

Cyclic Nucleotides as Mediators of Acinar and Ductal Function

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1. Cyclic Nucleotides and their Biosynthesis

Cyclic nucleotides, like other nucleotides, are composed of three functional groups: a ribose sugar, a nitrogenous base, and a single phosphate group. There are two types of nitrogenous bases: purines (adenine and guanine) and pyrimidines (cytosine, uracil and thymine). A cyclic nucleotide, unlike other nucleotides, has a cyclic bond arrangement between the ribose sugar and the phosphate group. There are two main groups of cyclic nucleotides: the canonical or well-established and the non-canonical or unknown function cyclic nucleotides. The two well-established cyclic nucleotides are adenosine 3',5'-cyclic monophosphate (cyclic AMP) and guanine-3',5'-cyclic monophosphate (cyclic GMP). Both cyclic AMP and cyclic GMP are second messengers. The non-canonical cyclic nucleotides include the purine inosine 3',5'-cyclic monophosphate (cyclic IMP), xanthosine 3',5'-cyclic monophosphate (cyclic XMP) and the pyrimidine cytidine 3',5'-cyclic monophosphate (cyclic cCMP), uridine-3',5'-cyclic monophosphate (cyclic UMP), thymidine 3',5'-cyclic monophosphate (cTMP) (128). An overview of the non-canonical cyclic nucleotides is provided in Section 3.

A cyclase enzyme (lyase) catalyzes the formation of the cyclic nucleotide from its nucleotide triphosphate precursor (**Figure 1**). Cyclic nucleotides form when the phosphate group of the molecule of nucleotide triphosphate (ATP or GTP) is linked to two different hydroxyl (3' and 5') groups of the ribose and forms a cyclic ring and releases

pyrophosphate. This cyclic conformation allows cyclic nucleotides to bind to proteins to which other nucleotides cannot. The reaction is an intracellular nucleophilic catalysis and requires Mg^{2+} as a cofactor, whose function is to decrease the overall negative charge on the ATP by complexing with two of its negatively charged oxygens. If its negative charge is not reduced, the nucleotide triphosphate cannot be approached by a nucleophile, which is, in this reaction, the 3' hydroxyl group of the ribose (162). Soluble AC prefers Ca^{2+} to Mg^{2+} as the coenzyme to coordinate ATP binding and catalysis (135).

2. Canonical Cyclic Nucleotide Signaling in the Exocrine Pancreas

Cyclic nucleotide signaling can be initiated by two general mechanisms. One mechanism is the binding of an extracellular ligand to a transmembrane G protein-coupled receptor (GPCR). The receptor protein forms seven transmembrane α helices connected by alternating cytosolic and extracellular loops. The N-terminus is located in the extracellular space, whereas the C-terminus is located in the cytosol. The extracellular domain has a ligand-binding site and the cytosolic domain has a heterotrimeric G protein-binding site (112). After ligand binding the GPCR, it activates a heterotrimeric G protein, which is composed of three subunits: a guanine nucleotide binding α subunit, and a $\beta\gamma$ heterodimer (88). Depending on the G protein, the transmembrane cyclase can be activated (Gas protein subunit) or inhibited (Gai protein subunit).

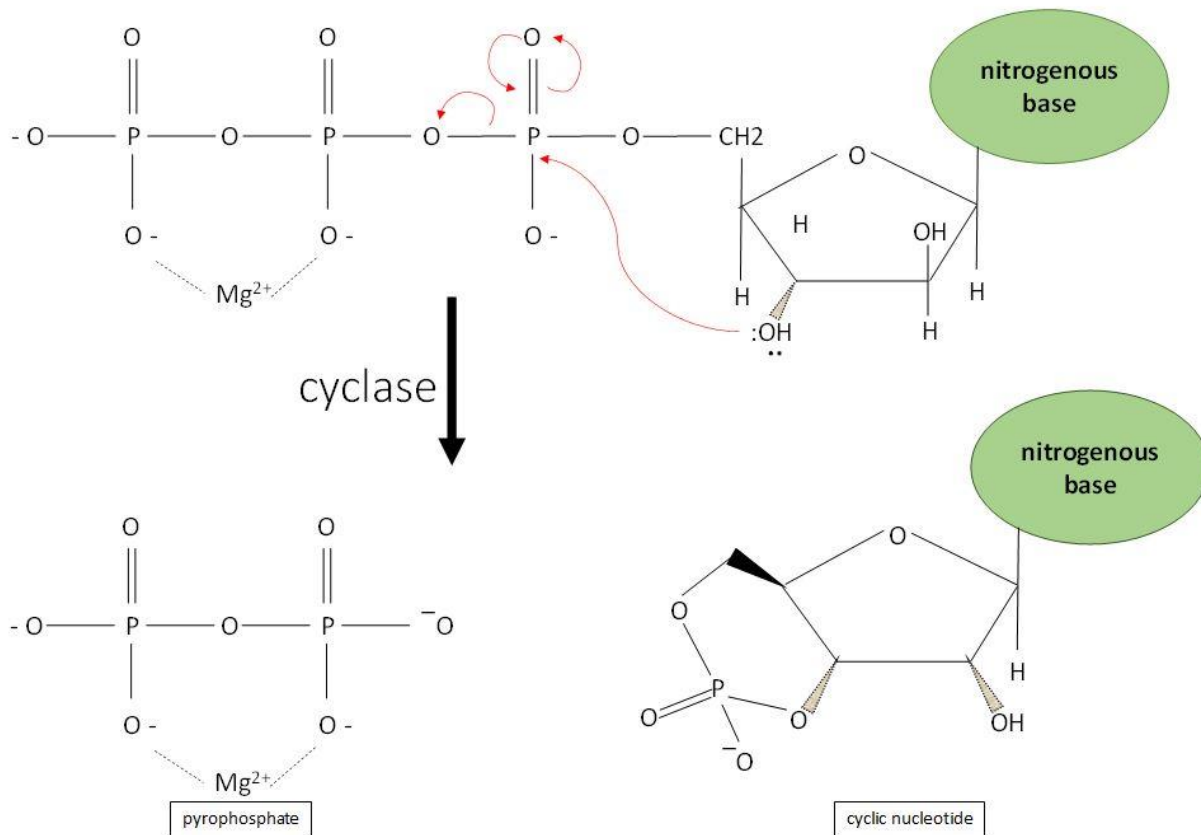


Figure 1. Two aspartic acid residues in the active site of the cyclase (AC or GC) promotes the binding of ATP. Two Mg^{2+} ions are required to decrease the overall negative charge on the ATP by complexing with two of its negatively charged oxygen. Mg^{2+} is involved in the deprotonation of the 3' hydroxyl group in the ribose ring of ATP. Soluble AC uses Ca^{2+} rather than Mg^{2+} as a coenzyme. This step is necessary for the nucleophilic catalysis on the 5' α -phosphate by the newly formed oxyanion. The end products of this catalytic reaction are a cyclic nucleotide (cyclic AMP or cyclic GMP) and a pyrophosphate group.

The second mechanism results from the binding of a signaling molecule to a soluble cyclase. The signal can be either extracellular, such as nitric oxide (NO) (102), or intracellular, such as bicarbonate (166). The signaling by an extracellular ligand is limited by its ability to cross the plasma membrane. In the cytosol, the signal binds the heme-binding domain of the soluble cyclase. The cyclase, in turn, increases the intracellular levels of cyclic nucleotides (74, 134).

In the exocrine pancreas, adenylyl cyclases can be activated by either extracellular or intracellular signals. The extracellular signals can be a neurotransmitter, such as vasoactive intestinal polypeptide (VIP), a hormone, such as secretin (143), or a gas, such as NO (160). Intracellular

signals include HCO_3^- (75). The increase in the cyclic nucleotide levels modifies the activity of downstream effectors such as kinases (59, 141), guanine-nucleotide-exchange factor (GEF) (32), RNA-binding protein (50), ion channels (69) and phosphodiesterases (27), which are discussed later in this chapter.

2.1 Adenylyl Cyclase/ Cyclic AMP Signaling

Cyclic AMP is formed from cytosolic ATP by the enzyme adenylyl cyclase. There are ten isoforms of adenylyl cyclases; nine are anchored in the plasma membrane, with its catalytic portion protruding into the cytosol, and one is soluble (140).

Transmembrane AC

The nine transmembrane AC isoforms are each coded by a different gene (**Figure 2**). The human *ADCY1* gene is located on chromosome 7 at p12.3, human *ADCY2* gene on chromosome 5 at p15.3, human *ADCY3* gene on chromosome 2 at p23.3, human *ADCY4* gene on chromosome 14 at q12, human *ADCY5* gene on chromosome 3 at q21.1, human *ADCY6* gene on chromosome 12 at q12-q13, human *ADCY7* gene on chromosome 16 at q12.1, human *ADCY8* gene on chromosome 8 at q24, human *ADCY9* gene on chromosome 16 at p13.3 (113). All of transmembrane AC isoforms share a high sequence homology in the primary structure of their catalytic site and the same three-dimensional structure. The AC structure can be divided in two main regions, transmembrane and cytoplasmic regions, and further divided into five different domains: 1) the NH₂ terminus, 2) the first transmembrane cluster (TM1), 3) the first cytoplasmic loop composed of C1a and C1b, 4) the second transmembrane cluster (TM2) with extracellular N-glycosylation sites, and 5) the second cytoplasmic loop composed of C2a and C2b. The transmembrane regions are composed of twelve membrane-spanning helices, which cross the plasma membrane in 2 clusters of 6 TM domains (TM1 and TM2), whose function is to keep the enzyme anchored in the membrane. The cytoplasmic regions C1 and C2 are approximately 40 kDa each and can be further subdivided into C1a, C1b, C2a, and C2b. Both C1a and C2a are highly conserved catalytic ATP-binding regions (28), which dimerize to form a pseudosymmetric enzyme, which forms the catalytic site. ATP binds at one of two pseudosymmetric binding sites of the C1-C2 interface. Two amino acid residues, Asn1025 and Arg1029 of AC2 are conserved among the C2 domains and critical for the catalytic activity of AC; mutation of either residue causes in a 30-100-fold reduction in the AC activity (157). A second C1 domain subsite includes a P-loop that accommodates the nucleotide phosphates and two conserved acid residues that bind to ATP through

interaction with two Mg²⁺; one Mg²⁺ contributes to catalysis, whereas the second one interacts with nucleotide β- and γ-phosphates from substrate binding and possibly also for leaving-group stabilization. Both C2a and C2b are less conserved than the C1 domain (28, 78). The C1b domain is the largest domain, contains several regulatory sites and has a variable structure across the isoforms. However, the C2b domain is essentially non-existent in many isoforms, and has not yet been associated with a function (164). The overall domain structure of each human transmembrane AC isoform is shown in **Figure 2** and a detailed comparison of the cytoplasmic domains (C1 and C2), transmembrane segments, acetylation, phosphorylation and ubiquitination sites of each isoform is indicated. **Figure 3** shows the three dimensional model of AC and its relation to heterotrimeric G protein α subunit.

Without stimulation, the enzyme AC is constitutively inactive. There are at least two heterotrimeric G proteins responsible for the regulation of transmembrane AC activity: G_s and G_i. When a secretagogue (for example: secretin, vasoactive intestinal polypeptide) binds to its GPCR, it causes a change in the conformation of the receptor that stimulates the G_sα subunit to release GDP and acquire a GTP, which causes GTP-G_sα to detach from the G_sβγ subunits and bind to the two cytoplasmic regions transmembrane AC (39). With GTP-G_sα in its structure, AC becomes active and converts ATP to cyclic AMP in a process involving release of water and a pyrophosphate. G_sα has shown to play an important role in the exocrine pancreas and G_sα-deficient mice show morphological changes in exocrine pancreas, as well as malnutrition and dehydration (153). Certain isoforms of transmembrane ACs are also positively (AC2, AC4, AC5, AC6, AC7) or negatively (AC1, AC3, AC8) regulated by the G_sβγ subunits, which also bind to the two cytoplasmic regions of transmembrane AC (39).

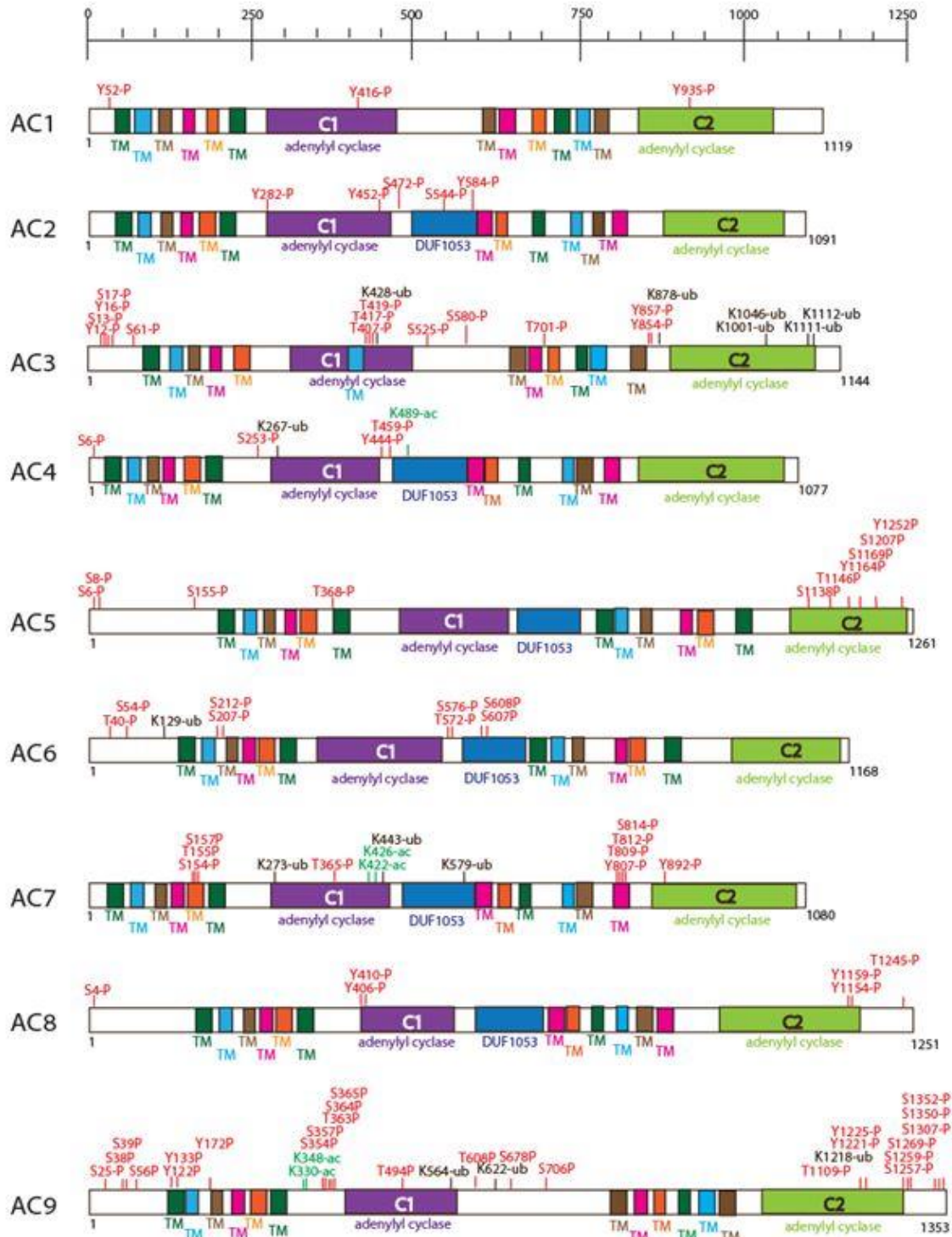


Figure 2. Schematic representation of the structure of the 9 human transmembrane AC isoforms. The number of amino acid residues is reported on the side of each structure. Modification sites and domains are represented with different color. The transmembrane AC isoforms share a common structure composed of two cytosolic domains (C1 and C2) and 6-transmembrane segments organized in two tandem repeats. Both C1 and C2 domains contribute to ATP binding and formation of the catalytic core. Abbreviations: TM: transmembrane segments; DUF: domain of unknown function; ac: acetylation; P: phosphorylation site; ub: ubiquitination; S: serine; K: lysine; T: Threonine; Y: Tyrosine. Data obtained from PhosphoSitePlus.

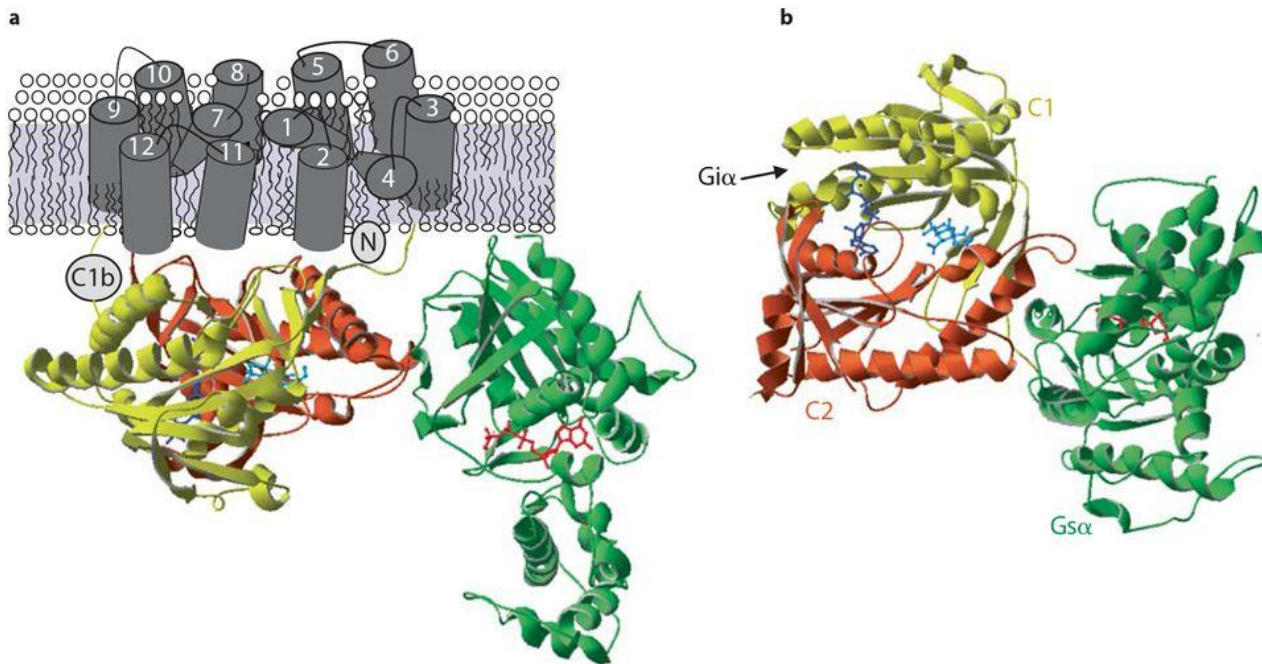


Figure 3: Crystal structure of Adenylyl Cyclase. a) The figure shows the catalytic domains of mammalian AC (C1 and C2) with $G_{\alpha s}$ (green) and $G_{\alpha i}$ (yellow). The location of forskolin (cyan) and P-site inhibitor (dark blue) is also appreciated. b) An alternate view from cytoplasmic side, showing forskolin and catalytic site. The interaction site of $G_{\alpha i}$ with C1 domain is indicated by an arrow. This figure was reproduced with permission from (122).

When a GPCR is coupled to the heterotrimeric protein G_i , $GTP-G_{\alpha i}$ binds to adenylyl cyclase and, unlike $GTP-G_{\alpha s}$, $GTP-G_{\alpha i}$ inhibits the activity of the enzyme, causing lower levels of cyclic AMP in the cells. In pancreas, somatostatin binds to its SS2 receptor and causes activation of $G_{\alpha i}$ subunit and inhibition of adenylyl cyclase (97, 132). Once the concentration of the ligand is below activation levels, the G_{α} subunit, which has an intrinsic GTPase activity, hydrolyzes GTP to GDP, re-associates with $G_{\beta\gamma}$ and becomes inactive. The cycle of GTP hydrolysis and inactivation occur within seconds after the G protein has been activated. Upon inactivation, G proteins are ready to be reactivated by another extracellular signal. Transmembrane ACs are classified into four groups based on their regulatory properties (**Table 1**):

- Group I, which consists of Ca^{2+} -stimulated isoforms: AC1, AC3, AC8.
- Group II, which consists of $G_{\beta\gamma}$ -stimulated isoforms: AC2, AC4, AC7.

- Group III, which consists of $G_{\alpha i}/Ca^{2+}$ -inhibited isoforms: AC5, AC6.
- Group IV, which consists of Ca^{2+} -, $G_{\beta\gamma}$ -insensitive isoform: AC9.

The expression profile of the transmembrane AC isoforms in intact mouse pancreas, isolated pancreatic acini and duct fragment has been established using RT-PCR. Five different transmembrane AC isoforms were identified in pancreatic exocrine cells: AC3, AC4, AC6, AC9 mRNAs were expressed in isolated pancreatic acini and sealed duct fragments, whereas AC7 mRNAs was only expressed in duct fragments (116). Using real-time quantitative PCR analysis, the relative expression of each isoform in pancreatic acini and ducts compared to the intact pancreas was assessed: isolated pancreatic acini were shown to have higher transcript levels of AC6 compared with intact pancreas, whereas isolated duct fragments were shown to have higher transcript levels of AC4, AC6 and AC7 compared with intact pancreas.

Table 1. Regulatory properties of Adenylyl Cyclase isoforms								
AC isoform	MW (kDa) ^a (mouse)	basal pI	G α s	G α i	G $\beta\gamma$	FSK	Calcium	Protein Kinases
Group I:							Calcium-stimulated	
AC1	123.37	8.77	(+)	(-)	(-)	(+)	(+, CaM) or (-, CaM kinase IV)	(+, PKC α)
AC3	129.08	6.15	(+)	(-)	(-)	(+)	(+, CaM) or (-, CaM kinase II) (+, CaM)	(+, PKC α)
AC8	140.1	6.53	(+)	(-)	(-)	(+)	(+, CaM) or (-, CaM kinase II) (+, CaM)	(=)
Group II:							Calcium-insensitive	
AC2	123.27	8.4	(+)	(=)	(+)	(+)		(+, PKC α)
AC4	120.38	7.31	(+)	(=)	(+)	(+)		(+, PKC) or (-, PKC α)
AC7	122.71	8.49	(+)	(=)	(+)	(+)		(+, PKC δ)
Group III:							Calcium-inhibited	
AC5	139.12	6.9	(+)	(-)	(+, β 1 γ 2)	(+)	(-, < 1 μ M)	(-, PKA ^b) (+, PKC α / ζ)
AC6	130.61	8.56	(+)	(-)	(+, β 1 γ 2)	(+)	(-, < 1 μ M)	(-, PKA ^b , PKC δ , ϵ)
Group IV:								
AC9	150.95	7.07	(+)	(-)	(=)	(=) or +, weak	(+, CaM kinase II) (-, calcineurin)	(-, novel PKC)
AC10	186/ 48 ^c	6.99	(=)	(=)	(=)	(=)	Calcium-stimulated	(=)

Table 1: Regulatory properties of AC isoforms. (+): AC activity is stimulated; (-): AC activity is inhibited; (=): AC activity is not modified. Data taken from (12, 33, 103, 150). (a) The molecular weight (MW) and basal isoelectric point (pI) data was obtained from PhosphoSitePlus from Cell Signaling Technology, Inc. (b) AC6 is directly phosphorylated by PKA at Ser674, and thereby inhibited (25). (c) 186 kDa: the full length form and 48 kDa: the truncated form.

Similar transcript levels of AC3 and AC9 were observed in pancreas, acini and ducts (116). In conclusion, several adenylyl cyclase isoforms are expressed in pancreatic exocrine cells, with AC6 being highly expressed in both pancreatic acinar and duct cells.

Soluble AC

Soluble AC, AC10, is unique in some many ways. The human *ADCY10* gene is located on chromosome 1 at q24. It not anchored in the plasma membrane. As indicated in the **Figure 4**, the catalytic domains C1 and C2 are located at the N-terminus and connected by a ~68 residue linker that forms a death domain like subdomain with the ~33 residue N-terminus of the protein. The C-terminal from this C1-C2 tandem of the full-length mammalian soluble AC comprises a ~1100 residue C-terminal region without a transmembrane region

(73). Unlike transmembrane AC, soluble AC has no transmembrane domain. Its catalytic domain sequence is more closely related to some bacterial ACs than mammalian ACs (12). For that reason, its location is in the cytosol, but can be associated with certain cellular organelles, such as the nucleus, mitochondria and microtubules (165). Unlike transmembrane ACs which are regulated by G proteins, forskolin and calmodulin among others, soluble AC is stimulated by HCO₃⁻ (166). The HCO₃⁻ ion induces a conformational change of the active site of soluble AC similar to that observed in transmembrane ACs upon stimulation with G α s (135). Using RT-PCR and Western-blotting, soluble AC has also been identified in acinar cells. By immunohistochemistry using a soluble AC antibody, AC10 has been localized just below the apical region of the cell in non-stimulated condition

and, after treatment with the CCK analog caerulein, a punctuate intracellular pattern was seen (75).

In pancreatic acini, the activation of soluble AC with HCO_3^- enhances secretagogue-stimulated cyclic AMP levels and inhibits secretagogue-stimulated zymogen activation and cell vacuolization (75).

2.2 Intracellular Targets of cyclic AMP

All the protein targets described below have a cyclic nucleotide-binding domain (CNBD) that has been conserved across a wide range of proteins, including the bacterial transcription factor catabolite activator protein (CAP) (146).

Protein kinase A

Cyclic AMP stimulates protein kinase A (PKA), which phosphorylates a number of cellular proteins by transferring a phosphate from ATP to a serine or a threonine located in sequence of residue of target protein. PKA contains two regulatory subunits, which possess the cyclic nucleotide binding domain (CNBD), and two catalytic subunits, which are responsible for the Ser/Thr phosphorylation. Upon binding of cyclic AMP to the two regulatory subunits, the two catalytic subunits are detached from the regulatory subunits and become active (141). The steps implicated in the activation of PKA by cyclic AMP are described in the **Figure 5**.

The presence of PKA in pancreas was first reported in acinar cells from guinea pig (66). PKA catalyzes the phosphorylation of regulatory

proteins associated with the pancreatic exocytotic process (14, 15). However, PKA does not appear to directly participate in pancreatic amylase secretion because the inhibitor of PKA, H-89, does not modify either basal or cyclic AMP-dependent secretagogues-stimulated amylase secretion from mouse pancreatic acini (115). Unlike in mouse acinar cells, in sealed mouse ducts PKA plays an essential role in the regulation of fluid secretion (116).

One of the important targets of PKA is the transcription factor cyclic AMP response element binding protein (CREB). Similar to other cell types, in pancreatic acini CREB phosphorylation at Ser133 increases upon PKA activation (115). The phosphorylation of CREB promotes the formation of a transcriptional complex on the cyclic AMP (cyclic AMP) response element (CRE) of certain promoters. The complex contains three proteins: 1) CREB, 2) the CREB-binding protein (CBP) and 3) CREB-regulated transcription coactivator 2. Its role is to stimulate the gene expression of certain proteins implicated in the regulation of metabolism, signaling, proliferation, differentiation, survival and oncogenesis.

Other important targets of PKA are the cystic fibrosis transmembrane conductance regulator (CFTR), 1, 4, 5-inositol trisphosphate receptor (IP3R), A-kinase anchoring proteins (AKAPs), ERK 1/2, and some isoforms of phosphodiesterase (PDE).

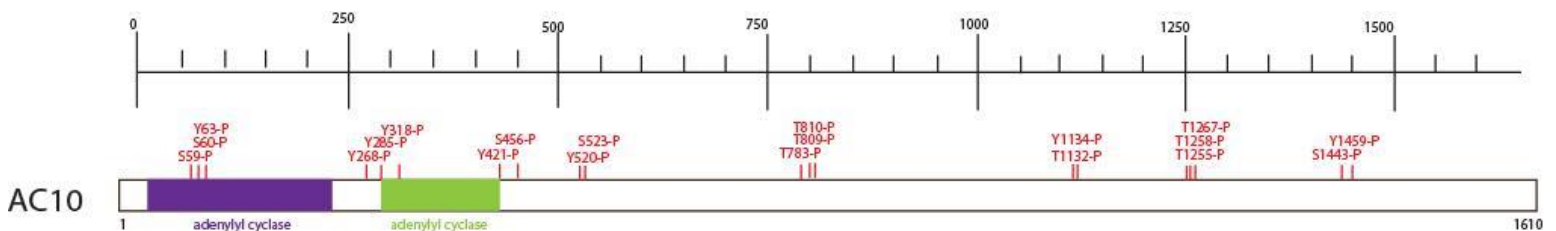


Figure 4: Schematic representation of the structure of the soluble AC isoform, AC10. The number of amino acid residues is reported on the side of structure. Modification sites and domains are represented with different color. Abbreviations: p: phosphorylation site; S: serine; K: lysine; T: Threonine; Y: Tyrosine. Data obtained from PhosphoSitePlus.

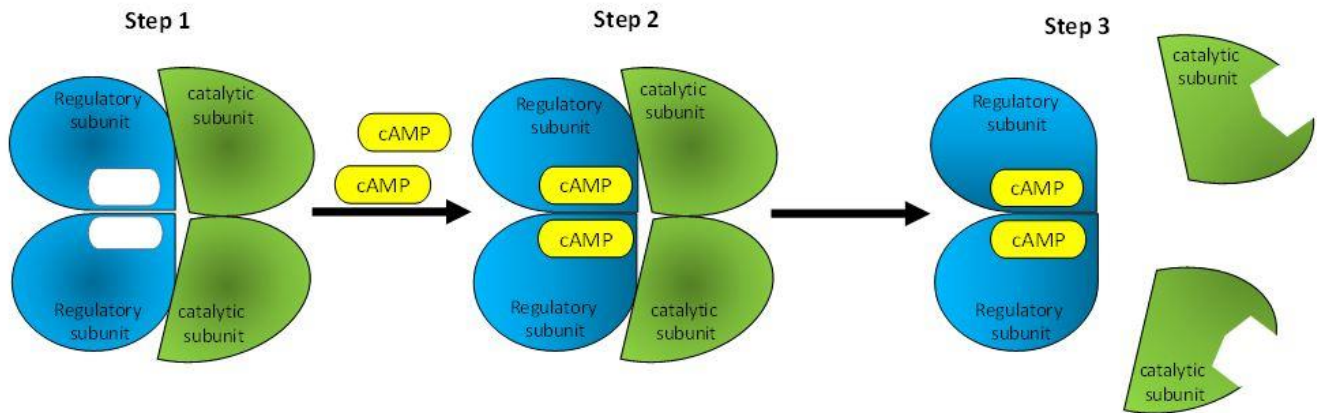


Figure 5: Schematic representation of the activation of protein kinase A (PKA) by cyclic AMP. PKA is composed of four subunits: two regulatory and two catalytic. In the absence of cyclic AMP, the regulatory subunit inhibits the catalytic subunit. Upon binding of cyclic AMP to regulatory subunits, the regulatory subunits change conformation. The catalytic subunits become detached and able to phosphorylate target proteins in the cell.

In pancreatic duct cells, PKA phosphorylates CFTR at the regulatory domain in the apical membrane, which then enables channel gating (opening and closing) and Cl^- secretion (3). Cyclic AMP evokes Cl^- currents of CFTR, which mediates fluid transport across the luminal surfaces of pancreatic epithelial cells (19). In pancreatic acinar cells, PKA phosphorylates only one of the three IP_3R isoforms, $\text{IP}_3\text{R}-3$ (84, 136). The phosphorylation of $\text{IP}_3\text{R}-3$ by PKA causes IP_3 -induced Ca^{2+} release, which is decreased in terms of the magnitude and kinetics of Ca^{2+} release (47, 136). Another important target of PKA are the A-kinase anchor proteins (AKAPs), which are a family of structurally related proteins consisting of more than 50 members (16). AKAP-150 has been shown to play a relevant role in the regulation of Na^+/K^+ ATPase pump activity in the homologous parotid gland (83, 123). Cyclic AMP increases the activity of phosphodiesterases PDE3, PDE4, and PDE5 through PKA-induced phosphorylation (27, 163). Both PDE3 and PDE4 are cyclic AMP-specific PDEs, whereas PDE5 is a cyclic GMP-specific PDE (27).

Exchange protein directly activated by cyclic AMP (Epac)

Cyclic AMP stimulates Epac (32). There are two isoforms of Epac: Epac1 and Epac2 (142). Both

isoforms are homologous proteins with Epac2 having a N-terminal extension. They share common domain structures within a N-terminal regulatory region and a C-terminal catalytic domain (Figure 6) (10, 48, 60). The N-terminal regulatory region possesses one (Epac1) or two (Epac2) cyclic nucleotide-binding domains (CNBD) and a DEP (Dishevelled, Egl-10, and Pleckstrin) domain responsible for its localization to the plasma membrane. The C-terminal region contains CDC25-homology domain, a REM (Ras exchange motif) domain required for stabilizing GEF activity, and the GEF domain, which exerts GEF activity toward the small G proteins Rap1 and Rap2 (22). Epac1 is found in both pancreatic acini and ducts (20, 115, 116) and participates in cyclic AMP-stimulated amylase secretion (20, 115). Epac activates Rap1 (32), which is a small G protein localized on zymogen granules as shown by both mass spectrometry and immunocytochemistry (23) and implicated in pancreatic amylase secretion (115). In addition to its role in pancreatic amylase secretion, Epac regulates exocytosis in pancreatic beta cells. Incretin-induced insulin secretion is mediated by Epac2, the primary isoform of Epac in pancreatic beta cells (68, 107, 129).

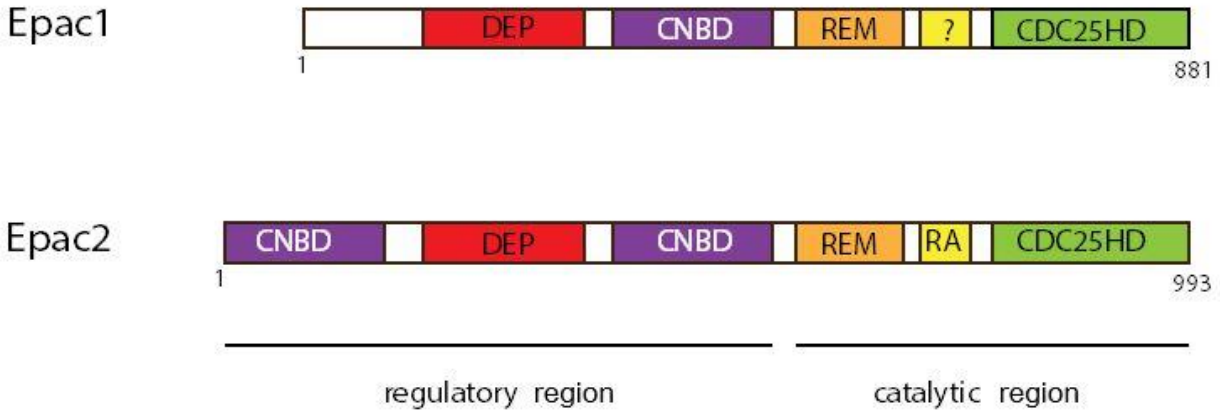


Figure 6: Domain structure of Epac1 and Epac2. Both Epac1 (881 amino acids) and Epac2 (993 amino acids) contain two regions: regulatory and catalytic regions. The regulatory region as the cyclic nucleotide-binding domain (CNB), the Desheveled-Egl-10-Pleckstrin (DEP), which is responsible for the membrane localization. The catalytic region has the CDC25-homology domain, which is responsible for the guanine-nucleotide exchange activity, the Ras exchange motif (REM), which stabilizes the catalytic helix of CDC25-HD, and the Ras-association (RA) domain, which is a protein interaction motif.

Cyclic nucleotide-gated channels (CNG)

CNG channels are nonselective tetrameric cation channels that mediate Ca^{2+} and sodium influx in response to direct binding of intracellular cyclic nucleotides (5, 8). The mammalian CNG channel genes fall into two different gene families. One of these subfamily consists of four members CNGA1, CNGA2, CNGA3 and CNGA4, which represent the principal subunits that, except for CNGA4, form functional channels (69). The core structural unit consists of six transmembrane segments, designated S1-S6, cyclic nucleotide-binding domains (CNBD) near the C-terminal region. A pore region of ~20-30 amino acids is located between S5 and S6. The S4 segment in CNG channels resembles the voltage-sensor motif found in the S4 segment of voltage-gated K^+ , Na^+ , and Ca^{2+} channels. Both N-terminal and C-terminal regions are located in the cytoplasmic side and a glycosylated segment connecting S5 to the pore region is extracellular (69). The functional role of CNGs is well-studied in retinal rod photoreceptors (94), sperm (147) central nervous system (36) and cardiac excitability (57). Studies of CNG channels in exocrine tissues have not been reported.

CNG channels belong to a heterogeneous gene superfamily of pore-loop cation channels that

share a common transmembrane topology and pore structure. Other members of this superfamily are the hyperpolarization-activated cyclic nucleotide-gated channel (HCN) (9), the ether-a-gogo (EAG) and human eag-related gene (HERG) family of voltage-activated K^+ channels (40). HCN channels are principally operated by voltage and permeable to both Na^+ and K^+ . Opening of HCN channels causes hyperpolarization of the membrane. Unlike CNG, in which cyclic nucleotides are strictly required to open the channel in HCN, cyclic nucleotides facilitate the opening by shifting the voltage dependence of activation to more positive values (9). Cyclic AMP has shown to modulate HCN channel activity through a PKA-dependent mechanism (11, 18).

The basolateral voltage-activated K^+ channels, which belong to the HCN channel subfamily, are necessary for the regulation of Cl^- secretion from pancreatic acini. In the rat pancreatic acinar cells, the presence of K^+ channels in the basolateral membrane causes a membrane hyperpolarization, which provides the driving force for Cl^- exit. In addition, the efflux of K^+ balances the K^+ uptake by the Na^+ , K^+ ATPase pump and other co-transporters (71). The functional and

pharmacological properties of these channels are conferred once KCNE1 co-assembles with KCNQ1 (145). Both KCNE1 and KCNQ1 genes are expressed in rodent pancreas (139, 159). Cyclic AMP (71, 85) and cabachol (70) increase the amplitude of the slowly activating voltage-dependent K⁺ channel current (IKs) in rat pancreatic acinar cells.

RNA-binding protein

The Ca²⁺-regulated heat-stable protein of 24 kDa (CRHSP-24, also known as CARHSP1) is a serine phosphoprotein originally identified as a physiological substrate for the Ca²⁺-calmodulin regulated protein phosphatase calcineurin (PP2B) (50). In pancreatic acini, cyclic AMP partially dephosphorylated CRHSP-24 on at least two sites (124) through the activation of a phosphatase inhibited by calyculin A and okadaic acid, namely a PP2A or PP4 (124).

2.3 Regulation of the Adenylyl Cyclase/Cyclic AMP Signaling.

The cytosolic levels of cyclic AMP are modulated by regulating GPCR activity, G protein activity, adenylyl cyclase activity, and cyclic AMP degradation.

1) *Receptor regulation:* GPCRs can be regulated in several ways. One way is through phosphorylation of specific amino acids in their cytosolic domain. When these amino acids are phosphorylated, the receptor becomes desensitized. G protein-coupled receptor kinase (GRKs) are proteins that specifically phosphorylate GPCRs. Two GRKs have been found in the pancreas: GRK5 (82) and GRK6 (6). PKA can also phosphorylate GPCRs. In mouse pancreatic acini, VPAC receptors appear to be regulated by PKA phosphorylation based on the inhibition of PKA activity using a PKA inhibitor (H-89) causing up to two-fold increase in VIP-stimulated cyclic AMP formation (116).

2) *At the G protein activity level:* G protein activity can be affected by various toxins, with the two best studied being *cholera toxin* and *pertussis toxin*. *Cholera toxin* in complex with NAD⁺ and GTP-bound ADP-ribosylation factor 6 (ARF6-GTP) catalyzes the ADP-ribosylation of the α subunit of Gs protein and prevents it from hydrolyzing its bound GTP, thereby locking the Gs protein in the active state, which causes the continuous activation of transmembrane AC (67). In guinea pig pancreatic acini *cholera toxin* increases cyclic AMP levels and amylase secretion (45). In rodent pancreatic acini, *cholera toxin* increases amylase secretion (31, 131). Its effect is potentiated by cholecystokinin and is less marked than in guinea-pig pancreatic acini (131). Unlike *cholera toxin*, *pertussis toxin* modifies the α subunit of Gi protein and locks the Gi protein in the inactive state, which causes the continuous activation of transmembrane adenylyl cyclases. The toxin catalyzes the ADP-ribosylation of a cysteine residue at position-4 from the C-terminal of the α subunit of Gi protein, inhibiting the interaction of this protein with the receptor and attenuating the intracellular transduction (1, 41, 77). In rabbit pancreatic acini, *pertussis toxin* enhances CCK-induced cyclic AMP levels without affecting cholecystokinin (CCK)-induced Ca²⁺ mobilization or amylase secretion (148). In rat pancreatic acinar cells, although the pretreatment with either *pertussis toxin* or *cholera toxin* does not modify CCK-stimulated intracellular Ca²⁺ levels or phosphoinositide hydrolysis (95), *pertussis toxin* increases the basal levels of cyclic AMP and amylase secretion (137). Regulators of G proteins signaling (RGS) molecules, which catalyze the GTP hydrolysis of heterotrimeric G proteins, have played a critical role in regulating the G protein activity. RGS1, RGS2, RGS4, RGS16 and GAIP, have been found in isolated pancreatic acinar cells using RT-PCR (93). Although their function in the regulation of Gs activity in exocrine pancreas is still unknown, in olfactory neurons RGS2 decreases Gs-stimulated cyclic AMP levels (133).

3) *At the adenylyl cyclase activity level:* Adenylyl cyclase activity can be regulated by distinct intracellular signals. As previously indicated in **Table 1**, transmembrane ACs are classified into four groups: Group I consists of Ca²⁺-stimulated (AC1, AC3, AC8); Group II consists of Gβγ-stimulated (AC2, AC4, AC7); Group III consists of Giα/Ca²⁺/PKC/PKA-inhibited (AC5, AC6); Group IV consists of Ca²⁺-inhibited (AC9), which is forskolin-insensitive (117, 121, 150). Recently, AC9 activity has also been shown to be inhibited by Gai/o proteins and PKC (30). The Ca²⁺-binding protein involved in the stimulatory effect of Ca²⁺ on the group I is calmodulin, which forms an active Ca²⁺-calmodulin complex. Calmodulin is present in pancreatic acini and activated by CCK (38). The Ca²⁺-calmodulin complex binds to the calmodulin-binding site present in the Group I isoform and increases its activity dramatically. AC9 is also stimulated by calmodulin (29). The Ca²⁺-binding protein involved in the inhibitory effect of Ca²⁺ on AC9 is calcineurin, which is a serine/threonine protein phosphatase activated by CCK (50, 52) and involved in amylase secretion from rat pancreatic acini (49), as well as caerulein-induced intracellular pancreatic zymogen activation (61). Forskolin is a diterpene extracted from the root of the plant *Coleus forskohlii* that directly activates all transmembrane AC isoforms, except AC9 (113, 127) by interacting with the two cytoplasmic domains (C1 and C2), that form the catalytic domain (138). The lack of effect of forskolin on AC9 may be accounted for by the residues Tyr1082 and Ala1112 (156).

Unlike transmembrane AC, AC10 is not activated by either G protein or forskolin. Its activation is dependent on the HCO₃⁻ levels (24), though it can also be activated by divalent cations, such as Ca²⁺, Mg²⁺ and Mn²⁺ (89). A combination of Ca²⁺ and HCO₃⁻ activates soluble adenylyl cyclase synergistically (89). AC10 is also activated by changes in intracellular pH (104).

The most common post-translational modification of AC isoform is the phosphorylation of a serine, threonine or tyrosine residue (**Figures 3 and 4**). Phosphorylation of AC1 and AC3 by Ca²⁺/calmodulin kinases inhibits the cyclase activity by blocking the binding site. Phosphorylation of ACs by either PKA or PKC causes an inhibition of the enzyme activity. Ubiquitination and acetylation are other modifications found in the human AC isoforms, though their consequences in AC activity are still unknown.

4) *At the cyclic AMP degradation level:* Cyclic AMP degradation is carried out by the enzyme phosphodiesterase (PDE), which is an exonuclease capable of hydrolyzing a phosphate ester and pyrophosphate bonds, and thereby, converting cyclic AMP into 5'AMP (27). Eleven PDE isoforms exist and each has unique biochemical properties. PDE1, which hydrolyzes both cyclic AMP and cyclic GMP, has been found in pancreatic acini using immunocytochemistry (100), whereas PDE7B, a cyclic AMP-specific PDE, has been found in whole pancreas using Northern blotting (58). PDE4, which is highly expressed in most immune and inflammatory cells and a cyclic AMP-specific PDE, is involved in the development of acute pancreatitis because the selective inhibitor rolipram attenuates the severity of acute pancreatitis in rats (98).

2.4 Role of Adenylyl cyclase/Cyclase AMP pathway in Pancreatic Exocrine Cells.

The exocrine pancreas is primarily composed of pancreatic acini and ducts. Pancreatic acini synthesize and release digestive enzymes into the duodenum, whereas pancreatic ducts release a HCO₃⁻-rich fluid to neutralize the acidic chyme released from the stomach. In this section the roles for adenylyl cyclase/cyclic AMP pathway are described.

Pancreatic duct HCO₃⁻-rich fluid.

Secretagogues, such as secretin and vasoactive intestinal polypeptide (VIP), increase cyclic AMP and stimulate HCO₃⁻-rich fluid secretion from pancreatic duct cells (62, 63, 111). An increase in the levels of cyclic AMP, through PKA phosphorylation, activates CFTR to recirculate chloride back into the glandular lumen, and thereby, depolarizes both luminal and basolateral membranes. Depolarization of the basolateral membrane increases the driving force of an electrogenic sodium-HCO₃⁻ co-transporter on the basolateral membrane leading to the entry of HCO₃⁻, which is then secreted at the apical membrane via the Cl⁻/HCO₃⁻ exchanger (3). AC6/cyclic AMP/PKA pathway has an important role in the physiological function of pancreatic ducts because *in vitro* the VIP-stimulated expansion of the lumen observed in pancreatic ducts from WT mice upon VIP stimulation was absent in duct fragments from AC6-deficient mice. *In vivo* collection of pancreatic fluid also showed a decrease in fluid secretion from AC6-deficient mice (116). The secretory effect is highly dependent on PKA activation because in isolated pancreatic ducts from AC6-deficient mice PKA activation was abolished in response to VIP, secretin, and forskolin (116, 117).

Several ion channels are affected by cyclic AMP/PKA pathway. PKA phosphorylates CFTR located in the apical membrane of the pancreatic duct cells (3). Elevation of intracellular cyclic AMP by stimulation with forskolin significantly inhibits the Na⁺/H⁺ exchanger (NHE) and this, like the stimulation of the apical anion exchanger, may occur through a direct physical interaction with CFTR (3). The basolateral Cl⁻/HCO₃⁻ exchanger (AE) does not seem to be directly activated by forskolin (86). For more details see Chapter XX "Molecular Mechanisms of Pancreatic HCO₃⁻ Secretion".

Pancreatic acini enzymatic-rich fluid.

Early work showed that a number of compounds that increase cyclic AMP levels stimulate amylase

secretion from pancreatic acini (2, 13, 26, 46, 97, 105, 106, 137). Phosphodiesterase inhibitors, such as 3-isobutyl-1-methylxanthine, increase pancreatic amylase secretion (44). *Pertussis toxin* catalyzes the ADP-ribosylation of a cysteine residue at position-4 from the carboxyl-terminal domain of the α -subunit of Gi protein, inhibiting the interaction of this protein with the receptor and impairing intracellular transduction. Treatment with *pertussis toxin* causes an increase in cyclic AMP levels and amylase secretion from rat pancreatic acini (148), where multiple *pertussis toxin*-sensitive G proteins have been found (e.g. Gi1, Gi2, Gi3 and Go) (125). Forskolin interacts with the two cytosolic domains C1 and C2 of transmembrane ACs, except AC9 (121). Forskolin slightly stimulates amylase secretion in rat (34, 72) and potentiates the response to Ca²⁺-dependent secretagogues (55). Recently, pancreatic acini from AC6 deficient-mice showed a reduction in stimulated amylase secretion and PKA activity (116). Because this inhibition was only partial, it is likely that other AC isoforms expressed in pancreatic acini and/or Epac1, which has participated in cyclic AMP-stimulated amylase secretion (20, 115) are also responsible for the secretory role of pancreatic acini. The result showing the deletion of AC6 does not affect the response to the Epac1 analog 8-pCPT-2'-O-Me-cyclic AMP on amylase secretion supports this hypothesis (116).

Differentiation, transdifferentiation and proliferation.

Cyclic AMP plays an important role in differentiation, transdifferentiation and proliferation of pancreatic cells. Isolated adult islets of Langerhans were able to transdifferentiate to duct epithelial-like cyst structures in presence of elevated cyclic AMP and a solid extracellular matrix (e.g. matrigel and collagen I) (144). The presence of intracellular cyclic AMP elevating factor, such *cholera toxin*, was also required for the proliferation and maintenance of pancreatic epithelial duct cells (155). However, transforming growth factor- β (TGF- β), which is an important regulator of growth and differentiation in the pancreas, can activate

PKA without affecting cyclic AMP levels in pancreatic acini (158). TGF- β -mediated growth inhibition and TGF- β -induced p21 and SnoN expression are mediated by PKA because both effects were blocked the PKA inhibitors H89 and PKI peptide (158). A physical interaction between a Smad3/Smad4 complex and the regulatory subunits of PKA has been shown in pancreatic acini (158).

Development of Pancreatitis.

Acute pancreatitis is an acute inflammatory disease of the pancreas. The disease appears to be initiated when a pathologic factor like alcohol or bile injuries the acinar cell and it responds by releasing inflammatory mediators and by activating digestive enzymes, especially proteinases, and restricting their secretion. These events initiate a cascade that leads to pancreatic inflammation and local and systemic tissue injury (154). The participation of cyclic AMP in the development of pancreatitis has been studied by the Gorelick lab. An early study showed that cyclic AMP-dependent secretagogues sensitizes the pancreatic acinar cells to zymogen activation induced by caerulein, a CCK analog (91). The same research group in a subsequent work shows that cyclic AMP, by enhancing the release of pancreatic enzymes from the acinar cell, can overcome the acinar cell injury induced by high concentrations of carbachol, a cholinergic agonist (21). Recently, the inhibition of soluble AC by KH7 was shown to enhance the activation evoked by caerulein of two important digestive enzymes chymotrypsinogen and trypsinogen, as well as caerulein-stimulated amylase secretion from rat pancreatic acini (75). Together these studies suggest a complex role for cyclic AMP in acute pancreatitis in which it may enhance some pancreatitis responses while simultaneously lessening the effects of others.

2.5 Guanylyl Cyclase/Cyclic GMP Signaling

Cyclic GMP is made from GTP through a catalytic reaction mediated by guanylyl cyclase (GC). Like AC, GC can be transmembrane or soluble. Unlike transmembrane ACs, all transmembrane GCs share a basic topology, which consists of an extracellular ligand binding domain, a single transmembrane region and an intracellular domain that contains a juxtamembranous protein kinase-homology domain (KHD), a coiled-coil amphipathic α -helical or hinge region, and the catalytic GC domain at its C-terminal end. The function of the KHD is still unknown. Although it binds ATP and contains several residues conserved in the catalytic domain of protein kinases, a kinase activity has not been found. In fact, it regulates the GC activity at the C-terminal end. The coiled-coil hinge region is involved in the process of dimerization, which is essential for the activation of GC domain (79). There are at least seven transmembrane guanylyl cyclases: GC-A, GC-B, GC-C, GC-D, GC-E, GC-F, and GC-G (**Figure 7**). Only GC-A, GC-B and GC-C have shown to regulate the function of exocrine pancreas.

Transmembrane GC

There are at least two groups of ligands for transmembrane GC:

- 1) *Natriuretic peptides*: There are three members of natriuretic peptide family: atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) (**Figure 8A**). The actions of natriuretic peptides are mediated by the activation of three transmembrane receptor subtypes: natriuretic peptide receptor type A (NPR-A, also known as GC-A), type B (NPR-B, also known as GC-B), and type C (NPR-C) (**Figure 8B**).

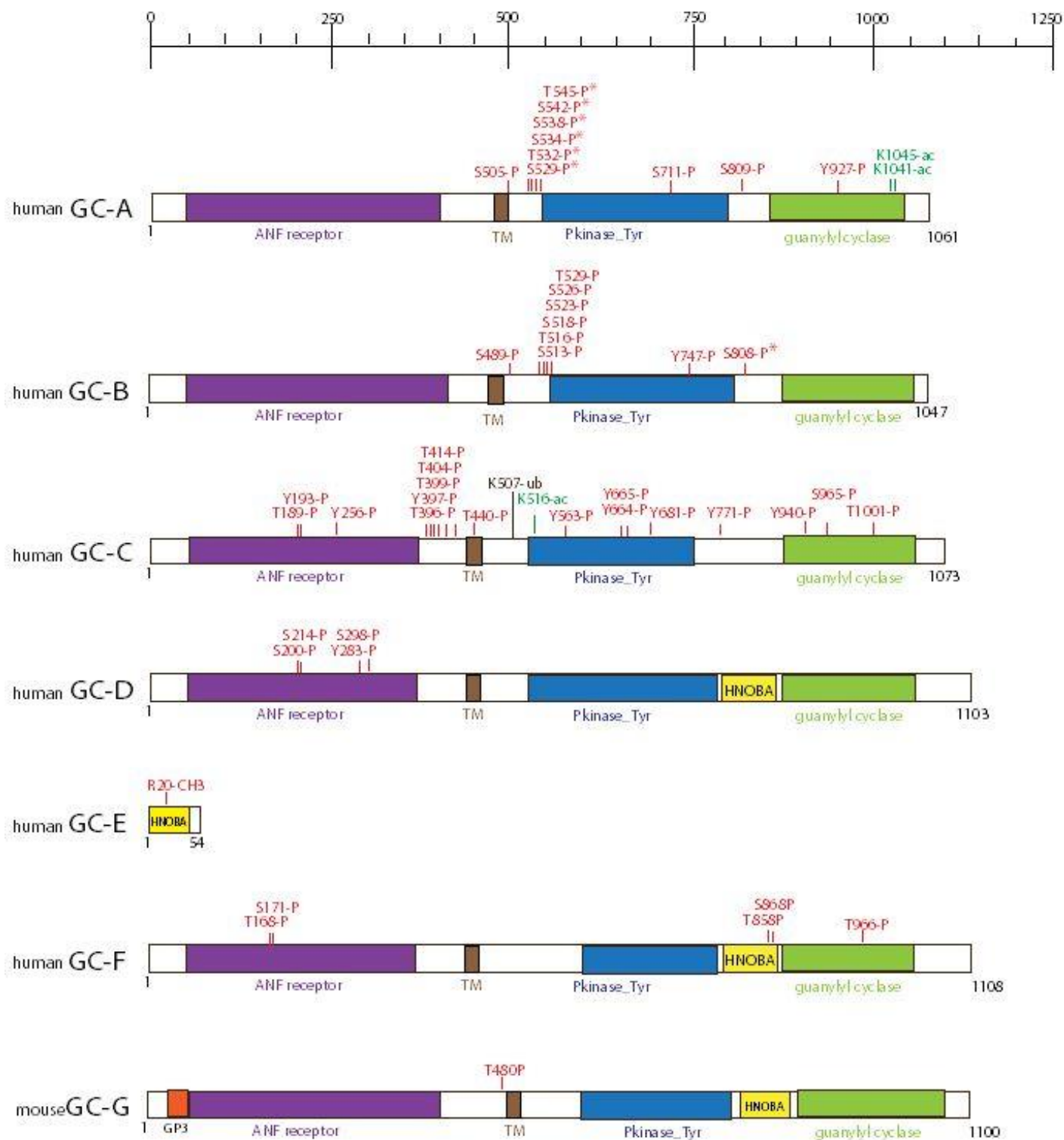


Figure 7. Schematic representation of the structure of the seven GC isoforms. The number of amino acid residues is reported on the side of each structure. Modifications sites and domains are represented with different color. The transmembrane GC isoforms share a common structure consisting of an extracellular ligand binding domain, a short transmembrane region (TM), and an intracellular domain that contains the catalytic region (GC) at its C-terminal end. Abbreviations: TM: transmembrane segments; ac: acetylation; P: phosphorylation site; ub: ubiquitination; CH3-: methylation; S: serine; K: lysine; R: arginine; T: threonine; Y: tyrosine. *: sites implicated in the activity of the enzyme. Data obtained from PhosphoSitePlus.

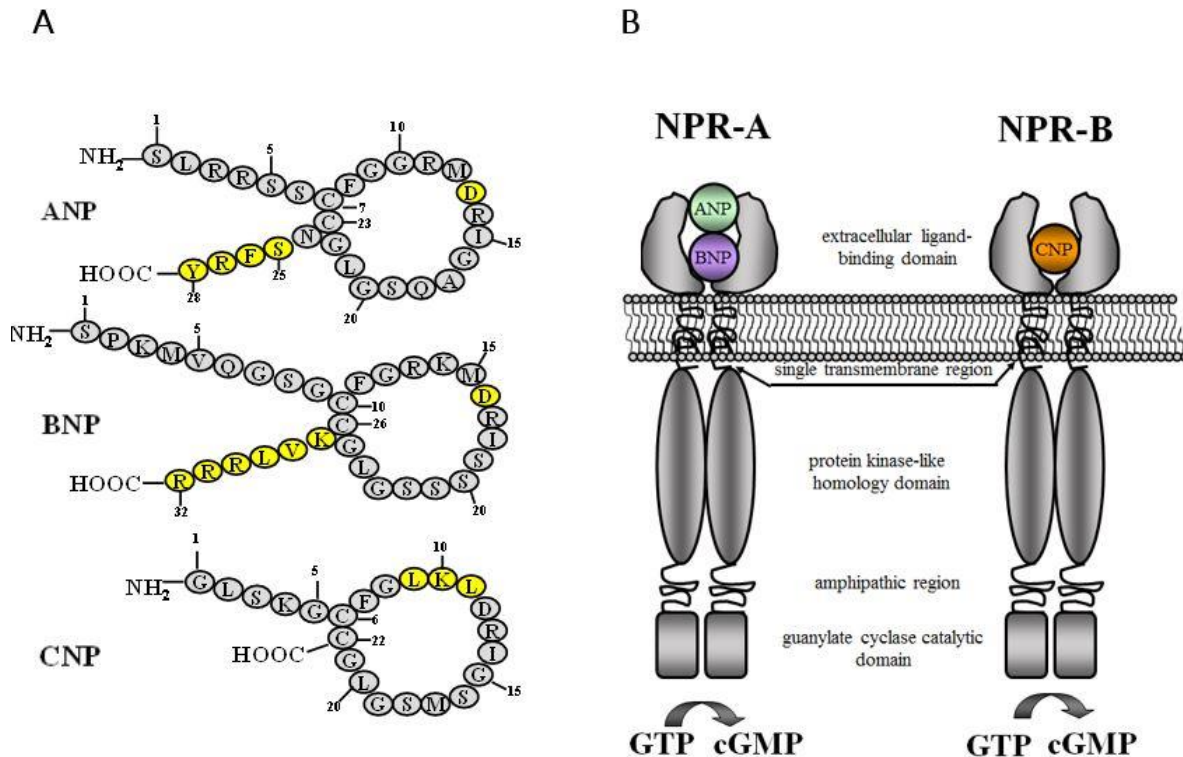


Figure 8: A: Schematic representation of the amino acid sequence and the structure of biological active natriuretic peptides ANP, BNP and CNP. The members of the natriuretic peptide family share a common structure, which consists of a 17-amino acid bonded loop bridge by intracellular disulfide bond required for the natriuretic and diuretic activity. Note the amino acids with a yellow color are also important for their activity. **B:** Schematic representation of natriuretic peptides receptors NPR-A and NPR-B. The structure of NPR-A and NPR-B receptors possesses three domains: the extracellular ligand-binding domain, the intracellular protein kinase-like homology domain, and GC catalytic domain.

The NPR-A and NPR-B receptors, whose relative molecular mass is 130-180 kDa, have a similar structure that contains four domains: an extracellular ligand-binding domain, a single transmembrane domain, an intracellular tyrosine-like domain, an amphipathic region and a GC catalytic domain. Upon ligand binding, the NPR-A and NPR-B receptors change their conformation which results in GC activation and cyclic GMP generation (108, 114).

2) *Guanylin and uroguanylin*: Guanylin and uroguanylin are peptides secreted from the intestine, which influence electrolyte and fluid transport in the intestine and kidney, respectively (42, 130). Their effects are mediated by GC-C receptor (GC-C), which is predominately expressed in the intestine.

Soluble GC

Soluble GC is a histidine-ligated hemoprotein that consists of two homologous subunits, α and β . The well-known isoform is the $\alpha 1\beta 1$ protein; $\alpha 2\beta 2$ subunits have also been identified (53, 161). Each soluble GC subunit consists of four domains, an N-terminal heme-Nitric Oxide Oxygen (H-NOX) domain (also called a SONO domain), a central Per-ARNT-Sim (PAS) domain, a coiled-coil domain and a C-terminal catalytic cyclase domain. The $\beta 1$ subunit contains a N-terminal heme-binding domain, a Per/Arnt/Sim (PAS) domain, a coiled-coil domain, and a C-terminal catalytic domain (17) as described in **Figure 9**.

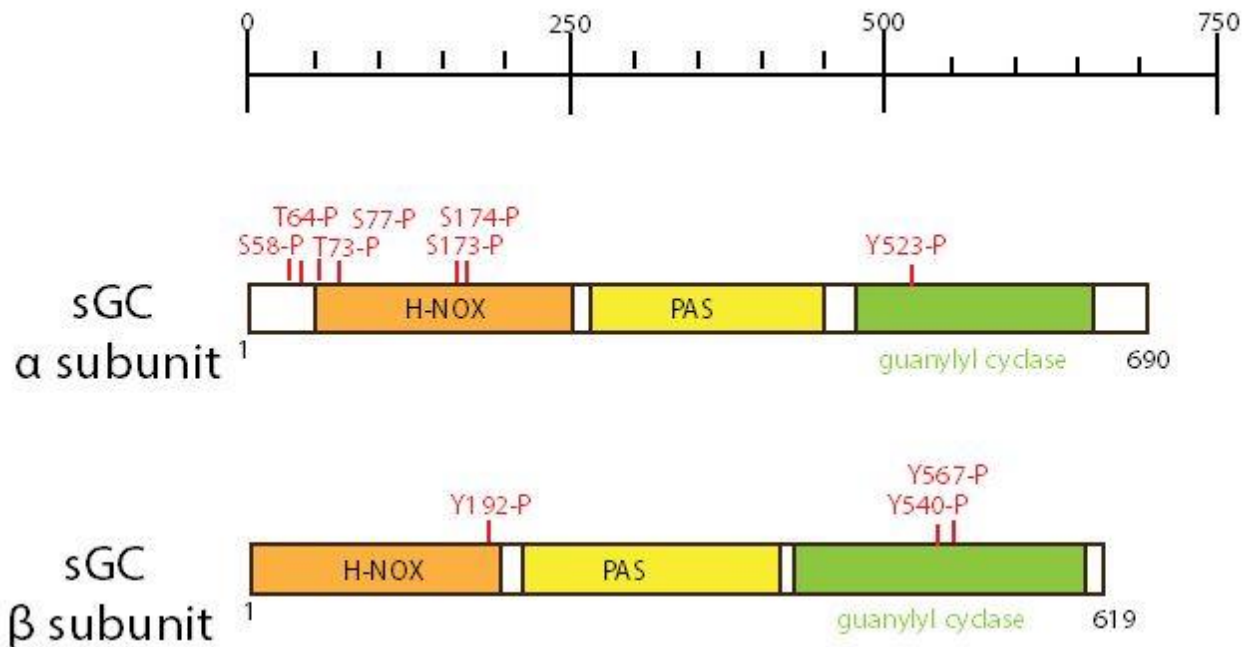


Figure 9: Schematic representation of the structure of soluble GC (α subunit and β subunit) is represented. The number of amino acid residues is reported on the side of each structure. Modifications sites and domains are represented with different color. Each soluble GC subunit consists of four domains, an N-terminal Heme-Nitric Oxide Oxygen (H-NOX) domain (also called a SONO domain), a central Per-ARNT-Sim (PAS) domain, a coiled-coil domain and a C-terminal catalytic cyclase domain. Abbreviations: P: phosphorylation site; S: serine; T: Threonine; Y: Tyrosine. Data obtained from PhosphoSitePlus.

PAS domain mediates protein-protein interactions and have often been found to bind heme, a flavin, or a nucleotide (99). The coiled-coiled domain, appears to be unique to soluble GC (35). The functions of PAS and coiled-coiled domains are still unknown. The catalytic domain is localized to the C-terminal 467-690 and 414-619 residues of the α 1 and β 1 subunits, respectively (151). The catalytic domains must form a heterodimer for cyclic GMP synthesis, and in the full length protein (35). The C-terminal regions of the α 1 and β 1 subunits are highly homologous to the particulate GC and AC catalytic domains (35). Soluble GC binds nitric oxide (NO), which is its primary activator (92), and can also be activated by carbon monoxide, but not oxygen (35). NO is a diatomic gas synthesized from L-arginine and oxygen by the enzyme NO synthase. NO binds to the heme cofactor of soluble GC. The binding of NO to soluble GC leads to an increase in cyclic GMP.

2.6 Intracellular Targets of cyclic GMP

Intracellular targets of cyclic GMP, like intracellular targets of cyclic AMP, have a cyclic nucleotide-binding domain (CNBD) in their structure.

Cyclic nucleotide-gated channels

Cyclic nucleotide-gated channels (CNG) have been described above (see 2.2. Intracellular Targets of cyclic AMP). The physiological significance of cyclic GMP as activating agent of CNG has been described in photoreceptors and olfactory receptors neurons, where CNG play an important role in sensory transduction (8). There are no reports of CNG function in an exocrine tissue.

Cyclic GMP-dependent protein kinase

The increase in the levels of cyclic GMP activates cyclic GMP-dependent serine/threonine protein kinase (PKG). Two genes *prkg1* and *prkg2* code for the two isoforms PKGI and PKGII (59). The

human *prkg1* gene is located on chromosome 10 at p11.2–q11.2 and has 15 exons. The N-terminus of PKGI is encoded by two alternative exons that produce the isoforms PKGI α and PKGI β . The human *prkg2* gene is located on chromosome 4 at q13.1q21.1 and has 19 exons. Its transcript yields a protein with an apparent mass of 87.4 kDa (59). Like PKA, PKG is composed of two functional domains: a regulatory domain and a catalytic domain. The regulatory domain is subdivided into the N-terminal domain and the cyclic nucleotide-binding domain (CNBD) containing the high and low cyclic GMP affinity binding pockets. The catalytic domain contains the Mg²⁺-ATP- and peptide-binding pockets. Upon binding of cyclic GMP to the two regulatory subunits, the two catalytic subunits are released from the regulatory subunits and become active (59). The substrates of this kinase are P240, P132 and phospholamban, though none of them is a specific PKG substrate (43, 87). The intracellular levels of cyclic GMP are regulated by PDE enzymes, which hydrolyze cyclic GMP into 5'GMP (43). In pancreatic acinar cells from guinea pig, the presence of PKG activity has been reported (66).

2.7. Role for Guanylyl Cyclase/Cyclic GMP Pathway in Pancreatic Exocrine Cells.

The role of GC/cyclic GMP in the regulatory function of pancreatic exocrine cells is still controversial. One of the first papers published on isolated pancreatic lobules from guinea pig and rabbit showed that carbamylcholine (carbachol), pancreozymin (now known as CCK), and caerulein all increased the levels of cyclic GMP without modifying the levels of cyclic AMP. The authors concluded that cyclic GMP is the second messenger involved in the process of stimulus-secretion coupling in the acinar cells of exocrine pancreas (54). Later, Ca²⁺ was shown to be an important mediator of the stimulus-secretion coupling process (149). Moreover, increased intracellular levels of cyclic GMP has a little or no effect on the stimulus-secretion coupling in pancreatic acinar cells (149, 160). However, cyclic

GMP has been involved in the Ca²⁺ entry across the cell membrane to replenish the intracellular Ca²⁺ stores (109, 110).

The function of NO, the ligand for soluble GC, has been studied in the exocrine pancreas. NO can increase endogenous cyclic GMP and rat pancreatic secretory activity (160). NO triggers an increase in intracellular Ca²⁺ levels via cyclic GMP and inositol trisphosphate in pancreatic acinar cells (101). NO is localized in intrapancreatic ganglionic cells and efferent nerve fibers (90) and implicated in the control of mesenteric circulation (96). NO inhibits pancreatic exocrine secretion in dogs (76), and rats (152). NO production regulates cyclic GMP formation and Ca²⁺ influx in rat and guinea pig isolated pancreatic acini (51). Blocking NO production by chemical inhibitors of NO synthase, NG-monomethyl-L-arginine or NG-nitro-L-arginine, abolished cyclic GMP formation induced by the cholinergic agonist carbachol in a dose-dependent manner (51). NO has shown to have a protective role in acute pancreatitis (37, 64, 65).

The functions of two ligands for transmembrane GCs have also been studied in exocrine pancreas:

- *Natriuretic peptides:* All of three receptors of natriuretic peptides are expressed in pancreatic acini (118) and both ANP and CNP increase intracellular levels of cyclic GMP in isolated pancreatic acini (56, 118). However, the action of ANP and CNP on pancreatic secretion is not mediated by an increase in cyclic GMP. Indeed, ANP and CNP increase pancreatic fluid and protein output through the NPR-C receptor activation/Ca²⁺ release (118-120).

- *Guanylin and uroguanylin:* In rat pancreatic acini, guanylin increases cyclic GMP levels, elicits a small amount of amylase secretion and a small Ca²⁺ transient (160). Guanylin is localized specifically to the centroacinar cells and proximal duct cells and released lumenally into the pancreatic ducts based on its presence in the pancreatic juice (80). Functional studies in two different human pancreatic duct cell lines revealed

that guanylin is an intrinsic pancreatic regulator of Cl^- current activation in pancreatic duct cells via cyclic GMP. Using whole-cell patch-clamp forskolin increased of Cl^- conductance mediated by cyclic AMP, while guanylin increased Cl^- conductance mediated by cyclic GMP, but not cyclic AMP (81).

The existence of both membrane and soluble GCs in pancreatic acini suggest that there are two distinct sources of cyclic GMP located in different compartments, which could have different effects in pancreatic acini.

3. Non- Canonical Cyclic Nucleotides

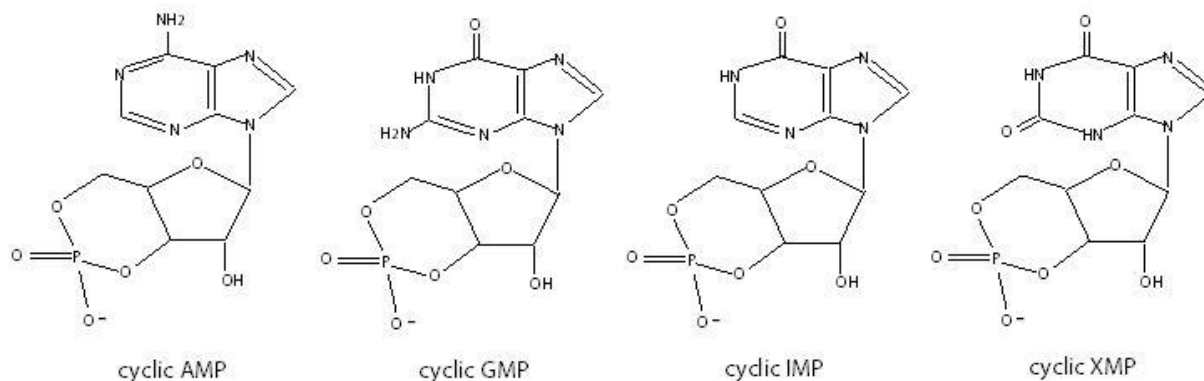
Cyclic IMP, cyclic XMP, cyclic CMP, cyclic UMP and cyclic TMP are cyclic nucleotides that are not well-characterized (**Figure 10**). Using HPLC-MS/MS spectrometry, both cyclic CMP and cyclic UMP have been found in numerous cultured cell types and in human urine. Cyclic CMP and cyclic UMP concentrations are regulated by the cell proliferation status because growth-arrest of cells resulted in preferential decrease of cellular cyclic CMP and cyclic UMP concentrations as compared to cyclic AMP and cyclic GMP concentrations. Previous findings suggest that cyclic CMP and cyclic UMP could play a role as second messengers because cyclic CMP and cyclic UMP-hydrolysing PDEs were found in mammalian tissues. Recently, soluble AC has shown to be responsible for the production of cyclic CMP and cyclic UMP in HEK293 and B103 cells because the soluble AC inhibitor KH7 decreased HCO_3^- -stimulated cyclic nucleotide levels in concentration-dependent manner. Forskolin, which is a stimulator of all transmembrane ACs except AC9, does not affect the levels of cyclic CMP and cyclic UMP. The authors conclude that soluble AC may likely have a distinct role in the regulation of cyclic nucleotide levels compared to soluble GC, membrane GC and membrane AC (128). In RFL6 lung fibroblasts endogenously expressing soluble GC, NO-stimulated cyclic UMP formation were

similar to cyclic GMP formation (4). In contract, to soluble GC, transmembrane GC do not induce cyclic UMP formation (7). Recently, cyclic CMP was found in several mouse tissues including pancreas as assessed by HPLC-MS/MS and HPLC-MS/TOF (126).

Unlike cyclic CMP and cyclic UMP, cyclic TMP, cyclic IMP and cyclic XMP levels are very low to be detectable in cultured cell lines (7). Cyclic IMP levels increase in a hypoxic environment probably as a result of ATP deamination, which becomes ITP, and by soluble GC activity, ITP becomes cyclic IMP (128).

Non-canonical cyclic nucleotides have been studied so far in cardiovascular system, central nervous system and reproductive system. A description of their roles in the regulation of these system can be found in (42). To the best of our knowledge, at the present there is no data available for the role of non-canonical cyclic nucleotides in the digestive system.

cyclic purine nucleotides



cyclic pyrimidine nucleotides

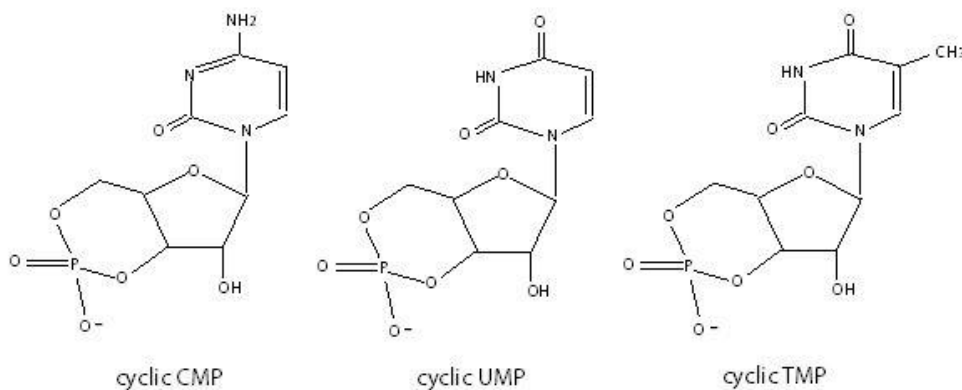


Figure 10. Schematic representation of cyclic purine (cyclic AMP, cyclic GMP, cyclic IMP, cyclic XMP) and pyrimidine (cyclic CMP, cyclic UMP and cyclic TMP) nucleotides.

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