the areas of detection, prevention, and therapy about the impact that the availability of these M. *tuberculosis* genome sequences would have on future research efforts to combat this ancient and reemerging killer.

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The Advent of Menopause

The article "Menopause: The aging of multiple pacemakers" by Phyllis M. Wise *et al.* ("Patterns of Aging," 5 July, p. 67) addresses a cardinal component of the ageing process, menopause, a normal physiological process that has a profound impact on more than half of the population. One of the principal theses advanced by Wise *et al.* to explain the advent of menopause is a reduction in frequency of the hypothalamic signal generator that occasions the rhythmic release of GnRH, the neuropeptide that controls secretion of the gonadotropic hormones and thus governs ovarian function. The only direct evidence cited in support of this far-reaching hypothesis, however, is an abstract published in 1994 (*1*).

Although a hypothalamic deficit is clearly responsible for the cessation of ovarian function in the ageing rat, as documented by Wise *et al.*, as well as by others, the preponderance of the evidence, *in women*, is that the frequency of the GnRH pulse generator is not diminished in menopause (*2*). Rather, an increase in luteinizing hormone (LH) pulse frequency and amplitude seems to occur as menopause is approached (*3*), a finding consistent with the decline in inhibin levels and ovarian follicle depletion (*4*).

Wise *et al.* do not acknowledge the fundamental differences between primates and rodents (*5*) in the control of ovarian function and have extrapolated findings in rats to women in the elaboration of their theory.

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Response: Knobil and Yen argue that changes in hypothalamic function are a consequence rather than a cause of the menopausal transition. One must examine mid-

dle-aged premenopausal women who have not yet experienced shortened or irregular menstrual cycles to test whether hypothalamic function is altered *before* the onset of shortened cycles. This is critical because Richardson *et al.* (*1*) showed that the loss of follicles is greatly accelerated by the time women display irregular cycles.

The papers cited by Knobil and Yen do not shed light on the validity of their point of view. Yen *et al.* (*2*) conclude that LH pulse frequency does not change in postmenopausal women on the basis of a sample size of only two women. Couzinet *et al.* (*3*) tested the effects of GnRH agonists and antagonists in postmenopausal women. No young women were included in this study, and thus one cannot derive from it information about the effects of age on pulsatile LH release. Reame *et al.* (*4*) found increased LH pulse frequency; however, the length of the menstrual cycle was shortened or, in some cases, cycle length in the compared groups was not clearly stated. The study of Burger *et al.* (*5*) included only middle-aged women.

We are aware of only one study (*6*) that focuses on middle-aged premenopausal women who have not exhibited shortened cycles and in whom the menopausal transition is imminent. Although this work is still only in abstract form (*7*), the data strongly suggest a desynchronization of hypothalamic signaling before the initiation of perimenopausal transition.

We suggested in our article that changes in the coordination of neurochemical signals that govern GnRH release might occur before the menopausal transition. This means that a change in frequency, regardless of direction, would lend support to our thesis. Thus, the increase in LH pulse frequency observed by Reame *et al.* (*4*) in the early stages of the menopausal transition supports our hypothesis that changes in hypothalamic function occur before the menopause.

Knobil and Yen state that fundamental differences exist between primates and rodents in the hypothalamic control of ovarian function and seem to infer that conclusions drawn from studies performed with rodents cannot be applied to primates. Similar mechanisms of neuroendocrine regulation of the menstrual-estrous cycle can be found in many species. For example, a surge of GnRH regulates the occurrence of the preovulatory surge of LH in all species tested, from rats to primates (*8*). Hormone profiles during the reproductive cycle of various species differ, the exact neuroanatomical location of GnRH cell bodies is not the same, the link between the circadian clock and the timing of the LH surge may be subtly different, and the extent to which and when during development the brain becomes sexually dimorphic may vary. However, we are impressed by the persistence of similar control and feedback mechanisms that regulate development of the reproductive system, puberty, and the maintenance of regular cycles in the adult throughout evolution.

We wish to reemphasize that the major point of our article was to move the discussion of menopause toward consideration of the complexity of the dynamic interactions of multiple deteriorating pacemakers. It was not to add fuel to the debate about which component of the reproductive axis deteriorates first.

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Programmed Cell Death

Jean Claude Ameisen's Perspective "The origin of programmed cell death [PCD]" (31 May, p. 1278) discusses possible evolutionary roots of cell suicide. In addition to the cited examples, sporulation in *Bacillus* is worth mentioning. In the final stage of sporulation, the spore matures and is released from the mother cell, which produces the coat proteins and then lysis, completing its terminal differentiation program. This is controlled by the σ^K sporulation factor, which is functional only in the mother cell. The coding sequence in *B. subtilis* is separated into two truncated genes that are brought together in-frame by a site-specific recombination event 3 hours after onset of sporulation. This leads to excision of a 48-kilobar element in between the two portions of the σ^K gene. Recent sequence analysis of this nonessential DNA indicates a possibility that the element is a cryptic remnant of an ancestral temperate phage (*1*). Microcycles, a reformation of spores just after germination when vegetative multiplication is disadvantageous because of suddenly changed conditions, were observed in sporulating microorganisms [reviewed in (*2*)]. Such reformation shows that PCD in microorganisms is precisely controlled to the advantage of the spore and that elaborate mechanisms preventing accidental activation of the pathways are in place.

Factors similar in function to those produced by eukaryotes controlling cell survival and development have also been found in prokaryotes (*3*). Some signals trigger cascades of preprogrammed events only at a certain growth stage, in the so-called preparatory phase (*2*). Depending on the signal, the consequence of intercellular communication may lead to the production of secondary metabolites, expression of virulence factors, or differentiation during which the fate of some cells is predetermined as fatal. Thus, we can perceive PCD as one of the wide repertoire of programs available in a genome.

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The figure in Ameisen's Perspective (p. 1279) suggests that multicellular plants diverged from multicellular animals 0.7 billion years ago. Surely this is a mistake. Virtually all sources derive the multicellular plants from green algae, which date back at least 1.5 billion years. Is Ameisen suggesting that the multicellular animals derive from green algae?

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Response: In adverse environmental conditions, *B. subtilis* undergoes asymmetrical cell division, the mother cell becoming terminally differentiated and undergoing PCD, while helping the other cell become a nonproliferating long-lived spore (the equivalent of a germ cell) (*1*).

Bacillus subtilis provides an example of how a major theoretical conundrum concerning the evolution in unicellular organisms can be solved: In order not to be counterselected, a cell suicide program has to be regulated in such a way that the death of some individuals in a unicellular colony will benefit (or at least will not impede) the survival of other individuals. As I proposed, the coupling of PCD regulation to that of cell differentiation and of intercellular communication represents one such possible mechanism. An important feature of the genetic regulation of sporulation and PCD in *B. subtilis* is asymmetrical cell division, which allows a complex intercellular regulation of the expression and activation of four transcription factors, σ^E to σ^K. This crisscross regulation ensures that the σ^K sporulation factor, which leads to spore differentiation and to mother cell death, is expressed only in the mother cell and only at a late stage of the forespore differentiation in order to prevent premature mother cell death (*1*). Such temporally and spatially regulated gene expression provides an example of how the coupling of PCD to intercellular communication can ensure a selective advantage to some cells of a colony at the expense of the sacrifice of others, thus avoiding the death of the whole colony