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Profiling human phosphodiesterase genes and splice isoforms

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Abstract

A mere 21 human phosphodiesterase (PDE) genes are responsible for modulating cellular levels of cAMP and cGMP in response to stimuli. Considering the importance of cAMP and cGMP to disparate physiological functions including visual response, smooth muscle relaxation, platelet aggregation, immune response, and cardiac contractibility, perhaps the 200 or more splice isoforms of PDE genes also play a major functional role. We profiled the human PDEs across 25 tissue samples using splice sensitive oligonucleotide microarrays containing probes for exons and exon–exon junctions. Our results suggest that PDEs exhibit tissue-specific differences in expression, as demonstrated by the high expression of PDE4B in skeletal muscle. At the splice variant level, the majority of PDE genes—notably 1A, 1C, 2A, 4C, 4D, 5A, 7A, 8A, 8B, 9A, 10A, and 11A—exhibited tissue-specific splicing with potential functional implications for PDE biology. This work validates expression of many EST transcripts, and confirms and expands on published findings based on PCR and cloning, illuminating some of the complexity of cAMP and cGMP processing.

Keywords: Phosphodiesterase; PDE; Splicing; Splice variant; Expression; Gene; Microarray; Array; Profile; Isoform

The human phosphodiesterase (PDE) gene family contains a mere 21 genes that are nonetheless critical determinants for modulating cellular levels of cAMP and cGMP. Grouped into 11 functional subfamilies, PDEs express perhaps 200 or more distinct gene products through alternate RNA splicing. Although many splice isoforms retain the catalytic domain, the presence or absence of other domains has been demonstrated to exert important conformational constraints on the catalytic domain with functional implications. For example, a long isoform of PDE4D contains a site that upon phosphorylation increases the V_{max} of the enzyme by threefold [1] and a PDE9A isoform appears to be found only in the cell nucleus [2]. In general, alternate splicing of PDE domains can alter phosphorylation, oligomerization, cellular localization, and interaction with other proteins, expanding the functional diversity of the small gene family by alternate splicing.

With the successful launch of the PDE5A inhibitor sildenafil citrate as treatment for erectile dysfunction, phosphodiesterases have joined the front ranks of therapeutic targets for a broad spectrum of disease indications. Gene knockout, gene inactivation, and genetic association studies have implicated PDEs in asthma [3], diabetes [4], infertility [5], depression [6], dementia, psychosis, and stroke [7]. Academics and pharmaceutical companies are evaluating-specific inhibitors of PDE4D, PDE4B, PDE3A, PDE3B, PDE10A, and PDE5A. Rolipram, a PDE4D-specific molecule, has been shown to be effective in animal models of memory.

Because of sequence homology among PDE genes, small molecular inhibitors often bind to homologues. Sildenafil notoriously binds not only to the target PDE5A but also to PDE6, a member of a different subfamily expressed in rod cells and whose inhibition can affect color vision. Due to similar lack of therapeutically tolerant specificity or restricted target profiles, researchers halted development of several cAMP-specific inhibitors for PDE3A and PDE4D [8]. Alternate splicing further complicates

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therapeutic specificity, while expanding the functional diversity of PDEs.

To identify splice isoforms, scientists have relied on PCR, cloning, and EST sequencing. Once identified, PCR can profile expression across multiple tissues. But splice sensitive oligonucleotide microarrays offer a scalable solution for profiling multiple splice isoforms for multiple genes simultaneously across tissues [9,10]. A broad and detailed picture of PDE expression using splice-sensitive microarrays may facilitate PDE-based drug development efforts.

Materials and methods

To identify PDE splice isoform sequences, we began with RefSeq mRNA sequences for all 21 human PDE genes, then mined the NR and EST sequence databases with BLAST. We aligned the resulting ESTs, cDNAs, and mRNAs to their corresponding genomic sequences with sim4 to obtain exon–intron structure, filtering out homologues and low quality sequences by requiring 96% identity between exons and genomic sequence, checking the acceptor and donor sites of introns, and eliminating partially processed pre-mRNA and genomic contaminants. A manual QC step using custom visualization software allowed further scrutiny of the automated alignments which resulted in 94 non-redundant candidate splice isoforms.

To visualize the relationships among all of the subfamilies, genes, and splice isoforms, we created a distance matrix for all pairwise comparisons of the isoform sequences based on BLAST expect score. Then we hierarchically clustered the sequences using the distance matrix as input to the yWorks software package. We plotted the results with cluster visualization software. (Supplemental Figure 5).

For each PDE splice isoform, we designed sense-strand oligonucleotide probes for each exon and exon–exon junction. To achieve a target melting temperature of 68 °C, probe lengths varied from 34 to 42 bases depending on GC content, Na^+ and Mg^+ concentrations, and base stacking enthalpy and entropy. For junction probes, the number of bases on each side of the junction varied to minimize half-binding. As negative controls, we designed a series of artificial exon–exon junction probes such that each half-oligo matched the RNA sequence, but the whole junction did not. Positive control oligos detected GAPDH exons and exon–exon junctions.

Oligonucleotides were synthesized by Sigma–Genosys with 5′ amine labels. Total RNA for 25 normal human tissues was obtained from Ambion. We used sample preparation, microarray spotting, and hybridization services provided by Mergen in late 2003. Oligos were spotted on ExpressChip™ activated glass slides with covalent chemistry. The sample preparation was then fairly standard, using T7 amplification to create 45 μl biotin labeled cRNA for each sample. To increase signal, the cRNA was sheared into 30–100 nucleotide lengths with 5 μl of 10× fragmentation buffer heated at 94 °C for 20 min. To this, 250 μl of hybridization buffer was added.

Microarrays were warmed to room temperature and dried. The Mergen hybridization buffer was heated to 50 °C for 5 min then vortexed to resuspend any precipitated material. The hybridization chamber was sealed over the arrayed area of each slide. Samples were hybridized overnight at 42 °C, then washed with 60 rpm constant shaking in preheated washing solutions of 2× SSC + 0.1% SDS at 42 °C for 20 min, of 0.2× SSC at 37 °C for 10 min, and of 0.1× SSC, also at 37 °C, for 10 min. Fluorescence was measured with Cy3 labeled streptavidin.

A gene expression level is an approximation of the sum of expression levels of a gene's splice isoforms. Therefore, we computed gene expression by log summing signals from each set of one or more mutually exclusive probes and log averaging all such sums for a gene. To determine splice isoform expression, oligos unique to each isoform were identified and plotted using background subtracted signals along with negative and positive controls (oligos from constitutively spliced regions). To identify tissue-specific regulated splicing, the log ratio or percent composition of mutually exclusive spliced regions was computed. Changes in log ratio or percent composition suggested regulated splicing even if the absolute signal intensity of a given isoform did not change.

In November 2005, we repeated the sequence database mining portion of the analysis using assembly hg17 of the UCSC genome database and BLAT alignments for genomic coordinates of candidate splice forms. We filtered the results to remove redundancies. This more recent effort yielded more splice forms because of additions to the public databases, including the extension of several RefSeq entries with 5′ exons, which enabled more ESTs to align and revealed more alternate splicing events. The updated version included 212 candidate splice variants.

Results and discussion

We profiled 21 human PDEs and their splice isoforms across 25 tissue samples using oligonucleotide microarrays containing exon and exon–exon junction probes. Results were compared with EST findings and, where possible, with the PDE literature. Based on hierarchical clustering analysis at the nucleotide level using expect value from a pairwise blastn, the high-confidence cluster of PDE1B splice variants could not be connected to that of PDE1A and PDE1C even within the expect value cutoff of 1.0e−10 perhaps because PDE1B gene is more divergent compared those of to PDE1A and PDE1C both in sequence and splicing. Similarly, high confidence cluster of PDE4B and PDE6C splice variants could not be linked to other members of their respective subfamilies perhaps suggestive of diversity in splicing. (Supplemental Figure S5). A phylogenetic tree of the 21 PDE genes generated by using translated sequences of just the catalytic domains confirms our finding with regard to PDE1B and PDE6C [11].

At the gene level, PDE expression from the exon and exon–exon junction microarray was largely consistent with literature findings, including other gene array platforms (Supplemental Figures S1 and S2), showing clear tissue specificity (Fig. 1). In general the most ubiquitous and highly expressed PDE was 9A. Overall, the highest expressed gene/tissue pairs were 4B in skeletal muscle (confirming [1]), 9A in colon, 1A in testis, and 9A in placenta. High expression of 4B is consistent with the PDE4 subfamily's implication in muscular disease, with pharmacological inhibitors demonstrating a major cAMP hydrolyzing activity [2].

At the splice isoform level, the majority of PDEs exhibited likely sample-specific splicing with potential functional implications. As a caveat, the sample preparation protocol, subsequently refined, introduced 3′ bias due to poly(A) amplification, leading to lower signal readings near the 5′ end of long genes. However, we were able to draw conclusions about alternate splicing even for the splice events at the 5′ end of genes such as 7A and 9A. Details of gene and splice variant expression for each PDE follow.

PDE1A: Ca^+ calmodulin-stimulated

Our data supported the high 1A gene expression levels already observed in testis, brain, kidney, and heart

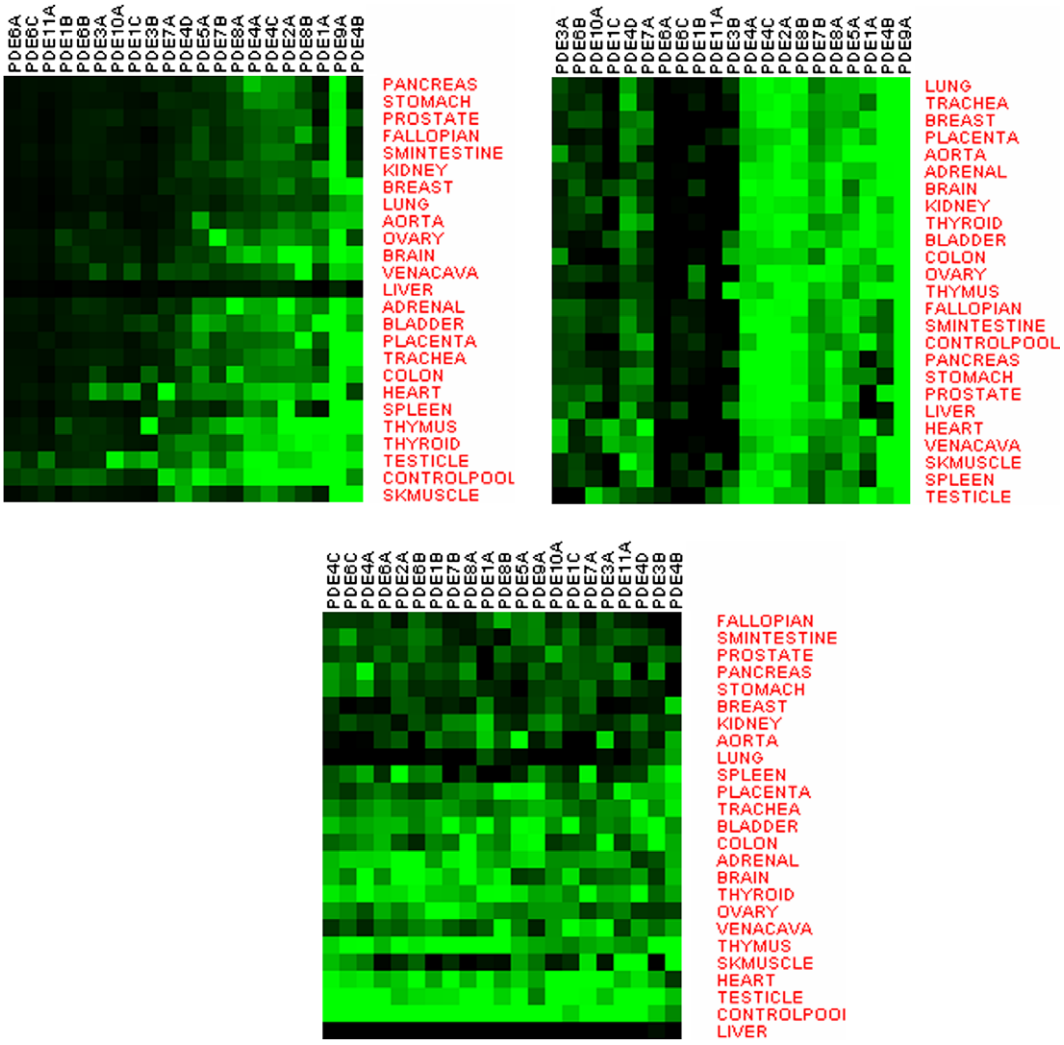

Fig. 1. Heat maps of PDE gene expression based on log average of exon–exon junction probes, taking the log sum of mutually exclusive splices: (upper left) absolute expression; (right) relative expression normalized by tissue to emphasize the genes highest expressed; (bottom left) relative expression normalized by gene to emphasize the tissues where highest expressed. (The liver sample had very low signal.)

[12,13], while also indicating high expression in aorta, bladder, thyroid, and thymus. Six splice isoforms of 1A have been reported, including two varying in the 3′ end and four varying in the 5′ end of the transcript [14]. EST mining identified sequence evidence for as many as 15 isoforms differing at both the 3′ end (such as U40370, called HSPDE1A3A, and BG190255) and 5′ end (such as AA757381, AL556442, and BI464993). Microarray results suggest that two 3′ isoforms, an isoform skipping one exon (U40370) and an isoform skipping two consecutive exons (NM_005019), were regulated in testis and thyroid relative to BG190255. Also, the wildtype isoform was up-regulated in thyroid, heart, and bladder, whereas testis expressed higher levels of the isoform including an alternate 5′ exon of perhaps 242 bases (AA757381).

PDE1B: Ca^+ calmodulin-stimulated

Of the PDE1 subfamily members, 1B was expressed at the lowest levels, in line with previous observations in mouse thymus, testis, lung, and spleen [15]. Two splice isoforms, PDE1B1 and PDE1B2, have been reported [16]. Data mining offers evidence of perhaps 7 isoforms. However, we did not observe significant differential expression in the tissues studied, perhaps due to the gene's low transcriptional activity.

PDE1C: Ca^+ calmodulin-stimulated

Heart tissue showed the highest expression of 1C (consistent with findings in rat [17]), followed by testis (seen in mouse [15]) and vena cava. Low levels of expression of 1C gene may have been present in bladder and brain. Several splice variants of mouse 1C have been reported, including PDE1C1–1C5 [18], and our data mining identified even more potential human isoforms (perhaps 10), with many alternate exons before and after the catalytic domain. The drop in expression in testis of the wildtype junction connecting exons of lengths 103 and 27 in testis may suggest an alternate promoter or alternate transcrip-

tion start, though probes for direct detection of such isoforms were not included at the time of probe design.

PDE2A: cGMP stimulated

Array results showed 2A to be expressed in most tissues, and at high levels in adrenal, spleen, thymus, thyroid, brain, and heart, expanding on a previous report of expression mainly in brain and heart [19]. PDE2A has also been reported in human venous and capillary endothelial cells [20]. The expression levels in several tissues confirm data from another microarray platform (GPL543–547 [10]). Less has been published about 2A splicing. EST mining suggest as many as 18 human splice isoforms, including an isoform with an additional 5′ exon of 62 bases (AK092278, taken from human tongue) and an isoform with an excised intron in the 3′ exon (AK095024). Microarray data suggest the splice isoform with the extra 5′ exon is in general more common, though the isoform skipping the exon is up-regulated in small intestine.

PDE3A: cGMP-inhibited and cAMP selective

The array data suggest that 3A may be primarily a heart-specific gene. Very low levels of expression were also observed in aorta, vena cava, and colon. We did not find splice isoforms of 3A through sequence data mining.

PDE3B: cGMP-inhibited and cAMP selective

3B was most highly expressed in thymus, testis, colon, and liver. Literature search and EST mining did not strongly suggest alternate splicing. However, the exonic structure of human 3B is considerably different from those of mouse and rat orthologues.

The PDE4 subfamily: cAMP-specific and cGMP-insensitive

From the drug development point of view, the PDE4 subfamily may be the most interesting. The range of illnesses implicated includes asthma, depression [21], Alzheimer's, emesis, stroke, and muscular disorders such as muscular dystrophy. The entire subfamily showed near ubiquitous expression in the samples we tested, though 4C and 4D were generally higher.

PDE4A

In pancreas, 4A shows up most, though the gene was expressed in a majority of the samples. Several 4A splice isoforms have been reported [1], and the public databases contained around 10 (Fig. 2), including a splice form missing two exons and sequenced from a fetal brain library (AL536669). This splice form was not highly expressed in any tissues we studied. Differential splicing for other novel splice forms occurred in skeletal muscle and thyroid, based on log ratios of probes relative to gene expression level.

PDE4B

According to our findings, 4B is more abundantly expressed in skeletal muscle than any other PDE in any of the 25 tissues (Fig. 3). This result is consistent with data deposited at NCBI by Rosetta using their exon–exon junction array [10]. It also shows up in spleen, breast, and lung. Of the PDE4 subfamily genes, only 4B was expressed in normal blood in a large microarray study (NCBI:-GEO:GPL564: Rosetta Blood Donor Experiment), perhaps making 4B a candidate diagnostic target for PDE4 inhibitors. We observed up to 21 splice forms, overwhelmingly located in the 5′ region preceding the RefSeq entry available in 2003. Subsequence sequence entries have only begun to address the likely complexity of splicing in 4B.

PDE4C

The 4C gene was ubiquitously expressed in the samples we tested, but highest in skeletal muscle, pancreas, small intestine, and stomach. Several splice isoforms have been reported in the literature [22]. EST mining yielded a similarly diverse range of splice events (around 15), including alternate 5′ exons (U88713), skipped exons (AA535428; U66348, called HSPDE4C2C; and U66349, called HSPDE4C2D), and retained introns (U66347, called HSPDE4C2B). Isoform AA535428, skipping a 50 base exon, was expressed in many tissues, while isoform 4C2C,

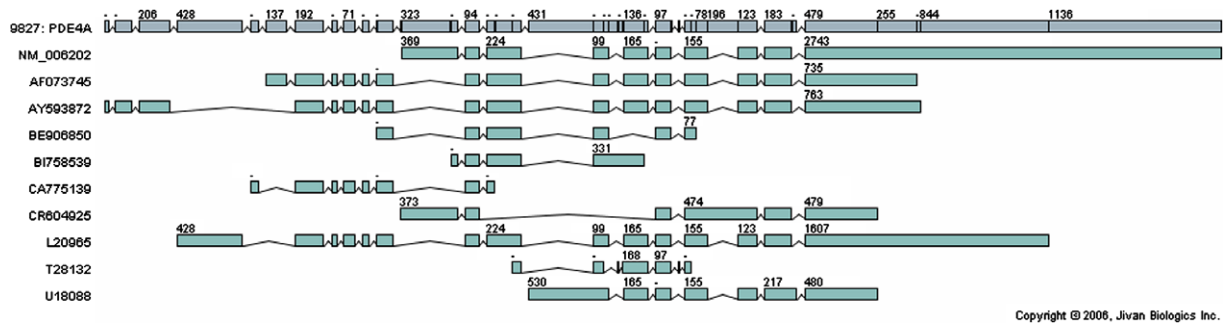

Fig. 2. Candidate splice isoforms of PDE4A, including novel 5′ exons.

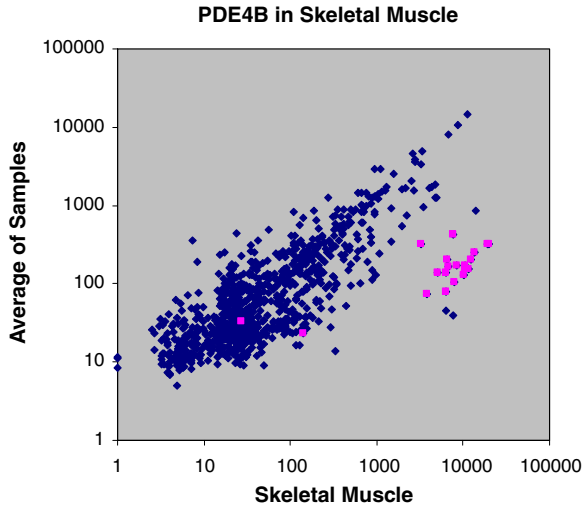

Fig. 3. All exon and junction probes for PDEs in skeletal muscle (*x*-axis) vs. the average of all samples (*y*-axis). Probes for 4B are highlighted.

skipping that exon and an additional 113 base exon, seemed not to be expressed in any tissue we investigated.

PDE4D

Like the other PDE4 genes, 4D was widely expressed, showing up highest in skeletal muscle. According to EST mining, 4D possesses many (around 16) potential splice forms. One widely expressed isoform (AF536975, also called PDE4D6) skips the first 6 exons of the wildtype, beginning with an alternate first exon. Another candidate splice form skips the first 4 exons of the wildtype and also starts with an alternate first exon (AF012074, called PDE4D2), but this isoform may be tumor-specific, based on separate microarray validation in kidney tumor. Still other 5′ isoforms were present in the EST databases, which await further study.

PDE5A: cGMP-specific

In accordance with the literature, we saw 5A most highly expressed in aorta, placenta, and testis [13,23], as well as in colon and bladder. RefSeq includes three splice forms with alternate 5′ exons (NM_001083, NM_033430, and NM_033437) and one with a trimmed 10th exon (NM__033431). EST mining revealed two more candidate splice forms with additional exons (AA926848 and BG771541). Microarray data showed that the 5′ isoforms were differentially regulated, with NM_033430 higher in aorta, bladder, placenta, and colon, and NM_001083 higher in adrenal, heart, and testis.

PDE6A: cGMP-specific

Across the full spectrum of tissues, 6A was highly expressed, notably in pancreas, liver, prostate, fallopian tubes, aorta, ovary, and skeletal muscle. Data mining discovered 7 candidate spliceoforms. A skin-specific splice form may exist (BG476062), including an inserted exon after the 13th exon of the wildtype (NM_000440). An alternate 3′ tail is possible (W22603), along a 214 base exon, extending the 48 bases of the wildtype.

PDE6B and PDE6C: cGMP-specific

These two PDEs are widely considered to be specific to retina, and our microarray results confirmed the status quo. EST mining identified a candidate splice isoform of 6C with a skipped penultimate exon (BG216846), and a similarly skipped penultimate exon of 6D (BI754373).

PDE7A: cAMP-specific, high affinity

Our microarray showed 7A expression in heart, skeletal muscle, spleen, thyroid, and placenta among the top tissues. A study of human 7A isoforms showed tissue specificity of alternate 5′ exons in heart and skeletal muscle [24]. Our microarray results validate the findings (Fig. 4). Differential expression of mouse and rat splice isoforms has also been reported [25]. From EST mining, we discovered a splice isoform with an additional exon of 104 bases in testis (BG717904), not confirmed by the array results. Another EST from human testis contained two novel exons (BG719532). The first of these exons was more highly expressed in pancreas. We found 9 candidate splice forms in total.

PDE7B: cAMP-specific, high affinity

This gene was most highly expressed in ovary, bladder, and thymus, but barely expressed in skeletal muscle, which differs from observations in mouse [26,27]. Although three 7B splice isoforms have been reported in rat—7B1 is wildtype, 7B2 skips exons 3 and 4 in ovary and bladder, ad 7B3 alters the splice site location between exons 4 and 5 in bladder—our EST mining of human sequences and microarray experiments did not strongly support the corresponding human splice forms. We did, however, discover a potential spliceoform with an alternate 3′ exon (AB209990).

PDE8A: cAMP selective

Both 8A and 8B hydrolyze cAMP. High expression levels of 8A were found in colon, adrenal, and liver. We observed gene expression in the majority of tissues. The public databases contain sequences for three human splice isoforms in addition to the wildtype 8A1: 8A4 skips exon 7 (AF388186); 8A2 skips exon 8 (AF388184); 8A3 skips both 7 and 8 (AF388185). Microarray results identified 8A3 in brain and testis, and 8A4 in adrenal, colon, testis and brain, but failed to find 8A2.

PDE8B: cAMP selective, IBMX insensitive

In thyroid, 8B showed the greatest expression (confirming [28]). Other tissues with more than high expression of

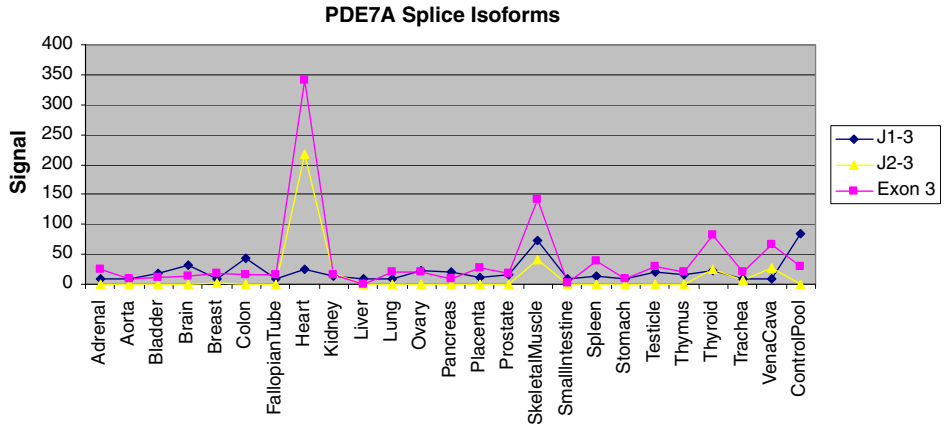

Fig. 4. Expression profiles of background subtracted signal intensities of two PDE7A splice isoforms that begin with different 5′ exons. J1–3 is in NM_002604, while J2–3 is in NM_002603.

8B were thymus, placenta, vena cava, aorta, brain, and Fallopian tube. Tissue-specific splicing of various 8B isoforms has been reported in human [29]. Our EST mining identified several splice variants, including 8B4 that skipped the 60 nucleotides of exon 2 (NM_001029853); an isoform that skipped the 141 bases of exon 8 (NM_001029854) in human brain as in mouse brain; 8B2 that skipped exons 8 through 10 (NM_001029851), also seen in mouse; 8B3 that skipped the 165 bases of exon 14 (NM_001029852); and one that possibly included a long exon between wildtype exons 14 and 15 (BI559575) in testis. Mining also revealed a likely earlier 3′ exon (BI548949), which if confirmed will throw off the standard exon numbering. Array results failed to confirm the testis isoform, but showed the wildtype NM_003719 to be the most common isoform in that variable gene region, highest in skeletal muscle, heart, and adrenal, with the alternate 8B2 up-regulated in thyroid, thymus, and vena cava.

PDE9A: cGMP-specific, IBMX insensitive

Of all the PDEs, 9A exhibited the highest expression levels in general, with ubiquity in our 25 samples. The highest 9A expression was in colon, consistent with Rentero et al. [30]. Splicing of 9A may be unusually complex, with several RefSeq splice forms and many more full length splice products with cassette exons near the 5′ end, numbered PDE9A1–PDE9A21 [30] (Table 1). EST mining further expanded the possibilities, with splice forms exhibiting a new and premature UTR (BF589752) in sciatic nerve; an additional exon and a short form exon (AW303609), two skipped exons and a trimmed exon in colon (BF002400); and a 12 nucleotide micro-exon formed by truncating a 96 base exon in kidney tumor (BI009803). Microarray results provided a glimpse into the complexity of 9A splicing, supporting the expression of many cassette exons in multiple combinations (Table 2).

Table 1
PDE9A splice isoforms

Splice isoform	5′ Exons included
PDE9A1	1–2–3–5–8
PDE9A2	1–2–3–5–9
PDE9A3	1–5–9–10
PDE9A4	1–5–10–11
SP-PDE9A5	1–3–5–9
BBRC-PDE9A5	1–2–3–4–5–10
PDE9A6	1–3–5–9
PDE9A7	1–9
PDE9A8	1–8
PDE9A9	1–2–5–9
PDE9A10	1–10
PDE9A11	1–5–10–12
PDE9A12	1–3–10–11
PDE9A13	1–3–5–10
PDE9A14	1–2–5–10
PDE9A15	1–10–12
PDE9A16	1–5–8
PDE9A17	1–3–5–8
PDE9A18	1–2–5–8
PDE9A19	1–5–7–9
PDE9A20	1–3–5–8–10
BF589752	1–2–3–5–6

SP abbreviates Schering–Plough, whose naming of PDE9A5 differs from a BBRC publication.

PDE10A: cGMP-sensitive, cAMP-selective

A dual substrate phosphodiesterase, human PDE10A, was expressed in several tissues according to our data, notably stomach, skeletal muscle, small intestine, testis (consistent with [31]), kidney, heart, and brain (as in mouse [32]). EST data mining identified possible novel exons, including a splice variant from brain with two exons, of lengths 97 and 60 nucleotides, included between wildtype exons 17 and 18 (BI520305). Array results show that the corresponding probe was up-regulated in testis.

Table 2
Expression of PDE9A exon–exon junctions in the first half of the gene

Junction	Isoforms containing junction	Samples where expressed
J1–2	9A1, 9A2, BBRC-9A5, 9A9, 9A14, 9A18	Placenta, Colon, Bladder, Adrenal
J1–3	SP-9A5, 9A6, 9A12, 9A13, 9A17, 9A20	Sk. Muscle, Heart, etc.
J1–5	9A3, 9A4, 9A11, 9A16, 9A19	Tumor Specific
J1–8	9A8	
J1–9	9A7	
J1–10	9A10	T-24, Sk. Muscle
J2–3	9A1, 9A2, BBRC-9A5	Lung, Trachea, Heart, Aorta, etc.
J2–5	9A9, 9A14, 9A18	Pancreas, etc.
J3–5	9A1, 9A2, SP-9A5, 9A6, 9A13, 9A17, 9A20	Colon, Placenta, Adrenal, Bladder
J3–4	BBRC-9A5	Pancreas, Thyroid, Trachea
J3–10	9A12	Control pool
J4–5	BBRC-9A5	Testicle, Ovary, Prostate, Heart, etc.
J5–6	BF589752	Adrenal, Colon, Placenta?
J5–8	9A1, 9A17, 9A18, 9A20	Colon, Placenta, Adrenal, Bladder
J5–9	9A2, 9A3, SP-9A5, 9A6, 9A9	Sk. Mus., Heart, Adrenal, Colon
J5–7	9A19	
J5–10	9A4, BBRC-9A5, 9A13, 9A14	T-24, Sk. Muscle, Heart, etc.
J7–9	9A19	
J8–9		Thyroid, Colon, Testis, etc.
J8–10	9A20	Thyroid, Testis, etc.
J9–10		Colon, Placenta
J10–11		Colon, Placenta, Bladder
J10–12	9A11 (exon deleted in catalytic domain)	Pancreas
J11–12		Colon, Bladder, Placenta

A novel exon 6 in BF589752 shifts downstream exon indexes compared to previous numberings. The sample column includes tumor samples run separately on the arrays.

PDE11A: cGMP-sensitive, dual specificity

The final PDE, 11A, showed up in skeletal muscle and thymus, but was below our threshold level of detection in most tissues. Three splice variants of 11A have been reported [33]; our data suggest isoform 11A1 (AJ251509) might be expressed in skeletal muscle. Another splice isoform (AI025081) with an extra exon of 86 bases compared to the wildtype (NM_016953) was found in testis. EST mining revealed additional possibilities at the 5′ end.

Conclusion

We profiled PDE genes and splice isoforms across a broad spectrum of tissue samples. The resulting data may provide a baseline for identifying disease-specific changes. Further validation, including replicate array experiments, quantitative PCR, and cloning, would add confidence to the many suggestive findings and novel discoveries. As new splice isoforms are identified, we have expanded the array with probes capable of detecting the novel splice sites. By also including related genes and splice forms, we hope to expand understanding of the cAMP and cGMP pathways as well as the avenues of potential therapeutic intervention. Patient-specific splicing may prove important for PDEs, given that heritable genetic mutations can lead to alternate splicing of genes, and given that drug treatment can induce splicing changes. In conclusion, splice variant arrays may provide valuable insight into the modulation of cAMP and cGMP in human biology and disease.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2006.08.180.

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