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GENETIC INVESTIGATION OF ADENYLATE CYCLASE: MUTATIONS IN MOUSE AND MAN

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INTRODUCTION

As a pivotal control point in cardiovascular, neural, endocrine, and metabolic regulation, hormone-sensitive adenylate cyclase serves as the target for drugs used in treating disorders of almost every organ system. How do drugs and hormones stimulate adenylate cyclase? The methods of molecular pharmacology and biochemistry have provided most of the experimental facts: Radioligand binding studies have allowed characterization of the molecular properties of membrane receptors and established that receptors are physically separable from the catalytic unit that synthesizes cyclic adenosine 3',5'-monophosphate (cAMP) from ATP (1–5). Purification of plasma membranes has allowed precise kinetic studies of regulation of cAMP synthesis, and has led to the discovery that guanine nucleotides regulate both cAMP synthesis and binding of agonists to receptors (6–10).

For detailed accounts of the biochemical characterization of adenylate cyclase and possible interactions of its components in membranes, we refer the reader to several recent reviews (11–13). Here we review results of an experimental approach, based on the concepts and techniques of somatic genetics, that has contributed significantly to our current understanding of adenylate cyclase. This approach has helped to define a membrane protein,

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hereafter called N,[1] that is required for functional coupling of drug and hormone receptors with catalytic adenylate cyclase. Quite recently the genetic approach has also provided evidence that the N protein is the target of a mutation that produces an inherited human disease, pseudohypoparathyroidism (18–20).

The mutant phenotypes we describe—both those isolated in tissue culture and those that occur in man—have elucidated the actions of drugs and hormones that work via cAMP as a second messenger. They also may provide useful hints for investigation of a variety of specific pharmacological problems that involve transduction of chemical messages across cell membranes. We hope that pharmacologists concerned with quite different problems will find it useful to review examples of the deliberate isolation of mutant cells for their potential value in understanding the actions of drugs.

N DEFICIENCY IN CYC⁻ S49 CELLS

Because the S49 mouse lymphoma cell dies upon exposure to glucocorticoids, cAMP, and hormones that stimulate cAMP synthesis, it is possible to isolate mutant S49 clones resistant to each of these agents. S49 mutants resistant to killing by glucocorticoids or cAMP have extended our knowledge of glucocorticoid receptors and cAMP-dependent protein kinase (21, 22). In this review we focus on S49 mutants with lesions that affect adenylate cyclase. These mutants were isolated by virtue of their resistance to killing by agents that stimulate cAMP synthesis in S49 cells, including β-adrenergic amines, prostaglandin-E_1 (PGE_1), and cholera toxin.

The first such S49 mutant, *cyc*⁻, was isolated by clonal selection in soft agar containing isoproterenol and the phosphodiesterase inhibitor RO 20–1724 (23).[2] Adenylate cyclase in *cyc*⁻ membranes fails to respond to five effectors that stimulate adenylate cyclase in the parental (wild-type) S49 line: β-adrenergic amines, PGE_1, cholera toxin, guanine nucleotides such as guanylyl-5'-imidodiphosphate [Gpp(NH)p] and guanosine-5'-0-(3-thio triphosphate) (GTPγS), and fluoride ion. *cyc*⁻ membranes have a normal

[1]We and others have called this protein N (11, 14). Other laboratories have referred to it as G (15), to indicate that it binds guanine nucleotides, or G/F (16, 17), and that it mediates stimulation of adenylate cyclase by guanine nucleotides and fluoride ion. All these names refer to the same protein.

[2]S49 clones that resulted from this selection were initially called *variants,* because evidence that they arose by mutation was lacking. Subsequent studies (24) indicate that these heritable phenotypes are stable, that they arise randomly in normal (wild-type) S49 populations, to indicate that their frequency is sharply increased by chemical mutagens. We therefore call them *mutants,* although rigorous proof that they are caused by structural alterations in DNA is still not available.

complement of β-adrenergic receptors, detected by radioligand binding (25), and also contain Mn^{2+}-stimulable adenylate cyclase activity similar to that seen in wild-type membranes (17, 26).

Elegant work by Ross, Gilman, and colleagues (16, 17, 27) has shown that *cyc*⁻ cells are not deficient in catalytic adenylate cyclase, but instead that *cyc*⁻ functionally lacks a third component, distinct from receptors (R) and catalytic cyclase (C). These workers mixed detergent extracts of wild-type S49 membranes with intact *cyc*⁻ membranes and succeeded in assembling an adenylate cyclase that was responsive to stimulation by hormone, guanine nucleotides, and fluoride ion. In this mixture *cyc*⁻ provides both the β-adrenergic receptors and the catalytic adenylate cyclase, as shown by experiments in which isoproterenol-sensitive adenylate cyclase could be assembled by mixing *cyc*⁻ with extracts of membranes that lacked either β-adrenergic receptors or catalytic cyclase. These membrane extracts provide the cyclase component missing in *cyc*⁻, now known to be the N protein.

Two procedures have been particularly useful in characterizing the N protein: (*a*) the in vitro complementation procedure devised by Ross and Gilman, which uses *cyc*⁻ as a "bioassay" for N protein's biochemical activity and (*b*) the use of cholera toxin and radioactive NAD to radiolabel peptide subunits of N. Cholera toxin stimulates adenylate cyclase by catalyzing transfer of ADP-ribose from NAD to the N protein, thereby increasing N's ability to stimulate cAMP synthesis by C in the presence of GTP (for details, see references 28–32). *cyc*⁻ membranes lack the 42,000 and 52,000 dalton peptide substrates of cholera toxin that are radiolabeled in wild-type S49 membrane incubated with toxin and ^{32}P-NAD (32).

Molecular properties of the N protein, whether derived from wild-type S49 membranes (33) or from human erythrocyte membranes (34), are remarkably similar: When solubilized in detergent, the protein is somewhat elongated and migrates as a particle with a molecular weight of about 126,000–130,000. N in the human erythrocyte is an intrinsic membrane protein oriented toward the cytoplasm (34, 35). Because only a small proportion of the N molecule's surface binds detergent in solution, it is likely that the protein's hydrophobic "stalk," which attaches it to the membrane, is similarly small in proportion to the fraction of the protein that protrudes into the cytoplasm (34). N is an oligomeric protein composed of at least two dissimilar subunits, although the stoichiometric relation of these subunits to one another is not yet clear. One subunit is the 42,000 dalton substrate of cholera toxin, present in both human erythrocytes and wild-type S49 membranes; the 52,000 dalton toxin substrate is not seen in human erythrocytes. Gilman's laboratory has recently reported purification of an N protein from rabbit liver: In addition to the two toxin substrate peptides,

this protein contains a 35,000 dalton subunit that is not a toxin substrate (36).

FUNCTIONS OF N

Several functions of the N protein can be inferred from comparison of the phenotype of wild-type S49 to that of the N-deficient *cyc*$^-$ mutant:

1. *Role of N in stimulation of adenylate cyclase by guanine nucleotides, cholera toxin, and fluoride ion, as well as by hormone-receptor complexes* All of these stimuli are ineffective in *cyc*$^-$, but may be rendered effective if exogenous N is added to *cyc*$^-$. N contains the guanine nucleotide binding site of adenylate cyclase, as can be inferred from the fact that N is protected by guanine nucleotides from thermal inactivation (17). In addition, Pfeuffer's laboratory has isolated an N-like protein from pigeon erythrocyte membranes by virtue of its binding to a guanine nucleotide affinity column. This protein contains a 42,000 dalton peptide that is ADP-ribosylated by cholera toxin (29). When the pigeon erythrocyte N protein is added to partially purified catalytic cyclase (C) prepared from the same membrane source, N and C associate in the presence of GTPγS, and C's adenylate cyclase activity is stimulated (15).

2. *Regulation of receptor affinity for agonists* Guanine nucleotides decrease the affinity of β-adrenergic receptors for binding agonists in many cell types, including wild-type S49. In contrast, β-adrenergic receptors of *cyc*$^-$ exhibit a low affinity for agonists in the presence or absence of guanine nucleotides (9). This effect of guanine nucleotides in wild-type cell membranes appears to be mediated through N, which binds to receptors (R) in the plane of the membrane; thus hormonal agonists and guanine nucleotides may be thought of as heterotropically displacing each other from binding to an RN complex (12). Limbird, Lefkowitz and colleagues have presented evidence consistent with this notion, obtained in experiments on β-receptors in frog erythrocytes and rat reticulocytes (37). They suggest that R occupied by agonist binds to N (detected by virtue of toxin-catalyzed radiolabeling of the 42,000 dalton N peptide) and that guanine nucleotides decrease the affinity of RN for agonists.

3. *Down-regulation of receptors* Exposure of wild-type S49 cells to isoproterenol causes a reduction in responsiveness of the cells to a second exposure to agonist. This agonist-induced refractoriness is associated with a reduction in the numbers of β-adrenergic receptors detectable by binding radioactive antagonist. Because this "down-regulation" of receptor number does not occur in *cyc*$^-$, it is likely that N is involved in the mechanism of down-regulation in wild-type S49 (38).

4. *Mg^{2+} transport* Isoproterenol decreases the rate of accumulation of $^{28}Mg^{2+}$ in wild-type S49 cells and in mutants deficient in cAMP-dependent

protein kinase, but not in *cyc*⁻ (39, 40). This suggests that N mediates regulation of Mg^{2+} transport by β-adrenergic agonists. It is intriguing to note that Mg^{2+} increases affinity of β-adrenergic receptors for agonists in wild-type but not in *cyc*⁻ membranes (41); perhaps this effect is due to promotion by Mg^{2+} of association of R and N molecules. No clear relation between the effects of Mg^{2+} on receptor affinity and the effects of agonists on Mg^{2+} uptake has been defined.

Finally, it should be noted that guanine nucleotides regulate the affinity of α-adrenergic (42) and opiate (43) receptors for binding agonists. These receptors mediate inhibition rather than stimulation of adenylate cyclase in some membrane systems. We may speculate that the effects of guanine nucleotides on agonist affinity of these receptors are mediated by N or an N-like protein. It has not been possible to explore this notion in the S49 system, because wild-type S49 cells do not exhibit obvious effects of α-adrenergic agonists or opiates on cAMP synthesis.

OTHER S49 MUTANTS

In addition to *cyc*⁻, five other mutant phenotypes (summarized in Table 1) have been selected by virtue of their resistance to killing by agents that stimulate cAMP accumulation in wild-type cells. We briefly discuss the

Table 1 S49 phenotypes with aberrant cAMP metabolism[a]

Characteristics	Wild type (9)	*cyc*⁻ (16, 17, 23, 26)	β_d (44)	*unc* (45)	N^{par} (24)	H21a (24)	K30a (24)
cAMP accumulation, stimulated by:							
β-Adrenergic agonist	+	–	↓↓	–	–	–	–
Cholera toxin	+	–	+	+	↓↓	–	↓↓
Adenylate cyclase, stimulated by:							
β-Adrenergic agonist	+	–	↓↓	–	↓	–	+
PGE_1	+	–	+	–		–	
Guanine nucleotides, NaF, cholera toxin	+	–	+	+	↓	–	+
Mn^{2+}	+	+	+	+	+	+	+
β-Adrenergic receptors:							
Normal number	+	+	↓↓	+	?	?	?
Regulation by guanine nucleotides	+	–	+	–	?	?	?
N activity:							
Complementation of *cyc*⁻	+	–		–/+	↓	–	+
Peptides ADP-ribosylated by cholera toxin	+	–		+	↓↓	+	+

[a] Numbers in parentheses refer to references.

phenotypes of each, with reference to their possible usefulness in understanding the mode of action of drugs and hormones that work via cAMP.

β_d Mutants

The isolation of *cyc*$^-$ clones resulted from a selection procedure that was in fact designed to isolate S49 cells deficient in β-adrenergic receptors. Because the *cyc*$^-$ phenotype occurs quite frequently, however, it was necessary to screen large numbers of clones resistant to killing by a β-adrenergic agonists before clones specifically deficient in β-adrenergic receptors were found (44).

Adenylate cyclase in β_d membranes responds normally to stimulation by PGE_1, cholera toxin, guanine nucleotides, and NaF. By the criterion of specific binding of [^{125}I]HYP, a β-adrenergic antagonist, β_d membranes exhibit a 80–90% decrease in β-adrenergic receptors.

Wild-type S49 cells exhibit a phenomenon often considered to suggest the existence of "spare" receptors: Isoproterenol stimulates adenylate cyclase maximally at a concentration well below that required to produce maximal occupation of β-adrenergic receptor sites (9). One interpretation of these observations is that only a small proportion of the β-adrenergic receptors are necessary for maximal stimulation of cAMP synthesis. The β_d phenotype directly contradicts this interpretation: The 80–90% decrease in number of β-adrenergic receptors is associated with a similar 80–90% decrease in maximal stimulation of cAMP synthesis by β-agonists. This implies that all or most of the β-adrenergic receptors in wild-type S49 cells are required for maximal responsiveness to β-adrenergic agonists. Because the concept of spare receptors does not account for the discrepancy between degree of receptor occupation by agonist and the degree of stimulation of cAMP synthesis, another explanation must be sought (44).

Unc Mutant

Haga et al (45) isolated an S49 mutant in which hormone receptors appear functionally uncoupled from N and from adenylate cyclase. *Unc* membranes exhibit a normal (wild-type) number of β-adrenergic receptors, and an adenylate cyclase that can be stimulated by cholera toxin, guanine nucleotides, and fluoride ion. However, β-adrenergic agonists and PGE_1 cause very little stimulation of cAMP synthesis in *unc* cells or membranes. β-adrenergic receptors in *unc* are uncoupled from N in another way: As in *cyc*$^-$, β-receptors in *unc* membranes exhibit low affinity for β-agonists, and this low affinity is unaffected by guanine nucleotides.

The *unc* phenotype raised the possibility of a fourth component in hormone-sensitive adenylate cyclase, required for functional coupling of receptors to N (and therefore to C). Two sets of findings suggest this is not the case, but that instead the *unc* lesion is intrinsic to the N protein:

1. The activity in detergent extracts of normal membranes that complements the N-deficiency of *cyc*$^-$ membranes comigrates with the activity that restores hormonal responsiveness to *unc* membranes (33, 46). In fact, highly purified N protein derived from rabbit liver membranes restores hormonal responsiveness to *unc* (36). This suggests that only a normal N molecule is required to restore *unc* to normal.

2. The 42,000 and 52,000 dalton cholera toxin substrates of *unc* membranes exhibit an alteration in electrical charge with respect to toxin substrates of wild-type membranes (47). Thus, peptide subunits of N in *unc* differ structurally from wild type.

These findings are compatible with two explanations for the functional abnormality of N in *unc* membranes: 1. The toxin substrate peptides could be products of a mutation that substituted an amino acid with a different electrical charge from the corresponding amino acid in wild type. 2. *unc* cells could lack the capacity for covalent modification of normal precursors of these peptides, by phosphorylation, methylation, or some other enzymatic process that results in altered charge of proteins.

Although neither possibility can be ruled out on the basis of available data, we favor the notion that the N peptides of *unc* are coded by a mutant gene. This is because hybrid cells formed by fusion of an *unc* and a *cyc*$^-$ parent express the *unc* rather than the wild-type phenotype (26). This result would not be expected if *unc* cells lacked a capacity for covalent modification of N. A simple interpretation of the N-deficient *cyc*$^-$ phenotype suggests that *cyc*$^-$ should possess the enzyme(s) necessary for covalent modification of N; if so, these enzymes should be able to produce normally modified N in *unc* X *cyc*$^-$ hybrid cells, and the hybrid cells would then be able to synthesize cAMP in response to hormones. If the covalent modification hypothesis is correct, *cyc*$^-$ cells will have to lack not only N activity, but also the putative modifying enzyme(s) lacking in *unc*.

Salomon & Bourne recently isolated three novel mutant S49 phenotypes, by selecting cholera toxin–resistant clones from independent wild-type sublines (24). The phenotypes of these mutants are not yet as well characterized as are the β_d, *cyc*$^-$, and *unc* phenotypes. We describe them briefly.

N^{par} *Mutants*

Membranes of *N*^{par} cells exhibit about 25% of the adenylate cyclase activity observed in wild-type cells, regardless of the effector used (hormone, guanine nucleotide, NaF, etc). Similarly, N activity in extracts of *N*^{par} membranes (assayed by its ability to complement the defect of *cyc*$^-$) is about 25% of that in wild-type extracts. N peptides in *N*^{par} membranes show only slight radiolabeling, in comparison to wild type, upon incubation with cholera toxin and ^{32}P-NAD.

We suspect that the N protein of *N*^{par} cells is qualitatively abnormal,

because extracts of N^{par} inhibit the *cyc*$^-$-complementing activity of wild-type extracts (24). Other explanations have not been ruled out, however.

H21a Mutant

Effectors that stimulate cAMP synthesis in wild-type S49 have little effect on H21a cells or membranes. In addition, extracts of H21a membranes do not complement the N deficiency of *cyc*$^-$ in vitro, which suggests that N in H21a is inactive. In contrast with *cyc*$^-$, however, H21a membranes contain 42,000 and 52,000 dalton peptides that are ADP-ribosylated by cholera toxin to an extent similar to that seen in wild-type membranes (24).

It is likely that the H21a lesion is due to a structural change in one of the subunits of N,[3] which causes functional "uncoupling" of N from the catalytic (C) unit of adenylate cyclase. It will be interesting to determine whether N in H21a membranes is "uncoupled" from receptors as well, by asking whether guanine nucleotides regulate the affinity of β-adrenergic receptors for binding agonists. As is the case with *unc,* the N lesion in H21a could be due to a structural change in the gene for one of the peptide subunits of N, or to altered post-translational modification of N.

K30a Mutant

Although K30a cells are resistant to both the killing and cAMP-elevating effects of cholera toxin, K30a membranes exhibit normal adenylate cyclase activity in response to all effectors, including the toxin. N in K30a membrane extracts complements *cyc*$^-$ normally, and its 42,000 and 52,000 dalton substrates are ADP-ribosylated normally by the toxin. The failure of intact K30a cells to accumulate cAMP upon treatment with cholera toxin and other effectors that stimulate cAMP synthesis appears to be due to increased capacity of K30a cells to degrade cAMP. Phosphodiesterase activity in K30a cells is four times higher than in wild type (24). Further investigation will be required to determine the molecular basis of this increase in phosphodiesterase activity.

PSEUDOHYPOPARATHYROIDISM

The demonstration that human erythrocytes contain a readily detectable N protein (48) provided an opportunity to search for mutations affecting the N protein that might cause inherited disease in man. Two laboratories have now reported (18–20) that N activity is reduced in erythrocytes of patients with one such disease, pseudohypoparathyroidism (PHP).

[3]It is unlikely that H21a membranes contain a normal N protein but lack a putative additional component required for complementing *cyc*$^-$, because purified N protein completely restores responsiveness of *cyc*$^-$ to all effectors (36).

In 1942 Fuller Albright and his colleagues (49) reported the first cases of PHP, and presented evidence that "the cause of the disturbance in each patient is the failure of the organism to respond to parathyroid hormone." PHP was the first endocrine disorder shown to be caused by primary resistance to a hormone, rather than by excess or deficit of the hormone.

Patients with PHP suffer from convulsions, tetany, and other symptoms of neuromuscular irritability associated with hypocalcemia. These symptoms are seen also in patients deficient in parathyroid hormone (PTH), owing to idiopathic or surgical hypoparathyroidism (HP). Many PHP patients exhibit a characteristic habitus that is quite rare in HP: They have short stature, short necks, round faces, and stubby fingers and toes (brachydactyly).

PHP can be diagnosed by showing that administration of PTH fails to raise serum calcium, lower serum phosphate, or stimulate urinary excretion of phosphate; PTH produces all of these effects in HP patients and in normal subjects. Serum PTH is high in PHP, low or undetectable in HP (50). Chase, Melson & Aurbach provided the first direct biochemical evidence of resistance of a target organ to PTH in this disorder: PTH administration causes little or no increase in urinary cAMP excretion in PHP patients, in comparison with normal or HP subjects (51).

PHP patients often exhibit endocrine abnormalities that cannot be simply explained on the basis of hypocalcemia or resistance to PTH. Many are hypothyroid, with elevated serum thyrotropin (TSH) (52). Some are resistant to the urine-concentrating effect of antidiuretic hormone (ADH) (53). Some show a blunted response of prolactin release to administration of thyrotropin releasing hormone (TRH) (54). Resistance to glucagon and gonadotropins has also been reported (55). Each of these abnormalities can be attributed to deficient cAMP synthesis, on the basis of evidence that cAMP acts as an intracellular second messenger for all these hormones, as well as PTH. Because each of the hormones acts by binding to a distinct class of receptors, it is likely that PHP patients bear a generalized defect in a postreceptor component that is essential for cAMP synthesis.

For this reason, N appeared a likely site of the biochemical lesion in PHP. In fact, adenylate cyclase in a renal biopsy of one PHP patient was observed to require exogenous GTP in order to synthesize cAMP at rates comparable to those observed in renal biopsies of normal patients (56). This finding could reflect abnormal affinity for GTP of the N component of adenylate cyclase, although other explanations cannot be ruled out.

Because routine renal biopsy is impractical, and because the hormone-response defect in PHP possibly is generalized, we tested the possibility that N is deficient in erythrocyte membranes of PHP patients. We used two assays: (*a*) measurements of the ability of detergent extracts of erythrocyte membranes to complement the *cyc*$^-$ defect in vitro and (*b*) measurements

of cholera toxin–catalyzed transfer of ^{32}P-ADP-ribose from ^{32}P-NAD to the 42,000 dalton peptide subunit of erythrocyte N.

By both assays, we found that erythrocyte membranes of 15 PHP patients contained reduced N activity, averaging about 50% of the activity in erythrocytes of normal or HP subjects (18, 19). A variety of controls indicated that the deficiency of N activity was not due to hypocalcemia (18). Using in vitro complementation assays, Levine et al (20) found decreased N activity in 12 of 13 PHP patients.

Thus deficient N activity in erythrocytes is a reliable biochemical marker of PHP. N activity is also deficient in platelets of PHP patients (57). The N defect probably reflects a primary, generalized defect in hormone-sensitive adenylate cyclase that accounts for the pathophysiology of the disorder. This hypothesis is consistent with the resistance of PHP patients to PTH, TSH, ADH, TRH, and other hormones that stimulate cAMP synthesis.

PHP is biochemically and genetically heterogeneous. We have studied two families in which PHP is *not* associated with decreased erythrocyte N activities (18, 19). These families show about the same incidence of hypothyroidism and other endocrine abnormalities as are observed in patients with deficient erythrocyte N. Thus it is likely that their primary defect lies in a protein that regulates hormone-stimulated cAMP accumulation in many tissues. Increased cAMP phosphodiesterase activity, like that found in the K30a S49 cell (24) described above, could account for the phenotype of these patients. PHP could also result from a lesion in the catalytic unit of adenylate cyclase, or from a lesion in N that is not detected by the methods we have used.

In some kindreds we have studied, erythrocyte N deficiency and the PHP phenotype show a dominant pattern of inheritance (19). This pattern would be seen if affected patients inherited one normal and one abnormal allele coding for a peptide subunit of N, and expressed 50% of normal N activity in their cells.

The pedigree of one PHP family shows a contrasting pattern: The disorder of hormone resistance and a 50% deficiency of erythrocyte N activity are present in two siblings, but absent in both their parents. The mechanism that underlies apparently recessive inheritance in this family has not been elucidated.

CONCLUSIONS

This review summarizes one example of the possible contributions of genetic analysis to understanding the actions of drugs and hormones. Many results of genetic analysis, of course, serve merely to confirm conclusions already obvious on biochemical grounds: For example, we did not need the

K30a mutant to tell us that elevated cAMP phosphodiesterase activity in a cell can decrease the effects of agents that act by stimulating cAMP synthesis. The *cyc*⁻ phenotype played quite a different role, because it could not easily be explained on the basis of the then current understanding of hormone-sensitive adenylate cyclase. The elegant in vitro complementation experiments of Ross, Gilman, and colleagues (16), followed by identification of peptides labeled by cholera toxin and ^{32}P-NAD in wild-type but not in *cyc*⁻ membranes (32), led to discovery of a previously undefined molecular component of adenylate cyclase.

Other S49 mutant phenotypes are serving to extend our knowledge of the transduction of chemical signals across membranes. The β_d mutants provide a stimulating counterexample that may lead to refinements in our concepts of spare receptors. N lesions in the *unc,* H21a, and N^{par} mutants are useful tools in investigating the interactions among receptors, catalytic cyclase, and the receptor-cyclase coupling protein in membranes. The increased phosphodiesterase activity in another mutant, K30a, should open avenues for studying alternative mechanisms that may modulate transmission of intracellular messages by cAMP.

Finally, it is gratifying that discovery of N should be followed so rapidly by evidence that N is the site of the primary genetic lesion of a human disease. Genetic analysis in mouse cells was essential for defining the N protein, and mutant mouse cells provided the complementation assay used to measure N in human cells.

Clinically, defining the role of N in pseudohypoparathyroidism will not make much difference. In part, this is because the disorder is so rare. The incidence of PHP is probably less than 1 per 100,000. In addition, the altered calcium homeostasis of PHP responds readily to therapy with vitamin D, and other problems (e.g. hypothyroidism) are for the most part quite amenable to treatment. At most, erythrocyte studies in affected families may be an aid in genetic counseling, and detection of the disorder early in life may alter management of some cases.

As experiments of nature, however, human mutations may be as useful as mutations in mouse cells in elucidating the structure and function of receptors and hormone-sensitive adenylate cyclase. In addition, the PHP phenotype should help in understanding the roles of cAMP, N, and hormone receptors in endocrine regulation of normal man.

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