Oncogenes in development

EILEEN D. ADAMSON
La Jolla Cancer Research Foundation, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA

Synopsis

(1) Introduction

Realization that the transforming oncogenes (v-onc) of the acutely oncogenic retroviruses are homologous to cellular genes (and were probably derived from them) brought several areas of research together with exciting prospects for advances in virology, carcinogenesis, evolution and development. The proto-oncogenes (c-onc) are likely to be crucially involved in growth regulation and/or differentiation because of their conservation throughout evolution and because of the well-known growth deregulation effects produced by the v-oncs. It was therefore reasoned that the normal counterpart of these genes should be active during embryonic development and that identification of a specific tissue or stage where c-oncs are expressed should help to identify their roles in all cells and provide a source of material to study the mechanisms of action. The preliminary results suggest growth modulatory roles for most oncogenes, and developmental studies have provided clues to c-onc roles that would not have been forthcoming from studies on cell lines.

Several c-onc products have now been identified with specific cellular proteins, and these have confirmed their importance to growth regulation. They are growth factor or hormone receptors (such as c-erb-B, or epidermal growth factor [EGF] receptor; c-fms, or colony stimulating factor-1 [CSF-1] receptor and c-erb-A, or thyroid hormone receptor) or growth factors (such as c-sis, or B chain of the platelet-derived growth factor [PDGF]). Table 1 lists the proto-oncogenes that have been studied in developing or differentiating systems. For general reviews, see Müller, 1983; Hunter, 1984; Weinberg, 1984; Varmus, 1984; Heldin & Westermark, 1984; Sinkovics, 1984; Klein & Klein, 1985; Bishop, 1985; and Müller, 1986.

(2) EGF receptor/c-erb-B

The best known of the tyrosine kinase family of c-oncs is the EGF receptor, which is a larger homologue of the v-erb-B protein that causes avian erythroblastosis in chickens infected with avian erythroblastosis virus (AEV) (Downward et al. 1984). The EGF receptor has been recognized as a cell-surface glycoprotein whose activity is triggered after binding EGF in the cellular environment. After receptor clustering and endocytosis, lysosomal compartments degrade both receptor and ligand. After this event there is very little known about how the cell receives the signal to commence DNA synthesis and to enter mitosis. Many steps of the process may be necessary, but after the discovery that the receptor also has a tyrosine phosphokinase enzyme activity (Ushiro & Cohen, 1985) that leads to the phosphorylation of itself and of other cellular substrates, the clearest clue...
Table 1. Some proto-oncogenes and their protein products

<table>
<thead>
<tr>
<th>c-onc</th>
<th>Protein identity, homology or size</th>
<th>Cell location and distribution</th>
<th>Activities</th>
<th>Possible roles</th>
<th>Chromosomal location</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>human/ mouse</td>
<td></td>
</tr>
<tr>
<td>I. Tyrosine kinase and related c-onc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-erb-B</td>
<td>EGFR receptor 170 K</td>
<td>Plasma membrane of mesodermal, ectodermal &amp; endodermal cells</td>
<td>EGF binding, TGFβ binding, Tyr. kinase</td>
<td>Signal transduction for mitogenesis and differ*, S1m* of tooth eruption, eye-opening, lung develop.</td>
<td>/7</td>
<td>1</td>
</tr>
<tr>
<td>c-neu</td>
<td>185 K Homology to EGFR-R</td>
<td>Plasma membrane</td>
<td>Tyr. kinase</td>
<td>Receptor for an unknown ligand</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>c-fms</td>
<td>CSF-1 receptor 140 K</td>
<td>Plasma membrane of macrophages &amp; extra-embryonic cells</td>
<td>CSF-1 binding, Try. kinase</td>
<td>Signal transduction for mitogenesis and differ*</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>c-src</td>
<td>60 K</td>
<td>Cytoplasmic face of membranes, Adhesion plaques</td>
<td>Tyr. kinase phosphorylates many cellular proteins, e.g., vinculin, vimentin, filamin, p36.</td>
<td>Neurone &amp; muscle development*</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>c-mos</td>
<td>37 K</td>
<td>Cytoplasmic, Embryonic testis &amp; ovary</td>
<td>Ser/thr kinase</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>c-abl</td>
<td>150 K</td>
<td>Plasma membrane</td>
<td>Tyr. kinase phosphorylates vinculin</td>
<td>B-cell differ*</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>c-fes</td>
<td>92 K</td>
<td>Cytoplasmic, plasma membrane</td>
<td>Tyr. kinase</td>
<td>Macrophage develop.*</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>c-fps</td>
<td>98 K</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II. GTPases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-Ki-raf-2</td>
<td>21 K</td>
<td>Membrane cytoplasmic face in a wide variety of cells</td>
<td>GTP + GTP binding, GTPase.</td>
<td>Adenylate cyclase regulation?</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>c-H-ras-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>N-ras</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>III Nuclear products</td>
<td>55 K</td>
<td>Nucleus of extra-embryonic tissues, haematopoietic cells &amp; macrophages All other cells at lower level.</td>
<td>DNA binding with an accessory protein</td>
<td>G0 to G1 transition, Differentiation</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>c-fos</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-myc</td>
<td>62 K</td>
<td>Nuclear matrix of most cells. In some tumours &amp; embryonic tissues.</td>
<td>DNA binding</td>
<td>Proliferation, Regulates DNA synthesis</td>
<td></td>
<td>8/15</td>
</tr>
<tr>
<td></td>
<td>66 K</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-myc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-myc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-myb</td>
<td>75 K</td>
<td>Nucleus of haematopoietic cells</td>
<td>DNA binding</td>
<td>Differentiation of haematopoietic cells?</td>
<td></td>
<td>6/11</td>
</tr>
<tr>
<td>IV. Others</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-erb-A</td>
<td>T3 receptor, homology to glucocorticoid receptor</td>
<td>Cytoplasmic &amp; nuclear</td>
<td>Birds thyroxine</td>
<td>Metabolic regulator?</td>
<td></td>
<td>17/12</td>
</tr>
<tr>
<td>c-sis</td>
<td>PDGF B chain 14 K</td>
<td>Secreted protein</td>
<td>Homo- or heterodimer binds to PDGF-R</td>
<td>Mitogenesis, Wound healing, Early embryonic growth factor?</td>
<td></td>
<td>22/13</td>
</tr>
</tbody>
</table>

K, 10^-3 M

References:

appeared to have been identified. This is because the proportion of phosphotyrosine, compared with phosphoserine and phosphothreonine in a normal cell, is very small, about 0.03% of the total. The mechanism of oncogenesis via tyrosine phosphorylation has been pursued vigorously, but the outcome so far has not given a clear picture of the roles of tyrosine phosphokinases in growth regulation. For reviews that
cover the enzymic and metabolic aspects of the EGF receptor, see Thompson & Gill, 1985; Herschman, 1985; Kris, Libermann, Avivi & Schlessinger, 1985; Carpenter & Zendoqui, 1986; Soderquist & Carpenter, 1986.

(2.1) The EGF-receptor gene

Normal tissues and cells appear to have a single gene for EGF receptor but differential splicing leads to the production of at least two mRNAs of about 6 and 10 kb (Ullrich et al. 1984; Lin et al. 1984; Xu et al. 1984). Both of these appear to code for full-length protein of 170 × 10^3 Mr (170 K) (Simmen et al. 1984). The gene is amplified in human A431 cells and in several types of tumours such as gliomas (Libermann et al. 1984), squamous cell carcinoma (Cowley et al. 1984) and retinoblastomas where the protein is also overexpressed. Presumably the expression of high levels of receptor provided a growth advantage to the tumour cells but it is not known if this was the original lesion.

(2.2) Distribution of EGF-binding activity in developing tissues

EGF receptors are present on a wide range of cell types including cells of ectodermal, mesodermal and endodermal origin (reviewed by Adamson & Rees, 1981). They are also present on embryonic/fetal tissues and extraembryonic tissues during murine development (reviewed by Adamson, 1983). But despite this, their role in development is not yet clear. EGF receptors increase in number during the gestation period so that the new-born's liver expresses more than any other tissue at any stage (Adamson & Meek, 1984). Crude membrane preparations from all fetal tissues (with the exception of the parietal yolk sac) contain EGF-binding activity, and the affinity of these receptors for ^125^I-EGF decreases somewhat during development. The earliest time of detection is on giant trophoblast cells of a 5-day blastocyst grown in vitro (Dekel & Sherizly, 1985), and postnatal and fetal development of rat gastric mucosa (Dembinski & Johnson, 1985; O'Loughlin et al. 1985; Conteas, DeMorrow & Majumdar, 1986) are influenced by EGF.

(2.3) Occurrence of EGF receptors in teratocarcinoma cells

The stem cell lines (embryonal carcinoma, EC) of murine teratocarcinomas appear to have very few (F9) or no EGF-binding sites (OC15, PC13, P19). When differentiation occurs, either in aggregate cultures (Adamson & Hogan, 1984) or in monolayers (Rees, Adamson & Graham, 1979; Mummery et al. 1985), receptors appear which respond to EGF by promoting cellular proliferation. Teratocarcinoma-derived differentiated cell line PSA5E (visceral endoderm-like) also has EGF receptors, while PYS and F9AC cells (parietal endoderm-like) do not. This distribution is in agreement with the tissue distribution determined from embryo studies. Surprisingly, OC15 EC cells have considerable EGF-receptor-kinase activity (equivalent to about 3-day differentiated cells), but it appears to be largely intracellular or possibly masked (Weller, Meek & Adamson, 1987). Therefore, we have to modify our original hypothesis that early embryonic ectoderm cells would be negative for this protein based on findings in EC cells. Indeed, a human EC cell-line (PA1) has been shown to express a low level of EGF receptors on the cell surface (Carlin & Andrews, 1985).

(2.4) Activation of the EGF-receptor oncogene protein during development

If the EGF receptor is to function it must receive signals by ligand binding to the extracellular binding domain. Two known growth factors bind to the EGF receptor, EGF/urogastrone and transforming growth factor α (TGFα). See reviews by Adamson (1983, 1986) and Roberts & Sporn (1985).
(2.4.1) EGF

This 53 amino-acid polypeptide (6 K) is synthesized and stored in very large amounts in male adult mouse (but not rat or human) submandibular salivary gland. It is detected in saliva, amniotic fluid, milk, urine, and most tissues of several species. Although EGF has been detected in fetal mice (Nexo, Hollenberg, Figueroa & Pratt, 1980) and fetal rats (Matrisian, Pathak & Magun, 1982), it seems unlikely to be derived from the fetus itself, and Popliker et al. (1986) have presented evidence that the EGF found in fetal mice is derived from the mother. The EGF gene occurs as a large gene encoding a precursor that contains several EGF-like peptides as well as EGF, together with a membrane-spanning domain (Scott et al. 1983). Using a cloned probe for prepro EGF mRNA, Rall et al. (1983) have shown high-level gene transcription in the mouse salivary gland and in kidney. Female kidney expresses about 2- to 4-fold higher levels than male, and this is a reversal of the salivary gland levels (Popliker et al. 1986; Gubits et al. 1986; Salido et al. 1986). However, only the salivary gland precursor appears to be processed to the active 6 K form. The kidney 130 K precursor form is not known to be an active mitogen. The importance of these reports is the realization that EGF is not synthesized until at least 2 weeks postpartum in the kidneys and even later (after weaning) in the male salivary gland. Then why does the fetus express EGF receptors?

(2.4.2) TGFα

Rat TGFα (5 K) has been purified, sequenced, synthesized, molecularly cloned and shown to have 35–40 % homology with EGF (Marquardt, Hunkapiller, Hood & Todaro, 1983; Tam et al. 1984; Lee, Rose, Webb & Todaro, 1985b). TGFα binds to EGF receptors and produces exactly the same effects, including mitogenesis and skin maturation. It can also synergize with TGFβ to stimulate anchorage-independent growth of normal fibroblasts. TGFα also occurs as a larger precursor form (Derynck et al. 1984) as a transmembrane protein. TGFα is found in normal rat tissues (Lee et al. 1985b; Stromberg, Pigott, Ranchalis & Twardzik, 1982; Proper, Bjornsson & Moses, 1982; Twardzik, 1985; Massagué, 1985). In contrast to EGF, TGFα has been detected as early as day 7 in mouse embryos using a specific radioimmunoassay procedure. The levels fall and then rise to a second peak in day-13 fetuses (Twardzik, 1985), in parallel with the levels of mRNA (Lee, Rochford, Todaro & Villareal, 1985a). Later in development, the placenta could be a major source of TGFα (Stromberg et al. 1982). These studies imply that early embryonic development could be affected by TGFα binding to EGF receptors, but the details of which embryonic tissues synthesize the growth factor and where TGFα finds its target are still to be discovered.

(3) CSF-1 receptor/c-fms

The v-fms product is the transforming protein of the McDonough strain of the feline sarcoma virus (SM-FeSV). Its proto-oncogene homologue is a membrane-inserted phosphorylated glycoprotein of approximately 165 K in mouse and 170 K in cat. The identity of the c-fms gene was first suggested by Sherr et al. (1985) who showed that it is very similar if not identical to the monocyte/macrophage cell surface receptor for CSF-1. The c-fms gene product is a tyrosine kinase, and the gene is therefore a member of the src family of related oncogenes (Rettenmier, Chen, Roussel & Sherr, 1985; Coussens et al. 1986).

The tissue expression of c-fms appears to be limited to the monocyte/macrophage lineage (spleen, bone marrow and fetal liver) and to developing extraembryonic tissues in mouse and human where it is expressed in a stage- and tissue-specific manner (Müller et al. 1983a). Highest levels of accumulated transcripts are present in 17th–19th day placenta with lower amounts in amnion, visceral yolk sac and chorion. The question arises whether this expression in extraembryonic tissues is due to high levels of macrophages found there. This will only be answered when in situ localizing methods are performed. However, evidence from a human teratocarcinoma cell line indicates that c-fms may be specific to placental cells. HT-H cells differentiate spontaneously in monolayer cultures to trophoblast-like cells that secrete hCGα sub-units and that express c-fms mRNA (Izhar et al. 1986). This phenotype is cell-type specific since undifferentiated stem cells and other types of cells that differentiate from HT-H cells in aggregates do not express c-fms and do not secrete hCG. BeWo human choriocarcinoma cells also express c-fms, further supporting localization in placental cells (Müller et al. 1983a,b).

(4) c-src

The src proteins were the first tyrosine kinases to be discovered and form the archetype for this group of oncogene products. v-src encodes the transforming protein of the avian Rous sarcoma virus and c-src encodes a similar 60 K protein. The demonstration that protein kinases are involved in oncogene-mediated transformation stems from the finding that pp60src is tightly associated with a protein kinase activity (Collett & Erikson, 1978; see review by
Wyke, 1983). The Rous SV product also phosphorylates phosphatidylinositol and diacylglycerol (Sugimoto, Whitman, Cantley & Erikson, 1984), and so its function is linked with other oncogene products which also affect phospholipid metabolism. A pp60^src related tyrosine kinase has been purified from bovine brain cerebral cortex (Neer & Lok, 1985) as a 61 K protein that can be phosphorylated on serine as well as tyrosine. It exhibits an autophosphorylating activity and also phosphorylates precipitating antibody (Resh & Erikson, 1985). Its location is on the cytoplasmic face of the cell as well as perinuclear. The amino terminus of pp60^src is myristylated, and this is needed both for localization to the inner face of the plasma membrane and for cellular transformation (Kamps, Buss & Sefton, 1985).

When the pp60^src gene is transfected into NIH 3T3 cells, gap junction communication between adjacent cells is inhibited and the activity of protein kinase C is enhanced (Chang et al., 1985). These activities suggest that if c-src has a similar function, it could be important to development. It was found several years ago that chick embryonic neural tissues express high levels of c-src (Cotton & Brugge, 1983). Immunostains show that most tissues have eight- to ten-fold lower levels of c-src than brain, retina and spinal ganglia. Immunocytochemical studies show highest levels in neural tube, brain and heart of stage-32 chicks, with lower levels in eye, limb bud and liver. A similar distribution is found in human fetuses with highest levels in cerebral cortex, spinal cord and heart (Levy, Sorge, Meymandi & Maness, 1984; Gessler & Barnekow, 1984). Thus the expression of c-src correlates strongly with the differentiation of electrogentic tissues when proliferation has ceased and expression persists in terminally differentiated neurons.

Recent immunocytochemical staining results of Maness, Sorge & Fields (1986) show that developing neural tissues in chick exhibit pp60^src expression at two different stages. On or before stage 4, transient localization is seen in the neural ectoderm. This declines by stage 12 (45-49 h) and later rises again in terminally differentiated neurons at about stage 21 (day 3-5) in the neural retina (Sorge, Levy & Maness, 1984) and stage 17 (day 2-5) in the cerebellum (Fults, Towl & Launder & Maness, 1985). Therefore it appears that c-src may play a role at proliferative stages also. An analogous pattern of c-src expression in Drosophila has been shown by in situ hybridization (Simon, Drees, Kornberg & Bishop, 1985). c-src mRNA is abundant in early embryos during gastrulation, low in larvae, and high in neural tissue and smooth muscle and pupae at later stages.

In vitro culture systems have been used to elucidate the location, effects and roles of c-src. Immunostaining shows that c-src is distributed all over cell bodies, processes and growth cones of chick dorsal root ganglion cultures (Maness, 1986). Primary cultures of neurons or astrocytes from rat brain contain 15- to 20-fold more c-src protein than fibroblasts, and the kinase specific-activity of c-src in neurons is 6- to 12-fold higher than in astrocytes (Brugge et al., 1985). Intriguingly, neuronal but not astrocytic c-src differs in size by a post-translational modification in the amino-terminal half. The murine teratocarcinoma cell line, PCC7, can be induced to differentiate into neuronal structures and a parallel elevation (3- to 5-fold) in c-src mRNA can be detected (Sejersen, Björklund, Sümegi & Ringertz, 1986). An 8- to 20-fold increase in src protein levels is observed after 5 days of induction of P195180A1 murine EC cells with retinoic acid, corresponding to the appearance of neuritic processes and other neuronal markers. The induced src protein is the slower mobility form associated with neurons, a finding that suggests the usefulness of teratocarcinoma cell model systems (Lynch, Brugge & Levine, 1986).

Studies with RSV infections or v-src introduced into cultured cells have also given results that suggest both proliferation and differentiation can be induced by the gene. For instance, marrow cultures are induced to greater selfrenewal of haematopoietic progenitor cells (Boettiger, Anderson & Dexter, 1984), while retrovirus carrying v-src introduced into PC12 rat phaeochromocytoma cells induces some features characteristic of differentiation (neurite extension) (Alema, Casalbore, Agostini & Tato, 1985). v-src-containing viruses suppress differentiation in chondroblasts as measured by the synthesis of chondrocyte-specific products (Alema, Tato & Boettiger, 1985). However, v-src has different properties to c-src and may also be regulated differently.

(5) c-abl

The Abelson leukemia virus (Ab-MLV) induces B and T cell lymphomas in mice by means of the expression of v-abl, which encodes a tyrosine kinase of 160 K. Cooperation with EGF receptors appears to be necessary for v-abl to transform murine fibroblasts into tumorigenic cells (Gebhardt, Bell & Foulkes, 1986). An homologous c-abl of 150 K is found in normal mouse cells (Goff, Gilboa, Witte & Baltimore, 1980). c-abl is actively transcribed during embryo and fetal development with high levels of transcripts in extraembryonic tissues as well as in the fetus proper (Müller et al., 1982). Its expression is highest on day 10 when organogenesis is progressing rapidly. Therefore, mRNA levels fall during gestation but are detectable throughout. Low levels have been detected in several teratocarcinoma cell lines (Sejersen, Sumegi & Ringertz, 1985).
vesicle breakdown (GVBD) is mediated, at least in oocytes.

Xenopus laevis genes were first identified as the viral oncogene \( v-kis \), which is the transforming gene of HZ4 feline retrovirus, has homologous regions to PDGF receptor and \( c-fms \), but is presumably a truncated version with no transmembrane domain (Besmer et al. 1986).

Distantly related to the tyrosine kinase encoding oncogenes is \( v-raf \), which has ser/thr kinase activity. The \( c-raf-1 \) gene has been cloned and utilized to evaluate expression in mouse embryos. mRNA is not detected, while a related but distinct gene, \( A-raf \), is transcribed in 14-day embryos with less in 18-day embryos and placenta. Moderate levels were found in certain adult tissues (Huleihel et al. 1986).

The cellular homologue of the transforming gene product, p37\(^{c-mos}\), of the Moloney murine sarcoma virus (Papkoff, Nigg & Hunter, 1983) was thought to be unexpressed in mouse tissues. By sensitive S1 nuclease and Northern assays, expression has been detected in whole embryos at moderate levels, in placenta, kidney and brain at very low levels, and at high levels in adult testes and ovary. Transcript sizes differ in different tissues and it was suggested that tissue-specific regulation of the size of \( mos \) transcripts could be transactivated, for example, by hormones, and could give rise to functionally different protein products (Propst & Vande Woude, 1985).

(6) Other oncogene kinases

Very little is known about the expression of the other tyrosine kinases in development. The proto-oncogene \( c-ras \) has features in common with the EGF receptor family and displays tissue-specific and developmentally regulated expression (Neckameyer, Shibuuya, Hsu & Wang, 1986). The oncogene \( v-kis \), which is the transforming gene of HZ4 feline retrovirus, has homologous regions to PDGF receptor and \( c-fms \), but is presumably a truncated version with no transmembrane domain (Besmer et al. 1986).

Distantly related to the tyrosine kinase encoding oncogenes is \( v-raf \), which has ser/thr kinase activity. The \( c-raf-1 \) gene has been cloned and utilized to evaluate expression in mouse embryos. mRNA is not detected, while a related but distinct gene, \( A-raf \), is transcribed in 14-day embryos with less in 18-day embryos and placenta. Moderate levels were found in certain adult tissues (Huleihel et al. 1986).

The cellular homologue of the transforming gene product, p37\(^{c-mos}\), of the Moloney murine sarcoma virus (Papkoff, Nigg & Hunter, 1983) was thought to be unexpressed in mouse tissues. By sensitive S1 nuclease and Northern assays, expression has been detected in whole embryos at moderate levels, in placenta, kidney and brain at very low levels, and at high levels in adult testes and ovary. Transcript sizes differ in different tissues and it was suggested that tissue-specific regulation of the size of \( mos \) transcripts could be transactivated, for example, by hormones, and could give rise to functionally different protein products (Propst & Vande Woude, 1985).

(7) The ras family of genes

The ras genes have been first identified as the viral oncogenes of the Harvey and Kirsten rat sarcoma viruses (Ellis, DeFeo, Futh & Scolnick, 1982). Cellular ras genes have been identified in most species, and they constitute a family of three human genes which encode a remarkably well-conserved protein, designated p21 (Defeo et al. 1981). The p21 protein is located on the cytoplasmic face of the plasma membrane in both normal and transformed cells. \( In vitro \), p21 binds GTP (Finkel, Der & Cooper, 1984), but the normal gene product hydrolyses GTP at a rate 8- to 10-fold higher than that of the transforming protein (Sweet et al. 1984). ras proteins are structurally and functionally analogous to the G proteins which are involved in adenylate cyclase regulation (Gilman, 1984; Hurley et al. 1984). The potential relationship between adenylate cyclase and p21 may be part of the control of cell division in \( Xenopus laevis \) oocytes. Progesterone-induced meiotic activation of germinal vesicle breakdown (GVBD) is mediated, at least in part, by inhibition of the oocyte adenylate cyclase (Finidori-Lepicard et al. 1981), but it appears not to involve the inhibitory guanine-nucleotide binding subunit G, Sadler, Schechter, Tabin & Moller (1986) showed that monoclonal antibodies to p21-ras inhibited adenylate cyclase activity and gave accelerated maturation of \( Xenopus laevis \) oocytes in a dose-dependent manner. It is possible either that p21 protein interacts with the pathway of normal cell division regulated by progesterone or that antibodies cross react with the oocyte G proteins. Ras proteins appear to interact with phospholipase C in a G protein-like manner (Fleischman, Chahwala & Cantley, 1986), and c-Ki-ras-2a has some homology with lipocortin and related proteins (Kretzinger & Creutz, 1986).

The ras gene products are clearly involved in an important aspect of cell proliferation since even normal p21\(^{c-ras}\) when expressed at elevated levels can result in the immortalization and transformation of mouse cells (but not human cells). c-Ha-ras and c-Ki-ras expression is detected at all stages of mouse embryonic development at almost unvarying levels (Müller et al. 1982; Müller, 1983; Slamon & Cline, 1984). This would suggest a general metabolic role in development. ras proteins are remarkably conserved evolutionarily, with homologous proteins occurring in yeast which have similar GTP-binding and hydrolytic properties (Temeles et al. 1985) and in \( Drosophila \) (Mozet, Marl, Parkhurst & Corces, 1985). Of the three v-Ha-ras-related cellular genes in \( D. melanogaster \), each is transcribed into two sizes of mRNAs. The larger is expressed at similar abundance during the life cycle stages, while the shorter transcript is more abundant in embryonic stages (Lev, Kimchi, Hessel & Segev, 1985). \( In situ \) hybridization was used to locate and identify active tissues. Distribution is uniform in embryos but is restricted to dividing cells in larvae. However, in the adult, both dividing and nondividing tissues contain high levels of ras transcripts, including ovaries, cortex of brain and ganglia, thus suggesting roles in growth and in differentiation (Segal & Shilo, 1986). An homologous p23 protein in \( Dictyostelium discoideum \) is expressed at highest levels in growing organisms, and this declines with the onset of differentiation (Pawson et al. 1985). Similarly, F9 murine teratocarcinoma cells express high levels of c-Ha-ras mRNA and this is moderately diminished during differentiation (Campisi et al. 1984). However, c-Ki-ras expression increases after 48 h of stimulation of differentiation of mouse erythroleukemic cells with DMSO. Other oncogenes are also induced, including fos, myb, and myc, while ten others remain unchanged (Todokoro & Ikawa, 1986).

The differentiation inducing properties of c-Ha-ras were tested by introducing sarcoma viruses carrying
ras oncogenes into PC12 cells (Noda et al. 1985) or by microinjecting purified normal or activated Ha-ras proteins (Bar-Sagi & Feramisco, 1985). There is no effect of normal c-ras on PC12 differentiation, but activated Ha-ras products induce differentiation in both cases. Antibody to p21-ras protein microinjected into PC12 cells inhibits nerve-growth-factor-induced differentiation (Hagag, Haleboua & Viola, 1986), and this indicates that c-ras does indeed play a role in differentiation.

The expression of c-Ki-ras appears to be cell cycle dependent in a chemically transformed mouse fibroblast cell line with highest expression in mid to late G0/G1 (Campisi et al. 1984). In addition, antibody to ras microinjected into quiescent NIH 3T3 cells prior to serum stimulation blocks a large population of cells from entering S phase later while control antibodies do not. A time course shows that ras activity is needed just before S phase or about 8 h after addition of serum (Mulcahy, Smith & Stacey, 1985). A similar time of Ha-ras gene activation is found after partial hepatectomy in rats, and this returns to normal after 3 days suggesting that proto-oncogene regulation is a normal regulated process in non-neoplastic growth processes (Goyette, Petropoulos, Shank & Fausto, 1984).

(8) c-fos

The FBJ and FBR murine osteosarcoma viruses rapidly induce osteosarcomas in mice, and a 55 K protein encoded by the transforming gene, v-fos, of the FBJ virus (75\textsuperscript{VRK}\textsuperscript{fos} from FBR-MSV) has been described (Curran & Teich, 1982; Curran & Verma, 1984). The cellular homologue, c-fos, also encodes a 55 K protein, which differs from the viral protein in the carboxy-terminal portion, but which, nevertheless, can transform normal fibroblasts (Miller, Curran & Verma, 1984). Both proteins are nuclear phosphoproteins and both are turned over rapidly in the cell, v-fos with a half life of 2 h and c-fos about 20 min. c-fos protein differs in the degree of post-translational modifications that can be detected soon after synthesis (Curran, Miller, Zokas & Verma, 1984). See reviews by Müller & Verma (1984) and Deschamps et al. (1985) for further details.

(8.1) c-fos expression in developing tissues and proliferating cells

A distinguishing feature of c-fos expression is that although transcripts are present at barely detectable levels in embryos and fetuses, the extrarenal tissues have very high levels (Müller et al. 1982). Day-7 murine conceptuses consisting predominantly of extrarenal tissues have very high levels of expression and, on further examination, a stage- and tissue-specific pattern is detectable (Müller, Verma & Adamson, 1983). In the mouse, amnion > visceral yolk sac > placenta, and a similar pattern is found in human tissues (Müller et al. 1983). In the mouse the level of c-fos mRNA rises to a plateau on the 16th to 17th day of gestation in extraembryonic tissues and also 14th day (haematopoietic) fetal liver, and later skin and bone/bone marrow contain high levels. Fetal liver and bone marrow probably express c-fos largely because of the population of macrophages and other haematopoietic lineages that express c-fos either constitutively or at some stage in their differentiation/maturaton processes (Müller, Müller & Guilbert, 1984; Müller, Curran, Müller & Guilbert, 1985). Macrophages cannot account for the high expression in very early extraembryonic tissues. In situ localization of c-fos protein (Adamson, Meek & Edwards, 1984) and mRNA (Deschamps et al. 1985) has clearly located fos expression in all the cells of the extraembryonic tissues. In addition, extraembryonic tissues have been shown to synthesize p55c-fos protein (Mason, Murphy & Hogan, 1985). Therefore, what is the role of c-fos gene expression in these tissues?

A brief survey follows of the three types of stimuli that induce c-fos expression, including growth, differentiation and stress stimuli. Quiescent, serum-starved mouse or rat fibroblasts in G0, stimulated to 'competence to divide' by PDGF, FGF (or serum), activate the fos gene within a few minutes; mRNA levels peak at 30 min and then fall to low levels in 60 min. This is caused by an increased rate of transcription and is accompanied by increased protein synthesis. The increased levels of mRNA and protein synthesis are both transient (Greenberg & Ziff, 1984; Müller, Bravo, Burckhardt & Curran, 1984; Krujier, Cooper, Hunter & Verma, 1984; Treisman, 1985; Zullo, Cochran, Huang & Stiles, 1985; Renz et al. 1985; Greenberg, Hermanowski & Ziff, 1986). In all cases c-fos mRNA accumulations are followed by an increase in the level of c-myc mRNA, although it is not known if these are linked responses. Epithelial and other cells respond to their specific mitogens with a similar transient increase in fos mRNA: EGF-stimulated A431 cells (Bravo, Burckhardt, Curran & Müller, 1985); PC12 cells (Greenberg, Greene & Ziff, 1985); and primary hepatocytes (Krujier et al. 1986); thymocytes stimulated with concanavalin A (Moore, Todd, Hesketh & Metcalfe, 1986); thyroid cells with thryrotropin (Colletta, Cipriani & Vecchio, 1986; Tramontano, Chin, Moses & Ingb, 1986); peripheral lymphocytes with phytohaemagglutinin and calcium ionophore (Reed, Alpers, Nowell & Hoover, 1986). Primary amnion cell cultures continue to express c-fos protein for 2 h after culture in vitro but this level declines to zero in 15 h. Addition of undefined factors in medium conditioned by placental or embryo
c-fos expression is not required since it is possible that a stable form of fos protein is present.

(8.3) c-fos activation by other stimuli such as heat shock

From the above examples, it can be seen that many different external stimuli seem to activate the transient expression of c-fos mRNA. When quiescent HeLa cells are changed from culture at 37°C to 40°C, a temperature-dependent, 5- to 20-fold increase in c-fos mRNA and protein levels follows in about 1 h (Andrews, Harding, Calbet & Adamson, 1987). Ionic signals such as calcium ionophore, benzodiazepines and elevated K+ (Curran & Morgan, 1985; Morgan & Curran, 1986), the cell division inhibitor mitomycin C and ultraviolet light (unpublished observations of S. Edwards and E. Adamson) all induce c-fos mRNA expression. Partial hepatectomy and even a sham operation elevate c-fos mRNA levels in rat liver (Krujier et al., 1986). Wounding a fibroblast monolayer results in rapid induction of c-fos (Verrier, Müller, Bravo & Müller, 1986). β-adrenergic stimulation of mice in vivo produces hyperplasia in parotid and submandibular salivary glands and stimulates transient c-fos expression (Barka, Gubits & Vander Noen, 1986). In summary, many kinds of external stimuli achieve a rapid cellular response in the form of c-fos gene induction and strongly suggests that fos is important in relaying extracellular signals to the nucleus, but the mechanisms remain unknown.

(8.4) c-fos expression in teratocarcinoma model systems of development

c-fos mRNA is expressed at very low levels in proliferating EC cells (Müller, 1983; Vilette, Emanoil-Ravier, Tobaly & Peries, 1985; Edwards & Adamson, 1986) and increases modestly during F9 aggregate differentiation to embryoid bodies, peaking on day 3 after retinoic acid addition (S. Edwards and E. Adamson, unpublished data; Müller, 1983). P19 EC aggregates stimulated with DMSO to differentiate into a mixture of cell types, including cardiac muscle express c-fos mRNA in increasing amounts peaking on the 12th day (Edwards & Adamson, 1986). These results indicate that c-fos expression can accompany teratocarcinoma cell differentiation and support the findings of Müller & Wagner (1984) and Rüther, Wagner & Müller (1985) who showed that the integration and expression of normal exogenous c-fos genes in F9 cells stimulates differentiation. P19 EC cells are less affected, however, and PC13 cells are not stimulated to differentiate by c-fos expression. Thus c-fos expression alone is not sufficient to trigger differentiation. Apparently contrary to the hypothesis that c-fos expression may be necessary (but not sufficient) for differentiation to occur, Mason et al. (1985) did not find elevated c-fos mRNA levels during F9 differentiation in monolayer cultures stimulated...
with retinoic acid and cAMP, but did detect a transient slight induction 60 min after RA addition.

An effective way to determine the function of a gene is to introduce complementary RNA into cells and determine the effect of the prevention of the expression of the protein product of that gene. The introduction of 'anti-sense' fos DNA into 3T3 cells has already shown to be effective in reducing growth rates and in reducing the frequency of such clones (Holt, Gopal, Moulton & Nienhuis, 1986). Our unpublished results indicate that anti-sense fos DNA expressed in F9 EC cells inhibits their ability to differentiate in response to RA. This experimental approach together with antibody injections, will likely become prominent in the near future. So far, the data support a hypothesis that fos may be important to development in two ways: one, to act as the 'second messenger' for a variety of external stimuli and second, to act in some part of the differentiation process, perhaps a step that is connected with growth restriction or with initiating a new programme of gene regulation associated with differentiated function.

(9) c-myb

c-myb is the homologue of the viral transforming gene in E26 and other avian viruses that produce myeloblastosis in birds. c-myb expression is restricted to, and is developmentally regulated, in haematopoietic tissue and is therefore thought to play a specific role in haematopoiesis (Duprey & Boettiger, 1985). c-myb is expressed in a differentiation stage-specific manner in pre-B cells lines (DeCino, Herbst, Lernhardt & Raschke, 1987). Five percent of the haematopoietic cells of the chick yolk sac contain all the detectable c-myb mRNA of that tissue. These cells were identified as M-CFC or the committed progenitors to the macrophage lineage. As the cells differentiate, the level of c-myb falls more than 100-fold. A similar fall accompanies WEHI-3B cell differentiation to macrophages (Gonda & Metcalf, 1984). Proto-oncogene c-myb expression is not restricted to macrophages since both T cells in the murine thymus and cells of the erythroid lineage also express c-myb, and this falls tenfold with increasing age (Sheiness & Gardinier, 1984). In situ hybridization confirms these lineages and demonstrates the presence of c-myb mRNA in rapidly proliferating precursors of the myeloid and erythroid lines in human bone marrow cells (Emilia et al. 1986).

(10) The myc gene family

c-myc is the genomic counterpart of the transforming gene of the avian myelocytomatosis virus (MC29) and, like c-myb and c-fos, its protein product moves rapidly to the nucleus after synthesis and is short-lived. Other homologous genes have been termed L-myc and N-myc corresponding to their activity detected in lung and neural tumours, respectively. The examples of c-myc expression in response to mitogens of many kinds have been listed in the section on c-fos and include thrombin, with insulin acting on Chinese hamster fibroblasts (Blanchard et al. 1985). In contrast, there are some exceptions where growth-stimulating factors do not induce c-myc mRNA levels: insulin (Kelly, Cochran, Stiles & Leder, 1983); B cell growth factor (Smeland et al. 1985), and adenovirus infection (Liu, Baserga & Mercer, 1985). In spite of the large variations seen in c-myc mRNA levels, its rate of transcription increases only modestly when cells move from G0 to G1 and then remains at the same lower level in all phases of the cell cycle (Thompson, Challoner, Neiman & Groudine, 1985; Rabbits et al. 1985; Kaczmarek, 1986) and differentiation (Dean, Levine & Campisi, 1986). Even more than for c-fos, post-transcriptional regulation is the major means by which the level of expression of c-myc is modulated. However, for growth stimulation of fibroblast cells by growth factors, an elevated transcription rate can occur depending on the cell type and the growth factor.

(10.1) c-myc expression and cell proliferation

c-myc, until recently, has been well correlated to various aspects of cell proliferative states (Birnie, Burns, Clark & Warnock, 1984). In transgenic mice, where levels of c-myc are elevated by introduction of the c-myc proto-oncogene coupled to the immunoglobulin μ or κ enhancer, frequent occurrence of lymphomas occurs within a few months of birth (Adams et al. 1985). Translocation of the c-myc gene to a chromosomal location that endows altered expression has been found in many leukemias in mouse and human. Although induction of the c-myc gene occurs when erythroleukemic cells are stimulated to differentiate, expression is transient and, in general, low levels of c-myc are present in differentiating haematopoietic cells (Gonda & Metcalf, 1984; Lachman & Skoultchi, 1984). In view of the association of c-myc with proliferation, it is not easy to understand why both mitotic and meiotic phases of germ cells have very low levels of c-myc transcripts (Stewart, Bellve & Leder, 1984). For somatic cells like quiescent Swiss 3T3 fibroblasts, DNA synthesis is stimulated by c-myc protein injected into the nuclei (Kaczmarek et al. 1985). Here c-myc acts like a competence growth factor to initiate the cell cycle so that cells progress into S phase. It now appears that at least one way that c-myc may act is by participating in the process of DNA synthesis, since the addition of
levels are sustained throughout the first trimester in cell-specific expression of with highest levels in c-myc during development. Stage-specific expression of c-myc mRNA was found by Pfeifer-Ohlsson et al. (1984) in human placenta with 30-fold variation in level. Four- to five-week placenta was the highest, and this declines to much lower levels by the end of the first trimester. The mRNA is located predominantly in the cytotrophoblast cells where [\textsuperscript{3}H]thymidine labelling also shows these cells to be rapidly dividing. The nondividing syncytiotrophoblast of the term placenta has 40-fold lower levels. Interestingly, not every cytotrophoblast cell contains c-myc transcripts, and it was suggested that a wave of mitotic activity moving down the placental villi may correlate with accumulated levels of c-myc mRNA. A most relevant observation is that coexpression of c-sis and c-myc occurs in the early placenta (Goustin et al. 1985). Since c-sis codes for a PDGF-like mitogen, secretion of this activity into the medium of cultured trophoblasts of first trimester placenta may not be coincidental (see section 11).

The human fetus proper also displays stage- and cell-specific expression of c-myc with highest levels in the rapidly proliferating epithelial tissues. These high levels are sustained throughout the first trimester in contrast to declining levels in the placenta during the second month of gestation. The distribution of c-myc levels implies more than mere association with proliferation; in 3- to 4-week embryos, c-myc expression is low, while in extraembryonic tissue c-myc mRNA levels are high (Pfeifer-Ohlsson et al. 1985). Furthermore, predominantly normal development occurs in transgenic mice that express varying levels of c-myc (Leder et al. 1986).

During murine development, c-myc mRNA has been observed at high levels throughout, but a related gene, N-myc, is highly expressed at very early stages (7.5 days of gestation) and continues at this level until 11.5 days when it declines thereafter (Jakobovits, Schwab, Bishop & Martin, 1985). Zimmerman et al. (1986) examined the expression of all three myc-related genes in murine postnatal development and observed that c-myc expression is present at all stages and in all tissues. c-myc levels decline to low levels in older adults in most tissues except adrenals, thymus, spleen, intestine and heart. Tissue- and stage-specific expression was demonstrated for L-myc and N-myc. L-myc expression is highest in newborn forebrain, hindbrain and kidney, at lower levels in lung and intestine, and absent in other tissues. L-myc expression is still present in adult lung but decreases in all other tissue so that a 17-day-old brain no longer expresses L-myc. N-myc was thought to be neuroectoderm-specific but in fact is found in a variety of newborn tissues. It is highest in newborn brain, kidney and intestine, and declines rapidly with increasing age. In addition, a striking differential distribution is observed in various B cell lines. N-myc is present in pre-B cells but not B cells or plasma cells, while c-myc is expressed in all of these lines, and L-myc is never expressed. Very high c-myc expression occurs in late fetal mouse cerebellum that declines and is succeeded by a second peak in postnatal days 3 to 10 (Ruppert, Goldwitz & Wille, 1986). The latter is localized in the mitotically active external granular layer and accompanies a change in the ratio of the two myc mRNAs to adult ratios. In general, the expression of myc family genes correlates with committed but still proliferating cells as well as with cells that are rapidly differentiating along the neural pathway. The only non-neural tissue in which a relatively high level of expression of N-myc and c-myc is observed is fetal and newborn kidney. If the myc family genes can all be stimulated by growth factors, possibly either the production and activity of prepro-EGF accounts for myc activation, or more likely, the constant exposure of the kidney to blood-borne growth factors stimulates high levels of expression of these oncogenes. N-myc is also expressed in mouse and human teratocarcinoma stem cells and in adult mouse testis (Tainsky, Cooper, Giovanella & Vande Woude, 1985; Jakobovits et al. 1985).

The myc gene is highly conserved through evolution and even Drosophila melanogaster genome contains sequences that hybridize with the v-myc probe, although there is little amino acid sequence homology in the products. Nevertheless, hybridizing transcripts are found in embryos, pupae, adults and in a Drosophila cell line (Kc), and stage-specific expression of several different-sized transcripts were recorded. The results also suggest that the transcripts found in early embryos are of maternal origin since they are found only in the ovaries (Madhavan, Bilodeau-Wentworth & Wadsworth, 1985).

(10.3) c-myc expression in teratocarcinoma cells
The steady-state levels of c-myc found in proliferating cells have been shown to decrease drastically (Dony, Kessel & Gruss, 1985) in F9 EC cells at a stage even preceding overt differentiation induced by retinoic acid 72 h later (Griep & DeLuca, 1986). Post-transcriptional mechanisms are apparently wholly responsible for reduced c-myc mRNA levels in F9 cells. In F9 cells stimulated to differentiate, the steady state of c-myc is reduced by as much as 50% within 3 h (Griep
& DeLuca, 1986). However, if F9 cells are blocked from differentiating in response to RA by the addition of 5 mM-sodium butyrate to the medium (Levine, Campisi, Wang & Gudas, 1984) the cells still respond to RA by decreased levels of c-myc. It appears that reduced levels of c-myc are not sufficient for differentiation but may precede differentiation or reduced growth rates. The undermethylation of the c-myc gene in F9 EC cells relative to differentiated teratocarcinoma cells and mouse liver DNA may account for its high level of expression and for its regulation (Griep & DeLuca, 1986). The second exon of the c-myc gene becomes methylated during F9 differentiation and this is remarkable in the face of global demethylation of every other gene tested (Young & Tilghman, 1984; Razin et al. 1984).

(11) c-sis

The predicted sequence of p28\textsuperscript{v-sis}, the transforming protein of the simian sarcoma virus (SSV), is strikingly homologous to the B chain of PDGF (Waterfield et al., 1983; Robbins et al., 1983; Chiu et al., 1984) or PDGF-2 (Rao et al., 1986). In SSV-transformed cells, p28\textsuperscript{v-sis} is proteolytically processed to generate a disulphide-linked dimer (Johnson, Betsholtz, Heldin & Westermark, 1986), and its presence and activity in the culture medium can be detected and neutralized by antibodies to PDGF. Using v-sis probes, transcripts of c-sis have been detected in many cell lines, tumours and tissues (Eva et al., 1982). Transformation of cells by a wide range of oncogenic agents appears to activate a cellular gene encoding a PDGF-like molecule and the induced expression of the c-sis gene by TGF\(\beta\) has been suggested as the intermediary step in the transformed phenotype induced by TGF\(\beta\) (Leof, Proper, Getz & Moses, 1986). Mouse embryo fibroblast cells stimulated with TGF\(\beta\) express activated the c-sis gene after 4 h with a peak at 12–16 h and release a PDGF-like activity into the culture medium. c-fos is also induced maximally at 4 h with the appearance of PDGF activity, while c-myc mRNA levels peak at 8–12 h suggesting that PDGF may have activated both genes. Since TGF\(\beta\) is present in most normal tissues (Roberts et al., 1981) and also in the placenta, serum (Stromberg & Twardzik, 1985) and blood platelets (Assoian et al., 1983), it is possible that fetal growth may be modulated, at least locally, by the release of TGF\(\beta\) and PDGF.

(11.1) c-sis in development

The developmentally regulated expression of the c-sis gene has been mentioned in section 10 since c-myc and c-sis expression occur together in the highly proliferative cytotrophoblast cells of the first trimester cytotrophoblast shell. What is interesting about this study (Goustin et al. 1985) is that placental explants secrete a PDGF-like activity into the medium in a similar developmental time course. In addition, cytotrophoblast-like cell lines established from early placentae display PDGF receptors with similar affinity for PDGF as fibroblast cells. These cells also respond to PDGF by tenfold elevation of c-myc mRNA levels in 2 h and by increased synthesis of c-myc protein (several-fold stimulation within 7 h). Autocrine stimulation of cytotrophoblast cells by the c-sis product that then results in c-myc and c-fos expression is an attractive hypothesis. However, the placenta produces a number of other growth factors such as IGFs, FGF and TGF\(\beta\), and these could also contribute.

PDGF is thought to mediate the proliferation of smooth muscle cells in injured arteries and may be involved in the pathogenesis of atherosclerosis. Although in injury, the main source of PDGF is the platelet, other cell types produce PDGF-like activity. For instance, rat aortic smooth muscle cells isolated from 13- to 18-day-old rat pups secrete a PDGF activity into the medium, and could account for autocrine stimulation of growth of these cells in culture. This activity is developmentally controlled since the same cell type isolated from adult rats does not secrete PDGF (Seifert, Schwartz & Bowen-Pope, 1984). c-sis transcripts have been detected at moderate levels in cultured human and bovine endothelial cells, at low levels in in vivo endothelium from human umbilical vein and at very low levels in bovine aortic endothelium in vivo (Barrett et al., 1984). These results also suggest that the sis gene is activated in certain cell types when removed from in vivo to in vitro conditions.

Model systems using murine EC cells have suggested that early embryonic stem cells may produce a PDGF-like activity (Gudas, Singh & Stiles, 1983; Rizzino & Bowen-Pope, 1985) but fail to bind this growth factor. It has not been definitively determined if these cells lack PDGF receptors, or if they are wholly down-regulated by the secreted PDGF. However, when F9, PC13 and PSA1 cells differentiate, they form cell types that are able to bind and respond to PDGF. If the corresponding embryonic cells, namely early embryonic ectoderm cells, produce PDGF-like factors, these could then stimulate the growth of the adjacent, more differentiated cell types, such as mesoderm cells which arise on the 7th day of gestation in mouse. PDGF also has a potent chemoattractant activity that stimulates the motility of certain types of cells and this could play a role in the migration of embryonic cells. Embryonic ectoderm cells could also produce other growth factors shown to be secreted by EC cells (Rizzino, 1982; Heath, Mahadevan & Foulkes, 1986).
(12) Other proto-oncogenes

The latest of the viral oncogenes to be identified with a cellular protein is v-erb-A, which has 89% homology to the thyroid hormone (T3) receptor (Weinberger et al. 1986). Thyroid hormone is known to be essential for tadpole metamorphosis and the switch to adult-type gene activities (Knowland, 1984). This receptor occurs widely in mammalian tissues but plays important roles in the liver and brain (reviewed by Oppenheimer, 1979). The receptors have higher affinity for T3 than T4, exist in two molecular forms (Casanova et al. 1984) and appear to be largely nuclear (Erkenbrack & Rosenberg, 1986). Since thyroid hormone is synthesized throughout gestation, presumably the receptor is also an early product in development.

The c-erb-A gene is related (22% at the amino acid level) to the glucocorticoid receptor (Weinberger et al. 1985; Hollenberg et al. 1985) which is also partly nuclear and partly cytoplasmic. The glucocorticoid receptor is expressed in many cell types and is active during embryogenesis in fetal liver, visceral yolk sac (G. Andrews, personal communication) and the secondary palate (Diewart & Pratt, 1981; Kim, Lauder, Joh & Pratt, 1984).

Two proto-oncogenes originally identified as mouse genomic sequences adjacent to integrated proviruses of the mouse mammary tumour virus (MMTV) in a large proportion of tumours in mice, int-1 and int-2 are unrelated genes. int-2 is transcribed in embryos from day 8.5 to 12.5 and also is seen in adult testis but not other adult tissues (Jakobovits, Shackleford, Varmus & Martin, 1986). This limited distribution should be helpful in determining the function of the int gene.

(13) Proto-oncogene expression in teratocarcinoma cells

Insufficient data have been accumulated to evaluate teratocarcinoma cells as model systems for studying proto-oncogene mechanisms in growth and differentiation, but the potential is great since the stem cells may be engineered to express experimentally introduced genes. The effect on the stem cell and on the pattern of differentiation produced should be highly instructive. The studies of Müller & Wagner (1984) and Rüther et al. (1985) have clearly suggested a role for c-fos in differentiation, for example. Once an EC cell line has been established that can express individual oncogenes, the cells may be aggregated with normal embryonic cells at the morula stage, or microinjected at the blastocyst stage, to follow its effect on the development of the resulting chimaeric animals. The proportion of engineered cells in each tissue of the chimaera will vary in individual animals and may allow a titration of dose versus effect.

To date, Table 2 summarizes what we know of the levels of oncogene expression in teratocarcinoma cells as transcripts that hybridize with DNA probes. As expected, the oncogenes most closely associated with proliferation are strongly expressed: these include c-src, c-abl, c-rasHa, c-rasK, c-myc, and N-myc. When rates of proliferation decline as differentiation occurs, several oncogenes decline in mRNA level: c-abl and c-ras decline modestly; c-myc and N-myc decline drastically. Neuronal differentiation is accompanied by significant increases in c-src (Sejersen et al. 1985; Lynch et al. 1986), but N-myc activity decreases to low levels (Sejersen et al. 1986). The nuclear oncogene, c-fos, is expressed transiently at some point during differentiation that may indicate a role in some general step of that process (Edwards & Adamson, 1986).

Some studies have centred on introducing viral or activated oncogenes into cell lines to observe effects on cell phenotype and differentiation. For instance, an activated ras gene, c-rasEU, introduced into P19 EC cells appears to have little or no effect and does not prevent the induction of differentiation in response to retinoic acid (Bell, Jardine & McBurney, 1986). However, viruses expressing v-fos (especially FBJ-MSV) are able to immortalize or transform mouse embryo-derived primary cell cultures of fibroblasts, myoblasts, and other cell types (Jenuwein, Müller, Curran & Müller, 1985), thus linking v-fos expression with increased growth potential in embryonic cells. Since c-fos can also transform established cell lines under the right conditions (Miller et al. 1984), the distinction, if any, between the properties of v-fos and c-fos proteins is not clear. The specific cell type expressing the c-fos protein may modulate the outcome. The specific processed forms of oncogene products such as c-fos, c-src and c-erb-A may differ among tissues and may give rise to differences in function.

(14) Conclusions

For most oncogenes, a specific function or activity has not been assigned, and data are still too sparse to hypothesize a mechanism in oncogenesis or to predict a role in development. In the cases of c-erb-B/EGF receptor, c-fms/CSF-1 receptor, c-sis/PDGF, an obvious niche in growth regulation can be deduced. Although these components are not direct gene regulatory molecules, they do have wide-ranging effects through their interconnections with a family of protein kinases and through their effects on phosphoinositide metabolism, ion fluxes and nutrient
## Table 2. Expression of c-oncs in teratocarcinoma cells

<table>
<thead>
<tr>
<th>c-onc</th>
<th>Cell line</th>
<th>Exp in ECC</th>
<th>Exp in differentiated cells</th>
<th>Reference</th>
</tr>
</thead>
</table>
| c-src             | PCC7              | +          | 3–5× inc. during neuronal diff
transport. Nevertheless, the role of these growth-related proteins is poorly understood. Largely this is due to their complicated interconnections with many cellular metabolic processes. Indeed, with a few exceptions such as PDGF, the oncogenes seem to be largely concerned with general metabolic processes as much as (if not more than) growth regulation per se. The proto-oncogenes that are known to have enzymatic activity, such as c-src, c-abl, the ras family, also fit into this metabolic framework. c-erb-A/thyroid hormone receptor is even more directly concerned with overall metabolic rates.

Table 2. Other Footnotes: RA = retinoic acid; cAMP = dibutyryl cyclic AMP; DMSO = dimethyl sulphoxide.
The nuclear-located proto-oncogenes are better placed for roles in growth/differentiation/gene regulation. They bind to DNA to various degrees, although specific binding sites have not been identified. The c-fos protein appears to bind to chromatin, particularly at deoxyribonuclease I-sensitive sites, thus indicating a role in the regulation of gene expression (Sambucetti & Curran, 1986; Renz, Verrier, Kurz & Müller, 1987). c-myc production is similarly activated but usually at a slightly later time. This has given rise to the possibility of chains of command leading to gene-stimulating events generated by signals from the environment. c-fos and c-myc may then activate sets of genes that lead to DNA synthesis and progression through the cell cycle, or to sets of genes that regulate differential cell-lineage pathways.

In reviewing the data here it becomes clear that almost without exception, the proto-oncogenes have been shown to play roles in differentiation as well as proliferation. Is this because directing the cell towards proliferation limits the cell’s resources to continue the previous commitment or direction towards a differentiated phenotype (e.g. c-myc?). Or is it a more direct process that facilitates differentiation (e.g. c-fos?). Only in one case is the function clear, that is, c-fms and this product appears late and may only be a marker and not a regulator of differentiation.

For considerations of the roles of proto-oncogenes in development, some specificity of action may reside in c-src since a neurone-specific isotype has been identified in brain during proliferation, commitment and expression of the neuronal phenotype. Other proto-oncogenes are less well defined to specific tissues. The placenta and other extraembryonic tissues (VYS, amnion, and chorion), however, do hold a special place in the high levels of expression of several c-onscs. In addition to c-myc, c-abl, c-ras, and c-src, very high levels of c-sis, c-fos, and c-fms occur, and these levels vary between the tissues mentioned and between the temporal stages of the gestational period. This makes it less likely that the undermethylated state of the DNA in these tissues (Rossant, Sanford, Chapman & Andrews, 1986) broadly dictates the levels of proto-oncogene expression. It is still possible that specific sites of methylated bases control the expression of these or other genes in a temporal and tissue-specific manner. The undermethylated state of the placenta could be necessary for the derepression of a wide range of growth-related genes that are known to be active in the placenta. In the presence of secreted ‘autocrine’ growth factors such as TGFα, TGFβ, PDGF, IGF-I, and IGF-II, receptors are continually down-regulated, synthesized and activating the chain of command. Although these tissues have been recognized as ‘pseudomalignant,’ they are not tumorigenic in nude mice and have somehow controlled the signal to proliferate in parallel to the needs of the growing fetus. Studies on modes of oncogene activation in carcinogenesis show that a quantitative change of expression is one mechanism of such activation (reviewed by Klein & Klein, 1984). Understanding the mechanisms of control in the placenta may therefore be instructive for future clinical applications (Conway, 1983).

Limitations of space did not allow recognition of all relevant references; for these omissions my apologies are offered. I am grateful to Drs C. Van Beveren, C. Der, and S. Edwards for comments on the manuscript. This work was supported by grants CA 28424, P30 CA 30199 and HD 21957 from the National Institutes of Health.

References


COHEN, S. (1962). Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid...


Oncogenes in development


retroviral oncoproteins (v-fes) provide evidence for a family of tyrosine-specific protein kinase genes. Cell 30, 775–785.


Krüger, W., Skelly, H., Batteri, F., van der Putten, H., Barber, J. R., Verma, I. M. & Leffert, H. L.


