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# A cluster of basic amino acids within an α-helix is essential for α-subunit recognition by the glycoprotein hormone *N*-acetylgalactosaminyltransferase

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**ABSTRACT** **The glycoprotein hormone *N*-acetylgalactosaminyltransferase is responsible for synthesis of Asn-linked oligosaccharides terminating with GalNAc-4-$SO_4$ on lutropin, thyrotropin, and the uncombined glycoprotein hormone α subunit. We previously established that a recognition determinant for the *N*-acetylgalactosaminyltransferase is contained within a 22-amino acid glycopeptide fragment of the α subunit. We proposed that the tripeptide Pro-Leu-Arg is an essential element of the recognition determinant. Using site-directed mutagenesis we have examined the role of individual amino acids in recognition by the glycoprotein hormone *N*-acetylgalactosaminyltransferase. Within the sequence $Pro^{40}$-$Leu^{41}$-$Arg^{42}$-$Ser^{43}$-$Lys^{44}$-$Lys^{45}$, $Lys^{44}$, and $Lys^{45}$, as well as $Arg^{42}$ of the tripeptide, are essential for recognition. Substitution of the $Leu^{41}$ with other amino acids can either increase or decrease the rate of GalNAc transfer over an 8-fold range, suggesting that the middle amino acid of the tripeptide plays a modulatory role in recognition. The critical $Leu^{41}$-$Arg^{42}$ and $Lys^{44}$-$Lys^{45}$ residues are present on the same surface of an α-helix, which projects from the surface of the α subunit. Our results indicate that an essential element of the recognition determinant consists of a cluster of basic residues and that neutral but not negatively charged residues are tolerated within this cluster.**

Establishing the mechanisms by which specific oligosaccharide structures arise on individual glycoproteins is central to our understanding of glycobiology. Asn-linked oligosaccharides arise from a common precursor, an oligosaccharide consisting of three Glc, nine Man, and two GlcNAc moieties, which is transferred en bloc from dolicholpyrophosphate to the nascent peptide chain. This oligosaccharide is remodeled by exoglycosidases and glycosyltransferases within the endoplasmic reticulum and Golgi in a series of steps called processing (1). As a result, Asn-linked oligosaccharides differ predominantly in the peripheral sugars that are added to a common core region. Since the identical oligosaccharide acceptors are utilized by many of the glycosyltransferases that add peripheral sugars, the presence of unique structures on individual glycoproteins or families of glycoproteins requires some form of protein-specific recognition by one or more glycosyltransferases. Three glycosyltransferases have thus far been demonstrated to be protein, as well as oligosaccharide, specific: UDPGlc glycoprotein glucosyltransferase (2, 3), lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase (4–6), and glycoprotein hormone *N*-acetylgalactosaminyltransferase (7–9).

Glycoprotein hormone *N*-acetylgalactosaminyltransferase and β1,4-galactosyltransferase add GalNAc and Gal, respectively, to the same oligosaccharide acceptor $GlcNAc_2Man_3GlcNAc_2Asn$. It is the glycoprotein hormone *N*-acetylgalactosaminyltransferase in combination with a GalNAc-4-sulfotransferase that accounts for the presence of Asn-linked oligosaccharides terminating with $SO_4$-4-GalNAc(31-4)GlcNAc on lutropin, thyrotropin, and the uncombined hormone α subunit rather than the sequence sialic acid(α2-3/6)Gal(β1-4)GlcNAc. The latter sequence is frequently encountered on the Asn-linked oligosaccharides of many glycoproteins including follitropin and chorionic gonadotropin (CG) (10–12). We previously demonstrated that the glycoprotein hormone α subunit and the human (h) CG β subunit both contain a peptide recognition determinant that reduces the apparent $K_m$ for GalNAc addition to the $GlcNAc_2Man_3GlcNAc_2Asn$ acceptor from 1–2 mM to <15 μM (8).

Reduction and carboxymethylation do not significantly alter the apparent $K_m$ for GalNAc addition to oligosaccharides on either the α or the hCG β subunit. This indicates that neither the tertiary structure of the hormone subunits nor features of their secondary structure that are dependent on disulfide bond formation are required for recognition (8). Furthermore, a 22-amino acid glycopeptide from the human α subunit has the same apparent $K_m$ for GalNAc addition as the native α subunit. Similar results were obtained with the CG β subunit (9). Alignment of the sequences of known *in vitro* and *in vivo* substrates implicated the tripeptide sequence Pro-Leu-Arg as the common feature. Support for the importance of this tripeptide sequence for recognition was obtained by chemical modification of Arg, which decreases the rate of GalNAc incorporation by 90% (9). This led us to propose that the sequence Pro-Leu-Arg is an essential element of the protein recognition determinant used by the glycoprotein hormone *N*-acetylgalactosaminyltransferase.

For this study, we have used site-directed mutagenesis to construct variants within the 22-amino acid sequence of the α subunit identified as sufficient for recognition, including the Pro-Leu-Arg motif, and compared them as acceptors for GalNAc addition *in vitro*. We have identified residues in addition to the Pro-Leu-Arg tripeptide that are essential for recognition and other residues that are not essential but modulate the rate of GalNAc transfer. In combination with the recently determined x-ray crystallographic structure of hCG (13, 14), our results provide a detailed picture of the recognition determinant on the α subunit.

## MATERIALS AND METHODS

**Materials.** Rabbit anti-mouse IgG (Fc) and biotinylated rabbit anti-sheep IgG were purchased from Pierce, anti-human α-subunit monoclonal antibody (MIH9821) was from Medix Biotech (Foster City, CA); sheep anti-human α-subunit was from BioDesign International (Kennebunkport, ME); *Wistaria floribunda* agglutinin was from Sigma; streptavidin was from Boehringer Mannheim; and biotinylated aequorin (Aqualite) was from SeaLite Sciences (Bogart, GA). Restriction enzymes,

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Abbreviation: hCG, human chorionic gonadotropin.

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modifying enzymes, and DNA polymerases were purchased from Boehringer Mannheim and Promega. pcDNA I/AMP was purchased from Invitrogen. CHO-Tag30A cell line was the generous gift of Peter L. Smith (Department of Pathology, University of Michigan).

**Construction of α-Subunit Variants.** α-Subunit constructs were made by PCR splice-by-overlap extension (15) with an α-subunit minigene (16) template. All mutations were contained within the 125-bp *Xba* I/*Hin*dIII fragment of the minigene. This fragment was excised from the second round PCR product isolated on a MetaPhor agarose gel (FMC), purified with Qiaex resin (Qiagen, Chatsworth, CA), and ligated into pcDNA I/Amp (polylinker *Xba* I and *Hin*dIII sites removed) containing the human α-subunit minigene with the *Xba* I/*Hin*dIII fragment excised. Mutations were confirmed by sequencing with the *fmol* sequencing kit (Promega). Qiagen Maxipreps were used for large scale plasmid preparations.

**Transfections.** Plates of CHO-Tag 30A cells (diameter, 100 mm) were transfected with 25 μg of LipofectAMINE (GIBCO/BRL) and 7.5 μg of DNA in serum-free medium for 6 h according to the manufacturer's protocol. Culture medium containing α subunit was harvested 72 h after transfection.

**Substrate Preparation.** Wild-type and variant α subunits were purified from cell culture medium by immunoaffinity chromatography with anti-α monoclonal antibody MIH9821 coupled to Affi-Gel 10 (Bio-Rad). Bound α subunit was eluted with 50 mM diethylamine (pH 11.5) and immediately neutralized with 0.1 vol of 0.5 M $NaH_2PO_4$. The eluants were dialyzed against 1% ammonium bicarbonate, lyophilized, resuspended in 10 mM sodium cacodylate (pH 6.0), and digested with neuraminidase (*Clostridium perfringens*) and β-galactosidase (*Streptococcus pneumoniae*) as described (8). α Subunits were buffer exchanged into 50 mM Hepes, pH 7.4/bovine serum albumin (10 mg/ml) on G-25 spin columns. The final concentrations of the α subunits were determined by a sandwich ELISA using monoclonal antibody MIH9821, sheep anti-human α subunit, and biotinylated aequorin as the reporter molecule.

***N*-Acetylgalactosaminyltransferase Assays.** Time course assays were performed as described (7, 17) using 2.36 microunits of *N*-acetylgalactosaminyltransferase and 100 ng of human α subunit per time point in a 100-μl reaction volume. Microplate analysis of GalNAc incorporation was done as described, except that microplate wells were coated with rabbit anti-mouse IgG (Fc) followed by anti-human α-subunit monoclonal antibody MIH9821.

## RESULTS

The apparent $K_m$ for GalNAc addition to the oligosaccharide at $Asn^{52}$ of the α subunit is the same for the 22-amino acid glycopeptide fragment hCG $\alpha^{35-56}$ and the intact α subunit, indicating that all of the information required for recognition by the *N*-acetylgalactosaminyltransferase is contained within this short sequence (9). We therefore examined the role of various amino acids within this region of the α subunit by preparing variants and comparing them as substrates *in vitro*. This required an expression system capable of producing sufficient quantities of α-subunit-bearing oligosaccharides that could be modified to be acceptors for the *N*-acetylgalactosaminyltransferase. To obtain high levels of expression, a minigene encoding the α subunit (16) was subcloned into pcDNA I/Amp, which contains the polyomavirus origin of replication and a cytomegalovirus promoter. Variants prepared by site-directed mutagenesis were transiently expressed in CHO-Tag 30A cells, a CHO cell line expressing polyoma large T antigen (18). Between 4 and 10 μg of α subunit is produced in 72 h per 100-mm culture plate. CHO cells do not express endogenous glycoprotein hormone *N*-acetylgalactosaminyltransferase activity (19) and have been shown to synthesize predominantly dibranched complex-type oligosaccharides with the terminal sequence sialic acid(α2-3)Gal(β1-4)GlcNAc(β1-2)Manα on recombinant α subunit (19). Therefore, the oligosaccharides of α subunit expressed in CHO-Tag 30A cells could be trimmed with neuraminidase and β-galactosidase to produce the synthetic intermediate $GlcNAc_2Man_3GlcNAc_2Asn$ used by the glycoprotein hormone *N*-acetylgalactosaminyltransferase.

We have developed a highly sensitive *N*-acetylgalactosaminyltransferase assay able to detect the α subunit product at the levels produced by these CHO cells. Monoclonal antibody is used to immobilize the product of the solution-phase transferase reaction onto a 96-well plate and the amount of terminal β-linked GalNAc present on the immobilized product is determined by using the lectin *W. floribunda* agglutinin and aequorin for detection (17). We compare the rate of GalNAc addition to each α-subunit variant with that obtained with α-subunit Pro-Ala-Pro-Leu-Arg (αPAPLR). This is done by fitting a line to each time course to determine the slope (GalNAc incorporation per 100 ng of α subunit per min) and dividing the slope obtained for the variant by that obtained for αPAPLR (Fig. 1).

The free α subunit, in contrast to α subunit associated with a β subunit, is subject to O-glycosylation at the Thr of the wild-type αPTPLR sequence (20). Because this Thr is adjacent to the Pro-Leu-Arg tripeptide we previously identified as an essential component of the recognition determinant, O-glycosylation could influence recognition by the glycoprotein hormone *N*-acetylgalactosaminyltransferase. Substitution of the Thr with an Ala prevents O-glycosylation of the free α subunit but does not affect either folding or secretion (21). Since the relative rate of GalNAc incorporation into wild-type αPTPLR does not differ significantly from that of αPAPLR (Table 1), the rate of GalNAc transfer in each instance is compared to that of αPAPLR to avoid any variation that might arise from O-glycosylation.

**$Arg^{42}$ Variants.** The effects of substituting Lys, His, Gly, and Glu for $Arg^{42}$ of the α-subunit Pro-Leu-Arg tripeptide are summarized in Table 1. The rate of GalNAc transfer does not significantly differ between αPAPLK and αPAPLR, indicating that either Arg or Lys can occupy this position. The relative rates of transfer to αPAPLH and αPAPLG are 58% and 22%, respectively, of αPAPLR. Since <50% of the His should be positively charged at pH 7.4, the pH used for the transferase reaction, and Gly has no charge, the progressive reduction in rate of transfer to αPAPLH and αPAPLG indicates a positive charge is required at this position for efficient transfer of GalNAc to the α subunit. Substitution of $Arg^{42}$ with Glu (αPTPLE) reduces the rate of GalNAc transfer to 11% of αPAPLR. The 11% relative rate of transfer to αPTPLE indicates that there is little if any enhancement of GalNAc transfer to this variant as compared to proteins such as transferrin or glycopeptides lacking the recognition determinant; this rate approaches the lower limits of detection for GalNAc transfer under the conditions used for the assay.

**$Leu^{41}$ Variants.** The relative rate of GalNAc incorporation can be either increased or decreased by substituting $Leu^{41}$ of the PTPLR sequence with amino acids that differ in the properties of their side chains. When Leu, a hydrophobic amino acid of intermediate size, is substituted with a bulky aromatic amino acid, the relative rate of GalNAc incorporation increases 170–230% (αPAPWR, αPTPYR, αPAPFR in Table 1 and Fig. 1*B*). Substituting a hydrophilic Gln (αPAPQR) for the hydrophobic Leu decreases GalNAc incorporation to 82% of the αPAPLR, whereas replacement of the hydrophobic side chain with a hydrogen (αPAPGR) increases GalNAc incorporation to 150% of αPAPLR.

The greatest change in relative rate occurs when $Leu^{41}$ is replaced with either positively or negatively charged residues. Replacement with Lys (αPAPKR) increases the rate of Gal-

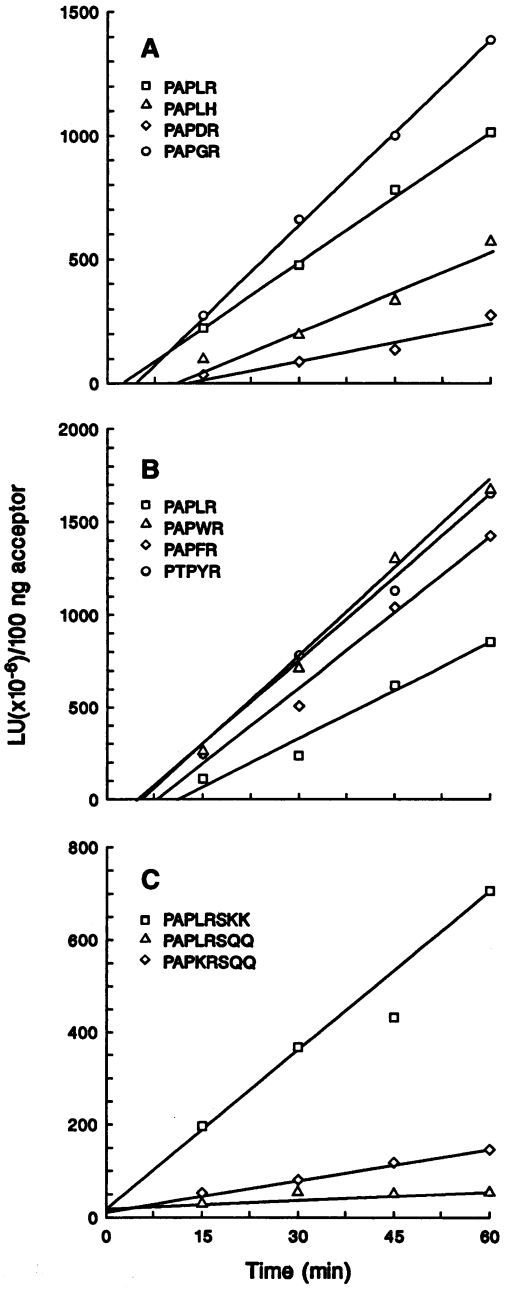



FIG. 1. Time courses for transfer of GalNAc to α-subunit variants. The agalacto α subunit, UDPGalNAc, and 2.36 microunits of partially purified *N*-acetylgalactosaminyltransferase were incubated at 37°C for the indicated times. For each time point, an 8-ng aliquot of α subunit was captured in triplicate and GalNAc incorporation was determined. (*A*) α-Subunit variants that both decrease and increase the relative rate of GalNAc transfer. (*B*) Aromatic amino acid substitution for $Leu^{41}$ improves the relative rate of GalNAc transfer. (*C*) Substitution of $Lys^{44}$-$Lys^{45}$ with $Gln^{44}$-$Gln^{45}$ abolishes recognition but is partially compensated by replacement of $Leu^{41}$ with Lys. LU, light units.

NAc incorporation 240%, whereas replacement with Asp (αPAPDR) decreases the relative rate to 30% of αPAPLR or 12.5% of αPAPKR. Thus, the relative rate of GalNAc incorporation can be modified over an 8-fold range by changing the middle amino acid of the Pro-Leu-Arg tripeptide. However, even though a negatively charged residue at this position decreases the rate of GalNAc transfer, it does not abolish peptide recognition by the *N*-acetylgalactosaminyltransferase as does replacement of $Arg^{41}$ with Glu.

**$Pro^{40}$ Variants.** Pro is unique among the amino acids in that it is a secondary amine, putting a bend in the peptide backbone. Since other glycoproteins that bear oligosaccharides terminating with GalNAc-4-$SO_4$ also contain the Pro-Xaa-Arg/Lys motif (22, 23), we expected the substitution of $Pro^{40}$ with Ala would abolish recognition. Although the rate of transfer is reduced, αPAALR maintains 47% of the relative rate of αPAPLR. Furthermore, when both Pro residues are substituted with Val and Ala, respectively (αVTALR), the rate of transfer increases to 120% of αPAPLR (Table 1). Thus, $Pro^{40}$ is not essential within the context of the native α subunit for recognition by the *N*-acetylgalactosaminyltransferase.

Table 1. Relative rates of GalNAc incorporation into human α-subunit variants within the Pro-Leu-Arg sequence

| Construct | Relative rate* | SEM |
|---|---|---|
| PAPLR | 1.00 | 0.00 |
| PTPLR† | 0.96 | 0.34 |
| Arg mutants | | |
| PAPLK | 1.20 | 0.33 |
| PAPLH | 0.58 | 0.01 |
| PTPLG | 0.22 | 0.08 |
| PTPLE | 0.11 | 0.06 |
| Leu mutants | | |
| PAPKR | 2.40 | 0.10 |
| PTPYR | 2.28 | 0.53 |
| PAPWR | 1.98 | 0.13 |
| PAPFR | 1.73 | 0.17 |
| PAPGR | 1.50 | 0.10 |
| PAPQR | 0.82 | 0.01 |
| PAPDR | 0.30 | 0.01 |
| Pro mutants | | |
| VTALR | 1.20 | 0.00 |
| PAALR | 0.47 | 0.07 |

*Relative rate of GalNAc incorporation equals the slope of the time course for the variant divided by the slope of the time course for αPAPLR.
†Wild-type human α-subunit sequence.

**Identification for Additional Amino Acids Essential for Recognition by the Glycoprotein Hormone *N*-Acetylgalactosaminyltransferase.** A characteristic of a number of the known acceptors for the *N*-acetylgalactosaminyltransferase is the presence of two or more positively charged amino acids in the immediate vicinity of the Pro-Xaa-Arg/Lys motif. For example, the tripeptide motif of the α subunit is followed by the sequence Ser-Lys-Lys, while the tripeptide motif of the hCG β subunit is followed by the sequence Pro-Arg-Cys-Arg. We therefore examined the contribution of $Lys^{44}$ and $Lys^{45}$ as summarized in Table 2.

Substitution of $Lys^{44}$ with Gln (αPAPLRSQK) reduces the relative rate of GalNAc incorporation to 50% of αPAPLRSKK, while substituting both $Lys^{44}$ and $Lys^{45}$ with Gln reduces the rate of incorporation to 9% of αPAPLRSKK. Further-

Table 2. Relative Rates of GalNAc Incorporation into human α-subunit variants adjacent to the Pro-Leu-Arg sequence

| Construct | Relative rate* | SEM |
|---|---|---|
| PAPLRSKK† | 1.00 | 0.00 |
| PAPLRSQK | 0.49 | 0.16 |
| PAPKRSQK | 0.92 | 0.13 |
| PAPLRSQQ | 0.09 | 0.05 |
| PAPKRSQQ | 0.27 | 0.07 |
| PAPLRSEK | 0.18 | 0.02 |
| PAPKRSEK | 0.24 | 0.05 |

*Relative rate of GalNAc incorporation equals the slope of the time course for the variant divided by the slope of the time course for αPAPLRSKK.
†Wild-type α-subunit sequence, except for Ala (Thr in wild type).

more, substitution of Lys[44] with Glu (αPAPLESEK) reduces the rate of transfer to 18% of control. This suggests that Leu[41]-Arg[42] and Lys[44]-Lys[45] represent distinct components of the recognition determinant that may influence the rate of GalNAc transfer independently. We examined this by determining the effect of substituting Leu[41] with Lys in the presence of either one or two Gln residues, αPAPLRSQK and αPAPLRSQQ, respectively. The rate of GalNAc incorporation increases 200–300% (compare αPAPKRSQK with αPAPLRSQK and αPAPKRSQQ with αPAPLRSQQ in Table 2 and Fig. 1*C*) for both variants. In contrast, the presence of a Pro-Lys-Arg sequence only improves the incorporation of GalNAc by 25% when Glu is substituted for Lys[44] (compare αPAPKRSEK to αPAPLRSEK; Table 2). Thus, modifications in Leu-Arg and Lys-Lys of the PLRSKK sequence can influence the rate of GalNAc transfer independently. Substitution with negatively charged amino acids is deleterious for each of these residues.

## DISCUSSION

The key feature of the recognition marker used by the glycoprotein hormone *N*-acetylgalactosaminyltransferase is the presence of basic amino acids. Based on our previous studies, we proposed that the tripeptide motif Pro-Xaa-Arg/Lys is essential for recognition (9). As predicted, Lys can substitute for Arg. However, substitution of His, which would be charged <50% of the time at pH 7.4, results in a decline in the rate of GalNAc transfer. Substitution with Gly results in further reduction in the rate of transfer, while substitution with Glu virtually abolishes peptide recognition.

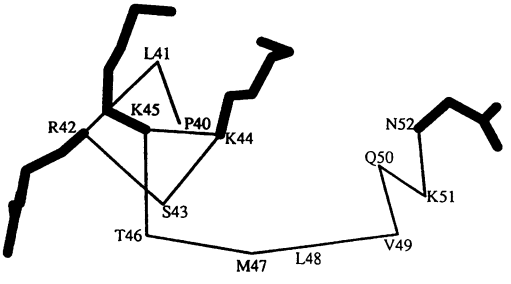
A number of amino acids are tolerated at the position of Leu[41] within the Pro-Leu-Arg motif. Asp reduces the rate of GalNAc transfer to 30% of wild type, whereas Lys increases the rate of transfer to 240% of wild type. Thus, the rate of GalNAc transfer to α-subunit oligosaccharides can be varied over at least an 8-fold range, determined solely by the characteristics of the Xaa residue of the Pro-Xaa-Arg/Lys motif. Amino acids with large, aromatic side chains enhance the rate of transfer, whereas Gly has little effect. The rank order for the variants tested is Lys > Tyr > Trp > Phe > Gly > Leu > Gln > Asp. Taken together, this suggests the amino acid at the Xaa position is not essential for recognition but instead plays a modulatory role, either enhancing or reducing the rate of GalNAc transfer.

Unexpectedly, Pro[40] is not essential for recognition by the glycoprotein hormone *N*-acetylgalactosaminyltransferase. Substitution of Pro[40] with Ala to produce αPAALR reduces the rate of GalNAc transfer to 50% of wild type, whereas substituting both Pro residues (αVTALR) actually enhances the relative rate of transfer as compared to wild type. This was not expected since all of the glycoproteins bearing oligosaccharides terminating with GalNAc-4-$SO_4$ examined to date, like those substrates that have been examined *in vitro*, contain a tripeptide motif including Pro (22, 23). Although the presence of Pro in the tripeptide motif is not essential for recognition, it may in some instances play a role in orienting the basic residues that are essential for recognition.

In addition to the Pro[40]-Leu[41]-Arg[42] tripeptide, the two basic amino acids Lys[44]-Lys[45], which are immediately adjacent to the tripeptide, are essential for recognition of the α subunit by the glycoprotein hormone *N*-acetylgalactosaminyltransferase. Substitution of Lys[44] with Gln reduces the rate of GalNAc transfer by 50%, whereas substitution of both Lys[44] and Lys[45] with Gln virtually abolishes recognition by the *N*-acetylgalactosaminyltransferase. Substitution of the Leu in the Pro-Leu-Arg sequence with Lys increases the rate of GalNAc transfer to constructs with a Lys[44]-Lys[45], Gln[44]-Lys[45], or Gln[44]-Gln[45] sequence to a similar extent, ≈200%. Substitution of Lys[44] with an acidic residue such as Glu reduces the rate of GalNAc transferase to a greater extent than substitution with Gln; 18% versus 50% of wild-type α subunit, respectively. Furthermore, replacement of Leu[41] with Lys does not increase the rate of GalNAc transfer when Lys[44] has been substituted with Glu (αPAPKRSEK). Notably, basic residues are also immediately adjacent to the tripeptide motif of the hCG βsubunit, which has the sequence Pro[4]-Leu[5]-Arg[6]-Pro[7]-Arg[8]-Cys[9]-Arg[10]. Introduction of a negative charge at the position of Cys[9] by reduction and alkylation with iodoacetic acid abolishes recognition by the glycoprotein hormone *N*-acetylgalactosaminyltransferase, whereas recognition is maintained after alkylation with iodoacetamide (9). This suggests that either or both of the Arg residues adjacent to the Cys in the β subunit contribute to recognition in the same manner as Lys[44] and Lys[45] of the α subunit and that introduction of a negative charge prevents this recognition by the transferase.

Recently, the crystal structure of hCG was solved to a resolution of 3.0 Å (13, 14). The basic amino acids that are essential for recognition by the glycoprotein hormone *N*-acetylgalactosaminyltransferase are located within two turns of an α-helix formed by amino acids 40–46 of the α subunit. Based on the crystal structure Leu[41], Arg[42], Lys[44], and Lys[45] are positioned on the same surface of this helix, forming a cluster of basic residues (Fig. 2). Pro[40] is at the base of the helix, which projects outward from the surface of the dimeric hormone, making it accessible to the glycoprotein hormone *N*-acetylgalactosaminyltransferase. Whether a helical structure is required for positioning of the basic residues on other glycoproteins recognized by the *N*-acetylgalactosaminyltransferase is not yet known. However, hCG β subunit, a good *in vitro* substrate, does not have any apparent helical structure within its recognition determinant, suggesting that other structures can produce the required cluster of basic amino acids. Although the basic residues we have identified are essential for recognition by the *N*-acetylgalactosaminyltransferase, other amino acids within the α subunit that have not yet been examined may also contribute to recognition.

A full understanding of the biochemical mechanism by which the *N*-acetylgalactosaminyltransferase recognizes and modifies acceptor substrates will require detailed kinetic analyses of α-subunit variants such as those we have prepared. Within the group of α-subunit variants presented here, the




AYPT**PLRSKK**TMLVQK**NV**TSE

FIG. 2. The 22-amino acid α-subunit fragment that contains all of the information required for recognition by the glycoprotein hormone *N*-acetylgalactosaminyltransferase. The spatial relationship of the side chains (boldface lines) of the basic amino acids within the α-helix, which are essential for recognition of the α subunit, are shown. Side chain but not the oligosaccharide found on Asn[52] is also shown. Pro[40] is at the base of the helix that projects out from the surface of the α subunit (13, 14). Numbering is based on the residue position in the primary α-subunit sequence. Linear sequence of the 22-amino acid fragment previously shown to have all the information essential for recognition (9) is also shown by single-letter code. Positions characterized in this study are numbered and shown in boldface letters. Asn[52] is glycosylated.

relative rate of GalNAc transfer varies over a 24-fold range. These variants provide a unique opportunity to examine how glycosylation is regulated *in vivo* since they can be expressed in cells known to contain the glycoprotein hormone *N*-acetylgalactosaminyltransferase to determine how *in vitro* kinetic parameters relate to glycosylation patterns observed *in vivo*.

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1. Kornfeld, R. & Kornfeld, S. (1985) *Annu. Rev. Biochem.* **54,** 631–664.
2. Trombetta, S. E. & Parodi, A. J. (1992) *J. Biol. Chem.* **267,** 9236–9240.
3. Sousa, M. C., Ferrero-Garcia, M. A. & Parodi, A. J. (1992) *Biochemistry* **31,** 97–105.
4. Lang, L., Reitman, M., Tang, J., Roberts, R. M. & Kornfeld, S. (1984) *J. Biol. Chem.* **259,** 14663–14671.
5. Baranski, T. J., Faust, P. L. & Kornfeld, S. (1990) *Cell* **63,** 281–291.
6. Baranski, T. J., Cantor, A. B. & Kornfeld, S. (1992) *J. Biol. Chem.* **267,** 23342–23348.
7. Smith, P. L. & Baenziger, J. U. (1988) *Science* **242,** 930–933.
8. Smith, P. L. & Baenziger, J. U. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7275–7279.
9. Smith, P. L. & Baenziger, J. U. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 329–333.
10. Green, E. D. & Baenziger, J. U. (1988) *J. Biol. Chem.* **263,** 25–35.
11. Green, E. D. & Baenziger, J. U. (1988) *J. Biol. Chem.* **263,** 36–44.
12. Baenziger, J. U. & Green, E. D. (1988) *Biochim. Biophys. Acta Rev. Biomembr.* **947,** 287–306.
13. Wu, H., Lustbader, J. W., Liu, Y., Canfield, R. E. & Hendrickson, W. A. (1994) *Structure* **2,** 545–558.
14. Lapthorn, A. J., Harris, D. C., Littlejohn, A., Lustbader, J. W., Canfield, R. E., Machin, K. J., Morgan, F. J. & Isaacs, N. W. (1994) *Nature (London)* **369,** 455–461.
15. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K. & Pease, L. R. (1989) *Gene* **77,** 51–59.
16. Matzuk, M. M. & Boime, I. (1988) *J. Cell Biol.* **106,** 1049–1059.
17. Mengeling, B. J., Smith, P. L., Stults, N. L., Smith, D. F. & Baenziger, J. U. (1991) *Anal. Biochem.* **199,** 286–292.
18. Smith, P. L. & Lowe, J. B. (1994) *J. Biol. Chem.* **269,** 15162–15171.
19. Dharmesh, S. M., Skelton, T. P. & Baenziger, J. U. (1993) *J. Biol. Chem.* **268,** 17096–17102.
20. Parsons, T. F., Bloomfield, G. A. & Pierce, J. G. (1983) *J. Biol. Chem.* **258,** 240–244.
21. Bielinska, M. & Boime, I. (1992) *Mol. Endocrinol.* **6,** 267–271.
22. Smith, P. L., Skelton, T. P., Fiete, D., Dharmesh, S. M., Beranek, M. C., MacPhail, L., Broze, G. J., Jr., & Baenziger, J. U. (1992) *J. Biol. Chem.* **267,** 19140–19146.
23. Skelton, T. P., Kumar, S., Smith, P. L., Beranek, M. C. & Baenziger, J. U. (1992) *J. Biol. Chem.* **267,** 12998–13006.