The Molecular Biology of Differentiation and Transformation: An Emerging Field Theory

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It appears to me that finally, a glimmer of insight is to be seen in the basic mechanism of oncogenic transformation. The emerging picture is based on fundamental observations from a large number of sources. We have been privileged to see the results of some of the currently brightest research in the mosaic during this workshop. The framework for the emerging picture is based on the recognition within recent years that cellular differentiation is a social event among cells in which one cell type triggers the differentiation of another. This principle hardly would have been a revelation to the early embryologists. Perhaps the foremost, technically accessible problem of biology at this time is to understand the specific biochemical mechanisms by which these processes are implemented within the cell.

The Emerging Field Theory

Certain cells exert their function in inducing differentiation in target cells either through cell-to-cell physical contact or from a distance. In both cases, specific substances, frequently glycoproteins, from one cell interact with highly specific receptors on the target cells. If the triggering substance is released from the cell it may be commonly known as a growth substance or hormone. These factors may be active at chemically incredibly low concentrations. At this meeting Malcolm Moore has reported that lactoferrin acts as a factor for the growth of colonies of myeloid cells and that the active component is effective at concentrations of 10^{-14} M! For the differentiation factors to be effective at these concentrations, the binding constant for interaction with the receptor must be extremely large. In most cases it appears that binding of no more than a few molecules per target cell is adequate to elicit the response. In general, a special system will be required to amplify the signal received at the cell surface to a level that it can be effective on intracellular reactions. It is not clear whether or not part or all of the differentiation factor molecules themselves must be somehow transported to the nucleus to effect transcriptional control. The relation of the cellular components involved are schematically depicted in Fig. 1.

A proper relation in time during development as well as space is implicit in the scheme. Differentiation may occur only during a transient period when cells exhibiting the appropriate recognition sites are exposed to the

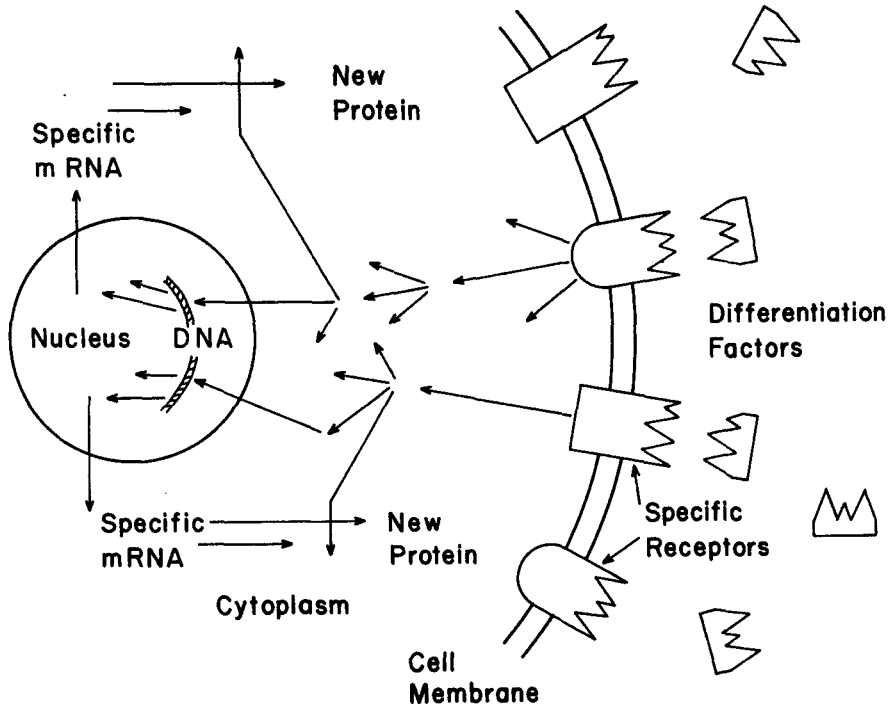

Fig. 1. Diagramatic representation of the cellular components and reactions involved in differentiation

proper signal molecules. Undifferentiated cells may be maintained in the adult organism, then triggered to differentiate into a mature or terminal cell type in which cell division is limited or completely stopped. Differentiation of blood cells provides a classical example of such a system. Thus the function of the cellular genome during differentiation might be equated more correctly with a library than a computer. Information stored in the DNA is expressed when the proper request is received rather than at a fixed point in a free-running program.

Recent evidence appears to indicate that at least in some and probably most situations, the immediate, causal alteration that results in oncogenic transformation involves a lesion in the information retrieval system rather than DNA itself.

Theoretically the lesion might occur at any point from the differentiation factors themselves to the synthesis of a new protein. A number of specific mechanisms can be envisioned. I divide them into four classes based on the location of the lesion:

I. Defective or Deficient Differentiation Factors

A differentiating cell might not be exposed to the proper factors, perhaps because of time-space relationships with other cells or because of a lesion in the cells producing the appropriate differentiation factor(s). In this case the oncogenic cell might be normal in its capacity to recognize and respond to differentiation factors which are not present in its environment. Certain teratocarcinoma appear to be excellent candidates for this class. Exciting

experiments involving implantation of normal embryonic and teratocarcinoma cells into pseudo-pregnant mice to form normal, chimeric or allophenic progeny strongly support such a model (K. Illmensee, and B. Mintz, 1976). Hopefully, additional successful experiments in this area will be reported shortly.

II. Defective Recognition Sites

Another theoretically possible situation might involve numerically inadequate or defective recognition sites on the target cell. Chemically inert, surface reactive materials such as asbestos may function as carcinogens by this mechanism. Such cells might be induced to differentiate if the concentration of the critical factors could be elevated to a level at which they could induce a non-proliferative state. There are a number of reports involving leukemias that may reflect this situation (L. Sachs, 1974; R.C. Gallo et al., 1977) or possible Type I defects. Certain neuroblastomas that can be stimulated to differentiate with cAMP (K.N. Prasad and A.W. Hsie, 1971) may fall into this category. Neuroblastomas that can be stimulated to differentiate in the presence of glia cells (C.P. Reynolds and J.R. Perez-Polo, 1975) and pheochromocytomas in which neurite proliferation can be stimulated with nerve growth factor (A.S. Tischler and L.A. Greene, 1975) might involve lesions at the level of either the differentiation factor or the cellular receptor.

III. Defective Intracellular Signal Transmission

The next level of lesion that can be envisioned involves transmission of the signal received at the cell surface to the transcriptional and translational machinery of the cell. Some virus-induced transformation appears to provide an example of a lesion at this level. Rat kidney cells that have been transformed by Rous sarcoma virus can be caused to revert to a normal phenotype by inhibiting protein synthesis, presumably by blocking the synthesis of an unstable product of the transforming gene (J.F. Ash et al., 1976). The src gene appears to code for a protein kinase that may interfere with intracellular control mechanisms as mentioned below and considered by Ray Erikson at this meeting. It appears that avian and murine leukemia virus carry transforming genes that are quite different from the src gene and code for other proteins that may disrupt the intracellular transmission of the signal in different ways. Murine erythroid cells transformed by Friend leukemia virus provide a system that may belong to this class. Differentiation can be induced in these cells by the simple expedient of growing them in tissue culture in the presence of dimethylsulfoxide (C. Friend et al., 1971) or a number of other aprotic solvents.

In all of the examples given above the transformed cells can be induced to undergo differentiation if they are exposed to the proper external stimulus. The block in differentiation can be overcome! In effect, the cells can be cured

of their "transformed" condition! The point of fundamental importance is that transformation has not involved an irreversible loss of genetic information or a structural change in DNA that precludes further differentiation.

IV. Defective Malignant Cell DNA

Yet another class of oncogenic transformation may involve structural alterations of DNA so that the affected cells lack genetic information required for terminal differentiation. Certain genetically determined cancers may fall into this class. However, it should be noted that some imaginal disc tumors of *Drosophila* that clearly are inherited according to the principles of Mendelian genetics may revert to a differentiating state (E. Gateff, 1978a). Genetically inherited imaginal disc tumors fall into two classes. Those that appear to be irreversibly transformed and others that are capable of differentiation when they develop in close contact with wild type cells (E. Gateff, 1978b). It appears likely that the latter class may involve a mutation that affects the production of a differentiation substance produced in non-malignant cells and thus probably should be classified as a Type I transformation.

X-ray induced leukemias may provide another example of a Type IV transformation in which radiation has resulted in damage of genes required for a late stage of differentiation which has occurred in an undifferentiated cell type. The damage may not be expressed for a relatively long time until the defective cells are induced to start along a differentiation pathway. Thus transformation resulting from changes in the DNA of the malignant cells fall into a fundamentally different category than the three classes considered above, in that they involve seemingly irreversible loss of essential genetic information.

Session Highlights

Peter Duesberg

Dr. Duesberg presented evidence from technically elegant experiments indicating that the transforming capability of avian acute leukemia virus MC29 and avian carcinoma virus MH2 is related to a specific 1,5–2,0 kilobase nucleotide sequence and that the sequence is not closely related to that of the src gene of Rous sarcoma virus. The nucleotide sequence appears to be near the *gag* gene which is located at the 5′-end of independently replicating virus. The results demonstrate clearly the technical capacity of existing techniques to study the structure of transforming genes. Dr. Duesberg suggested the intriguing possibility that transforming genes of these viruses may be host cell genes that have been integrated into the viral genome. They may code for regulating proteins, such as protein kinases, that no longer respond to the normal cellular control systems. Dr. Duesberg's findings were complimented by results presented by Thomas Graf who reported that a specific transforming gene in avian erythroblastosis virus appears to be distinct from the trans-

forming genes of both the avian acute leukemia virus and the Rous sarcoma virus. Dr. Graf also indicated that the transforming genes of avian erythroblastosis virus and avian myelocytomatosis virus may be acquired host cell genes that function in hemopoetic differentiation. He suggested that the product of these genes may induce leukemic transformation by a non-functional interaction with a cellular receptor, thereby competitively inhibiting the unmodified differentiation product of the host cell.

Ray Erikson

Perhaps the most exciting development in recent years in the area of the mechanism of transformation are the results from Dr. Erikson's laboratory, involving characterization of the src gene product. His group has used immunological procedures to detect the protein formed from the src gene of avian sarcoma virus. Of paramount importance is the observation that the protein appears to be a protein kinase that will phosphorylate IgG. It is likely that phosphorylation of IgG is an in vitro artifact. Thus far, there is no direct demonstration of what the substrate(s) in vivo for the kinase might be, however, there is strong indirect evidence suggesting that polymerization of cytoskeletal elements might be involved. Phosphorylation of a cytoskeletal protein appears to cause depolymerization of the cytoskeletal elements (W. Birchmeier and J. Singer, 1977) resulting in changes in the cell membrane that may trigger the physiological changes characteristic of the transformed state. An initiation factor of protein synthesis, eIF-2, also appears to be a possible candidate for the natural substrate of such a kinase. Clearly more work is required to firmly establish the src gene product as a protein kinase, however the data appear to be sufficiently reliable at this point to make this an extremely promising and no doubt intensely competitive area for future work.

Considered togehter, these and data from other sources appear to indicate that peptide(s) formed from specific nucleotide sequences, transforming genes, carried by certain types of virus is the immediate causal agent for oncogenic transformation. There appears to be nothing special about the virus itself or the transforming genes beyond their capacity to code for these special proteins. Furthermore, the nucleotide sequence of the transforming genes are different and apparently code for different peptides. It appears likely that these products will cause transformation by different specific mechanisms.

Gisela Kramer

Data indicating that the src gene product is a protein kinase, has evoked special interest in this area. Dr. Kramer has described cAMP-independent protein kinases that inhibit translation in Friend leukemia cells and rabbit reticulocytes. Activity of cAMP-dependent protein kinases is promoted by binding of cAMP to the regulatory subunit thereby causing it to dissociate from the catalytic subunit of the holoenzyme. However, virtually nothing is known about the molecular mechanism by which cAMP-independent kinases are regulated. The so-called heme-controlled repressor, HCR, from rabbit reticulo-

cytes is held in an inactive from in the presence of heme. Protein kinase activity with high specificity for an initiation factor of protein synthesis, eIF-2, and inhibitory activity for protein synthesis, is generated in the absence of heme both in vitro and in intact cells. An eIF-2 specific protein kinase that appears not to be regulated by heme has been isolated from Friend leukemia cells that have not been stimulated to differentiate by dimethylsulfoxide. It has been speculated that this kinase might be involved in the block in differentiation. It is not known whether or not the kinase is coded by the viral or host cell genome. After stimulation by dimethylsulfoxide, Friend cells appear to gain the capacity to be regulated by heme.

Ian Kerr

Dr. Kerr described what appears to be a different type of system to amplify the signal received at the cell surface. He has shown that interferon treatment of intact cells potentiates the synthesis of an adenine trinucleotide with a very unusual 2′- to 5′-phosphodiester linkage. Double-stranded RNA also is involved in the synthesis of this compound. Interferon is a species-specific glycoprotein that appears to have highly specific cell surface receptors. The target for the unusual adenine trinucleotide is not known but may be a ribonuclease that has been implicated frequently in interferon action. Also, interferon appears to activate a cAMP-independent, eIF-2 specific protein kinase that is physically distinguishable from the heme-controlled repressor. The physiological relation between the trinucleotide and the protein kinase is unclear. Experimentally interferon provides one of the most useful, and biochemically well-characterized examples of an intracellular regulatory system that is triggered by a specific interaction at the cell surface. The interferon system may come to serve as a model for this type of control.

Gebhard Koch

One of the fundamental conceptual problems of translational control involves specificity for the synthesis of specific proteins. How can phosphorylation of a peptide initiation factor that apparently is used during the translation of all mRNA species differentially affect the synthesis of specific proteins? A partial answer to this question has come from Dr. Koch's laboratory. His group has shown that different species of mRNA are translated with very different efficiencies. The relative proportion of products formed from different mRNA's can be altered by changing parameters such as salt concentration that affect the overall rate of the synthetic reaction. For instance viral mRNA typically is translated with high efficiency relative to cellular proteins. However, a reduction in the overall rate of protein synthesis frequently causes a dramatic reduction in the relative proportion of viral and host cell proteins that are formed.

The Future

I believe any detailed prediction of future developments in molecular biology and biochemistry are likely to be wrong or at least incomplete, probably to a major extent. However, there are key problems that must receive continual consideration, and several areas that appear to be ripe for investigation. The concepts inherent in the mechanism of normal differentiation reflected in Fig. 1 will be tested and retested in many systems in the forthcoming years. The most crucial problem for an understanding of normal differentiation is the molecular mechanism(s) by which signals received at the cell surface activate transcription from specific genes. Is part or all of the protein differentiation factor taken into the cell and used in the activation process itself, perhaps at the DNA level as appears to be the case with steroid hormones, or are intermediate reactions involved? For most systems, the physical and chemical characterization of the differentiation factors and their specific receptors presents a severe technical problem in working with the very small quantities that are available. In many cases the assay systems used to monitor isolation are not quantitative and are no more than marginally satisfactory. This presents a formidable problem, especially when the biological response depends on two or more specific components, as frequently appears to be the case. The development of better assay systems, especially in vitro systems involving specific biochemical reactions rather than the response of intact cells, is critical to satisfactory progress in this area.

With respect to the sequence of intracellular reactions triggered by growth substances, two problems or areas stand out as being both technically feasible and crucially important. The first problem involves the mechanism by which signals received at the cell surface are amplified and transmitted to target reactions in the cytoplasm and nucleus. It appears that there are likely to be a number of alternative mechanisms to cascade systems involving protein kinases for amplification of the signal received at the cell surface. The small nucleotide described by Ian Kerr that is produced as part of the interferon and double-stranded RNA system seems to be part of such a system. It is likely that other types of amplification mechanisms will be found. The second problem involves regulation of cAMP-independent protein kinases. A number and perhaps a great many cAMP-independent protein kinases may be involved in amplification and transmission of cell surface signals. The enzyme system that is activated by double-stranded RNA and interferon is an excellent example. What is the specific molecular mechanism by which such enzymes are activated and do they function in cascade sequences?

With respect to transformed cells, the search for differentiation factors and conditions with which transformed cells can be induced to either stop dividing or differentiate to a non-dividing form appears to be the key problem. However it is frought with technical limitations that may limit progress until they are resolved. The transforming genes and their products are ripe for investigation and an investigative effort will be made in a number of laboratories. Are transforming genes really cellular genes that have been integrated into a viral genome in such a way that they no longer respond to

the normal control systems within the cell? What is the biochemical mechanism by which the product of transforming genes disrupt differentiation and induce the physiological changes associated with transformation? It appears possible that the next Wilsede workshop may include hard answers to some of these problems and a consideration of substantiated models of the molecular mechanism by which oncogenic transformation occurs. Eventually, I believe such insight will provide the basis for a rational therapy to cure leukemia at the cellular level.

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