Feto-oncogenes

Retroviruses with two cell-derived oncogenes

J. Ghysdael1, J. Coll1, P. Martin1, C. Dozier2, B. Debuire2, G. Calothy3 and D. Stehelin1.1Institut Pasteur, Lille, France. 2I.R.C.L., Lille, France. 3Institut Curie, Orsay, France

The oncogenic properties of acutely transforming retroviruses are the result of the expression of viral oncogenes (v-onc). Viral oncogenes were derived from normal cellular genes (c-onc) and over 20 different c-onc genes have been identified in a variety of animal species.

Point mutations, amplifications and translocations of c-onc genes are observed in tumor cells from animal and human non-viral cancers. This suggests that activation of c-onc genes may represent at least some of the multiple steps involved in human carcinogenesis and that two or more distinct oncogenes can act in complementation.

The genome of three avian retroviruses was found to contain two distinct cell-derived oncogenes. 1) Avian erythroblastosis virus (AEV) contains the oncogenes erb A and erb B, 2) Avian erythroblastosis virus E26 contains the oncogenes myb and ets, 3) Avian carcinoma virus MH2 contains the oncogenes myc and mil.

The determination of the respective role of each of these v-onc genes in the properties of these different viruses provide a unique model for the study of the co-operation of two oncogenes.

Expression of the c-sis oncogene in early human placenta: implications for possible autocrine or paracrine control of cytotrophoblast proliferation

A. S. Goustin1, C. Betzholst2, S. Pfeifer-Ohlsson1, J. Rydner1, G. Holmgren1, C.-H. Heldin2, M. Bywater2, B. Westermark2 and R. Ohlsson1.1Departments of Applied Cell and Molecular Biology, Oncology, Gynaecology and Clinical Genetics, University of Umeå, Sweden. 2Departments of Pathology and Medical Chemistry, University of Uppsala, Sweden

The c-sis oncogene codes for the platelet-derived growth factor (PDGF), a factor capable of inducing proliferation of quiescent fibroblasts. Recently, it has been shown (Kelly et al. (1983) Cell 35, 603) that addition of PDGF to quiescent fibroblasts brings about a 40-fold increase in expression of the c-myc oncogene. In light of our recent finding implicating c-myc expression in the control of cytotrophoblast proliferation in human placenta (Pfeifer-Ohlsson et al., submitted), we screened for the presence of active c-sis oncogenes in early human placenta. Northern blot analysis of placental RNA reveals the presence of a 4.2 kb transcript similar in size and abundance to the transcript of glioma and osteosarcoma cell lines, actively producing PDGF. In situ hybridization analysis of thin placenta sections using 125I-labelled c-sis probes demonstrates that only proliferative cytotrophoblasts of the cytotrophoblastic shell harbour active c-sis oncogenes. The presence of abundant PDGF-receptors on cytotrophoblasts was demonstrated by the binding of 125I-labelled PDGF to cultured trophoblasts. We have also been able to demonstrate a 68 kd protein specifically-precipitated by PDGF antisera in media derived from the incubation of placental explants in 35S-cysteine. Using in situ hybridization to early passage cytotrophoblasts cultured on glass in microchambers (LabTek), we have seen a rapid transient appearance of abundant myc transcripts in response to PDGF. These findings will be discussed in relation to a function for PDGF in control of cytotrophoblast proliferation.

Supported by grants from the Swedish Cancer Society (RMC) and the Gustaf Vs Jubileum Fund of Sweden.
Feto-oncogenes

Role of 'ras' genes in cell transformation


Activated 'ras' oncogenes have been identified in a wide range of tumours. All examples of 'ras' gene activation in tumours so far result from amino acid substitution at either gly12 or gin61. In order to analyse the role of 'ras' genes in neoplastic transformation we have investigated whether reaction of a non-transforming c-Ha-ras-1 protooncogene with various ultimate carcinogen leads to the generation of a transforming oncogene when the reacted DNA is introduced into cells. Analysis of these activated genes will identify sites on the 'ras' gene which can lead to activation and allow the determination of the mutations which the carcinogens induce.

To investigate the role of 'ras' oncogenes in producing the transformed phenotype we have isolated flat revertants from tumour cells which contain activated 'ras' genes. These revertants can be retransformed when cloned 'ras' oncogenes are introduced into them demonstrating that transformed morphology of the tumour cells is under the control of the 'ras' gene product. To further investigate the role of the 'ras' genes we have studied the production of transforming growth factors (TGFS) by tumour cells that contain activated 'ras' genes and by N1H-3T3 cells transfected by 'ras' genes. Although little TGF activity, as measured by the stimulation of NRK cells to grow without anchorage, is produced by the original tumour cells, transfectants produce TGFS and have reduced EGF binding. These results show that the type of TGF elicited by the same 'ras' oncogene is highly dependent on the cell type in which the oncogene is present.

Transcripts regulated during normal embryonic development and oncogenic transformation share a repetitive element

David Murphy*, Paul Brickell, David Latchman and Peter W. J. Rigby, CRC Eukaryotic Molecular Genetics Research Group, Department of Biochemistry, Imperial College, London SW7 2AZ

We have isolated cDNA clones which correspond to RNAs elevated in Balb/c 3T3 fibroblasts upon transformation by Simian Virus 40. The clones of Set 1 detect RNAs of 1-6 kb, 0-7 kb and 0-6 kb; high levels of the 1-6 kb mRNA have been found in all transformed and tumour lines tested. The three Set 1 RNAs are related only by the presence of a B2 repetitive element. The repeat fails to detect transcripts in normal adult tissues but identifies a large number of RNAs in early mouse embryos and in pluripotent EC and EK cells. The expression of these RNAs is regulated during development; the majority of the transcripts decreasing in abundance upon in vitro differentiation of EC cells and as mouse development proceeds beyond 9-5 days in vivo. Experiments designed to elucidate the possible involvement of the repeat in the regulation of the Set 1 related genes, both in transformation and embryonic development, will be described.
Feto-oncogenes
Oncogenes and differentiation

M. Pillow and S. Bendix, Bendix Environmental Research, Inc., 1390 Market St., San Francisco, CA 94102, U.S.A.

Recent work on oncogenes has sparked interest in their possible role in normal cells. There is good evidence of a relationship between mechanisms of neoplasia and normal cellular developmental processes. Embryonic cells can be used to immunize animals against tumors induced by carcinogens and against tumors caused by various viruses, (Coggin, 1971) even though the viruses are in widely separated taxonomic groups. Animals immunized with cells transformed by a virus will resist growth of a tumor induced by chemical carcinogens (Whitmire, 1972). These observations can be related by our hypothesis (Pillow, 1984) that cancer results from the continuing expression of inappropriate embryonic gene sequences; and that oncogenic viruses use embryonic regulatory sequences involved in cell differentiation to accomplish the replication of viral gene sequences. The exact mechanism of interaction with cellular embryonic sequences differs, but it is the use of embryonic sequences which characterizes oncogenic viruses. RNA tumor viruses are cell lineage and stage specific, as is the DNA virus EBV. As Dawe points out, polyoma virus causes a variety of cancers in mice, and its range of target tissues is the same as that of anhidrotic ectodermal dysplasia, a human developmental malformation caused by an X-linked single gene defect (Dawe, 1981). Since these organs share a developmental regulatory gene, it appears that polyoma virus may induce neoplasia by interaction involving the same gene, which appears to be a developmental gene.

Other evidence is that when certain tumor cells are induced to differentiate, virus production increases until the cells are fully differentiated, at which point virus production ceases (Lieberman, 1978). Also, some neoplastic cells can be induced to become differentiated, benign cells when placed in an embryonic situation normal for cells of that lineage. Examples are teratocarcinoma, leukemia, and neuroblastoma cells (Pierce, 1982). Thus 'oncogenes' may be normal cellular regulatory genes, which viruses usurp for viral replication.


Temporal and spatial regulation of cellular myc oncogene expression during human placental development: implications for embryonic cell proliferation

S. Pfeifer-Ohlsson 1, A. S. Goustin 1, J. Rydner 1, L. Bjerse 1, D. Stehelin 2 and R. Ohlsson 1.

1Departments of Oncology, Gynaecology, Pathology and Applied Cell and Molecular Biology, University of Umeå, Umeå, Sweden. 2Laboratoire d’Oncologie Moleculaire, INSERM U 186, Institut Pasteur, 15 Rue C. Guerin, F-59019 Lille, France

The human placental trophoblast component derives from embryonal cells and is developmentally regulated; the tissue is highly proliferative and often described as pseudomalignant. Since cellular oncogenes have been implied to be involved in normal cellular proliferation and differentiation processes, we have studied c-myc oncogene expression in relation to progression of human placental development. Northern blot analysis of placental RNA preparations revealed the presence of a 2-4 kb c-myc gene transcript. The frequency of this transcript varies 20 to 30-fold over the course of placental development, showing a peak at 4–5 weeks after conception, where the myc transcripts comprise about 0.04 % by weight of the total placental mRNA. A clear decline in placental c-myc transcription is seen before the end of the first trimester of pregnancy. In situ hybridization to 125I-labelled myc probes demonstrates an unequal spatial distribution of myc transcripts in placenta with particularly high expression in the cytotrophoblastic shell of early placenta. Labelling of placental explants with 3H-thymidine, the localization of myc transcripts to cytotrophoblasts, and the temporal pattern of myc expression support a strong correlation between myc transcript abundance and cytotrophoblast proliferation. These findings will be discussed in light of a possible role for the c-myc gene in proliferation of normal cells in this tissue.
**Feto-oncogenes**

**Tissue-specific expression of cellular oncogenes in developing human fetus of the first trimester**

Susan Pfeifer-Ohlsson 1, Jan Rydnert 1, Anton Goustin 1, Erik Larsson 2 and Rolf Ohlsson*1. 1Departments of Oncology, Gynecology and Applied Cell and Molecular Biology, S-901 87 Umed, Sweden. 2Department of Pathology, University of Uppsala, Uppsala, Sweden

Primarily due to work on transforming retroviruses a set of cellular genes has been isolated with transforming properties when residing in a retroviral genome. These oncogenes have also been implied to play a role in human tumorigenesis in fashions reminiscent of the viral oncogenes. As the oncogenes appear to be conserved in evolution it has been argued that they play important roles in normal cell metabolism. Moreover, the transforming properties suggest a role for such oncogenes in the control of differentiation or proliferation of normal cells. We have therefore undertaken a survey of c-oncogene expression in normal embryonal cells for the following reasons: 1) a distinct congruence between the spatial and temporal pattern of a developmental event and the distribution of active c-oncogenes may provide insight into both the normal and transforming functions of a particular oncogene. 2) Once such functions have been defined, the active c-oncogenes may turn out to serve as endogenous markers for specialized functions and possibly as markers for different cell-cycle stages. Using in situ hybridization techniques to thin sections of formalin-fixed human fetus we have analyzed the distribution of active c-fos and c-myc oncogenes. The results can be summarized as follows:

a) the c-fos transcripts are found mainly in the developing bone tissue but also, to a lesser degree, in epidermis.
b) the c-myc transcripts are found mainly in the primitive epidermis but also in connective tissue and gut epithelial layers.

The data reveal further that the c-oncogene expression appear to be synchronized in only one plane. The results are consistent with a role for c-oncogenes in normal developmental processes.

**Temperature sensitive mutants of Avian Erythroblastosis Virus: their use in the study of transformation and differentiation of erythroid progenitor cells**

J. A. Schmidt*1, M. J. Hayman 1 and H. Beug 2. 1Viral Leukaemogenesis Laboratory, Imperial Cancer Research Fund Laboratories, St. Bartholomew’s Hospital, Dominion House, London EC1. 2European Molecular Biology Laboratories, Postfach 10.2209, Meyerhofstr. 1, 6900 Heidelberg, West Germany

Avian Erythroblastosis Virus (AEV), an acutely oncogenic retrovirus, causes erythroblastosis and sarcomas when injected into young chicks and transforms erythroblasts and fibroblasts in vitro. Oncogenicity is due to acquired DNA sequences of cellular origin (oncogenes) termed v-erbA and v-erbB and evidence suggests that the v-erbB gene is sufficient for transformation of both erythroblasts and fibroblasts, although the v-erbA gene appears to stabilise the transformed state. It has recently been shown that v-erbB is a truncated form of the mammalian EGF receptor.

By a number of criteria, erythroblasts transformed by AEV appear to be blocked in differentiation around the CFU-E cell stage of differentiation. This is most markedly demonstrated by the ability of erythroblasts transformed with temperature sensitive (ts) mutants of AEV to differentiate into erythrocytes simply by shifting cells to the viral non-permissive temperature (42 °C) under culture conditions required for normal maturation of CFU-E. Thus, ts AEV transformed erythroblasts offer a system to study (1) the mechanism of transformation by v-erbB and (2) the differentiation of erythroblasts to erythrocytes.

1) Inactivation of the transforming function of AEV in ts mutants is accompanied by a loss of cell surface expression of erbB as well as a block in carbohydrate processing of immature gp68v-erbB to mature gp74v-erbB protein. The use of glycoprotein processing inhibitors has discounted that correct processing to gp74 is necessary for maintenance of transformation and suggests that it is the plasma membrane localisation of v-erbB that is necessary for transforming activity.

2) We have isolated several mouse hybridoma cell lines which secrete monoclonal antibodies which recognise differentiation specific antigens on the surfaces of both ts AEV transformed erythroblasts and normal chicken bone marrow cells. The in vitro differentiation system is being used to characterise these antigens and study the control of their expression.
Feto-oncogenes

The developmental biology of an endogenous, ecotropic, murine leukemia provirus

John Wejman* and Allan Bradley, Department of Genetics, University of Cambridge, Downing Street, Cambridge

Lethal yellow, Ay, is a mutant allele of the agouti coat-colour locus in mice. It is a prenatal homozygous lethal, which terminates embryogenesis before 6.5 days post coitus. Post-natal Ay heterozygotes suffer from high incidences of a wide range of diseases: abnormal stimulation of body growth, a diabetes-like syndrome, male sterility, obesity and spontaneous and induced proliferative tumours. An endogenous, ecotropic murine leukemia provirus, emv-15, which expresses an infection-competent MuLV, is closely linked to Ay, but to no other allele of the agouti locus. In the light of the mutagenic properties of endogenous retroviruses, it is possible that emv-15 may be responsible for some or all of the pathological and developmental disorders with which Ay is associated.

We have established a pluripotential derived stem cell line which carried emy-15, and by implication Ay. Because the congenic inbred mouse strain, 129/SvJ-Ay/Ayb, from which this line has been derived, carries only the one ecotropic proviral locus, it is possible to use probes specific to the expression of endogenous ecotropic proviral loci to monitor the development of emv-15's activity during in vitro stem cell differentiation. The probes consist of a panel of monoclonal antibodies to endogenous, ecotropic MuLV antigens, and a hybridization probe to the ecotropic emv gene region. Similarly, the developmental biology of emv-15 can be followed and characterized throughout the course of pre- and post-natal in vivo mammalian development. We will discuss our initial findings.