CD1 in this location could act either as a target for the T cell receptor or as an antigen-presenting molecule for bacterial pathogens and toxins to regional T cells. Alternatively, mCD1 could be involved in the gastrointestinal localization of intraepithelial lymphocytes. Functional experiments elucidating the role of mCD1 in gastrointestinal epithelium should lead to a broader understanding of epithelial immunity.

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Down-Regulation of LFA-1 Adhesion Receptors by C-*myc* Oncogene in Human B Lymphoblastoid Cells

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The function of the c-*myc* gene and its role in tumorigenesis are poorly understood. In order to elucidate the role of c-*myc* oncogene activation in B cell malignancy, the phenotypic changes caused by the expression of c-*myc* oncogenes in human B lymphoblastoid cells immortalized by Epstein-Barr virus were analyzed. C-*myc* oncogenes caused the down-regulation of lymphocyte function–associated antigen–1 (LFA-1) adhesion molecules (α_L/β_2 integrin) and loss of homotypic B cell adhesion in vitro. Down-regulation of LFA-1 occurred by (i) posttranscriptional modulation of LFA-1 α_L-chain RNA soon after acute c-*myc* induction, and (ii) transcriptional modulation in cells that chronically express c-*myc* oncogenes. Analogous reductions in LFA-1 expression were detectable in Burkitt lymphoma cells carrying activated c-*myc* oncogenes. Since LFA-1 is involved in B cell adhesion to cytotoxic T cells, natural killer cells, and vascular endothelium, these results imply functions for c-*myc* in normal B cell development and lymphomagenesis.

THE C-*myc* PROTO-ONCOGENE IS involved in the control of cellular proliferation and differentiation; its deregulated expression, caused by chromosomal translocation, amplification, or retroviral insertion, is associated with tumorigenesis in different species (*1*). The precise function of the c-Myc protein in normal cells as well as in tumorigenesis is unknown. Consistent with the predicted structure of c-Myc (*2*), this function is presumably accomplished through the modulation of specific gene expression programs. However, only a few genes have been identified that may be physiologically regulated by c-Myc, either transcriptionally (*3*) or posttranscriptionally (*4*).

As an approach to elucidating the role of c-*myc* oncogene activation in Burkitt lymphoma (BL) (*5*), we studied the phenotypic changes induced by c-*myc* oncogenes in Epstein Barr virus (EBV)–immortalized B lymphoblastoid cell lines (LCLs), which may represent the natural target for c-*myc* activation during lymphomagenesis in vivo. The constitutive expression of c-*myc* oncogenes under the control of heterologous enhancer-promoter elements causes (i) the in vitro transformation of LCLs, which acquire the ability to be cloned in semisolid media, and (ii) tumors in immunodeficient mice (*6*). To identify further changes that are consistently associated with c-*myc* expression, we transfected several LCLs with a vector (pHEBoSVmyc2.3) that constitutively expresses a normal c-Myc protein (*7*) and studied the expression of molecules related to histocompatibility, stage of differentiation, and B cell function by cytofluorometric analysis with a panel of B cell–specific monoclonal antibodies (MAbs) (*8*).

Comparison of c-*myc*–transfected and control [pHEBoSV (SV)–transfected] LCLs (Table 1) shows that, in general, expression of most cell surface molecules is unchanged after c-*myc*–induced transformation, except for a slight increase in two activation markers (CD71 and CD38), consistent with the increased proliferative rate of c-*myc*–transformed LCLs. A consistent decrease in the expression of BA-2 (CD9) antigen (*9*) was also observed. The expression of transfected c-*myc* oncogenes consistently caused the down-regulation of the leukocyte-specific cell-adhesion molecule LFA-1 (lymphocyte function–associated antigen–1) (*10*), a member of the integrin superfamily of adhesion receptors (*11*) involved in homotypic B cell adhesion, cell conjugate formation between B cells and cytotoxic T cells or NK (natural killer) cells, and adhesion to vascular endothelium (*10, 12*). The reduction or absence of the cell surface expression of both LFA-1 chains in all c-*myc*–transfected LCLs was confirmed by immunoprecipitation of ^{125}I-labeled cell surface proteins with MAbs to α_L and β_2 chains (Fig. 1A). Analogous changes in surface LFA-1 expression were observed in nine distinct LCLs transfected with vectors carrying c-*myc* oncogenes. In all cases, the degree of LFA-1 down-regulation was pro-

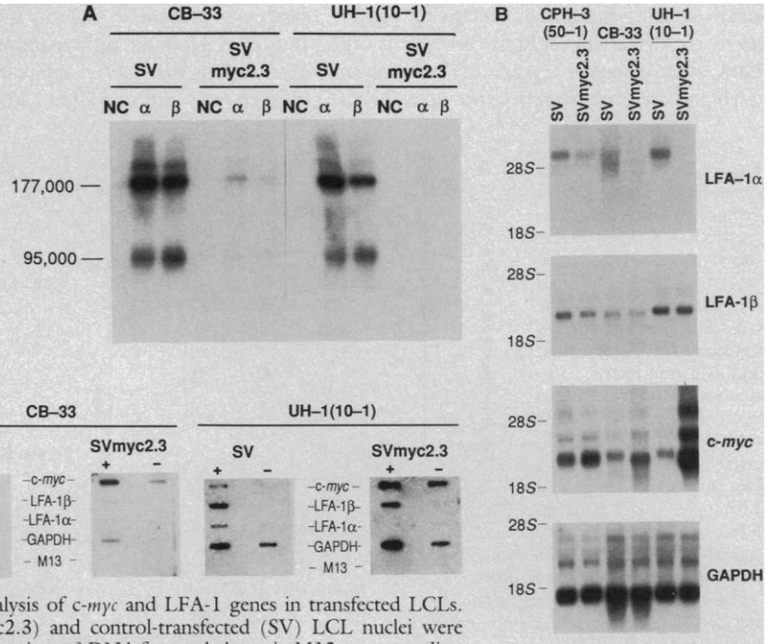

Fig. 1. (**A**) Immunoprecipitation analysis of cell surface LFA-1 α_L and β_2 molecules in c-*myc*–transfected LCLs. Equal quantities (counts per minute) of solubilized, ^{125}I-labeled (*36*) cell surface proteins from c-*myc*–transfected (SVmyc2.3) or control-transfected (SV) LCLs were precipitated (*36*) with MAbs to α_L (lanes α) or β_2 (lanes β), or with MOPC21 MAb (unrelated, isotype-matched, negative control, lanes NC). TS1/22 and TS1/18 (*10*) were used as MAbs to α_L and β_2, respectively. Both MAbs coimmunoprecipitate both the α_L (177-kD) and the β_2 (95-kD) LFA-1 chains (*10*). (**B**) Northern blot analysis of c-*myc*, LFA-1 α_L, and β_2 RNA in c-*myc*–transformed LCLs. Total cellular RNA (15 μg) from c-*myc*–transfected (SVmyc2.3) or control-transfected (SV) CPH-3, CB-33, or UH-1 LCLs was sequentially analyzed by Northern blot analysis with α_L, β_2, c-*myc*, or GAPDH (control for RNA amounts) probes as indicated. The α_L probe is represented by the 3R1 plasmid insert, which contains the 5′-most 1.8 kb of an α_L cDNA clone (*24*). The β_2 probe is represented by the 3.1.1 plasmid insert, which contains the 3′-most 1.8 kb of a β_2 cDNA clone (*22*). The diffuse α_L RNA band detectable in CB-33 cells has already been detected in other cells (*24*) and may be due to a polymorphism in the splicing pattern. Only a shorter c-*myc* RNA species (1.8 kb) derived from exogenous c-*myc* is detectable in SVmyc2.3-transfected LCLs due to down-regulation of endogenous c-*myc* expression (*6*). (**C**) Nuclear run-on transcription analysis of c-*myc* and LFA-1 genes in transfected LCLs. [^{32}P]RNA (1.5×10^7 cpm/ml) from c-*myc*–transfected (SVmyc2.3) and control-transfected (SV) LCL nuclei were hybridized to nylon filters carrying single-strand [(+), sense; (−), antisense] DNA from subclones in M13 corresponding to the indicated genes and derived from the plasmids described in (B). M13 DNA was used as a negative control. Preparation and ribonuclease (RNase) treatment of nuclei, the nuclear run-on procedure, and hybridization and washing procedures are as previously described (*35*).

portional to the levels of exogenous c-*myc* expression (*13*). Furthermore, in all cases low expression of cell surface LFA-1 molecules correlated with inefficient homotypic cell adhesion, detectable as a decrease or loss of the clumping pattern typical of LCLs growing in liquid suspension cultures (*6*). These changes represent a specific consequence of c-*myc* expression, since they were not seen upon transformation of LCLs with H-*ras*, N-*ras*, *bcl-2*, or c-*myb* (*14*).

To study the mechanism responsible for the modulation of LFA-1 expression in *myc*-transformed LCLs, we analyzed the main steps in the biosynthetic pathway of the LFA-1 complex. Analysis of LFA-1 α_L and β_2 mRNA expression by Northern blot hybridization showed that the steady-state levels of α_L mRNA were reduced in LCLs transformed by c-*myc*, whereas β_2 mRNA remained unchanged (Fig. 1B). To determine whether α_L mRNA expression was regulated transcriptionally or posttranscriptionally, we studied the rate of transcription of α_L by a run-on transcription assay (Fig. 1C). This assay showed that transcription of the α_L but not β_2 was reduced or absent in LCLs transformed by c-*myc*. Thus, α_L gene transcription is selectively modulated and is primarily responsible for the down-regulation of the LFA-1 complex in LCLs that stably express c-*myc*. The decreased surface expression of β_2 is likely secondary to the decreased availability of α_L, since only heterodimeric molecules can be expressed on the cell surface (*15*).

The kinetics of LFA-1 expression were studied in LCLs transfected with an inducible c-*myc* vector, pHEBoMTmyc2.3 (MTmyc2.3), in which the heavy metal–inducible metallothionein promoter (*16*) was linked to c-*myc* coding sequences (*17*). This vector, or a control vector pHEBoMT (MT) lacking c-*myc* sequences, was transfected into CB-33 LCLs, and antibiotic-selected transfectants were analyzed for LFA-1 RNA and protein expression after addition of zinc (Zn) ions to the cell culture medium. In MTmyc2.3-transfected, but not MT-transfected LCLs, Zn-mediated induction of c-*myc* expression caused a progressive decrease in cell surface LFA-1 α_L and β_2 (Fig. 2A) and in α_L RNA (Fig. 2B), detectable at 48 and 24 hours after Zn induction, respectively. Transcriptional down-regulation of the LFA-1 α_L gene is already detectable in MTmyc2.3-transfected LCLs before Zn induction because of leakiness of the MT promoter (Fig. 2C; α_L RNA and protein in MTmyc2.3-transfected cells were also reduced in Fig. 2, A and B). However, no down-regulation of α_L transcription was detected within the first 48 hours after Zn-mediated c-*myc* induction (Fig. 2C), indicating that, whereas LFA-1 down-regulation involves a shut-off at the transcriptional level at late stages, it may primarily involve a posttranscriptional mechanism, such as regulation of RNA transport, processing, or stability at early times. Because the down-regulation of LFA-1 molecules and α_L RNA is detectable within 24 hours and the half-lives for these molecules are >24 hours and >12 hours (*18*), respectively, the actual modulation of α_L expression must initiate early (<12 hours) after Zn-induced up-regulation of c-*myc* gene expression. Thus, LFA-1 down-regulation in c-*myc*–transformed LCLs is not a change related to cell culture selection or differentiation, but rather represents a rapid and specific consequence of c-*myc* expression.

The validity of the observed relation between c-*myc* and LFA-1 α_L expression was examined by studying LFA-1 gene expression in BL cells, which carry constitutively expressed c-*myc* oncogenes activated by chromosomal translocation (*4*). Because previous observations were limited to EBV-positive BL cell lines in which LFA-1 expression appears to change during in vitro passage (*19*), we analyzed cell surface LFA-1 in a panel of both EBV-positive and EBV-negative BL biopsies by cytofluorometric analysis and compared them to the ones observed in normal and c-*myc*–transformed LCLs. When tumor biopsies were analyzed, LFA-1 levels were uniformly low in both types of BL (Fig. 3), consistent with the role of c-*myc* in modulating LFA-1 expression in vivo, irrespective of the presence of EBV. Similar data were obtained in fresh biopsies of EBV-positive and EBV-negative acquired immunodeficiency syndrome (AIDS)–asso-

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ciated B cell lymphomas. Relatively higher levels are detectable only in certain BL cell lines after extensive (years of) culture in vitro and are likely to reflect the selection of genetic or epigenetic mutants that retain the ability to grow in tight clumps (*19*). The observations that LFA-1 expression is higher relative to differentiated (*20*) and quiescent (*18*) B cells (for example, cells that do not express c-*myc*), are also consistent with the notion that c-*myc* regulates LFA-1 expression in normal B cells.

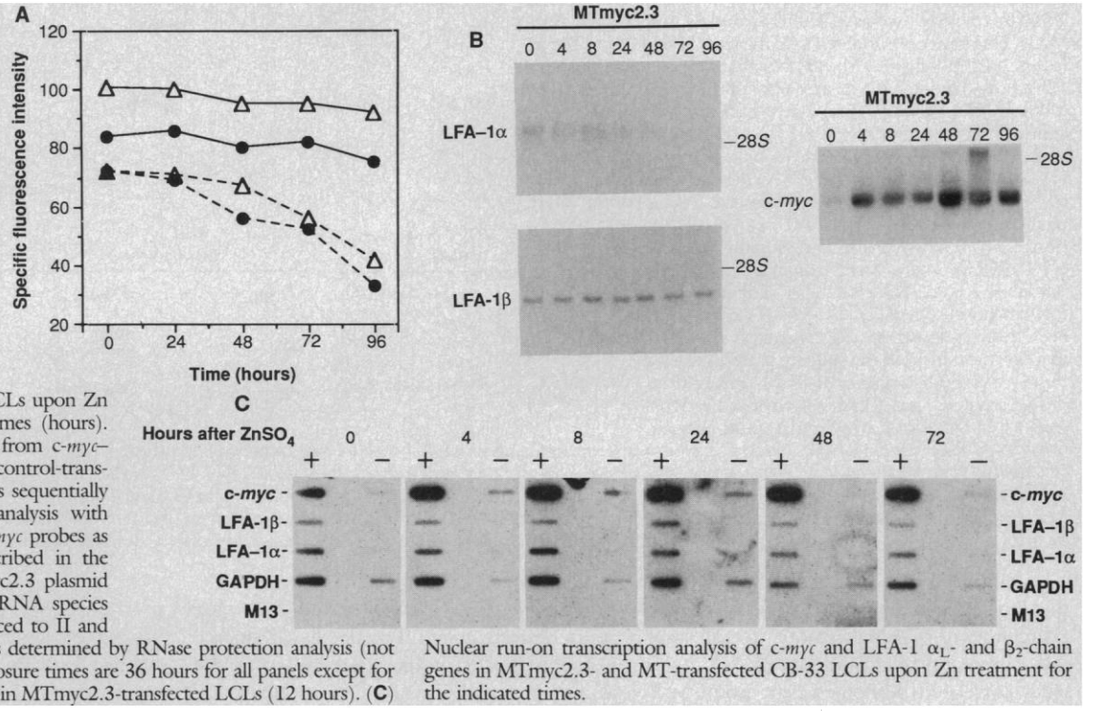

Fig. 2. Zinc-induced down-regulation of LFA-1 expression in MTmyc2.3-transfected CB-33 LCLs. (**A**) Cytofluorometric analysis of LFA-1 α_L (△) and β_2 (●) expression on the cell surface of MTmyc2.3-transfected (dashed lines) and MT-transfected (continuous lines) CB-33 LCLs upon Zn treatment (200 mM $ZnSO_4$) for the indicated times. (**B**) Northern blot analysis of c-*myc* and LFA-1 α_L- and β_2-chain RNA levels in MTmyc2.3- and MT-transfected CB-33 LCLs upon Zn treatment for the indicated times (hours). Total cellular RNA (15 μg) from c-*myc*–transfected (MTmyc2.3) or control-transfected (MT) CB-33 LCLs was sequentially analyzed by Northern blot analysis with LFA-1 α_L, LFA-1 β_2, and c-*myc* probes as indicated. Probes are as described in the legend to Fig. 1. The MTmyc2.3 plasmid codes for a shorter (1.8-kb) RNA species containing MT sequences spliced to II and III exon sequences of c-*myc* as determined by RNase protection analysis (not shown). Autoradiography exposure times are 36 hours for all panels except for the one relative to c-*myc* RNA in MTmyc2.3-transfected LCLs (12 hours). (**C**) Nuclear run-on transcription analysis of c-*myc* and LFA-1 α_L- and β_2-chain genes in MTmyc2.3- and MT-transfected CB-33 LCLs upon Zn treatment for the indicated times.

Table 1. Immunophenotypic characterization of c-*myc*–transfected LCLs. Control-transfected (SV) and c-*myc*–transfected (SVmyc2.3) CB-33 and UH-1(10-1) LCLs were analyzed for the expression of the indicated cell surface markers by cytofluorometric analysis. LCLs were established by EBV infection of peripheral blood mononuclear cells from healthy donors as described (*32*). LCL clones were obtained as previously described (*32*). Plasmids were transfected into LCLs by electroporation as described (*33*) with some modifications (*6*). After antibiotic selection, cells were subjected to flow cytometric analysis after staining by indirect immunofluorescence (*34*). Flow cytometric analysis was performed with a FACScan fluorescence-activated cell sorter (FACS). At least 2.5×10^7 events per sample were collected in list mode fashion, stored, and analyzed. Dead cells and debris were exlcuded by conventional scatter gating and propidium iodide (PI) staining (25 μg/ml). Emission signals were collected with appropriate filters at 530 and 575 nm. Specific fluorescence indicates the linear fluorescence intensity (FI) of the sample minus the linear FI value generated by an unrelated, isotype-matched MAb stained with the same FITC-conjugated antibodies (*35*). Transfected LCLs were analyzed in at least three different experiments and each analyzed in duplicate. Standard deviation was in all the cases less than 5% of the reported value.

Antigen	(CD)	Antibody	Specificity	Percentage of positive cells (specific FI)			
				CB-33		UH1 (10-1)	
				SV	SVmvc2.3	SV	SVmvc2.3
HLA-DR	(−)	L243	MHC	98 (92)	100 (94)	100 (160)	100 (180)
HLA-ABC	(−)	W6/32		100 (140)	100 (135)	97 (99)	98 (90)
cALLa	(CD10)	J5	Differentiation markers	14 (10)	11 (11)	1 (15)	1 (16)
gp45/55/65	(CD24)	BA-1		6 (18)	5 (12)	5 (15)	4 (15)
p24	(CD9)	BA-2		76 (66)*	7 (59)	87 (26)	2 (23)
B4	(CD19)	89B		90 (73)	90 (76)	91 (30)	71 (20)
B1	(CD20)	H299		99 (83)	98 (83)	92 (105)	96 (108)
T1	(CD5)	OKT1		17 (28)	92 (53)	.1 (15)	1 (14)
CR2r	(CD21)	OKB7		63 (60)	90 (70)	15 (25)	2 (15)
p90/TFr	(CD71)	OKT9	Activation markers	55 (60)	90 (72)	65 (55)	71 (60)
T10	(CD38)	OKT10		23 (51)	100 (73)	9 (17)	38 (41)
IL-2r	(CD25)	2A3		4 (10)	4 (7)	3 (16)	2 (15)
BLAST-2	(CD23)	EBVCS-5		100 (180)	100 (180)	100 (150)	100 (120)
Ki-1	(CD30)	BerH2		72 (60)	82 (64)	88 (46)	71 (31)
LFA-1α	(CD11a)	TS1/22	Cell adhesion antigens	99 (90)	88 (33)	99 (58)	5 (20)
LFA-1β	(CD18)	TS1/18		99 (100)	90 (23)	99 (56)	4 (17)
p150,95	(CD11c)	S-HCL-3		3 (15)	5 (9)	8 (15)	5 (16)
ICAM-1	(CD54)	RR1/1		99 (100)	100 (198)	77 (39)	98 (59)
LFA-3	(CD58)	TS2/9		100 (98)	100 (129)	99 (67)	99 (51)

*Underlined values were consistently changed in c-*myc*–transfected LCLs.

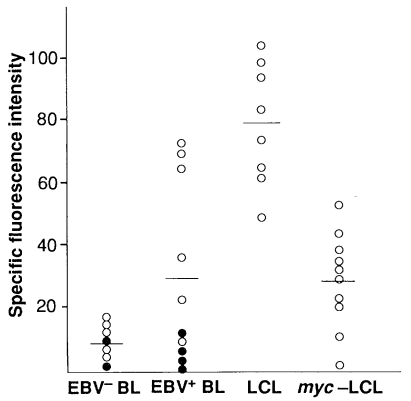

Fig. 3. Analysis of LFA-1 protein expression in BL. EBV-positive (EBV^+) and EBV-negative (EBV^-) BL cell lines (○) or biopsies (●), as well as control transformed (LCL) or c-*myc*–transformed LCLs (MYC-LCL), were analyzed for the cell surface expression of LFA-1 α_L molecules by cytofluorometric analysis as described for Table 1. Median values are indicated by the horizontal bars.

The LFA-1 α_L gene represents one of the very few genes for which regulation by c-*myc* has been demonstrated. In fact, although regulation by c-*myc* has been suggested for *heat shock* (*3*) and *HLA* genes (*21*), only in the case of the *plasminogen-activator inhibitor–1 (PAI-1)* gene has a close temporal relation between c-*myc* induction and *PAI-1* expression been demonstrated (*4*). The mechanisms by which c-*myc* down-regulates LFA-1 expression in B cells and up-regulates *PAI-1* in fibroblasts appear to be analogous, involving an early (in hours) posttranscriptional regulation upon acute c-*myc* induction and a transcriptional regulation in transfected cells constitutively expressing c-*myc* (*4*). As in the case of the *PAI-1* gene, however, the mechanism involved in the regulation of LFA-1 α_L gene by c-*myc* remains to be elucidated. C-*myc* does not act indirectly by regulating the expression of the LMP gene of EBV, which is known to regulate LFA-1 expression (*22*), since LMP expression was found unchanged after c-*myc*–induced transformation of LCLs (*23*). The dose-dependent, specific, and rapid effect of c-Myc on LFA-1 α_L expression suggests that there may be an interaction between c-Myc and LFA-1 α_L regulatory sequences. This interaction may be either direct or mediated by a cofactor molecule to form a heterodimeric regulatory complex, since c-Myc contains functional domains, such as helix-loop-helix and leucine zipper, capable of specific interactions with DNA or other regulatory proteins (*2*). The experimental system shown here, along with the availability of the cloned LFA-1 α_L sequences (*24*), offers the opportunity to directly address these questions.

Our data have implications for the specific role of the c-*myc* oncogene in the pathogenesis of BL. Burkitt lymphoma cells have little or no adhesion-related molecules, including LFA-1, intercellular adhesion molecule–1 (ICAM-1), and LFA-3 (*19*). Together with low expression of EBV latent gene products (EBNA-2 and LMP) (*23*) and histocompatibility antigen–1 (HLA-1) class I molecules, these features may be responsible for the resistance of BL cells to T cell–mediated cytotoxicity in vitro and possibly to immunosurveillance in vivo (*19, 25*). The mechanism responsible for these alterations is unknown, although they may be the result of immune selection in vivo or of intrinsic phenotypic characteristics of the putative normal counterpart of BL cells (*25*). Our data provide experimental evidence that the decrease in LFA-1, but not LFA-3, ICAM-1 (see Table 1), or EBNA-2 and LMP (*23*) expression represents a direct consequence of c-*myc* activation in vitro and suggests the occurrence of an analogous mechanism in vivo. We have already observed that c-*myc* transfection is also sufficient to make LCLs resistant to killing by autologous T cells in vitro (*26*), although the role of LFA-1 down-regulation in this phenomenon remains to be determined.

These results support the emerging notion that the loss of cell adhesion receptors may play a critical role in malignant transformation. This is suggested by the loss of specific integrin expression in cells transformed by various oncogenes in vitro (*27*), the reversion of the malignant phenotype in vitro after reexpression of adhesion receptors (*28*), and the structural similarities of a putative recessive oncogene, the loss of which is associated with colon carcinogenesis, to the adhesion receptor superfamily (*29*). Our data identify a molecular mechanism controlling both cell growth and adhesion in transformed cells and suggest the existence of a general regulatory pathway in which *myc* genes control adhesion related functions in different tissues. In fact, N-*myc* also appears to regulate integrin (LFA-1) expression in B cells (*30*), as well as the expression of integrin ligand molecules [neural cell adhesion molecule (N-CAM)] in other tissues (*31*). A second gene regulated by c-Myc is *PAI-1*, which is analogous to integrins in that it is involved in the control of interactions between cell and extracellular matrix (*4*). The elucidation of these regulatory pathways could lead to important insights into the mechanisms regulating normal cell and organ development, as well as into the specific biological roles of *myc* oncogenes in the pathogenesis of malignancy in different tissues.

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Table 1. Mutations in *pha-1*. Derivation of alleles is described in (*9*).

Allele	Mutagen	Embryos hatching (%)	Phenotype
e2123	EMS	4*	Strong‡, ts
t1001	EMS	5*	Strong‡, ts
e2468	EMS		Strong‡
e2275	EMS	5*	Strong at 25°C§
e2286	EMS	38*	Weak‖
t1002	EMS	95†	Weak‖
eDf20	X-ray		Weak‖
tDf2	X-ray		Strong‡

*All hatched embryos die as L1 larvae. †A few hatched individuals survive to late larval stages. ‡Pharynx cells are present and enclosed by a basement membrane; they are attached to the gut, but not extending to the tip of the head. No morphogenesis, differentiation, or pumping is visible. Prolonged incubation of mutant embryos beyond the time of normal hatching leads to gradual appearance of some differentiation markers, such as interior cuticle; ts, temperature sensitive. §Though lethal at any temperature, the phenotype of *e2275* is more severe at 25°C. ‖In embryos homozygous for weaker alleles, the pharynx is usually of full length but appears poorly differentiated. Pharyngeal pumping as it occurs in wild-type embryos shortly before hatching is generally not observed.

An Organ-Specific Differentiation Gene, *pha-1*, from *Caenorhabditis elegans*

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Embryonic lethal mutations in the nematode *Caenorhabditis elegans* were generated and screened for phenotypes that suggest regulatory functions in order to identify genes involved in the control of early development. In embryos homozygous for mutations in one such gene, *pha-1*, the pharynx fails to undergo late differentiation and morphogenesis. Early pharynx development is not affected; thus, *pha-1* controls the latter stages of this developmental process. All markers specific for differentiation in various pharyngeal cell types tested are affected, suggesting that *pha-1* acts in an organ-specific, rather than cell type–specific, manner. The temperature-sensitive phases of both temperature-sensitive mutations indicate that *pha-1* function is required solely during midembryogenesis, shortly before the onset of morphogenesis.

IN OUR STUDY OF THE EMBRYONIC development of the nematode *Caenorhabditis elegans*, we have attempted to identify developmental control genes by analyzing the phenotypes caused by embryonic lethal mutations; our experiments are similar to the work of Nüsslein-Volhard and Wieschaus in *Drosophila* (*1*). Here we report on one such developmental control gene, *pha-1*, which is specifically involved in the development of the pharynx, an organ required for the uptake and transport of food.

Shortly before the wild-type *C. elegans* embryo hatches (800 min after first cleavage) (*2, 3*), the pharynx has developed into a functional organ with characteristic morphology, enclosed by a basement membrane (*4*) (Fig. 1A). All pharynx cells, which come from a variety of unrelated lineages, are generated during the first half of embryonic development, the proliferation phase (0 to about 430 min). The pharynx primordium, a group of cells bounded by a basement membrane, becomes visible anterior to the gut toward the end of this phase. Subsequently, the pharynx elongates, attaches to the anterior tip of the embryo where a buccal cavity is formed, develops its characteristic morphology, and begins to pump shortly before hatching at 800 min (*2*). Embryos homozygous for mutations in *pha-1* (Table 1) lack a functional pharynx and instead have a group of cells anterior to the gut surrounded by a basement membrane (Fig. 1B). This structure is similar to that of the pharynx in wild-type embryos at an earlier developmental stage, the 1½-fold stage, just after initiation of morphogenesis (430 min), suggesting that pharyngeal development has arrested at this stage. The normal number, 80, of pharyngeal cells appears to be generated in mutant embryos as we counted 80 ± 4 nuclei (±SD, $n = 25$) by Nomarski microscopy in *pha-1(e2123)* embryos and 79 nuclei in a *pha-1(t1001)* embryo optically sectioned with a confocal microscope.

The pharynx of *C. elegans* contains five different cell types: muscle cells, marginal cells, epithelial cells, glands, and nerves. To determine whether the effect of *pha-1* mutations was limited to any single cell type, we stained mutant embryos with monoclonal antibodies (MAbs) to markers expressed in three of the five pharyngeal cell types. Antibody 3NB12 (Fig. 2A) recognizes an epitope expressed early (at about 400 min) in pharyngeal muscle cells, several hours before pharyngeal myosin is expressed (*5*). The staining pattern in terminal-stage *pha-1* embryos was similar to that in wild-type embryos of the 1½-fold stage (430 min) indicating, as suggested by the morphology observed in Nomarski microscopy (Fig. 1), that pharynx development in mutant embryos ceases at the 1½-fold stage of embryonic development. Furthermore, three late pharyngeal markers normally expressed during terminal differentiation—pharynx-specific myosin C, intermediate filaments, and a component of gland cells—were not observed in most *pha-1(e2123)* embryos (Fig. 2, B through D). Although we could not

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