for this antibody using a novel random PCR mutagenesis approach and expression screening in yeast.

The cDNA coding for rabbit GH receptor EC domain (1-240) was subjected to random PCR mutagenesis using a controlled nucleotide depletion protocol which gave mostly single and double mutations as determined by sequencing 55 clones after vector ligation. PCR products were ligated into the yeast expression vector YEpFLAG-1. This vector contains a sequence coding for a C-terminal epitope used to determine if the full length protein is being expressed. The mutagenesis products were transformed into yeast, and a library of 5000 colonies was picked and screened for MAb 263 reactivity using replica plating onto multiple nitrocellulose membranes. After blocking, these were screened with MAb 263 using HRP-conjugated second antibody and ECL. Colonies negative for 263 were then screened with the C-tag antibody, and only those which were full length but not 263 positive were sequenced.Sequences with single aminoacid mutations were used to map the epitope onto the structure of the trimeric complex for the homology modeled rabbit receptor.

The major part of the epitope is located on the side and top of domain 1, particularly the loop 79-96, and includes a small part close to the hormone binding site. Phe 225 and Pro 134 in the lower domain are also involved. This disposition would enable the MAb to grasp the two receptors in a scissors like movement, facilitating dimerization and any necessary conformational changes.

We next sought to determine if the main epitope, beta-turn loop residues 79-96 which are stabilized by a disulfide bond between 83 and 94, could be used to generate the GH receptor agonist antibody. The correctly folded peptide was coupled through its N-terminus to BSA or hemocyanin, and used to immunise young dwarf rats. While hGH administration induced a marked weight gain, immunization did not do so, despite the presence of antibodies to the peptide. It may be that the agonist activity of MAb 263 depends on a conformational change which aligns domains 1 and 2 at a precise angle, and antibodies to the upper domain alone are unable to do this.Supported by the NHMRC (Australia).

P3-267

Lit/Lit Mice Release Growth Hormone (GH) In Response to GH Releasing Peptide-2 (GHRP-2) Administration.

Cibele N Peroni*[1], Nanci Nascimento[1], Paolo Bartolini[1], César Y Hayashida[2], Cyril Y Bowers[3], Sergio PA Toledo[2]. [1]*Biotechnology Dept, IPEN-CNEN/São Paulo, São Paulo, SP, Brazil;* [2]*Endocrine-Genetics Unit, Univ of São Paulo Sch of Med, São Paulo, SP, Brazil;* [3]*Endocrine Sect, Tulane Univ Sch of Med, New Orleans, LA.*

We have recently reported a small but significant GH response to GHRP-2 administration in short individuals with GH deficiency due to an inactivating mutation in the GHRH-receptor (GHRHR) gene (Gondo RG et al, JCEM 2001;86:3279), indicating that GHRPs and probably other GH secretagogues (GHSs) can stimulate pituitary somatotrophs even in the absence of GHRH action. Literature data, however, report a lack of GH release in response to GHS administration in the lit/lit mouse, an animal model presenting a spontaneous mutation in the GHRHR gene. In normal C57BL mice, serum GH increased 2x over the basal levels after 1µg and 6-7x after 10µg ip administration of GHRP-2 (n=20 animals per group), with a peak at 10-30 min after injection. GHRP-2, 10µg, ip, were then injected in lit/+ and lit/lit mice (n=18 animals per group). A GH increment of 7x (GH peak of 34.5±9.7 ng/ml (mean± SD) over the base line was seen in lit/+ mice and, surprisingly, a 9x GH increase over the basal (9.3±1.5 ng/ml) was observed in the lit/lit mice. Finally, a chronic test (30 days) was performed in lit/lit, injecting 5 µg GHRP-2/animal/day, in comparison with a control group receiving saline (n=6 animal per group). Mice were weighed daily and blood withdrawn once a week. A small but significantly higher slope was seen in weight variation curves for the animals injected with GHRP-2 as compared to control ($p<0.05$)), whereas mouse GH and IGF-1 determinations did not present significant increase.

These data suggest that acute GHRP-2 administration was able to release significant amounts of GH in the lit/lit mice, as in humans with GHRHR mutation. The small but significant body weight gain could be due to the orexigenic effect of GHRP-2. Multiple daily doses or longer acting GHSs may be needed for chronic GH stimulation in mouse (and probably human) with GH deficiency due to inactive GHRHR.

P3-268

Opposite Effects of Unmodified Prolactin and a Molecular Mimic of Phosphorylated Prolactin on Morphology and the Expression of Prostate-Specific Genes in the Normal Rat Prostate.

Xiaolei Xu*[1], Wei Wu[1], Valencia Williams[1], Amy Khong[1], Yen-hao Chen[1], Ameae M Walker[1]. [1]*Biomed Scis, Univ of California, Riverside, CA.*

In the current study, we have investigated the individual roles of unmodified PRL (WT PRL) and a molecular mimic of phosphorylated PRL (S179D PRL) in the normal rat prostate.

In the first animal experiment, recombinant versions of unmodified PRL and S179D PRL were delivered to adult male rats at 14 µg/kg/day for 3 weeks. In the second animal experiment, two subcutaneous injections of long acting forms of 200 µg/kg of unmodified PRL or S179D PRL were given to adult male rats on day 1 and day 22 for a total of 5½ weeks of treatment.

Different forms of PRL have opposite effects on normal rat prostate independently of androgens. Unmodified PRL promoted morphological changes of prostate epithelium consistent with preparation for cell proliferation while S179D PRL produced morphological evidence of a more differentiated epithelium. Northern blot analysis of the two major prostate specific proteins, prostatein and probasin, showed that unmodified PRL decreased, while S179D PRL increased, the expression of these two proteins. At the same time, S179D PRL reduced both testosterone and dihydrotestosterone levels.

We conclude that PRL is an important factor in the normal rat prostate. Different forms of PRL have specific functions. Unmodified PRL induced a less differentiated state while the molecular mimic of phosphorylated PRL, S179D PRL, promoted epithelial cell differentiation.

P3-269

Enhancement of Transcriptional Activity by Intranuclear Somatolactogenic Hormones.

Michael A Rycyzyn*[1], Charles V Clevenger[1]. [1]*Pathology and Lab Med, Univ of Pennsylvania, Philadelphia, PA.*

The pleiotropic actions of the somatolactogenic hormones prolactin (PRL) and growth hormone (GH) are necessary for mammary growth and differentiation, lactation, in vitro lymphoid proliferation, metabolism regulation and whole body growth.. The proximal actions of these ligands are mediated by their cell surface receptors via associated networks. Our lab and others have demonstrated that the activation of PRL receptor associated signaling pathways coincides with the internalization of the ligand. We reasoned that if PRL acts directly within the nucleus it must be doing so through a binding partner, or chaperone, as this hormone lacks either an enzymatic activity or a nuclear localization signal sequence. Yeast two-hybrid analysis identified an interaction between PRL and cyclophilin B (CypB), a member of the immunophilin family of proteins containing peptidyl-prolyl isomerase (PPIase) activity. The exogenous co-addition of PRL and CypB to PRL-responsive cells potentiated PRL-induced proliferation over that seen with PRL alone. The increase in proliferation did not correspond to an enhancement of the signal being generated through the PRL receptor, but rather, coincided with a dramatic increase in nuclear translocated PRL. An enzymatically inactive form of CypB, termed CypB-PPI, was generated and found to competitively inhibit the potentiation of PRL-driven proliferation induced by CypB. Furthermore, a GH/CypB interaction was also confirmed with CypB enhancing GH-driven c-fos expression. Once in the nucleus, the PRL/CypB complex was found to associate with Stat5a and enhance Stat5-driven gene transcription and DNA-binding ability, thereby acting as a transcriptional inducer. This enhancement in Stat5 activity was further confirmed by transcriptional array analysis. The induction of Stat5 activity upon PRL/CypB binding was determined to be due to the release of the Stat repressor PIAS3, a previously uncharacterized interaction, which was dependent upon the PPIase activity of CypB. This repressive effect of PIAS3 on Stat5 was further confirmed by overexpression of PIAS3 in CHO cells, which inhibited Stat5 DNA-binding and transcriptional activity. Therefore, this represents a novel mechanism through which a peptide ligand functions directly within the nucleus to modulate transcriptional activity. This study was supported in part by NIH grants F32DK10043 (to M.A.R.) and R01CA69294 (to C.V.C.) and ACS grant RPG00307TBE (to C.V.C.)

P3-270 ⊗

A Novel and Functional Interaction between Cyclophilin (Cyp) A and the Prolactin Receptor.

Farhat Syed*[1], Michael A Rycyzyn[1], Charles V Clevenger[1]. [1]*Pathology and Lab Med, Univ of Pennsylvania, Philadelphia, PA.*

Prolactin (PRL) is involved in mammary tissue development and the pathogenesis of breast cancer via the prolactin receptor (PRLr), a member of the cytokine receptor superfamily. PRL acts as both an endocrine and autocrine/paracrine progression factor for mammary carcinoma in both rodents and humans. Several lines of evidence have indicated that the proline-rich Box 1 motif within the intracellular domain of the PRLr is necessary for PRLr- associated signaling, possibly undergoing a structural change during such transduction. We have demonstrated an interaction between the PRLr and cyclophilin (Cyp) A, a member of the cyclophilin family, possessing peptidyl prolyl cis/trans isomerase (PPI) activity both *in vitro* and *in vivo*. To test for direct interaction between CypA and the PRLr purified epitope-tagged CypA, obtained by RT-PCR of Jurkat mRNA was admixed to a purified chimera of GST and the intracellular domain of the PRLr (aa 235-599) in the presence or absence of 1 nM CsA. After extensive washing the Sepharose conjugates were subject to SDS-PAGE and immunoblotted with anti-V5 antibody. A direct interaction of the intercellular domain of PRLr and CypA *in vitro* in the presence and absence of CsA was observed. This suggests that the interaction does not involve the PPI pocket in CypA that interacts with CsA but instead a region of CypA outside of its core PPIase domain. The interaction between CypA and the PRLr was decreased by the inclusion of either divalent cation or reducing agent in the binding buffer. To further investigate the *in vivo* interaction between CypA and PRLr, human breast cancer cell (T47D) lysates stimulated with PRL (50 ng/ml) for zero and 15 minutes were immunoprecipated with anti-PRLr antibody. The immunoprecipitates were subject to immunoblot analysis with anti-CypA and anti-PRLr antibodies revealing a constitutive interaction between CypA and the PRLr. This confirms the *in vitro*