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Changes In c-*onc* Expression during Embryonal Carcinoma Cell Differentiation

by Thomas Sejersen,* Pei Jin,* Magnus Rahm,* and Nils R. Ringertz*

Protooncogenes expressed in murine embryonal carcinoma (EC) cells or their differentiated daughter cells include more or less ubiquitously expressed protooncogenes such as c-*myc*, c-K-*ras*, and c-*abl*, as well as c-*onc* genes with a very restricted expression pattern. Examples of the latter are N-*myc*, c-*mos*, and *int*-2. These c-*onc* genes are transcriptionally active in EC cells, as well as in germ cells and/or early embryonic cells. When EC cells are induced to differentiate some protooncogenes or oncogene-related products undergo changes in expression. Thus, EC cell differentiation has been associated with increased expression of c-*src*, c-*fos*, *int*-1, *int*-2, and the epidermal growth factor (EGF) receptor, whereas decreased expression has been observed for c-*mos*, c-K-*ras*, c-*myc*, N-*myc*, and platelet-derived growth factor. The relationships between these changes in expression and EC cell differentiation are not understood. They may be important for the differentiation process or for expression of a differentiated phenotype. They may, however, also be secondary events with no functional significance to EC cell differentiation.

Introduction

Protooncogenes (c-*onc*) are normal cellular genes homologous to the transforming retroviral oncogenes (v-*onc*). c-*onc* Genes are presumed to be of key importance in the control of cell proliferation and developmental processes. This notion first arose from demonstrations that transforming oncogenes can reduce growth requirements of cells and interfere with various differentiation pathways (*1*). More recent findings that some protooncogenes encode growth factors and growth factor receptors provided evidence that protooncogenes are involved in cellular growth control. Protooncogene products appear to function as growth regulators also as membrane-bound or intracellular mediators and as nuclear proteins. The importance of these genes for development and differentiation processes is, however, still largely unknown.

Teratocarcinoma cells provide interesting models for studies of the relationship between protooncogene expression, cell proliferation, and differentiation. Teratocarcinomas are malignant tumors derived from primordial germ cells, parthenogenetically activated oocytes, or from young, ectopic embryos (*2*). In the mouse, the tumors consist of malignant undifferentiated embryonal carcinoma (EC) cells, and a variety of differentiated cells. The EC cells closely resemble embryoblasts of the inner cell mass of the blastocyst. EC stem cells are small, have a high nucleo-cytoplasmic ratio, and contain few organelles. The differentiated cell types are derived from the stem cells (*3*). Among the cell types commonly found in differentiated teratomas are derivatives of more than one germ layer, e.g., nerve, skin, muscle, and cartilage (Fig. 1). The multipotency of EC stem cells has been demonstrated by showing that EC cells can differentiate into every tissue of perfectly normally developing chimeric mice derived from implanted blastocysts injected with EC cells (*4–6*). As the EC cells differentiate, they also loose their tumorigenic potential (*7*). The purpose of this paper is to review the present knowledge of how differentiation of embryonal carcinoma cells relates to changes in protooncogene expression.

c-*onc* Genes in EC Stem Cells

To date, studies of protooncogenes in teratocarcinomas at the DNA, RNA, or protein level have not revealed any consistent feature distinguishing the malignant embryonal carcinoma cells from their normal cellular counterparts. Two cases of *ras* gene derangements in teratocarcinoma cell lines have been reported. During passage

*Department of Medical Cell Genetics, Karolinska Institutet, S-10401, Stockholm, Sweden.
Address reprint requests to T. Sejersen, Department of Medical Cell Genetics, Karolinska Institutet, S-10401, Stockholm, Sweden.

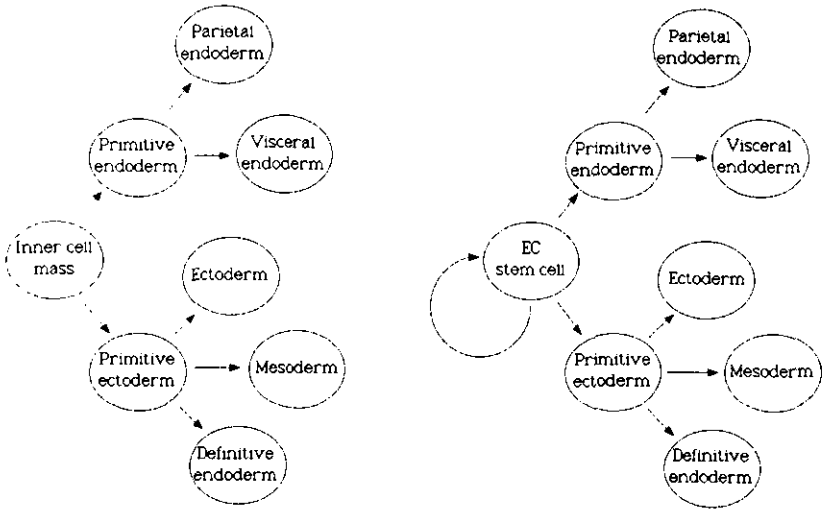


FIGURE 1. Simplified presentation of the earliest pathways of differentiation of the early embryo inner cell mass (left) and embryonal carcinoma (EC) stem cells (right).

of the human teratocarcinoma cell line PA1, N-*ras* has undergone a point mutation at the codon for amino acid twelve (*8*). In the other case, a 10- to 20-fold amplification of c-K-*ras* was detected in the genome of the murine embryonal carcinoma cell line PCC4 (*9*). This is not, however, a general feature of embryonal carcinoma cells, as such amplification is not found in other embryonal carcinoma cell lines (*10*).

c-*onc* Expression during EC Cell Differentiation

Oncogenes are commonly classified as belonging to one of four or five subgroups, depending on possible function and intracellular localization. In the following sections, differentiation-related changes in c-*onc* expression will be described for each class of oncogene. The protooncogenes described are listed in Table 1, summarizing the classification system used here.

c-*onc* Genes Encoding Protein Kinases

c-*src*

The expression of c-*src*, which encodes a plasma and cytoplasmic membrane-bound tyrosine kinase, is acti-

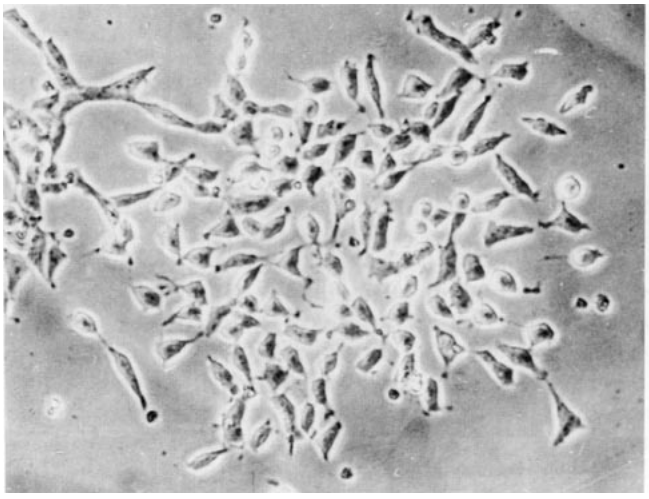
Table 1. *onc*-Related genes expressed in embryonal carcinoma cells before or after induced differentiation.

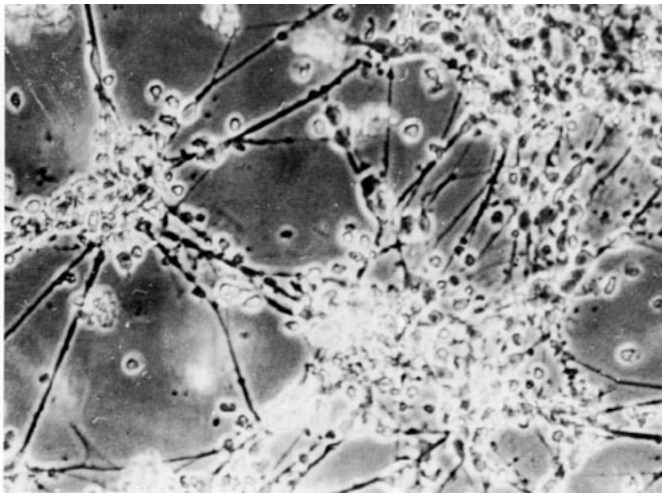
c-*onc*	Cellular location	Possible function	Expression	Reference
Protein kinases				
c-*src*	Plasma and cytoplasmic membranes	Tyrosine kinase	Transcriptional activation in nerve cells derived from P19 and PCC7	(*11,15*)
c-*abl*	Plasma and cytoplasmic membranes	Tyrosine kinase	Several EC cell lines; no association with differentiation	(*22,23*)
c-*erb*-B	Plasma and cytoplasmic membranes	EGF receptor Tyrosine kinase	Differentiated derivatives of several EC cell lines	(*26,27*)
c-*mos*	Cytoplasm	Serine/threonine kinase	311 EC cells; downregulated in differentiated 311 cells	(*36*)
Growth factors				
c-*sis*	Secreted	PDGF B-chain	PDGF-like activity secreted by EC stem cells	(*40,41*)
PDGF A	Secreted	PDGF A-chain		
GTPases				
c-H-*ras*	Plasma membrane	GTP binding and hydrolysis	Unaltered expressions in differentiating F9 cells	(*46*)
c-K-*ras*	Plasma membrane	GTP binding and hydrolysis	Downregulation in differentiating F9 cells	(*46*)
Nuclear proteins				
c-*myc*	Nucleus	?	Posttranscriptional downregulation in differentiating mouse EC cells	(*13,46, 56–57,59,69*)
N-*myc*	Nucleus	?	Posttranscriptional downregulation in differentiating mouse EC cells	(*13,51,59*)
c-*fos*	Nucleus	?	Induced in differentiating P19 cells	(*62*)
Other c-*onc* products				
int-1	Secreted ?	Segment polarity gene ?	Low expression in PSA-1 embryoid bodies	(*68*)
int-2	Secreted ?	Growth factor ?	Induced in F9 and PSA-1 endoderm	(*68*)

vated in PCC7 EC cells undergoing neuronal differentiation (*11*). PCC7 cells differentiate spontaneously into cholinergic neurons (*12*), but nerve formation is enhanced by culturing the cells in retinoic acid (RA), cAMP, and low (*2%*) serum content. PCC7 cells induced in this way express 4.0 kb c-*src* transcripts.

Activation of c-*src* expression can be explained by an increase in the transcription rate of the c-*src* gene, which is transcriptionally silent in PCC7 stem cells (*13*) (Fig. 2). It is not yet known if the *src* protein in these cells is of the modified type expressed at high levels in neural tissue of the central nervous system (*14*). The modified form of $pp60^{src}$ was, however, recently found to be induced in P19 embryonal carcinoma cells treated with retinoic acid to form neuronlike cells (*15*).

As a comparison with *in vivo* embryonic development of the mouse, c-*src* transcripts first accumulate to detectable levels approximately 10 days after fertilization (*16*). The biological function of c-*src* is not known, but it is interesting to note that expression of c-*src* is correlated to





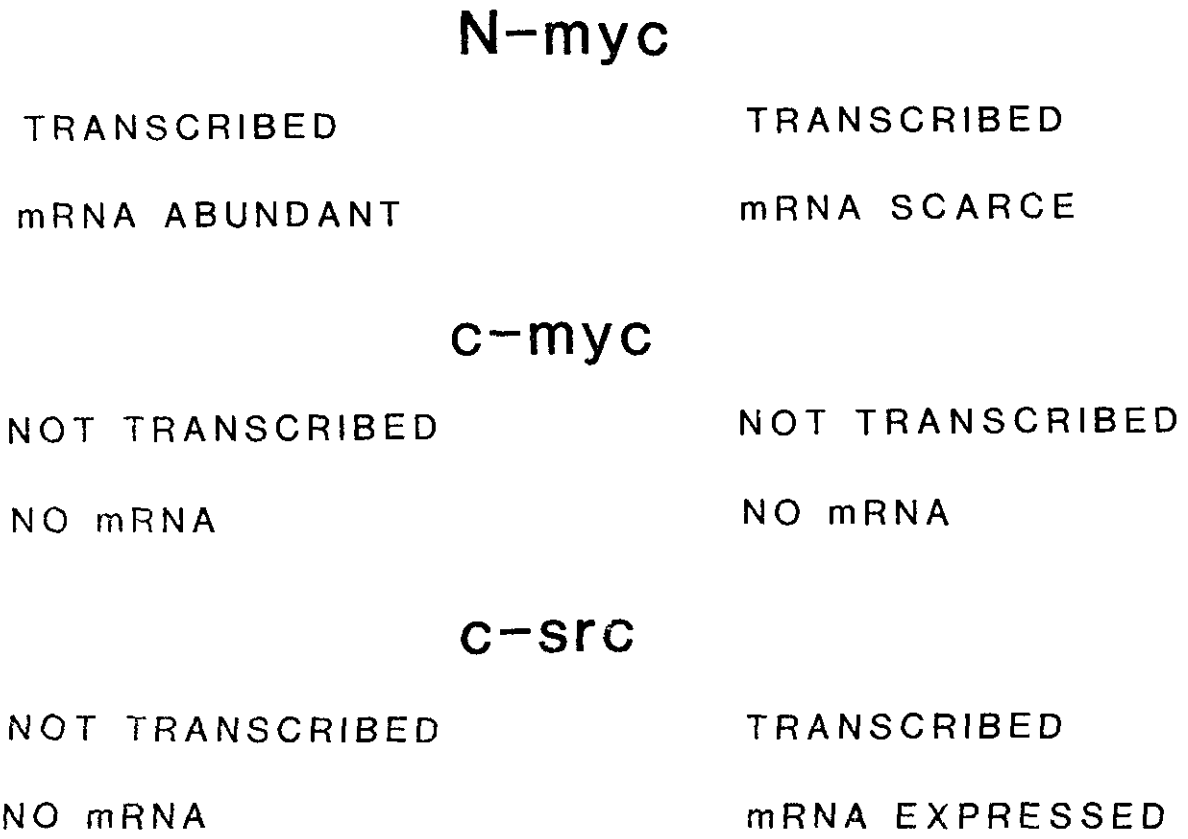


FIGURE 2. Regulation of N-*myc*, c-*myc*, and c-*src* in PCC7 stem cells (left) and in differentiated PCC7 nervelike cells (right). Differentiated PCC7 cells extend neurite outgrowths and accumulate neurofilaments, acetylcholinesterase, and choline acetyltransferase (*11,12*).

terminally differentiated neuronal cells, and not to rapidly dividing cells. The role of c-*src* may thus be to perform a specialized cellular function unrelated to cell growth. This function may differ in different cell types, just as the v-*src* product affects target cells differently. Thus, v-*src* inhibits myogenic (*17,18*), chondrogenic (*19*), and melanoblastic (*20*) differentiation, but induces pheochromocytoma cells to undergo neuronal differentiation (*21*).

c-*abl*

c-*abl* Encodes a tyrosine kinase closely related to the product of c-*src*. The c-*abl* gene is expressed both in EC stem cells and in visceral endoderm, parietal endoderm, trophoblastic cells, and myogenic cells derived from EC cells (*22*). No marked difference in c-*abl* expression has been observed between c-*abl* expression in EC stem cells and differentiated teratoma-derived cells (*23*). Similarly, c-*abl* expression is relatively constant during mouse prenatal development (*23*).

c-*erb-B*

Epidermal growth factor (EGF) receptors, like other growth factor receptors, show tyrosine kinase activity (*24*). The gene for the EGF receptor is now known to be identical to c-*erb-B* (*25*). During retinoic acid-induced endodermal differentiation of mouse EC cells, the number of unoccupied epidermal growth factor (EGF) receptors increases (*26,27*) (Fig. 3). Whether the apparent increase in EGF receptors on differentiating PCC4, PC13, and OC15S1 EC cells is due to unmasking of receptors occupied by growth factors produced by EC stem cells or whether the increase represents a true increase in the actual number of EGF receptors is not yet known. Nor has it been clearly established if EGF receptors are expressed by both parietal and visceral endoderm. Binding of EGF to its receptors on differentiated embryonal carcinoma cells catalyzes phosphorylation of tyrosine residues (*28*) and stimulates cell division (*26*). In early murine development, embryonic tissues bind EGF specifically from day 11 of gestation (*29*).

Similar to the situation with EGF receptors, PC13 EC stem cells express few, if any, receptors for insulin, whereas the differentiated derivative cells express a large number of insulin receptors and are stimulated to divide by insulin (*30*). F9 and OTT-6050 EC cells do, however, express receptors for both insulin and insulinlike growth factor II (IGF-II) (*31–33*). Insulin and IGF-II also promote the growth of these cells (*31–34*).

c-*mos*

Expression of c-*mos*, encoding a cytoplasmically located serine/threonine kinase, is suppressed in most differentiated tissues and was for some time believed to be incompatible with normal, untransformed cell growth. However, c-*mos* is now known to be expressed in mouse testes, ovaries, and term embryos (*35*). *mos*-Related transcripts

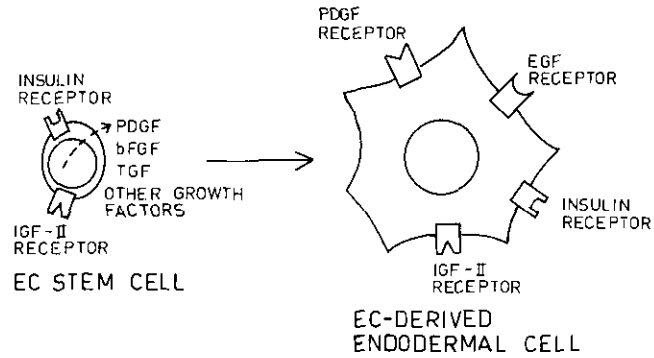


FIGURE 3. Simplified presentation of production of growth factors and expression of growth factor receptors by EC stem cells (left), and EC-derived endodermal cells (right). The illustration is based on findings in several different cell lines (see text), and is therefore not applicable to any particular EC cell line.

have also been detected in pluripotential murine 311 EC stem cells (*36*). After a 3-day lag period, c-*mos* expression is strongly inhibited when 311 EC cells are induced by retinoic acid treatment to undergo differentiation into a variety of cell types. The sizes of the major transcripts in embryo and in EC 311 cells differ, being 1.3 and 2.3 kb in the embryos and 6.1 and 4.6 kb in EC 311 cells. These sizes differ in turn from those of c-*mos* transcripts derived from testes (1.7 kb) or ovaries (1.4 kb). The reason for these differences is not known.

All of these different-sized transcripts are sufficiently long to cover the 1 kb open reading frame of c-*mos*. In EC cell lines other than 311, such as F9, PCC3, and PCC4, c-*mos* expression has not been detected (*22,23,36*). The role of c-*mos* in early embryonal cell growth and differentiation is not clear. An interesting observation is that infection with v-*mos*-carrying retrovirus enhances anchorage-independent growth of PCC4 EC cells but does not affect the ability of PCC4 cells to undergo epidermislike differentiation (*37*).

c-*onc* Genes Encoding Growth Factors

c-*sis* and PDGF-A Genes

Platelet-derived growth factor (PDGF) is a 30 kD polypeptide consisting of two chains, A and B, linked by disulfide bonds. PDGF is a potent mitogen for mesenchymal cells (*38*). There is approximately 40% amino acid homology between the A and B chain. The A chain is encoded by the PDGF A-chain gene, and the B chain is encoded by c-*sis*. Authentic platelet PDGF most likely consists of A-B heterodimers, but other cells are known to produce A-A or B-B homodimers. Although mitogenic, A-A homodimers are considerably less potent than authentic PDGF (*39*). PDGF-like activity has been found in medium conditioned by mouse embryonal carcinoma cells (*40,41*) (Fig. 3). EC cells appear to express PDGF as A-A homodimers (Charles Stiles, personal communication). PDGF B-chain-like RNA is, however, present both in EC

cells and in early mouse embryos (*16,22*). Differentiation of F9 and PC-13 EC cells into endodermal cells is paralleled by a drastic (< 90%) reduction in PDGF production. At the same time there is a 18- to 85-fold increase in PDGF binding activity (*41*) (Fig. 3). Expression of PDGF receptors, as well as a mitogenic response to PDGF, have been noted in a mesodermal cell line derived from P19 EC cells (*42*).

In addition to PDGF-like growth factors, undifferentiated EC cells also produce and secrete transforming growth factorlike activity (*43*), basic fibroblast growth factor activity (*44*), and three other unidentified stem cell growth factors (*45*).

c-*onc* Genes Encoding GTPases

c-*ras*

$p21^{ras}$ proteins constitute a large family of membrane-bound GTPases. Most studies of teratoma cells describe the abundance of c-K-*ras* and c-H-*ras* transcripts. F9 EC stem cells express both c-K-*ras* and c-H-*ras* mRNA (*46*). Retinoic acid- and cAMP-induced differentiation of F9 cells into parietal endoderm is accompanied by a moderate decrease in c-K-*ras* expression, but essentially unchanged levels of c-H-*ras*. However, F9 cells differentiated into visceral endoderm by growth as embryoid bodies in the presence of retinoic acid do not show altered mRNA levels of either c-K-*ras* or c-H-*ras* (*23*). These results indicate that $p21^{ras}$ proteins are not involved in EC cell differentiation processes.

A similar conclusion can be drawn from transfection experiments. P19 EC cells transfected with the EJ bladder carcinoma cell line oncogene (Ha-ras^{EJ}-1) differentiate into the same spectrum of cell types (neurons, astrocytes, and fibroblastlike cells) as the parental P19 cells (*47*). At the DNA level Vilette et al. (*10*) have found a 10- to 20-fold amplification of c-K-*ras* in the mouse EC cell line PCC4, and Tainsky et al. (*8*) have reported a point mutation of the N-*ras* gene in the human teratocarcinoma cell line PA1. As a comparison with normal mouse embryogenesis, expression of H-*ras* remains fairly constant during the prenatal development (*16*), whereas c-K-*ras* is downregulated (*48*).

c-*onc* Genes Encoding Nuclear Proteins

myc Family

The three best-characterized members of the *myc* family of protooncogenes are c-*myc*, N-*myc*, and L-*myc*. Expression of c-*myc* is nearly ubiquitous in proliferating tissue, whereas high-level expression of N-*myc* and L-*myc* appears to be very restricted with respect to tissue and stage of development (*49*). An interesting exception to the nearly ubiquitous expression of c-*myc* is found in mouse testes, where actively dividing premeiotic germ cells contain extremely low levels of c-*myc* transcripts (*50*).

In mouse EC stem cells, both c-*myc* and N-*myc* transcripts are expressed at relatively high levels (*11,46,51*). The same appears to be the case for c-*myc* and N-*myc* expression at the protein level (C. Waters, personal communication). The EC cell line PCC7 constitutes an exception, expressing N-*myc* but not c-*myc* at the RNA level (*11*), as well as at the protein level (Fig. 4). Thus, c-*myc* expression does not appear to be mandatory for rapid proliferation of EC cells. It can also be speculated that expression of a *myc*-like protein is required for proliferation of EC cells and that N-*myc* or some other member of the growing *myc* family can substitute for c-*myc*.

Both N-*myc* and c-*myc* expression have been found to be downregulated in murine EC cells following differentiation into visceral endoderm, parietal endoderm, or nervelike cells (*11,51*). Teratoma-derived myoblasts expressing c-*myc* but not N-*myc* also downregulate the level of c-*myc* transcripts during terminal differentiation (*11*). However, in a subclone of the human pluripotent teratocarcinoma cell line Tera-2, retinoic acid induced differentiation is not accompanied by decreased c-*myc* RNA levels (*52*). On the contrary, there appears to be a slight increase in the abundance of c-*myc* transcripts. Furthermore, the level of the $p62^{c\text{-}myc}$ protein appears to increase with increasing differentiation of human teratomas (*53*). The reason for the difference in c-*myc* regulation in Tera-2 cells and murine EC cells is not known, but it may to some extent reflect the differences in early embryonic markers between human and murine teratocarcinoma cells. In many respects murine EC cells resemble cells of the inner cell mass, while human teratocarcinoma stem cell lines express characteristics of preblastocysts, trophectoderm, and trophoblastic giant cells (*54,55*).

Differentiation of F9 EC cells into visceral endoderm leads to induction of α-fetoprotein expression, reduced DNA synthesis (80%), and decreased levels of c-*myc* and N-*myc* transcripts (85%) (*11*) (Fig. 5). The expression of both *myc* genes is reduced before transcription of the gene for the visceral endoderm marker α-fetoprotein is initiated. The downregulation of c-*myc* and N-*myc* mRNAs do, however, follow different kinetics. The reduction of c-*myc* mRNA precedes that of N-*myc* mRNA (*13*). Directing the differentiation of F9 EC cells toward parietal endoderm causes a similar reduction in c-*myc* and N-*myc* expression (*51*). The reduction in c-*myc* expression seems to be an early event also in this case (*56*).

PCC7 cells treated with retinoic acid and dibutyryl-cAMP (db-cAMP) will differentiate into aggregates of neurofilament positive cells with massive neurite outgrowths (Fig. 2). Very little DNA synthesis is observed in differentiated PCC7 cells. The level of N-*myc* mRNA is reduced by 85% in induced PCC7 cells over a 6-day period. The decrease in N-*myc* expression is rapid during the first 12 hr, preceding the reduction in DNA synthesis, activation of the c-*src* gene, and outgrowth of neurites (*11,13*). It is, therefore, tempting to speculate that the downregulation of N-*myc* expression is causally related to the differentiation process in retinoic acid treated PCC7 cells.

The complex events constituting differentiation include both reduced DNA synthesis and marked changes in phenotype. In order to examine the relationship between

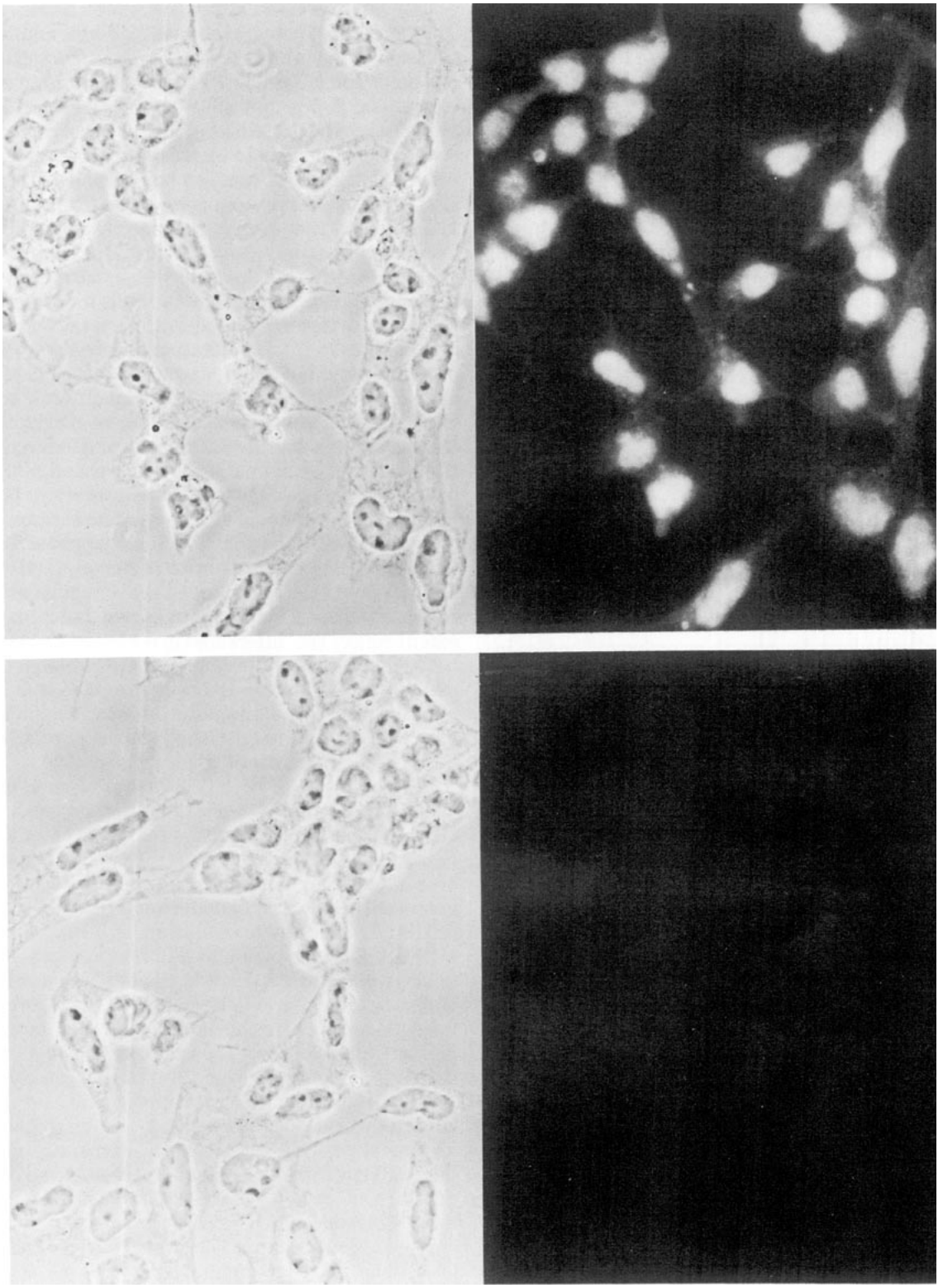
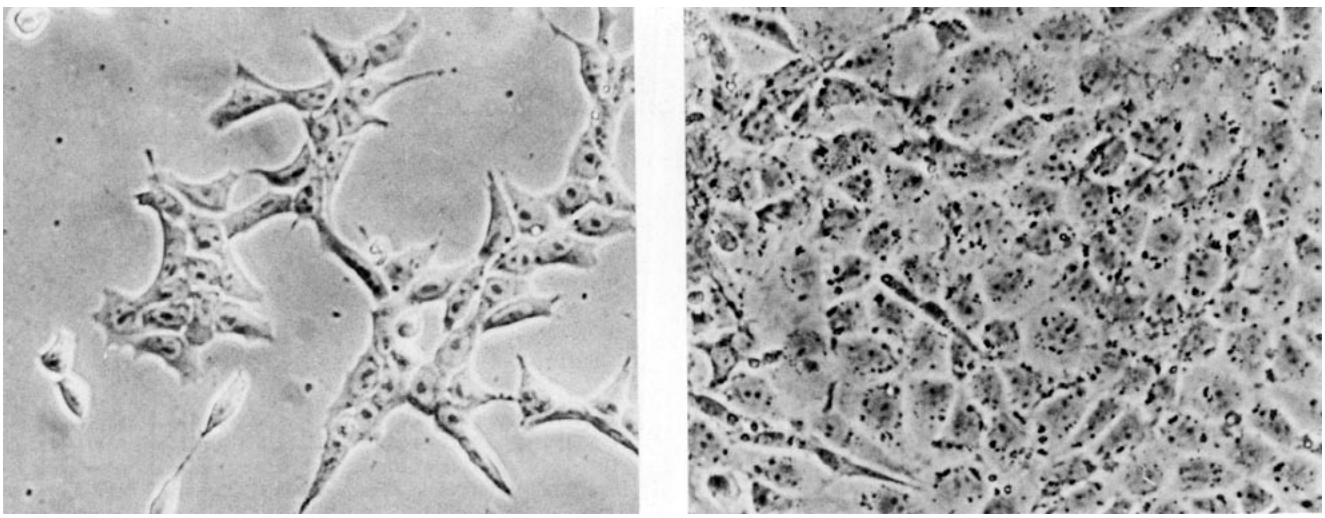


FIGURE 4. Expression of N-*myc* (upper right) and c-*myc* (lower right) immunoreactivity in PCC7 stem cells. Fixation procedures and exposure times were the same for both stainings. Corresponding phase contrast photographs are shown to the left.



N-myc

TRANSCRIBED

mRNA ABUNDANT

TRANSCRIBED

mRNA SCARCE

c-myc

TRANSCRIBED

mRNA ABUNDANT

TRANSCRIBED

mRNA SCARCE

c-src

NOT TRANSCRIBED

NO mRNA

NOT TRANSCRIBED

NO mRNA

FIGURE 5. Regulation of N-*myc*, c-*myc*, and c-*src* in F9 stem cells (left) and in F9-derived endoderm (right). F9-derived visceral endoderm express α-fetoprotein, have numerous microvilli and vacuoles, and intercellular junctional complexes (*70*). The F9 endoderm illustrated to the right is primitive endoderm.

myc expression and DNA synthesis, F9 and PCC7 EC stem cells can be partially growth arrested by serum deprivation. The abundance of both N-*myc* and c-*myc* transcripts is drastically decreased (90%) when F9 cells are subjected to serum deprivation, a treatment that causes a marked, but not complete, inhibition of DNA synthesis (*13*).

Although both c-*myc* and N-*myc* mRNA levels decrease radically following serum deprivation of F9 cells, the early kinetics of the reappearance of these transcripts upon growth stimulation differ markedly between the two genes. A marked increase in c-*myc*, but not N-*myc* expression is induced 4 hr after stimulation with either serum or a combination of insulin and transferrin. Isoleucine

deprivation, an alternative method to accomplish partial growth arrest of EC cells, has been reported by Dean et al. (*57*) to be accompanied by a similar decline in c-*myc* expression. The serum dependence of N-*myc* expression in F9 cells contrasts with the situation in PCC7 cells and a human neuroblastoma cell line. Serum deprivation of the latter cell types does not affect the level of N-*myc* transcripts (*11,58*).

The levels of N-*myc* and c-*myc* transcripts in the mRNA population is affected by the rate at which these genes are transcribed (transcriptional regulation), as well as by the stability of the transcripts (posttranscriptional regulation). *In vitro* nuclear run off transcription experiments have been carried out in order to distinguish between transcriptional and posttranscriptional regulation in EC cells in response to serum, mitogens, or induction of differentiation. The results indicate that both c-*myc* and N-*myc* are transcribed at a high level in F9 EC cells irrespective of the state of differentiation (Fig. 5) or presence of serum (*13,56,57,59*). Furthermore, Dony et al. (*56*) have demonstrated that the ability of cycloheximide, a protein synthesis inhibitor, to increase c-*myc* expression in F9 cells does not affect c-*myc* transcription and must, therefore, affect the mRNA level by stabilizing c-*myc* transcripts. It is therefore likely that these *myc* genes are regulated posttranscriptionally, in contrast to the gene for α-fetoprotein, which is subject to transcriptional control (*13*).

A quick and efficient posttranscriptional regulation involving the stability of c-*myc* and N-*myc* mRNA requires that the transcripts are short-lived. This has been shown to be the case for c-*myc* in a number of cell types (*60*). The half-lives of N-*myc* and c-*myc* mRNAs in actinomycin treated F9 stem cells were estimated to be 130 and 40 min, respectively (*13*). In PCC7 stem cells, the unexpected absence of c-*myc* transcripts appears to be due to lack of transcription, whereas the reduced levels of N-*myc* mRNA following *in vitro* neuronal differentiation is due to posttranscriptional events (Fig. 2). This can be contrasted with the c-*src* gene, which is subject to transcriptional activation.

It is not clear whether the decreased expression of *myc* genes is in any way causally related to endodermal differentiation, or if it merely reflects a general inactivation of genes unnecessary for the specialized visceral endoderm. It is interesting to note, however, that c-*myc* expression undergoes a drastic decrease in mouse erythroleukemia cells induced to undergo erythroid differentiation and that expression of transfected c-*myc* blocks this differentiation (*61*).

c-fos

c-*fos*, Another protooncogene encoding a nuclear protein with unknown function, is expressed at high levels in extra-embryonic membranes, fetal liver, and macrophages. Activation of the c-*fos* gene is a very rapid cellular response to serum and a number of growth factors and hormones.

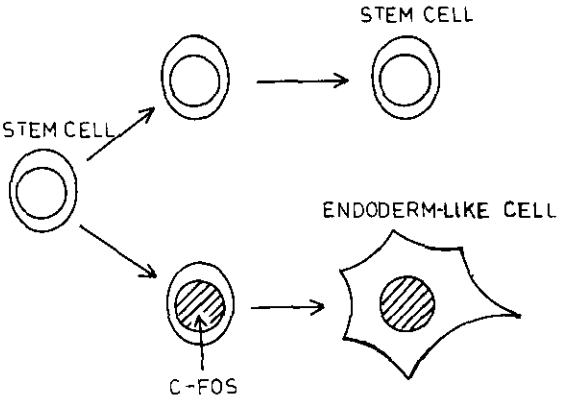


FIGURE 6. F9 stem cells rarely (> 1%) spontaneously give rise to differentiated derivatives (upper pathway). After transfection with c-*fos*, 10–70% of the colonies consist of differentiated cells (lower pathway) expressing intermediate filaments TROMA-1 and TROMA-3 as well as collagen type IV (*63,64*).

The regulation of c-*fos* expression in differentiating EC cells is not clear. Mason et al. (*62*) have found that retinoic acid and cAMP induction of F9 cells to form parietal endoderm is not accompanied by any large increase in c-*fos* RNA expression, although parietal endoderm at 13.5 days of murine development express c-*fos* at very high levels. Edwards and Adamson (*63*), on the other hand, discovered a large increase in c-*fos* expression as cells of a subline to P19 were induced to differentiate into a mixed popolution of cell types.

The relationship between c-*fos* expression and EC cell differentiation can also be analyzed by studying how elevated expression of transfected c-*fos* gene constructs affect the ability of EC cells to undergo differentiation. Rüther et al. (*64*) found that introduction of metallothionein promoter-driven c-*fos* genes into F9 cells lead to the appearance of c-*fos* expressing and spontaneously differentiating clones, using cellular morphology and presence of the surface antigens TROMA-1 and TROMA-3 as differentiation markers (Fig. 6). The phenotype of differentiated c-*fos*-transfected F9 cells is, however, not typical of either primitive, parietal, or visceral endoderm (*65*). In other EC cell lines, the differentiation-promoting effect of c-*fos* is less pronounced (P19) than for F9 cells, or absent (PC13). Thus, the role of c-*fos* in EC cell differentiation remains obscure.

Other c-*onc* Products in EC Cells

int-1 and *int*-2

int-1 and *int*-2 differ from the previously described oncogenes in that they were first isolated from DNA around proviral integration sites in mammary carcinomas, rather than from acutely transforming retroviruses. These sequences are not expressed at detectable levels in a variety of adult normal mouse tissues. *int*-1, Recently shown to be homologous to the Drosophila segment polarity gene that codes for the *wingless* phenotype (*66*), is ex-

pressed in postmeiotic germ cells and in the developing central nervous system of mid-gestation mouse embryos. This gene is not expressed in F9 or PSA-1 EC stem cells. Low levels of *int*-1 RNA appears in embryoid bodies of PSA-1 cells, but not in F9 cells induced to differentiate into parietal endoderm. *int*-1 Has no appreciable homology with *int*-2. The latter gene has recently been shown to be related to the fibroblast growth factor gene family (*67*). *int*-2 Transcripts are found in peri-implantation mouse embryos and at low levels in F9 and PSA-1 EC stem cells. In differentiated endodermal derivatives of F9 and PSA-1, the level of *int*-2 expression was found to be dramatically increased (*68*). The physiological functions of *int*-1 and *int*-2 proteins are still poorly understood.

Conclusion

Several protooncogenes are expressed in EC cells or their differentiated derivatives (Table 1). Although point mutations and amplifications of *ras* genes have been reported there is no evidence to link the malignant phenotype of EC cells to changes in protooncogene structure or expression. Protooncogenes expressed in germ cells and/or early embryonic cells are expressed also in EC cells. Examples of such genes are N-*myc*, c-*mos*, and *int*-2. Certain c-oncogenes or oncogene-related products undergo changes in the level of expression during induced differentiation of EC cells. These include c-*src*, c-*mos*, c-*erb*-B, PDGF, K-*ras*, c-*myc*, N-*myc*, c-*fos*, *int*-1, and *int*-2. The relationship of these changes to EC cell differentiation is not understood. Changes in protooncogene expression may be important for the differentiation process or for expression of the differentiated phenotype. The possibility that these changes are secondary events cannot, however, be excluded. So far, the only protooncogene that has been shown to affect differentiation of EC cells when expressed at elevated levels is c-*fos*. This result is difficult to interpret. We are still far away from understanding the role of protooncogenes in EC cell differentiation.

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