Methods

Protein expression and purification. Murine COX-2 cDNA fragments containing the coding sequence were cloned into baculovirus transfer vector pVL1393 and expressed in cultured Sf21 insect cells, as described for the human enzyme²⁶. The COX-2 enzyme was purified on an anion exchange column equilibrated with 20 mM Tris-HCl, 0.4% CHAPS, pH 8.1. The eluted enzyme was concentrated tenfold and further purified on a Sephacryl S-200 column equilibrated with 25 mM Tris-HCl, 150 mM NaCl, 0.3% β -octylglucopyranoside (β -OG). It was concentrated to 10 mg ml⁻¹ after reconstitution with hemin

Crystallization. The enzyme, containing 1 mM inhibitor, was dialysed overnight at 4 °C against 20 mM sodium phosphate, 100 mM NaCl, 0.6% β -OG, and an inhibitor concentration that is five times its IC_{50}. Crystals were grown at 18 $^\circ\text{C}$ using the vapour diffusion method by mixing an equal volume of protein solution and a reservoir solution containing 20-34% monomethyl PEG 550, 10-240 mM MgCl₂, 50 mM EPPS, pH 8.0. The final concentration of β -OG in the drop was adjusted to 0.6%. Crystals grew as hexagonal and rectangular rods over three weeks.

Data collection. The crystals of the native enzyme and the complexes with flurbiprofen, indomethacin and SC-558 belong to the space group P21212 (a = 180.0, b = 134.0, c = 120.0 Å) with two dimers in the asymmetric unit. The complex with SC-558 also crystallized in the space group /222 with similar lattice parameters, but with one dimer in the asymmetric unit. Crystals were flash frozen to -160 °C. Diffraction data (Table 1) were recorded using a 30-cm Mar Research image plate coupled to a Rigaku RU200 rotating anode X-ray generator fitted with mirror optics. The reflection intensities were integrated using the program DENZO and scaled using the program SCALEPACK²⁷

Structure determination and refinement. The structure of the COX-2 complex with flurbiprofen was determined initially by molecular replacement methods using the MERLOT package²⁸. The structure of COX-1 dimer was used as the search model⁸. The two crystallographically independent dimers in the asymmetric unit have the same orientation, as indicated by a single sharp solution (5.0 σ) to the cross-rotation function. The translation function searches yielded two unambiguous solutions, with the same y and z but different xcoordinates, corresponding to the positions of the two dimers. The vector relating the positions of the two dimers was determined in a subsequent intermolecular translation search (56 σ). The overall crystal packing of COX-2 closely resembles a pseudo-body-centred orthorhombic lattice (pseudo /222), as confirmed by a prominent peak in the Patterson function at x = 0.47, y = 0.5 and z = 0.5(peak height, 50% of the origin peak). In the initial maps, electron density was clearly visible for both the haem and the inhibitor that were excluded from the model. The model was improved by successive rounds of rigid-body, restrained positional, B-factor and simulated annealing refinements using X-PLOR with non-crystallographic symmetry restraints²⁹. This was followed by manual rebuilding using the program O³⁰ and further refinements with X-PLOR. Flurbiprofen was included in the final rounds of refinement. The refined COX-2 structure, omitting the flurbiprofen, was used as the starting model for refinements of the native structure and the complex with indomethacin and SC-558. The structures were refined using X-PLOR as described above. The structure of the COX-2 complex with SC-558 that crystallized in the /222 space group was also determined by molecular replacement methods. The search model consisted of the structure of COX-2 complex with the same inhibitor in the P2₁2₁2 space group but without the inhibitor. Rotation and translation function searches using the program MERLOT yielded unambiguous solutions. The model was refined using the same protocols.

Received 3 October: accepted 20 November 1996.

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ACKNOWLEDGEMENTS. We thank J. Edwards for growing crystals of COX-2; H.-S. Shieh for help with computations; C. Koboldt for measurement of IC50; and R. M. Garavito for providing coordinates of ovine COX-1

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ERRATA

Positional cloning of a global regulator of anteriorposterior patterning in mice

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Nature 383, 250-253 (1996)

THE amino-acid sequence was accidentally omitted from Fig. 1a. The complete figure is shown here.



MSEREVSTAPAGTDMPAAKKQKLSSDENSNPDLSGDENDDAVSIE PDTPTNTPNAPGRKSWGKGKWKSKKCKYSFKCVNSLKEDHNQPLFGVQFNWH SKEGDPLVFATVGSNRVTLYECHSQGEIRLLQSYVDADADENFYTCAWTYDS I7Rn51989SB N P I7Rn53354SB

T1031->A T1040->C 4

NTSHPLLAVAGSRGIIRIINPITMQCIKHYVGHGNAINELKFHPRDPNLLLS VSKDHALRLWNIQTDTLVAIFGGVEGHRDEVLSADYDLLGEKIMSCGMDHSL KLWRINSKRMMNAIKESYDYNFNKTNRPFISQKIHFPDFSTRDIHRNYVDCV RWLGDLILSKSCENAIVCWKPGKMEDDIDKIKPSESNVTILGRFDYSQCDIW YMRFSMDFWQKMLALGNQVGKLYVWDLEVEDPHKAKCTTLTHHKCGAAIRQT SFSRDSSILIAVCDDASIWRWDRLR

A transcriptional partner for MAD proteins in TGF- β signalling

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Nature 383, 691-696 (1996)

IN Fig. 1c of this Article, the shading of 1 H280 should have been striped as in the region of 1 H208 immediately above it, and not shaded grey as published.