PKA Subunits in the Human Pathogen *Paracoccidioides*: An *in Silico* Approach

Sestari SJ[1], Brito WA[1,2,3], Lacerda JGVC[1] and Salem Izacc SM[1]*

[1]Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Goiás, Brazil

[2]Unidade Universitária de Ciências Exatas e Tecnológicas, Universidade Estadual de Goiás, Brazil

[3]Centro Universitário de Anápolis, UniEvangélica, Brazil

*Corresponding author: Salem-Izacc SM, Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Goiás, Brazil, E-mail: silviaizacc@gmail.com

Abstract

Paracoccidioides brasiliensis and *P. lutzii* is the causative agent of paracoccidioidomycosis, a primary systemic mycosis with different clinical manifestations. Infection occurs when hypha-fragments or spores are inhaled by the host, an event that induces *Paracoccidioides* cells to switch to the yeast form. The cAMP-PKA signaling pathway is important in controlling the morphological transition, as well as several development processes in fungal pathogens. PKA holoenzyme is an inactive tetramer composed of two regulatory and two catalytic subunits. When cAMP binds to the regulatory subunits, the catalytic subunits are released and become active. These active subunits subsequently phosphorylate protein kinases, transcription factors, and other substrates. In this study, we show an *in silico* characterization of the PKA subunits from three strains of *Paracoccidioides*. The genome of each strain encodes a single regulatory, PbPKA-R, and two catalytic subunits, PbPKA-C1 and PbPKA-C2. The subunits are highly conserved among the strains analyzed. The computer-based prediction suggests that PKA can be found in more than one subcellular location: PKA-R and PKA-C2 are mainly localized in the nucleus, whereas PKA-C1 seems to act predominantly in the cytosol. Each PKA-R subunit presents two tandem copies of the cyclic nucleotide monophosphate-binding (CNMP) domain at the C-terminus and each PKA-C has a protein kinase and an AGC-kinase C-terminal domain. Alignment of human and *Paracoccidioides* PKA subunits reveals a huge discrepancy in the N-terminal sequences of these two organisms. This sequence divergence between human and *Paracoccidioides* subunits combined with the pivotal role that PKA plays in many cellular processes, makes this protein an interesting target for antifungal drugs.

Keywords: PKA; *Paracoccidioides; in silico*

Introduction

Protein kinases are key regulatory enzymes that play crucial roles in cellular biochemistry and physiology. These enzymes act as mediators in several signal transduction pathways by reverse phosphorylating specific amino acid residues [1]. Protein kinase A (PKA), a serine/threonine kinase that mediates cAMP signaling in eukaryotic cells, belongs to the AGC group of kinases. The holoenzyme is an inactive tetramer composed of two regulatory (PKA-R) and two catalytic (PKA-C) subunits. In response to external signals that increase intracellular cAMP levels, this second messenger binds to the PKA-R subunits triggering conformational changes that release the PKA-C subunits. The free PKA-C became active and subsequently phosphorylate protein kinases, transcription factors, and other substrates to control various physiological processes, such as cell growth and metabolism, DNA replication, cell division and actin cytoskeleton rearrangements [2,3].

Although evolutionary conserved, the PKA subunits can vary in number and isoforms among different species. Several variants of the PKA-C and PKA-R subunits have been identified in human cells. Four PKA-R subunits designated RIα, RIβ, RIIα, and RIIβ are transcribed from separate genes [4]. Five different PKA-C subunit genes have been identified in human: *PRKACA*, *PRKACB*, *PRKACG*, *PRKX*, and *PRKY*. Three of these genes, *PRKACA*, *PRKACB*, and *PRKX*, have been demonstrated to be transcribed and translated into functional protein kinases, termed PKA Cα, PKA Cβ, and PRKX, respectively [5]. In the nonpathogenic yeast *Saccharomyces cerevisiae* PKA consists of a PKA-R subunit encoded by a single gene, *BCY1*, and three PKA-C subunits encoded by the *TPK1*, *TPK2*, and *TPK3* genes. These Tpk isomers are functionally redundant for cell viability, but they regulate different processes [6].

The cAMP-PKA signaling pathway mediates the microbial response to changes in the environment. In yeast, PKA is activated in response to nutrients and subsequently regulates metabolism and growth [7]. This pathway is also associated with pathogenesis in several fungal species, regulating morphological changes and virulence of several plant and animal fungi [8-12]. In the human pathogenic fungus of the *Paracoccidioides* genus, PKA controls morphological changes that are imperative to the establishment of infection [13,14].

The *Paracoccidioides* genus comprises two closely related species: *P. brasiliensis* and *P. lutzii.* Both can infect humans, but they can vary in virulence and induce different immune responses by the host [15,16]. These thermally dimorphic fungi are the etiological agents of paracoccidiodomycosis, a mycosis that affects millions of people in Latin America [17]. Infection occurs when the host inhales the hypha-fragments or spores released from mycelium, which differentiates to the yeast form in the host lungs. This morphological switch is essential for the pathogenicity of these fungi since strains that are unable differentiate is often a virulent [18]. The cAMP-PKA pathway is important in controlling the morphological transition from mycelium to yeast [13,14]. Here we describe an *in silico* analysis of the PKA subunits encoded in the genome of two reference strains of *P. brasiliensis* (Pb03, Pb18) and one strain of *P. lutzii* (Pb01).

Methodology

Identification of the PKA subunits in the genome of *Paracoccidioides* and protein sequence analysis

Paracoccidioides PKA subunits were searched in the protein database available on the Broad Institute website [19,20], whose data have been relocated to the fungal ftpsite (http://archive.broadinstitute.org/ftp/pub/annotation/fungi/paracoccidioides/genomes/). The corresponding amino acid sequences were analyzed *in silico* as follows: similarity searches were performed using BLASTp [21]; the theoretical molecular weight and isoelectric point of each subunit were determined using theCompute pI/Mw tool, available at http://web.expasy.org/compute_pi/; the multiple alignment sequence analysiswere performed using the t-coffee program [22] at the http://www.ebi.ac.uk/Tools/msa/tcoffee/, under default settings; the protein domain identification were done using Prosite, a large database of protein domains, families and functional sites [23] and the cellular localization of the protein subunits was predicted using the PsortII program [24], recommended for yeast sequences.

Protein Sequence accession number

The uniprot accession numbers for the *human* proteins used in the multiple sequence alignment are: KAP0_HUMAN cAMP-dependent protein kinase type I-alpha regulatory subunit (P10644), KAP1_HUMAN cAMP-dependent protein kinase type I-beta regulatory subunit (P31321), KAP2_HUMAN cAMP-dependent protein kinase

type II-alpha regulatory subunit (P13861), KAP3_HUMAN cAMP-dependent protein kinase type II-beta regulatory subunit (P31323), KAPCA_HUMAN cAMP-dependent protein kinase catalytic subunit alpha (P17612), KAPCB_HUMAN cAMP-dependent protein kinase catalytic subunit beta (P22694), KAPCG_HUMAN cAMP-dependent protein kinase catalytic subunit gamma (P22612), PRKX_HUMAN cAMP-dependent protein kinase catalytic subunit PRKX (P51817) and PRKY_HUMAN Putative serine/threonine-protein kinase PRKY (O43930).

Results and Discussion

Overview of PKA subunits encoded in the *Paracoccidioides* genome

We identified and analyzed the PKA subunits encoded in the genomes of three phylogenetic species from *Paracoccidioides*. Each genome encodes a single PKA-R and two PKA-C subunits.

The *Paracoccidioides* PKA-R subunits are approximately 47.0 kDa, slightly acidic proteins, with pI ranging from 5.5 to 5.7 (table 1). Only the Pb01 strain present a PKA-R subunit annotated in the genome (locus number PAAG_07887). However, BLASTp analyses revealed hypothetical proteins in Pb03 (PABG_07470) and Pb18 (PADG_08191) strains that share over 97% amino acid identity with Pb01PKA-R subunit (figure 1). In addition to the strong conservation observed between these proteins in the three *Paracoccidioides* strains (figure 1A), they share the same predicted subcellular localization (table 2) and the same domain structures (figure 2), as described below. So, we believe that these computer-based analyses strongly indicate that Pb03

PABG_07470 and Pb18 PADG_08191 are, indeed, cAMP-dependent protein kinase regulatory subunits.

Each *Paracoccidioides* PKA-C strain presents two PKA-C subunits, which we called PKA-C1 and PKA-C2, according to the BLASTp best hit with either Tpk1 or Tpk2 from the model organism *S. cerevisiae*, respectively. The PKA-C1 subunits predicted mass are about 48kDa, with pI ranging from 5.5 to 6.0. The PKA-C2 are larger proteins, with 64.5 to 67.5 kDa, basic proteins, with pI between 8.1 to 8.8 (table 1). All PKA-C2 subunits exhibit large N-terminal regions that are not present at the PKA-C1 (S1 figure).

PKA-C1 subunits are highly conserved in the three strains. Pb01PKA-C1 shares 93% and 94% identity with Pb18 and Pb03 subunits, respectively; while the Pb03 and Pb18 subunits are 99% identical. *PKA-C2* subunits are also highly conserved in the three *strains analyzed, sharing over 91%amino acid identity (figure 1B). The respective guide tree (figure 1C) places PKA-C subunits from Pb03 and Pb18 closer to each other than to Pb01. This evolutionary evidence is also described for other molecular markers that place Pb01 as a separate Paracoccidioides species* [15,16]. *The extensive conservation of PKA-C1 and PKA-C2 protein sequences* among representatives of these three major phylogenetic species within the *Paracoccidioides* genus suggests that PKA is likely highly conserved in all *Paracoccidioides strains.*

We observe that PKA-C1 are clearly distinct from PKA-C2 subunits, sharing only 29% to 31% amino acid identity. This low identity between PKA-C1 and PKA-C2 subunits is due mainly to the N-terminus present only in the PKA-C2 sequences.

Name	Species (strain)	*Gene name	*Description	Type of subunit	Number of aminoacids	Molecular weight (kDa)	Isoeletricpoint
Pb01PKA-R	*P. lutzii* (Pb01)	PAAG_07887	cAMP-dependent protein kinase regulatory subunit	regulatory	445	47.49	5.75
Pb03PKA-R	*P. brasiliensis* (Pb03)	PABG_07470	Hypotheticalprotein	regulatory	440	47.07	5.55
Pb18PKA-R	*P. brasiliensis* (Pb18)	PADG_08191	Hypotheticalprotein	regulatory	440	47.04	5.64
Pb01PKA-C1	*P. lutzii* (Pb01)	PAAG_04050	Serine/threonine-protein kinase PRKX	catalyticty pe 1	417	47.77	5.56
Pb03PKA-C1	*P. brasiliensis* (Pb03)	PABG_03878	AGC/PKA proteinkinase	catalyticty pe 1	425	48.72	5.99
Pb18PKA-C1	*P. brasiliensis* (Pb18)	PADG_07326	AGC/PKA proteinkinase	catalyticty pe 1	426	48.87	5.99
Pb01PKA-C2	*P. lutzii* (Pb01)	PAAG_00108	cAMP-dependent protein kinase catalytic subunit pkaC	catalyticty pe 2	585	65.08	8.15

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Pb031PKA-C2	*P. brasiliensis* (Pb03)	PABG_0 5134	AGC/PKA proteinkinase	catalyticty pe 2	580	64.45	8.41
Pb018PKA-C2	*P. brasiliensis* (Pb18)	PADG_0 7154	AGC/PKA proteinkinase	catalyticty pe 2	606	67.48	8.74
*http://www.broadinstitute.org/annotation/genome/paracoccidioides_brasiliensis/MultiHome.html							

Table 1: Survey of the PKA subunits encoded in the genomes of *Paracoccidioides* species and strains.
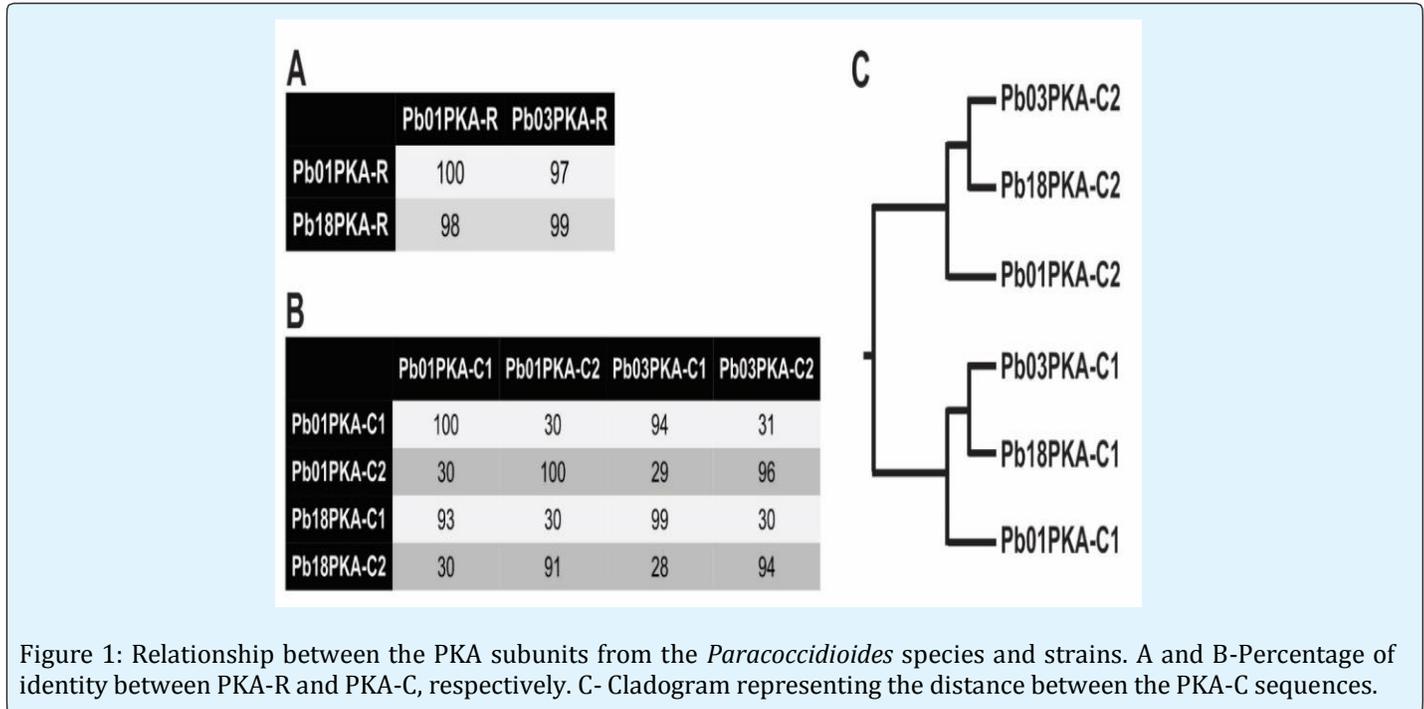
Figure 1: Relationship between the PKA subunits from the *Paracoccidioides* species and strains. A and B-Percentage of identity between PKA-R and PKA-C, respectively. C- Cladogram representing the distance between the PKA-C sequences.

Name	Predicted Localization	Name	Predicted Localization	Name	Predicted Localization
Pb01PKA-R	**78.3 %: nuclear**	Pb01PKA-C1	**60.9 %: cytoplasmic**	Pb01PKA-C2	**60.9 %: nuclear**
	13.0 %: cytoplasmic		26.1 %: nuclear		13.0 %: cytoplasmic
	4.3 %: cytoskeletal		8.7 %: mitochondrial		13.0 %: cytoskeletal
	4.3 %: mitochondrial		4.3 %: vacuolar		8.7 %: Golgi
Pb03PKA-R	**69.6 %: nuclear**	Pb03PKA-C1	**56.5 %: cytoplasmic**		4.3 %: plasma membrane
	17.4 %: mitochondrial		26.1 %: nuclear	Pb031PKA-C2	**73.9 %: nuclear**
	8.7 %: cytoskeletal		13.0 %: mitochondrial		13.0 %: cytoplasmic
	4.3 %: cytoplasmic		4.3 %: vacuolar		8.7 %: cytoskeletal
Pb18PKA-R	**69.6 %: nuclear**	Pb18PKA-C1	**47.8 %: cytoplasmic**		4.3 %: vesiclesofsecretory system

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		30.4 %: nuclear		**69.6 %: nuclear**
17.4 %: mitochondrial		13.0 %: mitochondrial	Pb018PKA-C2	13.0 %: mitochondrial
8.7 %: cytoplasmic		4.3 %: vacuolar		8.7 %: cytoplasmic
4.3 %: cytoskeletal		4.3 %: cytoskeletal		4.3 %: vesiclesofsecretory system

Table 2: Predicted cellular localization of the PKA subunits from *Paracoccidioides* according to the PSORT II program.
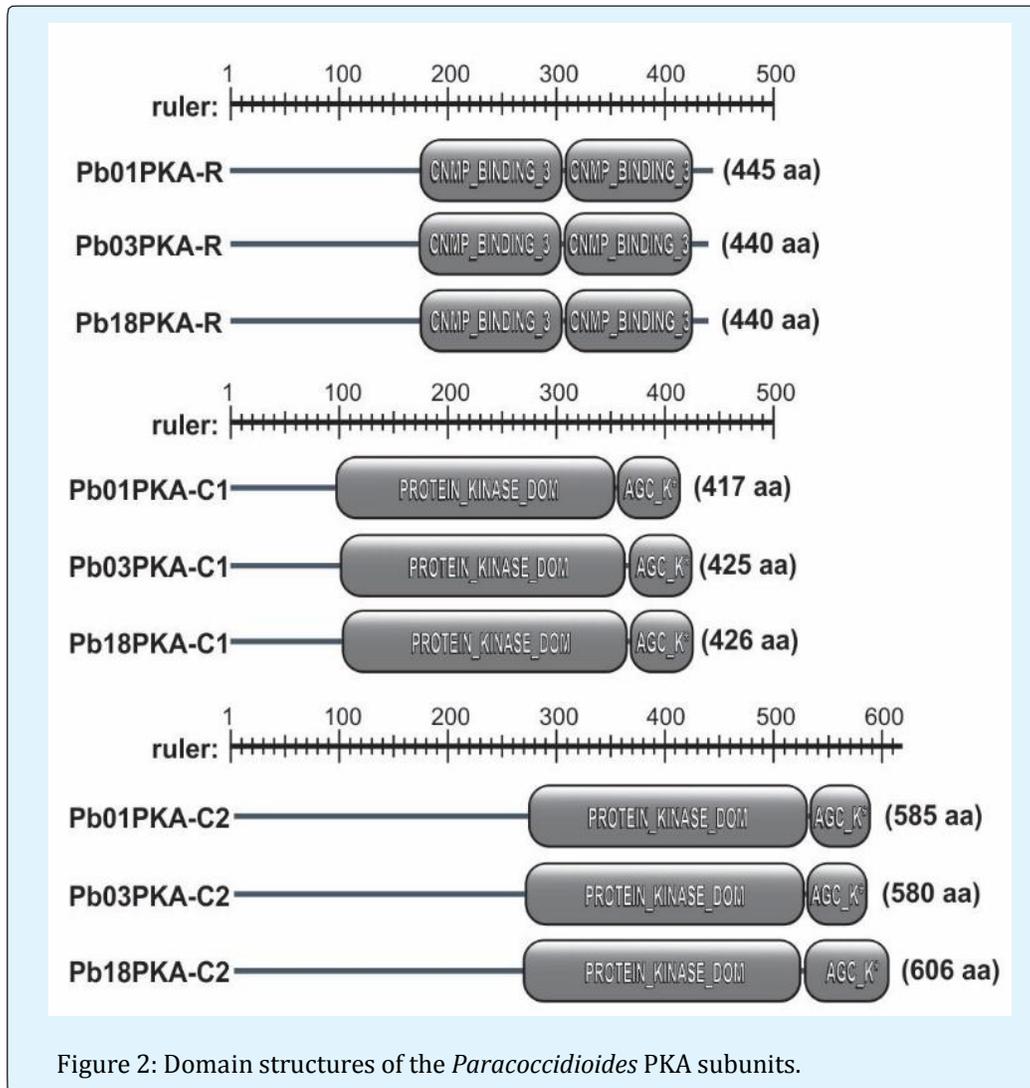

Figure 2: Domain structures of the *Paracoccidioides* PKA subunits.

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PRKACA      MGNAAA---AKK------------------------------------------GSEQE---------SVK------EFL--AKAK----------
PRKACB      MGNAAT---AKK------------------------------------------GSEVE---------SVK------EFL--AKAK----------
PRKACG      MGNAPA---KKD------------------------------------------TEQEE---------SVN------EFL--AKAR----------
PRKX        MEAPGLA--QAA------------------------------------------AAESD---------SR------KV---AEE----------
PRKY        MEAPGPA--QAA------------------------------------------AAESN---------SR------EV---TED----------
Pb01PKA-C1  MAAETI---QGQ------------------------------------SSTSYRP---------------ETDVPAEST---------
Pb01PKA-C2  MRGLGNLLKKKKKRTKDSRISRDLDLSASPVAESTSSSPGDNNNNIIINISSPLSQTTAATATAPATVTEASTTNTLLQHPAFTSPARDSSCKDPASSPG
Pb03PKA-C1  MAAETI---QGQ------------------------------------SSTSYRP---------------EIDVPAEST---------
Pb03PKA-C2  MRGLGNLLKKKKKRTKDSRISRDLDLSASPVAESTSSSPGDNNNNII-NNSSPLFLTTAATATAPATVTAAPTTNTLLQHPAFTSPARDSSCKDPASSPG
Pb18PKA-C1  MAAETI---QGQ------------------------------------SSTSYRP---------------EIDVPAEST---------
Pb18PKA-C2  MRGLGNLLKKKKKRTKDSRISRDLDLSASPVAESTSSSPGDNNNNII-NNSSPLFLTTAATATAPATVTAAPTTNTLLQHPAFTSPARDSSCKDPASSPG
            *                                                                              :.

PRKACA      ----------------------------ED---------FLK------------------------------------------
PRKACB      ----------------------------ED---------FLK------------------------------------------
PRKACG      ----------------------------GD---------FLY------------------------------------------
PRKX        ------------------------------------------------------------------------------------
PRKY        ------------------------------------------------------------------------------------
Pb01PKA-C1  --------------RDIDTVNKK------------------QQ-----QHQQQPQKKSNAEK-PNSL----
Pb01PKA-C2  TNKISSSSNPPASVFRTAIPTSTEPEGVASSPPTGSTTAGAITKIDSTHSAPMNPLQSSGPPSSNIAGDPSHFKQHQQQQQQPQQQSHHSQNVASIKNII
Pb03PKA-C1  --------------RDITVNKK-------------------KQQ-HHHHHHQHPQKKSNGEK-PNSLDLSL
Pb03PKA-C2  TNKISSSSNPPASVFLTAIPTSTKPEGVASSPPTGSTTAGAITKIDSTHSAPMNPLQSPAPPSSNIAGDPSHFKQHQQQQQQPQQQSHHSQNVASIKNII
Pb18PKA-C1  --------------RDIDTVNKK------------------KQQNHHHHHHQHPQKKSNGEK-PNSLDLSL
Pb18PKA-C2  TNKISSSSNPPASVFLTAIPTSTKPEGVASSPPTGSTTAGAITKIDSTHSAPMNPLHSPGPPSSNIAGDPSHFKQHQQQQQQPQQQSHHSQNVASIKNII
            .

PRKACA      ------------------------------KWES--------------P-----AQNTAHLDQFERIKTLGTGSFGRVMLVK---HKE-
PRKACB      ------------------------------KWEN--------------P-----TQNNAGLEDFERKKTLGTGSFGRVMLVK---HKA-
PRKACG      ------------------------------RWGN--------------P-----AQNTASSDQFERLRTLGMGSFGRVMLVR---HQE-
PRKX        -TPDGAPALCPSP-----------------EALSP-------------------EPPVYSLQDFDTLATVGTGTFGRVHLVK---EKT-
PRKY        -AADWAPALCPSP-----------------EARSP-------------------EAPAYRLQDCDALVTMGTGTFGRVHLVK---EKT-
Pb01PKA-C1  DLSLGLDLACPAS-----------------RPETPQDILENRERIRQRLP------AERLSIDDFDLLETVGTGTFARVWLARWAKRKEG
Pb01PKA-C2  NPPLNDEVAASSQRVGDGLHAAQQ-MHSQHSQQQQQQQQQQQQQQQQQQQQQQIQQSVPSVSRQTKGKYSLDDFTLQRTLGTGSFGRVHLVQ---SRH-
Pb03PKA-C1  DLSLGLDLSCPAS-----------------RPETPQDILENRERIRQRLP------AERLNIDDFDLLETVGTGTFARVWLARSVKRKEG
Pb03PKA-C2  NPPLNDEVAASPQPVGDGLHAAQQRMHSQHPQQQQ----QQQ-QQQQQQVQQQSVPSVSRQTKGKYSLDDFTLQRTLGTGSFGRVHLVQ---SRH-
Pb18PKA-C1  DLSLGLDLACPAS-----------------RPETPQDNLENRERIRQRLP------AERLNIDDFDLLETVGTGTFARVWLARLVKRKEG
Pb18PKA-C2  NPPLNDEVAASPQPVGDGLHAAQQRMHSQHPQQQQ----HQQQQQQQQQQQQQQVQQSVPSVSRQTKGKYSLDDFTLQRTLGTGSFGRVHLVQ---SRH-
            .                                     :: *:* *:*.** *.:

PRKACA      TGNHYAMKILDKQKVVKLKQIEHTLNEKRILQAVN-FPFLVKLEFSFKDNSNLYMVMEYVPGGEMFSHLRRIGRFSEPHARFYAAQIVLTFEYLHSLD-L
PRKACB      TEQYYAMKILDKQKVVKLKQIEHTLNEKRILQAVN-FPFLVRLEYAFKDNSNLYMVMEYVPGGEMFSHLRRIGRFSEPHARFYAAQIVLTFEYLHSLD-L
PRKACG      TGGHYAMKILNKQKVVKMKQVEHILNEKRILQAID-FPFLVKLQFSFKDNSYLYLVMEYVPGGEMFSRLQRVGRFSEPHACFYAAQVVLAVQVHSLD-L
PRKX        AKHFFALKVMSIPDVIRLKQEQHVHNEKSVLKEVS-HPFLIRLFWTWHDERFLYMLMEYVPGGELFSYLRNRGRFSSTTGLFYSAEIICAIEYLHSKE-I
PRKY        AKHFFALKVMSIPDVIRRKQEQHVHNEKSVLKEVS-HPFLIRLFWTWHEERFLYMLMEYVPGGELFSYLRNRGHFSSTTGLFYSAEIICAIEYLHSKE-I
Pb01PKA-C1  QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLLDYCPGGEVFTFLRRARCFSERTSQFYAAEIVLILEFLHEVHGV
Pb01PKA-C2  NQRFYAIKVLKKAQVVKMKQVEHTNDERKMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYLHDHH-I
Pb03PKA-C1  QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLLDYCPGGEVFTFLRRAHRFSERTSQFYAAEIVLILEFLHEVHGV
Pb03PKA-C2  NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYLHDHH-I
Pb18PKA-C1  QDGVYAIKILHKADVIKLKQVEHVRNEVRTLAAVSGHPFIISLISTFSDDQNLYMLLDYCPGGEVFTFLRRAHRFSERTSQFYAAEIVLILEFLHEVHGV
Pb18PKA-C2  NQRFYAIKVLKKAQVVKMKQVEHTNDERRMLQRVK-HPFLITLWGTFQDVKNLYMVMDFVEGGELFSLLRKSQRFPNPVAKFYAAEVTLALEYLHDHH-I
            :*:*:       ** :*     ** *  *:: .  .*::  :     ** :::::  ***:*: *:    **.*:: **:    ::**. .:

PRKACA      IYRDLKPENLLIDQQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH
PRKACB      IYRDLKPENLLIDHQGYIQVTDFGFAKRVKGR-TWTLCGTPEYLAPEIILSKGYNKAVDWWALGVLIYEMAAGYPPFFAD-QPIQIYEKIVSGKVRFPSH
PRKACG      IHRDLKPENLLIDQQGYLQVTDFGFAKRVKGR-TWTLCGTPEYLAPEVILSKGYNKAVDWWALGVLIYEMAVGFPPFFAD-QPIQIYEKIVSGRVRFPSH
PRKX        VYRDLKPENILLDRDGHIKLTDFGFAKKLVDR-TWTLCGTPEYLAPEVIQSKGHGRAVDWWALGILIFEMLSGFPPFFDD-NPFGIYQKILAGKIDFPRH
PRKY        VYRDLKPENILLDRDGHIKLTDFGFAKKLVDR-TWTLCGTPEYLAPEVIQSKGHGRAVDWWALGILIFEMLSGFPPFFDD-NPFGIYQKILAGKLYFPRH
Pb01PKA-C1  AYRDLKPENILLDAEGHLKLVDFGFAKQLWSRETYTLCGTPEYLAPEVIHNSGHGLAVDWWALGVLIYEFIVGQPPFWDQ-NPMRIYEQIVQGRLRFPVN
Pb01PKA-C2  VYRDLKPENLLLDRYGHLKITDFGFAKEVKDI-TWTLCGTPDYLAPEVVSSKGYNMSVDWWSLGILIFEMLCGFTPFWDGGSPMKIYENIIKCRVKYPSY
Pb03PKA-C1  AYRDLKPENILLDAEGHLKLVDFGFAKQLWSRETYTLCGTPEYLAPEVIHNKGHGLAVDWWALGVLIYEFIVGQPPFWDQ-NPMCIYEQIVQGRLRFPVN
Pb03PKA-C2  IYRDLKPENLLLDRYGHLKITDFGFAKEVKDI-TWTLCGTPDYLAPEVVSSKGYNMSVDWWSLGILIFEMLCGFTPFWDGGSPMKIYENIIKCRVKYPSY
Pb18PKA-C1  VYRDLKPENILLDAEGHLKLVDFGFAKQLWSRETYTLCGTPEYLAPEVIHNKGHGLAVDWWALGVLIYEFIVGQPPFWDQ-NPMCIYEQIVQGRLRFPVN
Pb18PKA-C2  IYRDLKPENLLLDRYGHLKITDFGFAKEVKDI-TWTLCGTPDYLAPEVVSSKGYNMSVDWWSLGILIFEMLCGFTPFWDGGSPMKIYENIIKCRVKYPSY
            :*******:*:*  *::::.******.:  .  *:******:*****::: ..*:. :****:**:**:*:  * .*:  .*: **::*:  :: :*

PRKACA      FSSDLKDLLRNLLQVDLTKRFGNLKNGVNDIKNHKWFATTDWIAIYQRKVEAPFIPKFKG-PGDTSNFDDYEEEI-------RVSINEKC---------
PRKACB      FSSDLKDLLRNLLQVDLTKRFGNLKNGVSDIKTHKWFATTDWIAIYQRKVEAPFIPKFRG-SGDTSNFDDYEEEDI-------RVSITEKC---------
PRKACG      LSSDLKHLLRSLLQVDLTKRFGNLRNGVGDIKNHKWFATTSWIAIYEKKVEAPFIPKYTG-PGDASNFDDYEEEEL-------RISINEKC---------
PRKX        LDFHVKDLIKKLLVVDRTRRLGNMKNGANDVKHHRWFRSVDWEAVPQRKLKPPIVPKIAG-DGDTSNFETYPENDW-----DTAAPVPQKD---------
PRKY        LDFHVKT----------------------------------------------------------------------------------------
Pb01PKA-C1  MPPAVRDIVSQLCTTNPSERLGYIKGGAARVKQHPFFKDINWDDIYFRRTKGPIVPRVDS-PTDTGNFEEYPDEPDSSLLTPYTKEMREKH---------
Pb01PKA-C2  MHPDAQDLLSQLITPDLTVRLGNLHGGSQDVKNHPWFAEVTWDRLARKDIDAPYIPPVKGGQGDASQFDRYPEETE-----PYGGSGDDPY---------
Pb03PKA-C1  MPSTARDIVTQLCTTNPSERLGYIKGGAARVKQHPFFKDINWDDIYFRRTKGPIVPRVDS-PTDTGNFEEYPDEPDSSLLTPYTKEMREKH---------
Pb03PKA-C2  MHPDAQDLLSQLITPDLTVRLGNLHGGSKDVKDHPWFAEVTWDRLARKDIDAPYIPPVKGGQGDASQFDRYPEETE-----PYGGSGDDPY---------
Pb18PKA-C1  MPSTARDIVTQLCTTNPSERLGYIKGGAARVKQHPFFKDINWDDIYFRRTKGPIVPRVDS-PTDTGNFEEYPDEPDSSLLTPYTKEMREKH---------
Pb18PKA-C2  MHPDAQDLLSQLITPDLTVRLGNLHGGSKDVKDHPWFAEVTWDRLARKDIDAPYIPPVKGGQGDASQFDRYPEETE-----PYGGSGDDPYVSPSFPSRL
            :      :

PRKACA      ---------------GKEFSEF
PRKACB      ---------------AKEFGEF
PRKACG      ---------------AKEFSEF
PRKX        ---------------LEIFKNF
PRKY        ----------------GRMM
Pb01PKA-C1  ---------------DDMFADF
Pb01PKA-C2  ---------------GNYFVDF
Pb03PKA-C1  ---------------DAMFADF
Pb03PKA-C2  ---------------GDYFVDF
Pb18PKA-C1  ---------------DAMFADF
Pb18PKA-C2  LQRRSFTNDVIDMAIISWTFEWG
```

S1 Figure: Multiple alignments of the *Paracoccidioides* and human PKA-C subunits. This analysis was performed using the t-coffee program at http://www.ebi.ac.uk/Tools/msa/tcoffee/. The N-terminus present only in PbPKA-C2 subunits is highlighted in yellow. The "*", "." and ":" indicate the conserved residues. Observe that human and *Paracoccidioides* PKA-C are highly divergent both in the N- and C-terminus (highlighted in color).

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Predicted cellular localization of the *Paracoccidioides* PKA subunits

Subcellular localization data gives important clues to understanding protein function. The cellular localization of PKA is decisive in determining which substrates are phosphorylated. The *in silico* prediction suggests that *Paracoccidioides* PKA subunits can be found in more than one subcellular location, as shown in table2. It seems that PKA-C1 subunits act mainly in the cytosol, while the PKA-C2 is transported to the nucleus. Psort II prediction also states a possibility that these subunits might be placed in other subcellular location, like mitochondria and secretory vesicles. However, these data need to be experimentally confirmed.

So far, there is only one study demonstrating the location of a PKA subunit in *Paracoccidioides.* It has been demonstrated that Pb01PKA-C2 goes to the nucleus where it phosphorylates transcription factors, altering the expression of genes that control the morphological changes in this fungus [14].

Several studies have demonstrated that the localization of PKA subunits may change depending on the external stimulus and also over lifecycle [25,26]. For instance, in *S. cerevisiae* cells cultured in the presence of glucose, during exponential growth, both Bcy1 and Tpk2 are localized in the nucleus, whereas Tpk1 and Tpk3 display a mixed pattern of nucleo-cytoplasmic localization; but in cells grown on glycerol and during stationary phase, the PKA subunits exhibited a cytoplasmic localization [26].
In multicellular organisms, the localization of PKA holoenzymes depends on the interaction with A kinase anchoring proteins (AKAPs). These proteins target PKA to specific subcellular locations, conferring spatio-temporal control of PKA signaling [27]. Nevertheless, AKAP proteins have not been identified in fungi and, so, the knowledge of the spatio-temporal control of PKA in these organisms is restricted.

Protein domains in the PKA subunits

The protein domains were scanned in the PROSITE database and the results are depicted in figure 2. The *Paracoccidioides* PKA subunits present the canonical domains both for thePKA-R and the PKA-C subunits.

Each PKA-R subunit presents two tandem copies of the cyclic nucleotide monophosphate-binding (CNMP) domain at the carboxyl terminus. The CNMP-binding is a structural domain of about 120 residues present in proteins that bind the cyclic nucleotides cAMP or cGMP. Prior to activation, PKA isoforms are maintained in an inactive state by the formation of an heterotetrameric complex consisting of two PKA-C bound to two PKA-R subunits. The cooperative binding of two cAMP molecules to the CNMP-binding domains mediate the allosteric activation of the holoenzyme by releasing the PKA-C subunits [28].

All the *Paracoccidioides* PKA-C subunits present 2 protein domains: a protein kinase and an AGC-kinase C-terminal domain. The protein kinase domain is a structurally conserved protein domain involved in the transfer of the gamma phosphate group to amino acid residues in proteins. This event results in conformational changes that turns on/off many cellular processes, like cell cycle progression, transcription, metabolism, cytoskeletal rearrangement, apoptosis, and differentiation [29]; The AGC-kinase C-terminal domain, where AGC stands for cAMP-dependent, cGMP-dependent and protein kinase C protein kinase family, is found in several protein kinases. AGC kinase proteins exhibit three conserved phosphorylation sites that regulate their function. These sites serve as phosphorylation-regulated switches to control both intra- and inter-molecular interactions [30] Although both PbPKA-C1 and PbPKA-C2 present the same domains, differences in the expression pattern, protein activity and subcellular localization may confer specificity of PKA signaling to different stimulus and environmental conditions.

Comparative alignment of human and *Paracoccidioides* PKA subunits

Multiple sequence analysis was used to create a branched tree that displays the relationship between *Paracoccidioides* and human PKA catalytic subunits (figure 3). This analysis clearly indicates a distinction between PKA in these two organisms. The multiple alignments shows that, although there is a moderate degree of conservation between the human and *Paracoccidioides* PKA subunits, a significant difference in both the N-and C-terminus can be observed (S1 figure). This divergence between human and *Paracoccidioides* PKA protein sequence, coupled with the central role that this protein plays in many cellular processes, makes PKA an attracting target for development of antifungal drugs.
To date, there are few studies on the *Paracoccidioides* PKA function and interactions. Further work is required to identify and characterize the expression, activity and cellular targets of PKA in these fungi.
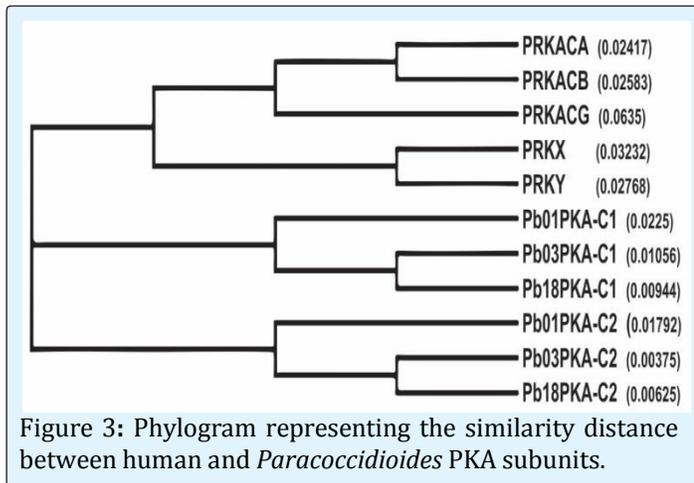
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Figure 3: Phylogram representing the similarity distance between human and *Paracoccidioides* PKA subunits.

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