material was eluted stepwise from the resin with 10 mM potassium phosphate (pH 6.5) containing 0.1 mM PMSF and 50 mM to 100 mM KCl. Fractions that were eluted with 90 mM KCl elicited HR.

12. The elicitor protein was eluted from SDS-polyacrylamide with an Elutrap apparatus (Schleicher & Schuell, Nashua, NH) at 150 V for 15 hours. The eluted protein (200 μg/ml) was dialyzed overnight against 2 liters of 5 mM potassium phosphate (pH 6.5) containing 0.1 mM PMSF.
13. Thin-layer IEF gels (Servalyte Precotes, pH 3 to 10, #42965 (Serva, Westbury, NY) and wide-range molecular weight markers (pI 4.6 to 9.6) (Bio-Rad) were used as directed.
14. Gel-permeation was carried out on a Sephadex G-100 (Pharmacia) column in 33 mM NaCl, 1 mM EDTA, 10 mM KPO_4 (pH 6.8), 1 mM β-mercaptoethanol, and 0.1 mM PMSF, at 4°C. HR-eliciting activity eluted in the void volume, as determined by elution of Blue Dextran (Pharmacia).
15. Antibodies were raised in rabbits in response to injection with harpin. Three injections of purified harpin (*19*) (100, 100, and 50 μg, respectively) were made at 2- to 3-week intervals. The antiserum was harvested after 8 weeks; immunoglobulin G was precipitated with ammonium sulfate and preabsorbed with sonicated *E. coli* DH5α(pCPP9) lysate. The specificity of the antiserum was confirmed by reaction in Western blots of harpin purified by high-performance liquid chromatography (*19*). No reaction was seen with pre-immune serum when Western blots containing resolved CFEP from DH5α(pCPP430) were hybridized.
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19. Harpin was purified to homogeneity from CFEPs by ion-exchange chromatography and then elution from a reverse-phase column (Yamamura model AQ-303) with a gradient of 20 to 80% acetonitrile in water containing 0.25% trifluoroacetic acid. Pure harpin (100 ng) was absorbed to a nylon membrane and the NH_2-terminal sequence determined by the Cornell University Biotechnology Program Protein Analysis Facility.
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21. pCPP1012, an 8.4-kb Bam HI subclone of pCPP430 in pBluescript M13+ containing *hrpN*, was mutagenized with Tn5tac1 (*28*). Two insertions in opposite orientations that mapped in the 1.3-kb Hind III fragment were marker-exchanged into pCPP430 in *E. coli* MC4100, and into *E. amylovora* Ea321. Mutant phenotypes were tested in the presence of 0.5 mM isopropylthiogalactoside. The location of the insertion in each of the resulting mutants was confirmed by probing Southern (DNA) blots with the 1.3-kb Hind III fragment.
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Increased Osteoclast Development After Estrogen Loss: Mediation by Interleukin-6

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Osteoclasts, the cells that resorb bone, develop from hematopoietic precursors of the bone marrow under the control of factors produced in their microenvironment. The cytokine interleukin-6 can promote hematopoiesis and osteoclastogenesis. Interleukin-6 production by bone and marrow stromal cells is suppressed by 17β-estradiol in vitro. In mice, estrogen loss (ovariectomy) increased the number of colony-forming units for granulocytes and macrophages, enhanced osteoclast development in ex vivo cultures of marrow, and increased the number of osteoclasts in trabecular bone. These changes were prevented by 17β-estradiol or an antibody to interleukin-6. Thus, estrogen loss results in an interleukin-6–mediated stimulation of osteoclastogenesis, which suggests a mechanism for the increased bone resorption in postmenopausal osteoporosis.

Loss of ovarian function in animals and humans, such as at menopause, causes a dramatic and precipitous loss of bone that can be prevented by estrogen replacement (*1*). However, the cellular and biochemical changes that mediate the adverse effects of estrogen deficiency on skeletal homeostasis have been difficult to elucidate. Nonetheless, it is now believed that osteoclasts arise from hematopoietic progenitors of the marrow, most likely the colony-forming units for granulocytes and macrophages (CFU-GM) (*2*), and that osteoclast development is under the control of the same paracrine cytokines that are responsible for CFU-GM formation. Among these cytokines, interleukin-6 (IL-6) plays a prominent role in the early stages of both hematopoiesis and osteoclastogenesis (*3*). Moreover, IL-6 production by bone marrow stromal cells and osteoblastic cells, both of which influence osteoclastogenesis, is inhibited by 17β-estradiol in vitro; and 17β-estradiol as well as a neutralizing antibody to IL-6 suppress osteoclast development in cultures of mouse bone cells (*4*). Prompted by this and by evidence for a role of IL-6 in the pathologic bone resorption associated with Paget's disease and multiple myeloma (*5*), we used ovariectomized mice to test the hypothesis that estrogen loss up-regulates osteoclastogenesis through an increase in the production of IL-6 in the microenvironment of the marrow.

Mice were killed at various times after ovariectomy, cells were obtained from the femur or spleen, and short-term cultures were established. The osteoclast progenitors in these ex vivo cultures were quantitated (*6*). In pilot experiments, ovariectomy led to a decrease in uterine weight and to a progressive loss of trabecular bone; and implantation of slow-release pellets containing 17β-estradiol to the ovariectomized animals prevented both of these effects (*7*). The minimum effective amount of 17β-estradiol was 10 μg per 30-g mouse, and this amount of 17β-estradiol was therefore used for estrogen replacement in the present studies.

The number of CFU-GM per femur and spleen was increased approximately twofold in mice killed 14 days after ovariectomy, compared to sham-operated animals (Fig. 1, A and B). This increase was prevented when ovariectomized mice were implanted with estrogen pellets. In parallel studies, the effect of ovariectomy and estrogen re-

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placement on osteoclast-like cells was examined in bone marrow cell cultures maintained for 9 days in the presence of 1,25 dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]. Osteoclast-like cells were identified by tartrate-resistant acid phosphatase staining and calcitonin binding, a combination of features unique to osteoclastic cells (8). Similar to the findings for CFU-GM, ovariectomized mice exhibited consistently greater numbers of osteoclastic cells, ranging from two- to tenfold higher, compared to sham-operated mice; and the increase was abrogated in the ovariectomized mice implanted with estrogen pellets (Fig. 2). This effect was observed at 14 (Fig. 2A) as well as at 28 days after ovariectomy (Fig. 2, B and C). The ovariectomy-induced upregulation of osteoclast formation was confirmed with a different method; 0.4×10^5, 1.0×10^5, or 2.0×10^5 marrow cells (approximately tenfold fewer cells than those used in the experiment in Fig. 2) from either sham or ovariectomized mice were cocultured with a constant number (4×10^5) of bone cells isolated from normal neonatal murine calvaria (9). Calvaria cells alone, or the marrow cells alone at these lower seeding densities, displayed minimal if any osteoclast-like cell formation. However, when the two cell preparations were cocultured, osteoclast-like cells formed, and their number was a function of the seeding density of the marrow cells. At each density of marrow cells, osteoclast-like cell formation was at least threefold higher in cocultures containing marrow cells from the ovariectomized animals compared to cocultures containing marrow from sham-operated animals, indicating that increased osteoclastogenesis was due to an increase in hematopoietic progenitors in the marrow.

To test the second part of our hypothesis, that is, that IL-6 mediates the ovariectomy-induced increase in the formation of CFU-GM and osteoclasts, we injected ovariectomized mice with 1 mg of a neutralizing rat monoclonal antibody to mouse IL-6 (20F3) at weekly intervals starting 1 to 2 days after ovariectomy. The dose and frequency of administration of this antibody were based on previous results (*10*). Efficacy of treatment with the antibody to IL-6 was

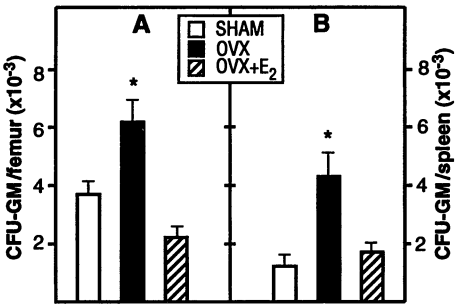

Fig. 1. Effect of ovariectomy and estrogen replacement on CFU-GM. Female Swiss Webster outbred mice (Taconic Farms, Germantown, New York), weighing 26 to 30 g and approximately 60 days old, were ovariectomized (OVX) or sham-operated (SHAM). Immediately after ovariectomy, a group was implanted subcutaneously with slow-release pellets containing 10 μg of 17β-estradiol (OVX + E_2) (Innovative Research of America, Toledo, Ohio). Animals (four per group) were killed and spleen and femur cell preparations were made. After determination of the total number of nucleated cells in each preparation, the total CFU-GM content of the (**A**) femur or (**B**) spleen was assessed in triplicate cultures established by plating 10^5 marrow cells or 10^6 spleen cells from each animal in 35-mm tissue culture dishes containing 1 ml of 0.3% agar (Difco, Detroit, Michigan) in McCoy's 5A medium supplemented with essential and nonessential amino acids (Gibco, Grand Island, New York), 10% heat-inactivated fetal calf serum (FCS) (Hyclone, Logan, Utah), and 10% pokeweed mitogen–stimulated mouse spleen cell conditioned medium (PWM-SCM) (*6*). Cultures were incubated at 37°C in a humidified atmosphere flushed with 5% CO_2 at lowered (5%) O_2 tension and scored after 5 days for colonies (>50 cells per group) and clusters (5 to 50 cells per group). Bars represent the mean number of CFU-GM (colonies plus clusters) per organ. Data were analyzed by analysis of variance (ANOVA) after establishing homogeneity of variances. *P < 0.02 versus both sham and OVX + E_2.

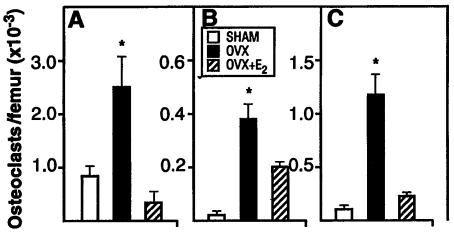

Fig. 2. Effect of ovariectomy and estrogen replacement on osteoclast formation. Either 14 days (**A**) or 28 days (**B** and **C**) after ovariectomy or sham operation, four animals per group were killed, and the marrow cells were obtained from the femur. In the 28-day experiment (B and C), the estrogen pellets were replaced at day 21. In the 14-day experiment (A), the cells from animals of each treatment group were pooled, and three to four replicate cultures were established. In the 28-day experiments, an aliquot of the cells from each animal was used to establish five to six separate replicate cultures (B); a second aliquot was used to prepare a pool from the group to establish four replicate cultures (C). Cells (2×10^6) were seeded onto 13-mm Thermanox tissue culture cover slips (Nunc, Naperville, Illinois), placed in the bottom of a 2-cm^2 well in 1 ml of α-MEM (minimum essential medium) containing 10% FCS, nonessential amino acids, and 1 nM (A), or 10 nM 1,25$(OH)_2D_3$ (B and C). Cultures were maintained for 9 days at 37°C, in 5% CO_2. Then, cells were processed for autoradiographic detection of ^{125}I-labeled calcitonin (^{125}I-CT) binding and tartrate-resistant acid phosphatase (TRAPase) as previously described (*16*), and cells exhibiting both features were analyzed with bright field and dark field microscopy. Specific binding of ^{125}I-CT was indicated by the absence of autoradiographic grains when the incubation with ^{125}I-CT was carried out in the presence of 300 nM unlabeled CT. Bars represent the mean number of osteoclasts per femur, which in (A) and (C), was calculated by using the average marrow cell yield. Data were analyzed by ANOVA as in Fig. 1. *P < 0.05 versus both sham and OVX + E_2.

Table 1. Effect of antibody to IL-6 or estrogen administration on the ovariectomy-induced increase of CFU-GM and osteoclast formation. Mice were either sham-operated or ovariectomized. Ovariectomized animals were then left untreated (OVX) or were implanted with 10-μg 17β-estradiol pellets (OVX + E_2) or received weekly intraperitoneal injections of 1 mg of the monoclonal rat anti–*Escherichia coli* β-galactosidase, GL113 (OVX + IgG), or 1 mg of 20F3 (*10*) dissolved in phosphate-buffered saline (OVX + IL-6 MAb). At 14 days after surgery animals were killed and bone marrow cells were obtained as described in Fig. 1. Cells from each animal were cultured separately. Cells from one femur were used to assess CFU-GM content as described in Fig. 1; marrow cells from the opposite femur were used to assess osteoclast formation in replicate cultures (n = 2 to 5) as described in Fig. 2B. For the CFU-GM determinations, three sets of cultures (each in triplicate) were maintained either in the presence of 10% PWM-SCM, 100U of rmGM-CSF per milliliter (Immunex, Seattle, Washington), or 100U of rmGM-CSF per milliliter + 50 ng of recombinant murine steel factor per milliliter (rmSLF, Immunex, also termed mast cell growth factor stem cell factor or *c-kit* ligand). SLF is a potent costimulator for early hematopoietic progenitor cells (*17*). Values shown are the mean ± SEM (n = 4); CFU-GM values shown were obtained by scoring colonies only. Data were analyzed by ANOVA as in Fig. 1.

Animal treatment	CFU-GM/femur (x10^{-3})			Osteoclasts/ femur
	PWM-SCM	GM-CSF	GM-CSF + SLF	
Sham	4.7 ± 0.7	4.7 ± 0.4	12.4 ± 1.2	162 ± 32
OVX	9.9 ± 1.6*	10.4 ± 2.0†	27.6 ± 2.1†	1419 ± 617
OVX + IgG	9.2 ± 2.1*	8.3 ± 1.6†	23.0 ± 4.2†	1634 ± 267*
OVX + IL-6 MAb	5.5 ± 1.2	4.7 ± 0.7	13.5 ± 0.4	603 ± 141
OVX + E_2	2.8 ± 0.4	3.0 ± 0.4	9.3 ± 1.1	892 ± 134

*P < 0.05 versus sham, OVX + IL-6 MAb and OVX + E_2. †P < 0.01 versus sham, OVX + IL-6 MAb and OVX + E_2.

Table 2. Effect of antibody to IL-6 or estrogen administration on osteoclast numbers in the secondary spongiosa of the tibia. Tibiae from the mice described in the experiment shown in Table 1 were fixed in 10% phosphate-buffered formalin, decalcified in 14% EDTA, and embedded in paraffin. Serial sections were cut and stained with Orange G, phloxine B, hematoxylin, and eosin, and three representative sections from each specimen were used for histomorphometric analysis of osteoclast numbers. We analyzed a standard segment of bone in the secondary (2°) spongiosa with a Bioquant Image Analysis System (R&M Biometrics, Nashville, TN) and a light microscope with a digitizing tablet and drawing tube attachment. The identity of the specimen was unknown to the examiner. Values are the mean ± SEM (n = 4 to 5). Data were analyzed by ANOVA after logarithmic transformation to achieve homogeneity of variances.

Animal treatment	Osteoclasts per millimeter of 2° spongiosa bone surface	Osteoclasts per square millimeter of 2° spongiosa bone
Sham	1.32 ± 0.39	68.6 ± 22.4
Sham + IL-6 MAb	1.13 ± 0.06	55.0 ± 6.3
OVX	2.51 ± 0.21*	125.8 ± 12.1†
OVX + IgG	2.70 ± 0.58*	110.4 ± 18.0†
OVX + IL-6 MAb	1.56 ± 0.28	69.0 ± 13.9
OVX + E_2	1.27 ± 0.23	37.0 ± 6.3

*P < 0.05 versus sham, sham + IL-6 MAb, OVX + IL-6 MAb, and OVX + E_2. †P < 0.01 versus sham, sham + IL-6 MAb, OVX + IL-6 MAb, and OVX + E_2.

confirmed by detection of IL-6 neutralizing activity in serum obtained as long as 28 days after initiation of treatment. Treatment with antibody to IL-6 reduced the number of CFU-GM from ovariectomized animals to the same extent as the treatment with 17β-estradiol (Table 1). CFU-GM from animals receiving injections of a monoclonal antibody to β-galactosidase of the same isotype [immunoglobulin G1 (IgG1)] as the antibody to IL-6 (at the same dose and frequency as the antibody to IL-6) were indistinguishable from those of the untreated ovariectomized mice. These findings were reproduced in three parallel sets of cultures in which CFU-GM numbers were assessed with either media from pokeweed mitogen–stimulated mouse spleen cells, or recombinant murine granulocytes and macrophage colony-stimulating factor (rmGM-CSF), or the combination of rmGM-CSF and recombinant murine steel factor. Similarly, administration of the antibody to IL-6, but not the immunoglobulin G control antibody, prevented the ovariectomy-induced increase in osteoclast numbers, seen in the untreated ovariectomized mice (Table 1). In two additional experiments (each using pools of marrow cells from four animals per group killed 28 days after ovariectomy), the mean (± SEM) number of osteoclasts per femur was 1186 ± 115 (n = 4) and 1738 ± 525 (n = 6), respectively; administration of antibody to IL-6 reduced these values to 154 ± 42 (n = 4; P < 0.01) and 447 ± 286 (n = 6; P < 0.05). In the same two experiments, estrogen replacement reduced osteoclasts to 225 ± 15 (n = 2; P < 0.01) and 503 ± 156 (n = 4; P < 0.05), respectively.

The amount of IL-6 [measured with the B9 bioassay (*4*)] in the media of marrow cell cultures from ovariectomized animals, killed 28 days after ovariectomy, was significantly higher (88 ± 48 pg/ml) than that from sham-operated animals (31 ± 14 pg/ml; P < 0.05; n = 6). In view of this finding, 35 μg of antibody to IL-6 per milliliter was added directly to cultures of pooled marrow cells from either ovariectomized or sham-operated mice (n = 4 per group). Marrow cells from ovariectomized mice cultured without antibody had 175 ± 17 (SD) osteoclasts per well, compared to 73 ± 6 (SD) osteoclasts per well when cultured with the antibody (P < 0.05; n = 4). However, the antibody had no effect on cultures from sham-operated mice [36 ± 6 (SD) compared to 49 ± 15; no significant difference; n = 4]. These results were reproduced in a second experiment.

To determine whether the number of osteoclasts in bone reflected the changes in marrow progenitors that were detected in the ex vivo marrow cultures, we fixed tibiae from sham-operated or ovariectomized mice (14 days after surgery) from the experiments described in Table 1 in formalin, then decalcified them, and subjected them to histomorphometric analysis (Table 2). In agreement with previous histomorphometric findings in mice as well as rats (*11*), ovariectomy caused a significant increase in the number of osteoclasts present in sections of the secondary spongiosa of the metaphysis of the proximal tibia, compared to sham-operated animals. Administration of the antibody to IL-6 (but not the control antibody) or 17β-estradiol to the ovariectomized mice prevented this increase. However, administration of the antibody to IL-6 had no effect on the osteoclast numbers in sham-operated animals.

Our findings are consistent with the in vitro data for an inhibitory effect of 17β-estradiol on IL-6 production (*4*), as well as with evidence that administration of IL-6 to mice, or engraftment of mice with bone marrow cells carrying transcriptionally active IL-6 genes, causes increased hematopoiesis in the marrow and spleen (*12*). They are also in line with evidence (*5*) that administration of antibody to IL-6 to a patient with plasma cell leukemia inhibited bone resorption. In addition, the evidence for up-regulation of osteoclastogenesis in the bone marrow and the actual number of osteoclasts attached to trabecular bone after estrogen loss is in line with evidence in humans that loss of ovarian function leads to an increase in bone resorption that cannot be balanced by a corresponding increase in the normally coupled bone formation and thus causes loss of bone mass (*13*).

In our studies administration of antibody to IL-6 resulted in a return of CFU-GM and osteoclast numbers to those of sham-operated animals, but it did not completely suppress CFU-GM or osteoclasts. Similarly, antibody to IL-6, added directly to the marrow cultures or administered to sham-operated animals, had no influence on osteoclast formation in the cultures or the number of osteoclasts present in trabecular bone. Thus, in the estrogen-replete state, IL-6 may not play a critical role in CFU-GM and osteoclast development, either because the system is redundant or because IL-6 levels in the marrow microenvironment are kept below a critical threshold relative to the sensitivity of myelopoiesis-osteoclastogenesis to this cytokine. Evidence that interleukin-1, tumor necrosis factor, and GM-CSF production by monocytic cells increases after ovariectomy in humans (*14*) raises the possibility that the production of other cytokines involved in osteoclast formation might also be influenced by loss of estrogens. In addition, evidence that estrogens have anabolic effects on osteoblasts (*15*) suggests that estrogen loss might have an adverse impact on bone formation as well. Hence, although IL-6 appears to be an important mediator of the up-regulation of the early stages of osteoclastogenesis precipitated by estrogen loss, it is reasonable to expect that IL-6 may not be the sole mediator of the cellular changes that ultimately lead to osteopenia after loss of ovarian function.

In conclusion, our results, together with the evidence for an inhibitory effect of 17β-estradiol on IL-6 in vitro (*4*), suggest the following mechanism for the pathophysiology of the osteopenia caused by estrogen deficiency. Bone and bone marrow cells produce cytokines, such as IL-6, which promote osteoclast development. In the estrogen-replete state, the production or action of some of these cytokines (or both) is inhibited by estrogens. In the estrogen-deficient state, the inhibitory effects of es-

trogens are removed, resulting in enhanced osteoclast development in the marrow, which is responsible for the increased bone resorption and hence the loss of bone.

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Expression of a Ryanodine Receptor–Ca^{2+} Channel That Is Regulated by TGF-β

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Ryanodine receptors (RyRs) are intracellular channels that release calcium ions from the sarcoplasmic reticulum (SR) in response to either plasma membrane depolarization (in skeletal muscle) or increases in the concentration of intracellular free Ca^{2+} (in the heart). A gene (β4) encoding a ryanodine receptor (similar to, but distinct from, the muscle RyRs) was identified. The β4 gene was expressed in all tissues investigated, with the exception of heart. Treatment of mink lung epithelial cells (Mv1Lu) with transforming growth factor beta (TGF-β) induced expression of the β4 gene together with the release of Ca^{2+} in response to ryanodine (but not in response to caffeine, the other drug active on muscle RyRs). This ryanodine receptor may be important in the regulation of intracellular Ca^{2+} homeostasis.

Extracellular stimuli, such as binding of hormones and neurotransmitters, cause a sustained increase in the concentration of intracellular free Ca^{2+} $[Ca^{2+}]_i$, which is mediated by the activation of channels that release Ca^{2+} from intracellular stores (*1*). One class of intracellular Ca^{2+} channel, the inositol trisphosphate ($InsP_3$)–operated Ca^{2+} channel (or $InsP_3$ receptor), is present in most cells (*2, 3*). A second class of intracellular Ca^{2+} channel, the ryanodine receptor, is sensitive to the plant alkaloids ryanodine (Ry) and caffeine and is present in the SR of striated muscle (*4*) as well as in smooth muscle, neurons, eggs, epithelial cells, and secretory cells (*5–13*). The two classes of intracellular Ca^{2+} channels are either present together in a single compartment or segregated, at least partially, in two distinct Ca^{2+} stores (*6, 7, 9, 14*) that may cooperate in complex processes such as the generation of Ca^{2+} oscillations and the propagation of Ca^{2+} waves (*2, 15, 16*). Other tissues and cell lines are not responsive to caffeine, the usual probe for RyRs.

To isolate genes whose expression is regulated by transforming growth factor beta (TGF-β) (*17*), we differentially screened a cDNA library prepared from TGF-β–treated mink lung epithelial cells (Mv1Lu) with the use of cDNA probes from either TGF-β–treated or untreated cells (*18*). One such cDNA clone (β4) was identified and further characterized. The amount of β4 mRNA that was expressed increased as early as 1 hour after the addition of TGF-β (*19*), and after 15 to 24 hours of continuous treatment had increased 30-fold (Fig. 1A). Constitutive and TGF-β–induced expression of β4 mRNA was observed in various cell lines. Induction of β4 mRNA by TGF-β was also observed in pPVU-O 1.5.3 cells (*20*), an Mv1Lu clone transformed with SV40 large T (LT) antigen (Fig. 1B). These cells no longer responded to the antiproliferative effect of TGF-β, but the induction of extracellular matrix proteins and nuclear transcription factors such as jun-B was maintained (*20*), which suggests that TGF-β induction of β4 mRNA levels was not a consequence of its effects on growth.

Treatment of cells with cycloheximide and TGF-β caused accumulation of greater amounts of β4 mRNA than treatment with either agent alone, whereas treatment with 2-aminopurine (2-AP), an inhibitor of serine-threonine kinases (*21*), abolished the effect of TGF-β, and treatment with phorbol myristate acetate left it unchanged (Fig. 1C). Therefore, induction of β4 mRNA (i) appeared to be a primary response to TGF-β because it did not require synthesis of new proteins, and (ii) appeared to be mediated by, or sensitive to, protein kinase or kinases that are different from protein kinase C but responsive to 2-AP. Results of nuclear run-on assays performed on nuclei isolated from control and TGF-β–treated cells indicated that TGF-β stimulated transcription of the β4 gene (Fig. 1D). The β4 mRNA was expressed in several tissues, including those of the spleen, lung, and kidney, but not of the heart (Fig. 1E).

The 2413-nucleotide sequence of clone β4 contained an open reading frame of 641 amino acids followed, after a TAA stop codon, by an untranslated region and a short polyadenylated [poly(A)$^+$] tail. The amino acid sequence of the protein deduced from the sequence of clone β4 was similar to that of the COOH-terminal sequences of human and rabbit RyRs. Two forms of the RyR, skeletal and cardiac, were cloned and are encoded by two different genes (*4*). In Northern (RNA) blots containing poly(A)$^+$ RNA (10 μg) isolated from TGF-β–treated Mv1Lu cells, clone β4 hybridized to a low-abundance mRNA of about 16 kb (*19*), a size similar to those observed for muscle RyR mRNA. The β4 clone thus appeared to be a partial cDNA that encoded a sequence with similarity to the COOH-terminus of skeletal and cardiac RyRs, including the last 7 (M4 to M10) of the 12 putative membrane-spanning regions of these proteins (Fig. 2). The β4

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