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25. All extraction procedures were done at 4°C. Cells were washed twice with phosphate-buffered saline, and each 10-cm dish of confluent cells was extracted in 300 μl of dilution buffer (*17*) containing 100 mM KCl and 5 mM $MgCl_2$, but only 0.05% v/v 2-mercaptoethanol (medium-salt buffer). DNA was sheared, and extracts were clarified by centrifugation (13,000*g* for 2 min); protein concentrations were measured with a Bio-Rad protein assay kit. Immunoblots for endogenous Ras or Raf-1 were done with monoclonal antibodies (products no. 02120 and no. R19120, Transduction Laboratories). For Ras-Raf complex analysis by immunoblot, endogenous Ras or Raf-1 was immunoprecipitated from ~15 mg of cellular protein with either 10 μg of Y13-238 or 10 μg of Raf-1 monoclonal antibody and probed by immunoblotting for Raf-1 or Ras. For assay of Ras-associated GSTMek-1 activation, Ras was immunoprecipitated from ~3.5 mg of cellular protein with Y13-238 (10 μg), washed four times with low-salt buffer, and assayed as described (*17, 24*). Ras immunoprecipitates were eluted with extraction buffer (40 μl), diluted with dilution buffer (160 μl) (*17*), and reprecipitated with Raf-1 monoclonal antibody (2 μg). Measurements of Ras-GTP were done as described (*23*). The cells were extracted in medium-salt buffer, and proteins (~3 mg) were absorbed to bacterially expressed GSTRBD. Ras proteins were revealed by immunoblotting. In control experiments using GSTR89LRBD, Ras-GTP was not detected (*13*).

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Integration of Environmental, Agronomic, and Economic Aspects of Fertilizer Management

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Nitrogen fertilization is a substantial source of nitrogen-containing trace gases that have both regional and global consequences. In the intensive wheat systems of Mexico, typical fertilization practices lead to extremely high fluxes of nitrous oxide (N_2O) and nitric oxide (NO). In experiments, lower rates of nitrogen fertilizer, applied later in the crop cycle, reduced the loss of nitrogen without affecting yield and grain quality. Economic analyses projected this alternative practice to save 12 to 17 percent of after-tax profits. A knowledge-intensive approach to fertilizer management can substitute for higher levels of inputs, saving farmers money and reducing environmental costs.

Agricultural intensification through the use of high-yielding crop varieties, chemical fertilizers and pesticides, irrigation, and mechanization—known as the "Green Revolution"—has been responsible for dramatic increases in grain production in developing countries over the past three decades. At the same time, intensification has had environmental consequences such as leaching of nitrate and pesticides, and emissions of environmentally important trace gases. We evaluated the economic and agronomic consequences, and the effects on N trace gas, of fertilizer management in irrigated spring wheat systems in the Yaqui Valley, Sonora, Mexico. This region is one of Mexico's major breadbaskets, so agricultural production and its environmental consequences are regionally important. In addition, as the "home" of the Green Revolution for wheat, the pattern of increasing fertilizer use in the Yaqui Valley provides a gauge of what is likely to occur in other high-productivity irrigated cereal systems of the developing world (*1*, *2*).

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Globally, application of fertilizer nitrogen (N) has increased rapidly in the last several decades, from 32 Tg N (32 million metric tons) in 1970 to around 80 Tg in 1990 (1 Tg = 10^{12} g); it is expected to increase to 130 to 150 Tg $year^{-1}$ by 2050, with two-thirds of that application in developing countries (*3*). Among the consequences of this change are increased losses of nitrate from soils to freshwater and marine systems and of N-containing gases to the atmosphere (*4*). Fertilized agriculture is the single most important anthropogenic source of N_2O, accounting for over 70% of the anthropogenic sources of this accumulating greenhouse gas (*5*, *6*). Likewise, fertilization results in elevated emissions of NO, a chemically reactive gas that regulates tropospheric ozone production and is a precursor to acid precipitation (*7*). Research in industrialized countries has shown that management practices can be used to control losses of N (*6*–*9*). However, integrated assessments of management alternatives in terms of their ability to reduce N trace gas fluxes and yet be feasible agronomically and attractive economically are wholly lacking. We carried out such an evaluation in the Yaqui Valley (*10*).

Using daily to weekly sampling frequencies during the 1994/1995 and 1995/1996 wheat cycles, we evaluated changes in soil nutrients and gas fluxes before and after fertilizer additions in experimental plots at the International Maize and Wheat Improvement Center (CIMMYT) field station (*11*). Several experimental conditions were studied: the conventional farmers' practice for the valley, as determined by farm survey (*12*); three alternative practices that were based on agronomist recommendations and that added less fertilizer N or fertilizer later in the crop cycle, or both (*13*); and a nonfertilized control. In our treatment that simulated the farmers' practice, 187 kg N/ha of urea were applied to dry soils 1 month beore planting, followed by preplanting irrigation; an additional 63 kg N/ha of anhydrous ammonia were applied ~6 weeks after planting.

After the soil was wetted by preplanting irrigation, ammonium (NH_4) levels increased markedly to over 600 μg/g (weighted average of bed and furrow positions) and then diminished to near zero as the microbially mediated process of nitrification converted NH_4 to nitrate (NO_3) (*14*, *15*). By the 1994 planting date, 116 kg/ha of NO_3-N were left in the top 15 cm of soil, with very little remaining in the NH_4 form. A similar pattern of transformation and loss was evident in the 1995/1996 wheat season.

Changes in N trace gas fluxes mirrored changes in the soil pools of inorganic N. The farmers' practice resulted in very large emissions of N_2O and NO in both years (Fig. 1), with preplanting gas fluxes summing to 5.6 and 4.6 kg N/ha in the 1994/1995 and 1995/1996 wheat cycles, respectively, and crop cycle fluxes summing to 6.61 and 11.3 kg N/ha, respectively (*16*, *17*). In the 1994/1995 study, average fluxes at midday in the bed positions (where most of the fertilizer was located) ranged up to 650 ng cm^{-2} $hour^{-1}$ for N_2O-N and 300 ng cm^{-2} $hour^{-1}$ for NO-N in the period before planting (*15*). In 1995/1996, which had less rainfall during the preplanting period, average N_2O and NO fluxes in the beds ranged up to 100 and 550 ng cm^{-2} $hour^{-1}$, respec-

tively, during the same period (*15*). These values are among the highest ever reported (6–8).

All but one of the alternative practices evaluated during the two study years resulted in significant reductions in crop-cycle N gas emissions as compared with the farmers' practice; the alternative in which 250 kg/ha N were applied, with 33% preplanting, 0% at planting, and 67% after planting, lost 6.93 kg/ha of N_2O plus NO-N, in contrast to 6.61 kg/ha lost in the farmers' practice. In both years, the alternative in which 250 kg/ha N were added, with 33% at planting and 67% after planting, lost at least 50% less N gas than the farmers' practice. In the "best" alternative with respect to reduced N_2O and NO emissions, a total of 180 kg N/ha were applied, with 33% at planting and 67% 6 weeks afterplanting (Fig. 1). In this treatment, total fluxes of N_2O and NO, summed over the 1995/1996 wheat cycle, were 0.74 kg N/ha.

The interplay between the timing of fertilization and irrigation was critical to inorganic N transformations and gas losses in this site. When fertilizer was added to dry soils, only very small changes in inorganic N concentrations or N gas emissions were measured. With irrigation, however, rapid conversion of urea to NH_4 was followed by nitrification of NH_4 to NO_3. High losses of N_2O occurred soon after irrigation, largely resulting from denitrification under waterlogged conditions (*18*). As the soils dried, NO emissions increased, produced during nitrification (*14*). Both N_2O and NO emissions dropped substantially by planting (Fig. 1) (*15*), when denitrification apparently was limited by a well-aerated soil environment and nitrification was limited by the low availability of NH_4. Process studies with ^{15}N-labeled NO_3 and NH_4 confirmed these patterns and controls (*19*).

Emissions of N_2O and NO under the farmers' practice were large relative to those observed in many other studies. However, they represent just two of several important pathways by which N can be lost from terrestrial ecosystems; others include ammonia volatilization, nitrate leaching, and dinitrogen gas flux (*20*). As seen by farmers, total loss of N is of more interest than specific trace gas losses, as it represents wasted fertilizer. To determine the total loss of fertilizer N, we applied ^{15}N-labeled urea (in place of the fertilizer) in isolated plots that were otherwise treated like the experimental plots; at the end of the crop cycle, the isolated plots were harvested and ^{15}N recovery in soil and plant components was measured (*21*). In the farmers' practice and in our best alternative, proportional recovery of the applied N in plants was 46 and 57%, respectively, and recovery in soil to a meter depth was 26 and 16%, respectively. Because less N was added in the alternative (180 kg/ha), quantitatively less N was lost than in the farmers' practice (48 kg/ha lost in the alternative versus 70 kg/ha in the farmers' practice).

Fertilizer use and loss are just one component of farm budgets, and farmers typically focus not only on costs but on the balance between costs and expected income under some degree of price and production uncertainty. For wheat farmers in the Yaqui Valley, yield of good-quality wheat provides the essential income. Yields reported in our socioeconomic surveys in 1994/1995 and 1995/1996 ranged from 3.1 to 7.3 tons/ha, with average values of 4.9 and 5.3 tons/ha for the two seasons, respectively (*12*). Mean yields in our simulated farmer practice were 6.08 ± 0.18 and 6.07 ± 0.28 tons/ha in 1994/1995 and 1995/1996, respectively (*22*). Our best alternative, in which 180 kg N/ha were added as compared with 250 kg N/ha in the farmers' practice, resulted in yields that were not significantly different (6.16 ± 1.3 tons/ha). Likewise, grain quality (estimated as the protein concentration in grain) in the alternative was not significantly different from the farmers' practice (14.87 versus 14.83%, respectively) (*22*).

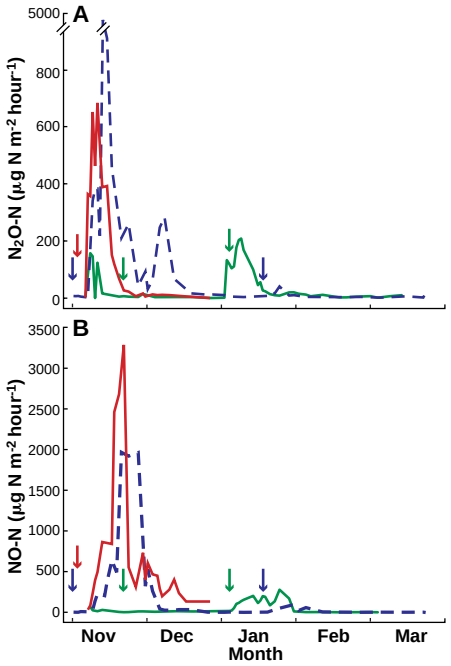

Fig. 1. Changes in the emissions of N_2O and NO from the soil surface in the farmers' practice over the 1994/1995 (in blue) and 1995/1996 (in red) wheat cycles, and for the best alternative (in green) in the 1995/1996 wheat cycle. Values are the area-weighted means (μg N m^{-2} hour^{-1}), on the basis of measurements taken in bed and furrow positions (*15*, *26*). (**A**) N_2O-N emissions. (**B**) NO-N emissions. Fertilizer applications in the different treatments and years are indicated by arrows (color-coded to match the flux data). For details on fluxes by field position, standard errors, and information on the timing of irrigation, planting, and harvest, see (*15*).

As in many high-productivity agricultural systems of the developing world, the dissemination of Green Revolution technologies initially provided farmers in the Yaqui Valley with modern seed varieties and highly subsidized N fertilizers. In recent years, however, the reduction of subsidies (in real terms) has been dramatic (*23*). Our economic analysis of farmers' costs and returns for both 1994/1995 and 1995/1996 wheat seasons indicates that fertilization has now become the highest direct production cost in the Yaqui Valley farm budgets (Table 1). During the 2 years of our study, fertilization exceeded even the costs of land preparation, which traditionally have represented the largest cost category in this highly mechanized system; just 5 years ago, the cost of land preparation was 50% higher than that of fertilization (*2*).

Given the importance of fertilizer in the Yaqui Valley farm budgets, we evaluated the extent to which increased fertilizer efficiency represented a significant budgetary savings to the farmers (*15*). In contrasting the farmers' practice with our best alternative, we found that the alternative resulted in a savings of new pesos (N$) 414 to N$571/ha, or U.S.$55 to U.S.$76/ha at then-existing exchange rates. These values, which resulted from lower fertilizer applications and reduced

Table 1. Costs and returns for the 1994/1995 and 1995/1996 wheat cycles. Data were collected during on-farm surveys (*12*).

Factors (N$/ha)*	1994/1995	1995/1996
Costs		
Land preparation	342	345
Planting	248	369
Fertilization	519	1041
Irrigation	96	300
Insect and weed control	177	238
Harvest	156	454
Other costs†	1710	1560
Total	3248	4307
Returns		
Gross revenue from yield (pre-income tax)	4294	8450
Profit		
Returns to management (after income tax)	1046	3413

*Costs, returns, and profits are shown in new pesos (N$) per hectare (current prices). The average exchange rates were N$3.2/U.S.$ in 1994, N$5.58/U.S.$ in 1995, N$7.55/U.S.$ in 1996. †Other costs include interest on credit, crop insurance, salary of the field manager, independent technical assistance, producer's organization fees, land rental, and taxes and subsidies (*25*).

loss of fertilizer, were equivalent to 12 to 17% savings of after-tax profits from wheat farming in the Yaqui Valley. Such potential cost savings may over time induce a shift in technology and management toward fertilization later in the wheat cycle; indeed, our on-going surveys indicate that some farmers are now postponing their first fertilizer application until planting. However, farmers may also face greater risks of low yields with our best alternative, particularly in years when late rains delay the second fertilizer application beyond the point of optimal plant response (*24*). The importance of such real or perceived risks, and the development of recommendations that are sensitive to them, are topics of our current research.

Our results demonstrate that alternative fertilizer practices can reduce trace gas and total losses of fertilizer and maintain yields. These alternatives, which require greater knowledge about efficient use of nutrients, can substitute for higher levels of those inputs and might ultimately allow Yaqui Valley farmers to remain competitive in an era of economic liberalization and expanding free trade. At the same time, they reduce the environmental costs of agriculture, some of which are directly felt in the Yaqui Valley, and others of which are globally important. An integration of agronomic knowledge of practical alternatives, economic analysis of their on-farm costs and benefits, and biogeochemical analysis of their consequences in soils and the atmosphere can provide the basis for the identification or development of win-win solutions.

REFERENCES AND NOTES

1. Fertilizer rates in the Yaqui Valley have grown over the past several decades, from 172 kg/ha (range: 0 to 280 kg/ha) in 1981 to ~250 kg/ha of fertilizer N (range: 151 to 396 kg/ha) in 1996 (*2*) and are now considerably higher than those in many of wheat systems of the United States and Europe. In contrast, many of the irrigated rice and wheat systems of Asia have half the fertilizer N application rates of the Yaqui Valley, and substantial increases in fertilizer use are expected there [International Fertilizer Industry Association (IFA)/Food and Agriculture Organization (FAO)/International Fertilizer Development Center (IFDC), *Fertilizer Use by Crop* (FAO, Rome, 1992); K. G. Cassman and P. L. Pingali, *GeoJournal* **35**, 299 (1995); P. R. Hobbs, K. D. Sayre, J. I. Ortiz-Monasterio, *Increasing Wheat Yields Through Agronomic Methods* (Natural Resources Special Report, CIMMYT, Mexico DF, 1997)].
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10. The Yaqui Valley is located near Ciudad Obregon in Sonora, Mexico (27°N109°W, 40 m above sea level), bounded on the west by the Gulf of California and on the east by the foothills of the Sierra Madres.
11. Field experiments during 1994/1995 and 1995/1996 wheat seasons (November to April) were established after summer rotations with soybean. The soil is a coarse, sandy clay mixed montmorillonite classified as a Typic Calciorthid. The site received 20 kg P/ha as triple superphosphate during initial land preparation. Seeds of bread wheat (*Triticum aestivum* L cultivar "Rayon F29") were planted in 75-cm-wide "beds" in two rows at the rate of 100 kg/ha.
12. Farm practices and budgets were determined by on-farm socioeconomic surveys conducted by the CIMMYT economics department (1981/1994) (*2*) and by the Institute for International Studies at Stanford University (1994/1996). The surveys consisted of a random sample of 58 farmers in 1994/1995 and 31 farmers in 1995/1996, stratified by operating size of farm units and land tenure (private versus collective landholders). Data were collected at two periods in each year; farm owners or managers relied on recorded input, yield, and price data to answer the questions. The surveys were conducted by Mexican nationals familiar with the Yaqui Valley.
13. In 1994/1995, four experimental conditions (in five block replicates) were established on 6 m by 50 m plots. These included a nonfertilized control, the farmers' practice (250 kg/ha N, with 75% applied as urea-N 1 month before planting, 0% at planting, and 25% as anhydrous ammonia-N 1 month after planting, designated as 75-0-25), and alternatives that applied 250 kg/ha N fertilizer at 33-0-67 and 0-33-67 allocations. Urea was applied in the beds, and anhydrous ammonia was bubbled into irrigation water in the furrows. All plots received identical land preparation, irrigation, tillage, pest control, planting, and harvesting; furrow irrigation was carried out within several days after preplanting fertilization as a weed-control strategy, twice after planting. In 1995/1996, experimental plots were 22 m by 27 m, and treatments included the nonfertilized control, the farmer practice (250 kg/ha at 75-0-25 as described above), one alternative that applied 250 kg/ha N at 0-33-67, and another that applied 180 kg/ha at 0-33-67.
14. In nitrification, NH_4 is oxidized to NO_3 under aerobic conditions; both NO and N_2O can be produced as by-products, but NO is typically dominant.
15. Supplementary material is available at www.sciencemag.org/feature/data/975726.shl.
16. We estimated gas emissions for 24-hour periods by multiplying the area-weighted mean fluxes (ng cm^{-2} $hour^{-1}$) from measurements taken during the midday period (*13*) by an equation that mathematically represents the average diel variation based on measurements of gas fluxes over 24 hours carried out four different times during the crop cycle. Daily fluxes for nonsampled days were estimated as a linear function of the 24-hour fluxes on the previous and subsequent sampling dates. Daily flux estimates were then summed over the period from first fertilization to planting (the preplanting flux) and first fertilization to harvest (the crop cycle flux); control values were subtracted from treatment values.
17. In the 1995/1996 wheat season, ~125 kg/ha, rather than 62.5 kg/ha N, were mistakenly applied by commercial applicators at the postplanting fertilization in the farmers' practice; thus, this treatment received 312.5 kg/ha over the entire cycle. Therefore, the postplanting flux in this site may be higher than is typical for farmers' fields.
18. Denitrification is the microbial reduction of NO_3 to N_2, N_2O, or NO under anaerobic conditions; under field conditions, NO is rarely emitted.
19. J. Panek and P. A. Matson, in preparation.
20. Ammonia volatilization and nitrate leaching both transport N between systems and have important consequences for other ecosystems; these loss pathways are being measured in this study and will be reported in later publications.
21. ^{15}N-labeled urea was added to replicated 1 m by 1.5 m plots (isolated with heavy plastic to 1 m in depth) to simulate the three 1995/1996 fertilizer treatments. At harvest time, the isolated plots were harvested by horizon down to a meter in depth, and ^{15}N recovery in plants, soil organic matter, microbial biomass, NH_4, and NO_3 were measured as described [P. A. Matson, P. M. Vitousek, J. J. Ewel, M. J. Mazzarino, G. P. Robertson, *Ecology* **68**, 491 (1987)].
22. Grain yields were estimated as weight of grain (at 12% moisture) after harvest with a plot combine. To determine grain quality, we measured total N concentration by Kjeldahl digestion and multiplied the value by 5.83 to estimate protein concentration.
23. In 1991, the world price for fertilizer was 31% greater than the domestic price (including transportation costs); by 1995, this price distortion had fallen to 6% [A. Puente, unpublished data].
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25. The indirect cost of interest on credit—much of it expended on fertilizer—exceeded direct fertilization expenses in the 1994/1995 season, when macroeconomic policy, including a 100% devaluation of the Mexican peso, caused lending rates for farmers in the valley to rise from 16 to 77% per annum during the 6-month wheat cycle. As interests rates fell to 35% per annum at the end of the 1995/1996 season, fertilization became the single most important cost component in the entire budget. Roughly 50% of the farmers in the 1995/1996 survey (*12*) obtained credit in U.S. dollars at 17% per annum with the agreement of selling their output in U.S. dollars through export contracts.
26. Gas samples were collected with two-piece 9-liter chambers as described (*8*). Nitrous oxide was analyzed with a Shimadzu 14A gas chromatograph Model 2 configured with an electron capture detector as described [A. R. Mosier and L. Mack, *Soil Sci. Soc. Am. J.* **44**, 1121 (1980)]. Standards (0.1, 0.5, and 1.0 parts per million; Scott Research Laboratory, Plumsteadville, PA) bracketed every 12 to 20 samples. Coefficients of variation of the standards were <1%. Fluxes were calculated as described (*8*). Minimum detectable flux was 0.1 ng cm^{-2} $hour^{-1}$. Within 1 hour preceding or after sampling for N_2O, NO was measured in the same rings with a Scintrex LMA-3 chemoluminescence detector modified for field measurements; methods are presented in detail in Matson *et al*. (*8*) and E. A. Davidson *et al.* [*J. Geophys. Res.* **96**, 15439 (1991)]. Standard curves (with dilution of a 0.1-ppm standard) were run in the

field before and after sets of 10 to 20 gas measurements. Minimum detectable flux was ~0.05 ng cm^{-2} $hour^{-1}$.

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Induction of Lens Differentiation by Activation of a bZIP Transcription Factor, L-Maf

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After the vertebrate lens is induced from head ectoderm, lens-specific genes are expressed. Transcriptional regulation of the lens-specific αA-crystallin gene is controlled by an enhancer element, αCE2. A gene encoding an αCE2-binding protein, *L-maf* (lens-specific *maf*), was isolated. *L-maf* expression is initiated in the lens placode and is restricted to lens cells. The gene product L-Maf regulates the expression of multiple genes expressed in the lens, and ectopic expression of this transcription factor converts chick embryonic ectodermal cells and cultured cells into lens fibers. Thus, vertebrate lens induction and differentiation can be triggered by the activation of L-Maf.

During development, the vertebrate lens is induced upon contact between the presumptive retina and head ectoderm (*1*). Differentiation of the ectoderm into lens cells is accompanied by the specific upregulation of crystallin gene transcription (*2*). We previously identified a lens-specific enhancer element, termed αCE2, in the chicken αA-crystallin promoter (*3*, *4*). The αCE2 sequence, located 100 base pairs upstream of the transcription start site, represents a lens-specific enhancer element that is conserved in the regulatory regions of many crystallin genes (*5*). The integrity of this αCE2 sequence to direct lens-specific transcription has been demonstrated in both cell culture and transgenic mouse experiments.

To identify a factor or factors that bind to the αCE2 sequence and are expressed during the formation of the lens, we screened an expression library prepared from chick embryonic lens with oligonucleotide probes encoding the αCE2 sequence (*6*). Positive cDNA clones were classified by their patterns of tissue distribution. Northern (RNA) blot analysis of 8-day-old chick embryonic tissues revealed a 3.6-kb mRNA that was expressed almost exclusively in the lens, with very weak expression in the brain (Fig. 1A). The spatial and temporal patterns of expression were examined by whole-mount in situ hybridization analyses (*7*). The transcripts were first detected in the lens placode at stage 11, when the head ectoderm makes contact with the optic vesicle (Fig. 1B). The expression remains restricted to the invaginating lens placode (stage 13, Fig. 1, C and D), and subsequently to the developing lens vesicle (stage 15, Fig. 1, E and F), where localized transcription of the αA-crystallin gene later occurs (stage 18, Fig. 1G). Early expression of this factor in the lens placode preceded the induction of the δ1-crystallin gene, one of the earliest lens markers.

Full-length cDNA was obtained and the sequence was determined. A single open reading frame encoding 286 amino acids predicted a putative transcription factor with a bZIP motif and additional sequences characteristic of the *maf* proto-oncogene family (Fig. 2A). Thus, we named this protein L-Maf (lens-specific Maf). The gene product, which represents a previously undescribed member of the family, can be classified with the large Maf subfamily including MafB, c-Maf, and NRL (Fig. 2B) rather than with the small Mafs such as MafK, MafF, and MafG (*8*). L-Maf most closely resembles MafB/Kreisler, which has been shown to be involved in segmentation of the hindbrain (*9*). Members of the large Maf family are expressed in the lens of the rat, mouse, chick, *Xenopus*, and zebrafish (*10*). Interestingly, *mafB* and *c-maf* or *L-maf* are detected in the lens epithelial and fiber cells, respectively, of the rat and *Xenopus*.

We next tested the ability of L-Maf to control transcription in transfection assays that used chicken primary culture cells and a reporter construct encoding the chick αA-crystallin promoter (−244 to +89) linked to a luciferase gene (Fig. 3A). Cotransfection of an L-Maf expression plasmid (pEFX–L-Maf) and the reporter into chick embryonic lens cell cultures (*4*, *11*) resulted in luciferase activity 10 times that caused by transfection with a control plasmid (pEFX) (*12*). Replacement of the αCE2 sequence, located in the promoter region between base pairs −119 and −99, with a Bam HI linker abolished this response, indicating that L-Maf activation occurs through the αCE2 sequence. Efficient transactivation of the αA-crystallin promoter by L-Maf was also observed in chick neural retina cell cultures. In addition, activation was observed when L-Maf was overexpressed in lung cultures; otherwise, activity of the αA-crystallin promot-

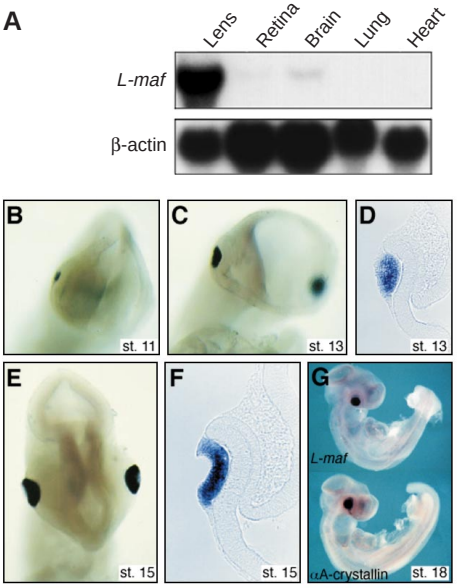

Fig. 1. Restricted expression of *L-maf* mRNA in the lens. (**A**) Northern blot analysis of mRNAs from 8-day-old chick embryonic tissues, including lens, neural retina, brain, lung, and heart tissues. In each lane, 10 μg of total RNA was blotted and hybridized with a randomly primed probe for *L-maf* (3.6 kb) or β-actin cDNAs. (**B** to **G**) Expression of *L-maf* during chick lens development. *L-maf* expression was analyzed by whole mount in situ hybridization from stages 11 to 18. Frontal views of embryos hybridized with antisense *L-maf* probes are shown for stages 11 (B), 13 (C), and 15 (E); coronal sections through the lens placodes are shown for stages 13 (D) and 15 (F). A lateral view of embryos at stage 18 (G) shows expression of *L-maf* and αA-crystallin.

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