Two proto-oncogenes that play dual roles in embryonal cell growth and differentiation

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ABSTRACT The function of growth factors in early development is reviewed. Special emphasis is on the epidermal growth factor and its receptor, and on the c-fos and its family of transcriptional factor proteins, which play an important role in modulating the growth and differentiation of early embryos and embryonal carcinoma cells.

KEY WORDS: *proto-oncogenes, embryonal carcinoma, EGF-R, c-fos*

Introduction

The dual nature of embryonal carcinoma (EC) cells has long been a fascinating feature for G. Barry Pierce. He came to the conclusion that «the teratocarcinoma is a caricature of early embryogenesis of the mouse» (Pierce *et al.*, 1978, 1983; Stevens, 1983). He is referring to the observation of many differentiated but disarrayed tissues in teratocarcinoma of mice. These tissues can be observed in teratocarcinomas derived from EC cells transplanted into the adult host as well as those derived from germ cell tumors of the ovaries of certain strains of mice. The remarkable fact that, in teratocarcinomas, malignant cells can spontaneously beget benign if not normal cells and that this is also true of many other types of tumor, was first demonstrated by Pierce *et al.* (1960). The culmination of the potential of EC cells was shown by Brinster (1974), Papaioannou *et al.* (1975, 1979) and Illmensee (1978) when it was shown that an EC cell injected into a blastocyst could take part in normal development to give a normal but chimeric mouse.

These observations led Dr. Pierce to make a study of how embryonic environments can regulate the tumorigenicity, growth, and differentiation of malignant EC cells (Pierce *et al.*, 1979; Wells 1982). One of the signals that is important for growth regulation is cell-cell contact of EC with normal embryo cells. More specifically, trophectoderm cells appear to be the important cell type (Pierce *et al.*, 1984). In essence an «embryonic field» can regulate embryonal carcinoma (Podesta *et al.*, 1984; Gerschenson *et al.*, 1986; Pierce *et al.*, 1986), and Dr. Pierce was interested in how to adapt this mechanism for non-toxic control of carcinoma in general. My interpretation of what constitutes an embryonic field is a semi-organized architecture of components including soluble secreted products such as growth factors, insoluble secreted products such as the extracellular matrix and membrane-inserted macromolecules such as cell adhesive molecules, precursor growth factors, growth factor receptors and modifying enzymes like alkaline phosphatase and glutaminyl transferase. This is the field that can promote controlled growth and differentiation.

Understanding the components necessary for cellular differentiation during development, therefore, must be of paramount importance in working towards therapeutic programs to control or manipulate normal or abnormal growth. Embryonic fields that control early normal growth must be understood before aberrations that push the wrong switches can be discerned.

Viruses have known for thousands of years how to push the wrong switches. Sufficient is known about viral oncogenes to be able to turn the tables and use this knowledge to understand the differences between normal and aberrant growth. Thus, studies and comparisons of the cellular and viral oncogenes have been useful in describing the more extreme aberrancies of cellular behavior and have opened a Pandora's box of related studies revealing the fine subtleties of the regulatory processes and multiple pathways that underlie the cell cycle, growth and differentiation. Two proto-oncogenes that have received a great deal of attention are c-erbB1 (the Epidermal Growth Factor Receptor, EGF-R, which can bind and respond to the ligands EGF and Transforming Growth Factor alpha, TGFα) and c-*fos* (one of a family of transcription factors which are components in the activating protein complex 1 [AP-1]). The EGF-R is a membrane-bound glycoprotein that can respond to extracellular ligands and thus is responsible for perceiving signals in the environment of a cell that will change the behavior of the cell. The c-Fos protein acts «downstream» of the signal and greatly increases as an early response to extracellular signals of many kinds. Within 5 min of a growth factor such as EGF binding to the EGF-R, increased

Abbreviations used in this paper: AFP, alphafetoprotein; DMSO, dimethyl sulfoxide; FGF, fibroblast growth factor; EC, embryonal carcinoma; EGF, epidermal growth factor; ICM, inner cell mass, PDGF, platelet-derived growth factor; RA, retinoic acid; TGFα, transforming growth factor-α.

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c-*fos* transcripts can be detected in responsive cells. Within 15 min, there can be observed in the nuclei of responding cells increased levels of newly-synthesized c-Fos protein, which, together with Jun proteins, elicits the response of a host of genes with AP-1 binding sites. Many of the complex molecular interactions involved in these first 15 min of activity are starting to be understood (reviewed by Gutman and Wasylyk, 1991) but multiple components and pathways are involved that cause multiple changes in the cell that can result in mitosis, cell differentiation or increased differentiated phenotypic behavior (Ben-Ari, 1991).

The very complexity of signal transduction mechanisms activated by an extracellular signal, such as EGF binding, underlies the duality (and even multiplicity) of the resulting cellular behaviors. Somewhere along the transduction pathway, choices are made and these are specific to the cell type being stimulated. The specificity of the response lies in the presence or absence, level of expression and degree of activity of all the components involved in the signal transduction. The degree of activity may be modulated by post-transcriptional modifications such as phosphorylation. Few of these complexities have been elucidated but the general outlines are being revealed.

It is not surprising, then, that the same signal applied to the outside of a cell can give rise to two opposite responses, cell division or cell differentiation, since responses depend on the composition of the intracellular environment. Each time a signal is transmitted, the primary responsive genes change the levels of the transcription factors in the cell and affect the secondary responsive genes which make further biochemical modifications. This alters the phenotype of the cell and the signal may gradually produce a change in responses. To illustrate this idea, I suggest that ubiquitously-active EGF-R and c-*fos* genes are important components in setting up the embryonic field and the perception and transduction of its signals. This article serves to highlight that these genes regulate the proliferation of cells as well as their specialization and thus have a profound influence on the developmental process.

How growth factor receptors may play key roles in early development

Embryonal carcinoma cells in general do not express cell surface EGF-Rs, do not bind EGF and do not respond to the addition of either EGF or TGFα to the medium. Since they need 10% serum to grow *in vitro*, and they do not become quiescent upon serum removal, they behave differently from most cultured cell types. Many EC cell lines have also been found to lack other growth factor receptors such as PDGF-R and FGF-R (Mercola and Stiles, 1988; Mummery, *et al.*, 1990) while they do express a range of growth factors including TGFα, PDGF-A and FGF (van Zoelen *et al.*, 1989). Since they do not express the corresponding receptors, EC cells cannot respond as an autocrine system. However, in the case of OC15, F9 and P19 cell lines, we have found that a low level of EGF-R protein is synthesized and it is degraded at very slow rates also (Weller *et al.*, 1987; Joh *et al.*, 1992). It is possible that there is some intracellular autocrine processing, or that the expression of the receptor at the cell surface is too low (except for F9 cells) to detect by binding assays and is rapidly internalized after binding to ligand (secreted either by the same cell or derived from serum in the medium). Based on these observations, our expectations were that the embryonic equivalent of EC cells, early inner mass cells or primitive ectoderm, would not express cell surface EGF-Rs.

Our results were not as predicted by the model system but were more complex. We assayed the EGF-R protein present from 1-cell to blastocyst stages by immunofluorescence and EGF-R mRNA by polymerase chain reaction of reverse transcription products (RT-PCR) (Wiley *et al.*, 1992). The receptor can be detected on all stages but with differences in the intensity of staining. Unfertilized oocytes and fertilized eggs both express very low reactivity to an affinity-purified anti-mouse liver EGF-R antibody. This is somewhat variable and not all eggs are reactive. This level remains variable until the late 4-cell stage when staining is more reliably observed. Thereafter, increasing levels are evident with a large rise during the 4 to 8-cell stage and in the late stages of blastocyst formation. Surprisingly, low but detectable levels of EGF-R antibody reaction were detected on the ICM cells at the blastocyst stage, but a much more intense reaction is seen on trophoblast cells. The latter result is supported by our findings that trophoblast cells in outgrowths and in whole blastocyst mounts respond to EGF by expressing higher levels of nuclear staining for the c-Fos protein 1 h after the addition of the signal to the embryo in serum-free culture medium (Adamson 1990; Adamson and Mercola, 1990). Further, blastocysts analyzed by SDS-PAGE both before and 2 h after the addition of EGF and ^{35}S-methionine express a 170 kDa radioactive protein that is increased by EGF and that is immunoprecipitated by the antibody to EGF-R. We interpret this result as the EGF-stimulated synthesis of the receptor protein, thus demonstrating further that the receptor present at this stage is active. Work by others supports the hypothesis that the receptor is active at stages as early as the 8-cell embryo when an EGF-binding activity is first detected (Paria and Dey, 1990). It has also been shown that EGF improves the development of the embryo (Wood and Kaye, 1989; Paria and Dey, 1990) and that a polyclonal antibody stimulates the development of the 8-cell embryo, presumably by imitating the binding of the ligand (Wiley and Adamson, unpublished data). The stimulatory activity of EGF on blastocyst embryos can be abrogated by the addition of a Tyrphostin compound that predominantly inhibits the tyrosine kinase activity of EGF-Rs (Paria *et al.*, 1991).

The presence of the mRNA for the receptor further authenticates the possibility that the EGF-R gene is transcribed at least as early as the late 4-cell stage embryo with large increases after that stage (Wiley *et al.*, 1992). Although transcripts can also be detected in the 1- and 2-cell embryo at a higher level than in the early 4-cell stage, we do not know if this is translated or if the receptor protein that can be detected at that stage is derived from the remains of the foot-processes of the follicle cells that surrounded the oocyte in the ovary. It is safe to say that the EGF-R protein that is present after the 8-cell stage is encoded by the embryonic genome and that the extent of this expression increases during the 3rd to 5th-day of pre-implantation development.

We noticed that after compaction in the 8-cell embryo, the concentration of EGF-R on the outer plasma membranes is increased while the receptor protein on the inner membranes is less (Wiley *et al.*, 1992; Fig. 1). It is possible to hypothesize that since the expression on the first epithelial cell formed during development is not located at the usual adult site (the baso-lateral surfaces of the epithelium) that this indicates a specific directional need or activity in the embryo. Namely, that the signaling ligand is largely found on the outside of the embryo. The ligand could be maternal EGF present in the uterine cavity, or TGFα secreted by the embryo. A further conjecture is that the increased concentration of the receptor on the outer cell surface of compacted embryos serves to alter the pathway

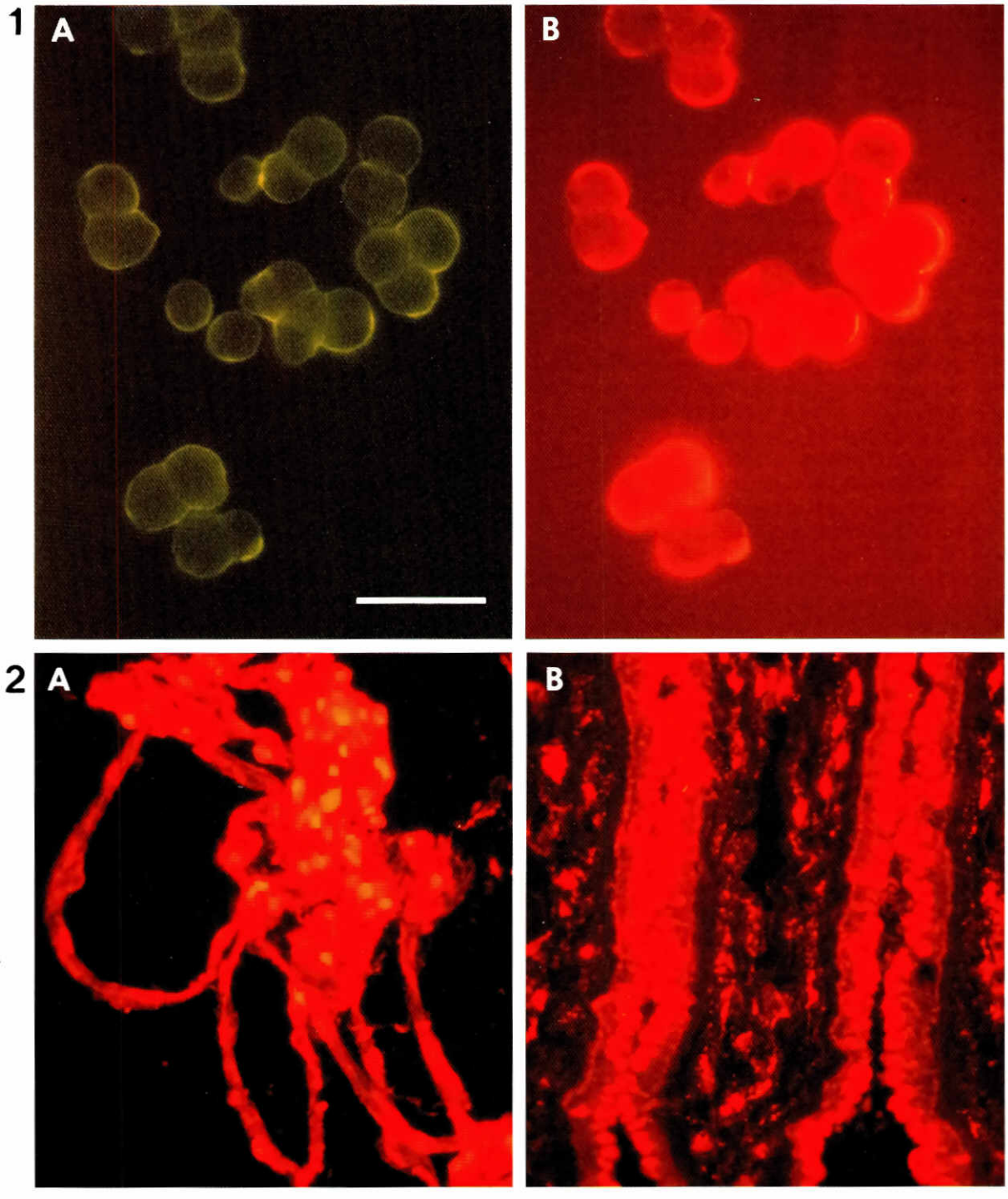

Fig. 1. Isolated blastomeres of morula embryos stained to show coincidence of apical domains (a) and EGF receptors (b). *Morula (16-cell) embryos were disaggregated in calcium-free medium for 20 min and were treated with fluorescein-labeled concanavalin A and rabbit antibodies to mouse EGF receptors and then with rhodamine-conjugated anti-rabbit goat IgG. The doubly-labeled blastomeres show a similar distribution of conA binding proteins in* **(a)** *with receptors in* **(b)** *. Since conA proteins are known to be concentrated on the apical surface of embryo cells, this demonstrates that receptors are unevenly distributed. This polarity is apparent in 8-cell embryos after compaction and the formation of apical tight junctions. The line indicates 40 μm (Wiley, et al., 1992, by permission).*

Fig. 2. Indirect immunofluorescent staining of c-Fos in amnion. *Cryostat sections of the extra-embryonic membranes, amnion, from* **(a)** *18 day mouse embryos,* **(b)** *human term conceptuses were fixed in methanol and immunohistochemically stained for c-Fos protein (Adamson, et al., 1985). Amniotic cells expressed the highest levels of c-Fos and EGF receptors compared with any other cell or tissue tested.*

of the signal such that the polarized cells now forming respond in a fashion that increases the expression of the polarity and makes the composition of the cell increasingly differentiated with respect to inside and outside. When the receptors bind ligand now, the result may be to increase the polarity of the blastomeres, perhaps by changing the levels of transcription factors that activate specific embryonic genes. As the outer layer of cells in the morula develops, the concentration of EGF-Rs increases rapidly and this contributes to the differentiation of the outer cell trophectoderm layer from the ICM. As mentioned above, the addition of EGF to embryos increases the synthesis of receptor protein and this is true also for many cell types in culture (Kudlow *et al.*, 1986; Earp *et al.*, 1988; Fernandez-Pol *et al.*, 1989; Thompson and Rosner, 1989). Therefore the presence of the ligand stimulates further receptor synthesis and perpetuates the process.

In order to have unlimited amounts of material to study the mechanism for the increased expression of the EGF-R gene as the embryonic cell differentiates, we turned to P19 murine EC cells that differentiate into three main pathways depending on the concentration of retinoic acid used. An additional necessary signal is the aggregation of the cells by culture in untreated bacteriological dishes so that the cells interact very closely. In the presence of >0.5 μM RA, P19 cells differentiate into neural and glial cells; at 10 nM RA (or 1% Dimethyl sulfoxide, DMSO) the products are mainly skeletal muscle; and with 1 μM RA (or 0.5% DMSO) the products include cardiac muscle, smooth muscle and visceral endoderm (Jones-Villeneuve *et al.*, 1983; McBurney *et al.*, 1982). We examined the level of expression of the EGF-R gene products during the ten-day process of differentiation.

As mentioned above, there is a very low level of transcription of the EGF-R gene in P19 cells. Similarly, the level of protein synthesized is low but detectable after metabolic labeling and immunoprecipitation. During the two days following RA induction, the level of mRNA actually falls. This is an important finding because it implies that the low activity of the gene in EC cells is unlikely to be due to the small number of spontaneously differentiating cells in the cultures. Further, it suggests that the direct effect of RA-activated RA receptors on the EGF-R gene in EC cells is that of inhibition of its activity (Hudson *et al.*, 1990). After further culture, especially after the cells are placed in anchorage-dependent growth in tissue culture dishes, the levels of transcripts rise markedly to reach levels 20 to 70 times higher at the end of the differentiation period. This occurs in all three pathways, but the rise is highest for the highest concentration of RA. The new transcripts are translatable, since the level of EGF-R protein also rises about 10-fold in each pathway, although this is somewhat variable depending on the culture conditions. Some of this remarkable rise in EGF-R expression may be attributed to increased transcription, but this is very difficult to ascertain because the rate of transcription is very low at all times. Both the mRNA and the EGF-R protein are long-lived and their stabilities also make a considerable contribution to the increased expression (Joh *et al.*, 1992). It is known that nearly all tissues express EGF-Rs and we hypothesize that the increase is achieved by increased transcription and by increased stability of the mRNA and protein. Presumably, there is also a mechanism for the removal of EGF-Rs during the formation of certain cell types such as myotubes.

The hypothesis that I would like to advance is that the progression or initiation of differentiation in EC cells could be by the polarization of cells, either in monolayers or aggregates, such that one surface of the cell gains an unequal number of EGF- and other growth factor receptors. In compacted aggregates or embryo blastomeres, the polarization may be initiated by the interaction of uvomorulin (E-cadherin) between apposing cell surfaces leading to the polarized distribution of cytoskeletal components. Cytoskeletal genes are among the earliest to be stimulated during embryogenesis and also in EC cells stimulated by RA induction (Oshima *et al.*, 1983) and these would rapidly change the architecture of the cells in a differential manner based upon the interaction of the neighboring cells. Mitosis of cells with an unequal distribution of cellular components would rapidly give rise to unequal daughter cells with different numbers and locations of EGF-Rs that would respond differently after ligand binding because they would activate different signal transduction pathways to different degrees.

Additional support to the idea that the EGF-R may be sufficient for the progression of differentiation is given by the work of den Hertog *et al.* (1991). When human EGF-R expression vector cDNA is transfected into P19 EC cells and stably-expressing cell lines are selected with a co-transfected neomycin-resistance gene, the resulting cells are now sensitive to the addition of EGF to the medium. The response of the cells is to differentiate to neural cells in the absence of retinoic acid, the agent that is normally required for the production of neural cell types in differentiating P19 cells. In this case, the over-expression of EGF-R protein is sufficient to generate all the multiple gene inductions known to occur during this process. The reciprocal experiment has not yet been performed wherein the EGF-R is prevented from expression to determine if the EGF-R is essential to the process of differentiation. This study is in progress in my laboratory.

In summary, the observations made on preimplantation embryos and EC cells suggest that the EGF-R could be an important component in both growth and differentiation of the early embryo.

The dual nature of the role of c-*fos* in embryonal cells

As discussed above, c-Fos and its family of transcription factor proteins is a well-tested early response product known to be a component in the generation of pleiotropic responses to mitogens. However, it is also a notable factor found in all the tissues of the extra-embryonic portions of the conceptus in vertebrate animals (Müller *et al.*, 1983a,b,c). Indeed, c-*fos* transcripts and protein are expressed at extremely low levels in the fetus compared to the levels in amnion (Fig. 2), parietal yolk sac, visceral yolk sac, trophectoderm, ectoplacental cone, chorion and placenta (Adamson *et al.*, 1983, 1985). Since these tissues are destined to be discarded and never form part of the embryo itself, and yet c-*fos* continues to be expressed highly in tissues that have ceased to grow, it is clear that the gene could have other roles than in the transduction of signals from mitogenic stimulation of cells.

The study of EC cells is again useful in determining the role of Fos in the formation of the extra-embryonic tissues. The EC cell line F9 is limited in its differentiation to two of the extraembryonic tissues, visceral and parietal endoderm. The pathway taken can be directed by the method of induction (Grover *et al.*, 1983a,b). When cultured as aggregates in the presence of 1 nM to 50 nM retinoic acid, F9 cells differentiate into embryoid bodies with an outer epithelial layer of visceral endoderm cells in 6 to 8 days. The protein that marks the differentiation of this cell type is alphafetoprotein (AFP), which is secreted into the medium as well as the increased production of extracellular matrix proteins such as laminin, type IV collagen and

chondroitin sulfate proteoglycans (Grover *et al.*, 1987). When the cells are cultured either as aggregates or monolayers in the presence of 0.1 to 1 μM retinoic acid and agents that increase the intracellular concentration of cyclic-AMP, the differentiated product 4-6 days later is parietal endoderm, characterized by a different set of markers (Grover and Adamson, 1985, 1986). The differentiation pathway leading to parietal endoderm is inhibited in F9 cells in which the level of expression of c-*fos* is reduced by the introduction of a cDNA expression vector for antisense RNA (Edwards *et al.*, 1988). This result indicates that the c-Fos expression is necessary for the differentiation of parietal endoderm. The formation of visceral endoderm is normal since the induction of AFP is unchanged in the cells over-expressing c-*fos* antisense RNA. The implication is that a certain level of c-Fos expression is needed for each of the two cell types and is higher for parietal differentiation than for visceral endoderm. Now that it is known that all members of the family of Fos proteins can be present in AP-1 and that members of the Jun family of factors must also be present, the consequences of altering the levels of c-*fos* in any cell might need a much more sophisticated explanation. However, the above experiment also illustrates the role of *fos* in the growth process, since the presence of antisense c-*fos* RNA in transfected cells markedly inhibits the ability of the cells to initiate growth into a colony. Very few colonies were produced in the presence of antisense *fos* and those that were able to thrive were able to produce some c-*fos* transcripts and protein.

c-Fos together with another ubiquitously-expressed transcription factor with close similarities, Egr-1 (early growth response gene-1, Sukhatme *et al.*, 1988) are expressed at increasing levels during the differentiation of P19 EC cells. In contrast to their ability to make rapid responses to mitogens and other stimuli, they clearly have other functions in differentiated cells and this may be concerned with the maintenance of the differentiated state. We have shown that c-*fos* and Egr-1 transcripts are present at different levels in RA-differentiated (neural) compared to DMSO-differentiated (cardiac muscle and visceral endoderm) tissues with higher levels in RA-differentiated tissues. In all differentiated tissues, both transcriptional and post-transcriptional mechanisms account for the increased expression. In addition, c-Fos and Egr-1 proteins are also more stable in differentiated tissues. These changes are highly suggestive that their role is not one that pertains in transiently-activated cells where the emphasis is on their rapid removal after an early peak in expression. As yet, the nature of the modifications needed to bring about the change in properties is obscure and the identification of their target genes is also unknown.

In summary, the total number of *fos*, *jun* and Egr-related genes that play roles in the regulation of differentiated genes during the complexly orchestrated process of spontaneous or induced differentiation of embryonic cells may not yet be known. They are likely to have some degree of overlap in function but also retain a degree of specificity based on their ability to interact with each other and with DNA. The ability of c-Fos to have multiple roles is not difficult to envision in the light of the existence of related family members, the complexity of their modulations and the resulting subtleties of their interactive properties. It appears that all of the proto-oncogenes that are transcription factors will likely have multiple roles in their regulation of the expression of target genes.

The simple idea of one gene for one function does not hold for the two genes discussed here. Studies of proto-oncogenes have focused on the genes that have the greatest impact on the cell, but in addition, this almost guarantees that the checks and regulations that govern important genes will be the most complex. It is not too much to expect that understanding these interactions among proto-oncogene products in detail will allow us to understand the course of normal development and how to harness these principles to reliably do as Dr. Pierce's fields do with facility — beget the benign from the malignant.

References

ADAMSON, E.D. (1990). EGF receptor activities in mammalian development. *Mol. Reprod. Dev. 27:* 16-22.

ADAMSON, E.D. and MERCOLA, M. (1990). Detection of EGF receptor protein and activities in embryo cells. *UCLA Symposium on Molecular and Cellular Biology 117*, pp. 47-52.

ADAMSON, E.D., MEEK, J. and EDWARDS, S.A. (1985). Product of the cellular oncogene, *c-fos*, observed in mouse and human tissues using an antibody to a synthetic peptide. *EMBO J. 4:* 941-947.

ADAMSON, E.D., MÜLLER, R. and VERMA, I. (1983). Expression of c-*onc* genes, c-*fos* and c-*fms* in developing mouse tissues. *Cell Biol. Int. Rep. 7:* 557-558.

BEN-ARI, E.T. (1991). All this and SH2. *The New Biologist 3:* 1040-1047.

BRINSTER, R.L. (1974). The effect of cells transferred into the mouse blastocyst on subsequent development. *J. Exp. Med. 140:* 1049.

DEN HERTOG, J. DE LAAT, A.W., SCHLESSINGER, J. and KRUIJER, W. (1991). Neuronal differentiation in response to epidermal growth factor of transfected P19 embryonal carcinoma cells expressing human epidermal growth factor receptors. *Cell Growth Differ. 2:* 155-164.

EARP, H.S., HEPLER, J.R., PETCH, L.A., MILLER, A., BERRY, A.R., HARRIS, J., RAYMOND, V.W., McCUNE, B.K., LEE, L.W., GRISHAM, J.W. and HARDEN, T.K. (1988). Epidermal growth factor (EGF) and hormones stimulate phosphoinositide hydrolysis and increase EGF receptor protein synthesis and mRNA levels in rat liver epithelial cells. *J. Biol. Chem. 263:* 13868-13974.

EDWARDS, S.A., RUNDELL, A.Y.K. and ADAMSON, E.D. (1988). Expression of c-*fos* antisense RNA inhibits the differentiation of F9 cells to parietal endoderm. *Dev. Biol. 129:* 91-102.

FERNANDEZ-POL, J.A., HAMILTON, P.D. and KLOS, D.J. (1989) Transcriptional regulation of proto-oncogene expression by epidermal growth factor, transforming growth factor B1, and triiodothyrosine in MDA-463 cells. *J. Biol. Chem. 264:* 4151-4156.

GERSCHENSON, M., GRAVES, K., CARSON, S.D., WELLS, R.S. and PIERCE, G.B. (1986). Regulation of melanoma by the embryonic skin. *Proc. Natl. Acad. Sci. USA 83:* 7307-7310.

GROVER, A. and ADAMSON, E.D. (1985). Roles of extracellular matrix components in differentiating teratocarcinoma cells. *J. Biol. Chem. 260:* 12252-12258.

GROVER, A. and ADAMSON, E.D. (1986). Evidence for the existence of an early common biochemical pathway in the differentiation of F9 cells into visceral or parietal endoderm: modulation by cyclic AMP. *Dev. Biol. 114:* 492-503.

GROVER, A., ANDREWS, P. and ADAMSON, E.D. (1983a). The role of laminin in epithelium formation by F9 aggregates. *J. Cell Biol. 97:* 137-144.

GROVER, A., EDWARDS, S.A., BOURDON, M. and ADAMSON, E. (1987). Proteoglycan-19, laminin and collagen type IV production is correlated with the levels of mRNA in F9 cell aggregates differentiating in the presence or absence of cyclic AMP. *Differentiation 36:* 138-144.

GROVER, A., OSHIMA, R.G. and ADAMSON, E.D. (1983b). Epithelial layer formation in differentiating aggregates of F9 embryonal carcinoma cells. *J. Cell Biol. 96:* 1690-1696.

GUTMAN, A. and WASYLYK, B. (1991). Nuclear targets for transcription regulation by oncogenes. *Trends Biochem. Sci. 7:* 49-54.

HUDSON, L.G., SANTON, J.B., GLASS, C.K. and GILL, G.N. (1990). Ligand-activated thyroid hormone and retinoic acid receptors inhibit growth factor receptor promoter expression. *Cell 62:* 1165-1175.

ILLMENSEE, K. (1978). Reversion of malignancy and normalized differentiation of teratocarcinoma cells in chimeric mice. In *Genetic Mosaics and Chimeras in Mammals* (Ed. L. Russell). Plenum Press, New York, p. 3.

JOH, T., DARLAND, T., SAMUELS, M., WU, J-X. and ADAMSON, E.D. (1992). Regulation of the epidermal growth factor receptor gene in murine embryonal carcinoma cells. *Cell Growth Differ. 3:* 315-325.

JONES-VILLENEUVE, E.M.V., RUDNICKI, M.A., HARRIS, J.F. and McBURNEY, M.W. (1983). Retinoic acid induced neural differentiation of embryonal carcinoma cells. *Mol. Cell. Biol. 3:* 2271-2279.

KUDLOW, J.E., CHEUNG, C-Y.M. and BJORGE, J.D. (1986). Epidermal growth factor stimulates the synthesis of its own receptor in a human breast cancer cell line. *J. Biol. Chem. 261:* 4134-4138.

McBURNEY, M.W., JONES-VILLENEUVE, E.M.V., EDWARDS, M.K.S. and ANDERSON, P.J. (1982). Control of muscle and neuronal differentiation in a cultured embryonal carcinoma cell line. *Nature* 299, 165-167.

MERCOLA, M. and STILES, C.D. (1988). Growth factor superfamilies and mammalian embryogenesis. *Development 102:* 451-460.

MUMMERY, C.L. and VAN DEN EIJNDEN-VAN RAAIJ, A.J.M. (1990). Growth factors and their receptors in differentiation and early murine development. *Cell Differ. Dev. 30:* 1-18.

MÜLLER, R., SLAMON, D.J., ADAMSON, E.D., TREMBLAY, J.M., MÜLLER, D., CLINE, M.J. and VERMA, I.M. (1983a). Transcription of c-*onc* genes C-*ras*^{k1} and c-*fms* during mouse development. *Mol. Cell. Biol. 3:* 1062-1069.

MÜLLER, R., TREMBLAY, J.M., ADAMSON, E.D. and VERMA, I.M. (1983b). Tissue and cell type-specific expression of two human c-*onc* genes. *Nature 304:* 454-456.

MÜLLER, R., VERMA, I.M. and ADAMSON, E.D. (1983c). Expression of c-*onc* genes: c-*fos* and c-*fms* transcripts accumulate to high levels during development of mouse placenta, yolk sac, and amnion. *EMBO J. 2:* 679-684.

OSHIMA, R.G., HOWE, W., KLIER, F.G., ADAMSON, E.D. and SHEVINSKY, L.H. (1983). Intermediate filament protein synthesis in preimplantation murine embryos. *Dev. Biol. 99:* 447-455.

PAPAIOANNOU, V.E., EVANS, E.P., GARDNER, R.L. and GRAHAM, C.F. (1979). Growth and differentiation of an embryonal carcinoma cell line (C145b). *J. Embryol. Exp. Morphol. 54:* 277.

PAPAIOANNOU, V.E., McBURNEY, M.W., GARDNER, R.L. and EVANS, M.J. (1975). Fate of teratocarcinoma cells injected into early mouse embryos. *Nature 258:* 70.

PARIA, B.C. and DEY, S.K. (1990). Preimplantation embryo development *in vitro:* co-operative interactions among embryos and role of growth factors. *Proc. Natl. Acad. Sci. USA 87:* 4756-4760.

PARIA, B.C., TSUKAMURA, H. and DEY, S.K. (1991). Epidermal growth factor-specific protein tyrosine phosphorylation in preimplantation embryo development. *Biol. Reprod. 45:* 711-718.

PIERCE G.B., DIXON, F.J., Jr. and VERNEY, E.L. (1960). Teratocarcinogenic and tissue forming potentials of the cell types comprising neoplastic embryoid bodies. *Lab. Invest. 9:* 583.

PIERCE, G.B., AGUILAR, D., HOOD, G. and WELLS, R.S. (1984). Trophectoderm in control of murine embryonal carcinoma. *Cancer Res. 81:* 7608-7611.

PIERCE, G.B., ARECHAGA, J., and WELLS, R.S. (1986). Embryonic control of cancer. *Prog. Clin. Biol. Res. 226:* 67-77.

PIERCE, G.B., LEWIS, S.H., MILLER, G.J., MORITZ, E. and MILLER, P. (1979). Tumorigenicity of embryonal carcinoma as an assay to study control of malignancy by the murine blastocyst. *Proc. Natl. Acad. Sci. USA 76:* 6649.

PIERCE, G.B., PODESTA, A. and WELLS, R.S. (1983). Malignancy and differentiation: the role of the blastocyst in control of colony formation. In *Teratocarcinoma Stem Cells* (Eds. L.M. Silver, G.R. Martin and S. Strickland). Cold Spring Harbor Laboratory, Cold Spring Harbor, pp.15-22.

PIERCE, G.B., SHIKES, R.H. and FINK, L.M. (1978). *Cancer: A Problem of Developmental Biology.* Prentice-Hall, Englewood Cliffs, New Jersey.

PODESTA, A.H., MULLINS, J., PIERCE, G.B. and WELLS, R.S. (1984). The neurula stage mouse embryo in control of neuroblastoma. *Proc. Natl. Acad. Sci. USA 81:* 7608-7611.

STEVENS, L.C. (1983). The origin and development of testicular, ovarian and embryo-derived teratomas. In *Teratocarcinoma Stem Cells* (Eds. L.M. Silver, G.R. Martin and S. Strickland). Cold Spring Harbor Laboratory, Cold Spring Harbor, pp.23-36.

SUKHATME, V.P., CAO, X., CHANG, L.C., TSAI-MORRIS, C.-H., STAMENKOVICH, D., FERREIRA, P.C.P., COHEN, D.R., EDWARDS, S.A., SHOWS, T.B., CURRAN, T., LE BEAU, M.M. and ADAMSON, E.D. (1988). A zinc-finger encoding gene co-regulated with c-*fos* during growth and differentiation, and after cellular depolarization. *Cell 53:* 37-43.

THOMPSON, K.L. and ROSNER, M.R. (1989). Regulation of epidermal growth factor receptor gene expression by retinoic acid and epidermal growth factor. *J. Biol. Chem. 264:* 3230-3234.

VAN ZOELEN, E.J.J., WARD-VAN OOSTWAARD, T.M.J., NIEWLAND, R., VAN DER BURG, B., VAN DEN EIJNDEN-VAN-RAAIJ, A.J.M., MUMMERY, C.L. and DE LAAT, S.W. (1989). Identification and characterization of polypeptide growth factors secreted by murine embryonal carcinoma cells. *Dev. Biol. 133:* 272-283.

WELLER, A., MEEK, J. and ADAMSON, E.D. (1987). Preparation and properties of monoclonal and polyclonal antibodies to mouse epidermal growth factor (EGF) receptors: evidence for cryptic EGF receptors in embryonal carcinoma cells. *Development 100:* 351-363.

WELLS, R.S. (1982). An *in vitro* assay for regulation of embryonal carcinoma by the blastocyst. *Cancer Res. 42:* 2736.

WILEY, L.M., WU, J-X., HARARI, I. and ADAMSON, E.D. (1992). Initiation of epidermal growth factor-receptor gene activity occurs at the two to four-cell preimplantation stage in murine development. *Dev. Biol.149:* 247-260.

WOOD, S.A. and KAYE, P.L. (1989). Effects of epidermal growth factor on preimplantation mouse embryos. *J. Reprod. Fertil. 85:* 575-582.