0013-7227/04/$15.00/0
Printed in U.S.A.

Endocrinology 145(12):5459–5464

doi: 10.1210/en.2004-0865

Minireview: *GNAS*: Normal and Abnormal Functions

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***GNAS* is a complex imprinted gene that uses multiple promoters to generate several gene products, including the G protein α-subunit ($G_s\alpha$) that couples seven-transmembrane receptors to the cAMP-generating enzyme adenylyl cyclase. Somatic activating $G_s\alpha$ mutations, which alter key residues required for the GTPase turn-off reaction, are present in various endocrine tumors and fibrous dysplasia of bone, and in a more widespread distribution in patients with McCune-Albright syndrome. Heterozygous inactivating $G_s\alpha$ mutations lead to Albright hereditary osteodystrophy. $G_s\alpha$ is imprinted in a tissue-specific manner, being primarily expressed from the maternal allele in renal proximal tubules, thyroid, pituitary, and ovary. Maternally inherited mutations lead to Albright hereditary osteodystrophy (AHO) plus PTH, TSH, and gonadotropin resistance (pseudohypoparathyroidism type 1A), whereas paternally inherited mutations lead to AHO alone. Pseudohypoparathyroidism type 1B, in which patients develop PTH resistance without AHO, is almost always associated with a *GNAS* imprinting defect in which both alleles have a paternal-specific imprinting pattern on both parental alleles. Familial forms of the disease are associated with a mutation within a closely linked gene that deletes a region that is presumably required for establishing the maternal imprint, and therefore maternal inheritance of the mutation results in the *GNAS* imprinting defect. Imprinting of one differentially methylated region within *GNAS* is virtually always lost in pseudohypoparathyroidism type 1B, and this region is probably responsible for tissue-specific $G_s\alpha$ imprinting. Mouse knockout models show that $G_s\alpha$ and the alternative $G_s\alpha$ isoform XLαs that is expressed from the paternal *GNAS* allele may have opposite effects on energy metabolism in mice. (*Endocrinology* 145: 5459–5464, 2004)**

GNAS: Normal Function

GNAS IS A COMPLEX imprinted gene that generates multiple gene products through the use of multiple promoters and first exons that splice onto a common set of downstream exons (exons 2–13; see Fig. 1) (1, 2). The major *GNAS* gene product, which is generated by the most downstream promoter (exon 1), is the ubiquitously expressed G protein α-subunit ($G_s\alpha$) that couples numerous hormonal and other seven-transmembrane receptors to adenylyl cyclase and is required for the receptor-stimulated intracellular cAMP production. The most upstream *GNAS* promoter generates transcripts for the chromogranin-like protein NESP55, which is structurally and functionally unrelated to $G_s\alpha$. The NESP55 coding region is limited to its specific first exon, and $G_s\alpha$ exons 2–13 form the majority of its 3′-untranslated region (3, 4). The next promoter generates transcripts encoding the neuroendocrine-specific $G_s\alpha$ isoform XLαs, which structurally is identical to $G_s\alpha$, except for the presence of an extra-long amino-terminal extension encoded by its specific first exon (5). NESP55 and XLαs are oppositely imprinted (6–8). NESP55 is only expressed from the maternally inherited allele, and its promoter is DNA methylated at CpG dinucleotides on the paternally inherited allele. In contrast, XLαs is only expressed from the paternal allele, and its promoter is methylated on the maternal allele. Both XLαs and NESP55 are expressed primarily in neuroendocrine tissues (9–12). Although the biological functions of these two gene products have not been fully established, it seems likely that NESP55 and XLαs deficiency have little effect in humans, but that XLαs deficiency leads to a severe phenotype in mice (see below). The XLαs promoter region probably contains a primary imprinting center in which maternal-specific methylation is established during gametogenesis and maintained throughout development (13). This region also generates paternally expressed antisense transcripts that may be important for NESP55 imprinting (14, 15), which is a secondary event that occurs during postimplantation development (16, 17).

The $G_s\alpha$ promoter lies 35 kb downstream of the XLαs promoter. The $G_s\alpha$ promoter is within a CpG island and is unmethylated on both parental alleles (16) despite the fact that $G_s\alpha$ is imprinted in a tissue-specific manner, being expressed primarily from the maternal allele in specific hormone target tissues (*e.g.* renal proximal tubules, thyroid, pituitary, and ovaries) (18–22). Tissue-specific $G_s\alpha$ imprinting in mice is associated with tissue-specific differences in the extent of histone H3 lysine 4 methylation in $G_s\alpha$ exon 1, an epigenetic modification that has been shown to be associated with transcriptional activity in lower species (23). Just upstream of the $G_s\alpha$ promoter is another differentially methylated region (DMR) that is methylated only on the maternal allele and contains a promoter and first exon (exon 1A or A/B) that generates paternally expressed untranslated mRNA transcripts (16, 24). The exon 1A DMR also appears to be a primary imprint mark, because its methylation is established during gametogenesis and is maintained throughout development (16). The exon 1A DMR can be deleted without affecting NESP55-XLαs imprinting (25) (Weinstein, L. S., and J. Liu, unpublished observations), indicating that the *GNAS* locus contains two independent imprinting domains (NESP55/XLαs and exon 1A DMR/ $G_s\alpha$). Both the human gene, located at 20q13, and the mouse or-

Abbreviations: AHO, Albright hereditary osteodystrophy; DMR, differentially methylated region; FD, fibrous dysplasia; $G_s\alpha$, G protein α-subunit; MAS, McCune-Albright syndrome; PHP1A, pseudohypoparathyroidism type 1A; PKA, protein kinase A.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

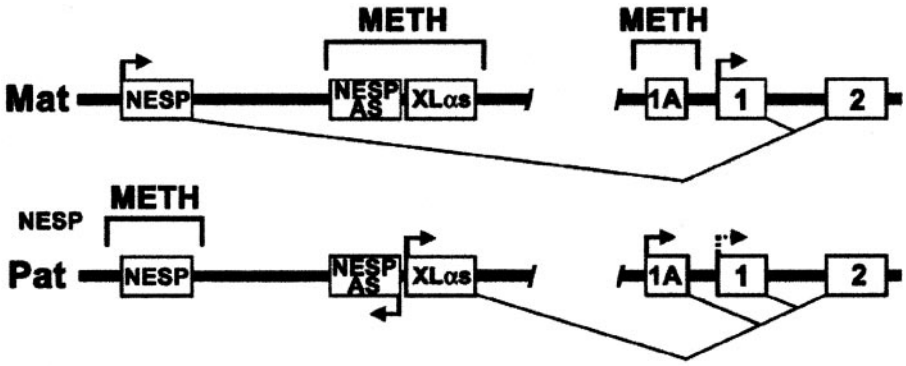

FIG. 1. General organization and imprinting patterns of the human *GNAS* (and mouse *Gnas*) locus. The maternal (Mat) and paternal (Pat) alleles of *GNAS* are depicted with alternative first exons for NESP55 (NESP), XLαs, untranslated (exon 1A), and $G_s\alpha$ (exon 1) mRNAs splicing to a common exon (exon 2). Common downstream exons 3–13 in the sense direction are not shown. Differentially methylated regions (METH) are shown *above*, and splicing patterns are shown *below* each panel. Transcriptionally active promoters are indicated by *horizontal arrow*s in the direction of transcription. Transcription from the paternal $G_s\alpha$ (exon 1) promoter is suppressed in some tissues, which is indicated with a *dashed arrow*. The XLαs promoter also generates paternally expressed antisense transcripts, and the first antisense exon is shown (NESPAS). The diagram is not drawn to scale.

tholog *Gnas*, which is located within a syntenic region in distal chromosome 2, have similar overall structure and imprinting patterns.

The most well characterized function of $G_s\alpha$ is as a signal transducer between seven-transmembrane receptors and generation of cAMP by adenylyl cyclase. cAMP is known to mediate many of its effects by stimulating protein kinase A (PKA), but more recently cAMP has been shown to have other effectors in certain cell types, such as cAMP-regulated guanine nucleotide exchange factors for Rap1 (26) and calcium channels (27). These diverse effectors lead to mitogenic and antimitogenic effects in different cell types (2). There is also evidence that $G_s\alpha$ itself may have other effectors, such as Src kinase (28) and calcium channels (29), and that $G_s\alpha$ may be activated by growth factor receptors (30, 31). In addition to residing on the inner leaf of cell membranes, $G_s\alpha$ is localized to intracellular membranes and therefore may have a role in membrane trafficking (32).

$G_s\alpha$, like all G protein α-subunits, consists of two domains: a GTPase domain that includes the sites for guanine nucleotide binding and receptor and effector interaction and a helical domain that may be important to maintain guanine nucleotide binding (33, 34). Long and short forms of $G_s\alpha$ result from alternative splicing of exon 3. In the inactive state, $G_s\alpha$ exists as a $G_s\alpha$-$\beta\gamma$ heterotrimer with GDP bound to its binding pocket. Ligand-bound receptors promote GDP release and binding of ambient GTP, which results in a switch to an active conformation and dissociation from $\beta\gamma$. GTP-$G_s\alpha$ directly activates adenylyl cyclase and its other effectors. The turn-off mechanism is an intrinsic GTPase that hydrolyzes bound GTP to GDP. The GTPase cycle may also be regulated by RGS proteins and effectors (35, 36). Unlike $G_s\alpha$, XLαs has a more limited tissue distribution, being primarily expressed in the pars intermedia of the pituitary, brain, adrenal, heart, and pancreatic islets (12). XLαs is able to bind to $\beta\gamma$-subunits and mediate receptor-stimulated cAMP production (37, 38).

GNAS-Activating Mutations

GNAS-activating mutations (also known as *gsp* mutations) are missense mutations that lead to amino acid substitution of either residue Arg^{201} or Gln^{227} within the long form of $G_s\alpha$ (39). These two residues are catalytically important for GTPase activity; therefore, these mutations cause constitutive activation by disrupting the turn-off mechanism. The exotoxin secreted by *Vibrio cholera* catalyzes a posttranslational modification (ADP ribosylation) of $G_s\alpha$ Arg^{201}, and the resulting increased cAMP in intestinal lining cells leads to severe secretory diarrhea. *Gsp* mutations are dominant acting, and such somatic mutations were originally found in approximately 40% of GH-secreting pituitary tumors, a small number of thyroid tumors, and rarely in other endocrine tumors (2, 40). Growth and hormone release in many endocrine glands are stimulated by trophic hormones that activate $G_s\alpha$-cAMP pathways. The GHRH-$G_s\alpha$-cAMP-PKA pathway stimulates growth and hormone release in somatotrophs via phosphorylation of cAMP response element-binding protein and induction of the somatotroph-specific transcription factor GH factor-1 (41, 42). The glycoprotein hormones TSH, ACTH, and gonadotropins stimulate growth and hormone release in their target tissues by both PKA-dependent and -independent mechanisms (2).

More widespread distribution of cells bearing Arg^{201} mutations, presumably due to somatic mutation occurring during early prenatal development, leads to the McCune-Albright syndrome (MAS). MAS is classically defined as the triad of gonadotropin-independent sexual precocity, café-au-lait skin lesions, and fibrous dysplasia (FD) of bone (43–45). Some MAS patients do not develop all three features of the triad, whereas others develop other characteristic endocrine and nonendocrine features, such as TSH-independent functional thyroid nodules, ACTH-independent adrenal hyperplasia with hypercortisolism, acromegaly, hypophosphatemia, cardiomyopathy, sudden death, and effects on other nonendocrine tissues (46). Somatic *gsp* mutations are also present in FD lesions from patients with or without other features of MAS (47, 48). MAS is virtually never inherited, and germline *gsp* mutations are considered lethal (49), although a possible germline Arg^{201} to Gly mutation was reported in one patient with severe manifestations (50). In the vast majority of patients with sporadic acromegaly or acromegaly associated with MAS, the mutation is present on the active maternal allele,

although there does not appear to be a parental allele bias in patients with MAS who do not have acromegaly (19, 51).

The hyperpigmentary lesions in MAS results from increased cAMP in melanocytes (52). cAMP production is normally stimulated in melanocytes by MSH, and cAMP induces the expression of tyrosinase, the rate-limiting enzyme for melanin production. FD results from hyperproliferation and incomplete differentiation of marrow stromal cells to osteoblasts, which is the direct result of excess cAMP in these cells (44, 53–56). cAMP stimulates the expression of Fos by PKA-dependent phosphorylation and activation of cAMP response element-binding protein. Fos overexpression in FD cells (57) inhibits the expression of osteoblast-specific genes and stimulates the expression of cytokines such as IL-6, which promotes bone resorption by osteoclasts (58). Recent studies show that hypophosphatemia in FD/MAS results from excess secretion of the phosphatonin fibroblast growth factor 23 from FD lesions (59).

GNAS-Inactivating Mutations

Heterozygous inactivating $G_s\alpha$ mutations result in Albright hereditary osteodystrophy (AHO), a congenital syndrome in which patients develop obesity, short stature, brachydactyly, subcutaneous ossifications, and neurobehavioral deficits (1, 60, 61). The severity of manifestations varies greatly, and some patients with mutations have minimal or no clinical features (62). Mutations that disrupt $G_s\alpha$ mRNA expression (*e.g.* premature stop codons and splice junction mutations) or missense mutations have been identified in all 13 $G_s\alpha$ coding exons, except exon 3, probably because splicing out this exon still produces a functional protein (63). Some missense mutations produce specific biochemical $G_s\alpha$ defects (1). These mutations result in $G_s\alpha$ haploinsufficiency (50% loss of expression or function) in many tissues, and this is the most likely molecular defect underlying the AHO phenotype. If NESP55 or XLαs deficiency were the underlying cause of AHO, one would expect different phenotypes resulting from mutations on the maternal and paternal alleles, respectively. Moreover, mutations in $G_s\alpha$ exon 1, which disrupt expression of $G_s\alpha$, but not XLαs or NESP55, also lead to AHO. Some patients with identical $G_s\alpha$ mutations develop a severe form of ectopic ossification known as progressive osseous heteroplasia, possibly resulting from inappropriate expression of the osteoblast-specific transcription factor Cbfa1/RUNX2 in ectopic locations (64, 65). The opposite skeletal effects of activating and inactivating $G_s\alpha$ mutations in FD and AHO strongly implicate $G_s\alpha$/cAMP as an important regulator of osteoblast differentiation.

AHO patients who inherit $G_s\alpha$ mutations from their mother also develop resistance to PTH, TSH, and gonadotropins [pseudohypoparathyroidism type 1A (PHP1A)], whereas patients who inherit mutations from their father inherit their mutations only develop AHO (also known as pseudopseudohypoparathyroidism) (1, 66). This is due to the fact that $G_s\alpha$ is primarily expressed from the maternal allele in the target tissues of these respective hormones (renal proximal tubules, thyroid, and ovaries) (18–22). Mutations in the active maternal allele lead to $G_s\alpha$ deficiency and hormone resistance, whereas mutation in the inactive paternal allele have little or no effect on $G_s\alpha$ expression or hormonal signaling (Fig. 2). Recent studies show these patients to also have GH deficiency due to GHRH resistance, although the short stature in these patients is probably the result of a primary skeletal growth plate defect rather than GH deficiency (67, 68). Olfactory defects and prolactin deficiency have also been reported in PHP1A patients (69, 70). Lack of $G_s\alpha$ imprinting in other hormone target tissues is one explanation for why PHP1A patients fail to develop resistance to other hormones that stimulate $G_s\alpha$/cAMP pathways (*e.g.* ACTH and vasopressin) (18).

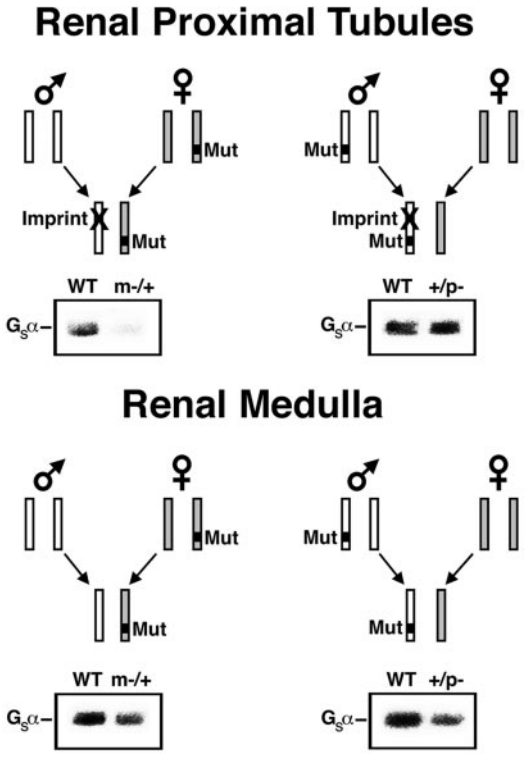

FIG. 2. Tissue-specific $G_s\alpha$ imprinting and the effects of heterozygous inactivating $G_s\alpha$ mutations. $G_s\alpha$ is paternally imprinted (denoted with X) in renal proximal tubules (*upper panels*). Mutation (Mut) on the active maternal allele (*left panel*) leads to $G_s\alpha$ deficiency and PTH resistance, whereas mutation on the imprinted paternal allele (*right panel*) has little effect on $G_s\alpha$ expression or PTH sensitivity. Immunoblots of renal cortical membranes isolated from mice with disruption of the *Gnas* maternal (m−/+) and paternal (+/p−) alleles, respectively, as well as their wild-type mice (WT) littermates confirm the predicted $G_s\alpha$ expression patterns (18). In most other tissues (*lower panels*), $G_s\alpha$ is not imprinted, and therefore, both maternal and paternal mutations lead to an approximately 50% loss of $G_s\alpha$ expression (haploinsufficiency), as shown in immunoblots of renal inner medulla membranes from the same mice. $G_s\alpha$ haploinsufficiency is probably the underlying molecular defect that leads to the AHO phenotype in patients with heterozygous $G_s\alpha$ mutations Figure adapted from Weinstein *et al.* (1).

GNAS Imprinting Defects

PHP1B patients have renal PTH resistance similar to that in PHP1A, but lack the AHO phenotype. In PHPIB, $G_s\alpha$ expression in tissues such as blood cells is normal, ruling out the presence of typical $G_s\alpha$-inactivating mutations. PHPIB results from a *GNAS* imprinting defect in which maternal-specific imprinting (methylation) of the exon 1A DMR is lost, with both alleles having a paternal imprinting pattern or epigenotype in this region (24). Presumably this leads to $G_s\alpha$

deficiency in renal proximal tubules due to the lack of an active maternal allele, but has no effect on $G_s\alpha$ expression in the majority of other tissues, where $G_s\alpha$ is normally expressed equally from both parental alleles. This is consistent with the fact that PHPIB patients have PTH resistance, but lack the AHO phenotype. Although TSH resistance was not considered to be a feature of PHPIB, it was recently shown that borderline to mild TSH resistance is present in almost half the patients with the imprinting defect (22).

In familial PHPIB, patients almost always have a deletion mutation within the closely linked *STX16* gene located upstream of *GNAS*, which has no effect when inherited paternally, but leads to the exon 1A DMR imprinting defect and PTH resistance when inherited maternally (71). Imprinting of NESP55/XLαs is unaffected in these patients. Presumably the deleted region contains *cis*-acting elements that are required to establish or maintain exon 1A DMR imprinting, but is not necessary for imprinting of the intervening NESP55/XLαs domain. Sporadic forms of PHPIB can be associated with loss of the maternal epigenotype within the exon 1A DMR alone or may also involve loss of the maternal epigenotype within the NESP55/XLαs imprinting domain as well (24, 72). Whether these imprinting defects in sporadic PHPIB are caused by underlying mutations or result from a random failure of the imprinting process is unknown. NESP55 expression in patients with the more global *GNAS* imprinting defect is absent due to methylation of the NESP55 promoter on both parental alleles (24). This subset of patients does not have a more severe phenotype, suggesting that NESP55 expression is dispensable in humans. PTH resistance has also been described in a patient with paternal uniparental disomy of 20q, which leads to a similar *GNAS* imprinting defect (73). In one family a PHPIB phenotype was caused by maternal inheritance of a $G_s\alpha$ missense mutation (deletion of Ile^{382}) that generates a $G_s\alpha$ protein, which is selectively uncoupled to the PTH/PTH-releasing peptide receptor (74).

We have proposed a model to explain how tissue-specific $G_s\alpha$ imprinting is controlled by the exon 1A DMR and how loss of exon 1A DMR imprinting leads to PHPIB (24). The fact that exon 1A-specific and $G_s\alpha$ mRNA transcripts have similar tissue distributions makes it unlikely that the exon 1A DMR controls $G_s\alpha$ by promoter competition or by a direct action of exon 1A-specific transcripts (16). Rather, the exon 1A DMR may contain a *cis*-acting, negative regulatory element (silencer or insulator) that is both methylation sensitive and tissue specific. In the example shown in Fig. 3, the exon 1A DMR has a silencer that binds a tissue-specific repressor protein. In proximal tubules, the repressor binds to the paternal allele and suppresses $G_s\alpha$ expression, but is unable to bind to the maternal allele because its site is methylated and heterochromatic (23), allowing $G_s\alpha$ to be maternally expressed. In most other tissues, the exon 1A DMR is methylated, but the repressor is not expressed; therefore, $G_s\alpha$ is biallelically expressed. In PHPIB, exon 1A DMR methylation is absent, allowing the repressor to bind to and suppress $G_s\alpha$ expression from both alleles in proximal tubules, leading to $G_s\alpha$ deficiency and PTH resistance. However, the methylation defect has no effect on $G_s\alpha$ expression in most other tissues because the repressor is absent. Support from this model comes from recent findings showing that paternal, but not maternal, deletion of the exon 1A DMR in mice leads to $G_s\alpha$ overexpression in renal proximal tubules and lower

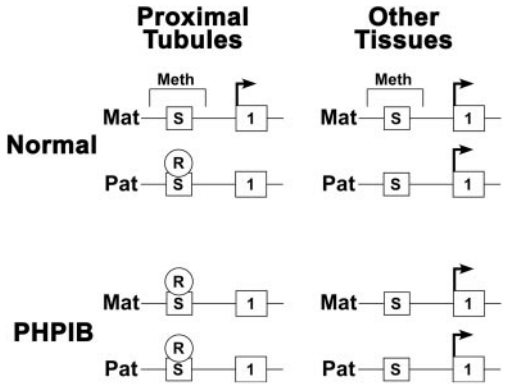

FIG. 3. Proposed model for the role of the exon 1A DMR in tissue-specific $G_s\alpha$ imprinting and the pathogenesis of PHP1B. Maternal (Mat) and paternal (Pat) alleles of the exon 1A DMR-$G_s\alpha$ exon 1 region are depicted. The exon 1A DMR is normally methylated on the maternal allele (Meth) and contains a *cis*-acting silencer (S; *upper panels*). In proximal tubules (*left panel*) a tissue-specific *trans*-acting repressor (R) binds to the silencer and suppresses $G_s\alpha$ expression on the paternal allele, but is unable to bind to the maternal allele due to methylation, allowing $G_s\alpha$ to be expressed from the maternal allele. In most other tissues (*right panel*) the repressor is not expressed, and therefore, $G_s\alpha$ is biallelically expressed. In PHP1B (*lower panels*), methylation is absent, allowing the repressor to bind to both alleles in proximal tubules, resulting in $G_s\alpha$ deficiency and PTH resistance. $G_s\alpha$ expression is unaffected in most other tissues because the repressor is absent.

serum PTH levels, a sign of increased PTH sensitivity (25) (Weinstein, L. S., and J. Liu, unpublished observations).

Gnas Knockout Models

Mice with heterozygous disruption of *Gnas* exon 2 on the maternal (m−/+) or paternal (+/p−) allele have distinct early phenotypes similar to mice with paternal uniparental disomy/maternal deletion and maternal uniparental disomy/paternal deletion of the distal chromosome 2 region including *Gnas*, respectively (18). These distinct phenotypes are consistent with the fact that *Gnas* has distinct maternally and paternally expressed gene products. Interestingly, m−/+ and +/p− mice have opposite metabolic phenotypes (18, 75–77). −/+ mice are obese, hypometabolic, and hypoactive, whereas +/p− mice are very lean, hypermetabolic, and hypoactive. Measurements of urinary norepinephrine suggest that the m−/+ and +/p− metabolic phenotypes may be secondary to decreased and increased sympathetic nerve activity, respectively (75). Interestingly, both groups of mice have increased whole body insulin sensitivity, although to a much greater extent in +/p− mice (76). Mice with paternal deletion of $G_s\alpha$ exon 1, a mutation that specifically disrupts expression of $G_s\alpha$, but not other *Gnas* gene products, lack the +/p− exon 2 phenotype and, in fact, have the opposite metabolic phenotype (obesity and insulin resistance) (Weinstein, L. S., and M. Chen, unpublished observations). Recently, XLαs knockout mice were shown to have a phenotype similar to that of +/p− exon 2 mice (78). These findings suggest that $G_s\alpha$ and XLαs have opposite effects on whole body metabolism, and that the +/p− exon 2 phenotype is primarily caused by XLαs deficiency. One possibility is that XLαs normally acts as a negative regulator of sympathetic nerve activity, and therefore, XLαs deficiency in +/p− mice leads to a hyperadrenergic state (77). The bio-

logical importance of XLαs may be species specific, because loss of XLαs expression in pseudopseudohypoparathyroidism patients does not result in a similar phenotype.

Conclusions

GNAS is a complex imprinted locus whose major product is $G_s\alpha$. Somatic $G_s\alpha$-activating mutations produce MAS, endocrine tumors, and/or fibrous dysplasia of bone primarily through the effects of increased cAMP levels, which in some cell types can lead to proliferation. Inactivating $G_s\alpha$ mutations leads to AHO and in some cases progressive osseous heteroplasia. The opposite effects of activating and inactivating $G_s\alpha$ mutations on osteoblast differentiation in these disorders suggest that $G_s\alpha$/cAMP pathways play a major role in regulating osteoblastogenesis. The AHO phenotype is most likely due to $G_s\alpha$ haploinsufficiency resulting from heterozygous $G_s\alpha$ mutations. $G_s\alpha$ is imprinted and expressed primarily from the maternal allele in various hormone target tissues, and therefore, maternal inheritance of $G_s\alpha$ mutations also leads to multihormone resistance (PHPIA). Hormone resistance in PHPIB can also result from loss of maternal imprinting of the exon 1A DMR region, a region within the *GNAS* locus that appears to contain elements important for establishing tissue-specific imprinting of $G_s\alpha$. However, these patients do not develop AHO, because the imprinting defect has no effect on $G_s\alpha$ expression in the vast majority of tissues where $G_s\alpha$ is normally expressed biallelically. *Gnas* knockout mouse models demonstrate that both $G_s\alpha$ and XLαs, a neuroendocrine-specific $G_s\alpha$ isoform expressed only from the paternal allele, have important roles in the regulation of energy metabolism.

Acknowledgments

Received July 7, 2004. Accepted August 17, 2004.

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