

Gonadotropin Beta Subunits Determine the Rate of Assembly and the Oligosaccharide Processing of Hormone Dimer in Transfected Cells

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Abstract. The glycoprotein hormones lutropin (LH) and chorionic gonadotropin (CG) share a common structure consisting of an identical α subunit noncovalently linked to a hormone-specific β subunit. While LH is produced in the anterior pituitary, CG is synthesized in placenta. To compare the assembly, processing, and secretion of human LH and CG in the same cell type, we have expressed their subunits, individually and together, in mouse C-127 mammary tumor cells. Analysis of transfected clones revealed an unexpected difference in the secretion of individually expressed subunits. Whereas α and CG β subunits were rapidly and quantitatively secreted, only 10% of newly synthesized LH β subunit reached the medium. The remaining subunit was found in an intracellular, endoglycosidase H (endo H)-sensitive pool that had a

turnover rate of ~ 8 h. Coexpression with α subunit resulted in "rescue" of LH β subunit by formation of LH dimer, which was efficiently secreted. However, combination of LH β with α was slow, with an overall efficiency of only 50% despite the presence of excess α . In contrast, CG β was rapidly assembled with the α subunit after synthesis. The two β subunits also differed in their influence on the N-linked oligosaccharide processing of combined α . The oligosaccharides of LH dimer were endo H resistant, while those of CG dimer remained partially endo H sensitive. Thus, despite a high degree of homology between LH β and CG β , the two subunits differ in their secretion as free subunits, their rate of assembly with α subunit, and in their effect on the N-linked oligosaccharide processing of combined α .

LUTROPIN (LH),¹ and chorionic gonadotropin (CG) are members of the glycoprotein hormone family that also includes follitropin (FSH) and thyrotropin. Each of these hormones is composed of two noncovalently linked subunits, α and β . Within a species, the α subunits of the hormones are identical in amino acid sequence, while the unique β subunits determine biological specificity (Pierce and Parsons, 1981; Sairam, 1983). LH, which is made in the pituitary, and CG, a product of the placenta, are the most closely related among the hormones in both structure and function. Amino acid homology of human LH β and CG β subunits exceeds 80%, including full conservation of cysteine residues (Shome and Parlow, 1973; Carlsen et al., 1973; Morgan et al., 1975; Keutmann et al., 1979). This close homology allows the β subunits, in concert with α subunit, to bind the same gonadal receptors (Rajaniemi and Vanha-Perttula, 1972; Haour and Saxena, 1974). Full biological ac-

tivity of LH and CG is dependent on the N-linked oligosaccharides present on both the α and β subunits (Moyle et al., 1975; Sairam, 1983).

Despite these similarities, human LH and CG bear N-linked oligosaccharides that are structurally distinct (reviewed by Green et al., 1986). The oligosaccharides of LH contain terminal N-acetylgalactosamine residues that are sulfated (Green et al., 1986) while those of CG terminate with galactose and sialic acid (Endo et al., 1979; Kessler et al., 1979; Mizuochi et al., 1979). Understanding the processing of LH and CG oligosaccharides is important in view of their role in the bioactivity of the hormones. Studies by Green et al., (1986) have established that the different structures of LH and CG are in part determined by different oligosaccharide processing enzymes in their tissues of origin. Thus, sulfation of LH in the pituitary is due to a sulfotransferase activity that is not found in the placenta (Green et al., 1984).

Underlying such tissue-specific differences in processing, however, there may be subunit-determined effects on oligosaccharide structure. LH and FSH are produced in the same

1. *Abbreviations used in this paper:* BPV, bovine papilloma virus; CG, chorionic gonadotropin; endo F, endoglycosidase F; endo H, endoglycosidase H; ER, endoplasmic reticulum; FSH, follitropin; LH, lutropin; MT, mouse metallothionein-I.

pituitary gonadotropes (Nakane, 1970; Phifer et al., 1973), yet they bear very different oligosaccharides (Green et al., 1985 and 1986). As suggested by Green et al. (1985), the processing of LH and FSH oligosaccharides may be influenced by their respective β subunits. A role of protein conformation in determining oligosaccharide processing has been established from studies of both viral and cellular glycoproteins (Burke and Keegstra, 1979; Weitzman et al., 1979; Nakamura and Compans, 1979; Rosner et al., 1980; Green, 1982; Sheares and Robbins, 1986). Whether such intersubunit effects are manifested in soluble, multisubunit complexes remains to be determined.

To investigate subunit-specific effects in the processing of glycoprotein hormones, we have established clones of transfected mouse C-127 mammary tumor cells that secrete human LH and CG. These clones eliminate the problem of tissue variability, allowing direct comparison of LH and CG synthesis in the same cell type. The clones show that the β subunits influence oligosaccharide processing of dimer. We have also isolated clones expressing α , LH β , and CG β subunits individually. Since secretion of LH β and CG β as free subunits is not observed in their native tissues (Franchimont et al., 1972; Prentice and Ryan, 1975), these clones provide the first opportunity to compare their processing and secretion. Comparison of LH β and CG β reveals unexpected differences in their secretion as free subunits, and in their rates of assembly with α subunit.

Materials and Methods

Materials

[³⁵S]Cysteine (1140 Ci/mmol) and L-[2,3,4,5-³H]arginine (40 Ci/mmol) were purchased from Amersham Corp. (Arlington Heights, IL). Cell culture media were prepared by the Center for Basic Cancer Research, Washington University School of Medicine. Fetal calf serum was purchased from KC Biological, Inc. (Lenexa, KS) or Hazleton Dutchland (Denver, PA), and dialyzed calf serum was from Gibco (Grand Island, NY). A preparation of *Staphylococcus aureus* cells (Pansorbin) was obtained from Behring Diagnostics (San Diego, CA). Rabbit anti-mouse Ig serum was from Pel-Freez Biologicals (Rogers, AR). Purified human LH was obtained from Dr. Salvatore Raiti, National Hormone and Pituitary Program, Baltimore, MD.

Vector Construction and Transfection

Construction of a bovine papilloma virus (BPV) vector for the expression of human α subunit (pMONI040) has been described (Ramabhadran et al., 1984). Construction of a vector for the expression of human CG β subunit will be detailed elsewhere (Corless, C. L., M. Bielinska, T. V. Ramabhadran, S. Daniels-McQueen, T. Otani, B. A. Reitz, D. C. Tiemeier, and I. Boime, manuscript submitted for publication). Briefly, a 3.7-kb fragment bearing the coding regions of a cloned CG β gene was inserted 3' to the promoter of the mouse metallothionein-I (MT) gene (Glanville et al., 1981), and the product of this fusion was cloned into a pBR322 plasmid carrying the entire BPV genome (Howley et al., 1983). The final BPV-MT-CG β vector (pMONI055) was used in single transfections or in transfections together with the BPV- α vector (pMONI040) to generate cell lines expressing CG β subunit or CG dimer, respectively (see below).

A pBR322 plasmid carrying a 1.25-kb genomic fragment that encodes human LH β subunit was obtained from Dr. John Fiddes, California Biotechnology, San Francisco, CA (gene CG β 4, Talmadge et al., 1984). Bam HI linkers (New England Biolabs, Beverly, MA) were inserted into the Bst EII site that lies 96 bp 5' to the AUG start of LH β and also into the 3' Eco RI site, allowing isolation of the LH β coding sequences on a single Bam HI fragment. The vector used in LH β transfections was adapted from a pSV2neo (Southern and Berg, 1982) construct in which was present the above MT-CG β fusion gene (Corless et al., manuscript submitted). The CG β sequences of this pSV2neo-MT-CG β plasmid were excised with Bgl II and

Bam HI, and were substituted by LH β coding sequences on the above Bam HI fragment. The resulting vector, pSV2neo-MT-LH β , contains LH β coding sequences under the control of the MT promoter (Fig. 1).

Cell Culture

C-127 clones were cultured in DME containing 4.5 g/liter glucose and 3.7 g/liter NaHCO₃, supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 7.5% heat-inactivated fetal calf serum. The cells were maintained at 37°C in a 5% CO₂ atmosphere.

Transfection and Selection of C-127 Clones

Isolation of C-127 clones expressing α or CG β subunit has been described (Ramabhadran et al., 1982; Corless, C. L., M. Bielinska, T. V. Ramabhadran, S. Daniels-McQueen, T. Otani, B. A. Reitz, D. C. Tiemeier, and I. Boime, manuscript submitted for publication). pSV2neo-MT-LH β was transfected into C-127 cells by the calcium phosphate method (Graham and van der Eb, 1973) as modified by Howley et al. (1983). 10 μ g of precipitated DNA was added to 6-cm dishes containing 8×10^5 cells, and a glycerol shock was performed 6 h later. 48 h after the shock, cells were trypsinized and replated at various dilutions. Stably transfected cells were selected in media containing 0.8 mg/ml of G418 (Gibco). G418-resistant colonies were picked after 14 d and screened for the expression of LH β by incubation in [³⁵S]cysteine, immunoprecipitation of lysates with β antiserum, and analysis by SDS PAGE (see below for details). To facilitate comparison with BPV-transformed clones, positive LH β clones were supertransfected with pBPV2308 (obtained from Dr. Peter Howley, National Cancer Institute, Bethesda, MD) and repicked as transformed colonies. LH β clones were maintained in media containing 0.5 mg/ml G418 and were checked by phase-contrast microscopy for the transformed phenotype before use in experiments.

Isolation of a C-127 clone expressing both α and CG β subunits (clone 2) will be detailed elsewhere (Corless, C. L., manuscript submitted for publication). Briefly, C-127 cells were transfected simultaneously with BPV- α (pMONI040) and BPV-CG β (pMONI055) vectors, and transformed colonies were screened for expression of α and CG β subunits in subunit-specific radioimmunoassays. Clone 2 secretes CG dimer that is bioactive (Ramabhadran, T. V., and K. M. J. Menon, unpublished results), and its phenotype has remained stable throughout 5 mo of continuous culture (data not shown).

Clones expressing LH dimer were obtained by transfecting a clone that stably expresses α subunit (clone α 20, Ramabhadran et al., 1984) with pSV2neo-MT-LH β . Transfection and selection of cells was carried out exactly as for the above LH β clones. Analysis of G418-resistant colonies from these transfections by labeling, immunoprecipitation and SDS PAGE showed that the colonies uniformly expressed α subunit. This confirmed a stable phenotype in the parental clone α 20. Expression of LH β subunit varied among the isolated colonies; however, in all colonies that were expanded and analyzed further the secretion of α subunit exceeded that of LH β (see Fig. 5). This imbalance in subunit secretion may reflect differences in the processing and stability of the mRNAs transcribed from their respective vectors. The LH dimer clones were maintained in media with 0.5 mg/ml G418 to ensure continued expression of the LH β subunit, and were inspected for the transformed phenotype (contributed by the BPV- α vector) before each experiment.

Cell Labeling and Sample Preparation

The labeling medium used was cystine-free DME supplemented with 20 μ M cysteine, 2 mM glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 5% dialyzed fetal calf serum. Subconfluent cultures were incubated with 20–125 μ Ci/ml [³⁵S]cysteine, depending on the experiment. The cells continued to grow while labeling under these conditions. After labeling, cells were rinsed twice with phosphate-buffered saline, lysed in 50 mM Tris (pH 7.8), 150 mM NaCl, 5 mM EDTA, 0.6% SDS, then diluted with 5 vol of the same buffer containing 0.6% Triton X-100 instead of SDS; the final volume was equivalent to the volume of media in which the cells were labeled. The viscosity of the lysates was reduced by aspiration through a 22-gauge needle five times. Media samples were adjusted to the following: 50 mM Tris (pH 7.8), 150 mM NaCl, 5 mM EDTA, 0.5% Triton X-100, 0.1% SDS. Phenylmethylsulfonyl fluoride (P7626; Sigma Chemical Co., St. Louis, MO) and iodoacetamide (I6125; Sigma Chemical Co.) prepared in *n*-propanol were added to media and lysate samples to a final concentration of 0.3 mg/ml.

Immunoprecipitation and Gel Electrophoresis

To reduce background, all samples were incubated with nonimmune rabbit serum for 4 h at room temperature or overnight at 4 C. The nonimmune rabbit serum was then cleared by addition of a suspension of *S. aureus* cells (10 vol/vol nonimmune rabbit serum) for 30–60 min at room temperature. hCG α - and hCG β -specific rabbit antisera were generously provided by Dr. Steven Birken and Dr. Robert Canfield (Columbia University College of Physicians and Surgeons, New York). Control experiments demonstrated that the α antiserum recognizes free α subunit as well as CG dimer and LH dimer, but does not react with free β subunits. Similarly, the β antiserum recognizes free LH β , CG β , and both dimers, but not free α subunit. Immune complexes were precipitated with *S. aureus* cells. Free β -specific B201 monoclonal antibody (Krichevsky, A., and R. Canfield, manuscript submitted for publication) was precipitated by incubating for 1 h at room temperature with rabbit anti-mouse Ig serum preabsorbed to *S. aureus* cells. Precipitates were washed twice in 50 mM Tris (pH 7.8), 150 mM NaCl, 5 mM EDTA, 0.5% Triton X-100, 0.1% SDS, then boiled in sample buffer and loaded on 20% SDS polyacrylamide gels as described (Maizel, 1971). Gels were soaked in 1 M sodium salicylate for 12 min (Chamberlain, 1979) before drying and exposure to pre-fogged film (Laskey and Mills, 1975).

Glycosidase Digests

After washing, immune complexes precipitated with *S. aureus* cells were resuspended in 40–50 μ l of 50 mM sodium acetate (pH 5.5) containing 0.5% Triton X-100, 0.1% SDS and 12.5 mM 2-mercaptoethanol. Samples were heated for 5 min at 95°C and centrifuged in a microfuge for 2 min. Supernatants were incubated at 37°C for 20–24 h with or without endoglycosidase H (endo H) (purchased from Dr. Frank Maley, NY State Department of Health, Albany, NY) or endoglycosidase F (endo F) (a gift of Dr. Jacques Baenziger, Washington University School of Medicine). After digestion, concentrated gel sample buffer was added and the samples were reheated to 95°C and loaded on 20% SDS polyacrylamide gels.

Results

Comparison of LH and CG processing and secretion in C-127 cells required clones expressing one or both subunits of each hormone. Clones expressing free α subunit were previously isolated (Ramabhadran et al., 1984). Here we describe clones expressing free LH β subunit and LH dimer (see below). Isolation of clones expressing free CG β subunit and CG dimer, and study of these clones in relation to placental CG synthesis will be described elsewhere (Corless, C. L., M. Bielinska, T. V. Ramabhadran, S. Daniels-McQueen, T. Otani, B. A. Reitz, D. C. Tiemeier, and I. Boime, manuscript submitted for publication). For comparison, subunits secreted by clones expressing free α , free CG β , and

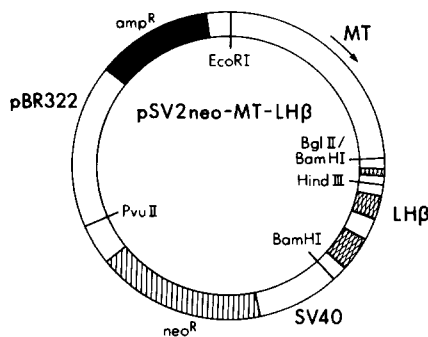


Figure 1. Expression vector for LH β subunit. The three coding regions (cross-hatched) isolated from the LH β gene were inserted onto a Bam HI–Bam HI fragment into pSV2-neo (Southern and Berg, 1982) containing the metallothionein-I (MT) promoter (see text for details). Genes encoding neomycin resistance (striped) and ampicillin resistance (solid) are noted.

CG dimer are shown in Fig. 2. The clones were incubated with [³⁵S]cysteine and the subunits immunoprecipitated and analyzed by SDS PAGE. A clone transfected with a BPV vector carrying an α cDNA linked to the MT promoter (Ramabhadran et al., 1984), secretes a large, heterogeneous form of α subunit that precipitates with α -specific antiserum (lane 1). A clone transfected with a BPV-CG β subunit expression vector secretes a protein with M_r 32,000 that is specifically recognized by β antiserum (lane 2). Coexpression of α and CG β subunits yields CG dimer (lane 5), which cross-reacts with antisera to both subunits (anti- β precipitate not shown), and is bioactive in a progesterone-stimulation assay (Ramabhadran, T. V., and K. M. J. Menon, unpublished results). An interesting feature of these C-127 clones is that the combined form of α subunit (M_r 21,000–22,000) is substantially smaller than the free form (M_r 25,000–27,500) (cf. lanes 1 and 5). This is due to differences in the N-linked oligosaccharides of the two forms of α (Corless, C. L., M. Bielinska, T. V. Ramabhadran, S. Daniels-McQueen, T. Otani, B. A. Reitz, D. C. Tiemeier, and I. Boime, manuscript submitted for publication).

Isolation of C-127 Clones Producing LH β and LH Dimer

To express human LH β subunit in C-127 cells, a genomic fragment bearing the coding regions of LH β was cloned adjacent the MT promoter in a derivative of pSV2neo (Fig. 1). Sequencing of the coding regions confirmed the correct sequence for LH β subunit (data not shown). Transfection and selection of C-127 cells in media containing G418 yielded multiple clones synthesizing a protein corresponding to glycosylated LH β (M_r 18,000 on SDS polyacrylamide gels), which was specifically precipitated by CG β antiserum (Fig.

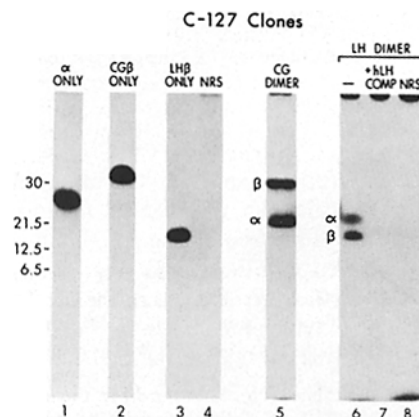


Figure 2. Comparison of C-127 clones. Flasks (25 cm²) of subconfluent C-127 clones were incubated for 12 h in 5 ml of cysteine-free labeling media (see Materials and Methods) containing 20 μ M cysteine and 20 or 50 μ Ci/ml [³⁵S]cysteine (depending on the clone). Subunits were immunoprecipitated from medium (lanes 1, 2, and clones 5–9) or lysate (lanes 3 and 4), and analyzed by SDS PAGE. Lanes shown are from the same fluorograph. (Lane 1) α clone 20 with α antiserum; (lane 2) CG β clone 55 with β antiserum; (lane 3) LH β clone 8–11 with β antiserum; (lane 4) LH β clone 8–11 with nonimmune serum; (lane 5) CG dimer clone 2 with α antiserum; (lane 6) LH dimer clone 8–12 with β antiserum; (lane 7) as in lane 6 with 30 μ g hLH added; (lane 8) LH dimer clone 8–12 with nonimmune serum. The migration of size markers is noted on the left (in kilodaltons).

2, lane 3) but not by nonimmune rabbit serum (lane 4). The protein was also recognized by antiserum to bovine LH β subunit, and by six different monoclonal antibodies generated against CG β subunit (data not shown). Such immunocross-reactivity of LH β and CG β reflects the extensive homology between the two subunits. The identity of the immunoreactive protein as LH β was confirmed by N-terminal sequencing. Biosynthetically labeled subunit showed [3 H]arginine at positions 2 and 6, and [35 S]cysteine at position 9, indicating that C-127 cells cleave the signal peptide of LH β at the correct site (Shome and Parlow, 1973; Keutmann et al., 1979).

The clones expressing α and CG β subunits were phenotypically transformed by the BPV vectors used to express the subunits. Since the pSV2neo vector used to express LH β subunit did not transform the cells, we transfected LH β clones with BPV DNA and repicked them as transformed colonies. This ensured a uniform phenotype among all the subunit clones, even though BPV transformation of LH β clones did not affect β processing or secretion (data not shown).

Clones expressing LH dimer were obtained by supertransfecting an α -expressing clone (clone α 20, Fig. 2, lane 1) with the LH β expression construct and selecting in G418-containing media. Immunoprecipitation of labeled media from one of the LH dimer clones with CG β antiserum shows both LH β and associated α subunit, indicating that the subunits are combined (Fig. 2, lane 6). This was confirmed in a modified receptor binding assay (Moyle, W., Jr., manuscript in preparation), in which LH dimer, but not the individually expressed subunits, was able to bind simultaneously to rat ovarian receptor and radiolabeled monoclonal antibody (data not shown). Precipitation of the secreted LH dimer was not observed with nonimmune serum (lane 8), and was completely blocked by the addition of purified hLH (lane 7). The LH dimer clones were maintained in media containing G418 to ensure continued expression of the LH β subunit.

Secretion of LH β

Characterization of the intracellular and secreted forms of the transfected subunits revealed an unexpected finding. Whereas most of the free α synthesized during a 12-h pulse with [35 S]cysteine was secreted into the media (Fig. 3 A), only trace amounts of free LH β were detected in the media of all clones examined, two of which are shown in Fig. 3 B. The remainder of the LH β was present in the cell lysate. Though intriguing, we were nevertheless concerned if this result was unique to these cells or whether it reflected a general property of LH β subunit. Unfortunately, study of LH β synthesis in its native tissue (human pituitary) is not feasible, and there are no LH secreting tumor cell lines available. We therefore transfected AtT-20 mouse pituitary cells, which secrete adrenocorticotropin (Orth et al., 1973), with the LH β expression construct used in C-127 cells (Fig. 1). An AtT-20 clone incubated for 12 h with [35 S]cysteine showed the same distribution of LH β in the lysate and media (Fig. 3 C) as seen in C-127 clones. Transfection of Chinese hamster ovary cells with LH β yielded identical results (not shown).

The lack of LH β subunit in the medium suggested that it is poorly secreted. However, the alternative possibility, that

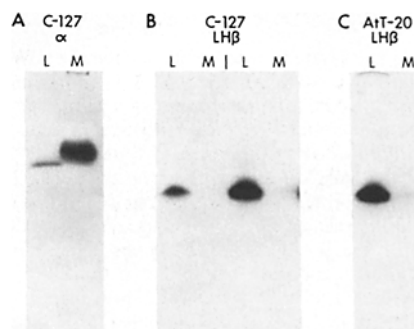


Figure 3. Secretion of LH β subunit. Clones were incubated for 12 h with [35 S]cysteine and samples immunoprecipitated from lysates and media were analyzed by SDS PAGE and fluorography. (A) Lysate (L) and medium (M) from C-127/ α clone 20, precipitated with α antiserum. (B) Lysate and medium from C-127/LH β clone 9-2 (left) and clone 9-13 (right), precipitated with β antiserum. (C) lysate and medium from AtT-20/LH β clone 5-9, precipitated with β antiserum.

the subunit is degraded in the medium, is difficult to exclude. In control experiments the small amount of LH β detectable in the medium appeared to be stable for several hours and was not influenced by the presence of serum (data not shown). Nevertheless, it is possible that this population was resistant to protease action while the majority of the subunit was rapidly degraded. However, this is unlikely for the following reasons: first, despite the use of three different polyclonal antisera, degradation products or fragments of LH β were not detected in the medium. Second, in pulse-chase experiments such as those discussed below, the majority of the pulse-labeled LH β remained cell associated throughout the chase. Third, the behavior of the subunit was identical in three cell lines that differ in species and tissue origin. It seems unlikely that all three cell lines secrete proteases that cause the same, very rapid degradation of LH β subunit. We propose, therefore, that the lack of LH β in the medium is due to inefficient secretion rather than to extracellular degradation.

Combination with α Rescues LH β Subunit

In contrast to LH β , LH dimer is readily detected in the media (Fig. 2, lane 6). To further examine the difference in secretion of combined and free LH β , and to compare secretion of the other subunits, pulse-chase experiments were performed. Clones were pulsed with [35 S]cysteine for 10 min and chased for various periods up to 5 or 25 h (Fig. 4). Secretion of pulse-labeled free α (Fig. 4 A) and free CG β (Fig. 4 B) was nearly complete in 5 h, and there was no loss of signal in either subunit. Free LH β subunit, however, did not accumulate in the media to appreciable levels even after 25 h of chase (Fig. 4 D). Scanning densitometry of data from Fig. 4 and from a second experiment was used to quantitate the rate of secretion for each subunit (Table I). The half-time of secretion was identical for free α and CG β . In the case of free LH β , the maximum secretion observed in three experiments was 12% after 25 h of chase. The remainder of the labeled LH β disappeared from the cells with a $t_{1/2}$ of 8.4 h. Thus, the majority of free LH β is degraded intracellularly rather than secreted.

When coexpressed with α subunit, the fate of LH β is al-

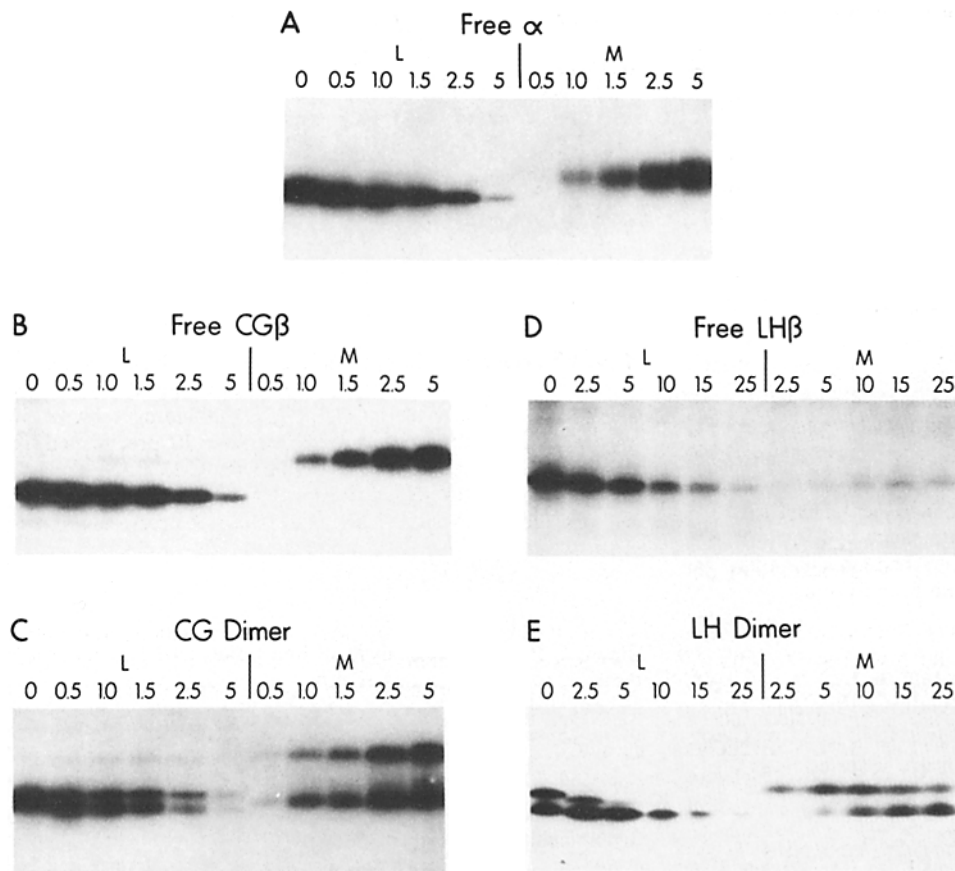


Figure 4. Pulse-chase analysis of C-127 clones. C-127 clones were plated onto 35-mm dishes (6×10^5 cells/dish) and cultured for 18 h. Cells were incubated in labeling medium for 1 h, then pulsed for 10 min with 0.4 ml of the same medium containing 125 $\mu\text{Ci/ml}$ [^{35}S]cysteine. At the end of the pulse, cells were rinsed twice with phosphate-buffered saline and chased in 1 ml normal culture medium supplemented with 1 mM cysteine for the indicated times. Lysate (L) and media (M) samples were immunoprecipitated with α antiserum (A and C) or β antiserum (B, D, and E), and analyzed by SDS PAGE. (A) α clone 20; (B) CG β clone 55; (C) CG dimer clone 2; (D) LH β clone 8-11; (E) LH dimer clone 8-12.

tered (Fig. 4 E). Scanning densitometry shows that 50% of pulse-labeled LH β reached the media as dimer during a 25-h chase. This rescue of LH β was reproducible, and was observed in two other independently isolated LH dimer clones (not shown). Thus, combination with α subunit permits exit of LH β from the cell. Secretion of CG dimer was quantitative, with a half-time that was slightly shorter than its free subunits (Table I).

Assembly of LH β with α Is Delayed

Based on the cysteine content of α subunit (10 cysteines), and LH β and CG β subunits (12 each), nearly equivalent labeling of the subunits in pulse-labeled dimer should be observed. This was the case for secreted CG dimer (Fig. 4 C), but not for LH dimer (Fig. 4 E). In pulse-labeled LH dimer cells,

labeled α subunit was chased into secreted dimer much sooner than labeled β subunit. Since β antiserum was used, the labeled α seen in early chase periods must be combined with predominantly unlabeled LH β subunit. This suggests the presence of an intracellular pool of free LH β , which dilutes newly labeled β and thereby slows its appearance in dimer. However, the existence of a pool of free LH β is inconsistent with the observation that the cells produce α in ~ 40 -fold excess over β (Fig. 5). The excess α subunit should combine with any and all LH β , unless the β subunits are unable to combine. The presence of a pool of unassociated LH β was not excluded by the experiment in Fig. 4 E, because the β antiserum used recognizes free and combined forms of β equally well. We therefore repeated the pulse-chase experiment using a monoclonal antibody specific for free β subunit, B201. As shown in Fig. 6, B201 antibody precipitated a substantial fraction of the total LH β (measured by β antiserum) present in LH dimer cells. Densitometry showed that at the end of the 10-min pulse, 95% of LH β was uncombined. This fraction declined to $\sim 50\%$ after 5 h of chase and leveled off thereafter, in good agreement with the 50% rescue of LH β observed in Fig. 4 E. Thus, despite the synthesis and secretion of excess α subunits by LH dimer cells, most of the newly synthesized LH β remains uncombined for a period of hours. About half of the β does not associate with α ; as expected, this free LH β is apparently degraded rather than secreted (Fig. 6).

The half-time of secretion for LH dimer, best judged by the appearance of labeled α in the media, was determined in short (5 h) pulse-chase experiments (not shown). LH dimer

Table I. Rates of Subunit Secretion

Phenotype	$t_{1/2}^*$ (average of two experiments)
	h
Free α	2
Free CG β	2
CG Dimer	1.4
Free LH β †	8.4
LH Dimer	
α subunit	2.1
β subunit‡	~ 10

* Time when signals in lysate and media are equal.

† Time when signal in lysate is diminished by half.

‡ Not corrected for degradation in lysate.

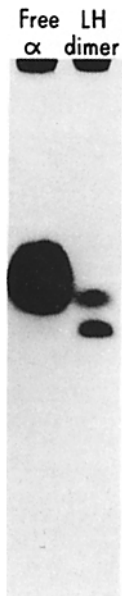


Figure 5. Excess α subunit secreted with LH dimer. LH dimer clone 8-12 was incubated with [35 S]cysteine for 12 h and samples were analyzed by SDS PAGE. Free α was precipitated from 0.3 ml of medium with α antiserum; LH dimer was precipitated from 1.2 ml medium (four times the volume for free α) with β antiserum.

was secreted at virtually the same rate as free α and free CG β , and only a little slower than CG dimer (Table I). Thus, despite the difference in secretion of free LH β and free CG β , the combined forms of these subunits are secreted with similar kinetics.

The β Subunits Affect Oligosaccharide Processing on Dimer

The N-linked oligosaccharides of gonadotropins are critical for bioactivity (Moyle et al., 1975; Sairam, 1983). To compare the extent of oligosaccharide processing on LH and CG dimer produced in the same cell type, we treated the dimers secreted by C-127 cells with endo H. This enzyme cleaves between the two core N-acetylglucosamine residues of high mannose and hybrid-type N-linked oligosaccharides, but does not release complex-type oligosaccharides (Tarentino and Maley, 1974), thereby allowing rapid classification of N-linked structures. endo F was used as a control for the migration of deglycosylated subunits, since this enzyme cleaves both high mannose and complex oligosaccharides (Elder and Alexander, 1982). Both subunits of secreted LH dimer were completely resistant to endo H (Fig. 7). As expected, endo F removed all oligosaccharides from the subunits (Fig. 7). In contrast to LH dimer, the subunits of CG dimer were partially sensitive to endo H. At least half of the dimer CG β was reduced in size by the loss of one N-linked oligosaccharide after endo H treatment (Fig. 7). Endo F removed both oligosaccharides from CG β . The same findings applied to the α subunit of CG dimer (Fig. 7). These results indicate that there is a difference in the interaction of α with LH β and CG β ; LH β allows for more complete N-linked processing, both on itself and associated α , than does CG β .

Since the efficiency of LH dimer and free LH β secretion is vastly different, we considered whether the oligosaccharide processing of free and combined LH β also differed. The poor secretion of LH β necessitated preparative labelings of an LH β clone, using increased amounts of [35 S]cysteine so that enough secreted material was obtained to allow analysis

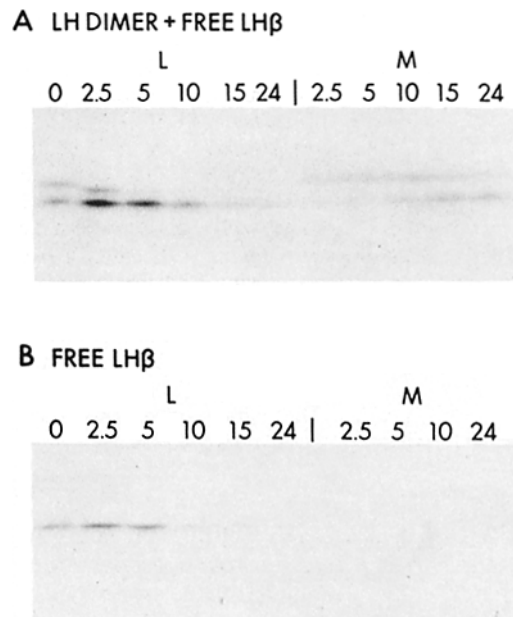


Figure 6. Pulse-chase analysis of free versus total LH β in an LH dimer clone. Cells from LH dimer clone 8-12 were plated into 60-mm dishes (9.5×10^5 cells/dish), and cultured for 18 h. A pulse-chase was performed as in Fig. 3, except that the volume of medium used was increased to 0.8 ml during the pulse and 3 ml during the chase. Lysate (L) and medium (M) collected at the indicated times were divided and immunoprecipitated with β antiserum (A) or monoclonal antibody B201 (B).

with endo H. Like the β in LH dimer, the secreted free LH β proved to be resistant to endo H (Fig. 8). Therefore, despite its inhibited secretion, LH β follows a normal secretory pathway in exiting the cell. Analysis of the intracellular form of LH β showed that it was sensitive to endo H (Fig. 8), suggesting that the majority of the subunit is present in the endoplasmic reticulum. The subunits of LH and CG dimer present within the cell were also endo H sensitive (data not shown), consistent with the view that assembly occurs predominantly in the endoplasmic reticulum (ER) (Hoshina and Boime, 1982; Magner and Weintraub, 1982; Peters et al., 1984).

Discussion

The LH β and LH dimer expressing clones described here, together with our CG expressing clones, allow the first direct comparison of the assembly and oligosaccharide processing of LH and CG within the same cell type. We are confident that the LH β clones express the correct product based on the following: (a) the sequence of the cloned gene was confirmed to encode LH β subunit; (b) expression of the gene yields a glycosylated protein of appropriate size and immunoreactivity for LH β ; (c) the expressed subunit has the correct N-terminal sequence; and (d) the subunit can combine with α to form LH dimer that binds LH receptor.

In examining the synthesis of LH β subunit, we were surprised that the uncombined form is very poorly secreted. Intracellular LH β was sensitive to endo H, suggesting that the block in secretion occurs in exit from the endoplasmic reticulum. The rate at which proteins are transported from the ER to the Golgi varies widely (Strous and Lodish, 1980; Fitting

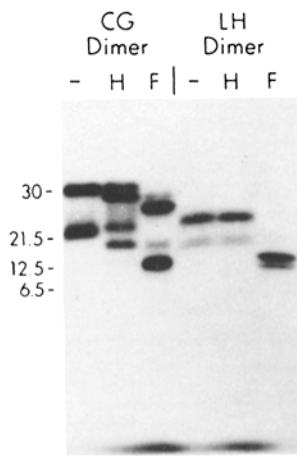


Figure 7. Endo H analysis of CG dimer and LH dimer. [³⁵S]-Cysteine-labeled CG dimer was precipitated from the medium of CG dimer clone 2 with α antiserum, while labeled LH dimer was precipitated from the medium of LH dimer clone 8-12 with β antiserum. The precipitates were incubated at 37°C for 20 h in the absence of enzyme (-), or in the presence of endo H (H) or endo F (F). The migration of size markers is noted (in kilodaltons).

and Kabat, 1982; Lodish et al., 1983; Scheele and Tartakoff, 1985), and is an important determinant of the overall rate of protein secretion from nongranulated cell types. Studies of mutant immunoglobulin (Ig) λ_2 chains, and of the Z variant of α_1 -antitrypsin, have shown that these altered proteins are unable to leave the ER (Wu et al., 1983; Hercz et al., 1978). In the case of LH β , the inhibition of secretion is not complete; $\sim 10\%$ of newly synthesized β reaches the media. The remaining subunit is degraded with a $t_{1/2}$ of ~ 8.4 h, a rather slow rate compared with the 80-min turnover of unassembled μ heavy chains in B lymphoma cells (Dulis et al., 1982). However, mutant Ig λ chains are degraded more slowly (3 h) than normal chains (2 h) (Winberry et al., 1980), so there may be little correlation between protein structure and the rate of degradation.

While free LH β is poorly secreted, LH dimer is secreted at a rate nearly equivalent to CG dimer. Evidently, assembly with α subunit rescues LH β , from which it follows that the formation of LH dimer occurs at a point in the secretory pathway prior to the block in free LH β secretion. It is known that assembly of glycoprotein hormones begins in the ER (Hoshina and Boime, 1982; Magner and Weintraub, 1982; Peters et al., 1984), and this is consistent with the above view that free LH β is blocked in exiting the ER. We therefore suggest that combination with α subunit allows LH β to bypass the ER block. The importance of assembly in regulating subunit secretion is clear from studies of other proteins. For example, assembly of fibrinogen subunits, which occurs in the ER (Kudryk et al., 1982), is a critical step in determining whether the subunits are secreted or degraded (Plant and Grieninger, 1986). Similarly, the α subunit of acetylcholine receptor must assemble with β , δ , and γ subunits to escape degradation in the ER (Merlie and Smith, 1986). In the synthesis of histocompatibility antigens γ subunit is required for processing of the α and β subunits of HLA-DR (Kvist et al., 1982), while β_2 -microglobulin is necessary for transport and expression of HLA-A and HLA-B antigens (Owen et al., 1980). Finally, covalent assembly with light chains allows processing and secretion of IgM heavy chains (Dulis et al., 1982; Bole et al., 1986). Thus, subunit combination is an essential feature in the expression of both membrane-bound (HLA antigens, acetylcholine receptor) and soluble proteins (fibrinogen, IgM, LH dimer).

While LH and CG dimers are secreted with similar ki-

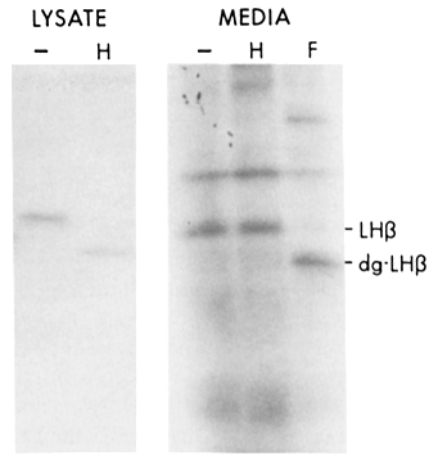


Figure 8. Endo H analysis of LH β subunit. [³⁵S]Cysteine-labeled LH β was immunoprecipitated from the lysate and medium of LH β clone 8-11. Precipitates were incubated at 37°C for 24 h in the absence (-) or presence of endo H (H) or endo F (F). The positions of LH β and deglycosylated (dg-LH β) are noted; the higher bands present in the media samples are nonspecific.

netics, the rates at which their subunits assemble are very different. Newly labeled α and CG β subunits are already combined at the end of a 10-min pulse. In LH dimer cells, however, pulse-labeled α is secreted in association with unlabeled LH β , while the labeled β remains within the cells as free subunit. The delay in the assembly of labeled LH β may be due to its dilution by a large pool of unlabeled, free subunit. Such pools of unassembled subunit have been observed in other systems (Tartakoff and Vassalli, 1979; Kvist et al., 1982; Ho and Springer, 1983). What is unusual in LH dimer cells is that the pool of free LH β subunit persists despite the presence of excess α subunit. This apparently reflects a delay in the ability of the β to combine with α . With time, approximately half of the LH β labeled during a short pulse is combined and thereby rescued. The other half of the β remains uncombined and is eventually degraded intracellularly.

Whether the delay in assembly of LH β is related to, or independent from, its low rate of secretion is unclear. The simplest view is that these phenomena are related. For example, there may be a specific binding protein for LH β that prevents it from assembling with α or exiting as free subunit. Such a protein was recently shown to prevent exit of unassembled Ig heavy chains from the ER (Bole et al., 1986), and similar effects on the expression of HLA antigens are mediated by the E3/19K protein of type 2 adenovirus (Burgert and Kvist, 1985). However, we have been unable to detect specific association of any proteins in the immunoprecipitates of labeled LH β . Another explanation for the behavior of LH β is that nascent α and LH β are somehow segregated into different regions of the rough ER, requiring time to reach a common ER region. However, there is no precedence for such segregation and it would be difficult to reconcile with the rapid assembly of α with CG β .

Perhaps a more likely explanation is that LH β precipitates in the ER, or else is associated with the ER membrane. Of the four human glycoprotein hormone β subunits, LH β is the most hydrophobic, particularly in the region between residues 75 and 90 (Krystek et al., 1985). In addition, there are

seven amino acids encoded at the 3' end of LH β coding sequence that are not found on the carboxyl terminus of β isolated from human pituitary (Keutman et al., 1979). The encoded carboxyl terminal sequence, -Leu-Ser-Gly-Leu-Leu-Phe-Leu, is quite hydrophobic. Perhaps this hydrophobic tail, or the region between amino acids 75 and 90, promotes self-aggregation of LH β and/or its interaction with membranes, thereby inhibiting both assembly and ER exit.

From the behavior of LH β in C-127 cells one might predict that its assembly with α is slow and inefficient in human pituitary gonadotropes as well. Unfortunately, there are no data available on the biosynthesis of LH β in its native tissue, nor are there suitable human tumor cell lines in which it might be examined. Studies of the synthesis of rat LH (Hoshina and Boime, 1982) and bovine LH (Corless, C. L., and I. Boime, unpublished results) subunits have not revealed a delay in the assembly of newly synthesized β . Thus, the findings reported here may be limited to human LH β . In this regard it is interesting that there is a species difference in the distribution of N-linked oligosaccharides on LH β subunits. The single glycosylated asparagine in rat, bovine, ovine, and porcine LH β subunits is located at position 13, while that of human LH β is at position 30 (Pierce and Parsons, 1981; Jameson et al., 1984). HCG β has glycosylated asparagines at both positions 13 and 30. Creation of a second glycosylation site in LH β by in vitro mutagenesis should allow us to determine whether an extra N-linked oligosaccharide can restore rapid assembly and/or secretion.

Endo H digestion of LH and CG dimer revealed a difference in the processing of their N-linked oligosaccharides: the oligosaccharides of secreted LH are complex (endo H resistant), but those on CG dimer remain partially endo H sensitive. The endo H-sensitive structures were present not only on dimer CG β , but were found on associated α subunit as well. These data provide the first conclusive evidence that gonadotropin β subunits can influence the oligosaccharide processing of noncovalently bound α subunit. Such intersubunit effects may account for differences in the N-linked structures of LH and FSH (Green et al., 1985), which are coexpressed in pituitary gonadotropes (Nakane, 1970; Phifer et al., 1973). In the case of LH and CG from C-127 cells, it will be interesting to determine whether the differing N-linked structures are limited to specific sites of glycosylation on the subunits. Expression of thyrotropin and FSH β subunits in C-127 cells may also contribute to our understanding of the assembly and processing of glycoprotein hormones.

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Note Added in Proof: Dahms and Hart (1986) have recently demonstrated that N-linked oligosaccharide processing on the β subunit shared in common by the cell surface glycoproteins Mac-1 and LFA-1 is determined by their unique α subunits.

References

- Bole, D. G., L. M. Hendershot, and J. F. Kearney. 1986. Posttranslational association of immunoglobulin heavy chain binding protein with nascent heavy chains in nonsecreting and secreting hybridomas. *J. Cell Biol.* 102:1558-1566.
- Burgert, H.-G., and S. Kvist. 1985. An Adenovirus type 2 glycoprotein blocks cell surface expression of human histocompatibility class I antigens. *Cell.* 41:987-997.
- Burke, D., and K. Keegstra. 1979. Carbohydrate structure of Sindbis virus glycoprotein E2 from virus grown in hamster and chicken cells. *J. Virol.* 29:546-554.
- Carlsen, R. B., O. P. Bahl, and N. Swaminathan. 1973. Human chorionic gonadotropin. Linear amino acid sequence of the β subunit. *J. Biol. Chem.* 248:6810-6825.
- Chamberlain, J. P. 1979. Fluorographic detection of radioactivity in polyacrylamide gels with the water-soluble fluor, sodium salicylate. *Anal. Biochem.* 98:132-135.
- Dahms, N. M., and G. W. Hart. 1986. Influence of quaternary structure on glycosylation. Differential subunit association affects the site-specific glycosylation of the common β -chain from Mac-1 and LFA-1. *J. Biol. Chem.* 261:13186-13196.
- Dulis, B. H., T. M. Kloppel, H. M. Grey, and R. T. Kubo. 1982. Regulation of catabolism of IgM heavy chains in a B lymphoma cell line. *J. Biol. Chem.* 257:4369-4374.
- Elder, J. H., and S. Alexander. 1982. endo- β -N-Acetylglucosaminidase F: endoglycosidase from *Flavobacterium meningosepticum* that cleaves both high-mannose and complex glycoproteins. *Proc. Natl. Acad. Sci. USA.* 79:4540-4544.
- Endo, Y., K. Yamashita, Y. Tachibana, S. Tojo, and A. Kobata. 1979. Structures of the asparagine-linked sugar chains of human chorionic gonadotropin. *J. Biochem.* 85:669-679.
- Fitting, T., and D. Kabat. 1982. Evidence for a glycoprotein "signal" involved in transport between subcellular organelles. *J. Biol. Chem.* 257:14011-14017.
- Franchimont, P., U. Gaspard, A. Reuter, and G. Heynen. 1972. Polymorphism of protein and polypeptide hormones. *Clin. Endocrinol.* 1:315-336.
- Glanville, N., Durmam, D. M., and R. D. Palmiter. 1981. Structure of mouse metallothionein-I gene and its mRNA. *Nature (Lond.)* 292:267-269.
- Graham, F. L., and A. J. van der Eb. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52:456-467.
- Green, M. 1982. Incorporation of amino acid analogs interferes with the processing of the asparagine-linked oligosaccharide of the MOPC-46B κ light chain. *J. Biol. Chem.* 257:9039-9042.
- Green, E. D., J. U. Baenziger, and I. Boime. 1985. Cell-free sulfation of human and bovine hormones: comparison of the sulfated oligosaccharides of lutropin, follitropin, and thyrotropin. *J. Biol. Chem.* 260:15631-15638.
- Green, E. D., I. Boime, and J. U. Baenziger. 1986. Differential processing of Asn-linked oligosaccharides on pituitary glycoprotein hormones: implications for biologic function. *Mol. Cell. Biochem.* 72:81-100.
- Green, E. D., J. Gruenebaum, M. Bielinska, J. U. Baenziger, and I. Boime. 1984. Sulfation of lutropin oligosaccharides with a cell-free system. *Proc. Natl. Acad. Sci. USA.* 81:5320-5324.
- Haour, F., and B. B. Saxena. 1974. Characterization and solubilization of gonadotropin receptor of bovine corpus luteum. *J. Biol. Chem.* 249:2195-2205.
- Hercz, A., E. Katona, E. Cutz, J. R. Wilson, and M. Barton. 1978. α_1 -antitrypsin: the presence of excess mannose in the Z variant isolated from liver. *Science (Wash. DC)* 201:1229-1232.
- Ho, M.-K., and T. A. Springer. 1983. Biosynthesis and assembly of the α and β subunits of Mac-1, a macrophage glycoprotein associated with complement receptor function. *J. Biol. Chem.* 258:2766-2769.
- Hoshina, H., and I. Boime. 1982. Combination of rat lutropin subunits occurs early in the secretory pathway. *Proc. Natl. Acad. Sci. USA.* 79:7649-7653.
- Howley, P. M., N. Sarver, and M.-F. Law. 1983. Eukaryotic cloning vectors derived from bovine Papillomavirus DNA. *Methods Enzymol.* 101:387-402.
- Jameson, L., W. W. Chin, A. N. Hollenberg, A. S. Chang, and J. F. Habener. 1984. The gene encoding the β -subunit of rat luteinizing hormone. *J. Biol. Chem.* 259:15474-15480.
- Kessler, M. J., M. S. Reddy, R. H. Shah, and O. P. Bahl. 1979. Structures of N-glycosidic carbohydrate units of human chorionic gonadotropin. *J. Biol. Chem.* 254:7901-7908.
- Keutmann, H. T., R. M. Williams, and R. J. Ryan. 1979. Structure of human luteinizing hormone beta subunit: evidence for a related carboxyl-terminal sequence among certain peptide hormones. *Biochem. Biophys. Res. Commun.* 90:842-848.
- Krystek, S. R., Jr., L. E. Reichert, and T. T. Anderson. 1985. Analysis of computer-generated hydropathy profiles for human glycoprotein and lactogenic hormones. *Endocrinology.* 117:1110-1124.
- Kudryk, B., M. Okada, C. M. Redman, and B. Blomback. 1982. Biosynthesis of dog fibrinogen. Characterization of nascent fibrinogen in the rough endoplasmic reticulum. *Eur. J. Biochem.* 125:673-682.
- Kvist, S., K. Wiman, L. Claesson, P. A. Peterson, and B. Dobberstein. 1982. Membrane insertion and oligomeric assembly of HLA-DR histocompatibility antigens. *Cell.* 29:61-69.
- Laskey, R. A., and A. D. Mills. 1975. Quantitative film detection of 3 H and

- ¹⁴C polyacrylamide gels by fluorography. *Eur. J. Biochem.* 56:335-341.
- Lodish, H. F., N. Kong, M. Snider, and G. J. A. M. Strous. 1983. Hepatoma secretory proteins migrate from rough endoplasmic reticulum to Golgi at characteristic rates. *Nature (Lond.)*. 304:80-83.
- Magner, J. A., and B. D. Weintraub. 1982. Thyroid-stimulating hormone subunit processing and combination in microsomal subfractions of mouse pituitary tumor. *J. Biol. Chem.* 257:6709-6715.
- Maizel, J. V., Jr. 1971. Polyacrylamide gel electrophoresis of viral proteins. *Methods Virol.* 5:179-246.
- Merlie, J. P., and M. M. Smith. 1986. Synthesis and assembly of acetylcholine receptor, a multisubunit membrane glycoprotein. *J. Membr. Biol.* 9:1-10.
- Mizuochi, T., and A. Kobata. 1979. Different asparagine-linked sugar chains on the two polypeptide chains of human chorionic gonadotropin. *Biochem. Biophys. Res. Commun.* 97:772-778.
- Morgan, F. J., S. Birken, and R. E. Canfield. 1975. The amino acid sequence of human chorionic gonadotropin. The α subunit and β subunit. *J. Biol. Chem.* 250:5247-5258.
- Moyle, W. R., O. P. Bahl, and L. Marz. 1975. Role of the carbohydrate of human chorionic gonadotropin in the mechanism of hormone action. *J. Biol. Chem.* 250:9163-9169.
- Nakamura, K., and R. W. Compans. 1979. Host cell- and virus strain-dependent differences in oligosaccharides of hemagglutinin glycoproteins of influenza A viruses. *Virology.* 95:8-23.
- Nakane, P. K. 1970. Classification of anterior pituitary cell types with immunoenzyme histochemistry. *J. Histochem. Cytochem.* 18:9-20.
- Orth, D. N., W. E. Nicholson, W. M. Mitchell, D. P. Island, M. Shapiro, and R. L. Byyny. 1973. ACTH and MSH production by a single cloned mouse pituitary tumor cell line. *Endocrinology.* 92:385-393.
- Owen, M. J., and A.-M. Kissonerghis. 1980. Biosynthesis of HLA-A and HLA-B antigens *in vivo*. *J. Biol. Chem.* 255:9678-9684.
- Peters, B. P., R. F. Krzesicki, R. J. Hartle, F. Perini, and R. W. Ruddon. 1984. A kinetic comparison of the processing and secretion of the $\alpha\beta$ dimer and the uncombined α and β subunits of chorionic gonadotropin synthesized by human choriocarcinoma cells. *J. Biol. Chem.* 259:15123-15130.
- Phifer, R. F., A. R. Midgley, and S. S. Spicer. 1973. Immunohistologic and histologic evidence that follicle-stimulating hormone and luteinizing hormone are present in the same cell type in the human pars distalis. *J. Clin. Endocrinol. Metab.* 36:125-141.
- Pierce, J. G., and T. F. Parsons. 1981. Glycoprotein hormones: structure and function. *Annu. Rev. Biochem.* 50:465-495.
- Plant, P. W., and G. Grieninger. 1986. Noncoordinate synthesis of the fibrinogen subunits in hepatocytes cultured under hormone-deficient conditions. *J. Biol. Chem.* 261:2331-2336.
- Prentice, L. G., and R. J. Ryan. 1975. LH and its subunits in human pituitary, serum and urine. *J. Clin. Endocrinol. Metab.* 40:303-312.
- Rajaniemi, H., and T. Vanha-Perttula. 1972. Specific receptor for LH in the ovary: evidence by autoradiography and tissue fractionation. *Endocrinology.* 90:1-9.
- Ramabhadran, T. V., B. A. Reitz, and D. C. Tiemeier. 1984. Synthesis and glycosylation of the common α subunit of human glycoprotein hormones in mouse cells. *Proc. Natl. Acad. Sci.* 81:6701-6705.
- Rosner, M. R., L. S. Grinna, and P. W. Robbins. 1980. Differences in glycosylation patterns of closely related murine leukemia viruses. *Proc. Natl. Acad. Sci. USA.* 77:67-71.
- Sairam, M. R. 1983. Gonadotropic hormones: relationship between structure and function with emphasis on antagonists. *In Hormonal Proteins and Peptides*. Vol. 11. C. H. Li, editor. Academic Press, Inc., NY.
- Scheele, G., and A. Tartakoff. 1985. Exit of nonglycosylated secretory proteins from the rough endoplasmic reticulum is asynchronous in the exocrine pancreas. *J. Biol. Chem.* 260:926-931.
- Sheares, B. T., and P. W. Robbins. 1986. Glycosylation of ovalbumin in a heterologous cell: analysis of oligosaccharide chains of the cloned glycoprotein in mouse L cells. *Proc. Natl. Acad. Sci. USA.* 83:1993-1997.
- Shome, B., and A. F. Parlow. 1973. The primary structure of the hormone-specific, beta subunit of human pituitary luteinizing hormone (hLH). *J. Clin. Endocrinol. Metab.* 36:618-621.
- Southern, P. J., and P. Berg. 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under the control of the SV40 early region promoter. *J. Mol. Appl. Gen.* 1:327-341.
- Strous, G. J. A. M., and H. F. Lodish. 1980. Intracellular transport of secretory and membrane proteins in hepatoma cells infected by Vesicular Stomatitis Virus. *Cell.* 22:709-717.
- Talmadge, K., N. C. Vamvakopoulos, and J. C. Fiddes. 1984. Evolution of the genes for the β subunits of human chorionic gonadotropin and luteinizing hormone. *Nature (Lond.)*. 307:37-40.
- Tarentino, A. L., and F. Maley. 1974. Purification and properties of an endo- β -N-acetylglucosaminidase from *Streptomyces griseus*. *J. Biol. Chem.* 249:811-817.
- Tartakoff, A., and P. Vassalli. 1979. Plasma cell immunoglobulin M molecules. Their biosynthesis, assembly and intracellular transport. *J. Cell Biol.* 83:284-299.
- Weitzman, S., M. Grennon, and K. Keegstra. 1979. Comparison of Sindbis virus and immunoglobulin glycopeptides in mouse myeloma cells. *J. Biol. Chem.* 254:5377-5382.
- Winberry, L., A. Marks, and R. Baumal. 1980. Immunoglobulin production and secretion by variant clones of the MOPC 315 mouse myeloma cell line. *J. Immunol.* 124:1174-1182.
- Wu, G. E., N. Hozumi, and H. Murialdo. 1983. Secretion of a λ_2 immunoglobulin chain is prevented by a single amino acid substitution in its variable region. *Cell.* 33:77-83.