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Aging: Noncycling Cells an Explanation

Cell and tissue aging is the result of transitions from cycling to noncycling cells.

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We have defined (*1–5*) three categories of potentially proliferating cells. The first is cycling cells that are actively moving through the cell cycle, $G_1 \rightarrow S \rightarrow G_2 \rightarrow M$ (where G_1 and G_2 are gaps, S is the period of nuclear DNA synthesis, and M is the period of mitosis). In addition there are two categories of noncycling cells, G_1 blocked and G_2 blocked, which are capable of moving into the cell cycle upon specific stimulation. All three populations of cells can exist within the same tissue. However, their relative proportions and transitions vary with changing environment, physiological conditions, and age.

Some of the phenomena explaining cell and tissue aging appear to be based upon transitions from cycling to noncycling cells (G_1 or G_2 blocked). In this article we present a model for viewing cellular aging in terms of these transitions. The model applies only to tissues capable of proliferation. It is supported both by our initial experiments and by results of others.

Concept of Cell and Tissue Aging

In Fig. 1 aging is depicted as an inevitable conversion of cycling to noncycling cells in tissues capable of proliferation. The term immature aging in vivo will apply to tissues that have completed their cellular aging transitions during embryogenesis or before completion of maximum adult growth of the entire organism. Mature aging in vivo will refer to cellular aging that occurs during the period of animal senescence. Immature and mature aging in vitro will apply to both the origin of the cell lines (that is, embryonic or adult) used to establish tissue cultures and to early or late periods in the life-span of a culture. Immature and mature aging transitions involve cycling cells becoming G_1 or G_2 blocked. The terms immature and mature aging also reflect chronological differences.

The transition to the noncycling state may be reversible; cells can be released from G_1 and G_2 blocks. This is depicted in Fig. 1 as release from aging. The degree and the rate of release may depend on whether cells are immature, adult, or aged, and on the particular tissue. Release from aging represents transitions from the noncycling to the cycling state. The model also implies that released cycling cells may in time revert to noncycling states.

In vitro Evidence for Cellular Aging

The first line of evidence to support the concept in Fig. 1 is drawn from tissue culture studies dealing with aging of human fibroblasts in vitro (*6–9*). These studies—primarily by Hayflick and Moorhead (*7*)—indicate that normal animal cells cannot be maintained in vitro indefinitely, but rather have a limited life-span. The life-span is expressed in the proliferative capacity of the cells in culture, and is also directly related to the age of the donor from which the cultured cells were taken. The maximum life-span of human diploid cells in vitro is about 10 months. This life-span represents approximately 50 cell population doublings, and it applies to cells taken from the youngest possible tissue, that is, from human fetal tissue. By comparison shorter life-spans and progressively fewer cell population doublings are observed in cultures originating from adult and old human tissues (*10*). These observations hold true only for normal, untransformed diploid cells. (Human fibroblast-like cells that have unlimited life-spans in vitro are abnormal, aneuploid cells with chromosomal anomalies.) Thus the significance of the human diploid tissue culture lies in its similarity to normal cellular aging in vivo. Consequently it provides a model system for studying both in vivo and in vitro aging of certain cell types.

The patterns of cell division of cultured cells undergoing aging in vitro coincide with and can be explained by the four aging transitions depicted in Fig. 1. Merz and Ross (*11*) observed individual cells throughout the life-span of a human diploid culture derived from fetal lung tissue. They showed that the proportion of nondividing cells gradually increased from 1 to 48 percent with time in culture, thus providing evidence that cellular aging represents a general transition from the cycling to the noncycling state. The studies of Macieira-Coelho and colleagues (*12, 13*) provide specific evidence for immature and mature aging transitions to both G_1 and G_2 blocked cells. Using [^{3}H]thymidine and autoradiography to demonstrate DNA synthesis, these investigators made detailed cell cycle analyses of both adult and embryonic human diploid cells during the various growth phases in vitro.

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They found that the decrease in proliferation associated with aging in vitro is due to cells becoming G_1 blocked (as shown by the decreased number of cells that take up [^{3}H]thymidine, that is, synthesize DNA) and to cells becoming G_2 blocked (as shown by the decline in the number of cells that enter mitosis after DNA synthesis). Moreover, the relative percentages of cells that become G_1 or G_2 blocked and the fact that these transitions occur in both embryonic and in adult tissues substantiate the designations of immature and mature aging in Fig. 1.

In vivo Evidence for Cellular Aging

The following are in vivo examples of immature aging, that is, of cells and tissues that move into the noncycling G_1 and G_2 blocked states before completion of development or of natural growth of the whole organism. During embryonic development of rat tongue muscle (*14*) there is a gradual transition from cycling to G_1 blocked cells (the autoradiographic labeling index decreases from 34 to 2 percent; the labeling index is a measurement of the percentage of cells that are synthesizing DNA, and is generally referred to as the DNA synthetic index). Similar observations have been recorded during early development of chick lens (*15*); in addition, measurements of DNA content in individual nuclei show that a small percentage of the chick lens cells become G_2 blocked cells. There is also autoradiographic evidence for early immature aging to the G_1 blocked state in the pancreas of the newborn rat (*16*), with practically all pancreatic cells withdrawing from the cell cycle (in G_1) during the transition from the embryonic to the postnatal state. Examples of immature aging in tissues of the growing rat and mouse include the detailed cell cycle analyses of Post and Hoffman (*17, 18*) for rat liver. These investigators provide conclusive evidence for the aging transition to G_1 blocked cells in growing liver, and indirect evidence for G_2 blocked cells in this tissue. Autoradiographic studies of mouse bone growth (*19*) also demonstrate the shift to G_1 blocked cells (the DNA synthetic index drops at 8 weeks of age and remains low afterward). Evidence that tubular epithelial cells in rat kidney become G_1 blocked by 3 months of age is provided by cytophotometric analysis of DNA content of individual nuclei (*20*). A study of the growth of rat lens epithelium during the first year of life (*21*) shows immature aging to both G_1 blocked cells and G_2 blocked cells at about 8 weeks of age. Analysis of mitotic activity and DNA contents (determined by cytophotometry) in the rat lacrimal gland (*22*) shows complete immature aging transitions to G_1 and G_2 blocked cells by 3 months of age. [At this age mitotic activity falls to zero, and diploid (G_1 blocked) and tetraploid (G_2 blocked) noncycling cells can be demonstrated afterward.] Immature aging transitions to noncycling cells can also be found in renewing tissues. Examples are mouse ear epidermis (*23*), which shows a sharp decrease in mitotic activity when animals reach 12 weeks of age; rapidly renewing mouse duodenum (*24*), which shows a drop in the number of crypt cells entering DNA synthesis when animals reach 8 weeks of age; and splenic lymphocytes of the rat (*25*), which also show a decrease in the number of cells entering DNA synthesis in animals at 8 weeks of age.

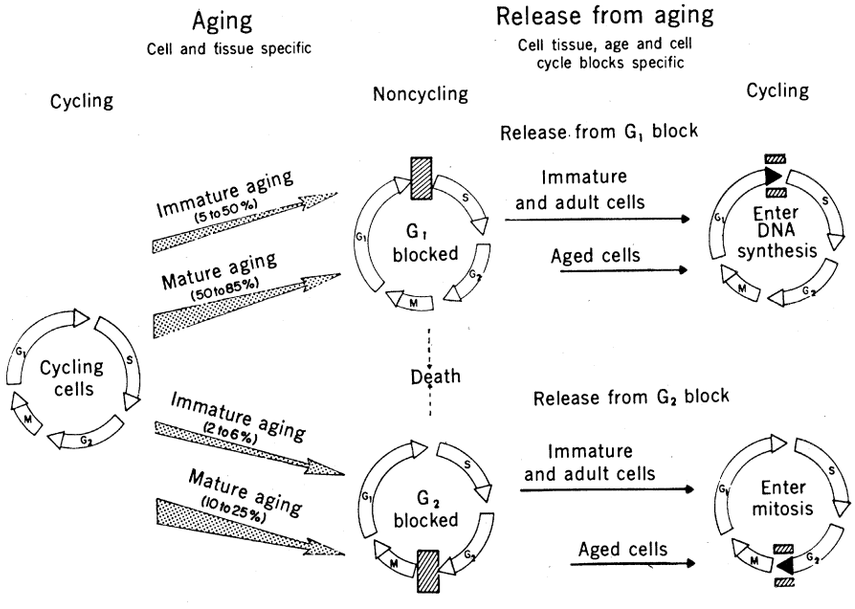

Fig. 1. Concept of cellular aging in tissues capable of proliferation.

Experimental evidence for the existence of G_2 blocked cells and for their behavior during animal aging in mouse kidney and epidermis is presented in Table 1. Cells blocked in G_2 were first described in mouse epidermis by Gelfant (*2, 3*). They exist in mouse kidney and duodenum (*26*), and also in a wide variety of plant, animal, and tumor tissues (*4*). Cells blocked in G_2 can be demonstrated by the experimental design in Table 1. Tissue-specific, compensatory growth stimuli are applied—unilateral nephrectomy, which produces a growth response in the contralateral kidney; and skin wounding, which causes repair and regeneration of epidermis. In both instances there is a delay of about 48 hours before kidney tubular or ear epidermal cells actively enter mitosis (*4, 27*). During this 48-hour period [^{3}H]thymidine is administered continuously; thus any epidermal or kidney cell that enters mitosis unlabeled must have been G_2 blocked before the experiment began. Colcemid is used during a 6-hour period to arrest mitotic cells in metaphase; significant numbers of cells in mitosis accumulate during this time. This procedure is highly sensitive, and it was specifically designed to demonstrate G_2 blocked cells in tissues showing a delayed mitotic response to growth stimuli [additional procedures are given by Gelfant (*4*)].

The results in Table 1 show that immature aging transitions from cycling to G_2 blocked cells occur in both the tubular epithelium of the kidney and in the epidermis of the ear. This manifestation of cellular aging occurs sooner in kidney than in epidermis. In both tissues, 5 to 6 percent is the maximum proportion of G_2 blocked cells that can be activated by compensatory growth stimuli (*28*). Thus these results support the concept in Fig. 1.

Table 1 also provides direct evidence for a tissue-specific, mature transition from cycling to G_2 blocked cells. In the epidermis of 30-month-old mice, the number of G_2 blocked cells is dou-

ble that of 3-month-old mice. This transition is tissue-specific, because no such change occurs in kidney tubular epithelium. Additional examples of cycling cells becoming noncycling G_1 or G_2 blocked cells during senescence are as follows. Mitotic activity in mouse ear epidermis drops from 1 percent at age 3 months to less than 0.1 percent at age 33 months (*23*). This observation substantiates the results in Table 1, which show an increase in noncycling G_2 blocked cells in mouse ear epidermis of the old animals. (The reported drop in mitotic index is probably also caused by mature aging transitions to G_1 blocked cells.) Proliferating mouse duodenum in senescent animals (*24, 29*) shows pronounced decreases in the number of cycling cells; the decreases are accounted for by decreases in the number of cells entering DNA synthesis (that is, cells that are not entering DNA synthesis are becoming G_1 blocked). There is diminished mitotic activity of myelocytes in the bone marrow of old rats (*30*); the mitotic index decreases from 2 percent in young animals to 0.05 percent in old animals. There also is a decrease in the number of antibody-producing cells in old mice (*31*); this reduced immunological capacity may be related to senescence and death of the animal. The last example comes from autoradiographic studies of replicating cells in rat liver (*17*). Adult liver is not considered to be a renewing tissue such as epidermal, gastrointestinal, and hematopoietic tissues (*32*), but has been described as a "discontinuous replicator" (*33*) that divides at a low "wear and tear" replacement rate (similar in this respect to kidney tubular epithelium and osteogenic tissue of bone). The low DNA synthetic index of rat liver was markedly decreased in the replicating diploid class of liver cells of 36-month-old rats.

Table 1. Immature and mature aging transitions to G_2 blocked cells in mouse kidney and epidermis [adapted from Pederson and Gelfant (*47*)]. The experimental design is as follows. (i) Kidney tubular epithelium is stimulated by unilateral nephrectomy. (ii) Ear epidermis is stimulated by wounding. (iii) [^{3}H]Thymidine is administered continuously for 54 hours in vivo. (iv) Mitoses are collected with Colcemid in vivo during the last 6 hours. (v) Unlabeled mitoses are evidence for release of G_2 blocked cells.

Age of mice	Unlabeled mitoses (%)*	
	Kidney epithelium	Ear epidermis
Immature aging		
2 weeks	3.0	2.5
8 weeks	6.0	3.2
12 weeks	5.6	6.5
Mature aging		
3 months	6.2	5.8
30 months	6.4	12.0

* Average of three C57BL/6J male mice used for each age group.

Release from Aging: In vitro Examples

The ideas of release from aging represented in Fig. 1 can also be supported by in vitro and in vivo results. The in vitro results are drawn from other sources in addition to studies of human diploid cell cultures, which were used above to support our views on in vitro cellular aging. For example, Castor (*34*), using time-lapse cinemicrography to study the effects of contact regulation of cell division in monolayer cultures of heteroploid mouse cells, showed that crowded, contact-inhibited cells could be released from inhibition merely by scraping a line across a confluent culture (thereby removing cells). Approximately 16 percent of the cells along the scrape edge divided within a few hours; this short time lapse indicates a release of G_2 blocked cells. Other cells were seen in mitosis after a latent period of about 20 hours, which indicates a release of G_1 blocked cells. In studies of myeloid cells in rat bone marrow, Balázs and Rappay (*30*) decribed the loss of dividing ability that occurs with aging during the differentiating stages of the myeloid series. The youngest myeloblasts, promyelocytes, and myelocytes synthesize DNA and proliferate; only 10 percent of the older metamyelocytes proliferate; and the oldest myeloid cells, the granulocytes, are unable to synthesize DNA or proliferate. In this instance the designations of immature and mature aging in vivo refer to the relative ages of cells in a differentiating series rather than to the age of the whole organism. These investigators report a release from aging in bone marrow cultures of cells that had lost their ability to synthesize DNA and divide (that is, G_1 and G_2 blocked cells). The cells transformed in tissue culture and began synthesizing DNA and proliferating—evidence of a release from G_1 and G_2 blocked states in aged cells.

Some observations of release from aging in the human diploid cell culture system raise questions about Hayflick's concept of genetically determined cell life-span and fixed number of cell divisions in vitro (*35, 36*). Macieira-Coelho *et al.* (*12*) provide conclusive evidence for the release from aging of G_2 blocked cells. Using [^{3}H]thymidine autoradiography, they demonstrated a transition to G_2 blocked cells occurring with increased culture cell density. The block is then released by seeding the cultures at lower cell densities. The released G_2 blocked cells then enter mitosis and proliferate during late passage stages, when normally there is very little or no cell proliferation. There also have been successful attempts to increase the life-span and the number of cell population doublings of human embryonic diploid cell strains by adding hydrocortisone or cortisone to the cultures (*9, 37*). An increase in the number of cells synthesizing DNA in the presence of hydrocortisone has been reported (*9*); this would represent a release from the G_1 block.

These effects of hydrocortisone, of increasing the life-span of cells by reviving their proliferative capacity in culture, require special comment because they so strongly support the concept of cellular aging in Fig. 1. Indeed, Cristofalo (*9*) interprets his results in terms of "retarding cellular aging" and "postponing cell death." There is additional evidence regarding hydrocortisone release of aged noncycling G_1 and G_2 blocked cells. DeCosse and Gelfant (*38*) show that hydrocortisone releases noncycling G_2 blocked Ehrlich ascites tumor cells into mitosis. It appears probable that release from aging by hydrocortisone in the human diploid cultures also involves a release from the G_2 blocked state. DeCosse and Gelfant (*5*) explain the mechanism of action of hydrocortisone in terms of immunosuppression by showing that other immunosuppressants, such as antiserum against lymphocytes, bring about mitosis of G_2 blocked Ehrlich ascites tumor cells. They introduce the idea that noncycling cells may be blocked because of an immune inhibition mechanism located on the cell surface of the tumor cells.

In vivo Examples of Release from Aging

The last line of evidence for release from aging deals mainly with in vivo observations of liver and kidney regeneration in animals of different ages. Compensatory cell proliferation is easily induced in these organs by sur-

gical or chemical removal of tissue (*39–41*). Studies of mitotic activity and DNA synthesis during regeneration show release of both G_1 and G_2 blocked liver and kidney cells (*42, 43*). Both the degree and the rate of release are related to chronological age; more liver and kidney cells proliferate and do so at a faster rate in young animals than in old animals (*41, 43*). The only difference between release in the two tissues is the time lag before release of G_1 blocked liver cells—but not kidney cells—in older animals (*44*). Thus the specific proliferative responses of regenerating liver and kidney in animals of different ages support the concept of release from aging.

The results in Table 1 also bear on the subject of regenerative release from aging. Percentages of unlabeled mitoses relative to labeled mitoses are given, with no information about the overall mitotic activity under the various experimental conditions. Our results on kidney epithelium confirm reports (*43*) that a similar qualitative response is obtained in both young and old animals after unilateral nephrectomy, although proliferative activity is much lower in older animals. Thus the relative percentage of G_2 blocked kidney cells entering mitosis (Table 1) is the same in animals aged 3 or 30 months, and this percentage reflects the proportions of cycling to noncycling cells within the tissue. In contrast to kidney tissue (which has undergone immature aging), renewing epidermis tissue shows an increase in the number of G_2 blocked epidermal cells in old animals because the epidermis is undergoing mature aging. These results further confirm the variety and complex manifestations of release from aging.

Reversal of Cell and Tissue Aging

The model in Fig. 1 implies that noncycling cells that are released from aging into a cycling state may again revert to noncycling states. Both liver and kidney cells revert back to noncycling states after completion of compensatory regeneration. Perhaps the best example of the flexibility of this reversal is the report by Ingle and Baker (*45*) that rat liver has the capacity to regenerate and to respond to partial hepatectomy 12 times in 1 year.

Samis *et al.* (*46*), studying mitotic activity and DNA synthesis in livers of rats of various ages, recorded a marked increase in both DNA synthesis and mitotic activity in an old rat (aged 1007 days). They attributed this response to an age-related increase in cell death, which produced a regenerative response (that is, release from aging) in a tissue capable of proliferation and reconstitution. Thus, as one can predict from our model, experimental rejuvenation of specific organs and tissues might be possible by selectively releasing noncycling cells.

Additional Evidence

Two recent articles provide additional proof for our model and confirm some of our ideas. Cameron (*36*) presents additional in vivo examples of cycling cells becoming noncycling G_1 blocked cells during animal senescence (that is, mature cellular aging). Mice of ages 3 to 19 months received [^{3}H]thymidine continuously (by repeated injections) for 4 days to label all cycling cells moving from G_1 into DNA synthesis during this extended period of time. There was a progressive decrease with animal age in the number of labeled nuclei in the epidermis of the ear and in the epithelium of the kidney, tongue, and esophagus; the decrease was most pronounced in the older animals. This again illustrates that the transition to the G_1 blocked state is a characteristic of cellular aging and that it occurs in tissues which are either slowly or rapidly renewing.

The following excerpt is quoted from the conclusions of a review article by Cristofalo (*9*).

> "Perhaps the most striking and best documented age-associated changes observed thus far are (1) the decrease in the fraction of cells in the population capable of dividing or capable of incorporating radio-labeled DNA precursor into their nucleic acids within a fixed period of time; of the cells which do incorporate label, older populations contain a smaller fraction that subsequently go on to complete cell division; (2) the ability of glucocorticoids, such as hydrocortisone, to retard aging of the population (hydrocortisone increases the total proliferative capability of the culture by increasing the fraction of cells capable of division)."

The first conclusion quoted supports our concept that cellular aging is a progressive conversion of cycling to noncycling G_1 and G_2 blocked cells in tissues capable of proliferation. The second conclusion highlights our suggestion that immune mechanisms may be responsible for cellular aging. We have suggested that hydrocortisone, an immunosuppressive agent, reverses cellular aging by releasing noncycling cells. If this is so, then an immune inhibition mechanism that keeps cells in the noncycling state may be involved in producing cellular aging.

Summary and Significance

Aging on a celluar level is described as a progressive conversion of cycling to noncycling cells in tissues capable of proliferation. Noncycling cells become blocked either in the G_1 or G_2 period of the cell cycle (periods in interphase during which DNA is not synthesized). They remain in these noncycling states until death or until they are recalled to proliferate in response to tissue injury. Some tissues complete their cellular aging transitions during embryogenesis or before completion of maximum growth of the entire organism; these tissues have undergone immature aging—in contrast to mature aging, which takes place in other tissues during animal senescence. The noncycling state (synonymous with cellular aging) may be reversible; cells can be released from the G_1 and G_2 blocks.

These ideas are presented in the form of a model explaining cell and tissue aging. The model is supported both by in vitro and by in vivo experimental findings. Immune mechanisms may be involved in cellular aging by keeping noncycling cells in restraint, and cellular aging may be reversed by immunosuppressive or by tissue-regenerative release of noncycling cells. This model and related suggestions offer a point of view for investigating cell and tissue aging.

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Fourier Transform Spectroscopy

New methods dramatically improve the sensitivity of infrared and nuclear magnetic resonance spectroscopy.

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In the last year or so a wealth of new information about the structure of molecular systems has become available as a result of the recent dramatic 10- to 100-fold increase in the sensitivity of infrared (IR) and nuclear magnetic resonance (NMR) spectrometers. The development that has made this possible is generally called Fourier transform (FT) spectroscopy; it is also sometimes termed interferometry or time-domain spectroscopy. Although interferometric methods in optical spectroscopy have been well known and understood since the early work of Michelson (*1*), the widespread application of these techniques has become possible only recently with the development of the necessary instrumentation and of fast, inexpensive computers.

In this article we describe the FT method, provide some insight into the elementary theory and instrumentation involved, and give some examples of the ways in which FT spectroscopy is beginning to make an impact on chemical and biochemical research. We shall first treat briefly FT-IR spectroscopy, since the concepts involved in optical methods are somewhat more familiar and easier to grasp. However, the major part of this article will be devoted to NMR (*2*).

The basic power of FT spectroscopy arises, quite simply, from the fact that it is very much faster than frequency-domain or conventional methods. In conventional ^{13}C NMR spectroscopy, for example, it would require about 5000 seconds to obtain a complete spectrum with a resolution of 1 hertz. The same basic spectral information can be obtained in 1 second with the use of FT methods. In addition, the general quality of the spectrum (especially line shape) obtained in this very much shorter time is appreciably better. This great saving in time may be exploited in improving the signal-to-noise ratio (S/N) by the coherent addition of signals, since random noise tends to cancel. Such coherent addition, or "time averaging," can indeed be carried out with conventional spectroscopy as well, but often only with an expenditure of time that makes the experiment prohibitive. In the ^{13}C

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