substitution may affect the positioning of the dimethylaminophenyl group in the 11β-pocket.

Approximately 1% of women do not respond to the antagonistic action of RU486 (*1*), but respond normally to progestins. In these cases a mutation at position 722 might be responsible for the failure of RU486 to induce abortion.

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7. The construction and expression of β-galactosidase fusion proteins with the cPR HBD has been described (*4*). Fusion proteins with chimeric or mutant cPR HBDs were constructed accordingly. To generate fusion proteins with the wild-type hPR, we inserted the Bcl I to Bgl II fragment of hPR1 (*16*) encompassing the hPR HBD into the Bam HI site of PUR (*4*). Extracts were prepared as described in (*4*), and binding affinities were determined by the incubation of receptors with 5 nM [^{3}H]RU27987 with or without various concentrations of competitor steroids for 24 hours at 0°C. Free ligand was removed by means of the dextran-coated charcoal method.
8. The Hind III fragment of cPR1 (*3*), encoding the cPR HBD, was cloned into a modified Bluescript KS+ (Stratagene), lacking all polylinker sites except Hind III. Silent mutations were introduced by site-directed mutagenesis (Amersham) generating unique restriction sites (Fig. 1B). Cassettes corresponding to the hPR fragments were constructed from synthetic oligonucleotides (sequences are available on request) and introduced into the corresponding sites of the vector. All mutations and sequences generated by oligonucleotides were sequenced and the chimeric cPR-hPR Hind III fragments were used to replace the corresponding cPR fragment in the expression vector cPR21 (*3*). HeLa cells at about 40% confluence were cotransfected with 1 μg of receptor expression vector, 1 μg MMTV-CAT reporter gene, and 3 μg of pCH110 [a β-galactosidase expression vector (Pharmacia) used as reference gene to normalize for variations in transfection efficiency]. Transfection, preparation of cell extracts, and quantitative determination of chloramphenicol transferase activity have been described (*23*).
9. Point mutations (verified by sequencing) were introduced by site-directed mutagenesis. The corresponding oligonucleotides are available on request.
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11. Total RNA (7.5 μg) from the uterus of estrogen-stimulated female hamsters was denatured for 5 min at 70°C in the presence of 100 ng of primer NT47 and 0.5 mM deoxyribonucleotide triphosphates (dNTPs) in a final volume of 30 μl. The mixture was placed on ice and 200 units of M-MLV reverse transcriptase, 25 units of RNasin and M-MLV reverse transcriptase buffer (Bethesda Research Laboratories) were added. After incubation for 45 min at 37°C in a final volume of 40 μl the reaction was terminated by boiling for 5 min. The polymerase chain reaction was done with 100 pmol each of primer NY39 and NY40 in 10 mM tris-HCl (pH 8.3), at 25°C, 50 mM KCl, 1.5 mM $MgCl_2$, and 200 μM dNTPs in a final volume of 100 μl, with 5 μl of the reverse transcription reaction and 2.5 units of AmplitaqTM DNA polymerase (Perkin-Elmer Cetus Instruments). The thermal conditions were as follows: 5 cycles of 40 s at 94°C, 2 min at 45°C, 2 min at 72°C; 30 cycles of 40 s at 94°C, 1 min at 65°C, 2 min at 72°C, and 7 min at 72°C. The major 500-bp amplification product was purified on a polyacrylamide gel (8%), digested by Not I and Xba I, and inserted into pBluescript II SK+ (Stratagene). Three recombinants of two different amplifications were sequenced using the Sanger dideoxy method, all inserts gave the sequence in Fig. 3B. The primers used were as follows: NT47, 5′-CATCATYTCNGGRAA; NY39, 5′-ATTCTAGAGAGAGTTATCTGGTCATC; NY40, 5′-ATGCGGCCGCGTHTTCTTYAARAGGGCAATG; R = A or G; Y = C or T, and H = A, C, or T.
12. The hGR (Gly^{567}-Cys) was constructed by site-directed mutagenes of the human glucocorticoid receptor expression vector HG1 (*17*). To assure that no mutations occurred outside the sequenced area, the sequenced Xho I to Bsp MI fragment, containing the point mutation, was reinserted into the corresponding sites of HG1. Western blots of extracts prepared from transfected cells confirmed that a protein of the expected size was expressed as efficiently as the wild-type receptor from HG0 (*17*).
13. No specific binding of tritiated dexamethasone or RU486 could be detected in extracts of COS cells transiently transfected with hGR(Gly^{567}-Cys), whereas specific binding was observed when transfecting HG0 (*17*).
14. The pattern seen for the agonistic or antagonistic properties of the various steroids with hPR(Gly^{722}-Cys) (Fig. 2B) was identical to that obtained with cPR.
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Selection of Intrinsic Horizontal Connections in the Visual Cortex by Correlated Neuronal Activity

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In the visual cortex of the brain, long-ranging tangentially oriented axon collaterals interconnect regularly spaced clusters of cells. These connections develop after birth and attain their specificity by pruning. To test whether there is selective stabilization of connections between those cells that exhibit correlated activity, kittens were raised with artificially induced strabismus (eye deviation) to eliminate the correlation between signals from the two eyes. In area 17, cell clusters were driven almost exclusively from either the right or the left eye and tangential intracortical fibers preferentially connected cell groups activated by the same eye. Thus, circuit selection depends on visual experience, and the selection criterion is the correlation of activity.

LONG-RANGE TANGENTIAL CONNECTIONS are a constituent feature of the intrinsic circuitry of neocortex (*1*). They consist mainly of axon collaterals of pyramidal cells (*2*), and in the visual cortex they interconnect discrete, regularly spaced groups of cells (*3*), which appear to show similar functional properties (*4*). It has been proposed that these horizontal pathways (i) form large receptive fields (*5*), (ii) mediate inhibitory and subliminal excitatory effects from outside the classical receptive field (*6*), and (iii) generate functionally coherent cell assemblies by synchronizing the responses of spatially distributed neurons (7). These functions require highly specific interactions, and it has been proposed that the tangential connections attain their specificity through experience-dependent selection (*8–11*). In kitten visual cortex, tangential connections develop mainly after birth (*9–13*), and final selectivity is achieved by pruning: either by elimination of inappropriate collaterals (*10, 12*) or transitory axons (*13*) or by decreases in the tangential extent and number of clusters (*11*). This pruning is influenced by visual deprivation (*9–12*). Here we test whether the selective stabilization of tangential connections is influenced by experience such that the selection criterion is the correlation of activity in interconnected cells (*14*).

To restrict correlated activity to defined

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cell groups, we raised kittens that were made strabismic at the age of 2 to 3 weeks by unilateral section of the medial rectus eye muscle (*15*). After this manipulation, the optical axes of the two eyes are no longer aligned, and the images on the two retinae cannot be brought into register. As a result, the responses mediated by anatomically corresponding retinal loci in the two eyes are no longer correlated. During a critical period of postnatal development the connections between the afferents from the two eyes and their common cortical target cells are malleable and become destabilized if their activity is not sufficiently correlated (*8, 16*). As a consequence, squint accentuates the segregation of the afferents from the two eyes in layer IV (*17*) and most of the cells in the visual cortex become responsive exclusively to stimulation of either the right or the left eye (*18*). Each of these monocularly driven cell populations is capable of subserving normal pattern vision. However, to avoid double vision, strabismics use only one eye at a time and suppress the signals from the other eye (*19*). Thus, in strabismics, the coherence of responses to visual patterns is likely to be the same as in normal animals for cells driven from the same eye, but much lower for cells driven from different eyes.

In area 17 of 2- to 3-month-old normal (n = 2) and strabismic kittens (n = 4), tangential intracortical connections were labeled with fluorescent tracers and the locations of cell groups activated by either the right or the left eye were visualized with the [^{14}C]2-deoxyglucose (2-DG) method (*15*). After monocular stimulation, regions of increased 2-DG uptake extend in a columnar fashion through all cortical layers (Fig. 1B). As revealed by intraocular injections of the transneuronal tracer [^{3}H]proline (*15*) in two additional strabismic kittens, these monocularly activated columns are in precise register with the termination zones of the afferents from the activated eye in layer IV (Fig. 1, A and B). The pattern of the 2-DG columns resulting from monocular stimulation thus shares the characteristics of ocular dominance columns as described in (*20*) (Fig. 1, C and D).

After two injections, one with red and the other with green fluorescent beads into striate cortex of a strabismic kitten (Fig. 2, A and B), retrogradely labeled cells were distributed in well-segregated clusters over a distance of up to 5 mm from the injection site (Fig. 2A). Comparison of these patterns with the corresponding 2-DG autoradiograph (Fig. 2, C and D) reveals that both the red- and the green-labeled cells are located preferentially within the territories activated by the same, in this case the right, nondeviated eye.

To identify the ocular dominance of the site of injection, we located the injections on the autoradiographs and in addition analyzed the laminar distribution of retrogradely labeled cells in the lateral geniculate nucleus (LGN). This was done because some injections produced lesions that appeared as inactive regions on the autoradiographs, mimicking territories of the nonstimulated eye. For the case illustrated in Fig. 2, both analyses indicate that both red and green tracers had been injected into right-eye territories (Fig. 2, D and E). Thus, tangential projections interconnect selectively territories served by the same eye (Table 1). In two other cases, similar results were obtained for injections into columns connected to the deviated eye. In the first, the investigated hemisphere was ipsi- and in the second it was contralateral to the eye stimulated for 2-DG labeling. In one other kitten, the injection was at the border between right- and left-eye territories and retrogradely labeled LGN cells were distributed over both laminae A and A1. In this case, retrogradely labeled cortical cells were found in both right- and left-eye columns.

Our analyses in the normally reared controls provided no evidence for an eye-specific selectivity of tangential connections. There were no consistent relations between the locations of the injections, the retrogradely labeled cells, and the 2-DG columns (Fig. 2F and Table 1). This agrees with other evidence that in normally reared cats

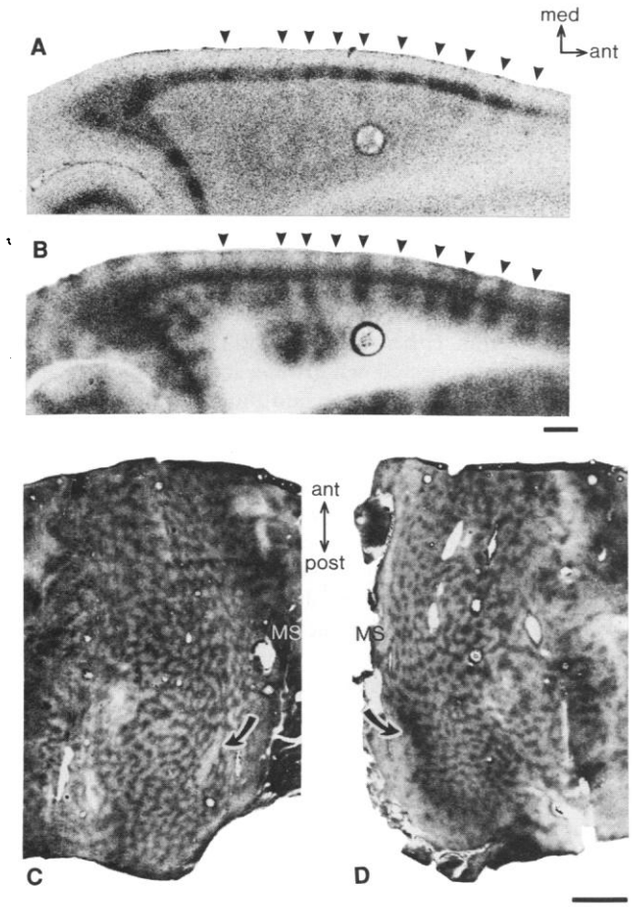

Fig. 1. Ocular dominance columns in the visual cortex of strabismic cats. (**A** and **B**) Comparison of ocular dominance columns in a horizontal section through the medial bank of right area 17 as revealed by transneuronally transported [^{3}H]proline injected into the right (normal) eye (A) and 2-DG labeling after right-eye stimulation (B). Both autoradiographs are from the same section. The columns of increased 2-DG uptake are in register with the right-eye territories in layer IV (arrows). (**C** and **D**) 2-DG autoradiographs of flat-mount sections from the unfolded left (C) and right (D) hemisphere of a strabismic cat that had been stimulated through the right eye. Note the sharp delineation of active and inactive territories. The optic disk representations of the stimulated and unstimulated eye are identifiable in the posterior third of both hemispheres as demarcated oval regions (arrows) that are solidly labeled ipsilateral (right) and unlabeled contralateral (left) to the open eye. The monocular segment (MS) is indicated by uniform labeling at the medial border of the contralateral area 17 and by the absence of labeling at comparable eccentricity on the ipsilateral side. The territories of the open eye tend to be larger in the contra- than in the ipsilateral hemisphere. Scale bars: 1 mm (A and B); 5 mm (C and D).

Table 1. Percentage of labeled cells in columns of the same and opposite ocularity as the injection sites.

Case	Percentage (and number) of labeled cells in		
	Same ocular dominance column	Opposite ocular dominance column	Transition zone
Strabismic 1 (Fig. 2D, red cells)	87 (880)	5 (51)	8 (77)
Strabismic 2 (Fig. 2D, green cells)	89 (60)	4 (3)	7 (5)
Strabismic 3	75 (375)	17 (85)	8 (40)
Strabismic 4	71 (436)	19 (117)	10 (61)
Control 1 (Fig. 2F)	49 (508)	38 (396)	13 (140)
Control 2	45 (88)	47 (92)	8 (16)

tangential connections are related to orientation but not to ocular dominance columns (*4*). Statistical analysis revealed that in squinting cats (unlike in controls) cell densities in columns of the same ocularity as the injection sites were significantly higher than those in columns of opposite ocularity (χ^2 test, $P < 0.001$) (*21*). These results suggest that the development of tangential intracortical connections depends on use-dependent selection mechanisms similar to those in the development of thalamocortical connections (*16*): neurons wire together if they fire together.

Electrophysiological recordings from area 17 of strabismic cats have revealed that the probability of response synchronization is very low between cell groups connected to different eyes, whereas it is close to normal between cell groups activated from the same eye (*22*). In conjunction with the present findings, this evidence provides support for the notion that tangential intracortical connections are responsible for response synchronization (7).

Psychophysical evidence indicates that strabismics are unable to combine the signals arriving from the two eyes to a single percept, even if these signals are made congruent by optical compensation of the squint angle (*23*). Together with the present results, this finding supports the conjecture that the tangential connections—by determining the probability of response synchronization—serve as substrate for feature binding and perceptual grouping (*8, 24*). As our data imply that the architecture of tangential connections reflects to some extent the activation conditions and feature constellations that have occurred during development, it follows that the criteria for perceptual grouping are at least partly acquired through experience. This, in turn, suggests that some of the perceptual deficits of amblyopic patients (*23*) may actually be due to errors in experience-dependent selection of corticocortical rather than of thalamocortical connections.

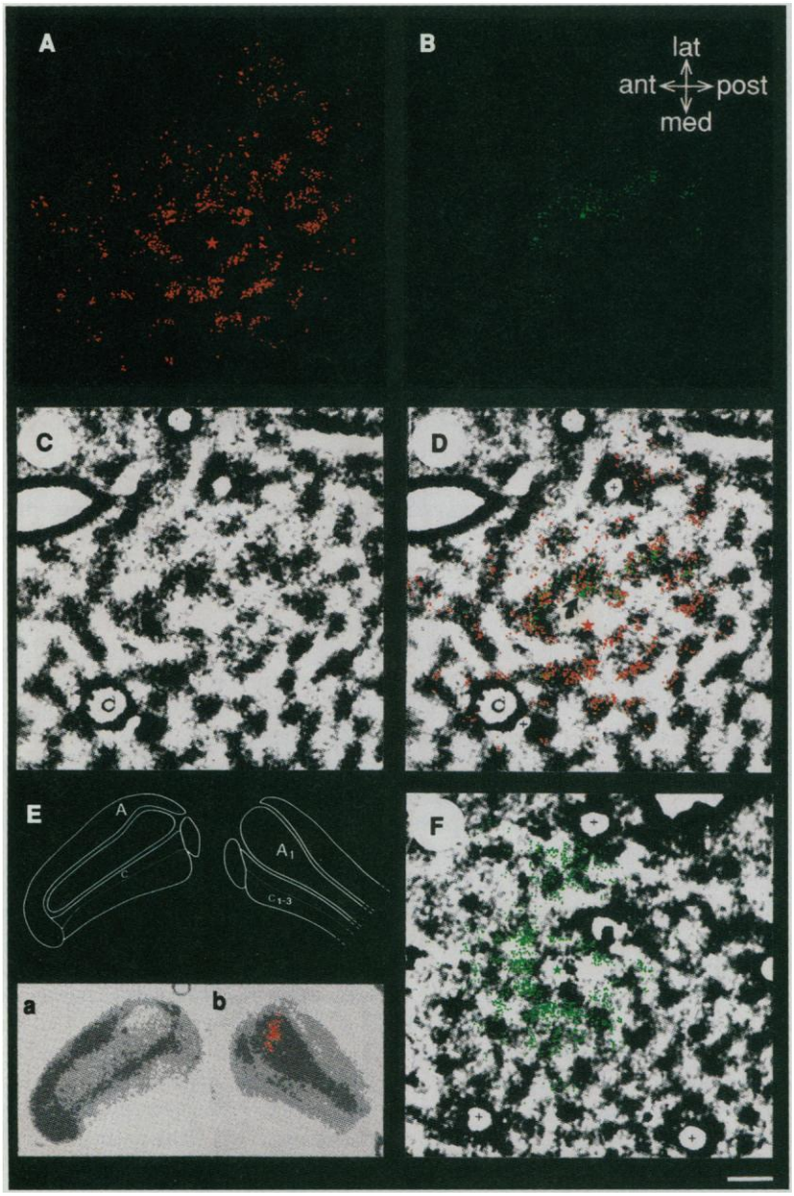

Fig. 2. Topographic relations between intrinsic horizontal connections and ocular dominance columns in area 17 of a strabismic cat (A through E) and a normally reared control animal (F). (**A** and **B**) Distributions of retrogradely labeled cells in the same tangential section after injections with red (A) and green (B) beads. Dots, the position of individual cells; asterisks, injection sites. Cortical axes refer to (A) through (D) and (F). (**C**) 2-DG pattern showing the topography of ocular dominance territories in the region containing the retrogradely labeled cells in (A) and (B). Enlarged detail of Fig. 1D. (**D**) Superposition of (A), (B), and (C). Crosses indicate two of the three landmarks used for superposition. Most of the retrogradely labeled cells are located within zones of high 2-DG uptake. The injection site of the green beads is within a dark, right-eye column (small green arrow). The injection of the red tracer had caused a small necrosis in the plane of the section, producing a patch of reduced 2-DG uptake in the close vicinity of a right-eye column. (**E**) Retrogradely labeled cells in the right LGN after the cortical injection of red beads (A). Autoradiographs of 2-DG distributions in frontal sections of the left (a) and right (b) LGN show the laminae activated by right-eye stimulation (left LGN: A and C; right LGN: A_1 and C_1). Laminar borders were derived from the same sections counterstained with cresyl violet. Labeled cells are confined to right lamina A_1, which is connected to the right eye. (**F**) Distribution of retrogradely labeled cells in area 17 of a normally reared cat after injection of green beads (green asterisk) into the territory of the nonstimulated left eye (retrogradely labeled LGN cells were concentrated in lamina A_1 corresponding to this eye). Scale bar, 1 mm.

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15. For the induction of strabismus and for tracer injections, anesthesia was induced with ketamine (10 mg per kilogram of body weight) and xylazine (2.5 mg/kg) intramuscularly, and maintained with ketamine intraveneously. In six kittens, we induced divergent strabismus at the age of 2 to 3 weeks by cutting the medial rectus muscle of the left eye. At age 2 to 3 months, pressure injections of red and green fluorescent latex microspheres ("beads") [L. C. Katz, A. Burkhalter, W. J. Dreyer, *Nature* **310**, 498 (1984); L. C. Katz and D. M. Iarovici, *Neuroscience* **34**, 511 (1990)] were made in the visual cortex of four squinting cats and of two normally reared control animals. Injection sites were typically 80 to 350 μm in diameter and confined to area 17 as evidenced by the distribution of retrogradely labeled cells in the LGN. One to 4 weeks later, one eye was occluded and a venous catheter implanted under halothane anesthesia. After full recovery from anesthesia, [^{14}C]2-DG (100 μCi/kg) was injected and the cats were allowed to freely move around in the laboratory for effective monocular stimulation. After 45 min, the animals were killed with pentobarbitone (180 mg/kg, intravenously). Flat-mounts of the occipital cortex were prepared and rapidly frozen. To provide landmarks for later superposition, three holes were made in the flat-mounts with warm needles. Subsequently, serial tangential sections were cut at −15°C and exposed to x-ray film for 3 to 4 weeks [S. Löwel, B. Freeman, W. Singer, *J. Comp. Neurol.* **225**, 401 (1987)]. The distributions of retrogradely labeled cells were mapped with a Zeiss fluorescence microscope (objective ×16) with the stage coupled to an analog *X*/*Y*-plotter. Thereafter cell plots and 2-DG autoradiographs from the same, representative supragranular sections were superimposed with the aid of the needle holes. For data presentation, we contrast-enhanced the 2-DG autoradiographs with an image-processing system (Imago II, Compulog) by expanding the gray values over the full modulation range: regions of lowest 2-DG uptake (unstimulated eye) are displayed in white, regions of highest 2-DG uptake in black. For transneuronal labeling of ocular dominance columns in layer IV, 2.5 mCi of [^{3}H]proline (in 50 μl of saline) were injected into one eye of the two remaining squinting cats 2 weeks before the 2-DG experiment. For the visualization of the [^{3}H]proline distributions, sections were postfixed in 4% paraformaldehyde, washed to remove all [^{14}C]2-DG, and then exposed to Ultrofilm (LKB) for 8 to 12 weeks [S. Löwel, H.-J. Bischof, B. Leutenecker, W. Singer, *Exp. Brain Res.* **71**, 33 (1988)].
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21. For statistical analysis of cell distributions, retrogradely labeled cells were classified into three groups depending on whether they were located within columns of the same (group a) or opposite ocularity (group b) as the injection sites or in the transition zone (group c) between bright and dark areas of the autoradiographs (Table 1). Subsequently, the relative areas of both ocular dominance territories were determined and cells in group c were assigned to groups a and b according to this ratio. The resulting distributions were compared with those expected if labeled cells distributed independently of ocular dominance territories according to the χ^2 test.
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Requirements for Phosphorylation of MAP Kinase During Meiosis in *Xenopus* Oocytes

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Mitogen-activated protein (MAP) kinases are activated in response to a variety of extracellular stimuli by phosphorylation on tyrosine and threonine residues. Xp42 is a *Xenopus laevis* MAP kinase that is activated during oocyte maturation. Modified forms of Xp42 that lacked enzymatic activity or either of the phosphorylation sites were expressed in *Xenopus* oocytes. When meiotic maturation was induced with progesterone, each mutant Xp42 was phosphorylated, indicating that at least one kinase was activated that can phosphorylate Xp42 on tyrosine and threonine. Phosphorylation of one residue is not strictly dependent on phosphorylation of the other.

MAP KINASES (*1*) ARE ACTIVATED in many cell types in response to mitogenic stimuli (*2, 3*). However, MAP kinases are also activated in specialized cells that are not mitogenically responsive, such as *Xenopus* and starfish oocytes undergoing meiosis (*4–6*), PC12 cells differentiating in response to nerve growth factor (*7–9*), adrenal cortical cells secreting catecholamines in response to nicotine or carbachol (*7*), and hippocampus undergoing a seizure response (*10*). The signaling pathways that trigger MAP kinase activation under these different conditions are initiated variously by tyrosine kinases, protein kinase C, or G proteins, and each pathway may activate MAP kinases by a common mechanism or by a distinct one.

Activated MAP kinase from mouse 3T3 cells is phosphorylated on threonine and tyrosine residues (*1*), and dephosphorylation of either or both residues reduces activity (*11*). Therefore, MAP kinases may be activated by the combined actions of tyrosine and serine-threonine kinases, by a single kinase able to phosphorylate threonine and tyrosine residues (*12*), by autophosphorylation, or by a combination of these possibilities. Both the phosphorylated residues are in kinase subdomain VIII (*13*), a region that contains autophosphorylated residues in other protein kinases (*14*). Either autophosphorylation or phosphorylation by other kinases could be induced by extracellular signals.

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Activities that induce serine, threonine, and tyrosine phosphorylation of MAP kinase have been detected in epidermal growth factor–stimulated mouse 3T3 cells and in nerve growth factor–stimulated PC12 cells (*15, 16*). These activators have no apparent protein kinase activity when assayed on other substrates. Studies of the MAP kinase ERK2 made in *Escherichia coli*, which lack tyrosine kinases, indicate that MAP kinases can autophosphorylate on tyrosine (*8, 17, 18*). Furthermore, phosphorylation of bacterially synthesized ERK2 is stimulated by the activator from 3T3 cells (*19*). The activators have been variously assumed to be activators of autophosphorylation (*19*) or specific "MAP kinase kinases" (*16*), but it is not clear which interpretation is correct. The activation of MAP kinase in *Xenopus* oocytes is also correlated with increased phosphorylation of the enzyme (*4, 5*). We now present evidence that this phosphorylation is catalyzed by one or more other kinases that are stimulated during oocyte maturation.

A cDNA for *Xenopus* MAP kinase Xp42 that is phosphorylated and activated during oocyte maturation has been cloned (*4, 20*), and antiserum has been raised to a COOH-terminal peptide (*21*). Xp42 was immunoprecipitated from oocytes and egg extracts and incubated with the substrates myelin basic protein (MBP) and γ-^{32}P–labeled adenosine triphosphate (ATP). Xp42 from eggs was enzymatically active and Xp42 from oocytes was inactive (Fig. 1A). Immunoblotting of the immunoprecipitates revealed that egg Xp42 migrated more slowly than oocyte Xp42 on gel electrophoresis (Fig. 1A). Xp42 from eggs, but not from