Published in final edited form as:
Endocr Dev. 2011 ; 21: 23–29. doi:10.1159/000328117.

Growth plate senescence and catch-up growth

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Abstract

Longitudinal bone growth is rapid in prenatal and early postnatal life, but then slows with age and eventually ceases. This growth deceleration is caused primarily by a decrease in chondrocyte proliferation, and is associated with other structural, functional, and molecular changes collectively termed growth plate senescence. Current evidence suggests that growth plate senescence occurs because the progenitor chondrocytes in the resting zone have a limited replicative capacity which is gradually exhausted with increasing cell division. In addition, recent experimental findings from laboratory and clinical studies suggest that growth plate senescence explains the phenomenon of catch-up growth. Growth-inhibiting conditions such as glucocorticoid excess and hypothyroidism delay the program of growth plate senescence. Consequently, growth plates are less senescent after these conditions resolve and therefore grow more rapidly than is normal for age, resulting in catch-up growth.

In humans, body length increases rapidly during fetal life and early childhood, but linear growth progressively slows and eventually ceases during adolescence. Similar declines in linear growth rates are also observed in other mammals. Although linear growth is highly regulated by endocrine factors, this decline in growth rate does not appear to be caused by a change in hormone levels. Indeed, there is no known growth-regulating hormone whose concentration changes in a pattern that would explain the slowing of linear growth. For example, circulating insulin-like growth factor-I concentrations rise during early childhood as growth is slowing [1]. Furthermore, growth plate transplantation experiments suggest that growth deceleration is not caused by any systemic mechanism; when growth plates are transplanted between rabbits of different ages, the growth rate of the transplanted growth plates depends on the age of the donor animal rather than the recipient [2], indicating that the decline in growth rate is primarily due to a mechanism intrinsic to the growth plates themselves.

The rate of longitudinal bone growth and hence the rate at which body length increases, is primarily determined by the rate of chondocyte proliferation, the size attained by the hypertrophic chondrocytes, and the rate of cartilage matrix secretion [3]. In fact, the rate of longitudinal bone growth can be approximated mathematically by the number of cell divisions occurring in each proliferative column per unit time, multiplied by the height of the terminal hypertrophic chondrocyte [4]. This formula has a simple theoretical explanation; the number of cell divisions per column equals the number of new cells

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produced per unit time, and the height of the last hypertrophic chondrocyte approximates the amount of linear distance that will eventually be provided by each of those new cells. Cartilage matrix synthesis, while not explicitly part of the formula, is required to expand the intercolumnar extracellular space in the direction parallel to the long axis of the bone at a rate commensurate with that of the cellular expansion within the columns.

The decline in linear growth rate in the rat is due both to a decline in the proliferation rate and the size of the terminal hypertrophic cells [5,6]. Of these, the greater fold change occurs in the rate of chondrocyte proliferation. Presumably, the rate of cartilage matrix synthesis is also declining to keep pace with the decline in cellular growth.

The progressive loss of function that occurs with increasing age in the growth plate is accompanied by a structural involution [6,9,10]. With increasing age, the overall height of the growth plate declines because of a decrease in the number of resting zone chondrocytes and the number of proliferative and hypertrophic chondrocytes per column. In addition, the columns of chondrocytes become more widely spaced with age. These senescent structural changes have been best studied in rodents and rabbits. In humans, the rate of longitudinal growth rate also declines with age, as does both the number of chondrocytes per cell column [7,8] and proportion of proliferative chondrocytes per column [7] decreased with age. However, unlike rats, the height of the terminal hypertrophic cells and the height of the resting zone in human growth plates remains relatively constant during the period of juvenile growth deceleration [8].

This postnatal development program, which includes both progressive loss of function and progressive structural involution, has been termed growth plate senescence [11,12]. In biology, the word senescence has two meanings [13]. First, it can refer to physiological changes that occur with increasing age and typically involve a loss of function. Second, the word can refer to a specific cellular program that was first identified in cultured cells and includes a cessation in proliferation [13]. The term growth plate senescence uses the first meaning and simply refers to a physiological program that involves a loss of function and involution with increasing age.

The fact that growth plate senescence occurs with increasing age suggests the possibility that senescence is driven by a timing mechanism within the growth plate, such as a biological clock; however several studies indicate that this is not the case. Conditions that slow growth in the growth plate also slow the entire development program of growth plate senescence, suggesting that the process of growth, rather than age per se, is the underlying driver of senescence. The specific experimental evidence for this conclusion comes from several animal models including growth inhibition by glucocorticoid excess in the rabbit [12], inhibition by hypothyroidism in rats [9], and inhibition by tryptophan deficiency (and consequently decreased food intake) in rats (unpublished observation). In each system, the experimental intervention slows longitudinal bone growth and also slows the changes in structural and functional markers of growth plate senescence. These observations, that slowing growth, using multiple different approaches, slows senescence, strongly imply that senescence is a function of growth rather than simply a function of age. However, the data do not exclude the possibility that age itself may also play some contributory role. How might growth drive senescence? One possibility is that chondrocytes might have some mechanisms to count cell cycles. Such cell-cycle counting appears to occur in some developmental processes. For example, the *Xenopus laevis* embryo undergoes exactly twelve synchronous cleavages before a period of slower asynchronous cell divisions [14]. This putative cell cycle counter in growth plate chondrocytes could involve some characteristic of the cell that is progressively altered with each cell division. As the cumulative number of cell divisions undergone increases, this putative counter might

advance and then turn off growth-promoting factors and/or turn on growth-inhibiting factors, causing proliferation to slow.

Whether or not a cell-cycle counter exists in the growth plate, it appears likely that this structure retains information about its prior growth history and that this prior growth history affects future growth. Theoretical considerations suggest that this information about the prior growth history is not stored in the proliferative or hypertrophic zone. The hypertrophic chondrocytes undergo apoptosis and are soon lost from the growth plate. The proliferative chondocytes under clonal expansion briefly but are not thought to be self-renewing but rather undergo terminal differentiation to become hypertrophic chondocytes and thus are subsequently lost to apoptosis. One could imagine that the information regarding prior growth history is somehow stored in these zones but passed on to surrounding cells so that it is not lost as each cell dies. However, another simpler explanation is that the prior growth history is retained in the resting zone. This zone appears to contain progenitor chondrocytes that can produce new columnar clones of proliferative and hypertrophic chondrocytes and are thought to persist, self-renewing by slow cell division, throughout the lifespan of the growth plate [15]. If this slow replication of progenitor cells in the resting zone were to gradually deplete their replicative capacity, then their clonal progeny in the proliferative zone might also show progressively diminished proliferative activity, leading to growth deceleration. Alternatively, if the progenitor cells were simply to become depleted numerically, the proliferative columns might be renewed less often. This might account for the decrease in column density that occurs with senescence as well as the decrease in proliferation within each column. In fact, there is evidence that the resting zone chondrocytes do become depleted both in number and proliferative activity as senescence progresses [16]. This qualitative and quantitative depletion might be interrelated; a loss of proliferative capacity in these cells might limit not only their ability to generate active proliferative clones but also their ability to self-renew, leading to their own numerical depletion. Interestingly, treatment with glucocorticoid excess *in vivo* slows chondrocyte proliferation in the resting zone and also slows the numerical depletion of these cells, consistent with the hypothesis that the numerical depletion of these cells is dependent on the cumulative number of cell divisions undergone [16].

Although the proliferative activity of resting zone chondrocytes declines during senescence *in vivo*, this decline may not necessarily be due to a cell-autonomous depletion of proliferative capacity. When resting zone chondrocytes are isolated from rabbits and cultured *in vitro*, they undergo a limited number of cell divisions before undergoing replicative senescence. However, the number of population doublings that these cells undergo seem to be fixed rather than dependent on whether the cells were obtained from fetal rabbits, rapidly growing young rabbits, or older rabbits that grow more slowly [17]. This finding suggests that the mechanisms responsible for limiting proliferation *in vivo* and the mechanisms that limit proliferation in cell culture are different. This observation also raises the possibility that the mechanisms limiting growth *in vivo* are not cell autonomous but rather require cell-cell interaction. As an example, slowing of proliferation might reflect decreasing expression of a paracrine growth factor. When cells are taken from the growth plate and placed in culture, their growth may no longer be limited by this factor but rather by growth factors added to the culture medium, allowing them to bypass the *in vivo* limit and continue growing until they reach a different limit, perhaps the same limit that prevents other cell types from growing indefinitely when placed in culture, the Hayflick limit [18].

The molecular mechanisms that slow growth *in vivo* are not well understood. However, recent studies suggest that there is a complex set of changes in gene expression that occurs in the growth plate during senescence that may contribute to this developmental process. Gene expression has been assessed in young rats, which are growing rapidly, and older rats,

which are growing more slowly [19]. This comparison was done by micodissecting the proliferative plus early hypertrophic zone from these rats, isolating RNA and then measuring specific mRNA levels by expression microarray analysis, followed by verification using real-time RT-PCR. This analysis has pointed to specific candidate genes, such as *Igf2*, a growth-promoting gene which is downregulated almost 1000-fold during senescence [20]. This analysis also suggests that FGF, Wnt, eicosanoid, p38-MAPK and vitamin D receptor signaling may be involved [19,21]. There is evidence that the decline in *Igf2* mRNA expression and a limited number of other changes in expression are driven by growth, rather than by time, suggesting that these changes are part of the senescence program [9]. Nevertheless, substantial additional investigation will be required to test whether these pathways are only temporally associated with growth plate senescence or they indeed play a causal role.

Our improved understanding of growth plate senescence has provided insight into the clinical phenomenon of catch-up growth. Catch-up growth is defined as body growth that occurs at a rate greater than normal for age, following a period of growth inhibition [22]. Clinically, catch-up growth has been observed after growth inhibition due to a variety of causes, including hypothyroidism, malnutrition, and glucocorticoid excess. Previously, catch-up growth was ascribed to a homeostatic mechanism within the central nervous system [23]. However, local growth inhibition within a single growth plate leads to local catch-up growth, suggesting that the mechanism responsible for catch-up growth resides, at least in part, within the growth plates themselves [24]. Subsequent studies have shown evidence that this local catch-up growth results from delayed growth plate senescence. Growth-inhibiting conditions slow senescence, including the decline in the chondrocyte proliferation rate, the size of the hypertrophic chondrocyte and, consequently, the rate of longitudinal bone growth [9, 12]. Therefore, when the growth-inhibiting condition resolves, the growth plates grow more rapidly than is normal for age, resulting in catch-up growth. Simply put, because growth-inhibiting conditions slow aging of the growth plate, after the condition resolves, the growth plates grow more rapidly like those of younger animals. The evidence for this mechanism comes from studies of catch-up growth after glucocorticoid excess in rabbits [12] and after hypothyroidism in rats [9].

Indirect evidence suggests that catch-up growth in humans is also due to delayed growth plate senescence. In children with celiac disease growth is often inhibited, and, when a gluten-free diet is initiated, catch-up growth occurs. In these children, the rate of growth during the first 4 years of treatment was greater than expected for age (indicating catch-up growth) but normal for a younger child, based on their height age or bone age at the time of treatment initiation [25]. This pattern of right-shift in the growth curve during catch-up growth was consistent with the hypothesis of that delayed senescence contributes to catch-up growth.

In summary, with increasing age, the growth plate undergoes programmed senescence, including both loss of function and structural involution. Growth plate senescence appears to be driven, not by time, which would suggest a biological clock mechanism, but by growth itself, suggesting the possibility of a cell-cycle counting mechanism. Theoretical considerations and limited empirical findings suggest that the information about the prior growth history is stored in the resting zone. The stem-like cells in this zone appear to be depleted during senescence, both quantitatively and also qualitatively, in terms of their proliferative activity. The molecular mechanisms that cause growth plate senescence are not well understood but may involve a complex set of changes in gene expression that occurs in the growth plate postnatally. Finally, growth plate senescence appears to explain an interesting clinical phenomenon; there is evidence that catch-up growth is due to delayed growth plate senescence.

Acknowledgments

This work was supported by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH.

Reference List

1. Zapf J, Walter H, Froesch ER. Radioimmunological determination of insulinlike growth factors I and II in normal subjects and in patients with growth disorders and extrapancreatic tumor hypoglycemia. J Clin Invest. 1981; 68:1321. [PubMed: 7028787]
2. Stevens DG, Boyer MI, Bowen CV. Transplantation of epiphyseal plate allografts between animals of different ages. J Pediatr Orthop. 1999; 19:398. [PubMed: 10344328]
3. Hunziker EB. Mechanism of longitudinal bone growth and its regulation by growth plate chondrocytes. Microsc Res Tech. 1994; 28:505. [PubMed: 7949396]
4. Sissons, HA. Experimental study on the effect of local radiation on bone growth. In: Mitchell, JS.; Holmes, BE.; Smith, CL., editors. Progress in radiobiology. Edinburgh: Oliver and Boyd; 1955. p. 436-452.
5. Kember NF, Walker KV. Control of bone growth in rats. Nature. 1971; 229:428. [PubMed: 4927000]
6. Walker KV, Kember NF. Cell kinetics of growth cartilage in the rat tibia. II. Measurements during ageing. Cell Tissue Kinet. 1972; 5:409. [PubMed: 4639299]
7. Sissons HA, Kember NF. Longitudinal bone growth of the human femur. Postgrad Med J. 1977; 53:433. [PubMed: 917957]
8. Kember NF, Sissons HA. Quantitative histology of the human growth plate. J Bone Joint Surg Br. 1976; 58-B:426. [PubMed: 1018028]
9. Marino R, Hegde A, Barnes KM, Schrier L, Emons JA, Nilsson O, Baron J. Catch-up growth after hypothyroidism is caused by delayed growth plate senescence. Endocrinology. 2008; 149:1820. [PubMed: 18174286]
10. Weise M, De-Levi S, Barnes KM, Gafni RI, Abad V, Baron J. Effects of estrogen on growth plate senescence and epiphyseal fusion. Proc Natl Acad Sci U SA. 2001; 98:6871.
11. Nilsson O, Baron J. Fundamental limits on longitudinal bone growth: growth plate senescence and epiphyseal fusion. Trends Endocrinol Metab. 2004; 15:370. [PubMed: 15380808]
12. Gafni RI, Weise M, Robrecht DT, Meyers JL, Barnes KM, De-Levi S, Baron J. Catch-up growth is associated with delayed senescence of the growth plate in rabbits. Pediatr Res. 2001; 50:618. [PubMed: 11641457]
13. Campisi J, dda di FF. Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol. 2007; 8:729. [PubMed: 17667954]
14. Newport JW, Kirschner MW. Regulation of the cell cycle during early Xenopus development. Cell. 1984; 37:731. [PubMed: 6378387]
15. Abad V, Meyers JL, Weise M, Gafni RI, Barnes KM, Nilsson O, Bacher JD, Baron J. The role of the resting zone in growth plate chondrogenesis. Endocrinology. 2002; 143:1851. [PubMed: 11956168]
16. Schrier L, Ferns SP, Barnes KM, Emons JA, Newman EI, Nilsson O, Baron J. Depletion of resting zone chondrocytes during growth plate senescence. J Endocrinol. 2006; 189:27. [PubMed: 16614378]
17. Nilsson O, Mitchum RD Jr, Schrier L, Ferns SP, Barnes KM, Troendle JF, Baron J. Growth plate senescence is associated with loss of DNA methylation. J Endocrinol. 2005; 186:241. [PubMed: 16002553]
18. Hayflick L. The limited in vitro lifetime of human diploid cell strains. Exp Cell Res. 1965; 37:614. [PubMed: 14315085]
19. Lui JC, Andrade AC, Forcinito P, Hegde A, Chen W, Baron J, Nilsson O. Spatial and temporal regulation of gene expression in the mammalian growth plate. Bone. 2010; 46:1380. [PubMed: 20096814]

20. Parker EA, Hegde A, Buckley M, Barnes KM, Baron J, Nilsson O. Spatial and temporal regulation of GH-IGF-related gene expression in growth plate cartilage. J Endocrinol. 2007; 194:31. [PubMed: 17592018]

21. Lazarus JE, Hegde A, Andrade AC, Nilsson O, Baron J. Fibroblast growth factor expression in the postnatal growth plate. Bone. 2007; 40:577. [PubMed: 17169623]

22. Boersma B, Wit JM. Catch-up growth. Endocr Rev. 1997; 18:646. [PubMed: 9331546]

23. Lozada-Nur F, Cram D, Gorsky M. Clinical response to levamisole in thirty-nine patients with erythema multiforme. An open prospective study. Oral Surg Oral Med Oral Pathol. 1992; 74:294. [PubMed: 1407989]

24. Baron J, Klein KO, Colli MJ, Yanovski JA, Novosad JA, Bacher JD, Cutler GB Jr. Catchup growth after glucocorticoid excess: a mechanism intrinsic to the growth plate. Endocrinology. 1994; 135:1367. [PubMed: 7925098]

25. Emons JA, Boersma B, Baron J, Wit JM. Catch-up growth: testing the hypothesis of delayed growth plate senescence in humans. J Pediatr. 2005; 147:843. [PubMed: 16356444]