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Control of Animal Cell Proliferation

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Present understanding of the control of animal cell proliferation is summarized briefly. Major gaps in present knowledge are listed. Models of growth control are discussed.

Key words: growth factors, growth inhibitors, models of growth control

Those interested in the control of growth of animal cells are concerned with two different but related questions. First, what are the factors outside the cell that control cell growth? Second, what happens inside the cell when the growth-controlling factors act? In this paper I plan to discuss both of these questions, placing the emphasis on problems that remain to be solved.

WHAT ARE THE FACTORS OUTSIDE THE CELL THAT CONTROL CELL GROWTH?

Many factors are known that can control the growth of animal cells in culture. The list of factors is striking both for its length and for its variety [1]. To simplify, the factors can be grouped into four general classes.

(1) Growth factors. There are many different growth-stimulating factors. Among them are a number of polypeptide growth factors, such as epidermal growth factor (EGF) and fibroblast growth factor (FGF), that are active at ng/ml concentrations [2, 3]. There are other, very different types of growth-stimulating factors, for example, prostaglandin $F_2\alpha$ stimulates the growth of some cells and is active at approximately 100 ng/ml [4].

(2) Nutrients. Various common nutrients stimulate or inhibit growth when their concentrations in the growth medium are raised or lowered. Included are amino acids [5], glucose [6], cations [7], and anions [8].

(3) Growth inhibitors. Many growth inhibitors have also been observed. These will be discussed later.

(4) Cell shape and surface area. The shape and surface area of cells often affect growth [9–11]. In general, growth is favored by increasing the amount of cell surface area exposed to the medium.

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As this summary suggests, enough is known at present to permit the culture of many cells in completely defined medium [12, 13]. The medium contains an appropriate set of growth factors, plus adequate concentrations of all of the necessary nutrients. Growth can be limited by growth inhibitors or by restriction of the surface area of the cells.

With this as background, let us consider the major gaps that remain in our knowledge of growth-controlling factors outside the cell. In my view they are the following.

Additional Polypeptide Growth Factors Remain to Be Identified

There is much evidence that additional polypeptide growth factors exist. Growth-promoting activity for many cells is found in biological fluids [1, 14] and in media conditioned by the growth of other cells [1, 15]. In many instances, the activity cannot be replaced by known growth factors. It seems likely that some of the unidentified growth factors have very important actions in vivo. The investigator is faced with the practical problem of deciding which growth-promoting activity to isolate, and also must choose a good source of the factor, as well as a good assay.

The Growth Factors That Act in Different Situations In Vivo Are Not Known

At present almost nothing is known about the identities of the growth factors that are required for the growth of the various tissues and organs in vivo. This is a very important problem. Until recently this problem has been difficult experimentally, but it seems to me that an approach is now available. The approach involves the use of monoclonal antibody prepared against a growth factor and injected into the animal to inactivate the growth factor in vivo. The approach is similar to that used by Levi-Montalcini and Booker [16] in their demonstration, in which they used rabbit antiserum to nerve growth factor (NGF), of an NGF requirement during the development of the sympathetic nervous system. The advantage of using monoclonal antibody instead of normal antibody is that a much higher concentration of antibody can be achieved. Arrest of growth of certain types of cells, as the result of inactivating a growth factor with monoclonal antibody, may limit growth or may cause developmental changes in a rapidly growing animal. By studying a variety of growth processes in the presence of monoclonal antibody, it should be possible to elucidate the action of a growth factor in different situations.

Many Growth Factor Interactions Remain to Be Studied

In cell culture, synergisms are often observed in the action of pairs of different growth factors [17, 18]. Some of these interactions have been studied, but many other possible interactions have not been investigated. One question of interest is whether specificity for control of growth of different cell types can result from highly specific interactions among low concentrations of different growth factors.

Interactions Between Different Growth Factors In Vivo Are Unknown

Based on observations in cell culture, it seems possible that specificity of growth factor action in vivo is achieved by the combined action of a specific set of growth factors, with a different set acting on each cell type. In principle, such interactions can be identified by inactivating combinations of growth factors in vivo with combinations of monoclonal antibodies. This will require a collection of different monoclonal antibodies.

Many Growth Inhibitors Remain to Be Purified

For technical reasons, growth inhibitors are less well studied than growth-stimulating factors. Nevertheless, there is evidence that suggests that growth inhibitors may be as numerous as growth factors, and it seems likely that growth inhibitors are important in vivo.

Recently we have purified a growth inhibitor that is produced by the BSC-1 cell line, a kidney epithelial cell line of African green monkey origin. Conditioned medium removed from crowded BSC-1 cells contains both low and high molecular weight growth inhibitors [19]. The low and high molecular weight inhibitors can be separated by ultrafiltration. The high molecular weight inhibitor has been concentrated 1,000-fold from serum-free conditioned medium, and has been purified by gel filtration followed by high-pressure liquid chromatography. This kidney epithelial cell growth inhibitor has the properties of a protein with a molecular weight of approximately 15,000. It arrests the growth of BSC-1 cells in the G_1 phase of the cell cycle. Present preparations give approximately a 50% inhibition of growth of 1 ng/ml. The action of the kidney epithelial cell growth inhibitor is reversible; that is, the cells resume growth after the inhibitor is removed. To the extent that it has been tested, it is highly specific for kidney epithelial cells. It has no interferon activity.

Preliminary evidence from this laboratory suggests that other epithelial cells also produce growth inhibitors.

The best studied growth inhibitors at present are ACTH [20] and interferon [21, 22]. They are growth inhibitory at approximately 1 ng/ml. A variety of other growth inhibitors of lower specific activity have been reported [23–28].

Thus far there is no information on the normal in vivo action of growth inhibitors. In principle, once inhibitors are available for the preparation of monoclonal antibodies, then these can be used to inactivate the endogenous growth inhibitors in vivo, and it will be possible to study the effect of the inhibitors on growth. It is possible that the growth inhibitors act as differentiation factors in vivo.

The Role of Cell Shape and Surface Area Require More Study

Much evidence indicates that increasing the surface area of cells favors growth. Decreasing the surface area inhibits growth [9–11]. Density-dependent regulation of growth is an example of this phenomenon. Cells become anchorage independent when they are able to grow with a minimum of surface area. Present evidence indicates that density-dependent regulation of growth has different causes in different situations [29], suggesting that the mechanisms by which cell shape and surface area act are complex. It is quite possible that different cells become anchorage independent for different reasons, depending on the factors that favor growth of the particular cell. Studies are needed of these phenomena with a number of different cells in a variety of growth situations.

WHAT HAPPENS INSIDE THE CELL WHEN THE GROWTH-CONTROLLING FACTORS ACT?

Turning to the question of how the growth factors act, it is clear that the polypeptide growth factors bind to specific cell surface receptors. Among the growth factors, EGF has been studied most extensively [2]. EGF binds to its specific receptor, the receptor–EGF

complex is internalized, and the EGF is degraded [2]. With EGF, the process of binding, internalization, and degradation continues, repeatedly, for many hours before the stimulated cells are committed to initiate DNA synthesis.

What Happens Inside the Cell?

It is clear that EGF and other growth factors have effects as soon as they bind to the cell surface receptors. Effects on transport processes [30], ion fluxes [31, 32], membrane composition (phosphorylation [33], phospholipase A_2 activation [34]), and cyclic nucleotide concentrations [35] are observed within minutes after binding of the factor. Internalization of the growth factor does not appear to be required for these early effects. However, there is no proof that these early events lead to the initiation of DNA synthesis. Is internalization of the growth factor required for the initiation of DNA synthesis? Various types of evidence (immobilization of the growth factor [36], inhibition of internalization [37]) suggest that little, if any, internalization is required. Nevertheless, there seems to be no way to exclude the possibility that the growth factor (or a fragment of it or of the receptor) also acts internally. Even one molecule arriving at the nucleus could be sufficient, and there is no way to exclude this possibility.

How Do External Nutrient Concentrations Act To Influence Growth Control?

It seems likely that the external concentrations influence internal concentrations of the nutrients. Whether these then act directly or indirectly, by influencing the concentration of other effectors, is unknown.

How Do Growth Inhibitors Act?

High molecular weight growth inhibitors such as the kidney epithelial cell growth inhibitor presumably act by binding to specific cell surface receptors. With ACTH, interaction with the cells has been studied extensively [20], and it seems likely that ACTH inhibits DNA synthesis in adrenal cells in culture by increasing the intracellular concentration of cAMP. The intracellular effect of the kidney epithelial cell growth inhibitor is unknown. The inhibitory action of the kidney epithelial cell growth inhibitor is counteracted by EGF, and vice versa. It appears that this takes place intracellularly, since there is no indication that the inhibitor affects EGF binding. The interaction of EGF and the inhibitor is an illustration of the complexity of growth control; growth appears to be under the simultaneous control of many different external factors.

Control of growth by cell shape and surface area is another example of the complexity of interacting growth-controlling factors. Cell surface area probably influences a variety of cell membrane and transport effects.

MODELS OF GROWTH CONTROL

The observation of simultaneous control of growth by numerous external growth-controlling factors is more or less puzzling depending on one's model of the events inside the cell that lead to DNA synthesis. There are two very different simple models of these internal events. At one extreme, the various external growth-controlling factors are considered to act on the cell and influence, directly, a series of processes that lead to the initiation of DNA synthesis. Alternatively, at the other extreme, the various external factors are considered to act on normal cellular processes, such as energy production,

protein synthesis, and RNA synthesis, among others, and it is the state of these cellular processes that controls the initiation of DNA synthesis. Present data can be explained with either model. Nevertheless, one's choice of model has a great influence on one's experimental approach to studies of internal events.

According to the first model, quiescent cells might be expected to be blocked at a specific place, a "restriction point" [38], corresponding to the first of the series of biochemical reactions, stimulated by the growth factor, that leads to the initiation of DNA synthesis. If one accepts this, studies of the early events after growth can be expected to lead to the identity of the "restriction point." According to the second model, the early events after growth stimulation affect general cellular processes, and the "restriction point" is, in a sense, an inactive metabolic state. The study of early events after growth stimulation will lead only to general cellular processes.

Though there seems to be no way to distinguish between the two models on the basis of present data, there is the possibility that the initiator of DNA synthesis can be identified directly and then pathways can be traced backward from it. Grummt has reported [39] that addition of Ap_4A to permeabilized cells leads to the initiation of DNA synthesis. Das [40] has reported the isolation of a protein fraction from stimulated cells that in turn stimulates the initiation of DNA synthesis in isolated Xenopus nuclei. Unfortunately, we do not know at this time whether either of these experiments represents the natural course of events. Nevertheless, the experiments do suggest that it may be possible to elucidate the series of internal events after growth stimulation by identifying the initiator of DNA synthesis and working backward from this.

Whatever model one favors, the series of internal events must be complicated, since there is typically a 12–15-hour period between the beginning of growth stimulation and the initiation of DNA synthesis. One's explanation of this long time period is influenced in turn by one's choice of model of the cell cycle.

In the classical model of the cell cycle, quiescent cells are considered to be in a special, G_0 state, which is in some way outside the normal cell cycle. The general explanation for the long delay before initiation of DNA synthesis in quiescent cells is that it takes a number of hours for a G_0 cell to return to the normal cell cycle.

In the Smith-Martin model [41] for the cell cycle, quiescent cells are primarily in an indeterminate, A, state, from which they leave with a very low transition probability. Stimulating quiescent cells with growth factors increases the transition probability, and it is the delay in changing the transition probability that causes the delay in changing the rate of initiation of DNA synthesis.

Both models of the cell cycle are in widespread use, and each has its appeals. The Smith-Martin model does have the advantage that it is consistent with the general observation that quiescent cell cultures usually have a significant labeling index and the quiescent cells are not strictly in a G_0 state. Also the Smith-Martin model might predict that there would be an additional 12–15-hour delay in increasing the transition probability when a culture that has had suboptimal growth stimulation is subjected to maximum growth stimulation.

An experiment of this type has been reported by Brooks [42]. He found that there is an additional 12-hour delay before the effect of the second increase in growth stimulation is observed. This experiment indicates that it takes approximately the same time to increase the rate of initiation of DNA synthesis whether the cell culture is already growing or not. This is consistent with general observations, but it is not what might be anticipated from simple theories of the cell cycle.

There are, however, experimental conditions that give different results. Jimenez de Asua et al report [18] that there is only a short delay after the second growth stimulation under some conditions with prostaglandin $F_2\alpha$ and with FGF. There may be experimental differences that lead to the differing results, or it may be that the pathways that lead to stimulation of the initiation of DNA synthesis in different situations are different. Whichever explanation one prefers, the observations suggest that the experimental situation is complex.

In summary, consideration of the various models, and the numerous unsolved problems, leads to an awareness that growth-control mechanisms are probably very complicated. Nevertheless, there is a strong drive to devise simple models. The dilemma is that the models may be misleading. Probably each of us is willing to tolerate being misled a little because we hope that our preferred model will be of more help than hindrance. Unfortunately, there is no way to know in advance which model will be a help and which will be a hindrance.

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