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2. R. M. May [*Nature* **296**, 803 (1982)] comments on the sparsity of information on mutualisms, especially the added complexity that emerges when the food web is expanded from simple two-species interactions.
3. S. Risch, M. McClure, J. Vandermeer, and S. Waltz [*Am. Midl. Natl.* **98**, 433 (1977)] first described the association between three species of *Piper* and their *Pheidole* inhabitants in Costa Rica and suggested that the ants provide nutrients to the plant by raising the CO_2 level inside the plant and by depositing excrement and ant carcasses inside the stem. I demonstrated an antiherbivore function of the ants [D. K. Letourneau, *Oecologia* **60**, 122 (1983)] by comparing insect egg removal and herbivore damage levels of leaves on plants with and without *Pheidole bicornis* colonies. This new discovery begs the question of the role of this custodial behavior by ants as an antiparasite behavior. Because the beetles deposit their eggs on the exterior of the plant, such egg removal may reduce beetle invasion.
4. A field study by S. J. Risch and F. R. Rickson [*Nature* **291**, 149 (1981)] showed that *Piper* plants produce food bodies when associated with *P. bicornis* ants but that congenerics do not stimulate food body production. In censuses of nearly a thousand plants in four *Piper* species, I have observed also many instances in which single petioles were inhabited by ants other than *P. bicornis*, but these petioles have never contained food bodies.
5. I have censused ants in *Piper* plants in nine forest sites throughout Costa Rica, including *P. cenocladum* at the La Selva Biological Station and in Gondoca de Talamanca; *P. fimbriulatum*, *P. sagittifolium*, and *P. obliquum* at Corcovado National Park (Sirena, Llorona, and Cerro de Oro), at Cañas Gordas, and at the Las Cruces Biological Station. Almost every plant with more than three full-sized leaves housed a *P. bicornis* ant colony. The few mature plants found to be vacant had obviously been inhabited in the past, as evidenced by chewed entrance holes into the sheathing leaf bases and hollow stems. Yet at Carara Biological Reserve, only 2 of 13 plants had *Pheidole bicornis* inhabitants.
6. I pried open each of the 56 petioles on the remaining seven plants enough to see its contents with a light. Twenty one chambers, distributed among all the plants, contained hundreds of food bodies whereas 35 interspersed petioles had no food body production.
7. In addition to the larvae found in the hollow chambers, nine beetle eggs were deposited one or two per petiole, and four pupae and one teneral adult were closed into the ends of hollow petioles by a partition.
8. *Phyllobaenus* and related genera are known to be predaceous as larvae and as adults. *Phyllobaenus* sp. *a* larvae collected from *Piper obliquum* at Carara were reared through the later instars on ant brood alone, and adult ants released into petioles with captive larvae were killed.
9. I reared this congeneric from *Piper cenocladum* at La Selva Biological Station, Heredia, Costa Rica. During censuses of petiole chambers over a 5-year period I have seen petiole chambers that contained a *Phyllobaenus* larva and ant carcasses at La Selva, Gondoca de Talamanca, and Cañas Gordas.
10. I estimated the leaf area missing from each leaf to obtain a mean herbivory index per plant for the four most recently produced, mature leaves. At Carara, all eight plants at the study site were used. At La Selva and Agua Buena, 8 plants were chosen randomly from a larger sample of 15 and 25 plants, respectively. The latter plants are control plants in studies of the effects of herbivory and ant occupancy on plant fitness.
11. Supported by University of California Santa Cruz Faculty Research Grants from the Division of Social Sciences and Academic Senate and University Research Expeditions Grants. I thank P. Barbosa, W. Barr, R. Chapman, J. Eisenbach, L. Fox, and M. Fusari for reviewing the manuscript; F. Arias G. R. Gomez M. L. Gomez M. L. Lobos, and P. Lockwood for excellent technical assistance; W. Barr and E. O. Wilson for describing the beetles and identifying ants, respectively; and D. Cole, the Organization for Tropical Studies, and the Costa Rican National Park Service for the use of their land and support.

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Differential Phosphorylation of c-Abl in Cell Cycle Determined by *cdc2* Kinase and Phosphatase Activity

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The product of the *c-abl* proto-oncogene (c-Abl) is phosphorylated on three sites during interphase and seven additional sites during mitosis. Two interphase and all mitotic c-Abl sites are phosphorylated by *cdc2* kinase isolated from either interphase or mitotic cells, with the mitotic *cdc2* having an 11-fold higher activity. Inhibition of phosphatases with okadaic acid in interphase cells leads to the phosphorylation of c-Abl mitotic sites, indicating that those sites are preferentially dephosphorylated during interphase. The differential phosphorylation of c-Abl in the cell cycle is therefore determined by an equilibrium between *cdc2* kinase and protein phosphatase activities. Treatment of interphase cells with okadaic acid leads to a rounded morphology similar to that observed during mitosis.

THE *cdc2* SERINE/THREONINE KINASE is required for both the G_1-S and the G_2-M transitions in the cell cycle of the fission yeast *Schizosaccharomyces pombe* (*1*). All eukaryotic cells examined contain a protein homologous to the *cdc2* kinase (*2–7*). In higher eukaryotes, *cdc2* has been shown to be required for entry into mitosis (*3, 4*). The protein kinase activity of *cdc2* is essential for its cell cycle function (*8*). At least two substrates are known to be phosphorylated by *cdc2* kinase during mitosis: histone H1 (*5*) and $p60^{c\text{-}src}$, a nonreceptor tyrosine kinase (*9, 10*). Mitotic phosphorylation of histone H1 is thought to cause chromatin condensation (*11*). Increased tyrosine kinase activity of $p60^{c\text{-}src}$ has been observed during mitosis, and the activation of $p60^{c\text{-}src}$ may be involved in the event of nuclear envelope breakdown (*9*). Further identification of the substrates phosphorylated by *cdc2* will help to show how *cdc2* controls cell cycle progression.

The c-*abl* proto-oncogene encodes a nonreceptor tyrosine kinase. There are two forms of c-*abl* protein, type I and IV: they share 1097 common amino acids, but differ at their NH_2-terminals (*12*). Both c-*abl* proteins (c-Abl) are expressed in all tissues and cell lines examined (*13*). The ubiquitous expression suggests the possibility of a fundamental function for c-Abl tyrosine kinase in cell physiology.

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Immunoblots of lysates from mitotic and interphase NIH 3T3 cells revealed that c-Abl from mitotic cells had a retarded mobility in SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1A). This was observed whether mitotic cells were obtained by metaphase arrest with nocodazole (*14*) or by mechanical shake-off in the absence of nocodazole. The Abelson murine leukemia virus protein $p160^{gag/v\text{-}abl}$ (Fig. 1A) and a Bcr/Abl fusion protein (*15*) also had a retarded mobility in mitotic cells. More than 95% of Abl exhibited a retarded mobility during mitosis, whereas 60% of the c-*src* protein was modified during mitosis (*9*).

The altered mobility of Abl is due to phosphorylation. Treatment of mitotic Abl in vitro with potato acid phosphatase caused it to comigrate with interphase Abl. The change in the phosphorylation state of c-Abl during interphase and mitosis was determined by two-dimensional phosphotryptic peptide maps. The c-Abl from interphase cells had three major spots: a, b, and c (Fig. 1B); c-Abl from mitotic cells had the same 3 spots and 12 additional spots (Fig. 1B). The numbered spots were reproducibly observed in multiple independent analyses.

A potential kinase for the mitotic hyperphosphorylation of Abl is the *cdc2* kinase, which is activated in mitotic cells (*6, 7*). To test whether *cdc2* kinase phosphorylates c-Abl, a coimmunoprecipitation kinase reaction was used. Antibody to *cdc2* (anti-*cdc2*) and anti-Abl were added to cell lysates, and their respective antigen-antibody complexes were precipitated on the same protein A–

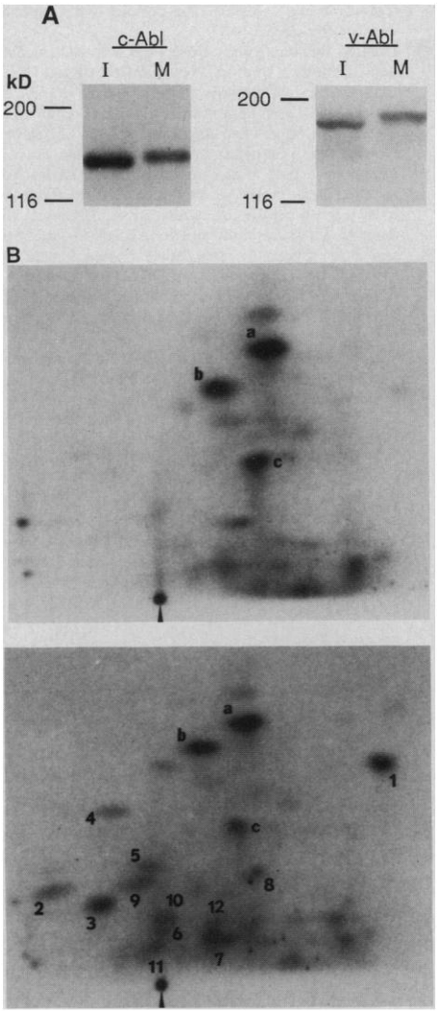

Fig. 1. Abl is hyperphosphorylated during mitosis. (**A**) Mobility difference between mitotic and interphase c-Abl and v-Abl. Immunoblots of mitotic (M) and interphase (I) cell lysates with anti-Abl monoclonal antibody 8E9 (*22*). Mitotic cells were prepared as described (*7*). Normal NIH 3T3 cells were used to examine c-Abl. NIH 3T3 cells transfected with temperature-sensitive v-Abl, DP (*22*), were used to examine v-Abl. (**B**) Phosphotryptic peptide maps of mitotic (lower panel) and interphase (top panel) c-Abl. NIH 3T3 cells overexpressing c-Abl type IV (*16*) were labeled in vivo with $H_3{}^{32}PO_4$ during interphase and mitosis (*23*); ^{32}P-labeled c-Abl was immunoprecipitated and processed for phosphotryptic peptide analysis as described (*24*). Electrophoresis was performed at *p*H 8.9 (1% ammonium carbonate). The anode is to the right. Ascending chromatography was performed in *n*-butanol–pyridine–acetic acid–H_2O (15:10:3:12). The origin is denoted by an arrowhead. The identity of spots was confirmed by two-dimensional phosphotryptic peptide maps of mixed mitotic and interphase samples.

Sepharose beads (Fig. 2). These beads were then incubated with [γ-^{32}P]ATP (adenosine triphosphate) under conditions that were favorable for *cdc2* kinase activity. When immunoprecipitated alone, c-Abl underwent autophosphorylation on tyrosine residues (Fig. 2, lane Abl). Coimmunoprecipitation with *cdc2* led to a significant increase in the phosphorylation of c-Abl on serine and threonine residues (Fig. 2, lane Abl/*cdc2*). Control reactions in which the immunoprecipitation of *cdc2* protein was blocked with *cdc2* peptide-immunogen produced no increase in the labeling of c-Abl (Fig. 2, lane Abl/*cdc2*/pep). Therefore, c-Abl was specifically phosphorylated by the *cdc2* kinase in vitro. In similar reactions the *cdc2* kinase also phosphorylated Gag/v-Abl proteins. This assay was later used to map the sites of phosphorylation by *cdc2* kinase.

By mixing in vitro and in vivo phosphotryptic peptide samples from c-Abl protein, it was found that all 12 of the mitosis-specific spots were phosphorylated by *cdc2* kinase in vitro (Fig. 3). None of these spots were phosphorylated when *cdc2* was left out of the in vitro kinase reaction. Spot z was generated by the autokinase activity of c-Abl (*16, 17*). Spot x was phosphorylated in vitro, but it was not found in vivo (Fig. 3). The *cdc2* kinase also phosphorylated spots a and b, which were present in c-Abl from both interphase and mitotic cells (Fig. 3).

When c-Abl was incubated with highly purified maturation promoting factor (MPF) from *Xenopus* oocytes (*18*), the *cdc2* kinase in MPF phosphorylated every c-Abl mitosis-specific spot. The two interphase spots a and b were also phosphorylated by MPF. The finding that two different sources of *cdc2* kinase phosphorylated c-Abl on its physiological sites strongly suggested that *cdc2* kinase was responsible for the phosphorylation of c-Abl in the cell cycle. The data, however, cannot rule out the possibility that c-Abl is phosphorylated in vivo by a kinase with substrate specificity similar to *cdc2*.

The 14 spots (a, b, and 1 to 12), which were consistently phosphorylated by *cdc2*, were redigested with trypsin to determine whether some of them represented partial cleavage products. The results showed that spot 8 was an incomplete cleavage of spot 1, spots 9 and 10 were incomplete cleavages of spot 4, and spots 11 and 12 were incomplete cleavages of spot 7. Thus, there were seven mitosis-specific phosphopeptides in c-Abl (numbered 1 to 7), and all of them could be phosphorylated by the *cdc2* kinase. Spots a and b were two distinct peptides that were phosphorylated by *cdc2* at all phases of the cell cycle. Four mitotic phosphopeptides, 1, 2, 3, and 6, contained phosphothreonine and the three others, 4, 5, and 7, contained phosphoserine (Fig. 3). The two interphase phosphopeptides a and b contained phosphoserine (Fig. 3).

The location of the *cdc2* phosphorylation sites in Abl were mapped by the in vitro and in vivo phosphorylation of truncation mutants of c-Abl and Gag/v-Abl proteins. Results of the mapping studies are summarized in Fig. 4. All of the sites phosphorylated by *cdc2* were COOH-terminal of amino acid 596, in a region outside of the tyrosine kinase domain. We have not observed a difference in the tyrosine kinase activity between the interphase and mitotic c-*abl* proteins when we used denatured enolase or casein as substrates in vitro.

Two of the *cdc2* phosphorylation sites in c-Abl have been precisely identified. Phosphopeptides 1 and a were mapped to a 38–amino acid region between amino acids 559 to 596 (of c-Abl type IV) (Fig. 4, compare ΔPvu II–Apa I and ΔApa I). The 38–amino acid region gives rise to two tryptic peptides: DAPDTPELLHTK and GLGESDA LDSEPAVSPLLPR. Because spot 1 contains phosphothreonine and spot a contains phosphoserine, spot 1 must be the first peptide, and spot a the second. By secondary digestion with V8 protease (*19*), Thr^{566} in peptide 1 (APDTPEL) and Ser^{588} in

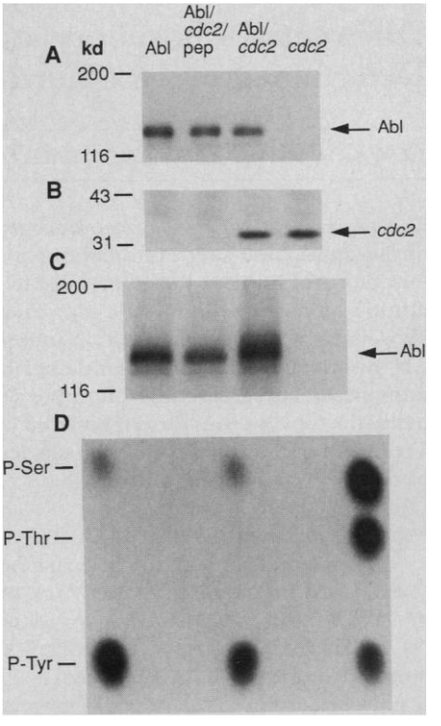

Fig. 2. In vitro phosphorylation of c-Abl by *cdc2* kinase. Coimmunoprecipitation kinase reaction is described in (*25*). Immunoprecipitation was with anti-Abl alone (lane Abl), anti-Abl plus anti-*cdc2* (lane Abl/*cdc2*), anti-Abl plus anti-*cdc2* and *cdc2* peptide immunogen (lane Abl/*cdc2*/pep), and anti-*cdc2* alone (lane *cdc2*). Immunoprecipitated samples were used in immunoblotting with anti-Abl (**A**) or anti-*cdc2* (**B**), to detect the immunoprecipitated proteins, or in kinase reactions (**C**) (*25*). (**D**) Phosphoamino acid analysis of c-Abl from (C) was performed as described (*7*). In all figures, an equal percentage of the original immunoprecipitations were used for each lane.

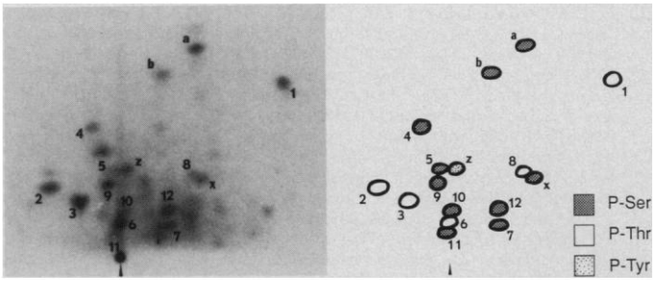

Fig. 3. Phosphotryptic peptide maps of c-Abl phosphorylated in vitro by *cdc2* kinase. In vitro kinase reaction was performed as described (*25*). Phosphotryptic peptide maps were performed as described in Fig. 1. In vitro and in vivo ^{32}P-labeled c-Abl type IV were mixed to confirm the identity of phosphopeptide spots generated in the in vitro reactions. A hand-drawn composite of different experiments summarizes the major phosphopeptide spots that were reproducibly observed. Phosphopeptides were eluted and analyzed for phosphoamino acid content (*7*). The identity of phosphoamino acid in each peptide is given in the hand-drawn composite.

peptide a (PAVSPLL) were determined to be the sites of phosphorylation in vivo and in vitro by *cdc2* kinase.

Two consensus amino acid sequences for phosphorylation by *cdc2* have been described for histone H1 and c-*src* proteins: basic-(Ser or Thr)-Pro-basic and (polar or basic)-(Ser or Thr)-Pro-X-basic (*9, 20*). The sequences of c-Abl phosphopeptides 1 and a do not match the consensus, other than that the site of phosphorylation is followed by proline. The (Ser or Thr)-Pro sequence may therefore be a fundamental determinant of substrate specificity for the *cdc2* kinase. Although this motif may be necessary for phosphorylation by *cdc2*, it is not sufficient, as evidenced by the presence of four Ser/Thr-Pro c-Abl sites that are not phosphorylated. The mitotic-specific *cdc2* phosphorylation sites appear to be flanked by polar or charged amino acids from the analysis of histone H1, c-*src*, and c-Abl sites. An interphase phosphorylation site in c-Abl, however, is surrounded by nonpolar amino acids (AVSPLL).

The differential phosphorylation of c-Abl in the cell cycle can be due to either of the following reasons: (i) The substrate specificity of *cdc2* kinase is altered between interphase and mitosis; or (ii) the specificity of *cdc2* kinase may not be changed, rather the mitosis-specific sites are more susceptible to phosphatases than the interphase sites. An increase in *cdc2* activity overwhelming the phosphatases during mitosis can lead to the accumulation of phosphate at the mitosis-specific sites. To determine if the substrate specificity of *cdc2* is altered in the cell cycle, interphase *cdc2* was used in the in vitro kinase reaction. We found on average an 11-fold reduction in interphase *cdc2* kinase activity compared to mitotic *cdc2* in the c-Abl phosphorylation reaction. Although interphase *cdc2* kinase activity was lower, it phosphorylated all nine sites (a, b, and 1 to 7) with no significant difference in the choice of c-Abl sites between the interphase and mitotic *cdc2*. These results do not support the idea of an alteration in substrate specificity of *cdc2* at various stages of the cell cycle, at least with respect to the phosphorylation of sites in c-Abl.

If the second alternative is correct, an inhibition of phosphatases during interphase would be expected to lead to the accumulation of phosphate on the mitotic sites of c-Abl. Okadaic acid is a potent inhibitor of protein phosphatases 1 and 2A and a weak inhibitor of phosphatase 2B (*21*). Within 20 min of the addition of okadaic acid to interphase cells, the majority of c-Abl had an altered mobility comparable to that observed with mitotic c-Abl (Fig. 5A). Phosphotryptic analysis showed that the mitosis-specific sites were phosphorylated in okadaic acid–treated interphase cells. The activity of *cdc2* did not increase in okadaic acid–treated interphase cells. Thus, inhibition of phosphatases alone could account for the mitotic phosphorylation of c-Abl in interphase cells. These results supported the idea that the lack of phosphate on the mitotic sites was due to their sensitivity to protein phosphatases. An increase in *cdc2* activity above that of the phosphatases as cells entered mitosis led to the accumulation of phosphate on the mitotic sites of c-Abl. The interphase sites a and b were less susceptible to phosphatases, possibly because of the surrounding sequences or the local conformation at those two sites.

Although treatment with okadaic acid led to the mitotic phosphorylation of c-Abl, it did not induce mitosis. Treated cells contained intact nuclear envelope and noncondensed chromatin. Thus, inhibition of phosphatases in interphase cells did not completely mimic activation of the MPF activity

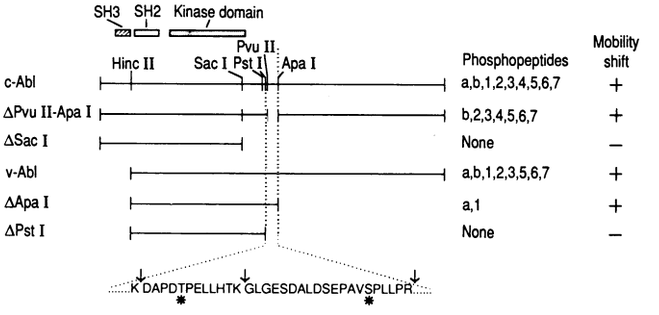

Fig. 4. Location of Abl phosphorylation sites. The line drawing represents Abl sequence; the kinase domain and *src* homology regions 2 and 3 (SH2 and SH3), which regulate the kinase activity of Abl (*16*), are denoted by boxes. Deletion mutants were constructed in the plasmid DP160 (*22*) or in pSVL/c-Abl type IV (*17*), and each was analyzed in vitro for phosphorylation by *cdc2* kinase by using the coimmunoprecipitation kinase assay (*25*). Peptides phosphorylated by *cdc2* kinase in each mutant protein are listed. Incubation of v-Abl with *cdc2* in vitro did not produce spot 4, for reasons that are not clear. The mobility shift of mutant proteins was determined by immunoblotting of lysates from mitotic cells stably expressing each mutant construct.

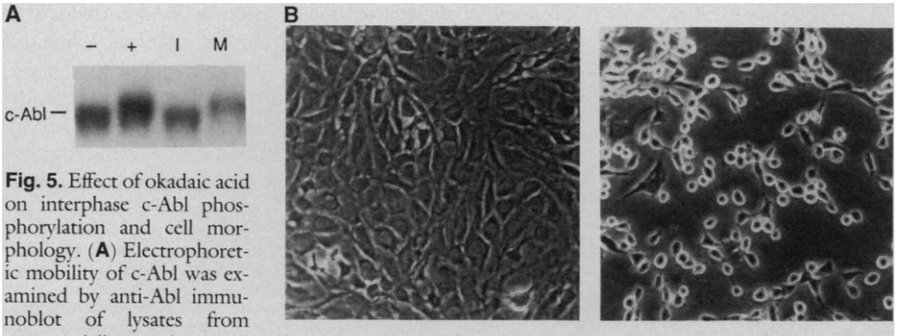

Fig. 5. Effect of okadaic acid on interphase c-Abl phosphorylation and cell morphology. (**A**) Electrophoretic mobility of c-Abl was examined by anti-Abl immunoblot of lysates from exponentially growing NIH 3T3 that were treated for 20 min with either 1 μ*M* okadaic acid (Moana Bioproduct, Honolulu, Hawaii) (+) or dimethyl sulfoxide (–) in MEM – P (plus 10% dialyzed calf serum). Regular interphase (lane I) and mitotic (lane M) NIH 3T3 cell lysates were run as controls on the same gel. (**B**) Rounding of okadaic acid–treated interphase cells. Photographs of exponentially growing NIH 3T3 cells with (right) or without (left) okadaic acid, as described in (A). Optical magnification was ×200.

of *cdc2* kinase. It is possible that certain mitotic substrates for MPF, unlike c-Abl, are not phosphorylated by interphase *cdc2*. Alternatively, critical substrates for MPF may only be present in late G_2 cells, and thus would not be available to *cdc2* kinase in the unsynchronized interphase cells used in our experiments.

Treatment of interphase cells with okadaic acid did induce a morphological change similar to that of mitotic cells (Fig. 5B). The rounding of mitotic cells is likely to be the result of Ser/Thr phosphorylation of certain proteins by MPF. The finding that okadaic acid induced similar morphological change in interphase cells suggested that the phosphorylation state of those MPF substrates might be regulated in the same manner as that of c-Abl. Cells transformed by the v-Abl tyrosine kinase have a rounded morphology. Hyperphosphorylation of c-Abl, although not affecting the measurable kinase activity in vitro, may alter its property in vivo to phosphorylate substrates that regulate cell morphology.

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23. Cells were grown in minimal essential medium lacking phosphate (MEM − P) with 10% calf serum and nocodazole (0.4 μg/ml) for 6.5 hours, then washed two times with MEM − P with 10% dialyzed calf serum; $H_3{}^{32}PO_4$ (12.5 mCi/ml) was added for 2.5 hours, then mitotic and nonmitotic cells were separated by gentle squirting of the cell monolayer.
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25. To obtain a sufficient amount of Abl for the in vitro kinase reaction, c-Abl was overexpressed in COS cells by transient transfection (*17*) and v-Abl was obtained from transformed NIH 3T3 cells (*16*). Cell lysate containing overexpressed Abl was mixed 1:1 with mitotic NIH 3T3 cell lysate. The c-Abl and *cdc2* proteins were coimmunoprecipitated with a mixture of monoclonal anti-Abl (8E9, 10 μg/ml) (*22*) and anti-*cdc2* (50 μg/ml) (*6*). Kinase reactions were performed with [γ-^{32}P]ATP in 50 m*M* tris (*p*H 5.5), 10 m*M* $MgCl_2$, 1 m*M* dithiothreitol, and 1 μ*M* ATP at 30°C for 30 min. Kinase reactions were then boiled in SDS sample buffer and diluted in immunoprecipitation buffer (*7*). Abl was reimmunoprecipitated with anti-Abl serum and analyzed by 5 to 10% SDS-PAGE autoradiography.

26. We thank J. L. Maller for purified MPF from *Xenopus* oocytes and A. O. Morla for the insightful observation of c-Abl mobility shift during mitosis. Supported by grants from NIH (CA43054) and NSF (DMB86-1378).

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A Class I Antigen, HLA-G, Expressed in Human Trophoblasts

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The α chain of the human histocompatibility antigen HLA-G was identified as an array of five 37- to 39-kilodalton isoforms by the use of two-dimensional gel electrophoresis. Both cell-associated and secreted HLA-G antigens are prominent in first trimester villous cytotrophoblasts and are greatly reduced in third trimester cytotrophoblasts. Allelic variation was not detected, an indication that HLA-G is not obviously polymorphic in cytotrophoblasts. Among the following choriocarcinoma cell lines studied, HLA-G is expressed in JEG but not in Jar or BeWo. Expression of endogenous HLA-G genes has not been found in normal lymphoid cells. Thus, HLA-G is subject to both cell type–specific and developmental regulation and is expressed in early gestation human cytotrophoblasts.

THE PLACENTAL TROPHOBLAST IS the only fetal cell type that is exposed to the maternal uterine decidua and blood. Because of their position at the maternal-fetal interface, trophoblasts are thought to shield the fetus from rejection by serving as a barrier to maternal effector cells (*1*), by eliciting the local production of maternal suppressor cells (*2*) or protective blocking antibodies (*3*), or by being nonrecognizable. The inability to HLA-type trophoblasts with standard typing allosera has suggested the absence of class I antigens as a possible explanation for maternal tolerance toward placenta of different HLA type. However, some trophoblasts bind reagents, such as the monoclonal antibody (MAb) W6/32 (*4*) or antibody to β-2-microglobulin (β-2m), that recognize monomorphic determinants on class I antigens (*5, 6, 7*). We now present data that may reconcile these apparently contradictory reports. These data show that an apparently nonpolymorphic class I molecule, HLA-G, is expressed by cytotrophoblasts and suggest a function for such molecules in protecting the placenta from rejection.

Human trophoblast cell types have different locations within the placenta and the amount of HLA class I expression appears to depend on location. Syncytiotrophoblasts line the external surface of the chorionic villi and are in direct contact with maternal blood; they do not bind MAb W6/32 (*8*). Beneath this layer are mononuclear cytotrophoblasts, an undifferentiated stem cell population that weakly binds MAb W6/32 (*8, 9, 10*). These cells either fuse to form the syncytia or invade the uterus as extravillous (intermediate) cytotrophoblasts (*11*), in which case they express class I–like proteins (*12*). In this work, we studied the mononuclear stem cell population from chorionic villi.

We and others (*13, 14*) have identified the α chains encoded by the HLA-E, -F, and -G class I genes that were isolated from human lymphoblastoid cell line (LCL) 721 (*15, 16*).

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