13q deletion is significantly less than in patients with heritable retinoblastoma. Matsunaga interpreted this difference as indicating that the major retinoblastoma gene probably is not located in the 13q14 region. In view of the close synteny between esterase D activity and retinoblastoma in instances of chromosome deletion Matsunaga's interpretation can be tested by linkage analysis of esterase D and retinoblastoma in families. Available data have been suggestive of linkage, but are not conclusive (*15*).

If the same genetic locus is involved in the autosomal dominant transmission of retinoblastoma and in the chromosome deletion, the difference observed in the frequency of bilateral tumors may be attributable either to the difference in the nature of the mutation as a primary event, or to some difference in the effect of the deletion on subsequent somatic events. Knudson (*13*) has suggested that a subsequent mutational event in the predisposed cell is essential to malignant transformation. If genetic change by mutation or recombination at the homologous locus is involved (*16*), a chromosome deletion might reduce the probability of recombination because of deletion of a critical "pairing site" (*17*), or might reduce the viability of the recombinant.

The central role of the 13q14 in retinoblastoma development is further emphasized by the findings of Dq- marker chromosomes, or more specifically 13q14 deletion or rearrangements in tumor cells from patients with unilateral and bilateral retinoblastoma and normal constitutional karyotypes (*18*). Although these data must be confirmed, the 13q deletion may be a common event in retinoblastoma development as a primary germline event, as in the present kindred, or as an acquired somatic event in patients with heritable or nonheritable retinoblastoma.

The finding of a chromosomal rearrangement in unaffected transmitting relatives demonstrates that a chromosomal mechanism may account for the apparent "lack of penetrance" and familial occurrence of retinoblastoma not explained by simple Mendelian inheritance. Although a chromosomal rearrangement or deletion may not be demonstrable in most patients with familial retinoblastoma and their unaffected transmitting relatives, the segregation ratio of 0.31 noted by Matsunaga (*2*) for such families is consistent with the 0.25 ratio expected of a chromosomal rearrangement and deletion. Further, the fraction of patients with bilateral tumors among all retinoblastoma patients with a chromosome deletion is 0.49 [see (*15*) and the data now reported], which is similar to the 0.54 fraction observed in such families. A chromosomal mechanism could also account for the consistent apparent autosomal dominant transmission of retinoblastoma to progeny of affected individuals.

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Progesterone Regulation of the Occupied Form of Nuclear Estrogen Receptor

Abstract. *Total concentrations of estrogen receptor in the uterine nuclear fraction are reduced rapidly after progesterone treatment of the proestrous hamster. Progesterone acts selectively on the occupied form of the nuclear estrogen receptor, with no effect on the concentration of an unoccupied form. This observation indicates that progesterone modulates the action of estrogen by controlling nuclear retention of the estrogen-receptor complex.*

Current models of steroid hormone action (*1*) include a schema whereby binding of the hormone to a specific receptor protein in the cytoplasm of the target cell leads to translocation of the steroid-receptor complex to the nuclear compartment. Within the nucleus, the steroid-receptor complex is believed to bind to acceptor sites, and this event induces changes in gene expression that lead ultimately to the biological response characteristic of the hormone. Thus, the steroid hormone–receptor complex is viewed as a transducer of the hormonal signal.

Unoccupied nuclear estrogen receptors are found in significant amounts in both normal (*2*) and abnormal (*3*) target tissues. A functional role for the unoccupied nuclear receptor has yet to be described. Our results demonstrate that, although significant amounts of unoccupied nuclear estrogen receptor are present, progesterone, a physiological modulator of estrogen action (*4*), selectively reduces the concentration of the occupied form of nuclear estrogen receptor. This finding supports the concept that progesterone modulates estrogen action by regulating the retention of the estro-

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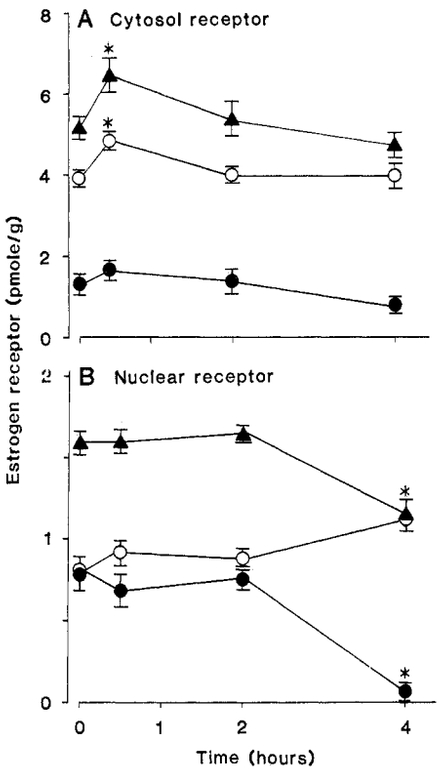

Fig. 1. Time course of (A) cytosol and (B) nuclear estrogen receptor response to progesterone treatment. Proestrous hamsters were treated with progesterone (5 mg per 100 g of body weight, subcutaneously) at time zero and killed at the indicated times. Controls were injected with corn oil vehicle at time zero. Uterine cytosol and nuclear KCl extract were prepared as detailed in (*4*). Receptor concentrations were determined by Scatchard plot analysis of specific binding data (*4, 13*). Portions (300 μl) of cytosol or nuclear KCl extract were incubated in a final volume of 500 μl with increasing concentrations of [^{3}H]estradiol (0.2 to 3 n*M*) for measurement of total radioactive ligand bound. Parallel sets of samples were incubated with a 200-fold excess of diethylstilbestrol to estimate nonspecific binding. Total estrogen receptor (▲) was determined by incubation of samples at 30°C for 1 hour, and unoccupied receptor (○) was determined by incubation at 2°C for 18 hours. After incubation, free steroid was removed by treating each sample with dextran-coated charcoal for 10 minutes (*4*). Specific binding data were obtained by subtracting nonspecific binding from total binding at each concentration of [^{3}H]estradiol. Occupied estrogen receptor (●) was computed as the difference between total estrogen receptor and unoccupied estrogen receptor. Each point represents the mean ± standard error for six assays. The asterisk indicates $P < .05$, compared to control.

gen-receptor complex in the nucleus of the target cell.

Progesterone rapidly and selectively reduces nuclear—as opposed to cytoplasmic—estrogen receptor concentrations in the uterus of the hamster (*5*) and the rat (*6*). The effect of progesterone on nuclear estrogen receptor levels does not depend on changes in either cytoplasmic estrogen receptor or serum estradiol levels. In addition, progesterone action is responsible for the rapid reduction of uterine nuclear estrogen receptor levels during the preovulatory period of the hamster estrous cycle (*4, 5*).

In previous studies (*4–6*), total concentrations of nuclear estrogen receptor were measured by a [^{3}H]estradiol exchange assay performed at an elevated incubation temperature (30°C). This exchange assay does not distinguish between receptor that is bound to hormone (occupied) from receptor that is not bound (unoccupied). To measure the quantity of nuclear estrogen-receptor complex, we utilized the temperature-dependent dissociation of bound hormone from receptor. At low temperatures (0° to 4°C), dissociation of bound hormone is negligible, and consequently incubation of cytosol or nuclear extract with [^{3}H]estradiol at low temperature gives an estimate of the amount of unoccupied receptor. At elevated temperatures (30°C), not only is there binding of ^{3}H-labeled hormone to unoccupied receptor, but previously bound nonradioactive hormone is exchanged with the added [^{3}H]estradiol, so that total (occupied and unoccupied) receptor can be measured. The difference between total receptor measured under exchange conditions and unoccupied receptor assayed at 0° to 4°C provides an estimate of the estrogen-receptor complexes present. A similar approach has been used by other investigators to distinguish between occupied and unoccupied receptor (*2, 3*). We had previously validated the assays we used for measurement of uterine estrogen receptor concentrations (*4, 5, 7*).

Early on the morning of proestrus (day 4) of the hamster estrous cycle, the serum estradiol titer is high, and it remains elevated until the time of the ovulatory surge of gonadotropin (*4, 5*). This estrogen priming in concert with the rise of serum progesterone during the preovulatory period of the cycle is necessary for eliciting mating behavior (*8, 9*). Estrogen and progesterone exert opposing effects on uterine luminal fluid during the estrous cycle; estradiol promotes luminal fluid accumulation, whereas progesterone induces fluid loss (*9*). Although hormonal interactions are tissue-specific, the physiological effects of progesterone on estrogen action may in part be a consequence of progesterone-induced modulation of the estrogen receptor system. Thus, our objective was to determine whether the progesterone-induced loss of nuclear estrogen receptor was accounted for by alteration of either occupied or unoccupied receptors in the proestrous hamster uterus. Our approach was to measure the response of occupied and unoccupied estrogen receptor in uterine cytosol and nuclear fractions at selected times after progesterone treatment of proestrous hamsters.

Following progesterone administration (5 mg per 100 g of body weight, subcutaneously), there were no significant changes in occupied cytosol estrogen receptor during the 4-hour period of observation (Fig. 1A). The concentration of unoccupied cytosol receptor increased transiently at 30 minutes and subsequently returned to values obtained before treatment. At all times, unoccupied receptor accounted for 75 to 80 percent of the total cytosol receptor. In conformity with results obtained previously (*5, 6*), total nuclear estrogen receptor concentration was reduced between 2 and 4 hours after progesterone treatment (Fig. 1B). Although unoccupied nuclear receptor constituted a large proportion (50 to 60 percent) of the total receptor population, progesterone did not significantly alter the amount of this form of receptor. However, the concentration of occupied nuclear receptor was reduced to the limit of detection between 2 and 4 hours after progesterone treatment. Differences in slope of the Scatchard plot (apparent affinity constant) could not explain these differences.

The demonstration that progesterone induces the preferential loss of estrogen-receptor complex from the target cell nucleus suggests that progesterone has a selective effect on the biologically active form of the nuclear estrogen receptor. Occupied and unoccupied estrogen receptors possess distinctive physicochemical properties (*1, 2*). In addition to the presence of steroid, features that differentiate occupied from unoccupied receptor include conformational changes in the protein acquired during activation, a phenomenon that produces an enhanced affinity of the receptor for nuclear acceptor sites (*10*). One or more of these characteristic physicochemical properties may account for the substrate specificity of progesterone action.

The mechanism by which progesterone selectively reduces the concentration of nuclear estrogen-receptor complex is unknown. However, we discovered recently that inhibitors of RNA synthesis (actinomycin D) and protein synthesis (cycloheximide) block the in-

hibitory effect of progesterone on nuclear estrogen receptor in vitro (*11*). Among other possibilities, we have hypothesized that progesterone modulation of nuclear estrogen receptor retention involves the induction or stimulation of a factor (estrogen receptor regulatory factor) that directly alters the nuclear estrogen receptor. The presence of a progesterone-induced modulator capable of altering the occupied form of estrogen receptor within the target cell nucleus may provide a fundamental mechanism for regulation of cellular response to hormone action. Furthermore, the regulation of such modulators by exogenous agents would provide a specific and selective means of controlling hormone-induced responses. Progestin therapy is useful in the management of certain types of endometrial cancer (*12*). Our results suggest that the responses of occupied nuclear estrogen receptor or of estrogen receptor regulatory factor to progestin may provide a novel approach for selecting patients with hormone-dependent endometrial carcinoma.

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Cellular Aging: Further Evidence for the Commitment Theory

Abstract. *A large, transient reduction in the population size of human fibroblasts in early passages significantly increases the variability of the life-spans of cultures in comparison to control cultures, as predicted by the commitment theory of cellular aging. The theory also predicts that a constant population of noncycling cells will appear in the later part of the culture life-span. This was confirmed by labeling the cells in culture with tritiated thymidine.*

Diploid human fibroblasts have a finite growth potential when they are cultured in vitro (*1*). Hayflick and Moorhead (*2*) defined three growth phases of cells in culture: phase 1 being the establishment of the primary culture, phase 2 being a long period of proliferation during which the cells are outwardly normal, and phase 3 being a period in which the growth rate slows down, cell morphology is abnormal, the yield of cells at confluence declines irreversibly, and the culture can no longer be propagated. The life-span of human fetal lung fibroblasts varies from about 40 to 70 population doublings (*3*).

A number of features of the growth of diploid cells and the existence of permanent lines led us to formulate the commitment theory of fibroblast aging (*3, 4*). We suggested that at some early stage, prior to the establishment of fibroblasts in culture, there exists a subpopulation of cells that can undergo an unlimited number of divisions (*5*). We call these "uncommitted" cells and assume that during their division they can become committed to finite growth with a probability of value P. Thus a committed cell is one that gives rise to a clone with strictly limited replicative potential. We also assume that each committed cell and its descendants multiply at the same rate as uncommitted ones through an incubation period, M, consisting of a fixed number of cell divisions, after which no further growth occurs. If P is sufficiently high (approximately .25) and M is sufficiently long (approximately 55 cell divisions), then for normal laboratory populations the cultures will eventually die out, since all the uncommitted cells will necessarily be lost by dilution from every culture. However, the exact time at which the last uncommitted cells are lost will fluctuate considerably from population to population, and this will greatly affect the final life-span of the culture in question.

The theory can thus explain the variability in life-span of parallel cultures set up at an early passage and grown under identical conditions. For example, among 24 such cultures of the fetal lung strain MRC-5, the average life-span was 57 passages, with a standard deviation of seven passages, which corresponds to more than a 100-fold difference in growth potential (*6*). In other experiments, populations of early-passage fibroblasts consisting of a 1:1 mixture of two heritable phenotypes were grown and then scored at intervals for the proportion of each type of cell. In many cases the ratio remained constant for many population doublings, showing that there was no direct selection for one type, but frequently there was a sudden transition to a predominance of one or other phenotype at the end of the life-span (*7*). This result is explained by the random and independent dilution of the two subpopulations of uncommitted cells. From studies of individual clones, it has also been concluded that there is an important underlying stochastic process in the aging of fibroblasts (*8*). The commitment theory predicts that population size can have an important effect on the longevity of fibroblast cultures, and evidence for this has been reported (*3*). We describe here further results that conform with other predictions of the commitment theory and we comment on the discordant report by Harley and Goldstein (*9*).

Since we assume that committed cells divide normally for many cell generations, initially all the cells in the culture will be viable. We refer to this as stage 1, and if the culture is finally to die out, all the uncommitted cells must be lost at some time during this stage. When the cells that first become committed cease growth, the viable cells in the culture must divide more than once to double the population number. Thus, the population growth rate will decrease. We call this stage 2 of culture growth. Our early experiments were with several populations of MRC-5 cells and one of these growth curves was published previously (*4*). In 29 cultures of another fetal lung strain, designated MG-4, the period of rapid growth was always followed by a period of constant slower growth (*10*) (Fig. 1). The same transition has also been observed in cultures of adult human skin (*11*). The reduction in growth rate in stage 2 is determined by the value of P (*4*). For MRC-5, MG-4, and adult skin fibroblasts we estimate from the change in growth rate that P is in the range of .25

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