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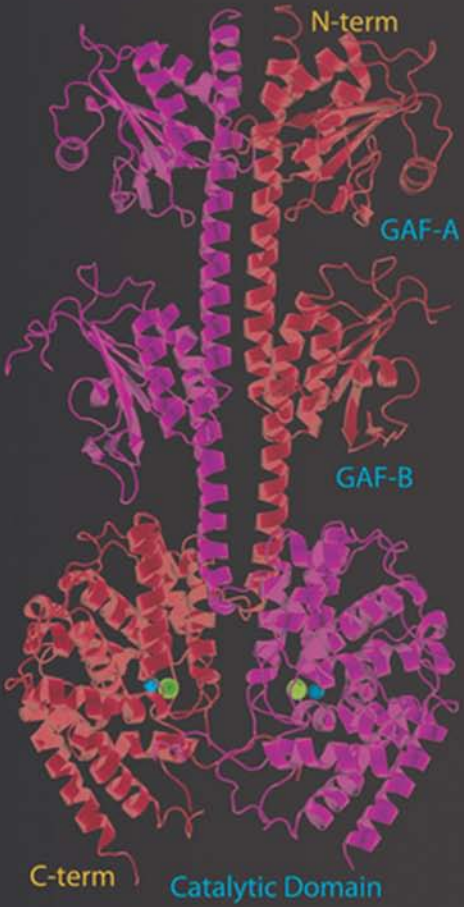
CYCLIC-NUCLEOTIDE PHOSPHODIESTERASES IN THE CENTRAL NERVOUS SYSTEM

From Biology to Drug Discovery


N-term
GAF-A
GAF-B
C-term
Catalytic Domain

Edited by

NICHOLAS J. BRANDON
ANTHONY R. WEST

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From Biology to Drug Discovery

Edited by

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PREFACE

Cyclic-nucleotide phosphodiesterases (PDEs) are critically involved in the regulation of cellular processes at work from cell birth to death. PDEs are produced by and operate within all cells of the body, and their key role in dampening or redirecting cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) signaling cascades makes them essential for cell health. In both the brain and spinal cord, PDEs show intricate patterns of cellular localization, both regionally and at the subcellular level. Such an infrastructure undoubtedly contributes to the tremendous computational power needed for the effective execution of sensorimotor, cognitive, and affective functions. On the flipside, we are entering a period of time when diseases of the central nervous system (CNS), which disrupt these essential functions, will affect more and more of us. For reasons described in this book, it is now clear that PDEs have enormous potential as targets for new medicines. In this book we have brought together the expertise of leading researchers from both basic and applied sciences to highlight the beautiful biology of the diverse superfamily of PDEs, as well as the medical potential of targeting PDEs for the treatment of disorders of the CNS. Indeed, numerous applications for small-molecule inhibitors selective for specific PDE isoforms are being investigated for the treatment of CNS diseases, including schizophrenia, depression, Alzheimer's disease, Parkinson's disease, Huntington's disease, spinal cord injury, and others. Drug discovery for disorders of the CNS is exceptionally difficult, but undoubtedly, our understanding of PDE biology and PDE-based therapeutics will continue to evolve and hopefully lead to the development of novel medicines of value for patients suffering from these devastating disorders.

We thank all of our wonderful colleagues who have contributed chapters to this book, as well as the numerous reviewers who have provided constructive criticism of its content. We hope that this work will render important insights into PDE biology and therapeutics that will inspire a new generation of researchers interested in this field.

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CHAPTER 1

PHOSPHODIESTERASES AND CYCLIC NUCLEOTIDE SIGNALING IN THE CNS

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INTRODUCTION

Discovery of PDEs, Historical Perspectives, and Progress in Understanding the Complexity of PDE Functions

Soon after the discovery of the second messenger cAMP by Sutherland and Rall [1], it was observed that cyclic nucleotides are unstable in tissue extracts. This observation paved the way for the identification of the enzymatic activities responsible for their destruction [1]. Sutherland and coworkers correctly attributed this activity to a Mg^{2+}-dependent, methylxanthine-inhibited enzyme that cleaves the cyclic nucleotide phosphodiester bond at the 3′-position, hence the name phosphodiesterase (PDE) (Figure 1.1). With the discovery of cGMP and the improvement of protein separation protocols [2], it also became apparent that multiple PDE isoforms with different affinities for cAMP and cGMP and sensitivity to inhibitors coexist in a cell (Figure 1.1). Only with the application of protein sequencing and molecular cloning techniques has it been realized that 21 genes code for PDEs in humans and that close to 100 proteins are derived from these genes, forming a highly heterogeneous superfamily of enzymes (Figures 1.1 and 1.2) [3].

Although PDEs were implicated early on in the control of intracellular levels of cAMP and cGMP and the termination of the neurotransmitter or hormonal signal, 30 additional years of research have been necessary to understand that PDEs are not simply housekeeping enzymes. The activity of PDEs is finely regulated by a myriad of regulatory loops and integrated in a complex fashion with the cyclic nucleotide signaling machinery and other signaling pathways. Blockade of PDE activity does not

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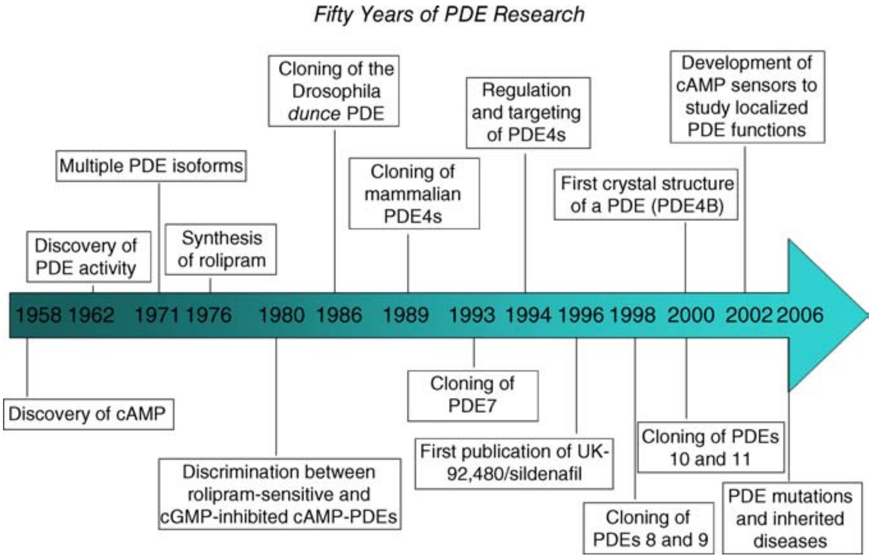
Fifty Years of PDE Research
Discovery of PDE activity
Multiple PDE isoforms
Synthesis of rolipram
Cloning of the Drosophila *dunce* PDE
Cloning of mammalian PDE4s
Regulation and targeting of PDE4s
First crystal structure of a PDE (PDE4B)
Development of cAMP sensors to study localized PDE functions
1958 1962 1971 1976 1980 1986 1989 1993 1994 1996 1998 2000 2002 2006
Discovery of cAMP
Discrimination between rolipram-sensitive and cGMP-inhibited cAMP-PDEs
Cloning of PDE7
First publication of UK-92,480/sildenafil
Cloning of PDEs 8 and 9
Cloning of PDEs 10 and 11
PDE mutations and inherited diseases

FIGURE 1.1 Timeline of the major discoveries related to the field of phosphodiesterases.

exclusively lead to an increase in cyclic nucleotides and a gain of function, as one would predict from the removal of cyclic nucleotide degradation. On the contrary, complex changes in cellular responses are associated with PDE inhibition, often causing loss of function, as documented by the phenotypes of natural mutations or engineered inactivation of the PDE genes [4–7]. These findings imply that PDEs and their regulation are indispensable to faithfully translate extracellular cues into appropriate biological responses. Indeed, in neurons as in other cells, the biological outcome of activation of a receptor is defined by the multiple dimensions of the cyclic nucleotide signal. This specificity of the response depends on the changes in concentration of the cyclic nucleotide, the time frame in which these changes occur, and the subcellular locale in which the nucleotides accumulate. Because cyclic nucleotide accumulation is dependent on the steady state of cAMP/cGMP production as well as hydrolysis, degradation by PDEs is a major determining factor of all three dimensions of the cyclic nucleotide signal.

In spite of seemingly comparable enzymatic functions, each of the several PDEs expressed within a cell appears to serve unique roles. This view is paradoxical because it implies, as fittingly summarized by L.L. Brunton, that “Not all cAMP has access to all cellular PDEs” [8]. As an extension of this concept, a PDE may play critical functions in a cell even if it represents a minor fraction of the overall hydrolytic activity, a view with considerable impact on pharmacological strategies targeting PDEs. The discovery of macromolecular complexes involving PDEs has confirmed this concept and added a new dimension to the function of these enzymes in signaling. In those complexes in which they are associated with cyclic nucleotide targets, it is likely that PDEs play an essential role in controlling or limiting the access of cyclic

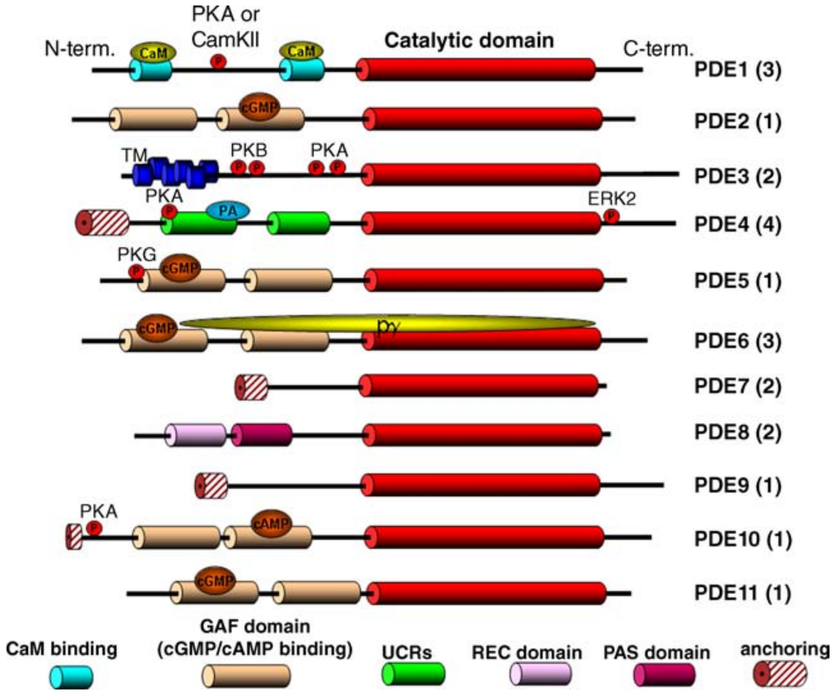
PKA or CamKII
N-term.
CaM
CaM
P
Catalytic domain
C-term.
PDE1 (3)
cGMP
PDE2 (1)
TM
PKB
PKA
PDE3 (2)
PKA
PA
ERK2
PDE4 (4)
PKG
cGMP
PDE5 (1)
cGMP
Pγ
PDE6 (3)
PDE7 (2)
PDE8 (2)
PDE9 (1)
PKA
cAMP
PDE10 (1)
cGMP
PDE11 (1)
CaM binding
GAF domain (cGMP/cAMP binding)
UCRs
REC domain
PAS domain
anchoring

FIGURE 1.2 The domain organization of the different families of phosphodiesterases. Domains are depicted as "barrels" connected by "wires" indicating linker regions. Phosphorylation sites are shown as red circles with the respective kinase phosphorylating this site listed above. PDEs are composed of a C-terminal catalytic domain (shown in red) and distinct regulatory domains at the N-terminus. These include Ca^{2+}/calmodulin (CaM)-binding sites (PDE1), GAF domains that function as cAMP or cGMP sensors (PDE2, PDE5, PDE6, PDE10, and PDE11), the UCRs that include a phosphatidic acid (PA)-binding site in PDE4, and the PAS domain (PDE8). The inhibitory gamma subunit of PDE6 is indicated as a yellow ellipse. Domains functioning as targeting sequences by mediating membrane–protein or protein–protein interactions are indicated as red striated barrels and the transmembrane (TM) domains of PDE3 are indicated in blue. The number of PDE genes belonging to each PDE family is indicated in parentheses beside the PDE family name.

nucleotides to their effectors. Since protein kinase A (PKA), protein kinase G (PKG), GTP exchange protein activated by cAMP (EPAC), and cyclic nucleotide-gated (CNG) channels are tethered to specific subcellular compartments, PDEs likely contribute to the compartmentalization of cyclic nucleotide signaling and to the spatial dimension of the signal. PDEs may also have scaffolding properties within these complexes, opening the possibility that PDEs serve functions beyond their catalytic activity and that a dynamic formation and dissolution of these complexes may contribute to the allosteric regulation of PDE activities.

THE PDE SUPERFAMILY

After several more PDE genes were discovered through homology screening of nucleotide sequence databases between 1996 and 2000 (PDE8, PDE9, PDE10, and PDE11), the completion of the Human Genome Project in 2001 eventually established that there are 21 PDE genes in humans [9]. Orthologs of all 21 genes are encoded in the genomes of rats and mice and might be present in the same number in other mammals. Metazoan model organisms such as *Caenorhabditis elegans* or *Drosophila melanogaster* express orthologs of some, but usually not all of the mammalian PDEs [3]. Based upon their substrate specificities, kinetic properties, inhibitor sensitivities, and, ultimately, their sequence homology, the 21 mammalian PDE genes are subdivided into 11 PDE families, each consisting of 1 to a maximum of 4 genes (Table 1.1). Most PDE genes are expressed as a number of variants through the use of multiple promoters and alternative splicing. The PDE6 genes, with only 1 transcript per gene reported, and PDE9A, for which more than 20 putative variants have been proposed, represent the extremes in the number of variants generated from individual genes. Together, close to 100 PDE proteins are generated in mammals, each likely serving unique cellular functions.

Nomenclature

Due to the large number of PDE variants present in mammals, an initial classification based on regulatory properties and inhibitor sensitivities of newly discovered enzymes as well as their order of discovery soon became inadequate. It was subsequently replaced with a consensus nomenclature in which the first two letters indicate the species followed by the three letters “PDE”, an Arabic numeral indicating the PDE family, a letter indicating the gene within the PDE family, and finally another Arabic numeral indicating the precise PDE variant. For example, HsPDE4D3 identifies the species as *Homo sapiens*, the PDE family as 4, the gene as D, and the variant as 3. This nomenclature was widely adopted in 1994 [27]. For a complete list of PDE genes and variants as well as information regarding the nomenclature used before 1994, please see http://depts.washington.edu/pde/pde.html or Ref. [28].

Overall Protein Domain Arrangement

Despite their multitude and diversity, all PDEs share several structural and functional properties. One of the most obvious is their modular structure consisting of a relatively conserved catalytic domain located in the C-terminal half of the protein and N-terminal domains that are structurally diverse, but all function to regulate enzyme activity (Figure 1.2). The C-terminal catalytic domain contains all residues required for catalysis and determines the enzyme kinetics unique to each PDE subtype. The characteristic features of the N-terminal regulatory domains are highly conserved modules such as Ca^{2+}/calmodulin-binding domains (PDE1), GAF domains (cGMP-activated PDEs, adenylyl cyclase, and Fh1A; PDE2, PDE5, PDE6, PDE10, and PDE11), UCR domains (upstream conserved regions; PDE4),

TABLE 1.1 The Properties of the Mammalian PDE Genes

Chromosome Region[a]	Gene Symbol	Name/Aliases	Predominant CNS Distribution[b]	Disease Association[c]	References
2q32.1	*PDE1A*	Phosphodiesterase 1A, Ca^{2+}/calmodulin-dependent	Cortex	Schizophrenia	[10]
12q13	*PDE1B*	Phosphodiesterase 1B, Ca^{2+}/calmodulin-dependent	Striatum, hippocampus		
7p14	*PDE1C*	Phosphodiesterase 1C, Ca^{2+}/calmodulin-dependent	Cerebellum		
11q13.4	*PDE2A*	Phosphodiesterase 2A, cGMP-stimulated	Striatum, hippocampus, cerebellum		
12p12	*PDE3A*	Phosphodiesterase 3B, cGMP-inhibited	Striatum, hippocampus	RR and QT interval	[11]
11p15.1	*PDE3B*	Phosphodiesterase 3B, cGMP-inhibited	Hippocampus		
19p13.2	*PDE4A*	Phosphodiesterase 4A, cAMP-specific (*Drosophila dunce* homolog, DPDE2, PDE2)	Cortex, hippocampus		
1p31	*PDE4B*	Phosphodiesterase 4B, cAMP-specific (*Drosophila dunce* homolog, DPD4, PDE4)	Cortex, striatum, hippocampus	Schizophrenia, bipolar disorder, depression, alcohol responses, multiple sclerosis	[12–15]
19p13.1	*PDE4C*	Phosphodiesterase 4C, cAMP-specific (*Drosophila dunce* homolog, DPD1, PDE1)	Low level in CNS		
5q12	*PDE4D*	Phosphodiesterase 4D, cAMP-specific (*Drosophila dunce* homolog, DPD3, PDE3, STRK1)	Cortex, hippocampus, brain stem	Stroke susceptibility, asthma, sleep disorders, neuroticism, schizophrenia	[10,16–21]
4q25–q27	*PDE5A*	Phosphodiesterase 5A, cGMP-specific	Cerebellum, hippocampus		

(*continued*)

TABLE 1.1 (*Continued*)

Chromosome Region[a]	Gene Symbol	Name/Aliases	Predominant CNS Distribution[b]	Disease Association[c]	References
5q31.2–q34	*PDE6A*	Phosphodiesterase 6A, cGMP-specific, rod, alpha	Retina	Retinitis pigmentosa	[5]
4p16.3	*PDE6B*	Phosphodiesterase 6B, cGMP-specific, rod, beta	Retina	Night blindness, congenital stationary type 3	[22]
10q24	*PDE6C*	Phosphodiesterase 6C, cGMP-specific, cone, alpha prime	Retina	Cone dystrophy	[7]
8q13–q22	*PDE7A*	Phosphodiesterase 7A, HCP1	Hippocampus		
6q23–q24	*PDE7B*	Phosphodiesterase 7B	Striatum, hippocampus		
15q25.3	*PDE8A*	Phosphodiesterase 8A	Cortex	Major depressive disorder	
5q13.3	*PDE8B*	Phosphodiesterase 8B	Striatum, hippocampus	Pigmented nodular adrenocortical disease	
21q22.3	*PDE9A*	Phosphodiesterase 9A	Cortex, cerebellum	Bipolar disorder	[23,24]
1q11	*PDE10A*	Phosphodiesterase 10A	Striatum	Autism	[25]
2q31.2	*PDE11A*	Phosphodiesterase 11A	Hippocampus CA1	Pigmented nodular adrenocortical disease	[26]

[a] Shown is the chromosomal localization of the PDE genes in humans.

[b] Expression data in human based on *in situ* hybridization; where not available, data from rat and mouse brain were used.

[c] The association with inherited diseases is based on published data, GWAS (http://gwas.nih.gov/) analysis, and OMIM (http://www.ncbi.nlm.nih.gov/omim) data.

REC domains (receiver; PDE8), and PAS domains (period, aryl hydrocarbon receptor nuclear translocator (ARNT), and single minded; PDE8), as well as phosphorylation sites, which mediate the regulation of enzyme activity by posttranslational modifications and/or ligand binding. As a result of this modular structure, truncated PDEs encoding only the catalytic domain not only retain enzyme activity, but also exhibit kinetic properties and substrate specificity similar to those of the holoenzyme, whereas regulation of enzyme activity is lost or is altered compared to full-length proteins [29–31]. In most cases, the inhibitor sensitivity of the full-length proteins is also retained in the catalytic domain constructs. There are some exceptions, however. The catalytic domain of PDE4, for example, exhibits an affinity for the prototypical PDE4 inhibitor rolipram that is about 100-fold lower compared to full-length proteins, whereas the sensitivity toward structurally unrelated compounds is not affected by the truncation [30,31]. Thus, catalytic domain constructs may have limited value for the development of PDE4 inhibitors.

The regulatory domains, in turn, may function in a similarly independent manner. The GAF domains, for example, are found in PDEs, adenylyl cyclases, and the *Escherichia coli* Fh1A protein. Chimeras between the GAF domains of different PDEs (PDE5, PDE10, and PDE11) and the catalytic domain of the cyanobacterial cyaB1 adenylyl cyclase were found to be fully functional in that the PDE-GAF domains sense cyclic nucleotide levels and mediate activation of the catalytic domain of the cyclase [32,33]. This finding, together with the similar domain organization of all PDEs, led to the proposal that despite their structurally distinct N-termini, the mechanism by which the N-terminal domains regulate enzyme activity is perhaps conserved among all PDE subtypes. The functional properties of the different N-terminal regulatory domains are discussed in greater detail later in this chapter.

The unique combination of catalytic domain kinetics and regulatory domain properties defines a PDE family and is conserved among its members. The variants generated from a single PDE gene, in turn, contain with few exceptions the identical catalytic domain, but often possess variant-specific N-termini. These variants are divided into those that encode the entire regulatory module characteristic of a given PDE family, such as GAF domains or UCR domains, and those that encode only a portion of the regulatory module or lack it altogether. The latter include so-called short PDE4 variants, which contain only a portion of the UCR module, and PDE11 variants that lack GAF domains. Underlining the critical role of the regulatory domains, variants that encode the entire regulatory module exhibit similar mechanisms of regulation, whereas variants that lack part or all of the regulatory domains are either insensitive to ligand binding or posttranslational modification *per se* or respond differently (Figure 1.2) [3,34]. The extreme N-termini unique to individual PDE variants are encoded by variant-specific first exons (Figure 1.3) and often mediate subcellular targeting through protein–protein or protein–lipid interactions, thus allowing the cell to specifically target PDE variants to subcellular compartments [35].

Although the mechanisms of regulation of PDE activity have been described biochemically in great detail, structural aspects of enzyme regulation had remained elusive (see Chapter 6 for a detailed discussion). Pandit et al. [36] recently provided a

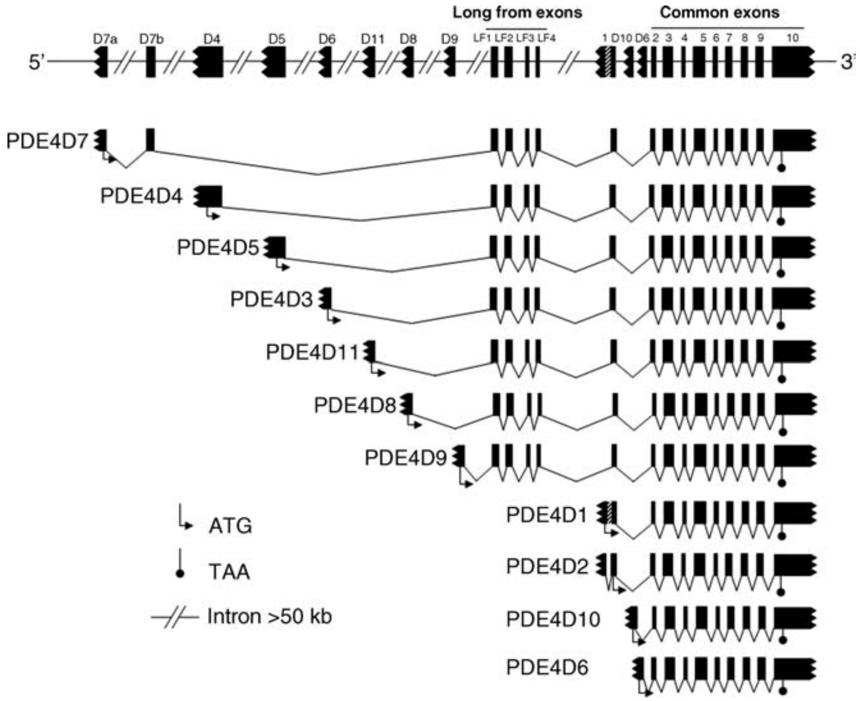

FIGURE 1.3 Structure of the PDE4D locus. Schematic representation of the structure of the mammalian PDE4D locus (top) and the encoded mRNAs. Exons are presented as filled bars, introns are drawn as solid lines, and a noncoding exon is depicted as a striated box. Indicated are variant-specific first exons, long form (LF) exons shared by all so-called long PDE4 isoforms, and common exons shared by most PDE4D variants. The scheme is not drawn to scale.

major breakthrough on the question of how modification of the N-terminal domains by posttranslational modifications and/or ligand binding exerts its effect on the conformation of the distal catalytic domain, thereby controlling PDE activity. The authors crystallized a PDE2A that, although lacking some N- and C-terminal sequence of the holoenzyme, contains the critical components of the PDE2 structure: a tandem set of GAF domains linked to the C-terminal catalytic domain. PDE2A crystallized as a linear structure that extends along a GAF-A/GAF-B/catalytic domain axis. The enzyme forms head-to-head dimers with the dimer interface spanning the entire length of the molecule with interactions between the GAF-A and GAF-A, GAF-B and GAF-B, and between the two catalytic domains of the individual monomers. When the GAF domains are unoccupied, the substrate-binding pockets of the two catalytic domains are packed against each other, essentially closing off access to the substrate. As a mechanism for the allosteric activation of PDE2, the authors propose that cGMP binding to the GAF-B domain results in a reorientation of the linker regions connecting GAF-B and the catalytic domain, which in turn leads to a disruption of the dimer interface between the two catalytic domains, thus promoting an "open" conformation of the enzyme that allows substrate access. Both the general organization of the PDE2 structure and the mechanism of PDE activation proposed by Pandit et al. [36] are in agreement with many structure–function relationships observed in other PDEs. Most PDEs have been reported to form homo- or heterodimers [29,34,37–41], and critical dimerization domains were identified in the N-terminal domains. The catalytic domains also retain some affinity as evidenced by the fact that several PDE catalytic domains form dimers in purified protein preparations as well as in crystal structures [42–44]. In addition, most PDEs also possess the elongated structure described for PDE2 as indicated by their high frictional ratios [39,45,46]. Electron microscopic images of PDE5 and PDE6 show an elongated structure highly similar to the atomic structure of PDE2, with points of contact between the GAF-As, the GAF-Bs, and the catalytic domains of the individual monomers [47,48]. Dimerization mediated by the N-terminal domains of PDE2 plays a critical role in stacking the substrate-binding sites at the catalytic domain against each other, thus preventing substrate access. This is in agreement with the observation that N-terminal domains in various PDEs exert an inhibitory constraint on the active site, which can be uncovered through deletion mutagenesis or proteolytic digest of full-length enzyme [49,50]. Taken together, these similarities suggest that the atomic structure of PDE2 might represent a model for a general organization of PDEs and a mechanism of enzyme activation.

However, Burgin et al. [51] recently suggested an alternative mechanism of how inhibitory constraint and regulation of enzyme activity is achieved in PDE4. In crystal structures of a truncated PDE4, substrate access to the catalytic site is prevented not by stacking of the catalytic domains against one another, as proposed for PDE2 [36], but by direct binding of a helix in the regulatory UCR2 domains to the substrate-binding pocket in the catalytic domain. PDE4 variants are divided into so-called long forms that contain the complete UCR1/2 module and short forms that lack UCR1 but still contain all or a portion of UCR2. The constructs crystallized by Burgin et al. lack UCR1 and encode only a portion of UCR2, thus encoding a PDE4 that resembles short forms. There are significant structural and functional differences between long and short forms including oligomerization, enzyme activation, and inhibitor sensitivity [40]. Thus, it remains to be determined whether the mechanism of enzyme inhibition/activation proposed by Burgin et al. [51] reflects properties of all PDE4 isoforms or whether this model describes properties inherent only to short PDE4 isoforms, whereas long isoforms are regulated differently. If the former is the case, PDE4 regulation of enzyme activity would be different from models described for PDE2, PDE5, and PDE6. This in turn would suggest that distinct modes of regulating PDE activity evolved for the different PDE families.

Catalytic Site Properties and Interaction with the Substrates

PDEs are divalent metal ion-dependent enzymes and share with other metal-dependent phosphohydrolases an HD(X2)H(X4)N motif, which defines residues forming the metal ion-binding site. Much progress has been made in understanding the structure and properties of the catalytic domains since crystal structures have become available in 2000 [43]. The catalytic domain consists of 16 α-helices folded into a compact structure. Whereas the sequence homology of the catalytic domains can be as low as 25% for members of different PDE families, the three-dimensional structure of the catalytic domain aligns all residues that are invariant or semiconserved among all PDEs to form the substrate-binding pocket. These residues include an invariant glutamine that forms hydrogen bonds with the 1- and/or 6-positions of the cyclic nucleotides, several residues that form a "hydrophobic clamp" that anchors the purine ring, and residues that form two metal ion-binding sites, termed M1 and M2, which are positioned at the bottom of the substrate-binding pocket. Based on biochemical data and X-ray diffraction, M1 is likely occupied by Zn^{2+}, whereas M2 is occupied by Mg^{2+} or Mn^{2+} in the native enzyme. The two metal ions function to activate the substrate phosphate and to coordinate a water molecule that acts as a nucleophile in the PDE reaction. Recent studies suggest that the water molecule coordinated by the metal ions may partially dissociate into a hydroxide ion [52–55], and that this hydroxide acts as the nucleophile on the phosphorus, eventually becoming part of the outgoing phosphate, whereas a nearby strictly conserved histidine assists with the protonation of the O3′ group.

Defining the first atomic structure of any PDE, Xu et al. [43] reported the crystal structure of the catalytic domain of PDE4B in 2000. Since then, crystal structures for the catalytic domain of most PDE families have been reported, providing further insight into the distinct mechanisms of inhibitor binding and substrate specificity [42,43,56–64].

On the basis of their substrate specificity, the 11 PDE families can be divided into three groups. PDE4, PDE7, and PDE8 selectively hydrolyze cAMP, whereas PDE5, PDE6, and PDE9 are selective for cGMP hydrolysis. The remaining PDEs (PDE1, PDE2, PDE3, PDE10, and PDE11) bind and hydrolyze both cyclic nucleotides with varying efficiency. An invariant glutamine residue that is conserved among all PDEs and that was shown to form hydrogen bonds with either AMP or GMP in crystal structures has been proposed as the major determinant of PDE substrate specificity. As both cyclic nucleotides were thought to bind to the substrate-binding pocket in the same conformation and the hydrogen-bonding character of the 1- and 6-positions of adenine and guanine is essentially reversed, a so-called glutamine switch mechanism was previously proposed to determine PDE substrate specificity [43,63]. According to this model, the amide group of the invariant glutamine can rotate by 180° to accommodate binding of either cAMP or cGMP. It was assumed that PDEs in which additional residues limit the mobility of the invariant glutamine are selective for one of the cyclic nucleotides, whereas PDEs that allow a free rotation of the invariant glutamine could bind both. However, several recent findings suggest that this cannot be the only mechanism that determines substrate specificity among PDEs. Mutation of this invariant glutamine in PDE5A, for example, did reduce affinity of the enzyme for its physiological substrate cGMP but did not enhance binding of cAMP [65]. In addition, mutation of an aspartic acid residue conserved among the cAMP-PDEs ablates the substrate specificity of PDE4 isoenzymes, suggesting that this residue represents an additional evolutionary conserved component of substrate specificity [66,67]. Most important are recent studies by Wang et al. [61,68] that demonstrate that PDE10 binds the substrates cAMP and cGMP in *syn*-conformation, whereas the PDE reaction products AMP and GMP dock in *anti*-conformation in crystal structures. This suggests that previous reports, which relied on the analysis of cocrystals of various PDEs with AMP or GMP, might not reflect the binding of substrates in the native enzyme. In addition, Wang et al. show that rotation of the invariant glutamine is unlikely the cause for the dual-substrate specificity of PDE10, as the position of this residue is locked through additional hydrogen bonds, and that PDE4 forms only one hydrogen bond with cAMP rather than two as suggested by the "glutamine switch model" [61,68]. Thus, in addition to the conserved glutamine residue, there are likely additional, perhaps PDE family-selective determinants of substrate specificity.

Properties of the Regulatory Domains

Three critical functions have been identified for the PDE sequences N-terminal to the catalytic domain. These are the regulation of enzyme catalytic activity, oligomerization, and subcellular targeting. These properties are examined in detail in the subsequent sections of this chapter.

THE PROPERTIES OF THE GENES ENCODING PDEs

Organization of the PDE Genes

A common feature of the PDE genes already present in the ancestral *dunce* PDE locus of the *D. melanogaster* is the complex arrangement of transcription start sites and exons. The *dunce* locus, coding for a PDE involved in learning, memory, and development, spans over more than 148 kb (1 kb = 10^3 bases or base pairs) of genomic DNA and includes at least 16 exons with exceedingly long introns that often contain exons of unrelated genes. This complex arrangement is further augmented in the mammalian PDE4 genes. For instance, the human *PDE4D* locus (5q12) spans more than a million base pairs with at least 25 exons and 10 different start sites (see Figure 1.3). The exons encoding the catalytic domain are clustered together, whereas the exons coding for the amino termini and the regulatory/dimerization domains (UCRs) are distributed over a large stretch of genomic DNA. Other PDE loci have similar characteristics. For instance, the *PDE10A* gene spans more than 200 kb and contains 24 exons, whereas the *PDE11A* gene covers approximately 300 kb of genomic DNA and contains 23 exons [69]. The four PDE11A variants have different amino termini due to separate promoters and transcriptional start sites [70–72]. The *PDE1A* gene spans over 120 kb and contains 17 exons, again with multiple promoters and start sites [73]. Comparison of *PDE1B1* genomic sequence with other PDE genes has indicated that two splice junctions within the region encoding the catalytic domain are conserved in rat *PDE4B* and *PDE4D*, as well as in the *Drosophila dunce*

PDE [74,75]. This commonality in the splicing junction strongly suggests that the catalytic domains of PDEs are derived from a common ancestral gene, a view further supported by the high conservation of the amino acid sequence of the catalytic domain.

PDE loci have been associated with several neurological disorders (see Table 1.1). These associations are derived from genome-wide association studies (GWAS), meta-analyses, or candidate gene approach studies. It should be noted that numerous single-nucleotide polymorphisms (SNPs) have been identified in PDE loci and that SNPs are in linkage disequilibrium in several studies of patient populations (Table 1.1). However, nonsynonymous SNPs in the coding regions are very rare. For example, the *PDE4D* gene has 1919 SNPs in introns, but none in exons.

Multiple Transcripts

The presence of multiple transcripts derived from each PDE gene was initially revealed by Northern blot analysis. For instance, multiple RNAs ranging in size from 4.2 to 9.6 kb were detected for the *Drosophila dunce* PDE [76,77]. PDE4D cDNA probes hybridize to at least three or four different transcripts ranging between 2.3 and 5 kb depending on the tissue used [78]. PDE11A is expressed as at least three major transcripts of ~10.5, ~8.5, and ~6.0 kb, thus again suggesting the existence of multiple subtypes [72]. Further studies using PCR and exon-specific primers have provided a better understanding of the exon composition of each transcript.

Most of the heterogeneity in transcript size is due to the presence of multiple transcription start sites. In general, these start sites are under the control of promoters that often function in a tissue- and cell-specific manner as described for PDE1 [79–81], PDE4 [82–84], PDE7 [85,86], and PDE9 [87]. This property provides one of several explanations for the complexity of the PDE genes, whereby different promoters have evolved to allow developmental and cell-specific expression of a given PDE gene. However, it is also clear that PDEs encoded in different transcripts exclude or include regions with regulatory function, and show differences in subcellular localization and interaction with other proteins [35,88]. Thus, multiple transcriptional units generate PDEs with distinct functions.

Alternate Splicing

Splicing and alternate exon usage has been reported for many PDEs and most of this splicing occurs at the amino terminus. PDE4 splicing variants have been extensively characterized and are generated mostly by alternate promoters and first exon usage. Most of the splicing variants predicted by transcript analysis have been confirmed with antibodies specific for the N-termini of PDE4s [84]. Different variants show differences in interaction with other proteins and in subcellular localization. The largest number of splicing variants has been reported for PDE9. Through PCR amplification and alignment of EST sequences, 20 N-terminal mRNA variants have been identified [87,89]. However, only the proteins encoded by PDE9A1 and PDE9A5 have been expressed and characterized [90]. Unlike PDE4, these variants use the same transcriptional start site but are alternatively spliced to produce unique mRNAs that are distinct at the 5′-end. The functional consequence(s) of these amino acid sequence changes is (are) unclear as the affected regions are outside the catalytic domain or any recognized regulatory domain. PDE1C is another example of alternative splicing where proteins with identical catalytic domain and divergent amino termini are generated. Although uncommon, splicing at the carboxyl terminus is also present, as shown for PDE1A, PDE1C, PDE7A, and PDE10A [91]. Several splicing variants have been detected for PDE10. Omori and coworkers have suggested that this splicing controls the PKA phosphorylation of the enzyme and possibly subcellular localization [92]. This possibility has recently been experimentally verified by Charych et al. [93] *as* detailed in Chapter 10.

The importance of transcript splicing is underscored by analysis of RNA processing of the *PDE6B* gene in patients with autosomal recessive retinitis pigmentosa [94]. An acceptor splice site mutation in intron 2 of the *PDE6B* gene leads to the accumulation of a pre-mRNA with intermediate lariats, generating a PDE6B transcript that is 12 nucleotides shorter than wild type. In the normal PDE6B mRNA, these 12 nucleotides code for four amino acids highly conserved in the putative noncatalytic cGMP-binding domain GAF-A of PDE6B and are probably important for the correct folding and function of the protein.

Promoter Regulation

Regulation of PDE protein synthesis was observed in the 1970s when it was shown that treatment of cultured cells with cAMP analogs produced a large increase in PDE activity, which was blocked by protein synthesis inhibition [95,96]. With the cloning of different PDE4 variants, it was demonstrated that transcriptional activation of specific PDE4s accounts for some of the increased PDE activities described. Using an endocrine model and hormones that regulate cAMP synthesis, Swinnen et al. were the first to show that the accumulation of mRNA for PDE4D1 and PDE4B2 is dependent on cAMP-mediated transcriptional activation of these open reading frames (ORFs) [78]. Subsequently, an intronic promoter controlling the transcription of PDE4D1 mRNA was identified and functionally characterized [97]. In cortical neurons, D'sa et al. demonstrated that dibutyryl-cAMP (db-cAMP) induces expression of PDE4B2 and PDE4D1/PDE4D2 [98], whereas the splice variants PDE4A1, PDE4A5, PDE4A10, PDE4B3, PDE4B1, PDE4D3, and PDE4D4, although present in these cells, were not regulated at the transcriptional level by db-cAMP. Dominant negative mutants of the cAMP response element-binding (CREB) protein suppress PDE4B2 promoter activity and a constitutively active form of CREB stimulates it, confirming CRE- and CREB-dependent cAMP-mediated transcription of the short PDE4 forms. Thus, cAMP-dependent regulation of PDE4 transcription is a negative feedback loop contributing to long-term adaptation of cAMP signaling. It should be pointed out that robust PDE4B2 synthesis is also induced by activation of Toll-like receptor 4 (TLR4) and signaling through NF-κB and related transcription factors, suggesting that additional signaling pathways control the expression of PDE4 short forms [6].

During the circadian rhythm and in synchrony with cAMP synthesis, an increase in PDE activity that peaks in the middle of the night has been reported in the pineal gland. This nocturnal increase in PDE activity results from a fivefold increase in abundance of PDE4B2 mRNA [99]. The increase in PDE4B2 mRNA is followed by increases in PDE4B2 protein and PDE4 enzyme activity. These changes are dependent on the activation of adrenergic receptors and require PKA activation. The findings in this pineal gland model are an additional demonstration that PDE4B2 expression is regulated by cAMP. They also document the involvement of this regulatory loop in the circadian rhythm, providing evidence that PDE induction is a physiologically relevant mechanism of feedback regulation.

Using promoter/reporter assays, CRE elements were also identified in the promoter region of PDE4D5 [100]. This PDE4D variant accumulates in response to increased cellular cAMP, although at much lower levels than that reported for the short PDE4D1/2 and PDE4B2 forms. Site-directed mutational analysis revealed that the CRE at position −210 from the transcription start site is the principal element underlying cAMP responsiveness. The authors further determined that cAMP induced PDE4D5 expression in primary cultured human airway smooth muscle cells, leading to upregulation of phosphodiesterase activity.

CREB-mediated and cAMP-dependent regulation of PDE expression is not restricted to PDE4. For instance, PDE7B, a cAMP-specific PDE that is predominantly expressed in the striatum, is also regulated at the transcriptional level by cAMP [101]. Transcriptional activation of rat PDE7B following activation of the dopaminergic system has been demonstrated in primary striatal cultures. RT-PCR analysis revealed that dopamine D_1 agonists, forskolin, or 8-Br-cAMP stimulated PDE7B transcription in striatal neurons, whereas D_2 agonists did not. The cAMP-dependent regulation of PDE7B transcription, like that of PDE4D and PDE4B genes, was also variant-specific, because only PDE7B1 transcription was activated by a D_1 agonist. Also in this case, functional CRE elements were identified in the promoter region.

With the above exceptions, little information is available about the mechanisms of transcriptional regulation of other PDE genes in spite of the fact that numerous reports show altered PDE expression in pathological conditions or after pharmacological treatments. For instance, alterations in PDE7 and PDE8 isozyme mRNA expression were observed in Alzheimer's disease brains examined by *in situ* hybridization [102]. McLachlan et al. [103] found that PDE4D isoforms 1–9 were expressed in the hippocampus of healthy human adults as well as a patient with advanced Alzheimer's disease. However, the expression for the majority of the PDE4D isoforms was reduced in the Alzheimer's disease patient compared to normal controls, whereas PDE4D1, a short isoform under the transcriptional control of cAMP, was increased twofold. PDE4D is also regulated by treatment with antidepressants [104] and chronic haloperidol and clozapine treatment causes altered expression of PDE1B, PDE4B, and PDE10A in rat striatum [105]. The above are mostly correlative studies but imply that PDE promoters are finely regulated by a variety of extracellular signals and that pathological conditions affecting cyclic nucleotide signaling also affect PDE expression.

PATTERN OF GENE AND PROTEIN EXPRESSION IN THE CNS

General Concepts

Many of the PDE variants encoded in the human genome are expressed in a tissue- and cell-specific manner. Among the various tissues examined, the CNS is remarkable in the fact that it expresses one of the highest amounts of PDE protein and activity; essentially all PDE genes are expressed in the CNS and most cells express a multitude of variants. This complexity documents the tight control of cyclic nucleotide levels in cells whose primary function is processing and integration of information. Chapter 2 shows this clearly with some original data sets, which document PDE expression in the adult mouse brain.

The majority of studies examining PDE expression patterns in the brain have focused on PDE genes rather than individual PDE variants. This information is sufficient when exploring the therapeutic potential of PDE inhibitors, which are currently designed to inactivate all members of a PDE family or, at least, all variants generated from a single PDE gene. PDE inhibitors were often developed to inactivate the major PDE subtype expressed in the target cells or tissues as a strategy that has been successfully applied in the development of PDE5 inhibitors for erectile dysfunction, PDE4 inhibitors for inflammatory airway diseases, and PDE3 inhibitors for heart disease [91]. However, with the idea of compartmentalized cAMP signaling gaining momentum (see Chapter 3), it should be realized that the amount of a PDE subtype expressed in a cell is not proportional to its functional significance or therapeutic potential. While the predominantly expressed PDEs are certainly important, one cannot discount that PDE variants that contribute only a minor fraction of the total PDE activity in a cell may yet play critical physiological roles and may well be targeted for therapeutic intervention. In this context, the focus on where a PDE is expressed most abundantly does not necessarily predict specific functions. Furthermore, the localization of PDE variants to specific subcellular compartments is thought to be necessary to stabilize cAMP microdomains of signaling (see below). As a result, displacement of a unique PDE variant from signaling complexes, rather than inactivation of catalytic activity, has been proposed for future drug development [106]. As subcellular localization is often unique to individual variants generated, understanding the nature of these complexes becomes a critical issue to develop new therapeutic strategies.

Individual PDE Gene Pattern of Expression

PDE1. The three *PDE1* genes, *PDE1A*, *PDE1B*, and *PDE1C*, are all expressed in the brain. *PDE1A* is widely distributed with high levels in cortex, hippocampus, cerebellum, olfactory bulb, and striatum. *PDE1B* message is also distributed widely, but is particularly enriched in striatum with PDE1B1 activity in mouse striatum being 3–17-fold higher than that in any other brain region. *PDE1C* is more selectively expressed in olfactory epithelium, cerebellum, and striatum [79–81,107–111]. *PDE1B* has received particular attention, as *PDE1B* knockout mice exhibit increased locomotor activity after D-amphetamine administration as well as spatial learning deficits. *PDE1B* knockout mice show increased levels of Thr34-DARPP-32 phosphorylation in response to dopamine D_1 receptor stimulation suggesting that elevated cyclic nucleotide levels resulting from *PDE1B* inactivation act synergistically to dopaminergic agonism [112]. Inactivation of PDE1 has been proposed as a possible therapeutic approach in Parkinson's disease because antiparkinson agents, such as amantadine or deprenyl, were shown to inhibit PDE1 *in vitro* [113–116]. In addition, SNPs in the *PDE1A* gene are associated with remission of antidepressant treatment response [117]. Chapter 7 explores the effect of PDE1B inhibition in memory disorders.

PDE2. Although the cGMP-stimulated *PDE2* is expressed in many tissues, the highest levels are found in the brain, where it is expressed in olfactory bulb and tubercle, cortex, amygdala, striatum, and hippocampus [108,118–122]. PDE2 inhibitors increase cyclic nucleotides in cortical and hippocampal neurons [123,124], promote enhanced long-term potentiation [123] and object recognition [125], and attenuate the learning impairment induced by acute tryptophan depletion [126]; all together suggesting that inactivation of PDE2 could boost memory functions. The potential of PDE2 inactivation in memory and cognition is presented in detail in Chapter 7.

PDE3. The two *PDE3* genes, *PDE3A* and *PDE3B*, are widely expressed throughout the brain, with PDE3A being enriched in striatal and hippocampal regions [127–129]. PDE3B has been suggested to play a role in hypothalamic leptin signaling [130].

PDE4. These enzymes are widely expressed throughout the body, but highest levels of activity and protein of any tissue are found in the brain, suggesting a critical role of PDE4 in cyclic nucleotide signaling in the CNS. Indeed, the mammalian *PDE4* genes are orthologs of the *D. melanogaster dunce* gene, whose ablation produces a phenotype of learning and memory deficits [131,132]. In addition, rolipram, the prototypical inhibitor defining the PDE4 family, was shown more than 25 years ago to produce antidepressant-like effects in rats and humans [133–135]. These studies have since been greatly expanded and PDE4 inhibitors are now investigated for potential therapeutic benefits for a plethora of CNS diseases, including Huntington's disease, Parkinson's disease, Alzheimer's disease, schizophrenia, and stroke [136].

While PDE4A, PDE4B, and PDE4D are all highly expressed in the brain, a detailed analysis reveals that their respective expression patterns are highly segregated, an observation that is further augmented if the expression patterns of individual variants, rather than the combined messages expressed from a PDE4 gene, are considered [82,98,104,137–148]. *PDE4A* is expressed at high levels in cortex, hippocampus, olfactory bulb, and brain stem. *PDE4B* displays a distinct pattern of accumulation with highest expression levels in striatum, amygdala, hypothalamus, and thalamus [12,83,149]. There is only a limited expression of *PDE4C* in the CNS, with some *PDE4C* found in cortex, some in thalamic nuclei and in the cerebellum in humans, but only in olfactory bulb in rat [148]. *PDE4D* is widely expressed with high levels in the cortex, olfactory bulb, and hippocampus [82].