particular it is known that phosphoenolpyruvate-dependent phosphotransferase system substrates are good substrates for growth in the absence of oxygen, but it is also known that cAMP is absolutely required for growth under such conditions (44). It seems that an appropriate control process ensures that a significant level of cAMP is produced when glucose is the carbon source and oxygen is absent. Modulation of adenyl cyclase synthesis might play some role (254), but it is most likely that the bulk of the control—which must operate rapidly under oxygen shift-down conditions—is through control of adenyl cyclase activity. A specific, feature of the protein might be relevant in this respect: enterobacterial adenyl cyclases are extremely rich in cysteine residues, an unusual feature for cytoplasmic proteins. A process involving formation and disruption of disulfide bridges, and which might use cofactors, could play some role in the anaerobic/aerobic control. As in the case for the catalytic domain, the regulatory domain is rich in cysteine residues, but only two of them are conserved in all the sequences that are known. Several stretches of amino acids are strongly conserved, perhaps indicative of the multiple interactions that could take place with this domain of the protein (including the tonic interaction with the catalytic domain). In this regard, sequence comparison with other cyclases could be revealing. Guanylyl cyclases, in their cytoplasmic form, bind a heme prosthetic group, which plays an important role in the regulation of the enzyme’s activity. The cysteine plus histidine content of class I cyclases may be well suited for such interaction. Besides, in enterobacteria there is an overlap in the region controlling transcription of cyclase and of heme biosynthesis genes. It seems therefore of some interest to investigate whether a heme does not play some function in control of class I adenyl cyclase activity.

**Calmodulin-Activated Toxic Cyclases**

Adenyl cyclase bacterial toxins are a spectacular illustration of the modular construction of proteins during evolution. Toxin evolution requires specific means for delivering the toxin to the host cell. This involves excretion of the protein (or of a fragment of the protein) in the external medium, followed by internalization in the host cell (34,204,246,313). Whereas this can be easily achieved in Gram positive organisms, as they possess only one membrane defining the interior of the cell, in Gram negative organisms, two membranes must be traversed by the protein which is secreted. This may explain the enormous difference in the domain structure of adenyl cyclases isolated from *B. anthracis* and *B. pertussis* (96,97).

As discussed earlier, a protein with a standard signal peptide at the aminoterminus is generated from the *B. anthracis* gene. However, the catalytic domain is located at the center of the 800 amino acid residues polypeptide, in contrast to other cyclases, where the catalytic domain is present at one
end of the polypeptide chain. A first domain of ~300 residues immediately follows the signal peptide, and lies upstream of the catalytic domain, which is followed by a ~100 residue third domain. The first domain has been shown to bear similarities with a domain of the lethal factor produced by *B. anthracis*, necessary for binding the protective antigen (PA) required for internalization of the toxin into the target cells (18, 66) (Fig. 11). The function of the third domain remains unknown, and it does not have significant similarities with proteins of known functions. Thus at least two factors, PA and calmodulin, may interact with adenyl cyclase.

*B. pertussis* is a Gram negative organism, possessing two membranes. Secretion of adenyl cyclase into the external medium, requires the crossing of both membranes, and is therefore a challenge for the organism. A secretary process, demonstrated by the hemolysins (142, 177, 211) or hemolysin-like proteins (277) of Gram negative organisms has been recruited for this purpose. The catalytic domain of the cyclase is anchored to a hemolysin-like domain acting as a carrier protein, permitting the passage of the chimera through the bacterial membranes (99, 177, 142). The carrier hemolysin portion consists of several domains, including a calcium-binding segment containing glycine rich repeats, and a carboxy-terminus that is necessary and sufficient for secretion (58, 90, 189). This apparatus comprises three proteins, two of

FIG. 11. Similarity between the lethal factor and adenyl cyclase of *B. anthracis* in the region binding to the protective antigen.
which are very homologous to counterparts utilized for hemolysin secretion, CyaB (51% identical to HlyB) and CyaD (32% identical to HlyD). CyaB is a protein that presumably binds ATP, and is very similar to a class of proteins, the eucaryotic multidrug resistance proteins (5). The third one, CyaE, has been found to be indispensable for secretion (90), has been shown to bear similarities with a protein required for protease secretion in Erwinia species, and is phylogenetically linked to the TolC protein of E. coli (308). This reconciles the secretion of all hemolysin-like proteins, and requires the presence of three proteins, HlyB-like, HlyD-like, and TolC-like for secretion. The actual process of secretion is however not yet well understood. Most models postulate the formation of a pore-like structure, composed of the HlyB and HlyD proteins, and which crosses both the cytoplasmic and the outer membranes. The energy required for secretion is obtained from hydrolysis of ATP bound to HlyB. The role of TolC-related proteins is unknown.

In all the above examples, GTP- and G-binding protein do not appear to play a role. Calcium may be involved in the regulation of hemolysin activity, but not only through its binding to calmodulin (101,234,317).

The Universal Class of Adenylyl Cyclases (Class III)

The class III adenylyl cyclases are of extremely diverse length, often comprising more than 1,000 residues (143), but the catalytic domain is only 250 to 300 residues long. This indicates that, as in the case of class I and class II, the catalytic domain is linked to polypeptides having other, presumably regulatory, functions. In addition to a polypeptide chain, which contains the catalytic center, most adenylyl cyclases are associated with a variety of subunits that control cAMP synthesis, as a function of the growth cycle or differentiation state of the cell.

Adenylyl Cyclases Activated by G-Proteins

GTP has long been known to regulate eucaryotic adenylyl cyclase. This is due to GTP binding and hydrolysis on subunits interacting with the catalytic domain of adenylyl cyclases. It is now known that different kinds of GTP-binding proteins modulate adenylyl cyclase activity according to the cell type. This will be discussed, not from the point of view of G-proteins (see 16, 86, 152, 167, 210, 218, 243, 269, 276, 278, 312) but from the point of view of the sequences in the catalyst which regulate its interaction with the appropriate G-proteins.

Interaction with the G-Protein Subunits

cAMP was discovered as a second messenger which mediates many hormonal responses in eucaryote cells (see 243). The hormonally regulated ade-
nyl cyclase type is an integrated structure comprising three well-defined functional units: (i) a hormone receptor, which interacts with (ii) a variety of G-proteins, and controls the activity of (iii) the catalytic subunit (see 168 for a review). The latter is composed of a polypeptide with a transmembrane domain coupling one or two catalytic centers. In the case of bovine brain adenyl cyclase two catalytic-like domains are present and similar to each other, but it is not yet clear whether both are functional (144). The G-proteins are trimers of the general type αβγ, where α can be of at least two distinct families, defined according to the way they modulate activity: αs, when they activate (Gx = αsβγ); αt, when they inhibit (Gi = αtβγ). The hormone receptor activates cyclase when interacting with a Gs type and inhibits cyclase when interacting with a Gi type. This implies that while the adenyl cyclases may not be identical in primary structure, they are strongly related in their general organization (thereby permitting similar interactions with a variety of G-proteins, whether inhibitory or activating). Adenyl cyclases of this general type are present in a number of tissues and are implicated not only in hormone regulation (69,86,111,167,168,180,243,275,279), but also in neurotransmitter reception and memory (56,68,115,137,138,282,323), taste (6,153,155), and olfaction (153–156,217). At present it is not possible to identify the sequence(s) in the catalytic polypeptide chain that are responsible for its interaction with G-proteins. Due to the lack of sufficient protein sequences, it is not yet possible to derive a phylogenetic tree for the regulatory portions of these adenyl cyclases.

Ras and Ras-Like Proteins

In view of the apparent universality in the organization of adenyl cyclases in higher eucaryotes, it was a surprise to discover that, while the yeast *S. cerevisiae* adenyl cyclase is membrane-bound (198) and stimulated by Mn²⁺-ATP as the mammalian adenyl cyclase (26,60,62), it is not regulated by Gi or Gs. In contrast, other GTP-binding proteins, similar to the ras oncogene products in higher eucaryotes, regulate the yeast enzyme. In *S. cerevisiae*, a cascade of reactions controls GTPase activity (62,241,286), and involves several other proteins, in addition to the RAS proteins. This variance with mammals (10,126) cannot be extended to the fission yeast *Shizosaccharomyces pombe* (61,77,179,129,310). The function of RAS in other yeasts and in vertebrates is not well known. It has been suggested that RAS controls meiosis in *Xenopus* oocytes (15). Mammalian RAS proteins stimulate yeast adenyl cyclase, under conditions where they appear to be incapable of stimulating vertebrate adenyl cyclase (10,22,47,184). *S. cerevisiae* contains two RAS genes, *RAS1* and *RAS2* which appear to only activate adenyl cyclase (22,46,232,285,294). The RAS-responsive domain is located in the leucine-rich repeats which are present upstream of the catalytic domain of
the protein, so that the first 605 amino terminal residues can be deleted and
the 308 residues interdomain region (linking the leucine-rich repeats to the
catalytic center) can be altered in sequence, without altering the response to
RAS (33,74,281). However, the direct interaction between RAS and adenylyl
cyclase was not unambiguously demonstrated. For this reason, Wigler and
coworkers purified a RAS-responsive adenylyl cyclase complex from S. cer-
veisiae, and isolated from this complex a cyclase-associated protein (acro-
nym: CAP) (73). The gene was cloned and found to encode a 526 residue
protein. A gene identical to CAP (called SRV2) was independently isolated
by Fedor-Chaiken et al. who also demonstrated that it was required for RAS
activation of adenylyl cyclase (70). Further work demonstrated that CAP is
a bifunctional component, activating the RAS-mediated response of adenylyl
cyclase at its amino-terminus and controlling several environmental re-
sponses of the cell at its carboxy-terminus (84). It is not entirely established
that RAS interacts directly with the leucine-rich segment of the protein,
although experiments involving mutant suppression substantiate this view
(71). Previous results of Uno and coworkers that demonstrate that a clone
of the yeast CYRI gene could synthesize cAMP in E. coli only in the presence
of the RAS2 gene product agree with this interpretation (303). In fact a RAS-
independent activity can be measured in vitro, and analysis of a variety of
mutants suggest that the segment in the region 1,300–1,400 linking the leuc-
ine-rich domain to the catalytic center could act as a tonic inhibitor of ade-
nylyl cyclase activity. Perhaps, by an internal interaction with the region
surrounding residue 1,651, RAS functions to alleviate this inhibition (71).
In this context it is interesting that the short protein described by Masson et
al. still retains this putative RAS-sensitive modulation domain, but that the
leucine-rich domain is dramatically truncated (187). It would therefore be
interesting to investigate the function of RAS when cells are grown in minimal
media. Taken together these results would suggest that several proteins are
required for full regulation of cyclase activity in yeast. In general, a model
emerges where yeast adenylyl cyclase is divided into three functional do-
 mains: (i) an amino-terminal domain resulting in the tonic inhibition of the
enzyme, which can be alleviated by RAS; (ii) a membrane-binding domain
located in the central part of the protein; and (iii) the catalytic domain located
near the carboxy-terminus. Most data substantiate this organization but ex-
periments from Bourne’s laboratory suggest that the mode of enzyme interac-
tion with the membrane is still not well defined (110). Efficient coupling to
the membrane must exist since external signals such as mating-type phero-
mones are known to modulate adenylyl cyclase activity (171). Finally, from
the phylogenetic point of view, the leucine-rich domain of S. cerevisiae ade-
nylyl cyclase has been found to be present in other proteins, where a distinct
regulatory function has been identified for this domain (249,260). Further-
more, this domain is also present in S. pombe, where no regulation by RAS
could be demonstrated. As in other cyclases, it seems that there has been
recruitment of various activities in a single polypeptide chain through a general process of domain shuffling. As a case in point, in *Trypanosoma equiperdum* two adjacent genes account for the adenylyl cyclase catalytic center and a leucine-rich putative regulatory gene (249).

**Calmodulin-activated Adenylyl Cyclases**

In some cells, calcium is required for full activation of adenylyl cyclase, in addition to GTP. In particular, in brain tissue, calcium and its binding protein, calmodulin, were required for appropriate adenylyl cyclase activation. However the mechanism of action of calmodulin in this process remains a puzzle. Is the effect of calmodulin related to the G-protein control process or is it independent from it? In general, it is implicitly admitted that the calmodulin effect is superimposed on the G-protein control. However there exists a large variety of adenylyl cyclases in a given mammalian tissue. There also exists a class of adenylyl cyclases directly activated by calmodulin (the microbial toxic cyclases, class II cyclases). The question can therefore be raised: Are the effects mediated by calmodulin due to yet another class of adenylyl cyclases, phylogenetically related to the toxin class, but distinct from the hormonally regulated class? The evidence in this respect is rather confusing, mainly due to the lack of purified adenylyl cyclases to permit well-controlled *in vitro* experiments.

A typical example of this is illustrated in the work of Natsukari et al. who describe a "synergistic" activation of brain adenylyl cyclase by calmodulin and GTP or catecholamines in synaptosomal membrane preparations (208). From the data one cannot resolve whether this is true synergy or superposition with allosteric activation of two distinct adenylyl cyclases. Rosenberg and Storm published that they could separate calmodulin-sensitive and calmodulin-insensitive adenylyl cyclases using polyclonal antibodies raised against the catalytic subunit of a calmodulin-insensitive adenylyl cyclase (248). Monneron and coworkers, using antibodies raised against the adenylyl cyclase from *B. pertussis*, were also able to separate, in brain tissue preparations, a calmodulin-sensitive form (MW 175 kDa) and a calmodulin-insensitive form (MW 125 kDa) (216). Gilman and coworkers, using a preparation of adenylyl cyclase derived from the bovine brain gene which they had cloned, were able to demonstrate some activation by calmodulin (287). What seems puzzling in this observation is that the latter enzyme is a class III enzyme, which has never been demonstrated to be directly activated by calmodulin *in vitro* in other organisms from which it has been isolated (e.g., the *R. meliloti* enzyme is not activated by calmodulin *in vitro*, our unpublished experiments, 1988) and does not resemble the toxic type calmodulin-regulated cyclase (see section on Phylogeny of the Catalytic Domain). However it should be noted that the brain enzyme studied by Gilman and cowork-
ers (287) corresponds to a protein having two putative catalytic sites in a single polypeptide chain. This unique organization of the protein may be needed for calmodulin-dependent activation. Furthermore, as discussed below, there exists a guanylyl cyclase enzyme, a member of class III cyclases, which is directly activated by a calcium-binding protein. Thus, the question of direct interaction and activation of class III adenylyl cyclase by calmodulin remains open.

Lipkin et al. published sequence information for a calmodulin-activated form of brain adenylyl cyclase, which had no significant relationship with any known cyclase, indicating either an error in the assignment of their clone, or the existence of still another cyclase class (172). The data obtained by Monneron and coworkers, suggesting that antibodies directed against class III enzymes fail to recognize the enzymes that are activated by calmodulin (but are recognized by antibodies directed against the calmodulin-activated toxic adenylyl cyclases) is an argument in favor of the existence of a further class, similar to the adenylyl cyclase toxic type, which is present in neural tissue (98.202,216).

**Guanylyl Cyclases**

Guanylyl cyclases are also modular proteins. As stated (see section on *Guanylyl Cyclases*), two major classes of guanylyl cyclase have been described. In all cases the catalytic center is typical of class III cyclases, but the domains linked to these catalytic centers differ widely. In fact, in the case of the membrane bound enzyme, guanylyl cyclase concentrates in a single polypeptide chain the various activities that are linked through quaternary interactions in most adenylyl cyclases. An amino-terminal receptor domain is situated on the outer face of the cytoplasmic membrane, and recognizes different signals (32,264). It is followed by a transmembrane domain made of a single short polypeptide segment. Furthermore, Singh et al. (1988) have demonstrated using a cDNA for guanylyl cyclase cloned from sea urchin testis, the protein is also homologous to protein kinases. It was later found that the carboxy-terminal end was similar to soluble forms of guanylyl cyclase (270). Using this cDNA as a probe, related genes were isolated from another sea urchin and from rat brain. These show similar structural features, namely an extracellular receptor domain, coupled through a unique transmembrane segment to a central cytoplasmic protein kinase domain, followed by the guanylyl cyclase catalytic center at the carboxy-terminal end of a 120 kDa polypeptide (264). On the other hand, guanylyl cyclase responsible for phototransduction was organized in a way strongly reminiscent of the G-protein dependent form of adenylyl cyclase. However, transducin, the retinal G-protein, is apparently not involved in the modulation of guanylyl cyclase activity, but in the light-induced modulation of cGMP-specific phosphodies-
terase activity (175). The actual structure of the enzyme responsible for cGMP synthesis in retinal cells is therefore still unknown (52). Finally, it has been demonstrated that calcium-dependent modulation of some guanylyl cyclase activity was due to a 26 kDa protein, in a way similar to calmodulin with the brain calmodulin-dependent adenylyl cyclases (150). Thus, guanylyl cyclases behave as subclasses of class III cyclases, which differ in their modular organization. Once again, this illustrates the very efficient way in which evolution has combined modules having many different structural or catalytic activities, to produce an integrated pattern for synthesis of the cyclic nucleotides.

SPACE AND TIME: PREREQUISITES FOR THE GENETIC STUDY OF TRANSIENT PHENOMENA

In addition to structural differences there is an immense variety in the efficiency of the enzymes that convert the nucleoside triphosphate into the cyclic nucleotide, some enzymes displaying extremely low efficiency (13,26, 49,238,252,287) whereas others—in particular the toxic enzymes—are extremely active (121,148,164,283).

To follow this line of reasoning is to assume a steady-state behavior of cyclic nucleotide synthesis in cells, which is precisely not the usual situation in events where cyclic nucleotides are involved. Could it be that it is not cAMP (or cGMP) which is the regulating molecule, but rather the shape of its time-dependent variation in concentration? I shall review a few observations that strongly suggest that cAMP does not have the same effect when it is delivered in a steady-state fashion, rather than as a pulse (or a series of pulses). I propose that the variety of cyclase catalytic and regulatory subunits is involved in a specific pattern of cAMP (or cGMP) flow, which is recognized, integrated, and amplified by specific enzymes. The variety is then explained by the very nature of the purpose that has to be achieved, synthesizing cyclic nucleotides in a way that can only be recognized by a given target system, in the presence of other similar systems. This would indicate that the structural analysis of adenylyl cyclases might be just the beginning of a new development in the genetics of transient phenomena.

cAMP in the Slime Mold Dictyostelium discoideum

The motile and aggregating amoeba Dictyostelium discoideum have been used as a model for cell differentiation where undifferentiated cells start to differentiate into specific tissues after starvation (51). cAMP secreted in the external medium has been identified as a signal for aggregation. The genetics, biochemistry, cellular biology, and physiology of the cAMP-mediated processes have been investigated in detail in this organism (11,131,297). cAMP
has been shown to control a cascade of events that are necessary not only for chemotaxis and aggregation but also for the expression of genes involved in differentiation (64, 74a, 75, 258). As in higher eucaryotes it was found that regulation of cAMP concentration was mediated by two enzymes, adenylyl cyclases (together with G-proteins (104, 130, 145)) and phosphodiesterases. In contrast with the situation with higher eucaryotes, however, cAMP and phosphodiesterase control operates not from the interior of the cell but from the external medium. This prompted detailed study of cAMP synthesis and degradation in the organism, and it was discovered that pulses of excreted cAMP were required for proper action of the molecule (170, 185). The pulses are generated by an appropriate coupling between adenylyl cyclase activity, phosphodiesterase activity, and diffusion. This particular example demonstrates that there exist enzyme systems that are able to recognize not a molecule, but the change in the concentration of this molecule. Several models can be proposed to account for such waves of cAMP, but they only require very simple enzyme properties (in particular standard nonlinear features, such as self-activation, and desensitization after saturating activity). The main observation is that variation in the cAMP pulse frequency changes the response of the cell (185). For example, this has been detailed in the study of so-called mnemonic enzymes, where there is hysteresis in the activity pattern of an enzyme, the present catalytic activity being affected by the past catalytic activity of the enzyme (233).

**cAMP and the Cell Cycle**

Acknowledging that there are instances where cAMP concentration varies in a way that can be correlated with important cell functions, it is interesting that cAMP might be coupled to the cell cycle.

Utsumi et al. have investigated cyclic AMP synthesis during the cell cycle of *E. coli* on synchronized cells. They have provided what seems to be an unambiguous demonstration that there was a strong correlation between cAMP synthesis and replication or cell division. This suggests that the cAMP molecule may play some role in the cell cycle (304). The importance of this observation has generally not been taken seriously because it is known that cells deficient for adenylyl cyclase or CAP are viable, indicative of a minor role for cAMP in the process. However Kohiyama and coworkers have shown that DnaA, a protein required for proper initiation of replication had a cAMP-binding site, was modulated by the nucleotide (118). In addition, it is known that cya strains are abnormal in many of their properties including proper division, and that they yield spherical cells instead of the usual elongated rods (for review see 299). Finally, d'Ari and coworkers have shown that cAMP plays a significant, if not absolutely indispensable, function in cell division (44). Taken together, these observations indicate that a specific
time-dependent change in the concentration of cyclic AMP is necessary for proper coordination of replication and division in E. coli. This could be achieved by modulation of cAMP synthesis in bacteria where it is well known that excretion of the nucleotide is very efficient, rather than coupling to the activity of a phosphodiesterase (123,299). In this respect, it seems worth noting that high concentrations of cAMP produced by the introduction of foreign adenyllyl cyclase genes in E. coli are not toxic till they reach a very high level (~ten-fold the normal concentration), whereas much lower concentration of cAMP produced by the endogeneous adenyllyl cyclase are toxic (Danchin et al., unpublished experiments, 1988). As the same chemical is synthesised in both cases, one is compelled to think that it is the time course of its synthesis which differs, and this is differently recognized by the cell.

In the same way, it has been found that intracellular and extracellular levels of cAMP differ during the cell cycle of S. cerevisiae. Using centrifugal elutriation, Smith et al. (1990) showed that the intracellular cAMP concentration followed the stages of the cell cycle, being highest during the division cycle and lowest immediately prior or just after cell separation; at the same time the external cAMP concentration did not vary (272). Therefore, in yeast as in E. coli it appears that the role of the external medium is to act as a sink for cAMP. Whether this variation in cAMP is the cause or the consequence of cell cycle events is not known. Nevertheless, these observations further demonstrate that, under normal conditions, appropriate enzyme systems can generate a specific time-dependent pattern of changes in cAMP concentration. As in the case of E. coli, S. cerevisiae adenyllyl cyclase is dispensible in mutants of the cAMP receptor, and in S. pombe adenyllyl cyclase is dispensable during vegetative growth (179). However, yeast cells that carry the mutation and are also deficient in adenyllyl cyclase harbor several growth defects. In this respect, the function of the time-dependent cAMP pattern could be to optimize transient processes, in particular cell division and chromosome segregation. It would be most interesting to see whether cells defective in adenyllyl cyclase and supplemented with cAMP behave differently from cells with adenyllyl cyclase, demonstrating that the time-dependent pattern of cAMP concentration could be specifically recognized.

**cAMP in Taste and Olfaction**

Nerve cells typically generate and are sensitive to transient signals. In this respect, it is important to note that cAMP (and also cGMP, 274), has been shown to be involved in taste and olfaction (6,7,151,153,155,156). Olfaction is mediated by specific sensory cells, each conveying some specific odor recognition (the nature and the concentration of the odorant). The role of the cyclic nucleotide is, at least in part, to modulate cyclic nucleotide-gated channel activity (97a). Cells then convert the signal that they receive into
action potentials that are further integrated before stimulating the brain centers involved in olfaction (154). Nobody would challenge the fact that specificity in neuronal recognition is given by the firing pattern, thus demonstrating that the time course of the underlying biochemical processes is meaningful. It has therefore to be said that the way receptors interact with the cell machinery is also apt to generate specific firing patterns. Modulation of adenylyl cyclase activity from sensory neurons is modulated (as are most other cyclases) by a variety of physicochemical signals (158,213,247,312). This certainly permits the generation of a variety of time-dependent patterns for cAMP synthesis, reflecting the environmental inputs as well as the molecular structure of the enzyme or its subunits. More integrated neural processes are also likely to use cyclic nucleotides (157a), as demonstrated in the analysis of learning and memory.

cAMP and Learning

A great deal of experimental data have demonstrated that cAMP acts as a mediator of learning and memory in *Aplysia* (209) and in *Drosophila melanogaster* (for a recent review see 56, 166a). Our knowledge of the learning processes indicates that time-dependent phenomena are very important for stabilization of appropriate connections, a general view being that synapses are created as labile entities and evolve in the following manner:

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   R
 / \
V   L
 |   S
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where virtual (V) synapses are created as transient entities (L) which can either regress (R) or be stabilized (S) (28,30). Accordingly, the evolution of the synaptic pattern is strongly dependent on the time-course of neurotransmitter delivery. Analysis of the minimal requirements for synapse stabilization suggests that the process of neurotransmitter release must be coupled to some other transient metabolic process in order to yield a stable geometry (29). In those cases where cAMP is involved, one can therefore speculate that the role of adenylyl cyclase is to trigger an appropriate biochemical process, when the proper time-dependent control of its activity is established (209,323). So, once again, it is not the cAMP concentration which is important, but, rather, the temporal variation of the cAMP concentration. In the process of learning, the regulation of adenylyl cyclase activity must therefore be exquisitely tuned to permit delivery of the molecule in the proper time-dependent manner.
cGMP

In the same way, cGMP has been demonstrated to be involved in a process leading to activation of specific cascades\(^\text{27}\) to control a specific process of neuronal activation, the sensitivity to light of retina photoreceptor cells\(^\text{175}\). As we have seen, the organization of guanylyl cyclase and its control elements is similar to—but distinct from—the organization of hormonally regulated adenylyl cyclase\(^\text{264}\). In particular, G-proteins regulate the cGMP phosphodiesterase rather than guanylyl cyclase. But this could also lead to a time-dependent pattern of the nucleotide concentration. In the case of the guanylyl cyclases that are controlled by atrial natriuretic peptide, modulation of cGMP synthesis is coupled—in a still poorly understood manner—to ion transport, a process that is generally linked to transient phenomena (depolarization pulses). The important observation here, which may well be related to the phylogeny of cyclic nucleotides is that on the one hand, ion pumps bear similarities with class III enzymes, and on the other hand that the cyclic nucleotides bind directly to specific ion channels, thereby controlling ion fluxes. In the case of vision, this type of interaction is directly responsible for the fine tuning of the firing pattern. The similarity that we have uncovered between ATP-driven ion pumps and class III cyclases (see also \textit{144} for a purely structural analysis) may also be relevant to this observation, and indicate a very early function of cyclic nucleotides in primitive cells (Fig. 8).

Time-Dependent Recognition and Gene Expression

Adenyllyl and guanylyl cyclases are involved in a wide variety of phenomena. We have listed above many of the processes which lead to cAMP production. In particular it can be seen that many phenomena in which discrimination is important yield cAMP or cGMP as a mediator. This may be perceived as somewhat puzzling: How is discrimination finally achieved? Taking into consideration the data presented above, a picture emerges, where specificity could come not from the molecule itself, but from the time-dependent pattern of changes in its concentration. This asks specific enzyme systems to recognize not the concentration of a molecule, but its variation in time. There are such examples in the case of neurotransmitter receptors, and it would therefore be interesting to investigate the components of cAMP receptor-mediated cascade with such discriminating properties\(^\text{323}\). A consequence of such interpretation is that ontogeny of differentiated cells should be strongly correlated with the structure of adenyllyl cyclases, and that mutants should exist where alteration of cyclase control is reflected by specific alterations of cell development. This type of correlation has certainly been observed (e.g., \textit{11, 128, 174, 276, 322}). Transient phenomena might therefore be of major importance in gene expression\(^\text{223}\).
The unexpected variety of adenyl cyclases, and, above all, of their regulatory subunits could reflect the variety of their cAMP-synthesis time-dependent pattern. Modular organization of the catalytic polypeptide might have evolved from the need for exquisitely tuned modulation of activity. This may be the ultimate explanation for the immense and puzzling variety in structure of adenyl cyclases and guanylyl cyclases.

ACKNOWLEDGMENTS

This work benefited from numerous critical discussions with O. Barzu, R. Kessin, M. Levinthal, A. Ullmann, and M. Véron, whom I gratefully thank for their invaluable help. Experiments from my laboratory benefited from grants from Centre National de la Recherche Scientifique (UA 1129), Ministère de l'Education Nationale (89-1540), and Institut Pasteur.

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