diverse, only one known angiosperm-type grain is reported, and it is monosulcate, spherical, tectate-columellate, and reticulate (*12*). This type occurs in *Saruma* (*20*) (Aristolochiaceae), Chloranthaceae (*21*), and Dioscoreales (*20*) (reticulum uneven).

The fossil clearly has a mosaic of states distributed in different extant families. The leaves are similar to the entire margined leaves of Saururaceae, Piperaceae, and Aristolochiaceae (some Aristolochiaceae have lobed leaves). Reproductive organs subtended by bract-bracteole complexes occur only in Chloranthaceae. Other early Cretaceous dispersed organs also show similarity to those in the same suite of taxa. Thus *Clavatipollenites*-type pollen is similar to that of Chloranthaceae and Aristolochiaceae. Contemporaneous dispersed leaves (*2*) share the intermediate pinnate to palmate primary venation found in these families, excluding Chloranthaceae, and some leaf cuticles are similar to those of Chloranthaceae (*6*). In addition, flowers similar to those of Chloranthaceae (*7*) are found in slightly younger sediments.

These data, combined with analyses of leaf (*16*), flower (*22*), ovule, and carpel (*23*) characters, support the hypothesis that the ancestral angiosperm was a small, rhizomatous perennial (with secondary growth), which had diminutive reproductive organs arranged cymosely and subtended by a bract-bracteole complex. Further support is indicated by recent phylogentic analyses of RNA sequences (*14*), and morphology and anatomy (*10*) of basal angiosperms. In the former the shortest or nearly shortest trees have members of Saururaceae or Piperaceae basal, whereas the latter has nearly shortest trees rooted near these families. This hypothesis explains the numerous similarities between the basal monocots (*18*) and these rhizomatous-herbaceous dicots, suggesting that they probably diverged quite early. It also suggests that the lack of pre-Albian fossil angiosperm wood (*4*) is due to their diminutive habit and that the failure to recognize protoangiosperm fossils results from their diminutive size and an incorrect search image.

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Expression of β-Nerve Growth Factor Receptor mRNA in Sertoli Cells Downregulated by Testosterone

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Nerve growth factor (NGF) is synthesized in male germ cells. The NGF receptor (NGFR) mRNA was found in the Sertoli cells of rat testis. Hypophysectomy increased both NGFR mRNA in testis and the number of NGFR hybridizing cells in seminiferous tubules. This was suppressed by treatment with chorionic gonadotropin or testosterone, but not with follicle-stimulating hormone. The NGFR mRNA also increased after destruction of Leydig cells or blocking of the androgen receptor. This suggests that NGF produced by male germ cells regulates testicular function in an androgen-modulated fashion by mediating an interaction germ and Sertoli cells.

TESTICULAR FUNCTION DEPENDS ON local cellular interactions that are influenced by pituitary secretions of luteinizing hormone (LH) acting on Leydig cells and secretions of follicle-stimulating hormone (FSH) acting on Sertoli cells. Leydig cells produce testosterone that regulates both Sertoli and germ cell function. Developing germ cells are supported by Sertoli cells that synthesize several essential components, including energy metabolites (*1*) and transport proteins such as transferrin (*2*), ceruloplasmin (*3*), and androgen-binding protein (ABP) (*4*). Several peptide growth factors have been detected in Sertoli cells, including insulin-like growth factors I and II (*5*), transforming growth factor β (*6*), and a testicular interleukin-1–like factor (*7*). However, the target cells in testis for these growth factors have not been identified, and their physiological roles in testis are poorly understood.

β-Nerve growth factor is a target-derived neurotrophic factor that is essential for the development and maintenance of sympathetic and sensory peripheral neurons, as well as central cholinergic neurons (*8*). Nerve growth factor (NGF) is also present at relatively high concentrations in bull semen and seminal vesicle (*9*). The NGF mRNA is detectable in testis, primarily in spermatocytes (*10*), and the NGF protein is in germ cells from primary spermatocytes to

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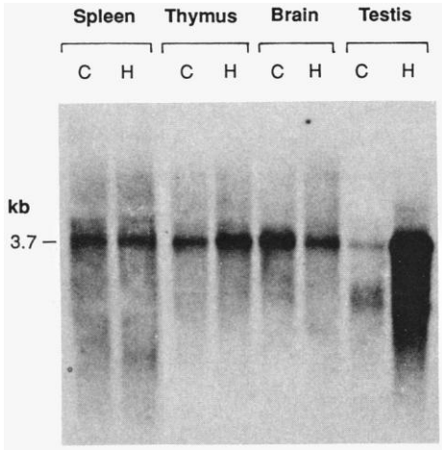

Fig. 1. Northern (RNA) blot of NGFR mRNA in rat tissues after hypophysectomy. Polyadenylated RNA was prepared (*12*) from the indicated tissues of 8-week-old Sprague-Dawley control (C) rats and from age-matched rats 2 weeks after hypophysectomy (H) performed transauricularly (*23*). The RNA (25 μg per slot) was separated on a formaldehyde-containing agarose gel, blotted onto a nitrocellulose filter, and hybridized (*12*) to a 3.4-kb rat NGFR cDNA clone (*13*). After hybridization, the filter was washed at high stringency and then was exposed to x-ray film.

mature spermatozoa in the seminiferous tubules of both mouse and rat testis (*10, 11*). The NGF receptor (NGFR) mRNA is also present in mouse testis (*10*). We detected NGFR mRNA in rat Sertoli cells and found its expression to be downregulated by testosterone. Thus NGF may participate in the interaction between germ cells and Sertoli cells, and this testicular function of NGF is modulated by testosterone from Leydig cells.

Polyadenylated RNA was prepared for blot analysis (*12*) from testis as well as from brain, thymus, and spleen, which are known to express NGFR mRNA (*12*). The tissues were from 8-week-old Sprague-Dawley rats that were controls or had been hypophysectomized 2 weeks earlier. A rat NGFR cDNA probe (*13*) hybridized to a 3.7-kb NGFR mRNA in testis, albeit to a lesser extent than in the other tissues analyzed. However, in the testis the amount of NGFR mRNA was greatly increased after hypophysectomy (Fig. 1). Densitometry scanning of autoradiograms obtained from three independent experiments showed that, after hypophysectomy, NGFR mRNA increased approximately 50 times in testes and 2 to 3 times in spleen and thymus, but decreased by approximately two-thirds in total brain. Due to the disappearance of maturating germ cells, the testis weight decreased by nine-tenths 2 weeks after hypophysectomy. However, the amount of mRNA for the Sertoli-specific gene product ABP only showed a small increase after hypophysectomy (*14*), suggesting that the increase in NGFR mRNA was not due to an enrichment of Sertoli cells, but rather reflected an increase of mRNA per cell.

A study of the kinetics for induction of NGFR mRNA in testis after hypophysectomy showed slightly increased amounts 2 days after the operation, 20 times the control level after 1 week, and an increase of approximately 50 times after 2 weeks. This increased amount was maintained for at least 6 weeks.

We next tried to identify the hormones involved in the control of NGFR mRNA expression by treating hypophysectomized rats daily with gonadotropins or testosterone. Although FSH alone had no effect, human chorionic gonadotropin (hCG), which interacts with the LH receptor and mimics the action of LH on Leydig cells, suppressed the increase in NGFR mRNA to control levels (Fig. 2A). A combination of LH and FSH, in the form of human menopausal gonadotropin (hMG), had the same effect. Testosterone treatment of hypophysectomized rats also suppressed the induction of NGFR mRNA (Fig. 2A).

Leydig cells, the testosterone-producing cells in the testis, were then specifically destroyed by a single subcutaneous injection of ethane dimethane sulfonate (EDS). As expected (*15*), this decreased the concentration ± SEM of testicular testosterone, which was measured by radioimmunoassay 6 days after the injection [1.2 ± 0.5 ng per gram of tissue in EDS-treated animals compared to 30.5 ± 4.5 ng per gram of tissue in EDS- and testosterone-treated animals ($n = 3$ in both cases)]. Blot analysis of mRNA prepared from such testes revealed a 50-fold increase in the concentration of NGFR mRNA, although there was little or no decrease in the testis weight. Daily treatment of EDS-injected animals with testosterone completely abolished this increase (Fig. 2B). A daily treatment of rats for 2 weeks with cyproterone acetate, which specifically blocks androgen receptors but no other steroid receptors (*16*), also increased testicular NGFR mRNA to a similar extent as seen after hypophysectomy or EDS treatment.

We detected testicular cells that produced NGFR mRNA by in situ hybridization histochemistry. In the control rat testis, few specifically labeled cells were observed in seminiferous tubules. The labeling was located at the periphery of the tubules close to the basal lamina, over cells that had the shape and nuclear size of Sertoli cells (Fig. 3A). A majority of tubules lacked specific labeling. The tubules that contained labeled cells were at stage VI to VIII of the seminiferous epithelial cycle (*17*), as evaluated by the shape and localization of late spermatids at the lumen border. No specific labeling was seen over peritubular cells or interstitial Leydig cells or over germ cells. No labeling was observed over Sertoli cells after hybridization to a control oligonucleotide probe complementary to the specific NGFR probe (Fig. 3B). Nonspecific labeling over late spermatids was occasionally seen with both control and specific probes. Two weeks after

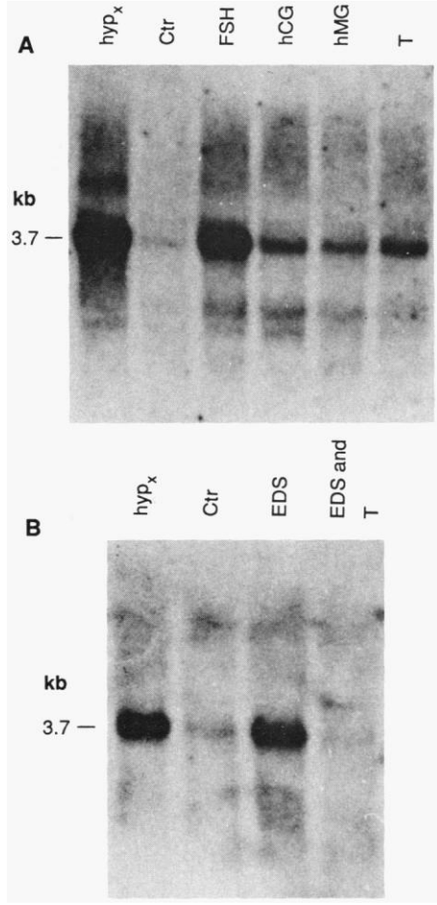

Fig. 2. Decrease of NGFR mRNA in the testis of hypophysectomized rats after injection of gonadotropins and testosterone. (**A**) Twenty-five 6-week-old Sprague-Dawley rats were hypophysectomized (hyp_x), and 1 to 14 days later four groups of five rats received daily subcutaneous injections of 7.5 international units (IU) of FSH (Fertinorm, Serono), 40 IU of hCG (Profasi, Serono), 40 IU of hMG (Humegon, Organon), or 10 mg of testosterone (T) (testosterone propionate in sesame oil). The rats were then killed together with five untreated rats (Ctr). Polyadenylated RNA was prepared from pooled testes from each group and analyzed as described in Fig. 1. (**B**) Polyadenylated RNA was also prepared from pooled testes from two groups of three 10-week-old rats which, 6 days earlier, had received a single injection of EDS (75 mg per kilogram of body weight). A group of age-matched untreated control rats was also included. One group of EDS-treated animals also received daily injections of 10 mg of testosterone, starting 1 day after EDS injection. RNA (25 μg) from each sample was analyzed as described in Fig. 1.

hypophysectomy, spermatogenesis was disrupted and most meiotic and postmeiotic sperm cells had disappeared from the tubules. Approximately 50% of the tubules then had dense label over their flattened wall (Fig. 3C), which mainly included spermatogonia and Sertoli cells. Given the abnormal morphology of the tubules, it was not possible to identify all labeled cells as Sertoli cells with certainty. When hypophysectomized rats had been substituted with hCG or hMG or supplemented with testosterone, the distribution of NGFR mRNA hybridizing cells in the testis was similar to that of control animals. However, the number of hybridizing cells was above normal in the first two cases, but lower than normal after testosterone treatment. A single injection of EDS resulted 6 days later in a large increase in the number of NGFR mRNA hybridizing cells, as seen after hypophysectomy (Fig. 3D). However, in this case the morphology of the tubules was less disturbed. The labeled cells were distributed as a ring toward the periphery of the tubules; when the morphology of the labeled tubules was preserved, most Sertoli cells expressed NGFR mRNA (Fig. 3D). In some cases, the accumulation of grains over the cytoplasm of the labeled cells showed the typical morphology of Sertoli cells (Fig. 3E). As after hypophysectomy, testosterone treatment of EDS-injected rats decreased the number of labeled cells to normal or lower than normal levels. Both after hypophysectomy and EDS treatment, the results of the in situ hybridizations showed an increase in the number of labeled cells in the tubules as well as an increase in density of labeling over individual Sertoli cells.

The results of the different lesions and hormonal treatments reported here indicate that testosterone, produced by Leydig cells, decreases the amount of NGFR mRNA in Sertoli cells. Because administration of an androgen receptor antagonist blocked this effect, it is likely that it was mediated directly by the steroid hormone–receptor complex. Moreover, the effect appears to be specific for androgens because testicular NGFR mRNA amounts did not change after adrenalectomy (*14*), which drastically reduces the concentrations of circulating adrenal steroids. The NGFR mRNA levels may also be regulated by testosterone in other tissues, because the amount of NGFR mRNA was slightly increased in both spleen and thymus after hypophysectomy. The small decrease observed in the brain may reflect an activation rather than a suppression of NGFR mRNA expression by testosterone in brain, suggesting the presence of brain-specific transcriptional factors that modulate the response to testosterone differently.

Testosterone mediates its physiological effects by interacting with a nuclear androgen receptor (*18*). The steroid-receptor complex then binds to a cis-acting DNA sequence that results in stimulation of transcription from androgen-responsive genes (*19*). Although no consensus motif that represents an androgen-responsive element has been identified, sequence comparisons between androgen-responsive genes have revealed short stretches of homology in regions upstream of the transcriptional initiation site (*20*). We found similar sequences upstream of the cap site in the human NGFR gene (*21*). In particular, the sequence TGGNTG found between positions −170 and −200 relative to cap site (+1) in seven rat and mouse androgen-responsive genes is also present in the human NGFR gene at position −174. However, in contrast to all other known androgen-responsive genes, expression of the NGFR gene in rat testis is decreased by the androgen rather than increased.

The demonstration of NGFR mRNA synthesis in rat Sertoli cells suggests that NGF exerts an effect on spermatogenesis through the interaction with Sertoli cells. As for NGF mRNA (*10*), the distribution of cells containing NGFR mRNA was not even through the testis. In control rats it was confined to regions of the seminiferous epithelium at stages VI to VIII, mostly VII, of its cycle where only a low percentage of Sertoli cells was labeled. The concentration of endogenous testosterone increases between stages VII and XI, with a peak at stage VIII, and concentrations of ABP are highest at stages VIII to X (*22*). Thus, NGFR mRNA synthesis in Sertoli cells is limited to a precise physiological state, which is soon followed by a testosterone-mediated suppression. If NGFR mRNA in Sertoli cells is not downregulated by testosterone, the number of NGFR-synthesizing cells would increase progressively as long as new Sertoli cells are induced to express NGFR mRNA. This is the situation in hypophysectomized or EDS-treated rats in which most Sertoli cells in some of the tubule sections were labeled.

The effect of NGF on Sertoli cells remains to be determined. One possibility is that NGF released from developing germ cells interacts with its receptor on Sertoli cells and thereby activates the synthesis of a set of cyclically expressed proteins, including cyclic protein 2, meiosis-inducing substance, insulin growth factor I, testicular interleukin-1, and plasminogen activator (*22*). The levels of these proteins peak between stage VII and IX during or shortly after NGFR mRNA expression in Sertoli cells. Hence, NGF-mediated interaction between germ cells and Sertoli cells could control two important steps in sperm cell formation, initiation of meiosis and spermiation.

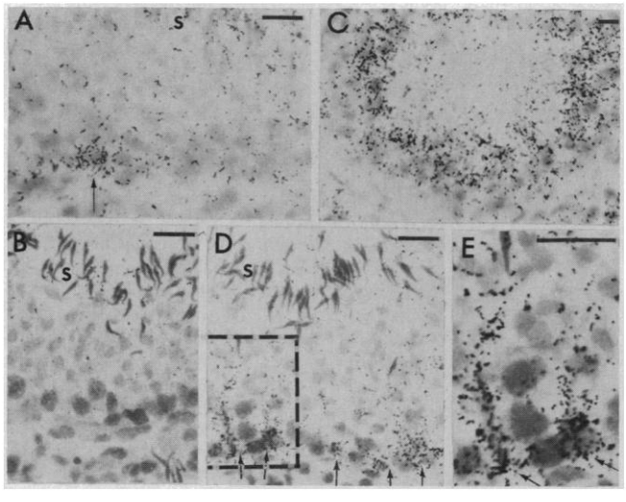

Fig. 3. Identification of NGFR mRNA–producing cells in rat testis by in situ hybridization. (**A**) Section of control 8-week-old rat testis hybridized to a 50-nucleotide oligonucleotide complementary to rat NGFR mRNA. The probe was 3′ end-labeled with [α-^{35}S]dATP (deoxyadenosine triphosphate) with terminal deoxyribonucleotidyl transferase, and the sections were hybridized as described (*24*). Few labeled Sertoli cells can be seen in a section of a seminiferous tubule at stage VII. (**B**) In a similar section, no hybridization can be seen by using a control oligonucleotide probe complementary to the specific NGFR probe. (**C**) Intense labeling, with the NGFR-specific probe, over rat seminiferous tubule 2 weeks after hypophysectomy. (**D**) Labeling, with the same probe, over numerous Sertoli cells in a rat seminiferous tubule prepared 6 days after a single injection of EDS. (**E**) Higher magnification of the boxed-in area of (D) showing densely labeled Sertoli cells. Arrows in (A), (D), and (E) indicate Sertoli cells. s, Spermatids. Scale bars, 20 μm.

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Induction of Cellular Senescence in Immortalized Cells by Human Chromosome 1

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The control of cellular senescence by specific human chromosomes was examined in interspecies cell hybrids between diploid human fibroblasts and an immortal, Syrian hamster cell line. Most such hybrids exhibited a limited life span comparable to that of the human fibroblasts, indicating that cellular senescence is dominant in these hybrids. Karyotypic analyses of the hybrid clones that did not senesce revealed that all these clones had lost both copies of human chromosome 1, whereas all other human chromosomes were observed in at least some of the immortal hybrids. The application of selective pressure for retention of human chromosome 1 to the cell hybrids resulted in an increased percentage of hybrids that senesced. Further, the introduction of a single copy of human chromosome 1 to the hamster cells by microcell fusion caused typical signs of cellular senescence. Transfer of chromosome 11 had no effect on the growth of the cells. These findings indicate that human chromosome 1 may participate in the control of cellular senescence and further support a genetic basis for cellular senescence.

Normal human fibroblasts in culture have a limited life span, beyond which the cells cease proliferation, enlarge in size, and undergo a process termed cellular senescence that results in cell death (*1*). The life span of human cells in culture decreases proportionately with the age of the donor (*1, 2*). Many, but not all, tumor cells can be grown indefinitely in culture and therefore have escaped senescence and are termed immortal (*3, 4*). Many carcinogenic agents, including chemical carcinogens, viruses, and oncogenes, can immortalize normal cells (*3–5*). Immortality is not sufficient for neoplastic transformation, but most immortal cells have an increased propensity for spontaneous, carcinogen-induced or oncogene-induced neoplastic progression (*3–5*). Therefore, escape from senescence can be a preneoplastic change that predisposes a cell to neoplastic conversion. Cellular senescence has been proposed as being one of the mechanisms by which tumor suppression occurs (*6*).

Two main theories of cellular senescence have been proposed. One is the error catastrophe model, which proposes that accumulation of random damage or mutations in protein and RNA synthesis results in the loss of proliferative capacity (*7*). A second hypothesis is that senescence is a genetically programmed process (*8*). The experimental evidence supporting the error accumulation hypothesis has been criticized (*1–9*), whereas recent support for a genetic basis of senescence has emerged (*10*). The majority of hybrids between human cells with a finite life span and immortal cells with an indefinite life span were found to senesce, indicating that senescence is dominant over immortality (*10, 11*). Certain hybrids between different immortal human cell lines senesce, indicating that different complementation groups exist for the senescence function lost in these cells (*10*). Four complementation groups were established, suggesting that multiple genes might be lost or inactivated during escape from senescence. With the use of hamster × human cell hybrids we have now mapped a putative senescence gene (or genes) to human chromosome 1.

For these studies, an immortal Syrian hamster cell line (10W-2) was fused with MRC-5 cells, which are normal, human, fetal lung fibroblasts previously used for studies of cellular senescence (*12*). The 10W-2 line was chosen because it has a near-diploid and a stable karyotype, and it is nontumorigenic (*13*). The MRC-5 cells were used at passage 25 and had undergone an estimated 40 population doublings in culture. When these cells were subcultured as controls, they reproducibly senesced after 21 to 24 additional population doublings in four independent experiments. The MRC-5 cells were fused with the 10W-2 cells as described in Table 1. From two independent experiments, 27 hybrid clones were isolated after fusion in medium containing HAT and ouabain. After 2 to 3 weeks in selective media, healthy colonies consisting of >1000 cells were isolated from cloning cylinders and passaged until the clones either ceased proliferation (that is, they senesced) or achieved >100 cell doublings as calculated from measurements of cell number at each passage. Fifteen of the 27 hybrid clones exhibited limited life spans. Each of these clones grew rapidly for the first three passages, underwent 15 to 20 total population doublings, and then displayed signs of cellular senescence characteristic of the parental MRC-5 cells at the end of their life span. Criteria for senescence included cellular enlargement and flattening, cessation of proliferation as measured by the failure to increase cell number during a 2-week period, failure to subculture, failure to form colonies at clonal density, and lack of significant incorporation of [^{3}H]thymidine [as measured by the presence of labeled nuclei (<2%) after autoradiography]. The limited life span of the hybrids indicates that cellular senescence is dominant in these hamster × human hybrids. A similar con-

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