM-FH medium (Grace's insect cell medium; Invitrogen) supplemented with 10% FBS. One-tenth milliliter of serial dilutions of virus stock was used for infection. The cells and virus were sedimented at 1000× g for 1 h at 28°C, and the supernatant medium was aspirated. Each well was overlaid with 0.5 mL 0.7% low-melting agar (Seaplaque®; CAM-BREX, Rockland, ME, USA) in medium containing 10% FBS, 50% TNM-FH (Grace's supplemented medium; Invitrogen), and 40% Grace's medium, Vaughn's modification (JRH Biosciences, Lenexa, KS, USA) after infection and incubated at 28°C in passively humidified containers. Plaques were counted three to four days after infection.

Comparison of ERβ Production between Different Insect Cell Lines

The viral titer determined by the plaque assay was used for the subsequent infection of the insect cell lines IPLB-Sf21AE (Sf21) and Tn368 (generously provided by Alan Wood, Boyce Thompson Institute for Plant Research, Ithaca, NY, USA), Sf9, and BTI-TN5b1-4. Viable cell counts were determined by Trypan blue staining. For time and cell density experiments, virus was added at a MOI of 10, and the mixture was diluted for an appropriate cell number in 3 or 3.5 mL/well (depending on the experiment and always the same within an individual experiment). Cells were plated at the indicated cell densities in six-well plates (9.5 cm² wells; Corning, Corning, NY, USA) and were infected with the ERβ baculovirus by sedimentation at 1000× g for 1 hour at 28°C. The time after infection was counted from the end of the centrifugation step. The inoculating medium was removed and replaced with fresh medium (3 mL/well). The plates were sealed with vinyl electrical tape, placed in pas-

sively humidified boxes, and incubated at 28°C for the indicated times.

For cell extract preparation, the cells were harvested and sedimented at 66× g for 5 min in a swinging-bucket rotor (Beckman Coulter, Fullerton, CA, USA). After resuspension, the cells were washed with room temperature isotonic saline (3.7 mM CaCl₂, 54.7 mM KCl, 11.2 mM MgCl₂, 11.3 mM MgSO₄, 7.3 mM NaH₂PO₄, 181 mM sucrose, pH 6.5), sedimented, and washed once with room-temperature hypotonic solution (10 mM Tris-HCl, pH 7.5, 10 mM KCl, 10% glycerol, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 2 µg/mL aprotinin, 2 µg/mL leupeptin, 2 µg/mL pepstatin, and 10 µg/mL E64 (Roche Applied Science, Indianapolis, IN, USA)). The cells were resuspended in 0.3 mL ice-cold hypotonic solution, frozen in liquid nitrogen, and stored at -70°C. Upon thawing, the KCl and Tris-HCl concentrations were adjusted to 600 mM and 50 mM, respectively, and the cells were disrupted using a sonicator (Branson Sonifier model 200; Branson Ultrasonics, Danbury, CT, USA) equipped with a cup horn cooled with ice water. Disruption was monitored with Trypan blue staining and examination with a phase-contrast microscope (Phase Contrast-2 ELWD 0.3; Nikon, Melville, NY, USA). After sonication, the extract was incubated on ice for 3 h and clarified by sedimentation in a TLS100.2 rotor (Beckman Coulter) at 279 000× g for 15 min. The supernatant solution was aliquoted, frozen in liquid nitrogen, and stored at -70°C.

For DNA binding assays, additional concentrated nuclear extracts were prepared. Three insect cell lines, IPLB-Sf21AE, Sf9, and BTI-TN5b1-4 were plated in 75 cm² plug-seal cell culture flasks (Corning). Following attachment, the medium was removed, and ERβ baculovirus was added at an MOI of 10 in a volume of 0.5 mL. The flasks were rocked slowly for 1 h, and then 15 mL TnM-FH medium (Grace's supplemented medium) and 10% FBS were added. The flasks were incubated at 28°C. Nuclear extracts were prepared from Sf21, Sf9, and BTI-TN5b1-4 cells at 1.5, 1.8, and 2.7 days after infection, respectively, for samples early in the infection cycle. For samples late in the infection cycle, Sf21, Sf9, and BTI-

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TN5b1-4 cells were harvested at 2.5, 2.5, and 4.7 days, respectively. At the start of infection, the cells were at densities (per cm²) of 77 330, 200 000, and 72 000 for Sf21, Sf9, and BTI-TN5b1-4, respectively.

For nuclear extract preparation, the cells were washed once with room-temperature isotonic saline, followed by a wash with room-temperature hypotonic buffer (described earlier). The cells were resuspended in two packed cell volumes of cold hypotonic solution and allowed to swell on ice for 20 min. A Dounce homogenizer was used to disrupt 90% of the cells. The homogenate was sedimented at 3200× *g* for 20 min. The nuclear pellet was resuspended in extraction buffer (hypotonic buffer with 50 mM Tris-HCl and 600 mM KCl as final concentrations) in a volume equal to that used for the homogenization. The samples were sonicated on ice with a sonicator equipped with a stepped microprobe horn. Disruption was monitored with Trypan blue staining and phase-contrast optics. After sonication, the extract was incubated on ice for 3 h and clarified by sedimentation in the TLS100.2 rotor at 279 000× *g* for 15 min. The clarified supernatant solution was aliquoted and stored at -70°C.

The concentration of ERβ in each sample was determined by a hydroxylapatite (HAP) assay (36). Cellular or nuclear extracts were incubated in TDPK111 buffer (40 mM Tris-HCl, pH 7.5, 1 mM DTT, 0.5 mM PMSF, and 111 mM KCl) containing 30 nM [³H]E₂ ([2,3,6,7-³H](N)]17β-estradiol, 74 Ci/mmol, NET-317; NEN® Life Science Products, Boston, MA, USA) for 1 h at 37°C. Following a 1-h incubation with a 10% HAP solution at 4°C, the HAP was sedimented and washed three times with 1 mL TDPK111. The HAP-bound [³H]E₂ was determined by liquid scintillation counting in Eco-scint™ A (National Diagnostics, Atlanta, GA, USA) in a Wallac 1409 scintillation counter (Turku, Finland). A 200-fold excess of unlabeled E₂ (300 nM) was used as a competitor with [³H]E₂ for ERβ binding as a control to determine nonspecific binding. The specific binding of the [³H]E₂ was used to calculate the ERβ concentration in mols of dimer, assuming that each ERβ

monomer binds one [³H]E₂ (27). The affinity of the E₂-ERβ interaction was determined by competition binding using the HAP assay (36), in which a fixed amount of ERβ (1.6 nmol) was incubated with 1.6 μM [³H]E₂ plus increasing concentrations (from 30 fmol to 1 μmol) E₂. Binding reactions were performed in triplicate within each HAP assay for each time point sample (22). The nonspecific binding did not vary significantly over the time course of infection or with differences in cell density. The data were analyzed by nonlinear regression analysis using GraphPad Prism® (GraphPad Software, San Diego, CA, USA).

Electrophoretic Mobility Shift Assay

Protein-DNA binding was measured by electrophoretic mobility shift assay (EMSA) as previously described (23,44–46). In brief, identical molar amounts of ERβ, based on the HAP assay results, were incubated with a fixed amount (50 000 dpm) [³²P]-labeled EREc38 (sequence: 5'-TCAGG-TCAGAGTGACCTGAGCTAAAATA-ACACATTTCAG-3') (24). ERβ-specific antibodies (Y19) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and ERβ-14C8 (14C8 or 14C) (GeneTex, San Antonio, TX, USA) (40) were included in selected reactions, as indicated in Figure legends. Dried EMSA gels were analyzed using an InstantImager and associated software, the Packard Imager for Microsoft® Windows® v2.04 as previously described (both from Packard Instrument, Meriden, CT, USA) (29). The competition EMSA method for determining the affinity of ERβ-ERE interaction has been previously reported (46).

Western Blot Analysis

The protein concentration of nuclear extracts was determined using the dextran-coated charcoal (Lowry) assay and BSA as a standard (Bio-Rad Laboratories, Hercules, CA, USA). For Western blotting, 30 μg protein from the nuclear extract of baculovirus-infected cells were separated on 10% SDS polyacrylamide gels and electroblotted onto a PVDF membrane. Following the transfer, the blots were

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probed with ER β polyclonal antiserum PA1-311 (Affinity Bioreagents, Golden, CO, USA) or monoclonal ER β antibody CWK-F12 that had been generously provided by Dr. Benita S. Katzenellenbogen (13). We used the Renaissance Enhanced Luminol Reagent (NEN Life Science Products) for immunochemical detection as previously described (23). Data were quantitated from scanned films using Un-Scan-ItTM Software ver. 5.1 (Silk Scientific, Orem, UT, USA) (23).

RESULTS

Maximizing BEVS ER β Production

We examined ER β production as a function of cell density and time after infection in four insect cell lines: Tn368, BTI-TN5b1-4, Sf9, and Sf21. Each insect cell line was infected at six different cell densities. The specific [³H]E₂ binding capacity was determined as a measure of functional ER β production. Preliminary observations

with all four cell lines [IPLB-SF21AE (Sf21), Sf9, Tn368, and BTI-TN5b1-4] revealed that Tn368 was a comparatively poor producer of recombinant rat ER β (data not shown).

We continued the investigation with Sf9 (the cell line used most often for the production of nuclear receptors), Sf21, and BTI-TN5b1-4. A series of densities of cells were infected at a MOI of 10. Phase-contrast microscope observations of the cells 24 h after infection revealed nearly synchronous in-

Figure 1. Quantitation of ER β production on a per well basis in insect cell lines. The indicated insect cell lines in panels A–C, representing BTI-TN5b1-4, Sf9, and Sf21 cells, respectively, were plated at the indicated cell densities and infected with ER β baculovirus, as described in the Materials and Methods section. Cells were harvested at the indicated number of days post-infection, and the ER β concentration was determined by [³H]E₂ binding. Values are $\bar{x} \pm$ SEM of two to three separate experiments in which each time point was performed in triplicate.

Figure 2. Comparison of ER β production on a per cell basis in insect cell lines. The data in Figure 1 are expressed on a per cell basis. Values are $\bar{x} \pm$ SEM of two to three separate experiments in which each time point was performed in triplicate.

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Table 1. Comparison of ERβ Production in Insect Cell Lines

Cell Line	Optimal Cells/Well and Time After Infection	Maximum ERβ Protein Production (pmol/well)	Maximum ERβ Protein Production (amol/cell)	Maximum ERβ Protein Production (pmol/mg protein)	ERβ-ERE Binding % of [³² P]ERE Bound/Total [³² P]ERE
BTI-TN5b1-4	7 × 10 ⁵ at 4.2 days	11.5	17	4	17
Sf9	1.9 × 10 ⁶ at 2.2 days	2.5	1.5	9	20
Sf21	7.5 × 10 ⁵ at 1.6 days	15	20	18	27

The peak ERβ protein production was compared between cell lines at the given cell densities and days after infection. ERβ protein production (pmol/well and amol/cell) was determined by HAP assay. ERβ-ERE binding was determined using EMSA. The details of this experiment are provided in the Materials and Methods section.

fection with the exception of the highest cell density in which the cells continued to divide, further increasing cell density. For BTI-TN5b1-4 cells (Figure 1A), ERβ production increased with cell density up to 7 × 10⁵ cells/well. At higher cell densities, ERβ production decreased. BTI-TN5b1-4 cells are sensitive to crowding, and protein production was expected to drop for the high cell densities (50).

For Sf9 cells (Figure 1B), the highest level of ERβ production was obtained for the 4.25 × 10⁶ cells/well at three to four days post-infection (Figure 1B). The rate of protein production at this density lagged behind other wells that peaked earlier (close to two days after infection). Even at the highest cell density, the maximum amount of ERβ produced by Sf9 cells was less than that produced by the BTI-TN5b1-4 cells.

The Sf21 cell line produced more ERβ at an earlier time after infection compared to either the BTI-TN5b1-4 or Sf9 cell lines (Figure 1C). The highest level of ERβ production was obtained for the 7.5 × 10⁵ cells/well, and this was achieved at 1.5 days after infection (Figure 1C). At the highest cell densities, the rate of ERβ production was lower. Like Sf9 cells, the highest density of Sf21 cells initially produced the lowest amount of ERβ, but at time points later in infection, more ERβ was produced at the higher cell densities compared to the lower cell densities.

Figure 2 expresses ERβ production on a per cell basis. For the BTI-TN5b1-4 cell line, ERβ production at all cell densities was similar, from 2.5 × 10⁵ to 7 × 10⁵ cells/well and decreased to between 7 × 10⁵ and 9 × 10⁵ cells/well (Figure 2A). These data and those in

Figure 1A indicate that between 5.5 × 10⁵ and 7 × 10⁵ cells/well is the best cell density for ERβ production in BTI-TN5b1-4 cells.

For the Sf9 cell line (Figure 2B), ERβ production was much lower at the highest cell density tested than at lower cell densities. One explanation for this decrease in ERβ production is that protein integrity and function might be compromised under these conditions. In the range of cell densities tested, Sf21 demonstrated a high sensitivity of ERβ production to cell density. On a per well basis, the peak ERβ production was observed at 7.5 × 10⁵ cells/well; but at that density, ERβ production was about half the peak ERβ at 3.5 × 10⁵ cells/well.

Figure 3 shows a comparison of ERβ production at the optimal cell densities determined in the preceding sets of experiments. These data show that Tn368 and Sf9 produce approximately 6-fold less ERβ than either the BTI-TN5b1-4 or Sf21 cell lines. Although ERβ production is comparable in BTI-TN5b1-4 and Sf21 cells, ERβ production in Sf21 cells peaks at an earlier time after infection (i.e., 1.6 days vs. 4 days for BTI-TN5b1-4). Therefore, it is more economical to produce large quantities of ERβ in Sf21 cells. An additional comparison of peak ERβ protein production is provided in Table 1. The affinity of E₂ binding to ERβ harvested from Sf21 cells was calculated to be a K_d = 2.6 ± 0.2 nM (data not shown).

ERE Binding Activity of Baculovirus-Expressed ERβ

In addition to ligand binding, DNA binding is the second biological activi-

Figure 3. Comparison of maximum ERβ production in different cell lines. The amount of ERβ produced per well at the indicated cell densities and time of harvest post-infection was determined by HAP assay. Values are $\bar{x} \pm \text{SEM}$ of triplicate determinations from a single experiment that are representative of two to three separate time course experiments, with the exception of Tn368 (first experiment). 5b1-4, BTI-TN5b1-4 cells.

ty essential for steroid receptor function. We employed EMSA to examine if the amount of ER β determined by the HAP assay correlated with the estrogen response element (ERE) DNA binding activity and to compare the ERE binding activity of ER β produced by the different insect cell lines. We compared ER β binding using a fixed amount, 89 fmol ER β [determined by [^3H]E $_2$ binding (36)], and a fixed concentration, 65 fmol [^{32}P]ERE (Figure

4A). We compared the binding of ER β produced early versus late after infection to examine whether ER β DNA binding capacity is labile over the course of viral infection. ER β bound specifically to [^{32}P]ERE as indicated by the supershift of the complex by ER β antibody Y-19. The ER β -14B8 antibody did not cause a supershift of the ER β -ERE complex, possibly indicating that its epitope is unavailable in the ER β -ERE complex. Quantitation of the data indicated that ER β produced by BTI-TN5b1-4 and Sf9 cells showed higher ER β -ERE binding capacity early in harvest compared to later

harvest (Figure 4B). The amount of ER β binding was comparable among the cell lines. However, ER β produced by Sf21 cells appeared to have greater ERE binding at the later harvest time point than BTI-TN5b1-4 and or Sf9 cells (Table 1).

Western Blot Analysis of Baculovirus-Expressed ER β

We also assessed immunoreactive ER β protein production by Western blot analysis using two different ER β -specific antibodies (Figure 5). The CWK-F12 epitope is aa (amino acid) 272–285 in the human ER β ligand binding domain, and PA1-311 was raised against aa 45–55 of rat ER β . Identical results were obtained with the two antibodies: two bands of 54 and 50

Figure 4. Recombinant ER β binds EREs. (A) EMSA of ER β -ERE binding. Identical ER β concentrations (89 fmol) in nuclear extracts harvested early (3.7, 1.8, or 1.5 days) or late after infection (4.7, 2.5, or 2.5 days) for BTI-TN5b1-4, Sf9, and Sf-21 cells, respectively, were incubated with [^{32}P]ERec38 as described in the Materials and Methods section. ER β -specific antibodies Y-19 (indicated as “Y”) and 14C8 (indicated as “14”) were added to the designated reactions. The arrow indicates the specific ER β -ERec38 band, and SS- indicates the supershifted complex formed between the ER β antibody Y-19 and the ER β -ERec38 complex. EMSA details are provided in the text. This autoradiograph is representative of four independent EMSA experiments that show similar results. (B) The data from panel A were quantitated as described in the text, and the [^{32}P]dpm converted was into percentages of the ER β -ERec38 complex. The solid bars indicate the amount of ER β -ERE complex formed with no added antibody, and the open and hatched bars indicate the amounts of ER β -ERE complex formed with the addition of ER β antibodies Y19 and 14C-8, respectively. 5b1-4, BTI-TN5b1-4 cells.

Figure 5. Western blot of ER β protein production. (A) Identical amounts of protein (30 μg) from cell extracts of rat ER β baculovirus-infected BTI-TN5b1-4, Sf9, and Sf21 cells harvested early (3.7, 1.8, or 1.5 days) or late after infection (4.7, 2.5, or 2.5 days), respectively, were separated by 10% SDS-PAGE. The resulting PVDF membrane was probed with ER β -specific antisera PA1-311 or monoclonal CWK-F12 ER β antibody (13) as described in the Materials and Methods section. (B) The blots shown in panel A were quantitated as described in the text. Shown here is the percent of maximal pixel density in each blot, using the pixel density detected in Sf21 L as 100%. These data are from a single representative experiment that has been repeated with similar results. 5b1-4, BTI-TN5b1-4 cells.

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kDa were observed. The calculated molecular weight of ER β was 54.2 kDa (27), which accounted for the 54-kDa band. We suggest that the 50 kDa band may be the result of incomplete translation, partial protein degradation during sample processing, or incomplete glycosylation or phosphorylation.

Use of Baculovirus-Expressed ER β to Measure ERE Binding Affinity

Competition EMSA (46) was used to calculate the affinity of baculovirus-expressed ER β binding to EREc38. ER β binds EREc38, which has a "perfect" palindromic ERE, with high affinity ($K_d = 0.72 \pm 0.20$ nM). These data indicate the utility of baculovirus-expressed ER β for in vitro DNA binding affinity determinations.

DISCUSSION

The production of recombinant steroid and nuclear receptor proteins in the BEVS has predominantly used AcMNPV and the Sf9 cell line. Although comparisons of recombinant protein production with BEVS both from large-scale suspension culture and for smaller scale production in disposable flasks has been performed for proteins such as secreted placental alkaline phosphatase and β -galactosidase (15), human muscarinic M2 (19) and dopamine D2 receptors (20), and DNA methyltransferase (38), this is the first comparison of the production of a nuclear receptor in different insect cell lines.

We examined ER β production in four insect cell lines: Tn368, BTI-TN5b1-4, Sf9, and Sf21. The Sf9 cell line is a subclone of Sf21 and offers no particular advantage over Sf21 in adherent culture conditions (35). The widely used BTI-TN5b1-4 cells were developed for recombinant protein production and are more efficient secretors of exported proteins (15).

ER β production was determined as a function of cell density and time after infection in Tn368, BTI-TN5b1-4, Sf9, and Sf21 cells. Our results show that Sf21 and BTI-TN5b1-4 cells produce more bioactive ER β protein, based on [3 H]E $_2$ and ERE binding activity in cell homogenates, as compared to Sf9 cells.

The ER β produced in Sf21 cells shows 2- and 4-fold the specific activity of ER β produced in Sf9 and BTI-TN5b1-4 cells, respectively. DNA binding activity, determined by ERE binding using EMSA, is more effectively preserved late in the infection cycle of Sf21 cells compared to the other two cell lines. An additional advantage of Sf21 cells is that they produced more immunoreactive ER β /mg protein than did Sf9 or BTI-TN5b1-4 cells. Moreover, because ER β production in Sf21 cells peaks at an earlier time after infection (i.e., 1.6 days), we conclude that it is more economical to produce ER β on this scale in Sf21 cells.

The maximum level of ER β produced in Sf21 cells reported here was 18 pmol ER β dimer/mg protein, which is 36 pmol ER β monomer/mg protein (Table 1). This result is comparable with the value of 32.8 pmol/mg protein for chicken ER α (8) but lower than the 230 pmol ER α monomer/mg produced 111 h post-infection in the Sf9 cells (34). Compared to other steroid and nuclear receptors, we observed higher ER β production in Sf21 cells than the 5–10 pmol androgen receptor/mg protein (21) or the 3.8 pmol hPR-A/mg protein (14) observed in Sf9 cells but lower than the 1200 pmol hRAR α /mg nuclear protein produced in Sf9 cells (39). Unfortunately, we cannot compare our results for the production of rat ER β with those for the short form (485 aa) of hER β in Sf21 cells because no quantitation was provided (17).

In summary, we have shown that the Sf21 cell line is superior to the commonly used Sf9 cell line for the production of biologically active recombinant ER β . Baculovirus-expressed ER β bound EREs with high affinity in vitro, indicating the utility of this method of ER production for functional measures of ER activity. The clear superiority of Sf21 cells for ER β production should encourage this type of comparison for other nuclear receptors by other investigators since the choice of cell type can dramatically influence yield and purity.

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