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Living and Dying for Sex

A Theory of Aging Based on the Modulation of Cell Cycle Signaling by Reproductive Hormones

Richard L. Bowen[a] Craig S. Atwood[b, c]

[a]Voyager Pharmaceutical Corporation, Raleigh, N.C., [b]Department of Pathology, Case Western Reserve University, Cleveland, Ohio, and [c]Section of Geriatrics and Gerontology, Department of Medicine, University of Wisconsin, Madison, Wisc., USA

Key Words
Aging · Hormone · Reproduction · Mitosis · Puberty

Abstract
A mechanistic understanding of aging has yet to be described; this paper puts forth a new theory that has the potential to explain aging in all sexually reproductive life forms. The theory also puts forth a new definition of aging – *any change in an organism over time*. This definition includes not only the changes associated with the loss of function (i.e. senescence, the commonly accepted definition of aging), but also the changes associated with the gain of function (growth and development). Using this definition, the rate of aging would be synonymous with the rate of change. The rate of change/aging is most rapid during the fetal period when organisms develop from a single cell at conception to a multicellular organism at birth. Therefore, 'fetal aging' would be determined by factors regulating the rate of mitogenesis, differentiation, and cell death. We suggest that these factors also are responsible for regulating aging throughout life. Thus, whatever controls mitogenesis, differentiation and cell death must also control aging. Since life-extending modalities consistently affect reproduction, and reproductive hormones are known to regulate mitogenesis and differentiation, we propose that aging is primarily regulated by the hormones that control reproduction (hence, the Reproductive-Cell Cycle Theory of Aging). In mammals, reproduction is controlled by the hypothalamic-pituitary-gonadal (HPG) axis hormones. Longevity inducing interventions, including caloric restriction, decrease fertility by suppressing HPG axis hormones and HPG hormones are known to affect signaling through the well-documented longevity regulating GH/IGF-1/PI3K/Akt/Forkhead pathway. This is exemplified by genetic alterations in *Caenorhabditis elegans* where homologues of the HPG axis pathways, as well as the daf-2 and daf-9 pathways, all converge on daf-16, the homologue of human Forkhead that functions in the regulation of cell cycle events. In summary, we propose that the hormones that regulate reproduction act in an antagonistic pleiotrophic manner to control aging via cell cycle signaling; promoting growth and development early in life in order to achieve reproduction, but later in life, in a futile attempt to maintain reproduction, become dysregulated and drive senescence.

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Richard L. Bowen, MD
Voyager Pharmaceutical Corporation
8540 Colonnade Center-Drive, Suite 409, Raleigh, NC 27615 (USA)
Tel. +1 919 846 4840, Fax +1 919 846 4881, E-Mail rbowenmd@voyagerpharma.com
Craig S. Atwood, PhD, csa@medicine.wisc.edu

Definitions

Mitogenesis – The process by which a cell divides to form two daughter cells.

Differentiation – The process by which a cell becomes specialized to perform unique functions.

Dyosis – The process by which dysregulated cell cycle signaling drives the biochemical and functional changes associated with senescence.

Definition of Aging

To uncover the answer(s) as to why we age we must first decide upon a definition of aging, i.e. to what are we seeking answers. A number of aging definitions have been put forward. An early definition that has lost favor is the probability of mortality definition that states that the older an organism is the greater the probability of its death. The major flaw with this definition is that the likelihood of death varies with age, i.e. there is an increased likelihood of death in humans during the first 3 months of life and in young men there is an increase in mortality during their late teens and early 20s compared to their 30s. The current, commonly accepted definition of aging – *a progressive decrease in functional ability over time* also has limitations [Austad, 1997]. An example given to support this definition is that regardless of how physically fit humans are at age 50 we are not able to run as fast as when we were age 20. This example is representative of neuromuscular function which begins to deteriorate in the mid to late 20s. However, there are numerous other functions that begin to deteriorate at much earlier ages. For example, immune function begins to deteriorate in the mid-teens, hearing begins to deteriorate after age 10, flexibility and vascular compliance begin to deteriorate almost immediately after birth. At least one function even begins to deteriorate prior to birth (fetal aging), the ability of female mammals to produce oocytes. Therefore many functions begin to deteriorate while other functions are still developing. Since senescence and growth and development are occurring simultaneously, in order for this definition to be meaningful one must specify to which function one is referring. Alternatively, one could argue that aging begins when *all* functions begin to deteriorate which typically does not occur until the seventh decade of life. These flaws illustrate the need for a better definition of aging.

Since from a reductionistic perspective all living organisms can be considered to be nothing more than a complex combination of chemical reactions, aging therefore has to be a result of changes in these chemical reactions. Therefore, we propose to define aging as *any change in an organism over time.* By defining aging as change over time, the *rate* of change could be used to define the *rate* of aging. By this definition, the time intervals during life associated with the greatest rate of change would correlate to intervals of the most rapid aging. This definition includes not only the changes associated with the loss of function (senescence) but also the changes associated with the gain of function (growth and development). If it were possible to stop all chemical reactions in an organism, no change would occur over time and aging would be halted. This has actually been achieved with the cryopreservation of embryos, some of which have been frozen for close to a decade [Machtinger et al., 2002] without exhibiting any loss of viability. In addition, spores that were encased in amber for over 250 million years have been successfully germinated [Vreeland et al., 2000]. In these situations the embryo and spore lack metabolic activity, and by the *loss of function* definition they would be dead since all functions have ceased. However, by the *change over time* definition they would not be dead, they would just not be aging. Again this definition can be illustrated with an example similar to the one mentioned previously, i.e, regardless of how physically fit we are at 50, *or at age 3*, we are not able to run as fast as when we are 20. In this vein, it is difficult to argue that a child does not age during the first 10 years of life. Therefore, if growth and development are also considered part of the aging process, the underlying mechanism(s) driving growth and development may very well be driving aging during all phases of life, including senescence.

The Reproductive-Cell Cycle Theory of Aging

Using the 'rate of change' definition of aging, the rate of aging in mammals is most rapid during the fetal period when organisms develop from a single cell at conception to a multicellular organism containing trillions of cells at birth. We would argue that increased cell proliferation, cell differentiation and cell death are the major changes that occur during this time and therefore define fetal aging. In other words, given that a neonate is born with well-differentiated tissues and organs, it is not only the number of cells (sum of cell proliferation and cell death) but also the type of cell (differentiation) that *changes* during this time. These changes during fetal life are controlled by a complex interaction of mitotic-differentiation-apoptotic stimuli henceforth termed 'cell cycle-signaling factors'. If cell cycle-signaling factors regulate aging during the fetal period, they may very well regulate aging during the rest of life.

Using humans as an example, the second most rapid period of aging corresponds to the first year of life, since there is a doubling in size and significant tissue remodelling from birth to 1 year. Thereafter, the rate of mitogenesis and differentiation is decreased throughout the remainder of childhood until the onset of puberty when it again increases significantly until reproductive maturity is reached. During this 5-year period of puberty (~13–18 years of age) the human doubles in mass from ~36–70 kg (for males) and the growth mitogenesis and development (differentiation) of the reproductive organs is completed resulting in humans becoming reproductively viable [Build Study, 1983]. We suggest the next 15 years, comprising the period of maximum reproductive function, represent a time when mitogenesis, differentiation, and cell death are in equilibrium and existing cells are replaced on a one-for-one basis with minimal change in their function. This is evidenced by the minimal change in the size and functional abilities of the organism. Therefore, the period of least change and slowest aging corresponds to the period of maximum reproductive function. As reproductive function begins to decrease, typically during the 4th decade of life, the rate of change in body composition and functionality, and therefore aging, begins to increase. This again might represent changes in the rates of mitogenesis-differentiation-cell death.

The question then becomes what factors are responsible for regulating mitogenesis, differentiation and cell death and therefore aging? Darwin [1859] argued that reproduction is the most important function of an organism with regard to the survival of its species. Therefore, an animal will attempt to maintain its ability to reproduce at all costs. Also from an evolutionary perspective, lifespan is dependent upon the requirement for an organism to develop the unique attributes that allow it to survive in an environment with finite resources while at the same time becoming reproductively viable. The species must then live sufficiently long to produce enough offspring to ensure perpetuation of the genome and yet not so long as to compete for resources with subsequent generations that are potentially more genetically appropriate for the current environment. In essence, since growth and development culminate with reproductive function and senescence begins with the loss of reproductive function, lifespan centers around reproduction. Considering this, the hormones that control reproduction, those of the hypothalamic-pituitary-gonadal (HPG) axis, are likely candidates for the signaling factors that regulate cell cycle processes. Hence, we will refer to this mechanism as the 'Reproductive-Cell Cycle Theory of Aging'.

Hormones That Regulate Reproductive Function – Hypothalamic-Pituitary-Gonadal Hormones

The hormones of the HPG axis are the principal hormones responsible for regulating reproduction and include centrally and peripherally produced hormones. In the human and many mammals the centrally produced hormones include gonadotropin-releasing hormone (GnRH) from the hypothalamus and the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), from the pituitary. Peripherally produced hormones include the sex steroids and inhibins that are primarily of gonadal origin, while activins and follistatin are produced in all tissues including the gonads [Carr, 1998]. The levels of each of these hormones are regulated by a complex feedback loop – activins from the periphery stimulate GnRH secretion from the hypothalamus which stimulates the anterior pituitary to secrete the gonadotropins, LH and FSH, which in turn bind to receptors in the gonads and stimulate oogenesis/spermatogenesis as well as sex steroid and inhibin production (fig. 1) [for review see Reichlin, 1998]. The sex steroids and inhibin then feedback to the hypothalamus and pituitary, resulting in a decrease in gonadotropin secretion [for review see Thorner et al., 1998]. GnRH secretion, and hence gonadotropin secretion, is modulated by activins which are produced in many tissues [Ling et al., 1986; Vale et al., 1986]. Activin signaling is in turn modulated by two different mechanisms, one mediated via inhibins and the other mediated via follistatin.

Inhibins bind to and inactivate activin receptors in a competitive manner and this inhibitory action is significantly enhanced in tissues whose cell membranes express β-glycan [Lewis et al., 2000]. Since inhibins are primarily produced in the gonads and their production is dependent on folliculogenesis/spermatogenesis [Knight and Glister, 2001], they are intimately involved in the regulation of the HPG axis and are a direct indicator of fertility. Since inhibin production correlates directly with gametogenesis, its primary function appears to be the regulation of gametogenesis, as opposed to steroidogenesis, allowing for sufficient, but not excessive, gamete production.

Follistatin irreversibly binds to activins and prevents them from binding to their receptors [DeKretser et al., 2002; Gray et al., 2002]. Follistatin is expressed in many different tissues and serum concentrations are known to change during pregnancy [Shang et al., 2003] and puberty [Foster et al., 2000], as well as with certain medical conditions such as polycystic ovary syndrome [Thorner et al.,

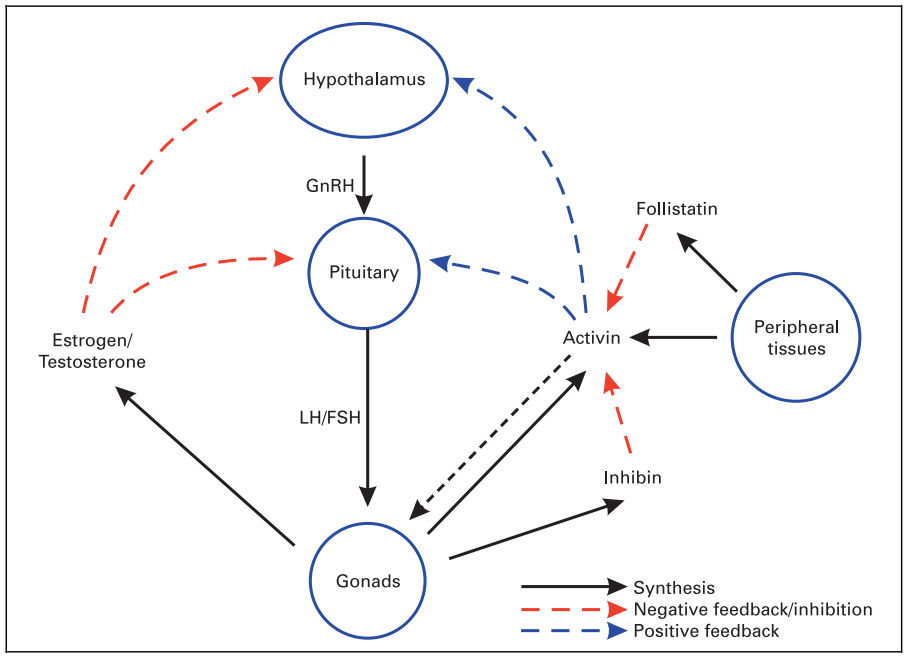

Fig. 1. The hypothalamic-pituitary-gonadal (HPG) axis. The concentration of each of the HPG axis hormones is regulated by complex feedback loops. The loop is initiated in periphery by activins which stimulate the hypothalamus to release gonadotropin-releasing hormone (GnRH). This in turn stimulates the anterior pituitary to secrete the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These then bind to receptors on the gonads and stimulate oogenesis/spermatogenesis, as well as sex steroid and inhibin production. The sex steroids feedback to the hypothalamus and pituitary, resulting in a decrease in gonadotropin secretion. Inhibin, produced primarily in the gonads in association with oogenesis/spermatogenesis, is known to bind to and lock activin receptors, thereby downregulating the reproductive axis. Follistatin, expressed in many different tissues also inhibits activins but appears to be more involved in non-reproductive functions of activin.

1998; Eldar-Geva et al., 2001]. Follistatin's role in the regulation of the HPG axis is quite complex and not fully understood at this time since it also likely functions to regulate some of the non-reproductive (mitogenic/differentiative) actions of activins in an autocrine/paracrine fashion. For instance, depending on the tissue, there is differential up- and down-regulation of follistatin and activins after injury [Inoue et al., 1994; Hubner et al., 1996; Wu et al., 1999; Kojima et al., 2001; Zhang et al., 2003; Armand et al., 2003] to allow for adequate healing. While there are significant gaps in our knowledge, it is possible that upregulation of follistatin could function not only directly in the healing process, but also in suppressing reproductive function until healing is complete.

The above paragraph describes how reproductive function is regulated in humans. The specific hormones involved in this reproductive axis may vary in other species. Nonetheless, we propose that an analogous feedback loop exists in all sexually reproductive life forms. While the members of the HPG axis are the direct regulators of reproduction, each member of the axis is also likely impacted by other factors that would be altered under adverse or favorable reproductive conditions. For example, epinephrine and corticotropin-releasing factor which are upregulated during stress suppress GnRH/gonadotropin secretion [Smith et al., 2003]. In other words, reproductive function is always regulated, it is not simply a constitutive process.

Life Changes in Hypothalmic-Pituitary-Gonadal Axis Hormones and Their Relationship to Physiologic Function in the Human

Starting with the fetal period, which is the time of greatest mitogenesis and tissue differentiation, most of the HPG hormones are significantly upregulated [Andersson et al., 1998; Boyar et al., 1972; Casey and MacDonald, 1998; Fisher, 1998] (see also figure 2 which represents changes in gonadotropins throughout life). hCG and LH have similar sequence homology, share a common receptor to which they bind with similar affinity [Fiddes and Talmadge, 1984], and in many circumstances have similar affects on cell function. During fetal life, LH/hCG concentrations are very high [Casey and MacDonald, 1998]. Serum concentrations of progesterone, inhibins, activins, hCG/LH and FSH then decrease at birth with the loss of the placenta [Fisher, 1998], but these hormones, except for progesterone, begin to rise within approximately 2 weeks [Boyar et al., 1972; Grumbach and Styne, 1998]. They continue to rise, peaking at approximately 3 months of age and then decline to childhood levels by 9 months of age [Thorner et al., 1998]. This pattern of reproductive hormone secretion mirrors the initial 2-week period of weight loss [for review see Itabashi et al., 1992; Smith et al., 1994] and the subsequent rapid rate of growth (mitogenesis) and development (differentiation) during the 1st year of life. Serum concentrations of these hormones, as

Table 1. Changes in circulating hormone concentrations in the human during senescence versus caloric restriction, a longevity-inducing situation. Hormones that regulate aging would be expected to show paradoxical changes between senescence and caloric restriction. Based on these findings, the gonadotropins and GH are the most likely age-regulating hormone candidates. While GH also displays a paradoxical change, no paradoxical change is seen for rodents (see table 2)

Hormone	Senescence	CR
Sex steroids	⇩ reviewed in Lamberts [2003]	⇩ Veldhuis et al. [1993], reviewed in Lamberts [2003]
GH	⬇ reviewed in Lamberts [2003]	⇧ Ho et al. [1988], Hartman et al. [1992], Veldhuis et al. [1993], Misra et al. [2003]
IGF-1	⇩ e.g. Hesse et al. [1994], Toogood and Shalet [1998], Elmlinger et al. [2003], reviewed in Lamberts [2003]	⇩ e.g. Misra et al. [2003]
PRL	⇩ Greenspan et al. 1990, Iranmanesh et al. [1999] No change Jacques et al. [1987], Elmlinger et al. [2003] ⇧ Feldman and Goldberg [2002]	⇩ Veldhuis et al. [1993]
TSH	No change Vosberg et al. [1976], Jacques et al. [1987] ⇩ Olsen et al. [1978] healthy elderly; Elmlinger et al. [2003], ↑ Olsen et al. 1978] sick elderly	⇩ Spencer et al. [1983]
LH	⬆ Larson [2002], Morley et al. [1997], Feldman et al. [2002], Elmlinger et al. [2003]	⇩ Veldhuis et al. [1993], Bergendahl et al. [1998]
FSH	⬆ Larson [2002], Morley et al. [1997], Feldman et al. [2002], MacNaughton et al. [1991], Elmlinger et al. [2003]	⇩ Hoffer et al. [1986] No change Travaglini et al. [1976], Veldhuis et al. [1993]
ACTH	No change Blicher-Toft and Hummer [1977], Lamberts [2003]	?
Cortisol	⇧ Bergendahl et al. [1998], Elmlinger et al. [2003]	⇧ Veldhuis et al. [1993], Bergendahl et al. [1998]
Activin	⇧ Harada et al. [1996], Loria et al. [1998], Baccarelli et al. [2001]	?
Inhibin	⇩ MacNaughton et al. [1991], Bohring and Krause [2003], Baccarelli et al. [2001]	?
Follistatin	⇧ Wakatsuki et al. [1996]	?

well as growth and development, remain comparatively diminished throughout the rest of childhood until the onset of puberty [Thorner et al., 1998].

With the onset of puberty (the initiation of which may also be explained by the Reproductive-Cell Cycle Theory; see Appendix 1) [Plant and Shahab, 2002] there is an increase in the secretion of all HPG hormones [Grumbach and Styne, 1998]. Some of these hormones likely contribute significantly to the rapid increase in the rate of growth (mitogenesis) while others may be responsible for the developmental (differentiation) changes during puberty. The completion of puberty marks the end of growth and development, and the beginning of the period of least change and therefore slowest aging (and the period of maximum reproductive function). From this point forward, most change/aging is no longer defined by an increase in function (growth/development), but by a decrease in function (senescence). During this reproductive period, the hormones of the HPG axis and, therefore, cell proliferation/differentiation are in balance. However, the age-related decline in reproductive function results in an imbalance of this hormonal axis (tables 1, 2, fig. 2). This is

Table 2. Changes in circulating hormone concentrations in the rodent during senescence versus situations known to extend longevity (Dwarf mice, CR and stress). Hormones that regulate aging would be expected to show paradoxical changes between senescence and longevity extending situations. Based on these findings, the gonadotropins are the most likely age-regulating hormone candidates. Data are for mice and serum unless indicated otherwise

Hormone	Senescence	Dwarf mice	CR	Stress
Sex steroids	⇩ Bronson and Desjardins [1977]	⇩ Hochereau-de Reviers et al. [1987] (Snell)	⇩ Compagnucci et al. [2002] rat; Baranowska et al. [2001]	⇩ Moshkin et al. [1993]
GH	⇩ Crew [1987] mRNA	⇩ Tang et al. [1993] (Ames)	⇩ Chan et al. [1993] pituitary; Koizumi et al. [1989]	⇩ Ruisseau et al. [1978], Tache et al. [1978] rat
IGF-1	⇩ van Beuningen et al. [1993], Kalu et al. [1998]	⇩ van Buul-Offers et al. [1994]	⇩ O'Sullivan et al. [1989]	?
PRL	⇩ Parkening et al. [1980], Crew [1987] mRNA	⇩ Tang et al. [1993] (Ames); de Reviers et al. [1984] (Snell)	⇩ Koizumi et al. [1989], Watanobe et al. [1999a, b]	⇩ Ruisseau et al. [1978], Tache et al. [1978] rat
TSH	⇩ Penzes [1991]	⇩ Tang et al. [1993] (Ames)	⇩ Shi et al. [1993] mRNA; Harris et al. [1978] rat	⇧⇩ Ruisseau et al. [1978], Tache et al. [1978], Fukuhara et al. [1996] rat
LH	⬆ Parkening et al. [1980] ⇩ Bronson and Desjardins [1977] see Appendix 2	⇩ Hochereau-de Reviers et al. [1987] and de Reviers et al. [1984] (Snell); Tang et al. [1993] (Ames); Chandrashekar and Bartke [1996]	⇩ Watanobe et al. [1999a, b], Badger [1985] rat	⇩ Ruisseau et al. [1978], Tache et al. [1978]
FSH	⬆ Parkening et al. [1980] No change Bronson and Desjardins [1977] see Appendix 2	⇩ de Reviers et al. [1984] and Hochereau-de Reviers et al. [1987] (Snell); Tang et al. [1993] (Ames)	⇩ Perheentupa et al. [1995]	⇩ Ruisseau et al. [1978] males only; Tache et al. [1978]
ACTH	⇧ Wang et al. [1997]	?	⇩ Suemaru et al. [1986]	⇧ Fukuhara et al. [1996], Goundasheva et al. [1994], Giagnoni et al. [1983]
Cortisol	?	?	?	⇧ Ishii and Nakagawa [2001]
Activin	?	?	?	?
Inhibin	?	?	?	⇩ Tohei et al. [1997] rat
Follistatin	?	?	?	?

represented by a decrease in gonadal inhibin production [Reichlin, 1998] causing an increase in activin signaling [Gray et al., 2002]. This increase in activins then causes an increase in secretion of GnRH and gonadotropins [MacConell et al., 1999; Schwall et al., 1988; Weiss et al., 1993]. In addition, the age-related decrease in sex steroid production causes a loss of hypothalamic feedback inhibition and also stimulates GnRH and gonadotropin production [Carr, 1998]. In women the loss of this negative feedback by estrogen and inhibins [Couzinet and Schaison, 1993] results in a 3- to 4-fold and a 4- to 18-fold increase in the concentrations of serum LH and FSH, respectively (fig. 2) [Chakravarti et al., 1976]. This increase in serum gonadotropin concentration is greatest during the onset of menopause and remains elevated over the next 4–5 years [Wide et al., 1973]. After this time, gonadotropin concentrations continually decline but never decrease to levels seen during the reproductive period. Men experience a much more gradual loss of reproductive function and a corresponding progressive increase in gonadotropins. This ultimately leads to a greater than 2- and 3-fold increase in LH and FSH, respectively (fig. 2) [Neaves et al., 1984].

Experimental and Environmental Evidence for the Reproductive-Cell Cycle Theory

The possibility that the hormones of the HPG axis play a central role in regulating longevity is supported by the fact that interventions conferring increased longevity also frequently result in a decrease in reproductive function [Wade et al., 1996]. This makes sense from an evolutionary perspective, since the single most important function of an organism relative to the survival of its species is reproduction. Evolutionarily, the neurons that comprise

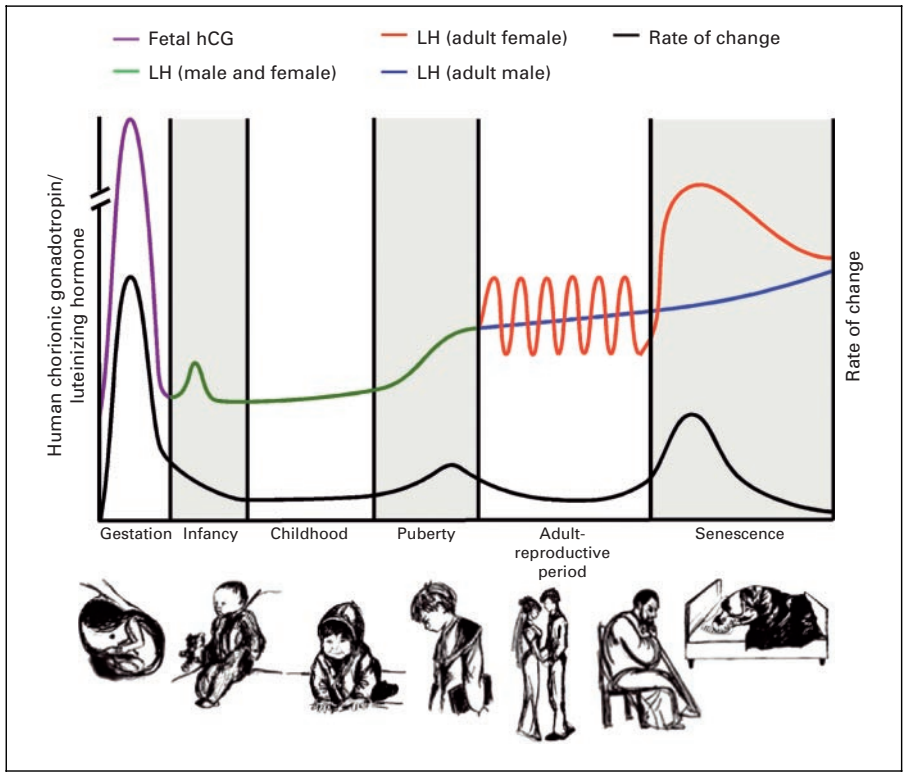

Fig. 2. Reproductive hormones drive change throughout life. For example, significant increases in LH/hCG occur during the growth and development of the mammalian fetus, infant, and pubertal adolescent. When the equilibrium between mitogenesis and differentiation-cell death becomes dysregulated during menopause/andropause, dyosis is initiated thus driving senescence (adapted after Boyar et al., 1972). Similarities between growth/development and senescence were even recognized by Shakespeare:

Jacques: All the world's a stage,
And all the men and women merely players;
They have their exits and their entrances,
And one man in his time plays many parts,
His acts being seven ages. At first, the infant,
Mewling and puking in the nurse's arms.
Then the whining schoolboy, with his satchel
And shining morning face, creeping like snail
Unwillingly to school. And then the lover,
Sighing like furnace, with a woeful ballad
Made to his mistress' eyebrow. Then a soldier,
Full of strange oaths and bearded like the pard,
Jealous in honour, sudden and quick in quarrel,
Seeking the bubble reputation
Even in the canon's mouth. And then the justice,
In fair round belly with good capon lined,
With eyes severe and beard of formal cut,
Full of wise saws and modern instances;
And so he plays his part. The sixth age shifts
Into the lean and slippered pantaloon
With spectacles on nose and pouch on side;
His youthful hose, well saved, a world too wide
For his shrunk shank, and his big manly voice,
Turning again toward childish treble, pipes
And whistles in his sound. Last scene of all,
That ends this strange eventful history,
Is second childishness and mere oblivion,
Sans teeth, sans eyes, sans taste, sans everything.
(As You Like It, 2. 7. 139–167)

the hypothalamus may have evolved from sensory neurons in more primitive species. This is supported by the fact that GnRH hypothalamic neurons originate from the olfactory placode [Schwanzel-Fukuda and Pfaff, 1989] which has a high concentration of mammalian chemoreceptors. The hypothalamus plays a crucial role in sensing and controlling the extracellular environment (blood) in the organism, in essence maintaining optimum osmolality, temperature, pH, oxygen tension and nutrients. Based on this information the hypothalamus also regulates reproductive function via modulation of the HPG axis through GnRH secretion. In this way, the hypothalamus senses the presence or absence of a reproductive friendly environment then appropriately regulates fertility and, according to the Reproductive-Cell Cycle Theory, aging. If environmental conditions for reproduction are not conducive to survival of the young, then fertility is reduced and aging is slowed [Holliday, 1989]. This allows the animal to preserve fertility and survive longer, awaiting conditions that are more advantageous for offspring and maternal survival. Examples of environmental settings that are not conducive for offspring survival and which

have been shown to increase longevity and decrease fertility include stresses such as an inadequate supply of food, water, and warmth [Holloszy and Smith, 1986; Wade et al., 1996; Weindruch and Walford, 1988].

Over 4 centuries ago Cornaro [1558] suggested that caloric restriction (CR; i.e. inadequate food supply) increases lifespan. He espoused CR as a means of prolonging life and these beliefs were advocated by many, including Thomas Edison, especially during the later years of his life [Benson, 1913]. Indeed, in all experimental situations in mammalian species studied to date, CR significantly increases lifespan by up to 40% [Lane et al., 2002; McCay et al., 1935; Weindruch and Walford, 1988]. This also holds true for lower organisms such as *Caenorhabditis elegans* where CR has been shown to increase longevity by up to 100% [Johnson et al., 1984; Weindruch and Walford, 1988] and in plants the restriction of available light energy (by pruning) results in the long-lived phenotype of the bonsai plant. It has been proposed that the mechanism by which CR extends lifespan is by decreasing the organism's metabolic rate and therefore its exposure to oxidative stress, i.e. the Metabolic Rate/Free Radical Theories of Aging [Harmon, 1956]. However, these theories are not supported by other studies where animals were provided food every other day (fed ad libitum 1 day and only water the next). In some of these studies, caloric intake is comparable between animals fed ad libitum every day and animals fed ad libitum every other day [Nelson et al., 1985]. Despite the minimal difference in caloric intake demonstrated by these two feeding regimens, every-other-day feeding still results in a significant increase in longevity [Goodrick et al., 1990]. These results suggest that it is not CR per se, but the lack of a *consistent* food supply that increases longevity. This also is supported by studies in rats that show continual exposure to cold results in increased food intake and metabolic rate yet has no affect on lifespan (and parameters of health) [Holloszy and Smith, 1986]. As would be expected, cold exposure in rats significantly increases oxidative stress (Kaushik and Kaur, 2003). These results are inconsistent with the Metabolic Rate/Free Radical Theories of Aging. However, these results are logical from a reproductive standpoint. A cold environment would compromise the survival of the young, but also the adult given the high metabolic demands of gestation, lactation and rearing. In an environment hostile to reproduction, hormones controlling reproduction (i.e. cell cycle-signaling factors) are suppressed allowing the animal to survive and preserve fertility until a more friendly reproductive environment exists [Holliday, 1989]. This is supported by many papers showing that pituitary hormones are extremely sensitive to environmental stressors such as decreased food intake, cold temperature, or injury (tables 1, 2) [for review see Wade et al., 1996]. Since environmental stresses such as CR and long-term cold are known to inhibit GnRH secretion [Badger, 1985; Hoffer et al., 1986; Kotaiah and Saxena, 1979; Wade et al., 1996] and decrease pituitary hormones and inhibin (tables 1, 2), it is logical that *the hormones that regulate fertility also regulate the rate of aging.*

There is ample experimental evidence supporting decreased pituitary hormones as the primary mechanism responsible for increased longevity. Early work by Arthur Everitt at the University of Sydney in the 1960s showed that the removal of the anterior pituitary (hypophysectomy) results in a decrease in reproductive function, delayed senescence and increased lifespan [Everitt, 1973; Everitt and Cavanagh, 1965; Everitt et al., 1968]. Deficiencies in pituitary hormones arising from hypophysectomy have been replicated more recently in mice with spontaneous genetic mutations and targeted knock-in/knock-out strategies that result in decreased pituitary hormone production. For example, Snell and Jackson dwarf mice [Camper et al., 1990; Li et al., 1990] have spontaneous mutations in the homeodomain of the Pit-1 gene [Ingraham et al., 1990] that result in pituitary glands that are hypoplastic and have significantly decreased populations of thyrotropes, somatotropes, gonadotropes and lactotropes and their respective hormones (table 2) [Yamaoka and Yamaguchi, 1988]. These mice have an increased mean longevity (25–50%), are infertile, show delays in age-dependent collagen cross-linking and in numerous age-sensitive indices of immune system status compared to background mice [Flurkey et al., 2001, 2002]. Likewise, the pituitary hormones are decreased in the Ames dwarf mice which have a spontaneous genetic mutation in the Prophet of pit-1 (Prop-1) gene [Sornson et al., 1996]. This gene encodes a paired-like homeodomain protein expressed specifically in embryonic pituitary that is necessary for Pit1 expression [Andersen et al., 1995; Wu et al., 1998]. This mutation results in a 50% and 64% increase (males and females, respectively) in longevity over wild-type siblings [Brown-Borg et al., 1996]. This mutation also results in significant decreases in gonadotropins (table 2) leading to decreased fertility or sterility [Chubb and Nolan, 1985; Chandrashekar and Bartke, 1993]. Although Dwarf mice are deficient in many hormones of the anterior pituitary including GH, prolactin and TSH, and have deficiencies in LH and FSH sufficient to induce sterility, most recent attention has focused on the GH/IGF-1 pathway as promoting longevity. This is largely based on stud-

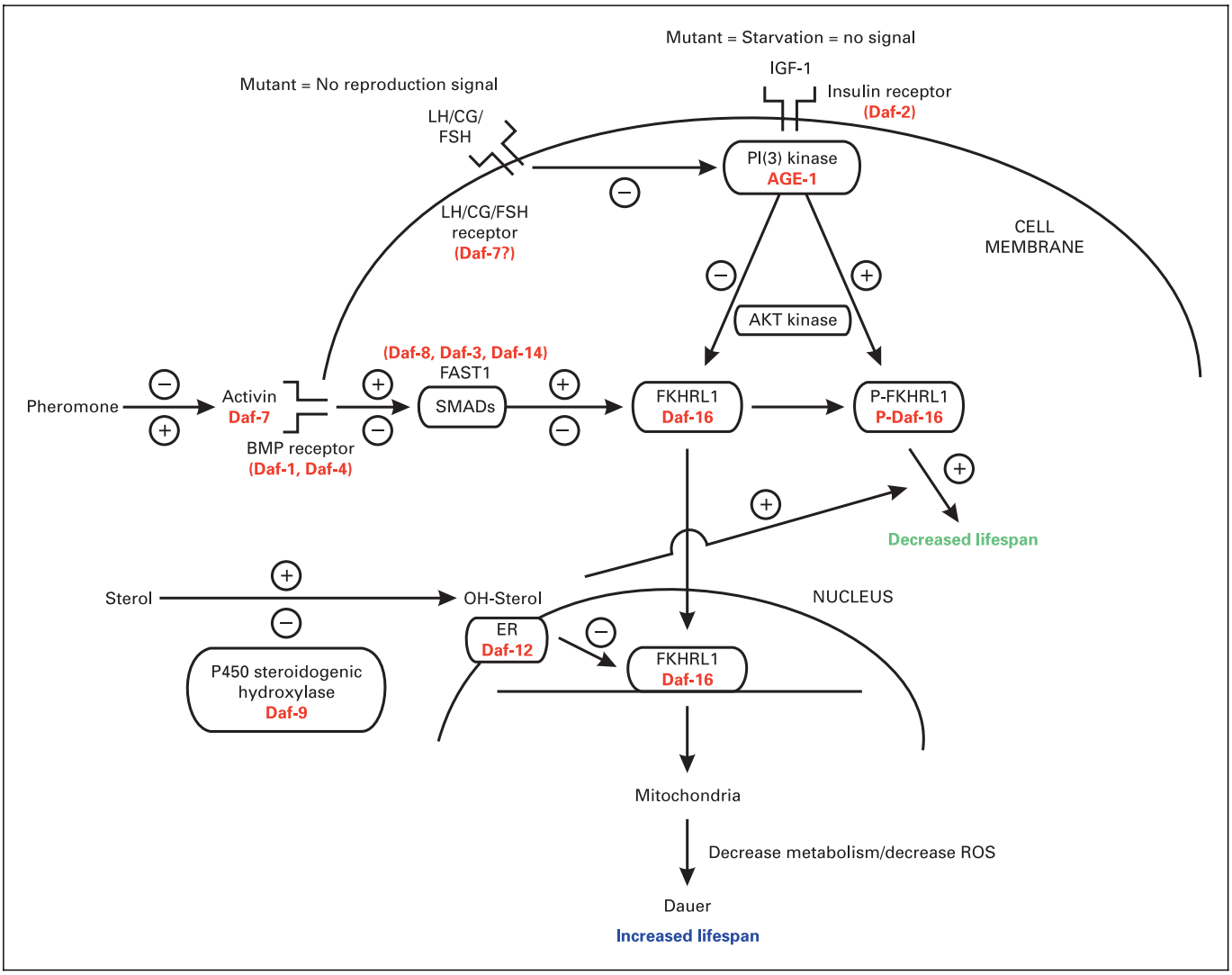

Fig. 3. Lifespan in *C. elegans:* homologous pathways in nematode and mammals. Null mutations in insulin/IGF-1 signaling pathway genes daf-2/age-1 and in steroidogenic pathway genes daf-9/daf-12 increase longevity and decrease fertility. Both pathways are dependent upon the transcription factor daf-16, the homologue of mammalian Forkhead that functions to regulate cell cycle events. Signaling through the daf-1 and daf-4 activin homolog receptors also modulates daf-16 nuclear localization. Thus, in both the nematode and in mammals, hormonal signals that determine aging exert their effects via the regulation of cell cycle events.

ies in *C. elegans* that have identified candidate genes via mutations that result in increased lifespan [Friedman and Johnson, 1988a, b; Kenyon et al., 1993]. The most well understood of these is the daf-2 gene which codes for an insulin/IGF-1 receptor (fig. 3) [Hsieh et al., 2002; Kenyon et al., 1993]. IGF-1 signaling through the daf-2 receptor is mediated via the PI3K/PDK1/Akt kinase signal transduction pathway [Franke et al., 1995; Hadari et al., 1992]. This leads to phosphorylation of the transcription factor, daf-16 (homologue of human Forkhead), preventing its translocation to the nucleus [Cahill et al., 2001; Paradis and Ruvkun, 1998] and results in a decrease in lifespan [Kops et al., 2002].

Further support for the GH/IGF-1 hypothesis are the findings that transgenic mice overexpressing GH and that acromegalic individuals who secrete large amounts of GH die prematurely [Laron, 2002]. The mice overexpressing GH [Debeljuk et al., 1999], and acromegalic patients [Damjanovic et al., 1996] and decreased fertility [Betea et al., 2002]. In addition, GH receptor knockout mice (pri-

mary IGF-1 deficiency) have been shown to display increased longevity compared to background controls [Coschigano et al., 2000]. Consistent with our theory, these animals exhibit quantitative deficits in various parameters of reproductive function [Zaczek et al., 2002]. These GH receptor knockout mice exhibit attenuated IGF-I signaling, increased serum prolactin concentrations, decreases in prolactin receptors and LH receptors and attenuated testosterone secretion in response to LH [Chandrashekar et al., 1999, 2001; Keene et al., 2002; Zaczek et al., 2002]. Taken together it is understandable why the prevailing hypothesis of aging contends that signaling through the IGF pathway controls longevity. However, since one of the last intermediates of the IGF-1 pathway is Forkhead, any signaling that affects Forkhead's translocation to the nucleus should also regulate longevity. Indeed, another longevity-modulating pathway has been identified in *C. elegans* that also converges on Forkhead (fig. 3). This pathway is regulated by the *C. elegans'* genes daf-9 and daf-12 [Gerisch et al., 2001; Larsen et al., 1995]. Daf-9 encodes an enzyme that is homologous to a human P450 steroidogenic hydroxylase, which functions in the biosynthetic pathway of steroids including estrogen and testosterone [Gerisch et al., 2001]. The product of daf-9 hydroxylation has been proposed to bind to the daf-12 orphan nuclear receptor [Gerisch et al., 2001; Jia et al., 2002]. In addition to being associated with the possible production of sex steroids, the daf-9 and daf-12 pathway is modulated by daf-1 and daf-4 which are homologous to human activin receptors [Estevez et al., 1993; Mathews and Vale, 1991; Ogg et al., 1997]. Daf-1 and daf-4 also modulate the daf-2 pathway and are involved with the *C. elegans'* response to pheromones [Gerisch et al., 2001; Ogg et al., 1997]. In addition to their association with daf-1 and daf-4, both pathways (daf-2 and daf-9/12) converge on, and their effect is dependent upon, daf-16 which, as mentioned previously, is homologous to the human Forkhead genes (FKHRL1, FKHR, and AFX) [Ogg et al., 1997]. Forkhead genes are known to function in the regulation of cell cycle related events (i.e. mitogenesis and differentiation) [for review see Kops and Burgering, 2000]. Indeed daf-16 plays a pivotal role in the growth of *C. elegans* as it progresses through the larval stages and determines whether the organism enters a hibernation-like state called dauer in the presence of environmental stressors [Vowels and Thomas, 1992]. Once reproductive maturity is reached at the end of larval stage 4 (puberty), division of somatic cells [Van Cleave, 1932] ceases but germ cells continue to proliferate. However, just as in mammals, the rate of germ cell proliferation (reproductive function) decreases with age [Dillin et al., 2002]. Decreased germ cell function in *C. elegans* is also associated with the daf-2 (IGF-1 receptor) mutation which has been equated to CR in mammals [Kimura et al., 1997]. Since the increased longevity of *C. elegans* daf-2 mutants is reversed in daf-2/daf-16 double mutants [Larsen et al., 1995], the changes associated with daf-2 mutations appear to be mediated through daf-16. Again, this gives credence to the idea that reproductive factors via their effect on Forkhead, either directly or indirectly through intermediates of the IGF-1 pathway, are primary regulators of longevity. The Reproductive-Cell Cycle Theory would suggest that senescence in *C. elegans* might also be driven by an attempt to maintain reproductive function resulting in hormonal changes and cell cycle dysregulation homologous to what we propose for mammalian senescence. No one to date has described such a phenomenon. Therefore, we propose that the hormones controlling reproductive function in this animal become dysregulated in an attempt to maintain germ cell proliferation and that this altered cell cycle signaling also affects somatic cells. An example of this phenomenon which we term 'dyosis' (dysregulated mitosis) is the polyploidy that occurs in terminally differentiated cells of *C. elegans*. Increased ploidy also has been reported in many other species during senescence [Flemming et al., 2000; Ly et al., 2000]. For example, polyploidy occurs in the terminally differentiated neurons in the Alzheimer's disease brain but not in age-matched controls [Herrup and Yang, 2001]. We would predict that ablation of germ cell precursors in *C. elegans* would result in an acceleration of polyploidy (dyosis) and may explain the increased size (giantism) observed in germ cell-ablated *C. elegans*. The increasing polyploidy of terminally differentiated cells has been termed 'endoreduplication' meaning that the cell duplicates itself within the confines of its cell membrane (does not undergo cytokinesis). This is supported by the fact that cytoplasmic volume and cytoplasmic organelle content increase proportionately to the cell's ploidy. Therefore, endoreduplication involves cell cycle activities and has been compared to cell proliferation [Larkins et al., 2001] and some have gone so far as to state 'the metabolic activity of a highly polyploid cell could be functionally equivalent to that of many diploid cells' [D'Amato, 1984; Larkins et al., 2001]. In certain cell populations such as megakaryocyte the functional consequences of endoreduplication may be desirable, however in terminally differentiated cells the consequences would be detrimental.

The manner in which a particular cell responds to dyotic signaling is likely a consequence of its state of differentiation, its particular receptor expression pattern for these hormones as well as the concentrations of the hormones to which the cell is exposed. For example, dyosis in a stem cell population might result in uncontrolled division (cancer) if the mitotic signal is high and the differentiation signal is low. In summary, the Reproductive-Cell Cycle Theory proposes that the loss of reproductive function triggers species- and tissue-specific dyotic signaling that is the cause of senescence.

In Search of the Human Mitogenic and Differentiation Factors

Thus far we have defined aging as any change in an organism over time and this includes growth and development as well as senescence. We have proposed that these changes are determined by changes in mitogenic and differentiation factors that regulate cell division, differentiation and cell death. Furthermore, these factors are likely members of the HPG axis hormones that control reproduction because all change/aging centers around reproduction. This assertion is supported by the abundant experimental and environmental evidence mentioned above. We will now discuss which hormones of the HPG axis likely represent the mitogenic factor(s) and differentiation factor(s). We will then go on to describe how interactions between these hormones dictate growth and development (i.e. onset of reproductive function and ultimate size of the organism) as well as senescence (i.e. loss of function and diseases of aging).

Serum concentrations of these factors would need to correlate with the rate of change during growth and development, and senescence, but also change in a predictable manner in situations known to induce longevity.

Mitogenic Factor(s)

The predicted profile of changes in serum concentrations of the mitogenic factors throughout human life are as follows: fetal +++++; infant ++++; childhood +/++; puberty +++; adult +, and senescence +++/++++. The profile of hormone concentrations during senescence would be expected to be the reverse of the profile seen in situations known to increase longevity such as CR as well as during other stresses that could be representative of hostile reproductive environments (table 1). The only hormones for which there are sufficient data to make a determination and that meet both the above criteria are LH, FSH [Burger et al., 1999; Kettel et al., 1996] and activins [Harada et al., 1996; Loria et al., 1998; Baccarelli et al., 2001]. It also is possible that GnRH meets the above criteria but there are no serum concentration data available during human senescence to make this determination. While activins have been shown to promote cell proliferation in some tissues, it is unlikely they would represent the mitogenic factor, since they more frequently inhibit cell proliferation and promote differentiation [Dalkin et al., 1996; Dignass et al., 2002; Engelse et al., 1999]. Therefore, the likely candidates for the mitogenic factor(s) are GnRH, LH, and/or FSH (fig. 2; Appendix 2). Evidence supporting a role for these hormones in driving cell proliferation includes: (1) FSH is associated with granulosa cell proliferation [El-Hefnawy and Zeleznik, 2001]; (2) hCG directly promotes the proliferation of myometrial and leiomyomal cells [Horiuchi et al., 2000]; (3) the basal proliferation of ovarian surface epithelium can be significantly increased by administration of pure recombinant gonadotropins FSH or LH [Davies et al., 1999]; (4) LH stimulates the growth of chondrocytes (cartilage cells) in rabbit epiphyseal growth plates [Webber and Sokoloff, 1981]; (5) LH receptors are expressed on lymphocytes [Lin et al., 1995] and both LH and FSH stimulate cell proliferation in lymphocytes [Athreya et al., 1993], and (6) LH increases BrdU incorporation into human neuroblastoma cells [Atwood, Bowen and Smith, unpublished observations]. The mechanism by which these hormones exert their mitogenicity is likely via signaling through members of the Forkhead family of transcription factors (human homologues of daf-16), phosphorylation of which stimulates mitosis [Richards et al., 2002]. This is based on: (1) recent evidence that FSH and LH regulate FKHR transcription [Richards et al., 2002]; (2) LH and FSH have been shown to increase signaling via the PI3K/AKT pathway (just as IGF-1 does) [Carvalho et al., 2003; Meroni et al., 2004] that is known to phosphorylate FKHR, and (3) in naive rodent granulosa cells, both FSH and IGF-1 stimulate rapid phosphorylation of FKHR at multiple sites causing its redistribution from the nucleus to the cytoplasm in a PI3K-dependent manner, thereby initiating mitogenesis [Biggs et al., 1999]. Additionally, in differentiated granulosa cells, FSH enhances phosphorylation of FKHR, PKB, and Sgk [Richards et al., 2002].

It also is possible that reproductive hormones promote mitogenicity by regulation of other growth factors. For example, GH is mitogenic [Thorner et al., 1998] and its secretion is inhibited by activin [Kitaoka et al., 1988; Yamaguchi et al., 1995; Bertherat et al., 1995; Bilezikjian et al., 1990; Struthers et al., 1992]. This may well explain

the suppression of GH during senescence due to the likely increase in bioactivity of activins as inhibin secretion decreases with decreased reproductive function [MacNaughton et al., 1991; Baccarelli et al., 2001; Bohring and Krause, 2003]. There appear to be direct interactions between GnRH and GH, although this interaction seems to be much more complex than that of activins [Amsterdam et al., 1982]. This aside, the potential importance of regulating cell proliferation in non-reproductive tissues is indicated by the widespread expression of receptors for these reproductive hormones. GnRH receptor is expressed in all tissues tested to date, including: pancreas, thymus, kidney, heart, brain, placenta, lung, liver, skeletal muscle, colon, ovary, small intestine, spleen, and pituitary [Chen et al., 1999]. LH receptor expression has been found in the skin, adrenal, vasculature, urinary bladder, duodenum, pancreas (rat), lung, brain (including neurons, astrocytes and microglia) and monocytes [Ascoli et al., 2002; Bukovsky et al., 2003]. To date FSH receptor expression in non-reproductive tissues has not been reported, and has only been documented as being localized to reproductive tissues: ovary, prostate, testis, corpus luteum, and placenta [Heckert and Griswold, 1991; Minegishi et al., 1997; Xing and Sairam, 2001]. If GnRH and/or LH are the mitogenic factors driving growth (cell proliferation), the question becomes what are the differentiating factors that limit growth and allow for cell specific function.

Differentiation Factor(s)

Changes in serum concentrations of activins fit the profile of an aging factor (table 1). They have been shown to regulate cell proliferation in reproductive and non-reproductive tissues, primarily functioning to promote differentiation [Asashima et al., 2000; Ball and Risbridger, 2001; Welt et al., 2002]. Therefore activins are likely to be the differentiation factor. They are important in tissue differentiation during fetal development in that they are required for endometrial receptivity, decidualization and implantation [Jones et al., 2002]. Moreover, activins regulate follicular development [Roberts et al., 1993]. Given that all cell types undergo differentiation, it would be expected that the receptors for the differentiation factor would be expressed in all tissues and such is the case with activin receptors [Baer et al., 1998; Baldwin et al., 1996; Dewulf et al., 1995; Kitten et al., 1999; Li et al., 2002; Schluns et al., 1995].

The manner by which activins [Nishimura et al., 1998] affect cellular function is extremely complex in that there are at least five different activin receptors; that these receptors share the same post-receptor signaling mechanism with at least 7 other bone morphogenetic protein receptors [for review see Kawabata et al., 1998; Miyazono, 2000] and by phosphorylating up to 8 different Smad proteins [Hoodless, et al., 1996; Nishimura, et al., 1998; Kawai et al., 2000]. Smads then participate directly in the regulation of gene expression by binding to DNA, interacting with transcription factors, and recruiting corepressors or coactivators to specific promoters [van Grunsven et al., 2002]. A further example of this complexity is exemplified by activin subunit interactions with one another. Activins and inhibins are dimeric proteins consisting of 2 non-covalently linked subunits which include 1 α-subunit and/or 5 β-subunits: A, B, C, D and E [Fang et al., 1996; Hotten et al., 1996; Oda et al., 1995; Vale et al., 1990]. The α-subunit is expressed primarily in reproductive tissues and is directly correlated to oogenesis and spermatogenesis, while β-subunits are expressed in reproductive and numerous other tissues [Hubner et al., 1999]. Inhibin A is composed of an α-subunit and a βA-subunit. Inhibin B consists of an α-subunit and a βB-subunit [Bernard et al., 2001]. Activin A is composed of 2 βA-subunits, activin AB is composed of 1 βA- and 1 βB-subunits, and activin B is composed of 2 βB-subunits [Halvorson and DeCherney, 1996]. Since β-subunits C, D and E have only recently been identified, very little is known about their interactions with the other subunits [Hotten et al., 1996; Mellor et al., 2000; O'Bryan et al., 2000]. Activins bind to specific receptors in the serine/threonine bone morphogenetic protein receptor family which, as mentioned previously, are expressed in all tissues thus far examined [Ethier and Findlay, 2001]. It remains to be determined if there are unique inhibin receptors; inhibin has, however, been shown to bind to activin type-2 receptors [Zimmerman and Mathews, 2001]. It appears inhibins function primarily to regulate the activity of activins by binding the activin receptor, blocking activin binding to its receptor [Bernard et al., 2001]. Even further complexity is evidenced by the fact that the inhibins' affinity or the activin receptor is greatly influenced by the presence or absence of the β-glycan content of the cell membrane.

The role of β:β dimers (activins) in regulating differentiation is well established by numerous studies in a wide range of species and tissues [Strahle et al., 1993; Dirksen and Jamrich, 1992; Kokan-Moore et al., 1991; Chertov et al., 1990]. Their potential role in the regulation of longevity is based primarily on studies in *C. elegans*. The *C. elegans* genes daf-1 and daf-4 are the homologues of human activin type-1 and activin type-2 receptors, respectively [Estevez et al., 1993; Mathews and Vale, 1991]. It has been shown that both the daf-2- and daf-9-signaling path-

ways are synergistically modulated by post-receptor signaling of daf-1/daf-4 [Ogg et al., 1997; Snow and Larsen, 2000]. The daf-1/daf-4 receptors are proposed to bind a TGFβ-type protein coded for by daf-7 [Estevez et al., 1993]. This results not only in decreased translocation of daf-16 to the nucleus [Lin, 2001], but also of the previously mentioned complex regulation of gene expression via Smads. It is probably through this complex regulation of gene expression that activins function in directing cellular differentiation into specific tissue phenotypes.

Molecular Interactions between Mitogenic and Differentiation Hormones Determine Cell Fate

In *C. elegans*, regulation of growth (mitogenesis) and development (differentiation) involves the common signaling pathway mediated via FKHR (fig. 3). We propose that in all species the ratio of mitogenic to differentiation hormones determines the phosphorylation state of FKHR and therefore its nuclear translocation. Differentiation signaling promotes non-phosphorylated FKHR proteins to translocate into the nucleus and mitogenic signaling promotes phosphorylation and prevents FKHR translocation into the nucleus. It is not clear whether a certain phosphorylation pattern among the plethora of FKHR proteins is responsible for determining whether a cell divides or differentiates or whether these FKHR proteins act in a tissue specific manner. Imprinting-associated DNA methylation patterns likely play a major role in the species tissue-specific outcome of this complex process. However, regardless of the complexity, it is logical that the translocation of FKHR into the nucleus is associated with transcription of genes that direct differentiation, while no translocation would be a 'default' signal to divide.

While the involvement of these hormones in reproduction and regulation of cell cycle events is evidenced by the information just presented, the Reproductive-Cell Cycle Theory proposes a logical mechanism that explains how these hormones go from promoting growth and development to regulating reproductive function, only to end up driving senescence.

Possible Mechanism by Which Interactions between Mitogenic and Differentiation Factors Regulate Growth and Development, Senescence, and Therefore Aging

In humans the concentrations of the proposed mitogenic factors (GnRH, LH and FSH) mirror serum concentrations of differentiation factors (activins) throughout life [Baccarelli et al., 2001; Temeli et al., 1985] and in this way growth (mitogenesis) is accompanied by development (differentiation). This allows for growth and development during childhood and puberty and allows for the maintenance of tissues in adult life. It would make sense that the same hormones that regulate growth and development would also regulate longevity since the period of growth and development leading up to reproductive maturity correlates well with lifespan [for review see Austad, 1997].

Rate of Growth and Determination of Size at Maturity

Regarding the importance of growth and development in determining lifespan, it is well established that, in general, large animal species have longer lifespans than small animal species [Finch, 1990]. However, there are many exceptions to this. For example the little brown bat lives 30 years [Austad and Fischer, 1991; Jurgens and Prothero, 1987], although it is half the size of the mouse that lives only 2 years [Austad, 1997; Miller et al., 2001; Weindruch and Walford, 1988]. The Reproductive-Cell Cycle Theory is able not only to explain the connection between body size and longevity but also the numerous exceptions (see Exceptions Section). By the theory, the rate of growth and ultimate size of an animal would be dependent upon the varying concentrations of mitogenic and differentiation factors and associated receptor expression patterns. We propose the mitogenic factors (human GnRH and gonadotropins) are produced centrally by the placenta during the fetal period and after birth by the pituitary [Casey and MacDonald, 1998; Thorner et al., 1998] while the differentiation factors (human activins) are produced peripherally by most all cell types [Welt et al., 2002]. To understand why the source of these hormones is important in the regulation of the ultimate size of the animal, a further understanding of activins is needed. As mentioned previously, 5 β-subunits have been identified thus far, possibly resulting in up to 32 (2^5) different dimeric combinations or unique activin molecules [Fang et al., 1996; Hotten et al., 1996; Mather et al., 1997; Oda et al., 1995; Vale et al., 1990]. We propose that as tissue-specific cell proliferation occurs, each individual cell produces a genetically predetermined species-specific amount of β-subunit.

If the expression of particular β-subunits is tissue-specific, which in some cases it appears to be, it could explain the regulation of organ size. For example, βE is primarily expressed in hepatic tissue [Fang et al., 1996, 1997]. Once synthesized, these subunits then dimerize and enter the circulation adding to the cumulative serum concentra-

Table 3. Animal size, growth rate and lifespan as determined by the Reproductive-Cell Cycle Theory

Rate of growth; Size of animal	Example	Weight	Reproductive maturity	Lifespan	Mitogen	Differentiation
Rapidly growing small animal	Mouse	28 g	2 months	2 years	95	95
Moderately growing small animal	Flying squirrel	85 g	1 year	15 years	50	95
Slowly growing small animal	Brown bat	20 g	1 year	30 years	10	95
Rapidly growing, medium-size animal	Sheep	90 kg	1 year	12 years	95	50
Moderately growing medium-size animal	Sloth bear	90 kg	3 years	30 years	50	50
Slowly growing medium-size animal	Human	68 kg	14 years	70 years	10	50
Rapidly growing large animal	Giraffe	690–1,900 kg	4 years	25 years	95	10
Moderately growing large animal	Hippopotamus	1,360–2,045 kg	7 years	50 years	50	10
Slowly growing large animal	Elephant	5,000–16,360 kg	13 years	70 years	10	10

The rate at which an animal matures and its size at maturity are strongly associated with species lifespan. In this table we assign hypothetical mitogenic and differentiation indexes to examples of animals of different sizes (small, medium, and large) and with different rates of growth (rapid, moderate, and slow). This illustrates how the hypothetical indexes determine the rate of growth, size at maturity and lifespan. The Reproductive-Cell Cycle Theory proposes that the rate of growth is determined by the centrally produced mitogen while the ultimate size at maturity is determined by the amount of differentiation factor (activin) produced by each individual cell.

tions of activins. The unique activin receptor expression pattern would then be responsible for promoting differentiation and halting cell proliferation *once circulating concentrations of its particular activin milieu reaches a critical level*. Once this critical level is reached, development of this tissue is complete. Again, in this way activins would be the primary regulators of organ/organism size. This is supported by work showing that activin A is a primary inhibitor of hepatic DNA synthesis and that blocking activin A after partial hepatectomy resulted in increased remnant weight and an increase in the rate of remnant growth [Kogure et al., 2000]. It is not known what regulates the production of activins, but if the function of activins is evolutionarily conserved from *C. elegans,* then it is likely regulated by many different environmental factors (see below).

The ability of this proposed mechanism to determine species-specific body size is exemplified in the following illustration. The species-specific rate of growth/cell proliferation, i.e. mitogenic index, and the rate of tissue differentiation (ultimately leading to the cessation of growth), i.e. differentiation index, is determined by the amount of mitogenic and differentiation factors produced and the sensitivity of cells to these factors. For example, if a particular species has a high mitogenic index (increased mitogenic signaling of central origin) and a high differentiation index (increased differentiation signaling of peripheral origin), the result will be a rapidly growing small animal such as the mouse (2-year lifespan; table 3). If, on the other hand, the species has a low mitogenic index, but still with a high differentiation index, the result will be a slowly maturing small animal such as the brown bat (30-year lifespan). Since the differentiation index determines the size of an organism, the mouse's differentiation index would be similar to that of the bat. Since the differentiation factors are proposed to be secreted by most peripheral cells, differentiation signaling would increase as the number of cells increases. Therefore, the point at which an organism reaches its ultimate size is dependent upon the overall number of peripheral cells and the amount of differentiation factors they secrete as well as their sensitivity to these factors.

This point can be further illustrated by comparing the rates of growth and development to lifespan in medium-sized animals such as the sheep and the human. Sheep reach reproductive maturity and adult size by 1 year, whereas it takes humans 14 years to reach reproductive maturity and a similar size (table 3). This corresponds to the differences in their respective longevities. Therefore, according to the Reproductive-Cell Cycle Theory, the mitogenic signaling from the sheep pituitary would be extremely potent (higher mitogenic index) compared to that of the human. The high potency of the sheep's mitogenic signaling could be a result of increased concentrations of mitogens and/or high receptor expression and/or high ligand-receptor affinity, etc. Since the differentiation index determines the size of an organism, the sheep's would be similar to that of the human.

Therefore, the Reproductive-Cell Cycle Theory proposes that the rate of cell proliferation, determined by the centrally produced mitogenic factors, combined with the rate of cell differentiation, determined by the peripherally produced differentiation factors, governs how quickly an animal matures and its size once maturity is reached, both of which are strongly correlated to species lifespan (table 3). The latter stages of this process typically coincide with the initiation of puberty which we propose is regulated by these factors (see Appendix 1).

Cost of Reproduction

Once maturity is reached, the rate of aging during the reproductive period would also be primarily determined by the cell cycle signaling factors that regulate reproductive function. In the long-lived bat for example, the GnRH/gonadotropins (proposed central mitogenic signaling factors) are briefly elevated only during the one time per year that they are fertile [Hayashi et al., 2002; Kawamoto et al., 2000]. This is also true for both sexes of the cockatiel which are exceptionally long-lived for their size, maximum lifespan 32 years. In these parrots the yearly increase in GnRH/gonadotropin (proposed central mitogenic signaling factors) concentrations in males is dependent upon witnessing nesting behavior of a female of the same species [Myers et al., 1989; Shields et al., 1989]. Therefore, the 'cost of reproduction' phenomenon can be explained by the fact that animals with a high rate of reproduction are exposed more often and to probably higher concentrations of these factors than animals with a lower rate of reproduction.

Body Size Paradox

In addition to their increased fertility, another factor that might contribute to the decrease in male lifespan compared to females is the fact that males are larger [Samaras and Elrick, 1999]. Interestingly, men and women of the same height have the same average age at death [Miller, 1990]. This also is supported by the fact that within a species, size and fertility have been shown to be inversely proportional to longevity [Comfort, 1961; Li et al., 1996; Miller, 1999; Miller et al., 2000, 2001; Samaras et al., 2002; Turturro et al., 1998]. However, the opposite is true for comparisons between species. A good example of this is the canine, a medium-sized species, that have more offspring per litter than larger species and also have much shorter lifespan [Li et al., 1996; Samaras and Elrick, 1999; Tedor and Reif, 1978]. In comparison, *within* the canine species, large-breed dogs generally have large litters and shorter lifespan than small-breed dogs [Tedor and Reif, 1978]. No studies have been performed comparing serum concentrations of the proposed reproductive cell cycle-signaling factors between breeds, however, the theory would predict that large breeds would have higher concentrations of the mitogenic factors and lower per cell production of differentiation factors than small-breed dogs. Interestingly, IGF-1 has been measured and found to be elevated in larger dogs within the same breed [Eigenmann et al., 1984a]. Similarly, small stature in different breeds of dogs has been explained on the basis of diminished IGF-1 production [Eigenmann et al., 1984b, c, 1988]. The Reproductive-Cell Cycle Theory would predict that the differences in IGF-1 are only partially responsible for the observed size. The difference in IGF-1 production could possibly be due to the indirect actions of activins on GH mentioned previously (activins inhibit the release of GH, large dogs would have less activins and therefore higher GH secretion resulting in increased concentrations of IGF-1). Activins also directly regulate the IGF-1 pathway via Smads (fig. 3). As previously stated increased signaling through the IGF-1 pathway is associated with a decrease in longevity. This explains why small dogs live longer than large dogs and an explanation of why the reverse is true between species was explained previously. The potential reconciliation of this well-known discrepancy between size and longevity within species compared to between species demonstrates the predictive power of the Reproductive-Cell Cycle Theory.

Exceptions – Cost of Food

In general large animal species live longer and have fewer offspring than small animal species. However, there are many exceptions to this generalization. These include animals that fly, such as the little brown bat and many birds, animals that have a shell (turtles), the naked mole rat that spends its entire life underground and certain fish. One explanation provided for this is that these species have developed specific attributes that allow them to evade predation. However, this doesn't hold for the poison dart frog (whose skin is covered with a poisonous mucous). Its lifespan is similar to other similar sized frogs that do not possess an attribute to avoid predation. Another example is the coral snake, one of the most poisonous snakes in the world which also possesses distinctive coloring. The maximum recorded lifespan is 7 years but the nonpoisonous garter snake lives 10 years and is of similar size. A further example is the flying fish that has evolved the ability to jump out of the water and glide up to *183 m*. This unique attribute is thought to assist in evading predators, however flying fish only live a year

which is much less than similarly sized fish. This brings into question the 'avoidance of predation hypothesis of longevity'. The Reproductive-Cell Cycle Theory may be better able to explain this phenomenon.

This is supported by the fact that species exhibiting exceptional longevity for their size usually produce fewer offspring than would be predicted. The longevity determining reproductive cell cycle-signaling factors are suppressed during CR. We propose that the reason these animals live longer is due to the well-known phenomenon that CR increases longevity but decreases fertility. Many of the attributes that allow these animals to evade predation are very expensive from a metabolic perspective. In essence, as the number of calories expended in the process of obtaining food increases, the more likely the animal will perceive a food-restricted, hostile reproductive environment. For instance in birds and bats the energy requirements of flight far exceed those of cows that only have to graze, i.e. they don't have to chase the grass! This also applies to turtles. While their shell does protect them from predation, it requires significant energy expenditure to carry it around. The shell also limits their mobility and agility making the task of acquiring food less energy efficient. These animals also exhibit decreased reproductive function: Loggerhead turtles only lay eggs every 2 years and it takes 2 nesting seasons to produce enough eggs to insure 1 loggerhead turtle will live long enough to reach maturity (only 1 egg/1,000 reaches maturity). The little brown bat, that consumes half its weight in food per day, likewise typically produces only 1 offspring/year, a time that coincides with the greatest food availability. As mentioned previously, the reproductive axis hormones in brown bats are suppressed the remainder of the time. With regard to turtles, the same species of turtle exhibits different growth rates and longevities depending upon the temperature of the water they inhabit. In turtles that migrate from cold to warm water, most of their growth occurs during the time they are in warm water. Interestingly, their reproductive axis hormones are suppressed while in cold water and are activated only when in warm water. In contrast, the poison dart frog and coral snake do not possess exceptional longevities because their strategies for avoiding predation do not require increased work for food. Regarding the flying fish, it is a filter-feeder that consumes plankton and therefore has abundant food availability. In summary, the Reproductive-Cell Cycle Theory would predict that the increased energy expenditure required to obtain food would better explain why certain species that have developed predator-evading attributes exhibit exceptional longevity and decreased fertility. In essence, you can't get your "money for nothin' and chicks for free" [Dire Straits, 1985].

Senescence

As eluded to previously, senescence can also be explained by examining changes in reproductive cell cycle-signaling factors. During the reproductive life of a human and many mammals, peripherally produced activins stimulate pituitary gonadotropin secretion [Ling et al., 1986] which regulate numerous gonadal functions, including the stimulation of inhibin α-subunit production [Franchimont et al., 1989]. The inhibin α-subunit then binds to a β-subunit, these α:β dimers then enter the circulation and bind to activin receptors blocking their activity [Muttukrishna, 2001] effectively downregulating the HPG axis [Padmanabhan and West, 2001]. However, with the decline in reproductive function, there is a corresponding decrease in α-subunit production [Baccarelli et al., 2001; Hughes et al., 1990; McLachlan et al., 1986; Tenover et al., 1988] despite increased GnRH/gonadotropin secretion. The net effect is that in a futile attempt to maintain reproductive function the HPG axis hormones become dysregulated, i.e. loss of inhibin α-subunit production would be expected to result in increased serum activin concentrations [Baccarelli et al., 2001] that would contribute to the well-documented increase in serum gonadotropin concentrations [Carr, 1998]. Since there are separate post-receptor-signaling pathways for gonadotropins and activins (fig. 3), the result could well be the previously predicted scenario of dyosis (dysregulation of cell cycle events). Similar changes in the HPG axis (reproductive cell cycle-signaling factors) described above occur in all mammalian species including humans. While there may be species specific variations in the dysregulation of the reproductive cell cycle-signaling axis, all sexually reproductive life forms that exhibit senescence undergo significant changes in the centrally produced mitogenic factors and peripherally produced differentiation factors as reproductive function declines. According to the Reproductive-Cell Cycle Theory, the unique changes in reproductive cell cycle signaling that occur between species determine the duration of the senescent period, the tissues which will be most affected, and therefore, the types of diseases to which a particular species is susceptible. In other words, not only are there differences between species, but there are differences between tissues in their response to the dyotic signaling. For instance, in humans, while skin cells (fibroblasts) do display decreased cellular proliferation during senescence [Dimri et al., 1995], cell types such as colonic and prostatic cells display increased

proliferation during senescence [Hermann and Berger, 1999; Holt et al., 1988; Saxena et al., 1995]. The different responses to the serum dyotic signal are due to tissue-specific hormone receptor expression patterns. That dyotic signaling drives senescence is supported by the following examples. (1) There is a strong positive correlation between serum LH concentrations and indicators of senescence (muscle strength and lean mass) in men [van den Beld et al., 1999]. (2) There are over 40 peer-reviewed publications indicating that Alzheimer's disease may be due to an aberrant reentry of neurons into the cell cycle [Raina et al., 2000; Yang et al., 2001]. It has been shown that individuals with Alzheimer's disease have elevated serum concentrations of gonadotropins [Bowen et al., 2000; Short et al., 2001], those regions of the brain most damaged by the disease exhibit elevated intraneuronal levels of LH [Bowen et al., 2002] and LH drives AβPP processing towards the amyloidogenic pathway, a significant component of Alzheimer disease neuropathology [Bowen et al., 2004]. (3) Osteoporosis is associated with osteoclast proliferation [Manolagas and Jilka, 1995]. The activin bone morphogenetic protein receptors are expressed in bone tissue [Suzawa et al., 1999], have been shown to promote osteoclast and osteoblast proliferation and differentiation [Gaddy-Kurten et al., 2002], are thought to contribute to altered cell differentiation, and may be associated with the increased bone resorption observed during senescence [Gaddy-Kurten et al., 2002]. (4) Recent evidence suggests that vascular smooth muscle cell proliferation is a primary cause of atherosclerosis [Lusis, 2000], and hCG/LH have been shown to promote angiogenesis [Zygmunt et al., 2002]. (5) The incidence of cancer increases with increasing age [Greenlee et al., 2001]. (6) The decline in immune function also occurs during pregnancy, another time when the serum concentration profiles of these hormones are significantly altered.

From the individual human perspective, all would agree that senescence is a negative event. While this is an area of contention, it has been argued that from an evolutionary perspective, senescence is an active process beneficial to the survival of the species. This is certainly the case in *Volvox carteri*, one of the most primitive multicellular organisms. After reproduction is complete in this organism, the remaining somatic cells synthesize a set of proteins that induce death and dissolution [for review see Gilbert, 2003; Pommerville and Kochert 1982]. Active senescence prevents the older less fertile animal from competing for finite resources with the younger more fertile and possibly more genetically appropriate animal. It also helps to protect the younger, more vigorous, more fertile members of the species from predation.

Based on their proposed actions in regulating growth and development, regulating reproduction, and driving senescence, the HPG hormones (reproductive cell cycle-signaling factors) fit the pleiotrophic antagonism model of aging [Kirkwood and Austad, 2000]. The obvious question is, what can be done to slow the rate of aging?

Interventions

Since the reproductive period is the time of least change in function, it would represent the time of slowest aging. Therefore, mimicking the levels of reproductive cell cycle-signaling factors during this time of life would be one possible intervention that would extend longevity. However, the rate of aging during this period can be slowed even further by exposure to a 'hostile reproductive environment', as seen with CR, cold temperature and excessive physical activity (increased work to get food/avoid predation) [Holloszy and Smith, 1986; Wade et al., 1996; Weindruch and Walford, 1988]. The Reproductive-Cell Cycle Theory would suggest that this is due to a decrease in reproductive cell cycle signaling. The mechanism by which this occurs is not known for mammals, but in *C. elegans* activin signaling is increased in the presence of a reproductive friendly environment, but decreased in the presence of environmental stressors [Nolan et al., 2002]. In the mammal, the decreased activin signaling would result in a decrease in all the remaining hormones of the HPG axis thereby leading to a decrease in the rate of aging. The dysregulation of HPG hormones during senescence results from the loss of inhibin α-subunit and sex steroid production that occurs with the loss of reproductive function [Burger et al., 2002; Muttukrishna et al., 2002]. Therefore, it would seem that the most physiologic intervention to slow aging in humans and many other mammalian species would be to administer exogenous inhibin α-subunit. While this type of intervention should be effective, the real question is why does reproductive function deteriorate in the first place? If there were a way to decrease the rate of germ cell depletion or increase the number of viable germ cells (i.e. decrease the rate of follicular atresia or increase oocyte reserves in women; decrease the rate of Sertoli stem cell loss or increase Sertoli stem cell reserves in men) one should be able to delay senescence without decreasing growth, delaying the onset of puberty or decreasing fertility. The first evidence that this may be possible was demonstrated in an intriguing experiment performed by Rogina et al. [2000]. Using *Drosophila melanogaster* with heterozygous mutations in the

Indy gene they produced a phenotype that was the same size as wild-type, without any delay in development, that lived 50% longer and was capable of producing more than twice as many offspring [Rogina et al., 2000]. This suggests that in Indy heterozygous mutants there is actually an increase in the number of offspring per unit time, indicating that this mutation may very well result in the desired increase in germ cells and/or a decrease in the rate of germ cell deterioration mentioned above. The same mechanism may be responsible for a similar phenotype exhibited by *D. melanogaster* that possess a heterozygous mutation of the EcR gene which codes for a sex steroid receptor [Simon et al., 2003]. Further research will no doubt determine whether these mutations actually result in the predicted changes in germ line cells. However, compelling evidence that increasing germ cell number extends longevity comes from a more recent study performed in mammals (rats) which revealed that transplanting reproductively viable ovaries from young rats into senescent rats significantly extented lifespan [Cargill et al., 2003].

Conclusion

We propose aging can be defined by the rate of change in an organism over time. This definition not only includes changes that result in the loss of function (senescence) but also changes that result in a gain of function (growth and development). The most basic change that occurs in an organism over time can be attributed to changes in the rate of cell proliferation and differentiation. The Reproductive-Cell Cycle Theory holds that in humans these changes in the rate of proliferation and differentiation in somatic as well as germ cells are primarily regulated by changes in the serum concentrations of the hormones of the HPG axis (reproductive cell cycle-signaling factors). Specifically, GnRH and the gonadotropins most likely represent the mitogenic agents and activins the differentiation agents. Mutational studies in *C. elegans* support these assertions; in that homologues of the human HPG hormones converge on daf-16, the homologue of human Forkhead that functions in the regulation of cell cycle events.

The Reproductive-Cell Cycle Theory is able to explain (1) two phenomena that are closely related to species lifespan – the rate of growth and development and the ultimate size of the animal; (2) the apparent paradox that size is directly proportional to lifespan and inversely proportional to fertility between species but vice versa within a species; (3) the simultaneous regulation of the rate of aging and reproduction as evidenced by the fact that environmental conditions and experimental interventions known to extend longevity are associated with decreased reproductive cell cycle-signaling factors, thereby slowing aging and preserving fertility in a hostile reproductive environment; (4) how differing rates of reproduction between species are associated with differences in their lifespan, and (5) an evolutionarily credible reason why and how aging occurs – these reproductive hormones act in an antagonistic pleiotrophic manner via cell cycle signaling, promoting growth and development early in life in order to achieve reproduction, but later in life, in a futile attempt to maintain reproduction, become dysregulated and drive senescence (dyosis). The Reproductive-Cell Cycle Theory explains aging in all sexually reproductive life forms. This would even include unicellular organisms such as yeast that secrete α-factor, a tridecapeptide mating pheromone that has extensive sequence homology with the hypothalamic decapeptide GnRH. α-factor has been shown to bind specifically to rat pituitary GnRH receptors and to stimulate the release of LH from cultured gonadotrophs [Loumaye et al., 1982]. Our contention is further supported by the fact that TGFβ-signaling pathways are evolutionarily conserved and are homologous to those found in plants [Jiang and Clouse, 2001]. In this connection, missense mutations in these signaling pathways in plants produces a phenotype of dwarfism and reduced fertility [Mussig et al., 2001] which are associated with increased longevity.

Therefore, since reproduction is the most important function of an organism, if reproductive cell cycle-signaling factors determine the rate of growth, determine the rate of development, determine the rate of reproduction, and determine the rate of senescence, then by definition they determine the rate of aging and thus lifespan.

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This idea was first presented at the Pennington Scientific Symposium on Mechanisms and Retardation of Aging in Baton Rouge, La. [Atwood et al., 2003] as the 'Mitogenic-Differentiation Theory of Aging'. Subsequent to the meeting the theory was renamed the 'Reproductive-Cell Cycle Theory of Aging' and further refinements added.

Appendix 1

Initiation of Puberty

The mechanism responsible for the initiation of puberty has thus far eluded scientists. Based on the Reproductive-Cell Cycle Theory of Aging, the authors predict the following scenario as a possible explanation for the initiation of puberty in humans. After birth, the mitogenic factors (possibly GnRH and/or gonadotropins) are secreted by the thymus [Batanero et al., 1992; Sabharwal et al., 1992] that also is very sensitive to the peripherally produced differentiation-apoptotic factors (possibly activins) [Tsai et al., 2003; Hager-Theodorides et al., 2002]. The thymus-derived mitogenic factors stimulate the rapid growth observed during the 1st year of life. However, as the size of the infant increases so do the serum concentrations of differentiation-apoptotic signaling factors (activins) secreted from the growing number of peripheral cells. This results in continued involution of the thymus, thereby causing a decrease in the production of the thymus-derived central mitogenic factors and a slowing in the rate of growth until puberty. During the remainder of childhood, growth is likely stimulated by basal insulin and IGF-1 secretion which then bind to the IGF-1 receptor and signal mitogenesis via Forkhead. As the child continues to increase in size so do the serum concentrations of activins. Since activins stimulate GnRH secretion, once the serum concentrations of activins reach a critical threshold, they activate hypothalamic secretion of GnRH which is thought to be the first event in the initiation of puberty. That growth hormone does not play an initiating role in puberty is evidenced by the fact that GH receptor knockout mice are fertile [pers. commun., Andrzej Bartke].

Appendix 2

Differences in Age-Related Changes in Serum Gonadotropins between Species

A significant age-related change in reproductive axis hormones is a universal phenomenon in organisms that exhibit aging, although the increase in gonadotropin levels during reproductive aging is not a universal phenomenon. For example, there is considerable evidence that in most strains of laboratory rats, gonadal function declines because gonadotropin release is reduced [Huang et al., 1976; Riegle et al., 1977; Steger et al., 1983]. The capacity of gonadotrophs to produce and secrete LH/FSH into the serum declines [Huang et al., 1976; Riegle et al., 1977; Steger et al., 1983] with age in the rat, despite normal adult GnRH production [Steger et al., 1983] and receptor expression [Sonntag et al., 1984]. Although there are differences in HPG hormone secretion between species with aging, they still result in dyotic signaling. Since the ovaries of old rats are capable of near normal function under appropriate gonadotropic stimulation, it has been suggested that the major cause for cessation of regular estrous cycles in old rats lies in altered LH/FSH secretion [Huang et al., 1978]. It is interesting that female rats typically die of pituitary adenomas that secrete prolactin [Goya et al., 1990] and almost all their male counterparts develop Leydig cell tumors and die from neoplasms [Amador et al., 1985]. The Leydig cell tumors produce large amounts of inhibin [Herath et al., 2001] that would explain the hypothalamic-pituitary suppression of gonadotropins. The inhibin would also block activin from binding to its receptor on other tissues as well. Since activins promote differentiation and activate cell cycle checkpoints, the loss of activin signaling could contribute to the fatal neoplasia in these animals. Therefore, as mentioned in the Senescence Section, dyotic signaling in the rodent results primarily in cancer whereas in the human it primarily results in artial disease. This is likely due to differences in hormone receptor expression. The pituitary and Leydig cell neoplasms mentioned above are an example of this and once established, these neoplasms distort the typical senescent-associated elevation in gonadotropins seen in most other species.

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