Int. J. Dev. Biol. 37: 67-74 (1993)



# Regulation of differentiation, proliferation and cancer suppressor activity

ROBERT E. SCOTT*, CHIN-YUAN TZEN, MICHAEL M. WITTE, STANLEY BLATTI and HANLIN WANG

*Department of Pathology, University of Tennessee Medical Center and Baptist Memorial Hospital, Memphis, Tennessee, USA*

**ABSTRACT This review suggests that carcinogenesis is linked to defects in linkages between cellular differentiation and regulation of cell proliferation. These linkages are discussed in terms of terminal and nonterminal states of differentiation and their relationship to the control of proliferation. The ability of differentiation to regulate proliferation potential proteins and cancer suppressor genes is also discussed, because these mechanisms may be important for cancer prevention and therapy.**

KEY WORDS: *differentiation, proliferation, SV40, 3T3T cell*

What is cancer? Is it a disease of growth control? Is it a disease of differentiation control? Is it a disease in the control of cellular invasive properties? In 1978, G. Barry Pierce published a book entitled *Cancer: A Problem of Developmental Biology* (Pierce *et al.*, 1978) that suggested that cancer results from defects in a complex array of biological and molecular mechanisms that control how cells regulate development, differentiation and proliferation, among other functions. For the past fifteen years, my research group has focused our studies on three topics that have resulted in data that expand Dr. Pierce's concepts. These topics include:

I. Linkages in the control of differentiation and proliferation
II. Mechanisms that control the terminal event in differentiation
III. Differentiation as a regulator of cancer suppression.

## Linkages in the control of differentiation and proliferation

For years, it had been known that a relationship existed between the control of cellular proliferation and differentiation even though it had not been clearly defined. We therefore initiated studies to investigate this question and established that definitive linkages exist in the control of proliferation and differentiation (Scott *et al.*, 1982b). These studies focused on 3T3T mesenchymal stem cells, which have the potential to differentiate into multiple cell types including adipocytes, chondrocytes and macrophage-like cells (Boone *et al.*, 1980; Krawisz *et al.*, 1981; Scott *et al.*, 1982a). Fig. 1 illustrates the linkages that are involved in *in vitro* adipocyte differentiation induced in low density cells by medium containing heparinized human plasma (Krawisz *et al.*, 1982). This figure shows that differentiation is a multistep process involving a series of reversible and irreversible states (Scott *et al.*, 1982b; Weir *et al.*, 1986). Linkages in the control of proliferation and differentiation are most evident at the Predifferentiation Growth Arrest (PGA) state and at the Nonterminal Differentiation (NTD) state; cells at both these states are quiescent, cells at the PGA state can either differentiate or they can exit the PGA state and return to the cell cycle and proliferate. The NTD state also serves to link the control of proliferation and differentiation because at this state cells can either undergo terminal differentiation, which is associated with the irreversible loss of proliferative potential, or cells at the NTD state can return to the cell cycle and proliferate in association with loss of the differentiated phenotype, i.e. dedifferentiation.

Cells at a PGA-like state *in vivo* include a variety of quiescent stem cells; cells at a NTD-like state *in vivo* include lymphocytes and hepatocytes that are highly differentiated cells that still retain their proliferative potential. Finally, cells at the TD state *in vivo* are typified by striated muscle and neuronal cells, which are highly differentiated cells lacking proliferative potential (Fig. 1).

Detailed *in vitro* studies on normal human keratinocytes have also documented the existence of a multistep process of differentiation involving mechanisms similar but not identical to those in 3T3T cells (Wilke *et al.*, 1988c). In this regard, we have recently found that the first steps in the process of keratinocyte differentiation, i.e. predifferentiation growth arrest, is associated with the stable overexpression of the *c-jun* protooncogene (Blatti and Scott, 1992).

*Abbreviations used in this paper*: CNS, central nervous system; $D^2CSA$, differentiation and dedifferentiation-induced cancer suppressor activity; FBS, fetal bovine serum; IFN, interferon; MIX, methyl isobutyl xanthine; NTD, nonterminal differentiation; nm23 antimetastasis suppressor gene; P2P, proliferation potential proteins; PDGF, platelet derived growth factor; PGA, predifferentiation growth arrest; PKC, protein kinase C; RA, retinoic acid; Rb-1, retinoblastoma suppressor gene product; RG, rapid growth; TD, terminal differentiation; TPA, 12-O-tetradecanoylphorbol-13-acetate; TGFß, transforming growth factor-beta.

***Address for reprints**: University of Tennessee Medical Center and Baptist Memorial Hospital, 800 Madison Avenue, Memphis, TN 38163, USA. FAX:

0214-6282/93/$03.00

Printed in Spain

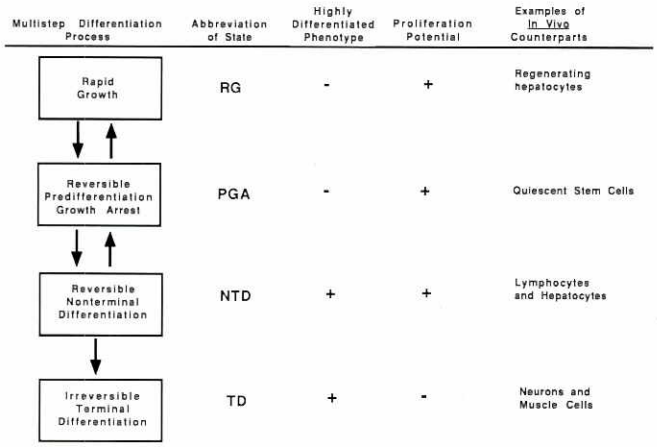


**Fig. 1. Model for the linkages of proliferation and differentiation in 3T3T mesenchymal stem cells.**

Most studies by other investigators have not been as detailed as those we published concerning 3T3T cells and keratinocytes. However, it has been shown that growth arrest, in general, precedes differentiation and that differentiation can ultimately result in the irreversible loss of proliferative potential, i.e. terminal differentiation (Sachs 1978; Marks *et al.*, 1982; Kuri-Harcuch *et al.*, 1983).

Such studies however do not explain why 3T3T stem cells or other stem cells differentiate along a specific lineage. In this regard, various determination genes have recently been described. One report suggested that an adipocyte determination gene had been identified (Chen *et al.*, 1989), but that work was superseded by the myriad of papers concerning determination genes for muscle cell differentiation, i.e. myoD-1; this topic has been extensively reviewed (Weintraub *et al.*, 1991). Although some evidence exists suggesting that the expression of determination genes can be modulated by carcinogenesis (Lassar *et al.*, 1989), there are very few clinical examples of human cancers resulting from changes in stem cell determination. For example, it is extremely rare for a sarcoma to be derived from a determined epithelial stem cell or for a carcinoma to be derived from a determined stromal stem cell. Carcinogenesis instead appears to result primarily from defects in differentiation, proliferation and related processes and not from changes in determination.

The fact that cancers of a particular cell lineage can express multiple biological phenotypes suggests that carcinogenesis is in fact associated with the development of multiple defects in the linkages that control differentiation and proliferation. This conclusion is supported by the fact that some cancers derived from a specific tissue can express a very slow growing, well-differentiated phenotype whereas other related cancers can express a rapidly growing, undifferentiated phenotype. The concept that cancer cells must show combined defects in the control of proliferation and differentiation is also supported by the fact that expression of sole defects in proliferation control, i.e. hyperplasia, and the expression of sole defects in differentiation control, i.e. metaplasia, result in benign processes. Fig. 2 illustrates examples of the multiple and complex phenotypes that can result from defects in the linkages in the control of proliferation and differentiation based on the 3T3T model presented in Fig. 1.

Cancer however results not only from defects in linkages between proliferation and differentiation but also from inappropriate invasive characteristics. Fig. 3 therefore expands the concept of biological and molecular linkages to suggest that the control of proliferation, differentiation, and invasive potential may all be linked.

With respect to Fig. 3 and its relationship to carcinogenesis, several important facts need to be emphasized. Cancer cells do not have to grow rapidly, cancer cells do not have to show completely defective differentiation and cancer cells do not have to be highly invasive and metastatic. In this regard, it needs to be pointed out that some of the fastest growing cells in the body (Quaroni *et al.*, 1979), i.e. small bowel mucosal cells, show very low frequencies of malignant transformation (Barclay and Schapira, 1983). Furthermore, many normal cells are invasive and/or metastatic; these include inflammatory cells and trophoblastic cells among others. The question therefore arises as to what combination of defects is required for carcinogenesis.

Table 1 presents several theoretical examples of various combinations of biological defects that can result in benign versus malignant lesions. This information is intended to suggest that for carcinogenesis to develop, defects in multiple biological processes must occur. These include defects in proliferation, differentiation and invasion.

As an experimental example of the multiple defects that can be associated with carcinogenesis, a brief summary of the effects that the transforming SV40 T antigen can have on the linkages of proliferation and differentiation is in order. Since these observations have been published in the past several years (Estervig *et al.*, 1989b, 1990; Scott *et al.*, 1989), the results will now only be

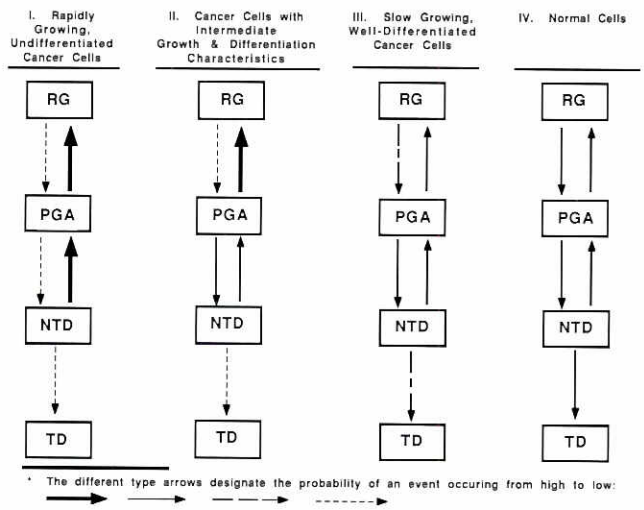


**Fig. 2. Examples of different cancer phenotypes explained by different defects in the linkages of proliferation and differentiation*.**

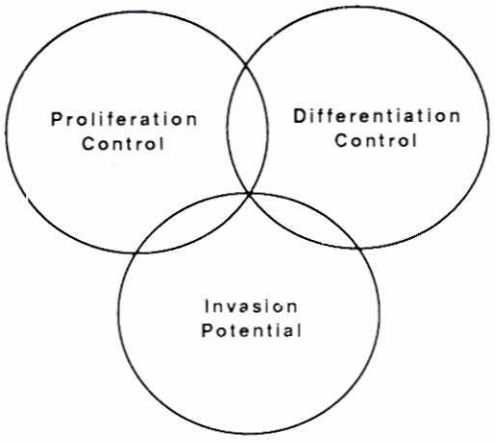


**Fig. 3. Model for important biological and molecular linkages.**

summarized. First, the SV40 T antigen antagonizes the ability of cells to undergo predifferentiation growth arrest. This is effected by making cells extremely sensitive to the mitogenic effects of various agents including those that are not complete mitogens for nontransformed cells, i.e. insulin and vanadate (Wang and Scott, 1991; Wang *et al.*, 1991). In fact, SV40 transformed cells at the PGA state can be induced to reenter the cell cycle and undergo DNA synthesis by mechanisms that do not require involvement of any of the following processes typically associated with mitogenesis in nontransformed cells: 1) PKC activation, 2) activation of a pertussis toxin sensitive G protein, 3) polyamine induction, or 4) *c-fos* or *c-myc* induction (Wang and Scott, submitted). SV40 transformed cells do however apparently require the induction of *c-jun* and *jun-B* to undergo DNA synthesis.

By using highly stringent culture conditions that are antiproliferative (Estervig *et al.*, 1990), it was possible to force SV40 transformed cells to undergo PGA and NTD (see Fig. 1). We thereby found that differentiation can overcome some but not all of the effects of the SV40 T antigen. For example, the process of differentiation can actually suppress T antigen expression at the transcriptional level (Estervig *et al.*, 1989b; Scott *et al.*, 1989). Preliminary results suggest that this effect may be mediated by modulating the expression of one or more transacting repressor factors.

Although the process of differentiation can suppress some of the effects of SV40 T antigen expression, other effects persist in NTD cells that prevent such cells from undergoing the terminal step in differentiation (Estervig *et al.*, 1990). Recent results have in fact established that transformed cells, including those containing the SV40 T antigen gene, produce autocrine anti-TD factors that block the terminal step of differentiation (Filipak *et al.*, 1988).

From this perspective, it is possible to conceive of new approaches for clinical cancer therapy. One such approach could be based on the data concerning anti-TD factors. If it proves to be possible to block the function or expression of anti-TD factors in cancer cells by using neutralizing antibodies or antisense oligonucleotides to anti-TD factors, it should then be possible to selectively induce cancer cells to terminally differentiate and lose their malignant character.

## Mechanisms that control the terminal event in differentiation

The idea that cellular differentiation is a multistep process that can be experimentally dissected is not well understood. Many biologists think that differentiation inexorably leads to the irreversible loss of proliferative potential and therefore that all differentiation is terminal. Nonetheless, a myriad of normal differentiated cells stably maintain their proliferative potential. These include different types of stem cells, such as hematopoietic stem cells and epithelial stem cells, which are determined/differentiated along a specific lineage. Other more highly differentiated cells, such as lymphocytes and hepatocytes, can also respond to specific mitogenic signals.

A nonterminal state of differentiation must therefore exist. This then implies that a distinct terminal step of differentiation must also exist. In this regard, our studies on human keratinocytes (Wille *et al.*, 1984, 1985; Pittelkow and Scott, 1986; Pittelkow *et al.*, 1986; Shipley *et al.*, 1986; Scott *et al.*, 1988; Wilke *et al.*, 1988a,b,c; Kasperbauer *et al.*, 1990) have established that during differentiation multiple steps occur that result in the irreversible loss of proliferative potential. Most of our studies have however employed 3T3T adipocytes at the NTD state which were then induced to convert to the TD state (Fig. 1). Numerous papers have been written on this topic in the past ten years (Florine *et al.*, 1982; Krawisz *et al.*, 1982; Scott *et al.*, 1982a,b, 1983; Wille *et al.*, 1982; Yun and Scott, 1983; Yun *et al.*, 1983; Hoerl *et al.*, 1984; Scott and Maercklein, 1985; Wier and Scott, 1985, 1986a,b, 1987; Sparks and Scott, 1986; Sparks *et al.*, 1986; Wille and Scott, 1986; Filipak *et al.*, 1988; Hoerl and Scott, 1989; Estervig *et al.*, 1990; Tzen *et al.*, 1990).

To begin this discussion, it is most important to characterize cells at the NTD and TD states. Table 2 presents a comparison of cells at these states and shows that NTD and TD adipocytes have essentially identical morphologies, but that they differ in their response to agents that induce proliferation and/or dedifferentiation.

NTD cells can be induced to dedifferentiate and proliferate, whereas TD cells cannot. That is, although cells at the NTD state are not as mitogenically responsive to growth factors as are quiescent

TABLE 1

**BIOLOGICAL CHARACTERISTICS OF DIFFERENT CELLULAR PHENOTYPES**

| Aberrant proliferation control | Aberrant differentiation control | Invasion potential | Phenotypes |
|---|---|---|---|
| – | – | + | Nonmalignant: inflammatory cells, fibroblast, etc. |
| + | – | – | Nonmalignant: hyperplastic cells |
| – | + | – | Nonmalignant: metaplastic cells |
| + | + | – | Nonmalignant: dysplastic cells |
| + | + | + | Malignant: cancer cells |

TABLE 2

**COMPARISON OF THE NTD AND TD PHENOTYPES**

| Phenotype | NTD | TD |
|---|---|---|
| I. Morphology | mature adipocyte | mature adipocyte |
| II. Proliferation potential | + | – |
| *Mitogenic responsiveness to specific growth factors | | |
| A. 30% FBS+50μg/ml insulin | + | – |
| B. 10 ng/ml PDGF+50μg/ml insulin | + | – |
| C. 5% FBS or 10 ng/ml PDGF | – | – |
| III. Potential to lose the differentiated phenotype (dedifferentiation) | + | – |
| *Response to agents that induce dedifferentiation | | |
| A. 30% FBS+50μg/ml insulin | + | – |
| B. TPA (100 ng/ml) | + | – |
| C. RA (10 μg/ml) | + | – |
| D. MIX ($2x10^{-4}$ M) | + | – |

undifferentiated cells, they can be stimulated to proliferate (Hoerl and Scott, 1989). This is illustrated by the fact that a high concentration of fetal bovine serum (30% FBS) and insulin (50 μg/ml) is required to induce mitogenesis in NTD cells, whereas undifferentiated quiescent 3T3T cells can be induced to proliferate by a serum concentration as low as 5% FBS.

As illustrated in Fig. 4, a great deal of important information has accumulated concerning the transition from the NTD to TD state. This process is induced by a distinct human plasma protein designated aproliferin which has been partially purified (Weir and Scott, 1986b); aproliferin has a molecular weight of ~45,000 D and an isoelectric point of ~7.0. To induce the terminal event, NTD cells need to be exposed to aproliferin for only approximately 6 h and thereafter this pulse treatment results in the irreversible loss of proliferative potential within the following 36 to 48 h (Weir and Scott, 1986a). The terminal event in adipocyte differentiation can be blocked by agents including DMSO, TNF and by inhibitors of protein synthesis, such as cycloheximide (Filipak *et al.*, 1988; Weir and Scott, 1986a). By using a variety of pharmacological agents and careful cell culture analysis, it has also been established that the transition from the NTD to TD state involves at least three distinct steps and thus multiple molecular mechanisms are implied (Weir and Scott, 1986a). Two dimensional gel electrophoretic analysis of cells at the NTD and TD states has in fact established that multiple differences in protein composition exist (Weir and Scott, 1987). The most important of these involves the disappearance from cells at the TD state of a distinct set of proteins, termed P2Ps for proliferation potential proteins. By comparison, P2Ps are readily expressed in cells at various reversible quiescent states and at the NTD state and in growing cells (Minoo *et al.*, 1989).

Table 3 summarizes the results of numerous studies that have analyzed the expression of P2P abundance by Western blotting using one or more antibodies that recognize P2P epitopes. These results show that in three model cell systems, including 3T3T cells, keratinocytes and melanoma cells, a strong correlation exists between the expression of proliferative potential and the expression of P2Ps and vice versa.

Cells can however be induced to lose their proliferative potential by mechanisms that do not require repression of P2P abundance. For example, treatment of human keratinocytes with the drug Razoxane induces irreversible growth arrest in the $G_2$ phase of the cell cycle without effecting P2P expression (Wang and Scott, unpublished observations). In addition, terminally differentiated neurons appear to contain P2Ps within their nucleus as determined by immunohistochemical localization of P2Ps. Therefore P2P-dependent and P2P-independent mechanisms for the irreversible loss of proliferative potential must exist.

P2Ps were initially identified as a group of three nuclear polypeptides with molecular weights between 34 to 38,000 D that contained epitopes in common with hsp90 (Minoo *et al.*, 1989). These polypeptides were also found to be recognized by antibodies developed against hnRNP core proteins, and P2Ps were found to be highly enriched in particulate nuclear subfractions. This and additional data support the conclusion that P2Ps comprise a subset of hnRNP proteins.

Based on this information, the cloning of the P2P cDNA was undertaken as was the *in vitro* translation of P2P peptides to generate additional anti-P2P monoclonal antibodies. These studies suggest that P2Ps may be derived from a larger precursor protein which is relatively abundant in embryonic tissue. Studies are currently in progress to establish biochemically how the purported P2P precursor generates P2Ps. Studies are also in progress to determine how the abundance of P2Ps is regulated during the terminal event in differentiation. Using a series of oligonucleotide probes complementary to the cloned P2P cDNA, it has been found that the abundance of P2P mRNA is markedly decreased during the terminal step of differentiation. This suggests a possible transcriptional regulatory mechanism for the control of P2P expression during terminal differentiation. It is however also possible that terminal differentiation may involve changes in P2P processing.

The model in Fig. 2 suggests that cancer cells can show defects in their ability to undergo the terminal step in differentiation and that this defect may be important in the process of carcinogenesis. Indeed, we and others have reported that many types of transformed cells including those derived from keratinocytes and 3T3T cells show defects in their ability to terminally differentiate (Rheinwald and Beckett, 1981; Weir and Scott, 1985; Scott *et al.*, 1988a; Estervig *et al.*, 1990). We have also reported that clones of SV40 transformed 3T3T cells that cannot terminally differentiate also

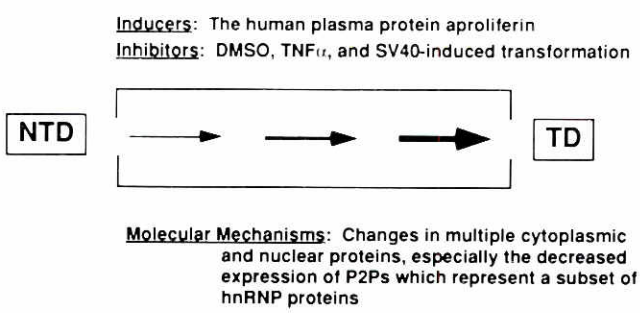


**Fig. 4. Characteristics of the terminal step in adipocyte differentiation.**

TABLE 3

**P2P EXPRESSION IN VARIOUS CELLS AT DISTINCT BIOLOGICAL STATES**

| Biological states | P2P expression |
|---|---|
| 3T3T mesenchymal stem cells | |
| A. Growth state | + |
| B. Quiescent state induced by serum deprivation | + |
| C. Quiescent state induced by contact inhibition | + |
| D. Quiescent state induced by predifferentation growth arrest | + |
| E. Nonterminal differentiation state | + |
| F. Terminal differentiation state | – |
| Normal human keratinocytes | |
| A. Growth state | + |
| B. Reversible quiescent state induced by TGFß, ethionine or growth factor deficiency | + |
| C. Irreversible growth arrest state induced by 2mM $Ca^{++}$ and growth factor deficiency or senescence | – |
| D. Terminal differentiation state | – |
| Immortalized human melanocytes* | |
| A. Growth state | + |
| B. Irreversible growth arrest and terminal differentiation state induced by various pharmacological agents | – |

*Performed in collaboration with Dr. Paul Fisher, Columbia University Medical Center, New York City, NY.

cannot repress P2P expression (Minoo *et al.*, 1989). If the relative abundance of P2Ps within cancer cells could therefore be pharmacologically regulated, such as by the use of antisense P2P oligonucleotides, it is possible that cancer cells could be induced to undergo irreversible growth arrest and/or terminal differentiation. If successful, such treatment could have the potential to represent another clinically useful new approach of differentiation therapy for cancer.

## Differentiation as a regulator of cancer suppression

Another important aspect of carcinogenesis concerns the role of suppressor genes. The field of cancer suppressor gene research is in its infancy; only approximately ten suppressor genes have been described so far. It appears that suppressor genes can either antagonize the function of specific oncogenes (Kitayama *et al.*, 1989) or they can have more general effects. The Rb-1 suppressor gene product, for example, regulates cell cycle progression at a critical $G_1$ restriction point and the SV40 T antigen transforming protein can bind to the Rb-1 gene product to negate its ability to regulate this process (Marshall *et al.*, 1991). Other suppressor genes may encode physiological pleiotropic regulatory molecules, such as interferon (Diaz *et al.*, 1989). Finally, suppressor genes may encode proteins that have been reported to control the invasive and/or metastatic properties of cells (Rosengard *et al.*, 1989).

We propose that multiple cancer suppressor genes may have related functions so that a defect in one suppressor gene can be compensated for by induction of another. This possibility is compatible with the existence of multiple complementation groups of cancer suppressor genes (Stanbridge *et al.*, 1982) and with our recent data to be reviewed subsequently which shows that the expression of at least one cancer suppressor gene can be physiologically regulated by cellular differentiation (Estervig *et al.*, 1989a,b; Scott *et al.*, 1989; Maercklein *et al.*, 1990; Tzen and Scott, 1990).

Fig. 5 depicts the concept that cancer suppressor genes may serve to regulate or maintain the linkages that normally control proliferation, differentiation and the invasive characteristics of cells. This figure suggests that as long as appropriate suppressor gene function is maintained, irrespective of which biological defects are induced by oncogenes on cell proliferation, differentiation and invasion, complete carcinogenesis need not develop. This concept is compatible with the evidence that suppressor gene deletion *per se* does not result in complete transformation but rather it predisposes cells to undergo transformation by failing to antagonize other carcinogenic events (Knudson, 1971; Comings, 1973).

These data emphasize the importance of defining the molecular mechanisms that mediate these linkages and if and how these linkages can be modulated so as to prevent or reverse carcinogenesis. In this regard, the induction of terminal differentiation would result in one form of cancer suppression.

A great deal of published data however suggests that it should also be possible to induce cancer suppressor activity without limiting a cell's proliferative potential. Evidence that cancer suppression can be mediated without limiting a cell's proliferative potential comes from a variety of developmental biology studies wherein individual cancer cells were transplanted by micromanipulation into the inner cell mass or other embryonic sites (Brinster, 1974; Mintz and Illmensee, 1975). The results of those studies showed that the cancer phenotype can be suppressed by embryonic microenvironments and that cells in these microenvironments continued to grow and divide and participate in normal embryological development (Mintz and Illmensee, 1975). This general experimental design has been shown to be effective for embryonal carcinoma cells (Mintz and Illmensee, 1975),

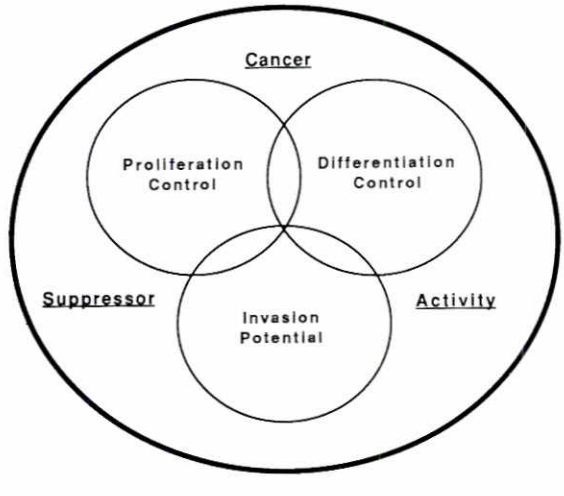


**Fig. 5. Model for the role of cancer suppressor activity in maintaining important biological linkages.**

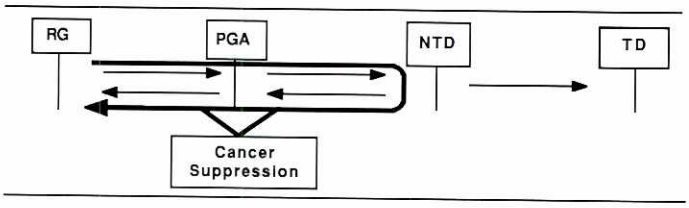


**Fig. 6. Model for the induction of cancer suppressor activity during differentiation and dedifferentiation.**

neuroblastoma cells (Podesta *et al.*, 1984), and myeloblastic leukemia cells (Gootwine *et al.*, 1982).

Based on this background, our research group undertook studies to determine if the process of nonterminal differentiation and dedifferentiation could stably induce cancer suppressor activity without limiting cellular proliferative potential (Scott *et al.*, 1988a, 1989; Estervig *et al.*, 1989a; Maercklein *et al.*, 1990; Tzen *et al.*, 1990). Two general types of studies were performed. Spontaneously transformed 3T3T cells were first induced to undergo nonterminal adipocyte differentiation. Individual adipocytes were then isolated and induced to reenter the cell cycle and dedifferentiate to produce stable cell clones. Assays were then performed to determine whether such dedifferentiated cell clones showed biological characteristics different from transformed predifferentiated cells. The results were striking. Whereas 100% of the cell clones derived from predifferentiated cells showed a transformed phenotype, only 50% of the clones derived from dedifferentiated cells were transformed and these results were obtained 20 population doublings after their initial cloning. Additional results suggest that the process of differentiation and dedifferentiation initially suppresses the transformed phenotype in >80% of cell clones (Scott *et al.*, 1988a, 1989; Estervig *et al.*, 1989a; Maercklein *et al.*, 1990; Tzen and Scott, 1990). This cancer suppressor activity which is induced by differentiation and dedifferentiation has been designated $D^2$CSA. $D^2$CSA was also shown to make nontransformed cells resistant to spontaneous transformation and to transformation induced by UV irradiation, *EJras* transfection and six different carcinogens (Estervig *et al.*, 1989a; Scott *et al.*, 1989). Finally, it was shown that $D^2$CSA can be stably expressed for up to 100 population doublings (Maercklein *et al.*, 1990). Fig. 6 summarizes these observations relative to our model for the multistep adipocyte differentiation. Another study that supports these data has also been reported (Harrington *et al.*, 1988).

Once the existence of $D^2$CSA was clearly established, the challenge was to determine what biochemical characteristics distinguished cells expressing $D^2$CSA from cells that do not express $D^2$CSA. Table 4 lists the various studies performed and the differences that were identified. Of all these studies only one clearly distinguished cells that express $D^2$CSA from cells that do not. In studies on three cell clones that do and four clones that do not express $D^2$CSA, $D^2$CSA was found to show an absolute correlation with the secretion of interferon (IFN) (Fig. 7) (Tzen *et al.*, submitted).

These observations are compatible with numerous previous reports suggesting that interferon is indeed a cancer suppressor agent both *in vitro* (Chany and Vignal, 1970; Brouty-Boye *et al.*, 1981; Samid *et al.*, 1985, 1987) and *in vivo* (Krown, 1987) and that deletion of the interferon gene occurs in acute leukemias (Diaz *et al.*, 1989).

Once the discovery was made that $D^2$CSA is associated with the secretion of interferon, an extensive series of additional studies was initiated. It has now been established that interferon expression is initially induced at the NTD state and that thereafter its expression is stably maintained as cells dedifferentiate and proceed to express $D^2$CSA. Preliminary evidence also suggests that IFN may mediate $D^2$CSA in association with other cancer suppressor factors and that $D^2$CSA may induce these factors in association with modulation of the expression of specific sets of transacting nuclear regulatory factors. It also appears that cells expressing $D^2$CSA produce a distinct type of interferon.

TABLE 4

**COMPARISON OF THE BIOLOGICAL AND MOLECULAR CHARACTERISTICS OF CELLS THAT DO AND DO NOT EXPRESS $D^2$CSA**

| | $D^2$CSA positive | vs | $D^2$CSA negative |
|---|---|---|---|
| Morphology | Same | | |
| Growth potential | Same | | |
| Cell cycle characteristics | Same | | |
| Differentiation potential | Same | | |
| Soft agar growth response to TGFß | Same | | |
| DNA repair mechanisms | Same | | |
| Cell-cell communication characteristics | Same | | |
| Interferon production | Distinct | | |

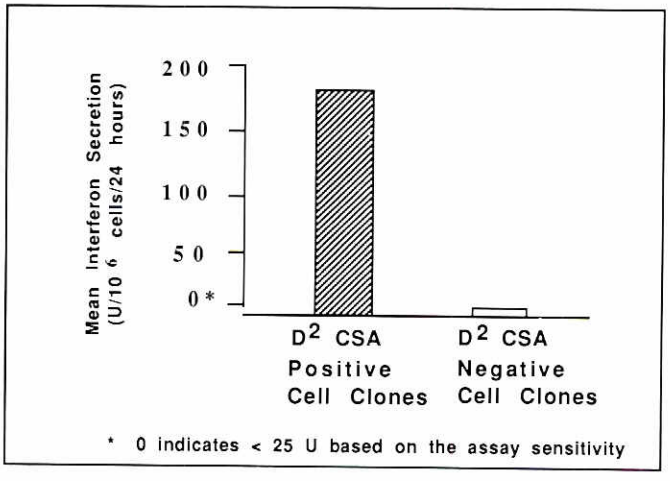


**Fig. 7. Interferon secretion correlates with the expression of cancer suppressor activity.**

## Conclusions

In summary, the data reviewed in this paper suggest that cellular differentiation regulates the phenotype of normal cells and that carcinogenesis is associated with the development of defects in the mechanisms by which differentiation is linked to the control of cell proliferation and the expression of cancer suppressor activity. Once we understand the molecular mechanisms by which differentiation mediates these effects, we anticipate that new approaches for cancer prevention and cancer therapy will result and that new forms of differentiation therapy will be possible.

*Acknowledgments*

*The authors thank W. Patrick for typing this manuscript and our numerous associates and technicians who have helped generate the results described herein. We also acknowledge the following support that funded these studies: NIH grants CA28240 and CA51715, CTR grant 2869 and the Muirhead Endowed Chair to RES.*

## References

BARCLAY, T. and SCHAPIRA, D. (1983). Malignant tumors of the small intestine. *Cancer 51:* 878-881.

BLATTI, S.P. and SCOTT, R.E. (1992). Stable induction of *c-jun* mRNA expression in normal human keratinocytes by agents that induce predifferentiation growth arrest. *Cell Growth Differ. 3:* 429-434.

BOONE, C.W. and SCOTT, R.E. (1980). Plate-induced tumors of BALB/3T3 cells exhibiting foci of differentiation into pericytes, chondrocytes, and fibroblasts. *J. Supramol. Str. Cell. 14:* 233-240.

BRINSTER, R.L. (1974). The effect of cells transferred into the mouse blastocyst on subsequent development. *J. Exp. Med. 140:* 1049-1056.

BROUTY-BOYE, D., WYBIER-FRANQUI, J., CALVO, C., FEUNTEUN, J. and GREESER, I. (1981). Reversibility of the transformed and neoplastic phenotype. IV. Effects of long-term Interferon treatment of C3H/10T1/2 cells transformed by methylcholanthrene and SV40 virus. *Int. J. Cancer 34:* 107-112.

CHANY, C. and VAGNAL, M. (1970). Effect of Interferon treatment on mouse embryonic fibroblasts transformed by murine sarcoma virus. *J. Gen. Virol 7:* 203-211.

CHEN, S, TEICHER, L.C., KAZIM, D., POLLACK, R.E. and WISE, L.S. (1989). Commitment of mouse fibroblasts to adipocyte differentiation by DNA transfection. *Science 244:* 582-585.

COMINGS, D.E. (1973). A general theory of carcinogenesis. *Proc. Natl. Acad. Sci. USA 70:* 3324-3328.

DIAZ, M.O., RUBIN, C.M., HARDEN, A., ZIEMIN, S., LARSON, R.A., LE BEAU, M.M. and ROWLEY, J.D. (1989). Deletions of Interferon genes in acute lymphoblastic leukemia. *New Engl. J. Med. 322:* 77-82.

ESTERVIG, D., MINOO, P., TZEN, C-Y, and SCOTT, R.E. (1990). Three distinct effects of SV40 T-antigen gene transfection on cellular differentiation. *J. Cell. Physiol. 142:* 552-558.

ESTERVIG, D.N., MAERCKLEIN, P.B. and SCOTT, R.E. (1989a). Resistance to neoplastic transformation induced by nonterminal differentiation. *Cancer Res. 49:* 1008-1013.

ESTERVIG, D.N., MINOO, P., TZEN, C-Y. and SCOTT, R.E. (1989b). Inhibition of SV40 T-antigen expression by cellular differentiation. *J. Virol. 63:* 2718-2725.

FILIPAK, M., SPARKS, R.L., TZEN, C-Y. and SCOTT, R.E. (1988). Tumor necrosis factor inhibits the terminal event in mesenchymal stem cell differentiation. *J. Cell. Physiol. 137:* 367-373.

FLORINE, D.L., HOERL, B.J. and SCOTT, R.E. (1982). Growth arrest of proadipocytes at a distinct predifferentiation GI state associated with cytotoxic responsiveness to 8-bromo cyclic AMP. *Cell Differ. 11:* 195-202.

GOOTWINE, E., WEBB, C.G. and SACHS, L. (1982). Participation of myeloid leukaemic cells injected into embryos in haematopoietic differentiation in adult mice. *Nature 299:* 63-65.

HARRINGTON, M., FELICIDAD, G. and JONES, P. (1988). Effect of cellular determination on oncogenic transformation by chemicals and oncogenes. *Mol. Cell. Biol. 8:* 4322.

HOERL, B.J. and SCOTT, R.E. (1989). Nonterminally differentiated cells express decreased growth factor responsiveness. *J. Cell. Physiol. 139:* 68-75.

HOERL, B.J., WIER, M.L. and SCOTT, R.E. (1984). Biological mechanisms for the loss of the differentiated phenotype by non-terminally differentiated adipocytes. *Exp. Cell Res. 155:* 422-434.

KASPERBAUER, J.L., HSU, B.M., NEEL, H.B., III and SCOTT, R.E. (1990). Proliferation and differentiation characteristics of normal human squamous mucosal cells of the upper aerodigestive tract. *Ann. Oto. Rhinol. Laryn. 99:* 29-37.

KITAYAMA, H., SUGIMOTO, Y., MATSUZAK, T., IKAWA, Y. and NODA, M. (1989). A *ras*-related gene with transformation suppressor activity. *Cell 56:* 77-84.

KNUDSON, A.G., Jr. (1971). Mutation and cancer: statistical study of retinoblastoma. *Proc. Natl. Acad. Sci. USA 68:* 820-823.

KRAWISZ, B.R. and SCOTT, R.E. (1982). Coupling of proadipocyte growth arrest and differentiation. I. Induction by heparin medium containing human plasma. *J. Cell Biol. 94:* 394-399.

KRAWISZ, B.R., FLORINE, D.L. and SCOTT, R.E. (1981). Differentiation of fibroblast-like cells into macrophages. *Cancer Res. 41:* 2891-2899.

KROWN, S.E. (1987). Clinical trials of Interferon in human pathology. In *Mechanisms of Interferon Action* Vol. II (Ed. L.M. Pfeffer). CRC Press Inc., Boca Raton, pp. 143-178.

KURI-HARCUCH, W. and MARSCH-MORENO, M. (1983). DNA synthesis and cell division related to adipose differentiation of 3T3 cells. *J. Cell. Physiol. 114:* 39-44.

LASSAR, A.B., THAYER, M.J., OVERELL, R.W. and WEINTRAUB, H. (1989). Transformation by activated *ras* or *fos* prevents myogenesis by inhibiting expression of myodD-1. *Cell 58:* 659-667.

MAERCKLEIN, P.B., ESTERVIG, D.N. and SCOTT, R.E. (1990). Stability of anticancer activity induced by cellular differentiation. *Lab. Invest. 62:* 196-201.

MARKS, P.A., RIFKIND, R.A., GAMBARI, R., EPNER, E., CHEN, Z. and BANKS, J. (1982). Commitment to terminal differentiation and the cell cycle. *Curr. Top. Cell Regul. 21:* 189-203.

MARSHALL, C.J. (1991). Tumor suppressor gene. *Cell 64:* 313-326.

MINOO, P., SULLIVAN, W., SOLOMON, L.R., MARTIN, T.E., TOFT, D.O. and SCOTT, R.E. (1989). Loss of proliferative potential during terminal differentiation coincides with the decreased abundance of a subset of hnRNP proteins. *J. Cell Biol. 109:* 1937-1946.

MINTZ, B. and ILLMENSEE, L. (1975). Normal genetically mosaic mice produced from malignant teratocarcinoma cells. *Proc. Natl. Acad. Sci. USA 72:* 3585-3589.

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

PITTELKOW, M.R. and SCOTT, R.E. (1986). New techniques for the *in vitro* culture of human skin keratinocytes and perspectives on their use for grafting of patients with extensive burns. *Mayo Clin. Proc. 62:* 771-777.

PITTELKOW, M.R., WILLE, J.J., Jr. and SCOTT, R.E. (1986). Two functionally distinct classes of growth arrest states in human prokeratinocytes that regulate clonogenic potential. *J. Invest. Dermatol. 86:* 410-417.

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.

QUARONI, A., WANDS, J., TRELSTAD, R. and ISSELBACHER, K. (1979). Epithelioid cell cultures from rat small intestine. *J. Cell Biol. 80:* 248-265.

RHEINWALD, J.G. and BECKETT, M.A. (1981). Tumorigenic keratinocyte lines requiring anchorage and fibroblast support cultured from human squamous cell carcinomas. *Cancer Res. 41:* 1657-1663.

ROSENGARD, A.M., KRUTZSCH, H.C., SHEARN, A., BIGGS, J.R., BARKER, E., MARGULIES, I.M.K., KING, C.R., LIOTTA, L.A. and STEEG, P.S. (1989). Reduced Nm23/*awd* protein in tumor metastasis and aberrant *Drosophila* development. *Nature 342:* 177-180.

SACHS, L. (1978). Control of normal cell differentiation and the phenotypic reversion of malignancy in myeloid leukemia. *Nature 274:* 535-539.

SAMID, D., CHANG, E.H. and FRIEDMAN, R.M. (1985). Development of a transformed phenotype induced by a human *ras* oncogene as inhibited by Interferon. *Biochem. Biophys. Res. Commun. 126:* 509-516.

SAMID, D., FLESSATE, D. and FRIEDMAN, R.M. (1987). Interferon-induced revertants of *ras*-transformed cells: resistance to transformation by specific oncogenes and retransformation by 5-Azacytidine. *Mol. Cell. Biol 7:* 2196-2200.

SCOTT, R.E. and MAERCKLEIN, P.B. (1985). An initiator of carcinogenesis selectively and stably inhibits stem cell differentiation: a concept that initiation of carcinogenesis involves multiple phases. *Proc. Natl. Acad. Sci. USA 82:* 2995-2999.

SCOTT, R.E., EDENS, M., ESTERVIG, D.N., FILIPAK, M., HOERL, B.J., HSU, B.M., MAERCKLEIN, P.B., MINOO, P., TZEN, C-Y., WILKE, M.R. and ZSCHUNKE, M.A. (1988a). Cellular differentiation and the prevention and treatment of cancer. In *The*

*Status of Differentiation Therapy of Cancer* (Eds. S. Waxman, G.B. Rossi and F. Takaku). Raven Press, New York, pp. 3-16.

SCOTT, R.E., ESTERVIG, D.N., TZEN, C-Y., MINOO, P., MAERCKLEIN, P.B. and HOERL, B.J. (1989). Nonterminal differentiation represses the neoplastic phenotype in spontaneously and simian virus 40-transformed cells. *Proc. Natl. Acad. Sci. USA 86:* 1652-1656.

SCOTT, R.E., FLORINE, D.L., WILLE, J.J., Jr. and YUN, K. (1982a). Coupling of growth arrest and differentiation at a distinct state in the $G_1$ phase of the cell cycle: GD. *Proc. Natl. Acad. Sci. USA 79:* 845-849.

SCOTT, R.E., HOERL, B.J., WILLE, J.J., Jr., FLORINE, D.L., KRAWISZ, B.R. and YUN, K. (1982b). Coupling of proadipocyte growth arrest and differentiation. II. A cell cycle model for the physiological control of cell proliferation. *J. Cell Biol. 94:* 400-405.

SCOTT, R.E., PFEFFER, L. and TZEN, C-Y. (1993). Di

SCOTT, R.E., WILKE, M.S., WILLE, J.J., Jr., PITTELKOW, M.R., HSU, B.M. and KASPERBAUER, J.L. (1988b). Human squamous carcinoma cells express complex defects in the control of proliferation and differentiation. *Am. J. Pathol. 133:* 374-380.

SCOTT, R.E., YUN, K. and FLORINE, D.L. (1983). Differential mitogenic effects of methyl isobutyl xanthine and a tumor growth factor on $G_1$ arrested 3T3 T proadipocytes at the predifferentiation GD state and the growth-factor deficiency GS state. *Exp. Cell Res. 143:* 405-414.

SHIPLEY, G.D., PITTELKOW, M.R., WILLE, J.J., Jr., SCOTT, R.E. and MOSES, H.L. (1986). Reversible inhibition of normal human prokeratinocyte proliferation by type-ß transforming growth factor-growth inhibitor in serum-free medium. *Cancer Res. 46:* 2068-2071.

SPARKS, R.L. and SCOTT, R.E. (1986). Transforming growth factor type-ß is a specific inhibitor of 3T3 T mesenchymal stem cell differentiation. *Exp. Cell Res. 165:* 345-352.

SPARKS, R.L., SEIBEL-ROSS, E.I., WIER, M.L. and SCOTT, R.E. (1986). Differentiation, dedifferentiation, and transdifferentiation of BALB/c 3T3 T mesenchymal stem cells: Potential significance in metaplasia and neoplasia. *Cancer Res. 46:* 5312-5319.

STANBRIDGE, E.J., DER, C.J., DOERSEN, C-J., NISHIMI, R.Y., PEEHL, D.M., WEISSMAN, B.E. and WILKINSON, S.E. (1982). Human cell hybrids: analysis of transformation and tumorigenicity. *Science 215:* 252-259.

TZEN, C-Y. and SCOTT, R.E. (1990). Differentiation can convey resistance to transformation by activated *ras* oncogene. *Anticancer Res. 10:* 1329-1334.

TZEN, C-Y., FILIPAK, M. and SCOTT, R.E. (1990). Metaplastic change in mesenchymal stem cells induced by activated *ras* oncogene. *Am. J. Pathol. 137:* 1091.

WANG, H. and SCOTT, R.E. (1992). Induction *c-jun* independent of PKC activation, pertussis toxin-sensitive G protein and polyamines in quiescent SV40-transformed 3T3T cells. *Exp. Cell Res. 203:* 47-55.

WANG, H. and SCOTT, R.E. (1991). Insulin-induced mitogenesis associated with transformation by the SV40 large T antigen. *J. Cell Physiol. 147:* 102-110.

WANG, H., WANG, J-Y., JOHNSON, L.R. and SCOTT, R.E. (1991). Selective induction of *c-jun* and *jun-B* but not *c-fos* or *c-myc* during mitogenesis in SV40-transformed cells at the predifferentiation growth arrest state. *Cell Growth Differ. 2:* 645-652.

WEINTRAUB, H., DAVID, R., TAPSCOTT, S., THAYER, M., KRAUSE, M., BENEZRA, R., BLACKWELL, T.K., TURNER, D., RUPP, R., HOLLENBERG, S., ZHUANG, Y. and LASSAR, A. (1991). The myoD gene family: nodal point during specification of the muscle cell lineage. *Science 251:* 761-766.

WIER, M.L. and SCOTT, R.E. (1985). Defective control of terminal differentiation and its role in carcinogenesis in the 3T3 proadipocyte stem cell line. *Cancer Res. 45:* 3339-3346.

WIER, M.L. and SCOTT, R.E. (1986a). Regulation of the terminal event in cellular differentiation. Biological mechanisms of the loss of proliferative potential. *J. Cell Biol. 102:* 1955-1964.

WIER, M.L. and SCOTT, R.E. (1986b). Aproliferin — A human plasma protein that induces the irreversible loss of proliferative potential associated with terminal differentiation. *Am. J. Pathol. 125:* 546-554.

WIER, M.L. and SCOTT, R.E. (1987). Polypeptide changes associated with loss of proliferative potential during the terminal event in differentiation. *J. Cell. Biochem. 33:* 137-150.

WILKE, M.S., EDENS, M. and SCOTT, R.E. (1988a). Ability of normal human keratinocytes that grow in culture in serum-free medium to be derived from suprabasal cells. *J. Natl. Cancer Inst. 80:* 1299-1304.

WILKE, M.S., HSU, B.M. and SCOTT, R.E. (1988b). Two subtypes of reversible cell cycle restriction points exist in cultured normal human keratinocyte progenitor cells. *Lab. Invest. 58:* 660-666.

WILKE, M.S., HSU, B.M., WILLE, J.J., Jr., PITTELKOW, M.R. and SCOTT, R.E. (1988c). Biologic mechanisms for the regulation of normal human keratinocyte proliferation and differentiation. *Am. J. Pathol. 131:* 171-181.

WILLE, J.J., Jr. and SCOTT, R.E. (1986). Suppression of tumorigenicity by the cell-cycle-dependent control of cellular differentiation and proliferation. *Int. J. Cancer 37:* 875-881.

WILLE, J.J., Jr., MAERCKLEIN, P.B. and SCOTT, R.E. (1982). Neoplastic transformation and defective control of cell proliferation and differentiation. *Cancer Res. 42:* 5139-5146.

WILLE, J.J., Jr., PITTELKOW, M.R. and SCOTT, R.E. (1985). Normal and transformed human prokeratinocytes express divergent effects of a tumor promoter on cell cycle-mediated control of proliferation and differentiation. *Carcinogenesis 6:* 1181-1187.

WILLE, J.J., Jr., PITTELKOW, M.R., SHIPLEY, G.R. and SCOTT, R.E. (1984). Integrated control of growth and differentiation of normal human prokeratinocytes cultured in serum-free medium: clonal analyses, growth kinetics, and cell cycle studies. *J. Cell. Physiol. 121:* 31-44.

YUN, K. and SCOTT, R.E. (1983). Biological mechanisms of phorbol myristate acetate-induced inhibition of proadipocyte differentiation. *Cancer Res. 43:* 88-96.

YUN, K., HOERL, B.J. and SCOTT, R.E. (1983). Efficient differentiation of proadipocyte stem cells on nonadherent surfaces: evidence for differentiation without DNA synthesis. *J. Cell. Physiol. 117:* 249-256.