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EFFECTS OF CYCLIC AMP AND CALCIUM IONS ON VIRUS-INDUCED FUSION OF EHRLICH ASCITES TUMOR CELLS

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Summary

The effects of cyclic nucleotides and Ca^{2+} on the fusion of Ehrlich ascites tumor cells induced by HVJ (Sendai virus) were studied.

1. Addition of inhibitors of cyclic nucleotide phosphodiesterase increased the frequency of fusion up to 2 fold. None of these drugs affected the fusion and haemolysis of human erythrocytes.

2. Cyclic AMP content reached a maximum (about 2 fold) after 2 min from the start of the fusion reaction and the increase in cyclic AMP was almost proportional to the dose of the virus added.

3. Reagents which might increase intracellular cyclic GMP, such as insulin and carbamylcholine partially inhibited the fusion process (40% inhibition at 6 μM).

4. The stimulatory effect of theophylline was dependent on the extracellular concentration of Ca^{2+} with an optimal concentration of 0.5 mM.

5. Theophylline partially antagonized the inhibition of the fusion reaction by cytochalasin D (0.1 μg/ml or 0.5 μg/ml).

The inhibition of the fusion reaction by cytochalasin D was influenced by the extracellular concentration of Ca^{2+}.

6. However, fusion of human erythrocytes which required neither Ca^{2+} nor ATP was not affected by cyclic AMP and cytochalasin D. Therefore, fusion of Ehrlich ascites tumor cells has more complex regulatory systems than human erythrocytes. A possible mechanism of the regulation is discussed.

Introduction

Fusion of biological membranes is an important and fundamental event in cell biology. At the subcellular level, membrane fusion is widely observed in

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many tissues and cells [1]. Membrane fusion also occurs between cells, and in this case multinucleated cells are formed [1].

Although the mechanisms of these various membrane fusions at cellular and subcellular levels may not be completely the same, there will be a common mechanism in the fusion process. The factors which control both cellular and subcellular membrane fusion may be important in this connection. For example, Ca^{2+} and ATP are well known as control factors in many membrane fusion processes [1,2].

HVJ (Sendai virus)-induced fusion of Ehrlich ascites tumor cells was originally described by Okada [4]. It requires adequate concentrations of Ca^{2+} and ATP which is regenerated by oxidative phosphorylation [5,6] and glycolysis [7]. Thus, membrane fusion which occurs both in vivo and in virus-induced cell fusion have common factors that regulate the fusion process. However, very little is known about the precise role of Ca^{2+} and ATP on the fusion process in both cases of membrane fusion. In the course of survey of reagents, which might affect membrane phenomena, it was found that reagents which elevated the intracellular concentration of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) also stimulated the fusion process [6]. It has been well-known that there is a close relation between Ca^{2+} and cyclic nucleotides in several cellular events such as secretion [2,9]. Microfilaments which require ATP and possibly Ca^{2+} for their function were also thought to participate in the virus-induced fusion process as reported elsewhere [10]. Therefore, relationship between Ca^{2+}, cyclic AMP and microfilaments in the fusion process of Ehrlich ascites tumor cells was studied.

In this report, the elevation of the intracellular cyclic AMP level, effectively stimulated HVJ-induced fusion of Ehrlich ascites tumor cells, and a requirement for Ca^{2+} for this stimulatory effect is described. Some results, which suggest the involvement of microfilaments in the cyclic AMP-dependent process will also be described.

Materials and Methods

Ehrlich ascites tumor cells were planted in the abdomen of ddo mice and grown for 7 to 10 days. The cells were freed from contaminating erythrocytes and leucocytes by washing three times with a balanced salt solution consisting of 140 mM NaCl, 54 mM KCl, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4 buffered with 10 mM Tris · HCl at pH 7.6 (BSS). The washed cells were suspended in BSS.

HVJ (Sendai virus), Z strain, was grown in embryonated eggs, purified by differential centrifugation, and suspended in BSS [5]. The dose of the virus was expressed in terms of its hemagglutination unit (HAu), which was determined by Salk's pattern method according to Okada by using 0.5% chicken red blood cells [5].

Cell fusion was measured as follows. A desired dose of HVJ was added to the tumor cells suspended (to a desired density) in BSS containing 2.0 mM $CaCl_2$ (final volume, 1.0 ml) at 0°C and the mixture was allowed to stand for 15 min in ice to complete the virus-induced agglutination of the cells. The fusion reaction was then started by raising the temperature to 37°C and terminated after

15 min of incubation by cooling rapidly in an ice bath. The number of cells was counted before and after the fusion reaction by the method of Okada and Tadokoro [11]. Since one fusion event results in a decrease of the total cell number by one, the efficiency of cell fusion was expressed as "fusion frequency", i.e. percent decrease in cell number.

For determination of intracellular level of cyclic AMP, the cells were rapidly sedimented at 4°C from 2.0 ml of suspension and extracted with ice-cold 0.4 ml of 10% $HClO_4$.

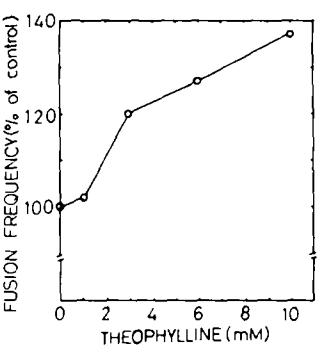
The extract was neutralized with 3 M K_2CO_3 and the resulting $KClO_4$ precipitates were removed by centrifugation. The cyclic AMP content in the supernatant was determined by the competitive binding method using cyclic [^{3}H]-AMP as a competitor [12]; a bovine adrenal extract prepared as described by Brown et al. [13] was used as a cyclic AMP-binding protein preparation.

Intracellular ATP level was measured as previously described [14]. 2 ml of the cell suspension were extracted with perchloric acid and neutralized with K_2CO_3 as described in the case of cyclic AMP. The ATP content of the neutralized extract was measured by firefly luciferase method [15] with a Packard Tri-Carb Model 3330 liquid scintillation spectrometer. Cytochalasin D was kindly supplied by Sionogi Co., Osaka.

Results

Stimulation of cell fusion by increasing intracellular cyclic AMP level

As shown in Fig. 1, HVJ-induced fusion of Ehrlich ascites tumor cells was enhanced by theophylline, a well-known inhibitor of cyclic AMP-phospho-



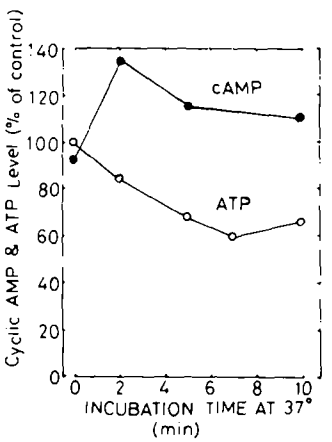


Fig. 1. Effect of theophylline on HVJ-induced fusion of Ehrlich ascites tumor cells. Cells suspended in a balanced salt solution containing 2 mM Ca^{2+} ($1.84 \cdot 10^7$ cells/ml) were preincubated with theophylline for 20 min at 37°C then cooled before the addition of 1000 HAu of HVJ. The fusion frequency was determined as described in Materials and Methods, except that theophylline was added as indicated.

Fig. 2. Cyclic AMP and ATP content of Ehrlich ascites tumor cells during the course of cell fusion. The reaction mixture was similar to that described in the legend to Table I except that $3.68 \cdot 10^7$ cells in 2.0 ml of suspension were used and 10 mM of theophylline was included in the system. Cells were rapidly cooled at the indicated time, centrifuged and nucleotides were extracted. Cyclic AMP and ATP content was determined as described in Materials and Methods. Cyclic AMP and ATP level was indicated as a percent of the control cells which were incubated without virus.

diesterase, at concentrations similar to those used for studies of other cyclic AMP-dependent cellular events [16]. Brief pre-incubation of the cells with theophylline (5 min at 4°C) was needed to obtain maximal activation. Cell fusion was also stimulated by dibutyryl cyclic AMP, isobutylmethylxanthine (IMX), prostaglandin E_2 (PGE_2) as well as theophylline as reported previously [6]. All these reagents are known to elevate the intracellular concentration of cyclic AMP. The degree of enhancement by these drugs was also influenced by the extent of fusion of the control samples. In other words, if fusion frequency of the control is higher, stimulation by these drugs becomes lower. Therefore, conditions that resulted less than 50% decrease in cell number in control samples were usually employed.

To see if the stimulation is really caused by an increase in the intracellular concentration of cyclic AMP, the nucleotide content was measured during the course of cell fusion. It was found that in the presence of 10 mM theophylline cyclic AMP content was maximal at 2 min after the initiation of fusion reaction by raising the temperature of virus-cell aggregates to 37°C, and then it was decreased gradually (Fig. 2). No such increase of cyclic AMP after 2 min of incubation at 37°C was observed in control cells. Thus it is reasonable to assume that this increase of intracellular cyclic AMP level was caused by the addition of HVJ. Since the fusion reaction of ascites tumor cells require high ATP levels [5] and the cyclic AMP-dependent process will consume ATP, the intracellular level of ATP was determined during the fusion reaction. As depicted in Fig. 2, the ATP level decreased, and a maximum decrease was observed at about 7 min after the initiation of fusion reaction (Fig. 2).

As shown in Fig. 3, cyclic AMP content was increased by raising the virus dose from 500 HAu/ml to 2000, although no increase was observed when the virus dose was raised further to 8000 HAu/ml.

We would like to propose that HVJ could modify the ascites tumor cell membrane, resulting in the increase of intracellular level of cyclic AMP.

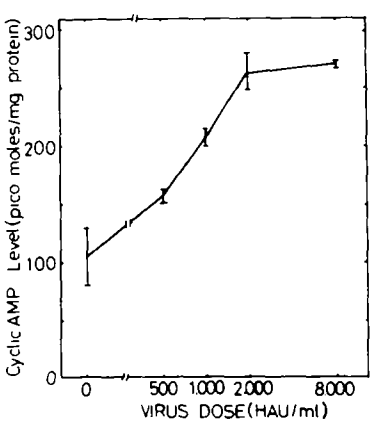


Fig. 3. Virus dose response of the elevation of intracellular cyclic AMP level at 2 min after the initiation of the fusion reaction. The condition of fusion reaction and the determination of cyclic AMP content was the same as for Fig. 1. Cyclic AMP content was determined at 2 min after the initiation of the fusion reaction at 37°C.

TABLE I

EFFECTS OF THEOPHYLLINE, IMX AND DIBUTYRYL CYCLIC AMP ON FUSION OF HUMAN ERYTHROCYTES

Human erythrocytes were preincubated with the tested reagents for 30 min at 37°C, and then allowed to stand for 10 min at 0°C. 100 HAu of HVJ were added to the cells suspended in a balanced salt solution (final volume, 1.0 ml) at 0°C and then the mixture was allowed to stand for 25 min in ice to complete the virus-induced agglutination of the cells. The fusion reactions was performed for 40 min at 37°C and terminated by the addition of four volumes of cold balanced salt solution. The cell number was counted by a Coulter counter before and after the fusion reaction.

Additions	Fusion frequency (% of control)	Hemolysis (%)
10 mM theophylline	109	101
0.5 mM IMX	100	100
2.0 mM dibutyryl cyclic AMP	100	99
None	(100)	(100)

Effects of cyclic AMP on fusion of human erythrocytes

Human erythrocytes which have no cytoplasmic organelles [17] have an ability to fuse under the influence of HVJ, but they show no requirement for ATP and Ca^{2+} for the fusion reaction [18]. To see whether these reagents which stimulate the fusion of Ehrlich ascites tumor cells also exhibit a stimulatory effect on fusion of human erythrocytes, the influence of these reagents on human erythrocytes was examined. As shown in Table I, HVJ-induced hemolysis of human erythrocytes, a partial reaction of virus-induced fusion of erythrocytes [19], was not affected by these compounds. The fusion process was also little affected by IMX and dibutyryl cyclic AMP (Table I), thus the results were in accord with no requirement for ATP or Ca^{2+} in this system. By using the virus-containing spin-labelled lecithin, fusion of the viral envelope to the cell membrane could be studied as described previously [20]. No stimulation of virus-cell fusion was observed in the presence of drugs which increase intracellular cyclic AMP (Maeda, T. and Ohki, K., unpublished observation).

Effects of cholinergic agents

Since it has been well-known that insulin decreases the concentration of cyclic AMP in many tissues, and exhibits the opposite effect to cyclic AMP

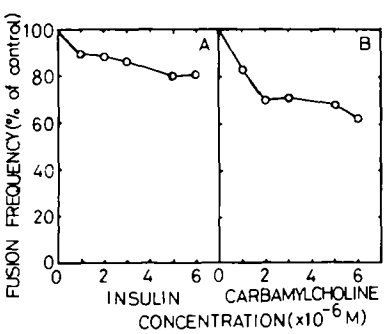


Fig. 4. The inhibition of the fusion reaction by insulin and carbamylcholine. The condition of the fusion reaction was the same as for Table I, except that insulin or carbamylcholine was added 5 min after the addition of virus at 0°C.

[21,22], its effect on the fusion reaction was studied. As shown in Fig. 4(A), insulin lowered the fusion frequency, although only 20% of inhibition was obtained at 6 μM. Recently, it was reported by Illiano et al. that insulin can act as a cholinergic drug, and increase the intracellular concentration of cyclic GMP in tissues [23]. If this is so, the inhibition of the cell fusion by insulin could be due to the increase in concentration of cyclic GMP in the cells. A cholinergic drug, carbamylcholine which is known to increase the intracellular cyclic GMP level [24,25] was used to see the effects on virus-induced fusion. As shown in Fig. 4(B), carbamylcholine inhibited effectively the fusion reaction at low concentrations, and its inhibition reached to 40% at higher concentrations of the drug. Thus, inhibition observed by the addition of insulin or carbamylcholine could be due to either the decrease of cyclic AMP or the elevation of cyclic GMP.

Role of Ca^{2+} on cyclic AMP-dependent stimulation

As stated in introduction, function of cyclic AMP has a close relationship with Ca^{2+} [3]. It became necessary to see whether there is a relation between cyclic AMP-dependent stimulation of the fusion reaction and the extracellular concentration of Ca^{2+}. As depicted in Fig. 5, the maximal stimulation was obtained at a concentration of 0.4 mM, when the Ca^{2+} concentration was either raised to 2.0 mM or lowered to 0.2 mM, the stimulation was decreased, and at a higher concentration (5.0 mM) the stimulation was abolished. Since the intracellular concentration of cyclic AMP was almost equally elevated by theophylline regardless of the presence or absence of Ca^{2+} as reported previously [6], the abolishment of the stimulation of cell fusion was caused by difference in concentrations of extracellular Ca^{2+}. It is well known that extracellular Ca^{2+} is required for several cyclic AMP-mediated processes [3]. Cittadini et al. reported that the Ca^{2+} uptake by the ascites tumor cells was inhibited by ruthe-

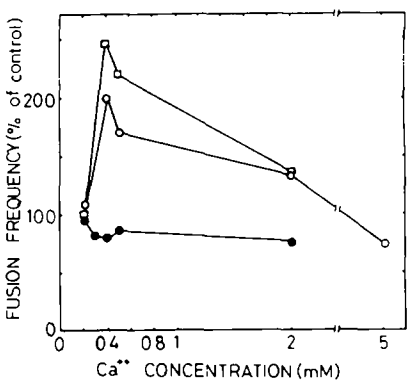


Fig. 5. Effect of Ca^{2+} concentrations on theophylline stimulation of HVJ-induced cell fusion, and the effect of ruthenium red and La^{3+} on theophylline stimulation. Fusion was performed in Tricine-buffered medium (135 mM NaCl, 5.4 mM KCl, and 40 mM Tricine/NaOH buffer (pH 7.8) [7] instead of a balanced salt solution to avoid precipitates formation at higher Ca^{2+} concentrations. The dose of HVJ was decreased to 400 HAu in these experiments, since a higher dose of HVJ causes lysis of the cells at low Ca^{2+} concentrations. The cells were preincubated with 10 mM theophylline (○———○), 10 mM theophylline and 30 μM ruthenium red (●———●), and 10 mM theophylline and 5 μM $LaCl_3$ (□———□) for 10 min at 37°C.

nium red but not by $LaCl_3$, whereas Ca^{2+} uptake by mitochondria isolated from the same cells was repressed by both ions [26]. Based on these facts, it was examined if the influx of Ca^{2+} into the cells is required for the appearance of the stimulatory effect of theophylline. When ruthenium red at a concentration of 30 μM was added to 10 mM theophylline, the stimulation of cell fusion by theophylline was completely abolished at all of the Ca^{2+} concentrations tested; on the other hand, the addition of 5 μM. $LaCl_3$ did not inhibit the stimulation of cell fusion by theophylline (Fig. 5). Since the stimulatory effect was abolished by ruthenium red, it could be assumed that stimulation of the fusion reaction was only exhibited when accompanied by Ca^{2+} influx.

Relationship between cytochalasin inhibition and theophylline stimulation

Some cytoplasmic components, such as microfilaments and microtubles may participate in membrane fusion as in the case in secretion [27] and phagocytosis [28]. Recently, virus-induced cell fusion was found to be strongly inhibited by cytochalasin D [10,29], and cytochalasin B [30], a microfilaments inhibitor. From this and other supporting evidence, it is very likely that microfilaments participate in the virus-induced fusion of Ehrlich ascites tumor cells [10]. Since Ca^{2+} is a well-known effector of the contractile system [31], effects of extracellular Ca^{2+} concentration on cytochalasin inhibition were studied. As depicted in Fig. 6, an increase in extracellular Ca^{2+} concentration above 0.5 mM partially antagonized the inhibitory effect of cytochalasin D. In other words cytochalasin D inhibited more effectively the cell fusion at lower Ca^{2+} concentrations. Because microfilaments could be a possible site of action of cyclic AMP, an experiment was designed to see if the cyclic nucleotide could modify the sensitivity of microfilaments toward this drug. As shown in Fig. 6, cytochalasin D completely inhibited the fusion reaction at concentrations of 0.1 μg/ml and 0.5 μg/ml with 0.5 mM Ca^{2+} in the reaction medium. Prior treatment of cells (10 min at 37°C) with 10 mM theophylline resulted in the protection against the inhibition of cell fusion by cytochalasin D at both concentrations. When cells were pretreated with theophylline, the inhibition was reduced to 60% and 74% at concentrations of 0.1 μg/ml and 0.5 μg/ml, respectively (Table II).

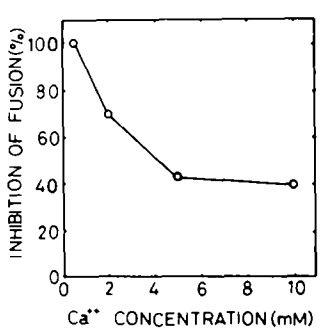


Fig. 6. The effect of Ca^{2+} concentrations on cytochalasin inhibition of HVJ-induced cell fusion. The fusion reaction was performed as described in Table I except that at higher Ca^{2+} concentration (5 and 10 mM) Tricine-buffered medium was used. Cytochalasin D (0.5 μg/ml) and about 800 HAu of HVJ were used as indicated.

TABLE II

THE EFFECT OF THEOPHYLLINE ON THE INHIBITION OF CELL FUSION BY CYTOCHALASIN D

The fusion reaction was performed in a balanced salt solution containing 0.5 mM $CaCl_2$ and with 800 HAu of HVJ. To observe the protection, the cells were preincubated with 10 mM theophylline for 10 min at 37°C, cooled, and then cytochalasin D was added.

Addition	Fusion frequency (% of control)	
	— theophylline	+ theophylline
None	(100)	168 (100)
Cytochalasin D (0.1 μg/ml)	0	68 (40)
Cytochalasin D (0.5 μg/ml)	0	44 (26)

Discussion

Stimulatory effect of cyclic AMP on HVJ-induced fusion of Ehrlich ascites tumor cells became evident from the results reported in this paper. All the reagents that are known to increase the intracellular level of cyclic AMP were stimulatory and reagents which either decrease the intracellular cyclic AMP level [21,22] or increase the cyclic GMP level [23] such as insulin and carbamylcholine (a cholinergic drug) were inhibitory to the fusion process (Fig. 4). Although an increase of intracellular cyclic AMP level was observed when HVJ was added to the cells and this increase was dependent on the dose of the virus employed (Figs. 2, 3), these results do not necessarily mean that this increase of the nucleotide is acting as a trigger for the fusion process. For example, similar enhancement by theophylline and other related reagents was not observed with HVJ-induced fusion of human erythrocytes. Therefore, it is probable that the nucleotide stimulates a controlling step or the steps of the fusion of Ehrlich ascites tumor cells.

Agglutination of the tumor cells by HVJ seems to represent the initial step of the overall cell fusion process. Since this step was not appreciably influenced by the addition of theophylline (data not shown), it is likely that cyclic AMP exhibits its action at a later step or steps of the whole process. A step at which cyclic AMP might affect its activity will be discussed below. It was recently found that HVJ-induced fusion of human erythrocytes is unique in a sense that it requires neither energy nor a bivalent cation [18]. In this connection, it is interesting that the stimulation of fusion reaction by cyclic AMP (Table I) and inhibition by cytochalasin D [19] was not observed in fusion of human erythrocytes. Thus, the regulatory mechanisms of fusion of Ehrlich ascites tumor cells may differ from that of human erythrocytes, although fundamental mechanisms of fusion reaction should be the same. Therefore, it could be postulated that cyclic AMP and also cytochalasin D may affect the part of fusion reaction at which Ca^{2+} and ATP are required.

As shown in Fig. 5, the stimulation of fusion by theophylline depended on an extracellular Ca^{2+} concentration and ruthenium red abolished this stimulation. Thus, the mere elevation of intracellular cyclic AMP level by theophylline could not induce the stimulation of cell fusion. Accordingly, it is likely that either co-presence of cyclic AMP and Ca^{2+} is required for stimulation or Ca^{2+} is

needed for the enhancement and cyclic AMP may be acting as the stimulant of Ca^{2+} influx. The other possibility of the action of cyclic AMP is the phosphorylation of cytoplasmic components, such as microfilaments. It has been reported that myosin light chain was phosphorylated by an endogenous protein-kinase [32], and concentrations of cyclic nucleotides were changed during the contraction-relaxation process [8]. Further supports for the participation of microfilaments for a cyclic AMP-dependent process in the fusion process may come from the facts that cytochalasin inhibition of fusion reaction was antagonized either by increasing the extracellular Ca^{2+} concentration (Fig. 6) or preincubating with 10 mM theophylline (Table II) both of which indicate mutual interaction of microfilaments, cyclic AMP and Ca^{2+}. Inhibition of cytochalasin D-induced zeiosis by cyclic AMP was reported recently [33]. Antagonism of concanavalin A-induced capping by dibutyryl cyclic AMP and testololactone was also reported [34]. From recent studies of fusion of phospholipid vesicles [35], somatic cell fusion by polyethylene glycol [36], it could be hypothesized that the bare membrane of phospholipid bilayers fuse easily to each other when the composition of the bilayer is appropriate and other requirements are fulfilled. Thus, regulatory mechanisms of cell fusion could be a process that affect the molecular distribution on the surface of cell membrane [37] and as a result, the modification of the membrane allows the contact of a fusable parts of phospholipid bilayers. In the fusion of Ehrlich ascites tumor cells, therefore, cyclic AMP and microfilaments may act on this regulatory system. To pinpoint the site of action of cyclic AMP and Ca^{2+} in this complex system, will require further study. Isolation of components of a cellular contractile system and their interaction with each other and with the membrane component(s) are now under progress in our laboratory.

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References

1 Poste, G. and Allison, A.C. (1973) Biochim. Biophys. Acta 300, 421–465
2 Berridge, M.J. (1976) in Advances in Cyclic Nucleotide Research (Greengard, P. and Robison, G.A., eds.), Vol. 6, pp. 2–98, New York, Raven Press
3 Okada, Y. (1958) Biken's J. 1, 103–110
4 Okada, Y. and Murayama, F. (1966) Exp. Cell Res. 44, 527–551
5 Okada, Y., Murayama, F. and Yamada, K. (1966) Virology 28, 115–130
6 Ohki, K., Nakama, S., Asano, A. and Okada, Y. (1975) Biochem. Biophys. Res. Commun. 67, 331–337
7 Yanovsky, A. and Loyter, A. (1972) J. Biol. Chem. 247, 4021–4028
8 Brooker, G. (1973) Science 182, 933–934
9 Smith, R.J. and Ignarro, L.J. (1975) Proc. Natl. Acad. Sci. U.S. 72, 108–112
10 Asano, A. and Okada, Y. (1977) Life Sci., 20, 117–122
11 Okada, Y. and Tadokoro, J. (1962) Exp. Cell Res. 26, 108–118

12 Walton, G.M. and Garren, L.D. (1970) Biochemistry 9, 4223–4229
13 Brown, B.L., Ekins, R.P. and Tampion, W. (1970) Biochemistry 120, 8
14 Asano, A., Coehn, N.S., Baker, R.F. and Brodie, A.F. (1973) J. Biol. Chem. 248, 3386–3397
15 Stanley, P.E. and Williams, S.G. (1969) Anal. Biochem. 29, 381–392
16 Zor, U., Kaneko, T., Lowe, I.P., Bloom, G. and Field, J.B. (1965) J. Biol. Chem. 244, 5189–5195
17 Juliano, R.L. (1973) Biochim. Biophys. Acta 300, 341–378
18 Peretz, H., Toister, Z., Laster, Y. and Loyter, A. (1974) J. Cell Biol. 63, 1–11
19 Sekiguchi, K. and Asano, A. (1976) Life Sci. 18, 1383–1390
20 Maeda, T., Asano, A., Ohki, K. Okada, Y. and Ohnishi, S. (1975) Biochemistry 14, 3736–3741
21 Flawia, M.M. and Torres, H.N. (1973) J. Biol. Chem. 248, 7417–7426
22 House, P.D.R., Poylis, P. and Weidemann, M.J. (1972) Eur. J. Biochem. 24, 429–437
23 Illiano, G., Tell, G.P.E., Siegel, M.I. and Cuatrecasas, P. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2443–2447
24 Goldberg, N.D., O'dea, R.F. and Haddox, M.K. (1973) in Advances in Cyclic Nucleotide Research (Greengard, P. and Robison, G.A., eds.), Vol. 3, pp. 155–213, New York, Raven Press
25 Goldstein, I., Hoffstein, S., Gallin, J. and Weissman, G. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2916–2920
26 Cittadini, A., Scarpa, A. and Chance, B. (1973) Biochim. Biophys. Acta 291, 246–259
27 Buther, F.R. and Goldman, R.H. (1974) J. Cell Biol. 60, 519–523
28 Mimura, N. and Asano, A. (1976) Nature 261, 319–321
29 Asano, A., Ohki, K., Sekiguchi, K., Tanabe, M., Nakama, S. and Okada, Y. (1974) Symposia Cell Biol. (Okayama) 26, 39–45
30 Pasternak, C.A. and Mickelem, J.J. (1973) J. Memb. Biol. 14, 293–303
31 Nachimas, Y. and Asch, A. (1974) Biochem. Biophys. Res. Commun. 60, 656–664
32 Adelstein, R.S., Conti, M.A. and Anderson, Jr., W. (1973) Proc. Natl. Acad. Sci. U.S. 70, 3115–3119
33 Godman, G.C., Miranda, A.F., Deitch, A.D. and Tanenbaum, S.W. (1975) J. Cell Biol. 64, 644–667
34 Storrie, B. (1975) J. Cell Biol. 66, 392–403
35 Papahadjopoulos, D., Poste, G., Schaeffer, B.E. and Vail, W.J. (1974) Biochim. Biophys. Acta 352, 10–28
36 Davidson, R.L. and Gerald, P.S. (1976) Somatic Cell Genetics, 2, 165–176