Mechanisms of Disease

The Expanding Spectrum of G Protein Diseases

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DISEASE-causing mutations often reveal key pathways of physiologic regulation and their underlying molecular mechanisms. Mutations in the trimeric guanine nucleotide-binding proteins (G proteins), which relay signals initiated by photons, odorants, and a host of hormones and neurotransmitters, cause many diseases. For the most part, the diseases are confined to a set of fascinating but rare endocrine disorders (Table 1).[1] A recent study suggests that mutations in G proteins can also lead to essential hypertension.[2] If this study is correct, hypertension may be one of several common disorders caused by defects in this ubiquitous family of signaling molecules.

This review focuses primarily on recent discoveries that help us understand the pathogenesis and pathophysiology of diseases caused by G protein mutations. We also discuss the underlying molecular mechanisms of G protein signaling, a topic that has recently been reviewed in detail elsewhere.[3-6]

Mutations that alter G protein activation may cause disorders characterized by either insufficient or excessive transmission of signals (Table 1). Decreased transmission of signals — loss of function — results from mutations that impair the ability of the G protein to become activated by hormone receptors. Increased transmission of signals — gain of function — results from mutations that mimic or augment the activation of receptors.

STRUCTURE AND FUNCTION OF G PROTEINS

G proteins relay signals from each of more than 1000 receptors to many different intracellular effectors, including enzymes and ion channels.[5,6] The G protein is composed of an α subunit that is loosely bound to a tightly associated dimer made up of a β subunit and a γ subunit (Fig. 1A); each of the three subunits is encoded by a separate gene, selected from 16 α, 6 β, and 12 γ genes, respectively. Various Gα proteins define different G protein trimers (G$_s$, G$_q$, G$_i$, G$_t$, and so on), each of which regulates a distinctive set of downstream signaling pathways (Table 2).

The activity of a trimeric G protein is regulated by the binding and hydrolysis of guanosine triphosphate (GTP) by the Gα subunit (Fig. 2). An α subunit to which guanosine diphosphate (GDP) is bound is inactive and associates with the $\beta\gamma$ dimer. Activation of a receptor by a ligand causes the $\alpha\beta\gamma$ complex to release GDP. This release is followed by the binding of GTP to the α subunit, after which the α subunit–GTP complex dissociates from $\beta\gamma$ and from the receptor. This complex or the free $\beta\gamma$ dimer then activates downstream effectors.[6] The conversion of bound GTP to GDP terminates the signal, because α subunit–GDP complexes lack the capacity to regulate effectors and inactivate $\beta\gamma$ by binding to it.

G protein defects can cause disease in several ways. The production of a G protein that cannot hydrolyze GTP and terminate the signal results in persistently elevated activity of the downstream effector, even in the absence of extracellular stimuli (Fig. 2). Decreased production of a normal G protein or production of an unstable G protein can reduce the normal response to hormonal stimulation. Abnormalities affecting the ability of a G protein to switch to the "on" state may result in an increase or a decrease in the downstream signal. An increase results when the defective protein releases GDP and binds GTP more rapidly than normal, and a decrease results when the protein releases GDP more slowly or binds GTP less avidly (Fig. 2).

DEFECTS IN THE TERMINATION OF G PROTEIN SIGNALING

To turn off the signal, a G protein must cleave the γ-phosphate from GTP in a reaction that requires association of the γ-phosphorus atom with the oxygen of a water molecule. To catalyze this reaction, the in-

TABLE 1. DISEASES CAUSED BY DEFECTS IN THE PRODUCTION OR ACTIVITY OF G PROTEINS.*

CAUSE AND DISEASE	PROTEIN	MOLECULAR MECHANISM	DISTRIBUTION
Caused by defective signal termination — signal excessive			
Cholera	$G_s\alpha$	ADP-ribosylation of Arg201 inhibits GTP hydrolysis.	Intestinal epithelium
Adenomas of the pituitary and thyroid	$G_s\alpha$	Point mutation Arg201 or Gln227 inhibits GTP hydrolysis.	Sporadic (somatic mutation)
Adenomas of the adrenal and ovary	$G_{i2}\alpha$	Point mutation Arg179 inhibits GTP hydrolysis.	Sporadic (somatic mutation)
McCune–Albright syndrome	$G_s\alpha$	Point mutation Arg201 inhibits GTP hydrolysis.	Mosaic (mutation in early embryo)
Caused by absent or inactive $G\alpha$ — signal deficient			
Pseudohypoparathyroidism type Ia	$G_s\alpha$	One null $G_s\alpha$ allele decreases response to hormones (parathyroid hormone, thyrotropin).	Germ-line mutation
Pseudohypoparathyroidism type Ib	Possibly $G_s\alpha$	The sole defect is decreased response to parathyroid hormone.	Germ-line mutation
Night blindness	$G_t\alpha$	Point mutation Gly38Asp; mechanism is not known.	Germ-line mutation
Caused by abnormal signal initiation — signal inadequate or excessive			
Pertussis	$G_i\alpha$	ADP-ribosylation of a cysteine blocks activation by receptor and decreases signal.	Bronchial epithelium
Pseudohypoparathyroidism type Ia	$G_s\alpha$	Point mutation Arg385His blocks activation by receptor and decreases signal. Point mutation Arg231His impairs GTP binding and decreases signal.	Germ-line mutation
Testotoxicosis with pseudohypoparathyroidism type Ia	$G_s\alpha$	Point mutation Ala366Ser accelerates GDP release, enhances signal at 34°C (testis) or inactivates $G_s\alpha$ at 37°C (pseudohypoparathyroidism type Ia).	Germ-line mutation
Essential hypertension	$\beta3$	Short $\beta3$ enhances receptor activation and increases signal.	Germ-line mutation

*ADP denotes adenosine diphosphate, GTP guanosine triphosphate, and GDP guanosine diphosphate.

volved atoms must be arranged precisely adjacent to neighboring amino acids of Gα (Fig. 3). When the ability of these amino acids to stabilize the precise arrangement of atoms is impaired, GTP hydrolysis is slowed and termination of the signal is delayed, causing disorders characterized by persistent and excessive signals generated by the abnormal G protein.

Cholera

The watery diarrhea caused by *Vibrio cholerae* results from the secretion of salt and water into the intestine, a secretion stimulated by increased concentrations of cyclic AMP (cAMP) in mucosal cells.[27] The bacterium's pathogenic exotoxin, an enzyme, attaches the adenosine diphosphate (ADP)–ribose moiety of intracellular nicotinamide adenine dinucleotide to the side chain of a key arginine residue (at position 201) in G$_s\alpha$, the stimulatory regulator of adenylyl cyclase. Like a finger steadying a delicate piece of machinery, the side chain of this arginine accelerates GTP hydrolysis by holding an oxygen atom of the γ-phosphate in exactly the right place (Fig. 3). The attachment of ADP–ribose to the side chain markedly slows GTP hydrolysis and locks G$_s\alpha$ into its active GTP-bound form. Similar toxins are responsible for the traveler's diarrhea caused by certain strains of *Escherichia coli*.[28]

Acromegaly

The first oncogenic Gα mutants were found in the G$_s\alpha$ genes of pituitary tumors from patients with acromegaly.[25,29] The mutant G$_s\alpha$ oncogene, dubbed *gsp* (for G stimulatory protein), is found in 40 percent of the somatotropic tumors in such patients.[29,30] The proteins encoded by *gsp* have the same defects in signal termination that occur in cholera — the inability to hydrolyze GTP and the persistent stimulation of adenylyl cyclase. The protein encoded by *gsp* is oncogenic because it mimics the intracellular signal triggered by growth hormone–releasing hormone, which normally stimulates a receptor that activates G$_s$ and cAMP synthesis; like the releasing hormone, *gsp* stimulates the secretion of growth hormone and increases the proliferation of somatotrophs. As with most oncogenes, *gsp* results from somatic mutations that occur in the affected tissue; indeed, a germ-line *gsp* mutation would almost certainly be lethal.

Most mutations in *gsp* substitute other amino acids for the arginine residue that is a target of cholera toxin in gut cells; a few mutations replace a nearby
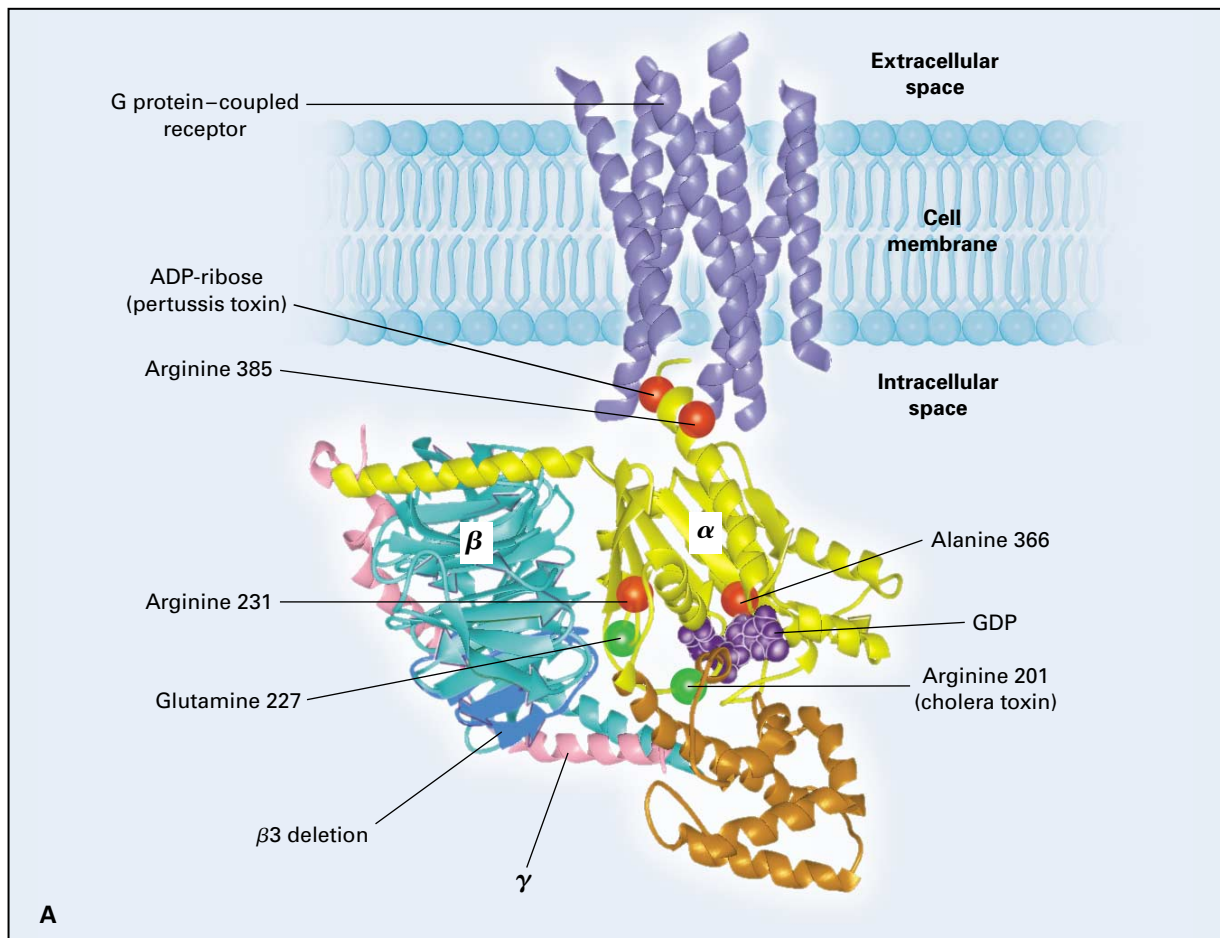
Figure 1. Structure of G Proteins.

In Panel A, the $\alpha\beta\gamma$ trimer is shown in its probable orientation relative to a G protein–coupled receptor and the cell membrane.[7,8] GDP (dark purple) is cradled between two domains (yellow and brown) of Gα; the yellow domain binds Gβ (cyan), which is tightly associated with Gγ (pink). Colored arrows and amino acids indicate residues at which point mutations cause disease; red indicates inhibition of the transmitted signal, and green indicates augmentation. In G$_s\alpha$, these residues include mutations of arginine at position 201 and glutamine at position 227, which inhibit GTP hydrolysis, prevent signal termination, and generate an augmented signal,[3,5,6] and mutations that replace arginine at position 385 and arginine at position 231, which are found in different families with pseudohypoparathyroidism type Ia[9-11] and block receptor activation of G$_s\alpha$ by different mechanisms. Mutational replacement of alanine at position 366 with a serine residue causes both pseudohypoparathyroidism type Ia and testotoxicosis.[12] Pertussis toxin catalyzes ADP-ribosylation of a cysteine in the C terminal of G$_i\alpha$ proteins, preventing activation of G$_i$ by receptors.[5,6] Panel B shows the surface of the G$\beta\gamma$ subunit that binds to Gα. With respect to Panel A, the structure has been rotated 90 degrees about the vertical axis. In both panels, the dark blue region demarcates the 41 amino acids deleted in the β3 chain of some patients with hypertension.[2]
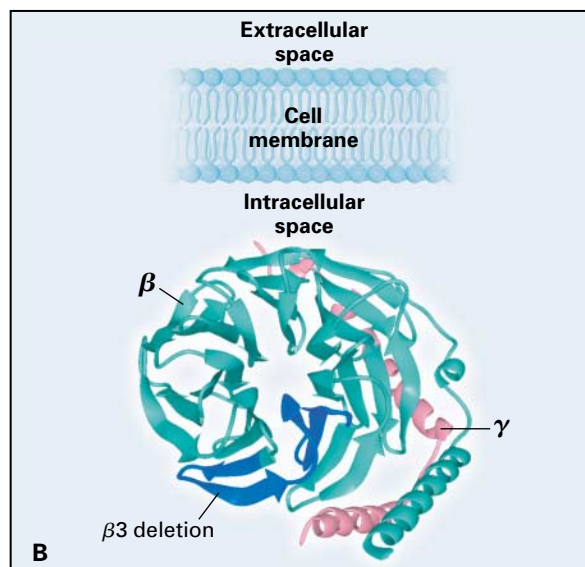
1014 · April 1, 1999

TABLE 2. G PROTEIN FAMILIES AND THEIR FUNCTIONS.

| Gα SUBTYPE	DOWNSTREAM SIGNAL	PHYSIOLOGIC RESPONSES MEDIATED	PHENOTYPES OF TRANSGENIC MICE
$G_s\alpha$	Increases cAMP synthesis	β-Adrenergic amines, parathyroid hormone, thyrotropin, glucagon, corticotropin, many other hormones	Knockout lethal; heterozygotes have condition similar to pseudohypoparathyroidism type Ia, with apparent imprinting of paternal $G_s\alpha$ allele[13]* Cardiac overexpression causes hypertrophy[14] Thyroid overexpression causes thyroid adenoma[15]
$G_i\alpha$	Inhibits cAMP synthesis Closes Ca^{2+} channels Opens K^+ channels	Acetylcholine, α-adrenergic amines, many neurotransmitters, chemokines	$G_{i2}\alpha$ knockout: ulcerative colitis, adenocarcinoma[16] $G_o\alpha$ knockout: abnormal calcium-channel regulation (heart),[17] motor and sensory abnormalities (brain)[18]
$G_t\alpha$	Increases cyclic GMP breakdown	Photons (in rod and cone cells)	
$G_q\alpha$	Increases phosphoinositide synthesis, intracellular Ca^{2+}	Acetylcholine, α-adrenergic amines, many neurotransmitters	$G_q\alpha$ knockout: platelet dysfunction,[19] cerebellar dysfunction[20] $G_{11}\alpha$ knockout: normal[21] $G_q\alpha/G_{11}\alpha$ double knockout: severe cardiac hypoplasia, craniofacial defects[21] Cardiac overexpression of $G_q\alpha$ causes hypertrophy, congestive heart failure[22]
$G_{13}\alpha$	Stimulates exchange of Na^+ and H^+, cytoskeletal rearrangement	Thrombin, other agonists	Knockout lethal; impaired angiogenesis, decreased motility of embryonic fibroblasts[23]

*Heterozygous loss of a *Gα* gene, except for $G_s\alpha$,[13] is not known to cause an abnormal phenotype.

glutamine.[25] Both the arginine and glutamine "fingers" maintain rapid GTP hydrolysis by stabilizing the γ-phosphate of GTP (Fig. 3).

Other Activating Mutations in Gα

Mutations in *gsp* can be found in any tumors derived from a cell in which cAMP stimulates cell proliferation. The McCune–Albright syndrome is a striking example of the effects of these mutations. This syndrome is characterized by polyostotic fibrous dysplasia, scattered regions of hyperpigmented (café au lait) skin, and autonomous hyperfunction of one or more endocrine glands; it results in gonadotropin-independent precocious puberty, hyperthyroidism, Cushing's syndrome, or acromegaly. The affected tissues of patients with this syndrome contain *gsp* mutations that encode substitutions for the arginine finger that is also mutated in pituitary tumors (Fig. 3).[26] All manifestations of this congenital (but not heritable) syndrome reflect a *gsp* mutation that occurred early in the development of the embryo, in the DNA of a cell whose progeny will contribute to the mosaic distribution of affected cells later in life.[26,31]

Intracellular cAMP, like cAMP-elevating hormones, normally stimulates the proliferation of cells in the glands (thyroid, adrenal cortex, ovary, and pituitary) that give rise to hyperfunctioning tumors in patients with the McCune–Albright syndrome. The cutaneous hyperpigmentation does not result from excessive proliferation of cells, but from the mimick-
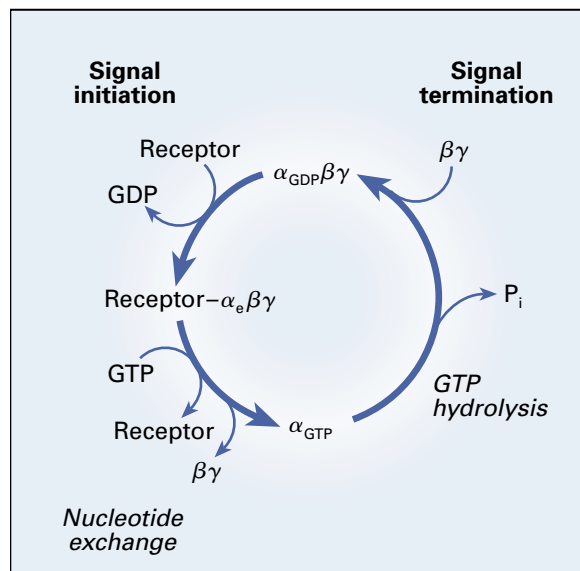
Figure 2. The Cycle of GTP Binding and Hydrolysis Responsible for Initiating and Terminating Signals Transmitted by Trimeric G Proteins.

The initiation of signals by receptors activates G proteins (left-hand side of the figure) by releasing GDP from the αβγ trimer; this release allows GTP to bind to the empty guanine nucleotide pocket of Gα (denoted α_e), which results in dissociation of the α subunit–GTP complex from βγ and the receptor. The α subunit–GTP complex and βγ dimer transmit the signal until GTP is hydrolyzed, which allows the α subunit–GDP complex to bind and inactivate βγ. Bacterial toxins and mutations cause diseases in humans by interfering with regulation of this cycle. P_i denotes inorganic phosphate.
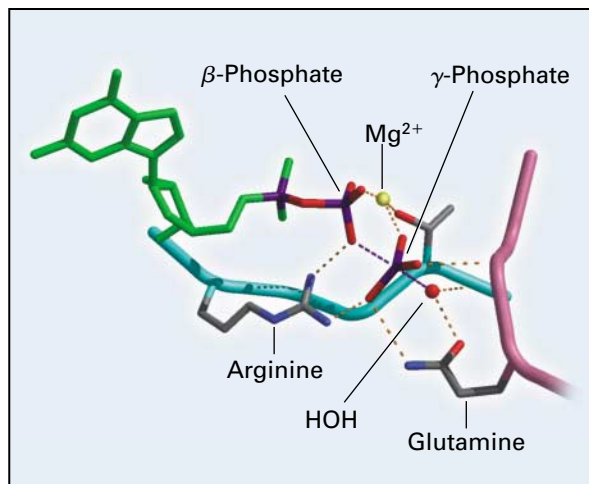
Figure 3. Hydrolysis of GTP.
The likely arrangement of GTP and key amino acids in the high-energy transition state of the GTPase reaction is shown.[24] The γ-phosphate (including a central phosphorus atom and three surrounding oxygen atoms) is shown as simultaneously leaving GDP (green) and associating with a water molecule (HOH) to form inorganic phosphate. Gα promotes GTP hydrolysis by precisely stabilizing the γ-phosphate so that a straight line, perpendicular to the plane of the γ-phosphate, connects the water, the γ-phosphorus, and the oxygen leaving the β-phosphate. The arginine residue stabilizes both the β- and γ-phosphates, is the target for ADP-ribosylation by cholera toxin, and is mutated in hyperfunctioning adenomas of the pituitary and thyroid and in the McCune–Albright syndrome.[1,3,25,26] Mutation of the glutamine residue has been reported in growth hormone–secreting pituitary adenomas.[25] Atoms shown in red and dark blue are negatively and positively charged, respectively.

ing of the normal effect of melanocyte-stimulating hormone on receptors that use G$_s$ to increase cAMP synthesis in melanocytes. The pathogenesis of the bone lesions is not clear. G$_s\alpha$ activated by mutations may exert its pathogenic effect on osteoclasts or fibroblasts, mimicking the normal regulatory effects of parathyroid hormone, calcitonin, or other hormones. Patients with the McCune–Albright syndrome also have an increased incidence of arrhythmia and sudden death in infancy[32]; perhaps elevated levels of G$_s\alpha$ activity in cardiac myocytes and conducting fibers produce the same arrhythmogenic effects as β-adrenergic amines.

A few autonomously functioning thyroid adenomas harbor *gsp* mutations,[29] and *gsp*-like mutations in the gene encoding the α subunit of G$_{i2}$ have been found in a few adenomas of the adrenal cortex and endocrine tumors of the ovary.[29] Other Gα subunits activated by mutations can trigger neoplastic transformation of cultured cells but have not been found in tumors in humans.[33] The study of transgenic mice that overexpress normal or mutationally activated Gα subunits in specific tissues may prove useful in

predicting Gα diseases in humans.[14,15,22] For example, overexpression of G$_q\alpha$ in the heart results in severe cardiac hypertrophy and subsequent congestive heart failure.[22]

Regulators of GTP Hydrolysis

Diseases are likely to result from mutations in genes encoding the recently discovered RGS (regulators of G protein signaling) proteins.[34] Members of this large protein family (encoded by 16 or more genes) accelerate the hydrolysis of GTP by G$_i$, G$_t$, G$_q$, and G$_{13}$, thereby terminating signals in milliseconds rather than seconds.[34,35] RGS proteins bind to Gα and stabilize the arginine and glutamine fingers in the active site of GTPase.[36]

The G proteins regulated by RGS proteins mediate fast physiologic responses, including vagal slowing of the heart rate (G$_i$), retinal detection of photons (G$_t$), and contraction of vascular smooth muscle (G$_q$). The RGS proteins probably play key parts in terminating the intracellular signals that mediate each of these responses.[34] Thus, mutations affecting genes that encode RGS proteins may be involved in diseases associated with gain of function, which affect vision, cardiovascular regulation, and other functions.

GENETIC INACTIVATION OF G PROTEIN SUBUNITS

Pseudohypoparathyroidism Type I

Pseudohypoparathyroidism type I is caused by genetic loss of G$_s\alpha$, the α subunit of G$_s$. The clinical features resemble those of hypoparathyroidism but are caused by inherited resistance of target tissues to parathyroid hormone rather than by lack of the hormone.[37] The inactivation of one allele of the G$_s\alpha$ gene causes the best-understood form of this disease, pseudohypoparathyroidism type Ia (Table 1). It is characterized by resistance to the effects of parathyroid hormone and sometimes thyrotropin and other hormones that use G$_s$ and adenylyl cyclase to generate cAMP as a second messenger in cells. Affected patients also have a distinctive habitus — short stature, round head, and short fingers and toes (brachydactyly) — called Albright's hereditary osteodystrophy.[37] The activity of erythrocyte G$_s$ is reduced by 50 percent in patients with pseudohypoparathyroidism type Ia.[38,39] All patients are heterozygous, with one normal G$_s\alpha$ allele; the mutant alleles lead to production of inactive G$_s\alpha$ or to small amounts of active G$_s\alpha$.[37]

The dominant pattern of inheritance of pseudohypoparathyroidism type Ia and the variable severity and diversity of its clinical manifestations are puzzling. Dominant inheritance has been attributed to so-called haploinsufficiency of the G$_s\alpha$ gene, meaning that the protein produced by a single normal G$_s\alpha$ allele cannot support normal function, although it may suffice for survival. (Other haploinsufficient genes

are those encoding the low-density lipoprotein receptor and the calcium-sensing receptor.[40,41]) With the exceptions of the responses to parathyroid hormone and thyrotropin, most responses to hormones that depend on cAMP synthesis (responses, for instance, to corticotropin, glucagon, or β-adrenergic amines) are clinically unaffected in pseudohypoparathyroidism type I. For the responses of the latter hormones, 50 percent of the normal complement of $G_s\alpha$ suffices. The inference that haploinsufficiency of $G_s\alpha$ is tissue-specific explains the selective resistance to hormones and the characteristic habitus of patients with pseudohypoparathyroidism type Ia.

Tissue-specific haploinsufficiency can explain only part of the variability, however. In a single family, some patients with a defective $G_s\alpha$ gene have resistance to parathyroid hormone, whereas others share with them the habitus of Albright's hereditary osteodystrophy but are not resistant to parathyroid hormone; the latter are said to have pseudopseudohypoparathyroidism.[37] An analysis of pedigrees of families that included patients with either pseudohypoparathyroidism or pseudopseudohypoparathyroidism revealed that 60 offspring who had inherited the defective gene from the mother had pseudohypoparathyroidism type Ia, whereas 6 offspring who had inherited the gene from the father had pseudopseudohypoparathyroidism.[42] This striking pattern suggests that the paternal $G_s\alpha$ gene is normally not expressed, owing to genomic imprinting[43]; that is, the modification of paternally inherited DNA sequences in the fertilized egg selectively prevents expression of $G_s\alpha$ encoded by the paternal gene in the progeny. Thus, patients who inherit the defective gene from the father have pseudopseudohypoparathyroidism because the mutant gene is not expressed and because the product of a single maternally inherited $G_s\alpha$ gene suffices for normal responses to parathyroid hormone and thyrotropin. This pattern of imprinting has been found in heterozygous transgenic knockout mice that were carrying one normal and one defective $G_s\alpha$ gene (Table 2): only mice that had inherited the defective gene from the mother were resistant to parathyroid hormone.[13]

The occurrence of Albright's hereditary osteodystrophy in patients with pseudopseudohypoparathyroidism, however, indicates that one $G_s\alpha$ gene is not sufficient in all tissues. This paradox can be explained only if imprinting alters the expression of genes in a cell-specific fashion in the progeny, as has been described for several other genes.[44] Thus, the different phenotypes probably result from tissue-specific combinations of haploinsufficiency and paternal imprinting (exclusive expression of the maternally inherited $G_s\alpha$ gene). The functional defects found only in patients with pseudohypoparathyroidism type Ia depend on G_s activity in cells in which the $G_s\alpha$ gene is subject to paternal imprinting but

haplosufficient (one allele normally suffices, for instance, in parathyroid hormone–responsive cells of the kidney). The phenotype of Albright's hereditary osteodystrophy results from deficient signaling function in cells in which the $G_s\alpha$ gene is haploinsufficient but not imprinted (both alleles are normally expressed). Finally, cAMP-dependent functions that are not affected in either pseudohypoparathyroidism type Ia or pseudopseudohypoparathyroidism are performed by cells in which the $G_s\alpha$ gene is haplosufficient and not imprinted.

Other Potential Mutations in G Proteins

Patients with pseudohypoparathyroidism type Ib have hypocalcemia and hyperphosphatemia as a result of resistance to the renal actions of parathyroid hormone (Table 1).[37] They lack the habitus of patients with Albright's hereditary osteodystrophy, however, and have no resistance to thyrotropin or hormones other than parathyroid hormone. This disorder is inherited as an autosomal dominant trait, but mutations have not been found in the $G_s\alpha$ or parathyroid hormone–receptor genes[37,45]; the mutant gene has not been identified. A recent study that involved gene mapping raised the provocative possibility that the defect in patients with pseudohypoparathyroidism type Ib alters tissue-specific expression of $G_s\alpha$. In four kindreds, the unknown gene was paternally imprinted, like $G_s\alpha$, and was mapped to a small region of chromosome 20q13.3, very near (but possibly separate from) the $G_s\alpha$ gene itself.[45] The simplest interpretation of these results is that patients with pseudohypoparathyroidism type Ib inherit a mutant promoter or enhancer that has lost the ability to support expression of $G_s\alpha$ in the kidney but not in other tissues.

Affected patients in a family with dominantly inherited congenital night blindness were reported to carry a point mutation in the gene for $G_t\alpha$, which mediates rod-cell responses to photons; the biochemical mechanism is unknown.[46] Otherwise, $G_s\alpha$ is the only Gα known to be genetically inactivated in a human disease. This may be explained by the phenotypes of transgenic mice that lack specific Gα genes, so-called Gα-knockout mice (Table 2). In accord with the disease in humans, knockout of the $G_s\alpha$ gene results in resistance to parathyroid hormone and evidence of paternal imprinting of the gene. In contrast, knockout of other Gα genes — $G_{i2}\alpha$, $G_o\alpha$, $G_q\alpha$, or $G_{13}\alpha$ — results in abnormalities only in animals with homozygous knockout; heterozygotes are normal (Table 2). This striking discrepancy probably reflects the fact that $G_s\alpha$ is the only member of its subfamily expressed in most cells; most cells usually express several different members of each of the other subfamilies. This functional redundancy is unlikely to be complete in every tissue; therefore, inactivating mutations in $G_i\alpha$, $G_q\alpha$, and

other human genes may contribute to subtle, tissue-specific regulatory dysfunction in homozygotes and perhaps even in heterozygotes. The phenotypes of homozygous knockouts in mice suggest that $G\alpha$ mutations may be found in inherited disorders affecting the gastrointestinal tract, heart, brain, platelets, and blood vessels (Table 2).[16-20,23] Other disease-causing mutations will surely be found in genes encoding the $G\beta$ and $G\gamma$ subunits.

ABNORMAL INITIATION OF THE G PROTEIN SIGNAL

Pertussis

Pertussis, a common infectious disease known as whooping cough, is caused by *Bordetella pertussis* organisms living in the tracheobronchial tree. The infection causes a paroxysmal cough that can persist for weeks and may be complicated by secondary infection and death. The principal pathogenic toxin of *B. pertussis*, like that of cholera, is an enzyme that catalyzes attachment of ADP-ribose to the side chain of a specific amino acid. In this case the amino acid is a cysteine located in the C-terminal tails of $G\alpha$ subunits belonging to the $G_i\alpha$ family. The attachment of ADP-ribose in this region (Fig. 1A) profoundly reduces responsiveness to receptor activation, because the C terminal of $G\alpha$ interacts directly with hormone receptors.[5,6]

Knowledge of the molecular mechanism of the toxin has not yet led to understanding of the cellular pathogenesis of pertussis. The pulmonary symptoms have not been traced to impaired activation of any of the many effectors regulated by the toxin's targets, the G_i proteins (Table 2). The molecular mechanism, however, probably does explain the persistence of the paroxysmal cough long after *B. pertussis* is gone from the respiratory tract; this persistence probably reflects the irreversibility of toxin-catalyzed covalent modification and a slow turnover of key target cells in the respiratory epithelium (in contrast to the rapid turnover of the intestinal epithelium exposed to cholera toxin).

Specifically Impaired Activation of $G_s\alpha$ in Pseudohypoparathyroidism Type Ia

Two point mutations of the $G_s\alpha$ gene, found in different families with pseudohypoparathyroidism type Ia, result in the production of normal amounts of defective signaling proteins.[9,10] The substitution of histidine for arginine at position 385 in the C-terminal tail of $G_s\alpha$ (Fig. 1A) selectively prevents G_s from being stimulated by receptors.[9] The substitution of histidine for a different arginine (at position 231) (Fig. 1A) also prevents $G_s\alpha$ from responding to receptor stimulation,[10,11] but by a different mechanism. In the latter case, a salt bridge[47,48] between the arginine and another residue normally serves as an intramolecular hasp, locking GTP into

its binding pocket and enabling the $G\alpha$–GTP complex to dissociate from $G\beta\gamma$.[10] By breaking the hasp, the Arg231His mutation prevents receptor-induced binding of GTP and causes pseudohypoparathyroidism type Ia.

The commonly used biochemical reconstitution assay for G_s fails to detect G_s deficiency in 10 to 20 percent of families that have the full clinical phenotype of pseudohypoparathyroidism type I.[10,49] As with cases involving the Arg231His mutation, many of these cases may reflect the relative insensitivity of the reconstitution assay to specific impairment of receptor activation.[50]

Testotoxicosis with Pseudohypoparathyroidism Type Ia

Testotoxicosis — autonomous production of testosterone — results in precocious puberty in boys. It is usually caused by a mutation involving a gain of function in the receptor for luteinizing hormone[51]; this mutation stimulates G_s, resulting in excess production of testosterone by testicular Leydig cells. Because patients with pseudohypoparathyroidism type Ia may have resistance to luteinizing hormone and primary testicular insufficiency,[52] it was surprising to find testotoxicosis associated with pseudohypoparathyroidism type Ia in two unrelated boys.[12] In both boys the disorder resulted from identical point mutations in the guanine nucleotide-binding pocket of $G_s\alpha$ (at position 366) (Fig. 1A), which caused simultaneous loss and gain of $G_s\alpha$ function. Pseudohypoparathyroidism type Ia, characterized by a loss of $G_s\alpha$ function, results from thermal inactivation of the mutant protein at body temperature. Testotoxicosis, a gain of $G_s\alpha$ function, results from accelerated (receptor-independent) release of GDP and consequent activation by binding of GTP; the lower temperature of the testis (34°C) protects the mutant protein from thermal inactivation.

Essential Hypertension

A mutation in the gene encoding a β subunit of the G protein may be pathogenic in some patients with essential hypertension.[2] The detection of increased G_i-dependent signaling in cells cultured from patients with hypertension led to the discovery of an apparent mutation involving a gain of function in the gene encoding the $\beta3$ member of the $G\beta$ family.[2,53] The mutation results in aberrant splicing of $\beta3$ messenger RNA and the production of $G\beta3$-s, a short protein that lacks 41 residues in the middle of the amino acid sequence (Fig. 1B); $G\beta3$-s is found in platelets and cultured cells of affected patients.[2] Although the presence of $G\beta3$-s in cultured cells correlates well with increased G_i-dependent hormone responses, the underlying molecular mechanism is not known.[54]

In a case–control study with more than 400 subjects in each group, the $G\beta3$-s allele was found in 53

percent of patients with hypertension and 44 percent of normotensive subjects.[2] If this small difference is confirmed, it suggests that the mutation could account for the elevated blood pressure of 15 percent of the patients with hypertension[54]; in this case, Gβ3-s would represent a balanced polymorphism whose selective advantage in evolution is balanced by the deleterious effect of hypertension.

CONCLUSIONS

Because G proteins play key parts in regulation, genetic analysis is likely to reveal additional mutations in G proteins that cause disease in humans. Some of these mutations will cause rare diseases, like pseudohypoparathyroidism type I or acromegaly, but they will nonetheless teach us lessons about basic regulatory mechanisms. In other cases, such as the β3 mutation in hypertension, balanced polymorphisms may be found to contribute to other common chronic disorders.

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