Int. J. Dev. Biol. 35: 389-397 (1991)

Review

The F9-EC cell line as a model for the analysis of differentiation

ANGEL ALONSO*, BEATE BREUER, BARBARA STEUER and JÜRGEN FISCHER

Deutsches Krebsforschungszentrum, Heidelberg, Germany

CONTENTS

***Address for reprints**: Deutsches Krebsforschungszentrum, Im Neuenheimer Feld-280, D-6900 Heidelberg 1, Germany. FAX: 6221-401.271.

0214-6282/91/$03.00

Characteristics of the F9 cell line

Differentiation is a process characterized by a reorganization of gene expression which results in the appearance of a new phenotype. Between undifferentiated cells and their differentiated counterparts, it is possible to postulate an intermediate stage, the committed cells. In this stage, the cells have already determined the differentiated phenotype they should adopt, but this phenotype is still not recognizable. The characterization of the commitment stage can only be made, therefore, *a posteriori*.

Mouse teratocarcinomas are tumors which can be experimentally induced by ectopically implanting embryos in the peritoneal cavity or the testis. Embryonal carcinoma cells (EC-cells) are the stem cells of teratocarcinomas, cells which still retain their capacity to differentiate into derivatives of one or more embryonic layers. For this reason, EC-cells are considered to be similar to embryonic cells, and are able to develop *in vitro* some of the early embryonic developmental stages (Martin, 1980). Some EC-cells can differentiate into several tissues and are able to take part in the normal embryo development when injected into blastocysts, whereas others are unable to do so. The F9 cell line is characterized by the inability to differentiate spontaneously, and is therefore a so-called nullipotent cell line. Nevertheless, upon treatment with several agents, F9 cells differentiate into endodermal-like derivatives, depending on the culture conditions and the type of agent added to the culture medium (Strickland and Mahdavi, 1978; Strickland *et al.*, 1980; Hogan *et al.*, 1983; Steuer *et al.*, 1990).

The F9 cell line was isolated by Bernstine *et al.* (1973) as a subline of the teratocarcinoma OTT6050, established by implanting a six-day-old embryo in the testis of a 129/J mouse (Stevens, 1970). When cultured in gelatinized dishes, the cells grow adherent forming foci composed of tightly packed polygonal cells of small size and prominent nuclei (Fig.1).

The F9 cell line has been widely used in many laboratories during the last decades as a model for the analysis of the molecular mechanisms of differentiation (Lehtonen *et al.*, 1989). This has been facilitated by the fact that their karyotype has remain fairly stable during this time. The cells cultured in our laboratory are pseudodiploid, with 39 chromosomes, 38 acrocentric and 1 metacentric, produced by a Robertsonian translocation (Rb(2.8)). This chromosome is characteristic for the F9 cell line and can be used as a marker in experiments involving cell fusion. Furthermore, the cells possess only one sex-chromosome (karyotype 39,X0), a monosomy of chromosome 8 and an insertion of unknown origin at chromosome 4 (Fig.2), being therefore slightly different from the cells karyotyped by Hogan *et al.* (1986).

The F9 cells grow very rapidly with a doubling time of about 8-10 hours in the exponentially growing phase. The cell cycle is characterized by a very short G1 phase and an S-phase of about 8 hours (Rosenstrauss *et al.*, 1982). Induction of differentiation produces a lengthening of the G1 phase and a decrease in the proliferation fraction visible from 30 hours after addition of the differentiating agent. This lengthening finally results in growth cessation and death, characteristic for differentiated cells (Linder *et al.*, 1981).

Differentiation of the F9 cells

As already mentioned, F9 differentiate spontaneously only rarely. Nevertheless the addition of retinoic acid (RA) and dibutyryl cAMP (dbcAMP) to cells growing under adherent conditions triggers the adoption of a new phenotype, with changes in gene expression involving an increased synthesis of tissue-type plasminogen activator, extracellular matrix proteins (laminin, entactin and collagen type IV), cytokeratins, low levels of alkaline phosphatase activity, and the disappearance of the stage-specific embryonic antigen (Solter and Knowles, 1978), all of which are specific markers for the extraembryonic parietal endoderm (Fig. 3; Sherman and Miller, 1978; Strickland *et al.*, 1980; Hogan *et al.*, 1983). Morphologically, the cells are now more rounded, have moved apart from each other and some of them occasionally display large processes resembling neural derivatives (Fig.4). This kind of differentiation seems to be determined very soon after RA treatment, since culture of the cells for 8 hours in the presence of the inducer is sufficient to promote their irreversible differentiation (Levine *et al.*, 1984; Dong *et al.*, 1990). Treatment with RA alone triggers the cells to differentiate into a primitive endoderm-like stage, which may be subsequently modulated into either parietal or visceral endoderm (Hogan *et al.*, 1981). Careful analysis of the primitive-endoderm-like cells allows one to distinguish two different cell populations: one still conserves a polygonal morphology and the cells have the tendency to remain in small groups attached to each other; the second population shows cells with large cytoplasmic processes, separated from each other and adopting sometimes a neuron-like morphology (Moore *et al.*, 1986). Analysis of the expression of the markers reveals that both phenotypes express the endoderm-type markers differently. Cytokeratins are demonstrable earlier in the former population, which also shows large amounts of intracellularly located laminin. In contrast, most of the synthesized laminin is found extracellularly in the second population (Breuer, 1989). Whether both types of cell are able to differentiate into visceral endoderm is not known, but addition of dbcAMP to the culture medium allows the distinction of only one differentiated cell-type population, suggesting that both primitive endoderm-like types are able to follow the same differentiation pathway further.

Culture of small clumps of cells in bacteriological dishes generates the appearance of aggregates growing in suspension. Addition of RA to the medium leads the cells located at the outer surface of the aggregates to differentiate into visceral endoderm, as demonstrated by the synthesis of alpha-fetoprotein (AFP) (Hogan *et al.*, 1981). This seems to be a reversible process, since after seeding the aggregates on gelatinized dishes in the presence of dbcAMP the synthesis of AFP ceases with the subsequent appearance of

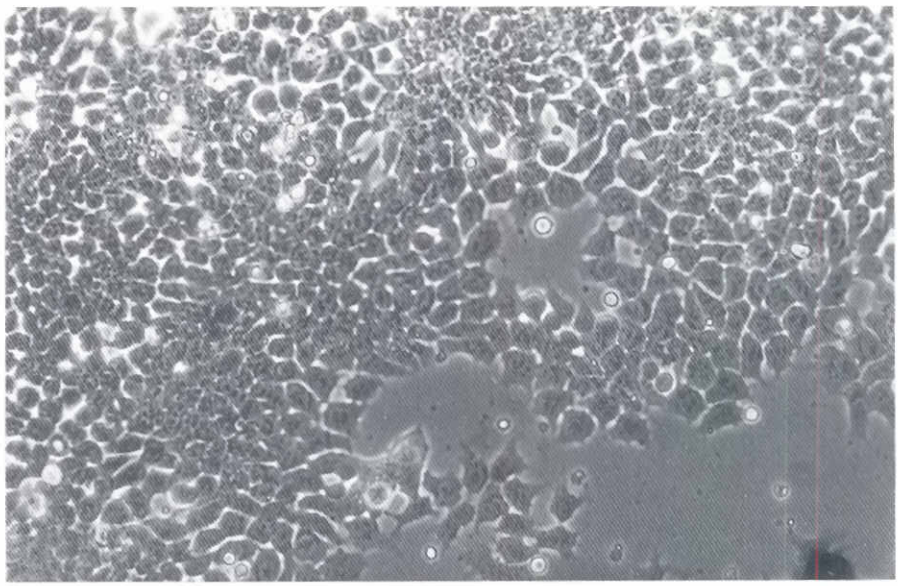

Fig. 1. Morphology of exponentially growing F9 cells *(x 200).*

markers typical for parietal endoderm. Thus, it seems that whereas the parietal endoderm pathway is irreversible, the visceral pathway can be reversed according to the culture conditions employed (Grover and Adamson, 1986).

Treatment with 10^{-6} retinoic acid and 10^{-3} dbcAMP differentiates more than 95% of the cells into parietal endoderm. The first morphological changes can be observed 24 hours after addition of both inducers and the fully differentiated phenotype is observed 72 hours after. The differentiated cells are able to grow further for several days; this growth slows down in the next days until no proliferation is observed in the fully differentiated cells. At this point, the differentiated cells begin to be overgrown by a small proportion of undifferentiated cells, which were refractory to the action of RA and which will colonize the culture dish completely; retreatment of these cells with RA triggers the differentiation program resulting in a large proportion of differentiated cells and again a small number of refractory cells.

The induction of differentiation

Besides RA/dbcAMP other agents have been used to differentiate F9 cells *in vitro*. N'-N'-dimetylacetamide and 5-bromodeoxyuridine have been used as differentiating agents (Moore *et al.*, 1986) and it has also been observed that in the presence of the fluorescent dye Hoechst 33342 the F9 cells stop growth and express markers characteristic of parietal endoderm (Steuer *et al.*, 1990).

RA-treated F9 cells sometimes adopt a neural-like morphology, with large processes which seem to contact the neighboring cells. Although it has been reported that these cell types express characteristics of nerve cells, this has not been confirmed in later studies using the same differentiation protocol (Liesi *et al.*, 1983; Tienari *et al.*, 1987). F9 cells plated at clonal density in the presence of STO-conditioned medium and RA differentiate into derivatives of all three germ layers, suggesting that they may in fact be pluripotent (Koopman and Cotton, 1986, 1987). A similar conclusion can be drawn from the experiments of Kellermann *et al.* (1987), who demonstrated that F9 cells transfected with a recombinant containing the SV40 large T antigen driven by the E1A promoter are able to differentiate into derivatives of all three germ layers when injected into animals, the differentiated cells being immortalized. Thus it seems probable that the F9 cells can differentiate into derivatives of all three germ layers, their differentiation potential therefore not being restricted to endodermal-derivatives. This differentiation pattern seems to be dependent on the culture conditions, since only one phenotype is observed when the cells are plated at normal or high densities.

Differentiation has also been attained after transfection of c-fos sequences into undifferentiated F9 cells (Müller and Wagner, 1984). c-fos-transfected cells express characteristics typical for parietal endoderm and have lost their proliferative capacity, subsequently dying similarly to RA-treated cells. Nevertheless, the fact that only some of the parietal endoderm markers are expressed in some of the cells argues in favour of a different differentiation pathway from that followed by RA, or for a mechanism intrinsic to the F9 cell line, not directly correlated with the process of induction of differentiation. This conclusion is supported by results showing that transfection of F9 cells with antisense c-fos sequences only partially inhibits differentiation to endoderm (Edwards *et al.*, 1988).

Furthermore, F9 cells transfected with c-myc sequences cloned in the anti-sense position also express parietal-endoderm-like characteristics. It seems that transcription of these sequences allows neutralization of the endogenous c-myc RNA sequences resulting in development of a differentiation program, in accordance with the finding that c-myc is down-regulated shortly after treating F9 cells with RA/cAMP (Griep and De Luca, 1986, 1988) and that this down-regulation may play an important role in the process of differentiation. These results remain controversial, as other authors have been unable to reproduce these results (Nishikura *et al.*, 1990). It seems likely therefore that expression of some oncogenes known to have a role in the control of proliferation is sufficient to trigger the cells into a differentiated stage. Experiments involving transfection of H-ras sequences further corroborate this possibility (Yamaguchi-Iwai *et al.*, 1990). Controversial results have been also obtained after transfection of the E1a gene of adenovirus. Whereas some authors described a clear effect on differentiation, others claim the contrary, the E1a gene products being sufficient to stop

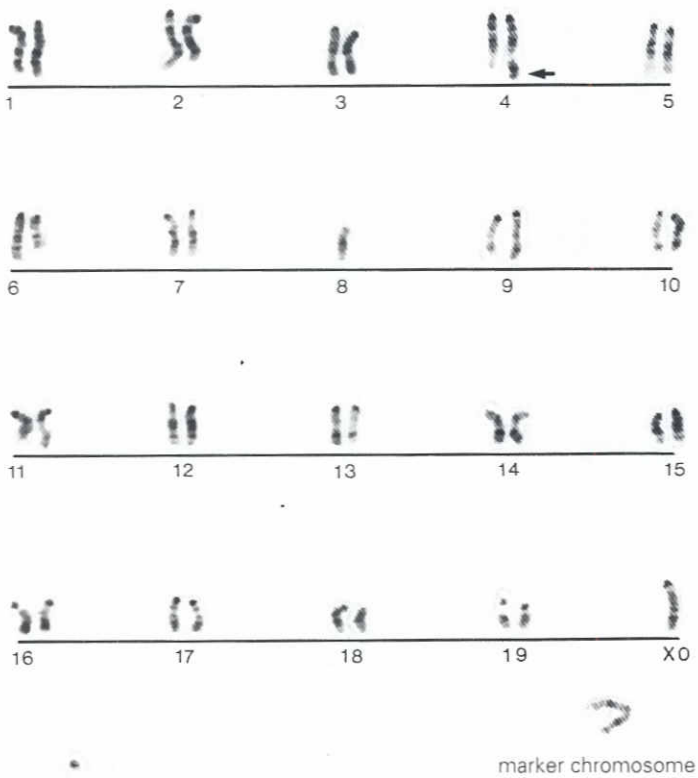

Fig. 2. G-banded karyotype of F9 cells. *Exponentially growing F9 cells were treated with Colcemid, spread on glass slices and stained with Giemsa stain after Trypsin treatment. Typical for F9 cells is the presence of a metacentric marker chromosome and an insertion on chromosome 4 (arrow). A small percentage of the cells contain a minute chromosome (see bottom left) of unknown origin.*

or even reverse F9 cell differentiation (Montano and Lane, 1987; Young *et al.*, 1989). A critical point common to all these experiments remains the fact that the differentiation stage is deduced from the analysis of only a few markers, making it difficult to assess the true differentiated stage of the transfected cells in an unambiguous manner.

Gene expression and differentiation

Differentiation of F9 cells is accompanied by a reorganization of gene expression with the synthesis of new products and the shut-off of others. Although a great number of gene products which change their expression after differentiation to PE or VE have been identified, most if not all of these products are the results of the differentiation rather than their cause (Wang *et al.*, 1985). So far, no genes have been isolated which can be made responsible for the launch of the differentiation program. Table 1 shows those gene products identified so far whose expression is modulated during differentiation to endodermal derivatives.

One strategy for identifying genes that may be directly involved in the control of differentiation is to search for sequences with a differential expression at the first stages of RA-treatment. Unfortunately, very few gene products have been identified during the first 8 hours of RA-treatment, a time that has been considered as the commitment period. Transcription of the myc oncogene has been shown to drop shortly after addition of RA (Griep and De Luca, 1986) but no direct evidence exists that may link the action of the myc-protein to the launch of differentiation, despite the fact that neutralizing the c-myc RNA results in cell differentiation (Griep and De Luca, 1988). Similarly, a small increase in the synthesis of c-fos with a maximum at 60 minutes after RA-treatment has been reported (Manson *et al.*, 1985). It may very well be that both oncogenes turn on a cascade of events, one of which is connected in some way to channels leading to differentiation but without having a direct, specific effect on the initial launch of differentiation. Also, very early after RA-treatment a homeobox-containing sequence, the Era1 gene, shows a strong transcriptional stimulation (La Rosa and Gudas, 1988). This sequence seems to be related to the homeobox-containing sequences already described as modulating their expression at late times during F9 differentiation (Colberg-Poley *et al.*, 1985a,b). Interestingly, all these sequences are known by their DNA-binding properties, and their role, therefore, may rely on the modulation of transcription of responsible genes, but the question remains open whether these transacting factors act on the primary target point(s) responsible for the start of differentiation.

The action of RA on differentiation is probably mediated by the RA-receptors (RARs) present in the nucleus of most cells. This action is carried out through the binding of the receptors to DNA sequences located in the promoter regions of genes responsive to RA. Thus it seems probable that treatment with RA produces the activation of a series of genes containing RA-responsive elements. Such elements have been located in the promoter regions of genes whose transcription is modulated by RA, such as laminin, ß-RAR or histone H1° (Alonso *et al.*, 1988; Vasios *et al.*, 1991; Breuer *et al.*, 1989; De The *et al.*, 1990). The interpretation of these results is nevertheless complicated by the fact that undifferentiated F9 cells contain alpha- and gamma-receptors and that their number remains constant during RA treatment. Only the ß-RAR increases their steady-state in RA-treated cells, reaching a 20-fold induction 48 hours after RA-addition. It may be speculated that treatment with RA not only controls transcription of the RARs but perhaps secondarily modifies pre-existing receptors, allowing them to become active immediately after addition of the inducer. Another possibility is that RA after being metabolized in the cell triggers the synthesis of other products which follow a different pathway than the receptors.

Curiously, RA-responsive elements have been found to date in gene products whose expression takes place rather late during differentiation, with the exception of Histone H1°. The reason for this behavior is completely unknown. It may be argued that the affinity of the RARs for the RA-responsive elements analyzed is very low, and that some time is needed until the receptors increase to a large number and are able to bind to the element. Nevertheless, analyses of the effect of RA on the number of receptors do not support this point of view. Another possibility is that the transcription of the RA-element-containing genes is not only dependent on the presence of receptors but also on other transcription factors that are synthesized later during differentiation. Although no experimental evidence exists to prove this point, the tremendous complexity of the promoter regions of some RA-regulated genes (like H1° gene) argues in favor of this interpretation.

Most of the sequences differentially transcribed in RA-treated cells and identified so far account for the specific markers of

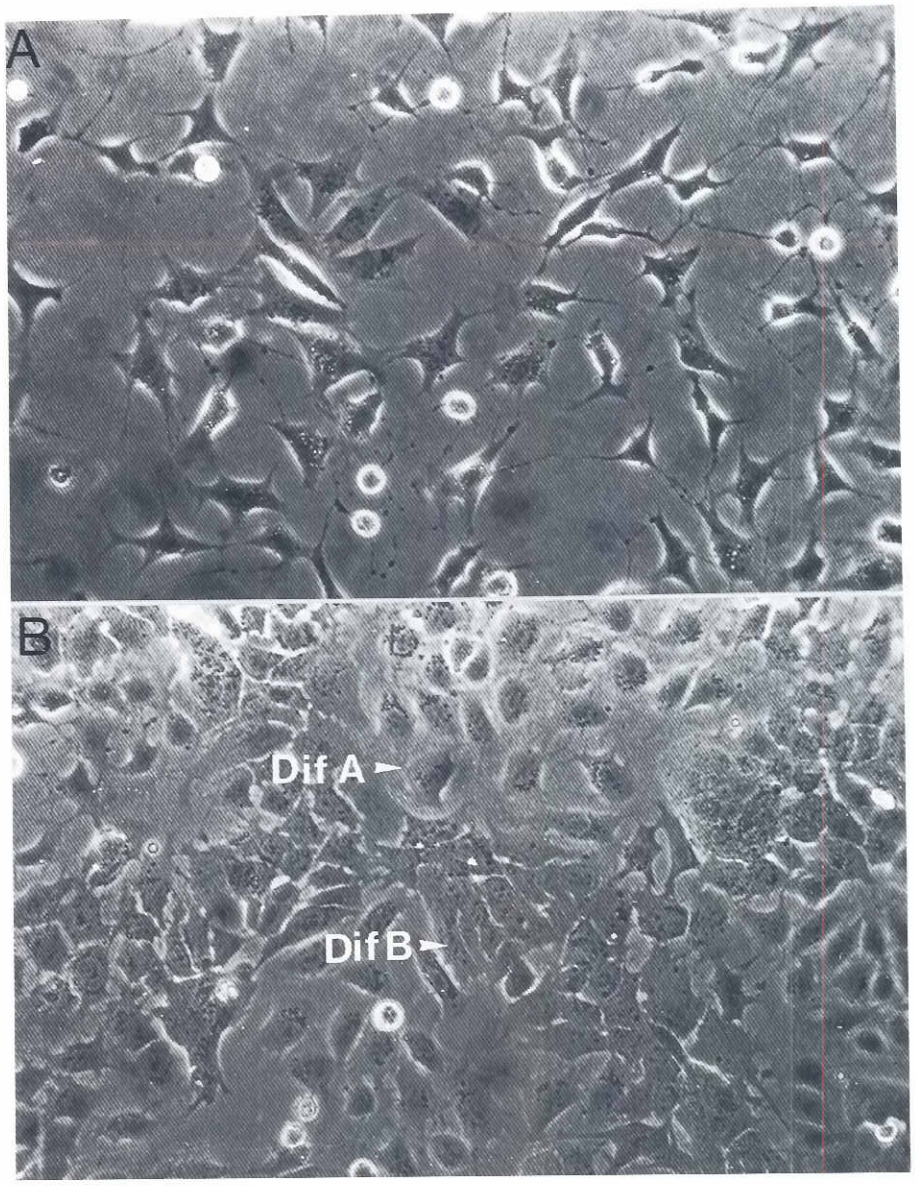

Fig. 3. Morphology of *in vitro* differentiated F9 cells. *F9 cells were seeded at an initial density of 600.000 cells/10 cm plate on gelatinized tissue culture dishes. Cells were treated either with RA and dbcAMP (A) or with RA alone at final concentrations of 10^{-7} and 10^{-3} respectively. The cells were passaged every second day during the differentiation time. DifA and DifB gave the two differentiated phenotypes appearing during treatment with RA as described in the text (x 200).*

endoderm. A large number of the components of Reichert's membrane are present in differentiated cells, but their expression is not always homogeneously distributed, independent of the differentiation protocol used. The role of cAMP is not fully understood, because together with reports showing that the role of cAMP is only to enhance the effect of RA, others have been published indicating that cAMP *per se* is able to differentiate F9 cells. Nevertheless, it seems probable that the only effect of cAMP is to modulate the response of the cells. Furthermore, the same biological result can be obtained when the intracellular concentration of cAMP is raised without addition of exogenous cAMP. The fact that cAMP is in fact able to modulate differentiation either to parietal or visceral endoderm makes it a key point, at least in the pathway to be followed during differentiation in suspension cultures. The cellular response concerning the number of markers synthesized is nevertheless related to the differentiation protocol used. Whereas treatment with RA alone triggers the production of a well-developed cytoskeleton in F9 cells, addition of dbcAMP to the culture medium reduces the proportion of cells expressing cytokeratins 8 and 18, which are responsible for the production of the intermediate filaments. Similarly, the cells down-regulate the increased synthesis of ß-RAR observed after RA-treatment when dbcAMP was included in the differentiation protocol. Thus it is evident that cAMP is able to modulate individually the gene expression of different sequences.

Retroviral promoters are negatively regulated in F9 cells

An interesting property of the F9 cells is their inability to use viral promoters efficiently. Differentiation of the cells releases this block and restores transcription of the viral sequences (Kelly and Condamine, 1982). This characteristic is even more pronounced in retroviruses. Although retroviruses efficiently integrate into the

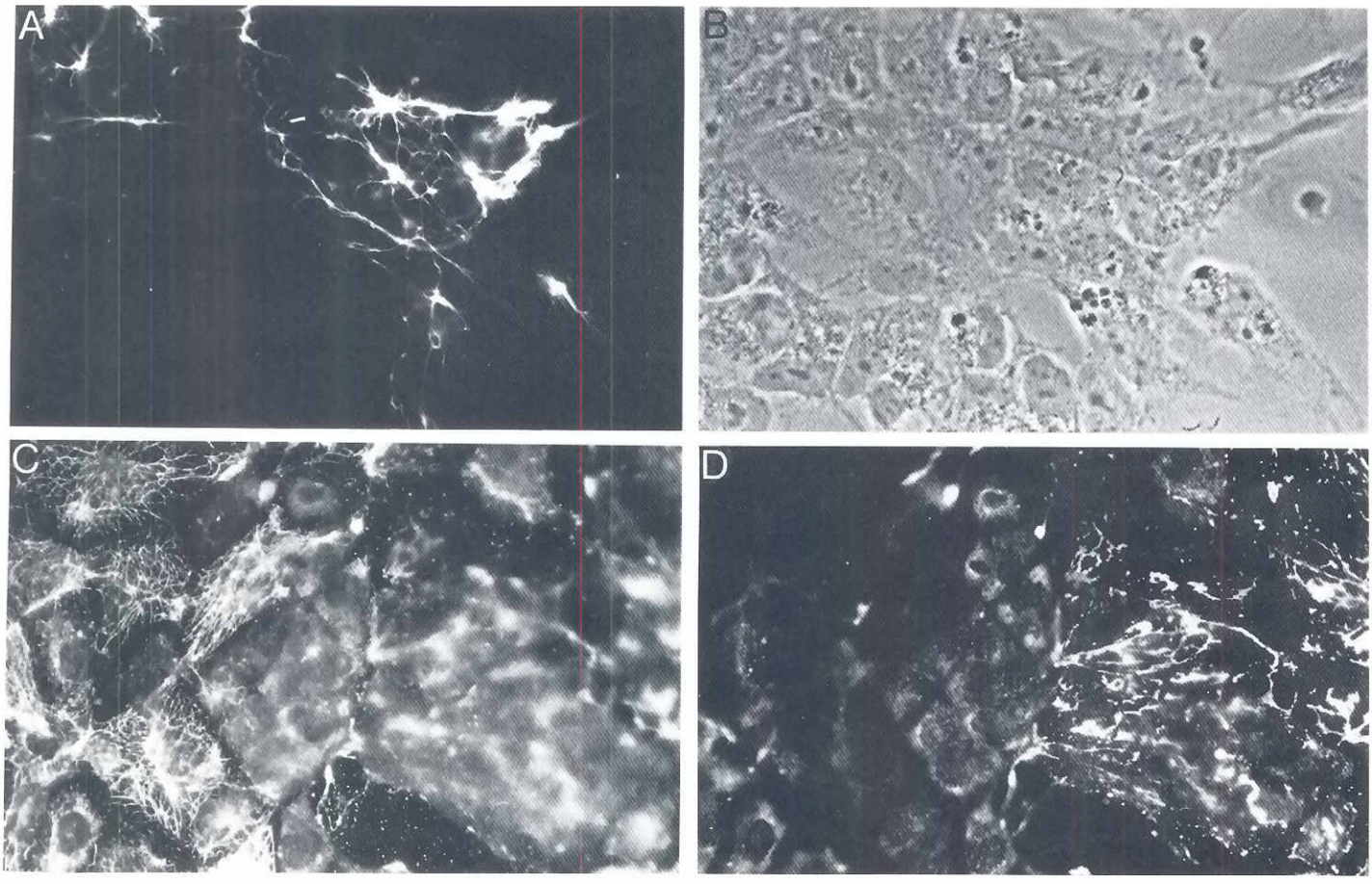

Fig. 4. Immunolocalization of cytokeratins and laminin during differentiation. *Cells were treated for 3 days with RA and dbcAMP* **(A,B)** *or for 5 days with RA alone* **(C,D)** *using the culture conditions as described in Fig. 1. The cells were fixed and stained with antibodies specific for cytokeratins (A and C) or laminin (D) using indirect immunofluorescence. (B) is the phase contrast picture to (A). (x 1500).*

genome of EC-cells, the proviruses are transcriptionally inactive. This effect seems to be due to an inefficient function of the enhancer, since mutations in this region relieve the block and allow transcription in undifferentiated F9 cells (LaThange and Rigby, 1987, 1988; see also Silver *et al.*, 1983). This block relies at least partially on the existence of transacting factors — absent in F9 but existent in their differentiated counterparts — which are able to bind to the enhancer region of the LTR (Speck and Baltimore, 1987).

Similarly, other promoters are also very inefficient in undifferentiated F9 cells (Sleigh, 1985; see also Silver *et al.*, 1983). For example, the early promoter of SV40 as well as of polyoma is only weakly used in F9 cells, whereas differentiation of the cells produces an effect similar to that observed for retroviral promoters.

The methylation degree of some promoter sequences also seems to play a role in controlling gene expression. Methylation has been associated with a repressed state of the gene, sequences which are actively transcribed being undermethylated in relation to their inactive counterparts. Transfected retroviral sequences are rapidly methylated in early mouse embryos and in ES cells. This methylated stage is accompanied by an inactivation of transcription. Nevertheless, whether this methylation is the cause of the repression still remains to be elucidated. Experimental evidence indicates that methylation accompanies repression but is not its primary cause (Stewart *et al.*, 1982; Gautsch and Wilson, 1983).

The aforementioned results support the general conclusion that the F9 cells are restricted in their transcriptional properties and mimic the situation found in early embryonic cells. This conclusion is supported by the finding that several transcription factors are absent in undifferentiated cells, in a similar manner to early embryonic cells, but appear after differentiation. So, a PEA2 and a PEA1 activity (similar to the human AP1 activity) have been found in differentiated but not in stem cells, despite the fact that fos-protein has been identified in untreated F9 cells (Kryszke *et al.*, 1987; Cáceres *et al.*, 1990). In this context, it should be mentioned that transfection of c-jun sequences into P19 teratocarcinoma stem cells triggers differentiation, but whether this is also the case in F9 cells remains to be demonstrated (De Groot *et al.*, 1990). A series of oct-binding activities has also been described in F9 cells and their synthesis has been shown to be developmentally regulated (Lenardo *et al.*, 1989; Schöler *et al.*, 1991 and references therein). Interestingly, whereas some general transcription factors are present in F9 cells, like SP1 or AP2 activity, others like NF1, are missing. Thus, F9 cells also represent a well-suited system for experiments

TABLE 1

IDENTIFIED GENE PRODUCTS WITH REGULATED GENE EXPRESSION DURING F9 DIFFERENTIATION

	F9	F9 diff.
Alkaline phoshatase (Bernstine *et al.*, 1973)	+	-
Apolipoprotein E (Baasherruddin *et al.*, 1987)	+	-/++*
Clones with reduced expression (Levine *et al.*, 1984)	+	-
Cytokeratins 8 and 18 (Oshima, 1981)	-	+
EGF receptors (Adamson and Hogan, 1984)	+	++
Entactin (Cooper *et al.*, 1983)	-	+
Era-1 (La Rosa and Gudas, 1988)	-	+
Fetomodulin (Imada *et al.*, 1990)	-	+
H-2Kd (Knowles *et al.*, 1980)	-	+
Hox-1.3 (Murphy *et al.*, 1988)	+	++
Laminin (Grover *et al.*, 1987)	+	++
Lamins A and C (Lebel *et al.*, 1987)	-	+
Oncogene expression (Sejersen *et al.*, 1985; Locket and Sleigh, 1987)	+/-*	+/-*
Parathyroid hormone-like protein (Chan *et al.*, 1990)	-	+
Proteoglycan-19 (Grover *et al.*, 1987)	-	+
Retinol-binding protein (Soprano *et al.*, 1988)	-	+
Rex-1 (Hosler *et al.*, 1989)	+	-
Sparc (Mason *et al.*, 1986)	-	+
SSEA 1 (Solter and Knowles, 1978)	+	-
ß-Interferon (Francis and Lehman, 1989)	-	+
TGFß2 (Mummery *et al.*, 1990)	-	+
Tissue type plasminogen activator (Sherman *et al.*, 1976)	-	+
Transferrin receptors (Adamson and Hogan, 1984)	+	++
Transthyretin (Soprano *et al.*, 1988)	-	+
Type IV collagen (Grover *et al.*, 1987)	-	+

F9 diff. corresponds to cells differentiated either to parietal or to visceral endoderm.
+: presence of a specific gene product
-: absence of a specific gene product
*: varying amounts

concerning the molecular mechanisms controlling gene expression at the transcriptional level.

Somatic cell genetics of the F9 cell line

A very interesting analysis of the properties of the F9 cells can be obtained from the results of cell fusion experiments. Hybrids between F9 and a mouse immortalized, parietal endoderm-like cell line, the PFHR9 cells, display a differentiated phenotype and are also immortalized (Howe and Oshima, 1982). Thus the differentiated phenotype is dominant over the EC-characteristics. The same holds true for fusion experiments involving PYS-2 cells, another mouse parietal-like permanent cell line. On the contrary, fusion experiments involving RA/dbcAMP-treated F9 cells (4-day treatment) and their undifferentiated counterparts always displayed an undifferentiated phenotype and were immortalized. This can be interpreted either as rescue of the differentiated phenotype by the F9 cells (Littlefield and Felix, 1982) or as the result of the non-viability of the fusion products between RA-treated and untreated F9 cells. Our results support the second possibility, suggesting that fusion products between RA-treated and untreated F9 cells die shortly after fusion, similarly to differentiated F9 cells (Wrede, 1989). Furthermore, micronuclei prepared from PFHR9 cells which have been superinfected with retroviruses carrying the Neomycin resistance gene were fused with undifferentiated F9 cells and the phenotype of the resulting Neomycin resistant fusion products was analyzed. Some of the resulting clones displayed all markers characteristic for parietal endoderm and karyotyping demonstrated a chromosome 4 trisomy in all of them. This trisomy was never found in the undifferentiated fusion products so far analyzed (Steuer, 1991). Furthermore, quantitation experiments following a mathematical model demonstrated that one chromosome alone cannot be sufficient for conferring the differentiated phenotype and that at least one other, as yet unidentified, chromosome must be involved. Thus these results suggest that differentiation of F9 cells is probably not accomplished by a single sequence and that this process can be switched on in hybrids in the absence of most of the differentiated cell chromosomes.

Summary

The teratocarcinoma stem cell line F9 has been widely used as a model for the analysis of molecular mechanisms associated with differentiation. This cell line has been considered to be nullipotent and able to differentiate into endodermal-like derivatives upon treatment with retinoic acid. Nevertheless, under definite culture conditions, F9 cells are able to differentiate into derivatives of all three germ layers. The F9 cells express characteristics of early mouse embryonal cells and possess all repression factors known to be present in cells of the early mouse embryogenesis. Induction of differentiation can be achieved not only by adding chemical agents to the culture medium but also by transfection of several oncogenic sequences. In somatic cell genetic experiments, immortalized, differentiated F9-like cells have been shown to express dominantly genes responsible for the appearance of the differentiated phenotype.

KEY WORDS: *teratocarcinoma cells, embryogenesis, development, cell culture, in vitro differentiation, induction of differentiation.*

Acknowledgments

This work has been supported by a grant from the Deutsche Forschungsgemeinschaft to Angel Alonso.

References

ADAMSON E. D. and HOGAN B.L.M. (1984). Expression of EGF receptor and transferrin by F9 and P13 teratocarcinoma cells. *Differentiation 27:* 152-157.

ALONSO, A., BREUER, B., BOUTERFA, H. and DOENECKE, D. (1988). Early increase in histone H1° mRNA during differentiation of F9 cells to parietal endoderm. *EMBO J. 7:* 3003-3008.

BAASHEERUDDIN, K., STEIN, P., STRICKLAND, S. and WILLIAMS, D.L. (1987). Expression of the murine alipoprotein F gene is coupled to the differentiated state of F9 embryonal carcinoma cells. *Proc. Natl. Acad. Sci., USA 84:* 709-713.

BERNSTINE, E.G., HOOPER, M.L., GRANDCHAMP, S. and EPHRUSSI, B. (1973). Alkaline phosphatase activity in mouse teratomas. *Proc. Natl. Acad. Sci. USA 70:* 3899-3903.

BREUER, B. (1989). Analyse der Genregulation in der F9 Teratokarzinomazellinie im undifferenztierten sowie differenztierten Zustand. Retrovirale Vektoren und das endogene Histon H1° Gen. Ph. D. Thesis. Universität Heidelberg.

BREUER, B., FISCHER, J. and ALONSO, A. (1989). Cloning and characterization of the mouse histone H1° promoter region. *Gene 81:* 307-314.

CHAN, S.D.H., STREWLER, G.J., KING, K.L. and NISSENSON, R.A. (1990). Expression of a parathyroid hormone-like protein and its receptor during differentiation of embryonal carcinoma cells. *Mol. Endocrinol. 4:* 638-646.

COLBERG-POLEY, A.M., VOSS, S., CHOWDHURY, K. and GRUSS, P. (1985a). Structural analysis of murine genes containing homeobox sequences and their expression in embryonal carcinoma cells. *Nature 314:* 731-738.

COLBERG-POLEY, A.M., VOSS, S.D. and GRUSS, P. (1985b). Structure and expression of two classes of mammalian homeobox-containing genes. Cold Spring Harbor Symposia on Quantitative Biology. Vol. 50. Cold Spring Harbor, New York, pp. 285-290.

COOPER, A.R., TAYLOR, A. and HOGAN, B.L.M. (1983). Changes in the rate of laminin and entactin synthesis in F9 embryonal carcinoma cells treated with retinoic acid and cyclic AMP. *Dev. Biol. 99:* 510-516.

CÁCERES, J., GLIKIN, G., BRAVO, R. and WEINMANN, R. (1990). c-fos mediated stimulation of sn AP-1 DNA binding activity in undifferentiated teratocarcinoma cell lines. *Oncogene 5:* 59-67.

De GROOT, R.P., KRUYT, F.A.E., van der SARG, P.T. and KRUIJER, W. (1990). Ectopic expression of c-jun leads to differentiation of P19 embryonal carcinoma cells. *EMBO J. 9:* 1831-1837.

De THE, H., VIVANCO-RUIZ, M.M., TIOLLAIS, P., STUNNENBERG, H. and DEJEAN, A. (1990). Identification of a retinoid acid responsive element in the retinoic acid receptor ß gene. *Nature 343:* 177-180.

DONG, J.M., LI, F. and CHIU, J.F.(1990). Induction of F9 cell differentiation by transient exposure to retinoic acid. *Biochem. Biophys. Res. Commun. 170:* 147-152.

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

FRANCIS, M.K. and LEHMAN J.M. (1989). Control of ß-interferon expression in murine embryonal carcinoma F9 cells. *Mol. Cell. Biol. 9:* 3553-3556.

GAUTSCH, J.W. and WILSON, M.C. (1983). Delayed *de novo* methylation in teratocarcinoma suggests additional tissue-specific mechanisms for controlling gene expression. *Nature 301:* 32-37.

GRIEP, A.E. and De LUCA, H.F. (1986). Decreased c-myc expression is an early event in retinoic acid-induced differentiation of F9 teratocarcinoma cells. *Proc. Natl. Acad. Sci. USA 83:* 5539-5543.

GRIEP, A.E. and De LUCA, H.F. (1988). Antisense Myc sequences induce differentiation of F9 cells. *Proc. Natl. Acad. Sci. USA 85:* 6806-6810.

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 cAMP. *Dev. Biol. 114:* 492-503.

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

HOGAN, B.L.M., BARLOW, D.P. and TILLY, R. (1983). F9 teratocarcinomas as a model for the differentiation of parietal and visceral endoderm in the mouse embryo. *Cancer Surv. 2:* 115-140.

HOGAN, B.L.M., CONSTANTINI, F. and LACY, E. (1986). Manipulating the mouse embryo. Cold Spring Harbor Laboratory, New York.

HOGAN, B.L.M., TAYLOR, M. and ADAMSON, E.(1981). Cell interaction modulate embryonal carcinoma cell differentiation into parietal or visceral endoderm. *Nature 291:* 235-237.

HOSLER, B.A., La ROSA, G.J., GRIPPO, J.F. and GUDAS, L. (1989). Expression of Rex-1, a gene containing zinc finger motifs, is rapidly reduced by retinoic acid in F9 teratocarcinoma cells. *Mol. Cell. Biol. 9:* 5623-5629.

HOWE, W.E. and OSHIMA, R.G. (1982). Coordinate expression of parietal endodermal functions in hybrids of embryonal carcinoma and endodermal cells. *Mol. Cell. Biol. 2:* 331-337.

IMADA, S., YAMAGUCHI H. and IMADA, M. (1990). Differential expression of fetomodulin and tissue plasminogen activator to characterize parietal endoderm differentiation of F9 embryonal carcinoma cells. *Dev. Biol. 141:* 426-430.

KELLERMANN, O., BUC-CARON, M.H. and GAILLARD, J. (1987). Immortalization of precursors of endodermal, neuroectodermal and mesodermal lineages, following the introduction of the simian virus (SV40). early region into F9 cells. *Differentiation 35:* 197-205.

KELLY, F. and CONDAMINE, H. (1982). Tumor viruses and early mouse embryos. *Biochim. Biophys. Acta 651:* 105-141.

KNOWLES, B.B., PAN, S., SOLTER, D., LINNENBACH, A., CROCE, C. and HUEBNER, K. (1980). Expression of H-2, laminin and SV40T and TASA on differentiation of transformed murine teratocarcinoma cells. *Nature 288:* 615-617.

KOOPMAN, P. and COTTON, R.G.H. (1986). The response of embryonal carcinoma cells to retinoic acid depends on colony size. *Differentiation 31:* 55-60.

KOOPMAN, P. and COTTON, R.G.H. (1987). Pluripotent differentiation of single F9 embryonal carcinoma cells. *Exp. Cell Res. 168:* 567-571.

KRYSZKE, M.H., PIETTE, J. and YANIV, M. (1987). Induction of a factor that binds to the polyoma virus A enhancer on differentiation of embryonal carcinoma cells. *Nature 328:* 254-256.

La ROSA, G.J. and GUDAS, L.J. (1988). Early retinoic acid-induced F9 teratocarcinoma stem cell gene ERA-1: alternate splicing creates transcripts for a homeobox-containing protein and one lacking the homeobox. *Mol. Cell. Biol. 8:* 3906-3917.

La ROSA, G.J. and GUDAS, L.J. (1988). An early effect of retinoic acid: cloning of an mRNA (Era-1) exhibiting rapid and protein synthesis-independent induction during teratocarcinoma stem cell differentiation. *Proc. Natl. Acad. Sci. USA: 85:* 329-333.

La THANGUE, N.B. and RIGBY, W.J. (1987). An adenovirus E1a-like transcription factor is regulated during the differentiation of murine embryonal carcinoma stem cells. *Cell 49:* 507-513.

La THANGUE, N.B. and RIGBY, W.J. (1988). Trans-acting protein factors and the regulation of eukaryotic transcription. In *Transcription and Splicing* (Eds. B.D. Hames and D.M. Glover). IRL Press, Oxford, pp. 1-42.

LEBEL. S., LAMPRON, C., ROYAL, A. and RAYMOND, Y. (1987). Lamins A and C appear during retinoic acid-induced differentiation of mouse embryonal carcinoma cells. *J. Cell Biol. 105:* 1099-1104.

LEHTONEN, E., LAASONEN, A. and TIENARI, J. (1989). Teratocarcinoma stem cells as a model for differentiation in the mouse embryo. *Int. J. Dev. Biol. 33:* 105-116.

LENARDO, M.J., STAUDT, L., ROBBINS, P.,KUANG, A., MULLIGAN, R.C. and BALTIMORE, D. (1989). Repression of the IgH enhancer in teratocarcinoma cells associated with a novel octamer factor. *Science 243:* 544-546.

LEVINE, R.A., CAMPISI, J., WANG, S. and GUDAS, L. (1984). Butyrate inhibits the retinoic acid-induced differentiation of F9 teratocarcinoma stem cells. *Dev. Biol. 105:* 443-450.

LEVINE, R.A., La ROSA, G.J. and GUDAS, L.J. (1984).Isolation of cDNA clones for genes exhibiting reduced expression after differentiation of murine teratocarcinoma stem cells. *Mol. Cell. Biol. 4:* 2142-2150.

LIESI, P., RECHARDT, L. and WARTIOWAARA, J. (1983). Nerve growth factor induces adrenergic neural differentiation in F9 teratocarcinoma cells. *Nature 306:* 265-267.

LINDER, S., KRONDAHL, U., SENNERSTAM, R. and RINGERTZ, N.R. (1981). Retinoic acid-induced differentiation of F9 embryonal carcinoma cells. *Exp. Cell. Res. 132:* 453-460.

LITTLEFIELD, J.W. and FELIX, J.S. (1982). Rescue of terminally differentiating teratocarcinoma cells by fusion to undifferentiated parental cells. *Somatic Cell Genet. 6:* 743-757.

LOCKETT, R.J. and SLEIGH, M.J. (1987). Oncogene expression in differentiating F9 mouse embryonal carcinoma cells. *Exp. Cell Res. 173:* 370-378.

MANSON, I., MUROHY, D. and HOGAN, B.L.M. (1985). Expression of c-fos in parietal endoderm, amnion and differentiating F9 teratocarcinoma cells. *Differentiation 30:* 76-81.

MARTIN, G.R. (1980). Teratocarcinomas and mammalian embryogenesis. *Science 209:* 768-776.

MASON, I.J., MURPHY, D., MÜNKE, M., FRANCKE, U., ELLIOTT, R.W. and HOGAN, B.L.M. (1986). Development and transformation-sensitive expression of the *Sparc* gene on mouse chromosome 11. *EMBO J. 5:* 1831-1837.

MONTANO, X. and LANE, D.P. (1987). The adenovirus E1a gene induces differentiation of F9 teratocarcinoma cells. *Mol. Cell. Biol.7:* 1782-1790.

MOORE, E.E., MITRA, N.S. and MORITZ, E.A. (1986). Differentiation of F9 embryonal carcinoma cells. Differences in the effect of retinoic acid, 5-bromodeoxiuridine and N-N-dimethylacetamide. *Differentiation 31:* 183-190.

MUMMERY, C.L., SLAGER, H., KRUIJER, W, FEIJEN, A., FREUND, E., KOORNNEEF, I. and Van den EIJNDEN-VAN RAAIJ, A.J.M. (1990). Expression of transforming growth factor $ß_2$ during the differentiation of murine embryonal carcinoma and embryonic stem cells. *Dev. Biol. 137:* 161-170.

MURPHY, S.P., GARBERN, J., ODENWALD, W.F., LAZZARINI, R.A. and LINNEY, E. (1988). Differential expression of the homeobox gene *Hox-1.3* in F9 embryonal carcinoma cells. *Proc. Natl. Acad. Sci. USA 85:* 5587-5591.

MÜLLER, R. and WAGNER, E.F. (1984). Differentiation of F9 teratocarcinoma stem cells after transfer of c-fos proto-oncogenes. *Nature 311:* 438-442.

NISHIKURA, K., KIM, U. and MURRAY, J.M. (1990). Differentiation of F9 cells is independent of c-myc expression. *Oncogene 5:* 981-988.

OSHIMA, R.G. (1981). Identification and immunoprecipitation of cytoskeletal proteins from murine extra-embryonic endodermal cells. *J. Biol. Chem. 256:* 8124-8133.

ROSENSTRAUSS, M.J., SUNDELL, C.L. and LISKAY, R.M. (1982). Cell-cycle characteristics of undifferentiated and differentiating embryonal carcinoma cells. *Dev. Biol. 89:* 516-520.

SCHÖLER, H.R., CIESIOLKA, T. and GRUSS, P. (1991). A nexus between Oct-4 and EiA: implications for gene regulation in embryonic stem cells. *Cell 66:* 291-304.

SEJERSEN, R., SÜMEGI, J. and RINGERTZ, N.R. (1985). Expression of cellular oncogenes in teratoma-derived cell lines. *Exp. Cell Res. 160:* 19-30.

SHERMAN, M.I. and MILLER, R.A. (1978). F9 embryonal carcinoma cells can differentiate into endoderm-like cells. *Dev. Biol. 63:* 27-34.

SHERMAN, M.I., STRICKLAND, S. and REICH, E. (1976). Differentiation of early mouse embryonic and teratocarcinoma cells *in vitro:* plasminogen activator production. *Cancer Res. 36:* 4208-4216.

SILVER, L.M., MARTIN, G.R. and STRICKLAND, S. (1983). Teratocarcinoma stem cells. Cold Spring Harbor Conferences on Cell Proliferation. Vol. 10, CSHL, New York.

SLEIGH, M.J. (1985). Virus expression as probe of regulatory events in early mouse embryogenesis. *Trends Genet. 2:* 17-21.

SOLTER, D. and KNOWLES, B.B. (1978). Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). *Proc. Natl. Acad. Sci. USA 75:* 5565-5569.

SOPRANO, D.R., SOPRANO, K.J., WYATT, M.L. and GOODMAN D.S. (1988). Induction of the expression of retinol-binding protein and transthyretin in F9 embryonal carcinoma cells differentiated to embryoid bodies. *J. Biol. Chem. 263:* 17897-17900.

SPECK, N.A. and BALTIMORE, D. (1987). Six distinct nuclear factors interact with the 75-base-pair repeat of Moloney murine leukemia virus enhancer. *Mol. Cell. Biol. 7:* 1101-1110.

STEUER, B. (1991). Differenzierung der embryonalen Karzinomzellinie F9 zu parietalem Endoderm mittls Transfer genetischer Information. Ph. D. Thesis. Universität Heidelberg.

STEUER, B., BREUER, B. and ALONSO, A. (1990). Differentiation of EC cells with the fluorescence dye Hoechst 33342. *Exp. Cell. Res. 186:* in press.

STEVENS, L.C. (1970). The development of transplantable teratocarcinomas from intratesticular grafts of pre- and postimplantation mouse embryos. *Dev. Biol. 21:* 364-382.

STEWART, C.L., STUHLMANN, H., JÄHNER, D. and JAENISCH, R. (1982). *De novo* methylation, expression, and infectivity of retroviral genomes introduced into embryonal carcinoma cells. *Proc. Natl. Acad. Sci. USA 79:* 4098-4102.

STRICKLAND, S. and MAHDAVI, V. (1978). The induction of differentiation in teratocarcinoma stem cells by retinoic acid. *Cell 15:* 393-403.

STRICKLAND, S., SMITH, K.K. and MAROTTI, K.R. (1980). Hormonal induction of differentiation in teratocarcinoma stem cells: generation of parietal endoderm by retinoic acid and dibutyryl cAMP. *Cell 21:* 347-355.

TIENARI, J., VIRTANEN, I., SOINILA, S. and LEHTONEN, E. (1987). Neuron-like derivatives of cultured F9 embryonal carcinoma cells express characteristics of parietal endoderm cells. *Dev. Biol. 123:* 566-573.

VASIOS, G., MADER, S., GOLD, J.D., LEID, M., LUTZ, Y., GAUB, M.P., CHAMBON, P. and GUDAS, L. (1991). The late retinoic acid induction of laminin B1 gene transcription involves RAR binding to the responsive element. *EMBO J. 10:* 1149-1158.

WANG, S.A., LA ROSA, G.J. and GUDAS, L.J. (1985). Molecular cloning of gene sequences transcriptionally regulated by retinoic acid and diobutyryl cyclic AMP in cultured mouse teratocarcinoma cells. *Dev. Biol. 107:* 75-86.

WREDE, U. (1989). Expression parietal-endodermaler Differenzierungsmarker in Hybridzellen aus differenzierten und undifferenzierten EC-Zellen. Ph.D. Thesis. Universität Heidelberg.

YAMAGUCHI-IWAI, Y., SATAKE, M., MURAKAMI, Y., SAKAI, M., MURAMATSU, M. and ITO, Y. (1990). Differentiation of F9 embryonal carcinoma cells induced by the c-jun and activated c-Ha-ras oncogenes. *Proc. Natl. Acad. Sci. USA 87:* 8670-8674.

YOUNG, K.S., WEIGEL, R., HIEBERT, S. and NEVINS, J. (1989). Adenovirus E1a-mediated negative control of genes activated during F9 differentiation. *Mol. Cell. Biol. 9:* 3109-3113.

Accepted for publication:September 1991