ential inhibition of the class I- but not the class II-directed cytotoxicity of CTL 23, 27 and 47 by the anti-CD8 mAb is not consistent with a simple additive contribution of the CD8 interaction with class I antigen to the avidity of the CTL for the target cell, because all targets used in this study simultaneously expressed class I and class II antigens. The present results can be coherently interpreted by postulating that stabilization of the interaction between the T-cell receptor and MHC antigen by CD8 is accomplished through the binding of this molecule to a different epitope on the same class I molecule bound by the antigen receptor. Anti-CD8 mAbs would inhibit lysis by disrupting this ternary complex. In this model, a physical association of the T-cell receptor and the CD8 molecule is not required in the absence of an MHC antigen on the target cell to which both the receptor and CD8 can bind. Since CD8 would not bind to class II antigens, the anti-CD8 antibody would not have any disruptive effect in the class II-directed lysis of target cells by these CTL clones and would not inhibit lysis of those cases.

In conclusion, the results show that: (i) among alloreactive CTL clones with identical or very similar fine specificity, lysis of HLA-B27 (B*2705)$^+$ murine transfectants correlates with the avidity of the CTL clone for the target cell; (ii) for the same CTL clone, lysis of murine target cells is more dependent on CD8 than lysis of human cells; (iii) in CTL clones with dual specificity for class I and class II HLA antigens, CD8 is essential for their class I- but not for their class II-directed cytotoxicity, and (iv) this suggests a co-operative role of CD8 with the T-cell antigen receptor, which may be achieved by binding to a non-polymorphic site of the same class I molecule bound by the T-cell receptor.

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Activation of phosphatidylcholine-specific phospholipase C in cell growth and oncogene transformation

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Introduction

The control of cell proliferation stimulated by growth factors can take place at three different levels, according to the scheme outlined in Fig. 1. In the first level are the growth factors themselves which upon interaction with their respective receptors trigger, in most cases, a tyrosine kinase activity [1]. This enzymic activity has been shown to be necessary for DNA synthesis [2]. Thus, mutations in the ATP-binding site of platelet-derived growth factor (PDGF) and epidermal growth factor receptors yield mutants that bind normally the growth factor, but are unable to transduce the signals generated by this interaction [3].

The second level of control of the proliferative response are the second messengers which amplify and carry out the information from the membrane to the intracellular milieu. The phosphodiesteratic hydrolysis of a minor set of phospholipids, the polyphosphoinositols, has revealed a rich source of second messenger molecules [4]. The existence of an association between phosphoinositide turnover activation and cell growth has long been known. Thus, as early as in 1968, Fisher & Mueller [5, 6] discovered that haemagglutinin potently activated this phospholipid degradative pathway in lymphocytes.

Abbreviations used: PGDF, platelet-derived growth factor; DAG, diacylglycerol; PKC, protein kinase C; PLC, phospholipase C; PtdChO, phosphatidylcholine; PC, phosphoryl choline.

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Phosphoinositide-derived signals produced by growth factors

The addition of certain growth factors, like PDGF or bombesin, induces the phospholipase C (PLC)-mediated degradation of polyphosphoinositols [7, 8] yielding diacylglycerol (DAG) and inositol phosphates. Some of the inositol phosphates have been shown to be involved in the control of intracellular Ca^{2+} levels [9–11]. The cytosolic concentration of this cation has been suggested to be important in the regulation of the proliferative response [12, 13].

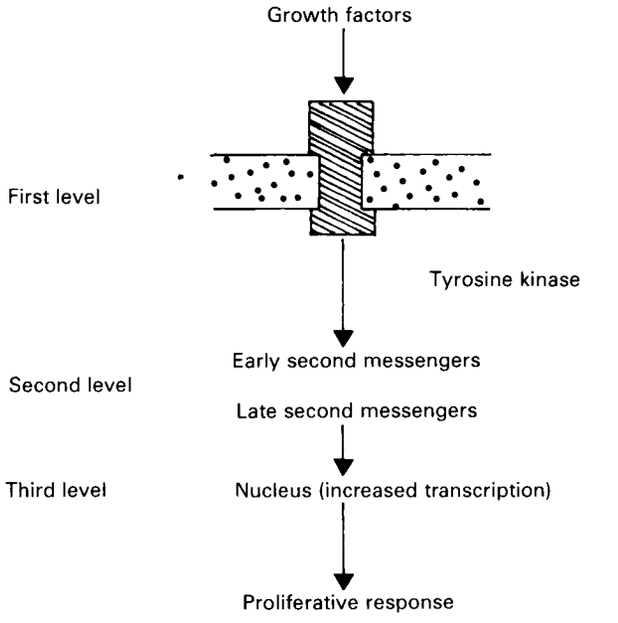

Fig. 1. *Different levels of control of the proliferative response*

DAG, on the other hand, is a key cofactor necessary for protein kinase C (PKC) activation [14]. This kinase is the target of pharmacological analogues of DAG which possess tumour-promoting properties [15]. This led to the suggestion that PKC activation could be an important step in the control of cell growth. More recently, experimental evidence which strongly supports this assumption has been produced. Thus, transfection of fibroblastic cell lines with plasmids harbouring PKC cDNA clones, under the control of strong viral promoters, yields cell lines which display alterations in growth regulation. These alterations include a marked reduction in serum dependency and enhanced tumorigenicity [16, 17].

Furthermore, the presence of PKC has been shown to be necessary for DNA synthesis induced by certain oncogene products [18].

Phosphoinositide turnover activation is not sufficient for cell proliferation

Although some growth factors do not activate phosphoinositide turnover, there is some circumstantial evidence that this pathway may be important for mitogenesis. Thus, mutations that impair the ability of PDGF to initiate DNA synthesis are associated with the loss of the ability of this growth factor to activate the release of inositol phosphates [19].

Furthermore, an antibody raised against phosphatidylinositol 4,5-bisphosphate, which apparently does not cross-react with other polyphosphoinositols, blocks DNA synthesis in response to PDGF when microinjected in NIH-3T3 fibroblasts [20]. All these results suggest that phosphoinositide turnover activation is a required event for proliferation stimulated by at least some growth factors.

Whether or not stimulation of phosphoinositide turnover is sufficient to activate cell proliferation is the next question to be answered when the importance of this pathway is being established. The classical approach followed to study the involvement of phosphoinositide turnover products, namely Ca^{2+} and PKC activation, in the early response to agonists [21, 22], is not applicable to DNA synthesis because of the toxic effects, in long-term incubations, of the drugs used to mimic the actions of the second messengers released during phosphoinositide turnover activation. However, recent work by Escobedo & Williams [23] has shed new light on this problem. Thus, they obtained a PDGF receptor mutant which upon binding of its ligand induced the release of inositol phosphates to an extent similar to that produced by the wild-type receptor. Interestingly, the addition of PDGF to cells expressing that mutant did not give any DNA synthesis when challenged with PDGF [23]. It appears that phosphoinositide turnover activation by this growth factor may be necessary, but is not sufficient for mitogenesis. Therefore, metabolic cascades other than phosphoinositide turnover have to be turned on for DNA synthesis to occur in response to growth factor addition. Among these other signals, a novel phosphatidylinositol 3-kinase recently discovered by Cantley's group could play a role in these processes [2, 24]. Also the stimulation of the phosphodiesteratic hydrolysis of phospholipids distinct from polyphosphoinositols could be important in the maintainance of PKC activation long after phosphoinositide metabolism challenge has taken place [25]. Actually, the study of the effect of oncogene transformation on the phospholipid metabolism has revealed the possible importance of these novel pathways in cell growth and transformation [26, 27] and J. Moscat, T. Fleming, M. T. Diaz-Meco & S. A. Aaronson (unpublished work).

Role of oncogene products in mitogenic signalling

The studies on the mechanisms of growth control have been given further impetus by the discovery that proto-oncogenes, genes that when altered give rise to oncogenes, are involved in mitogenic signal transduction. Thus, some proto-oncogenes code for growth factors [29–32] or growth factor receptors [33–35], while others, like the *ras* oncogene product [36] appear to be linked directly or indirectly in post-receptor mitogenic signalling pathways.

Ras oncogenes are activated by point mutations at one of two sites in their coding sequences [36]. The product of this oncogene, *ras*p21, has a molecular mass of 21 kDa, binds guanine nucleotides and has intrinsic GTPase activity [37, 38]. The fact that microinjection of bacterially expressed transforming *ras*p21 into NIH-3T3 fibroblasts induces DNA synthesis [39, 40] suggests that, by analogy with known G-proteins, *ras*p21 may be regulating steps involved in the transduction of signals that activate mitogenesis.

One intriguing aspect of this hypothesis is the possible involvement of *ras*p21 in the mitogenic cascade at the phosphoinositide turnover level [41, 42]. To address this point in a reliable way, the stimulation of phosphoinositide specific PLC should be measured in a system activatable by *ras*p21 at different times. The *Xenopus laevis* oocyte is a suitable experimental model for two reasons: (i) microinjection of a neutralizing anti-*ras* antibody inhibits hormone-dependent cascades [43], which indicates that this oncogene product is normally operating in signal transduction in the oocyte; (ii) because of its large size, the oocyte allows quantification of phospholipid degradative products at different times after microinjection of *ras*p21. Therefore, DAG levels were measured in oocytes microinjected with either transforming or normal H-*ras*p21. Results shown in Fig. 2, indicate that microinjection of transforming, but not normal, *ras*p21 dramatically activates the release of DAG in oocytes. Surprisingly, the production of DAG was associated with little or no change in inositol phosphate levels [26]. This suggests that sources other than phosphoinositides are activated upon microinjection of *ras*p21 in the oocyte system. To prove that this effect is not specific to this experimental model and that

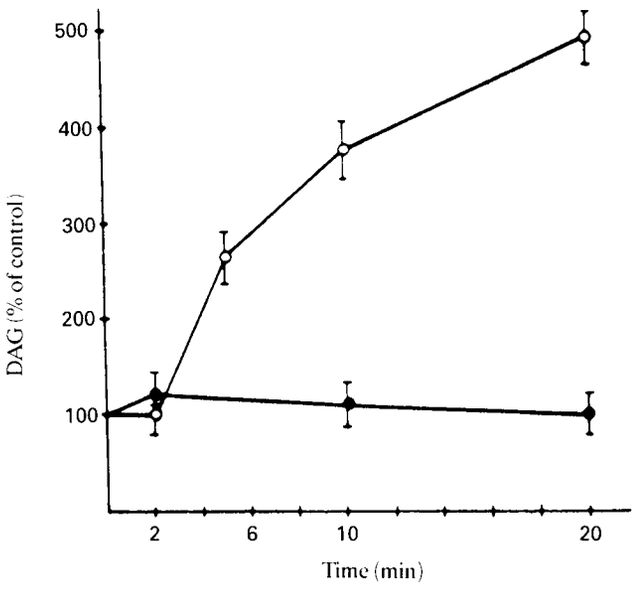

Fig. 2. *Effects of microinjected rasp21 proteins on DAG levels in X. laevis oocytes*

[^{3}H]Glycerol-labelled oocytes were injected with either normal (●) or transformant *ras*p21 (○), and DAG levels were estimated at different times. Results are means ± S.D. of three independent experiments with incubations in duplication. From Lacal *et al.* [26].

Table 1. *Steady-state levels of [2-^{3}H]glycerol-labelled DAG, myo-[2-^{3}H]inositol-labelled inositol phosphate (InsP) and [^{14}C]methylcholine-labelled PtdCho in normal and H-ras-transformed NIH-3T3 fibroblasts*

Results are expressed as stimulation over control values for NIH/3T3 cells, and represent the means ± S.D. of three independent experiments with incubations in duplicate.

NIH/3T3 cell line	Transformed phenotype	PLC products (fold stimulation/control)		
		DAG	InsPs	PC
Control	−	1.00	1.00	1.00
+PDGF (1 h)	−	1.25 ± 0.08	4.10 ± 0.11	0.90 ± 0.08
H-*ras*	+	1.54 ± 0.14	1.02 ± 0.03	2.47 ± 0.02

Table 2. *Fatty acid composition of DAG from different cell lines*

Results are expressed as percentage of total fatty acids in DAG from each cell line, and are mean values ± S.D. of three independent experiments with incubations in duplicate.

Fatty acid	Cell lines		
	NIH/3T3	NIH/3T3 + PDGF (1 h)	H-*ras*
Palmitic	37.0 ± 0.1	30.0 ± 0.9	31.0 ± 0.8
Stearic	14.0 ± 0.3	20.2 ± 0.3	13.8 ± 0.2
Oleic	20.0 ± 0.1	12.8 ± 0.1	25.2 ± 0.1
Linoleic	14.1 ± 0.1	3.0 ± 0.2	22.5 ± 0.2
Arachidonic	15.2 ± 0.2	35.1 ± 0.1	8.0 ± 0.3

this intriguing finding is also present in mammalian cell lines, DAG levels were investigated in either normal NIH-3T3 fibroblasts or those transformed by the *ras* oncogene. Results shown in Table 1 indicate that H-*ras* transformants gave a substantial elevation of DAG compared with control cells. Cells stimulated with PDGF also displayed a significant elevation in DAG. Interestingly, whereas PDGF-stimulated cells gave a four-fold increase in inositol phosphates, no alterations in this parameter were detected in H-*ras* transformants. This is in good agreement with the results obtained in the oocyte, and supports the notion that a different DAG source is being activated by *ras* transformation. If this is true, and bearing in mind that phosphoinositide-derived DAG is rich in stearic and arachidonic acid, whereas other phospholipids are not [44], *ras* transformants should have a different DAG fatty acid composition than PDGF-activated NIH-3T3.

To test this possibility, DAG from PDGF-stimulated NIH-3T3 or from H-*ras* transformants was isolated and its fatty acid composition was determined by gas–liquid chromatography. Table 2 shows that, as expected, DAG from cells stimulated by PDGF gave increased levels of stearic and arachidonic acid as compared with normal or *ras*-transformed cells. Cells transfected with the H-*ras* oncogene showed DAG rich in oleic and linoleic acid which are fatty acids abundant in phosphatidylcholine (PtdCho). Therefore a possible source for this DAG is the PLC-mediated degradation of PtdCho. If this were the case, increased steady-state levels of phosphorylcholine (PC), which along with DAG is produced after the phosphodiesteratic hydrolysis of PtdCho, should be observed in *ras* transformants. Results shown in Table 1 confirm that enhanced release of PC is associated with *ras* transformation.

Relatively little is known concerning the physiological importance of the phosphodiester hydrolysis of PtdCho. A number of agonists have been shown to induce a rapid stimulation of this pathway [28, 44–48]. We have not detected significant increases in PC levels shortly after the addition of PDGF to NIH-3T3 cultures (Table 1). This might indicate that the effects of PDGF and *ras* transformation on phospholipid metabolism differ qualitatively. However, it has been demonstrated that the *ras* oncogene product is a required step in the signal transduction pathway activated by PDGF [49]. Therefore, if PtdCho hydrolysis is important in mitogenic signalling, it should be activated by at least some growth factors. One possibility is that this pathway is a late event in the cascade triggered by mitogens. In this regard, it should be noted that cell-cycle-dependent phosphorylations, involving some oncogene products, appear to be important events controlling cell proliferation [50–53]. This novel DAG generated upon *ras* action may be activating PKC isoenzymes which could be potentially involved in these phosphorylation events. All these possibilities are interesting aspects for future research.

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Structure, control and assembly of a bacterial electron transport system as exemplified by *Paracoccus denitrificans*

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The cytoplasmic membrane of *Paracoccus denitrificans* has attracted increasing interest since 1975 when John & Whatley [1] pointed out some of the functional similarities between its electron transport proteins and those from mammalian mitochondria. Subsequently, knowledge of these similarities has been extended and reinforced by evidence of relatedness at the structural level. Information about the subunit structures and sequences, together with redox centre compositions for NADH dehydrogenase [2–5], succinate dehydrogenase [6], the cytochrome bc_1 complex [7, 8] and cytochrome aa_3 oxidase [9, 10], has all added to the overall picture of similarity. An important difference between the mitochondrial and bacterial proteins was found for the cytochrome bc_1 complex and cytochrome aa_3. In each case, the bacterial system possesses fewer polypeptides than the mitochondrial counterpart. The cytochrome aa_3 oxidase of *P. denitrificans* is recognized to contain three polypeptides [11, 12], although only two are found in some preparations, whereas the mitochondrial enzyme has up to 12 subunits [9]. This relative structural similarity aids assignment of function to individual polypeptides. The cytochrome bc_1 complex from *P. denitrificans* has three polypeptides and lacks several polypeptides that do not bear redox centres and yet are found in the mitochondrial enzyme [7]. Interestingly, two of the latter polypeptides have recently been implicated in the assembly of the mitochondrion [13]. The definite subunit structure of the *P. denitrificans* NADH–ubiquinone oxidoreductase is awaited. There is evidence that the enzyme may fragment more readily than its mitochondrial counterpart [2–5]. As the latter has as many as 26 polypeptides a simpler and fully functional bacterial NADH dehydrogenase would considerably aid the understanding of this enzyme.

The electron transport system of *P. denitrificans* alters according to the growth conditions of the cells. Aerobic growth on methanol or methylamine requires the presence of periplasmic dehydrogenases for these substrates together with periplasmic redox proteins that are suggested to connect the dehydrogenases to the respiratory chain in the manner shown (Fig. 1).

Under denitrifying conditions, four additional reductases are synthesized. These are thought to connect to the underlying electron transport system as shown in Fig. 1 and thus allow conversion of nitrate to nitrogen. Reductases for nitrate [14], nitrite [15] and nitrous oxide [16] have been recognized for some years and all have been purified and characterized. More recently established is the role of nitric oxide reductase. Trapping experiments have shown that nitric oxide is formed as a free intermediate between nitrite and nitrous oxide [17, 18], while the high activity of an NADH–nitric oxide oxidoreductase activity, which is associated with cytoplasmic membrane vesicles and inhibited by myxothiazol, establishes that nitric oxide reductase is a membrane-bound system that is connected to the respiratory