Review

Physiology and pathophysiology of prostanoid receptors

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Abstract: Prostanoids, consisting of prostaglandins (PGs) and thromboxanes (TXs), are oxygenated products of C_{20} unsaturated fatty acids. They include PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and TXA_2. Given that aspirin-like nonsteroidal anti-inflammatory drugs exert their actions by suppressing prostanoid production, prostanoids have been implicated in processes inhibited by these drugs, including inflammation, fever, and pain. Prostanoids also contribute to vascular homeostasis, reproduction, and regulation of kidney and gastrointestinal functions. How prostanoids exert such a variety of actions had remained unclear, however. Prostanoids are released outside of cells immediately after their synthesis and exert their actions by binding to receptors on target cells. We have identified a family of eight types or subtypes of G protein–coupled receptors that mediate prostanoid actions. Another G protein–coupled receptor was also identified as an additional receptor for PGD_2. Genes for these receptors have been individually disrupted in mice, and analyses of these knockout mice have not only elucidated the molecular and cellular mechanisms of known prostanoid actions but also revealed previously unknown actions. In this article, I review the physiological and pathophysiological roles of prostanoids and their receptors revealed by these studies.

Keywords: prostaglandin, thromboxane, cyclooxygenase, G protein–coupled receptor

Prostanoids, consisting of prostaglandins (PGs) and thromboxanes (TXs), are oxygenated products derived from C_{20} unsaturated fatty acids (Fig. 1). γ-Homolinolenic acid, arachidonic acid, and 5,8,11,14,17-eicosapentaenoic acid are the precursor fatty acids for the series 1, 2, and 3 prostanoids, which contain the 13-trans double bond, the 5-cis and 13-trans double bonds, and the 5-cis, 13-trans, and 17-cis double bonds, respectively. Given that arachidonic acid is the most abundant among these precursor fatty acids in most mammals, including humans, the series 2 prostanoids predominate in these organisms. These precursor fatty acids are esterified to the sn-2 position of glycerophospholipids in cell membranes and are liberated from the membrane phospholipids by the action of phospholipase A_2 in response to various physiological or pathological stimuli.[1)] Arachidonic acid thus liberated is metabolized by the action of cyclooxygenase (COX) to PGH_2, which is then converted to various PGs by the respective PG synthases (Fig. 1). There are two isoforms of COX; one is COX-1 that is constitutively expressed in many tissues, and the other is COX-2, expression of which is induced by various inflammatory as well as other stimuli and suppressed by glucocorticoids such as dexamethasone.[2)] Similarly, there are two major isoforms of PGE synthase, cytosolic PGE synthase (cPGES) that is constitutively expressed and membrane-bound PGE synthase-1 (mPGES-1) that, like COX-2, is induced by various proinflam-

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Abbreviations: PG: prostaglandin; TX: thromboxane; COX: cyclooxygenase; NSAID: nonsteroidal anti-inflammatory drug; GPCR: G protein–coupled receptor; cAMP: cyclic adenosine monophosphate; Th: T helper; ACTH: adrenocorticotropic hormone; LPS: lipopolysaccharide; IL: interleukin; TNF: tumor necrosis factor; CRF: corticotropin-releasing factor; DRG: dorsal root ganglion; Ig: immunoglobulin; OVA: ovalbumin; SNP: single nucleotide polymorphism; DNFB: dinitrofluorobenzene; IBD: inflammatory bowel disease; DSS: dextran sodium sulfate; PTH: parathyroid hormone; RANKL: receptor activator of NF-κB ligand.

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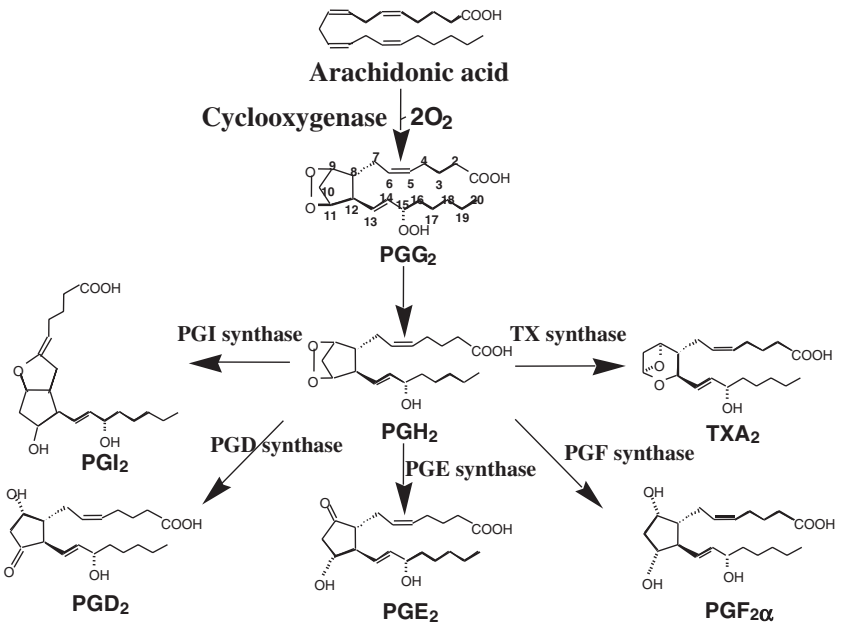

Fig. 1. Pathway of prostanoid biosynthesis. Arachidonic acid is metabolized by the action of cyclooxygenase (COX) first to prostaglandin endoperoxide (PGG_2) and then to PGH_2, which is subsequently converted to various PGs and TXA_2 by respective synthases. Aspirin-like nonsteroidal anti-inflammatory drugs (NSAIDs) exert their effects by inhibiting COX and thereby suppressing prostanoid biosynthesis.

matory stimuli and suppressed by glucocorticoids.[3] Prostanoids thus formed are released outside of cells immediately after their synthesis. PGH_2, PGI_2, and TXA_2 are chemically unstable and are degraded to inactive products under physiological conditions, with half-lives of 30 s to a few minutes. Other PGs, although chemically stable, are metabolized rapidly. For example, they are inactivated during a single passage through the lung. Prostanoids are therefore thought to act locally, in the vicinity of the site of their production.

Aspirin-like nonsteroidal anti-inflammatory drugs (NSAIDs) exert their pharmacological actions by inhibiting COX and suppressing prostanoid production. The actions of prostanoids have therefore been examined either by the addition of exogenous prostanoids to cells, tissues, or the body or by analysis of the effects of aspirin-like drugs on various physiological or pathophysiological processes. The typical actions of prostanoids revealed by these studies include induction of the relaxation or contraction of various types of smooth muscle. Prostanoids also modulate neuronal activity by inhibiting or stimulating neurotransmitter release or sensitizing sensory fibers to noxious stimuli, and they exert central actions such as induction of fever or sleep. They regulate secretion and motility in the gastrointestinal tract as well as transport of ions and water in the kidney. They play roles in apoptosis, cell differentiation, and oncogenesis. They also regulate the activity of blood platelets both positively and negatively, and they contribute to vascular homeostasis and hemostasis. Prostanoids thus exert a wide variety of actions in various cells and tissues and the body. However, the mechanisms underlying such actions remained a mystery until the receptors responsible were identified, their cDNAs cloned, and their roles analyzed. Furthermore, whereas methods for the chemical synthesis of natural prostanoids and related compounds have been developed for reproduction of or interference with the various bioactivities of prostanoids, they have been of limited use without knowledge of the prostanoid receptors and their properties.

Structure, signal transduction mechanisms, and distribution of prostanoid receptors

Given that prostanoids are produced from fatty acids and were therefore generally regarded as

hydrophobic compounds, it was initially thought that they were incorporated into the cell membrane and exerted their actions by perturbing lipid fluidity. However, prostanoids are not as hydrophobic as originally thought and do not incorporate into or permeate the cell membrane.[4] Furthermore, comparison of the potencies of various prostanoids as determined by bioassays in various tissues revealed that each prostanoid possesses a unique activity profile, indicating that each compound has a specific site of action. Moreover, various synthetic TXA_2 agonists and antagonists were developed in the late 1970s to early 80s, and these compounds facilitated the pharmacological characterization of a receptor for TXA_2.[5] Biochemical evidence also supported the existence of prostanoid receptors. Beginning in the mid-1960s, the actions of prostanoids were repeatedly found to be associated with changes in the levels of intracellular second messengers such as cyclic adenosine monophosphate (cAMP), phosphoinositides, and Ca^{2+}. In addition, the availability of radioisotopically labeled prostanoid derivatives in the early 1970s allowed the demonstration that many tissues and cells possess specific high-affinity binding sites for prostanoids.[6–8]

These various studies culminated in the proposal of a pharmacological classification of prostanoid receptors into those specific for TX, PGI, PGE, PGF, or PGD, designated TP, IP, EP, FP, and DP receptors, respectively, with EP receptors being further divided into four subtypes designated EP1, EP2, EP3, and EP4.[9,10] However, none of these receptors had been isolated or cloned until we purified the TXA_2 receptor from human blood platelets in 1989[11] and cloned its cDNA in 1991.[12] The TXA_2 receptor thus revealed is a G protein–coupled, rhodopsin-type receptor with seven transmembrane domains (Fig. 2A). Homology screening of cDNA libraries prepared from mouse and other mammals subsequently resulted in the identification of the primary structures of the remaining seven types or subtypes of prostanoid receptors.[13] All are G protein–coupled, rhodopsin-type receptors with seven transmembrane domains and each is encoded by a distinct gene. Several variants of EP3 that are generated by alternative splicing of exons encoding the carboxyl-terminal tail were also identified. The three EP3 splice isoforms in mouse, α, β, and γ, contain carboxyl-terminal tails of 30, 26, or 29 amino acids, respectively, that do not share any structural motifs or hydrophobic features.[13–15] These isoforms show similar ligand binding characteristics but distinct signal transduction properties, as described below. Such multiple splice isoforms for EP3 have also been identified in other species including rat, rabbit, cow, and human.[11] In addition to this family of prostanoid receptors, there is a distinct type of receptor for PGD_2.[16] This receptor, designated CRTH2, was originally cloned as an orphan receptor expressed in T helper (Th) 2 lymphocytes and has recently been shown to bind PGD_2 with an affinity as high as that of DP, although its binding profile for other PGD analogs differs from that of DP. CRTH2 belongs to the family of chemokine receptors and mediates chemotaxis of Th2 lymphocytes as well as of eosinophils and basophils toward PGD_2.

Signal transduction pathways linked to prostanoid receptors have been studied by examination of the effects of agonists on the intracellular concentrations of second messengers such as cAMP, Ca^{2+}, and inositol phosphates in cells transfected with expression vectors for each receptor. These studies revealed that the eight types or subtypes of prostanoid receptors can be divided into three groups on the basis of their modes of signal transduction (Fig. 2B): IP, DP, EP2, and EP4 mediate an increase in the intracellular level of cAMP and have been designated "relaxant" receptors; TP, FP, and EP1 induce elevation of intracellular Ca^{2+} and have been denoted as "contractile" receptors; and EP3 elicits a decrease in the intracellular concentration of cAMP and has been termed an "inhibitory" receptor.[13] This functional classification of prostanoid receptors is consistent with the phylogenetic tree derived from the nucleotide sequences of the corresponding genes.[17] These studies indicate that the prostanoid receptors originated from a primitive PGE receptor; this primitive receptor gave rise to the PGE receptor subtypes, and the receptors for other PGs and TX subsequently evolved from functionally related PGE receptor subtypes by gene duplication. It should be noted, however, that this functional grouping of prostanoid receptors is based on the coupling of each receptor to only one of three signaling pathways, increase or decrease in the intracellular cAMP level or elevation in the intracellular Ca^{2+} concentration; it does not exclude the possibility that these

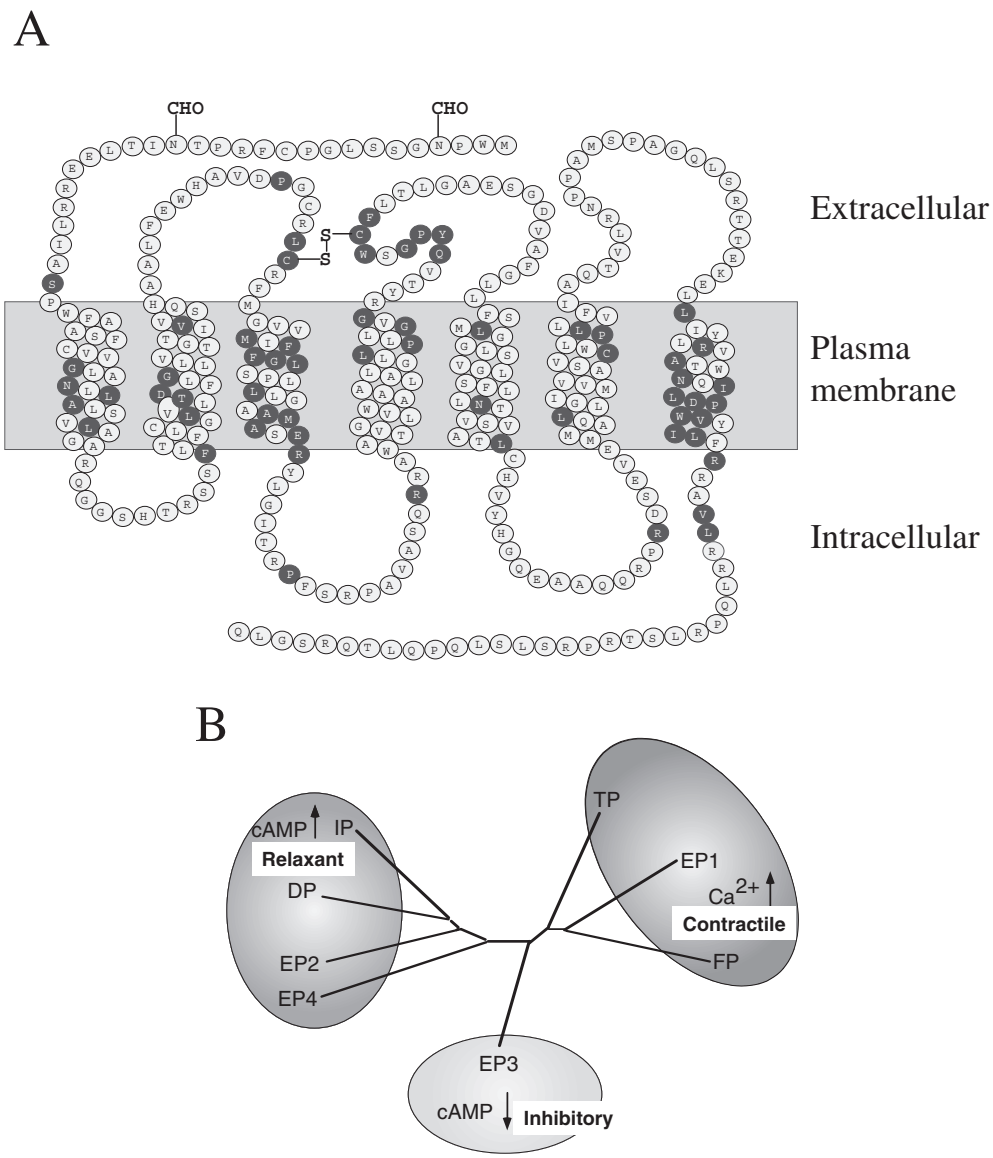

Fig. 2. (**A**) Membrane topology model for the human TXA_2 receptor (modified from ref. 118 with permission). Each amino acid is represented by the single-letter code within a circle. White letters in black circles indicate residues that are conserved among members of the prostanoid receptor family. The model is based on hydrophilicity analysis of the amino acid sequence. Asparagine residues at positions 4 and 16 are glycosylated. (**B**) Phylogenetic tree and functional grouping of prostanoid receptors (modified from ref. 118 with permission).

receptors are able to couple to more than one G protein and signal transduction pathway.

The wide range of prostanoid actions had suggested that prostanoid receptors might be ubiquitously distributed throughout the body. The cloning of the receptor cDNAs made it possible to examine the tissue distribution and cellular localization of each member of the prostanoid receptor family. Northern blot analysis revealed that each receptor gene shows a specific pattern of expression in the mouse body, with expression levels differing among tissues (Fig. 3A).[18)–25)] In situ hybridization further revealed that, even within the same organ, transcripts of different receptor genes were distributed differentially. In the kidney, for example, the EP3 gene is expressed in the tubular epithelium of thick ascending limb of the Henle loop, and collecting ducts in the outer medulla, the EP1 gene

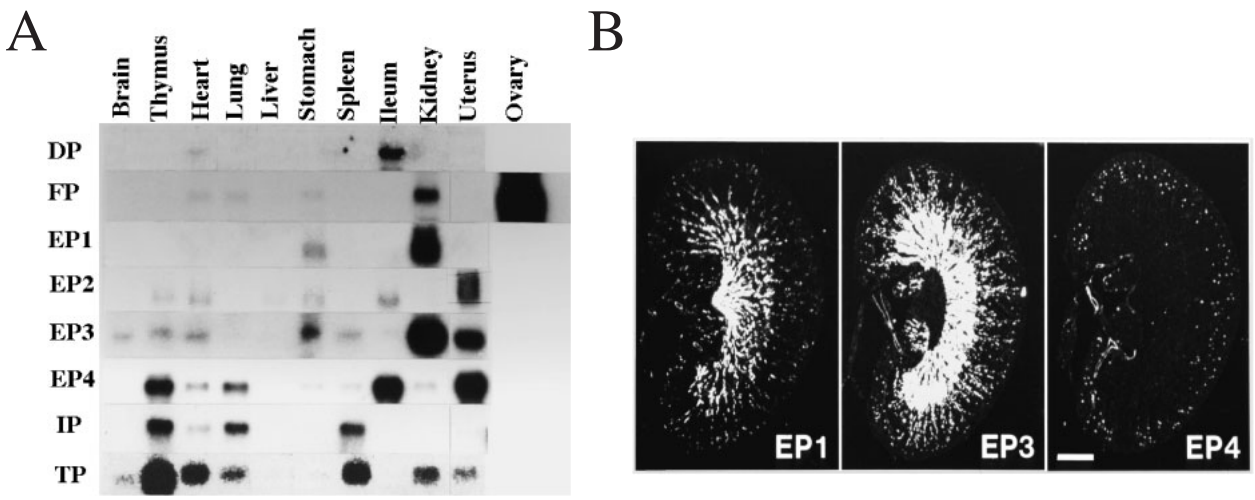

Fig. 3. (**A**) Northern blot analysis of transcripts derived from the eight types or subtypes of prostanoid receptor in mouse tissues (from ref. 119 with permission). (**B**) In situ hybridization of mRNAs for the EP1, EP3, and EP4 subtypes of the PGE receptor in mouse kidney (from ref. 26 with permission). Scale bar, 1 mm.

in the papillary collecting ducts, the EP4 gene in the glomerulus[26] (Fig. 3B), the IP gene predominantly in blood vessels such as the interlobular arteries and afferent glomerular arterioles,[27] and the FP gene in the distal convoluted tubule and cortical collecting ducts.[28] Some of the results of these analyses are consistent with known functions of prostanoids in corresponding tissues. For example, the distributions of EP3, EP1, and EP4 in the kidney correlate with the PGE_2-mediated regulation of ion transport, water reabsorption, and glomerular filtration, respectively, and that of IP in the glomerular arterioles may be related to prostanoid-induced renin secretion associated with reduced renal perfusion. However, other results, such as the observed enrichment of TP and IP in the thymus, were unexpected and are indicative of functions of prostanoids yet to be clarified.

Development of prostanoid receptor type- or subtype-selective agonists and antagonists

The cloning of eight members of the prostanoid receptor family has facilitated both the reassessment of previously developed receptor-active compounds and the development of new drugs. Examination of the binding properties of a series of PGs and available PG analogs with a panel of CHO cells stably expressing each cloned receptor revealed that most of these compounds exhibited no absolute selectivity.[29] For example, two PGI_2 analogs, iloprost and carbacyclin, indeed bound to IP with relatively high affinity, but they also bound to EP3 (both drugs) or EP1 (iloprost) with similar or higher affinities (Table 1). 17-Phenyl-PGE_2, which has been used as an EP1 agonist in many analyses, was found to bind to EP3 with a higher affinity and to FP with a similar affinity relative to that with which it binds EP1. Such cross-reactivity of conventional PG analogs likely reflects the fact that, during their development, they were screened with bioassays based on native tissues and cells that express more than one type of prostanoid receptor. A new generation of compounds has since been developed that was screened with a panel of cloned prostanoid receptors (Table 1).[30)–32] These agents are highly selective for each receptor type or subtype. Indeed, they have been used in combination with knockout mice deficient in each receptor type or subtype to elucidate the physiological roles of the corresponding receptor, and they have been shown to exert pharmacological actions consistent with the phenotypes of these knockout mice.

Generation and analysis of knockout mice deficient in each type or subtype of prostanoid receptor

The roles of prostanoids in various physiological and pathophysiological processes were initially suggested by comparison of the effects of aspirin-

Table 1. Binding specificity of prostanoid analogs developed before and after cDNA cloning of the prostanoid receptor family. Dissociation constants (nM) for binding of each compound to the eight types or subtypes of mouse prostanoid receptors are shown. The lower values for each compound are shown in bold letters (from ref. 117 with permission).

Compound	DP	EP1	EP2	EP3	EP4	FP	IP	TP
Compounds developed before receptor cloning								
Iloprost	>10,000	**21**	1,600	**22**	2,300	>10,000	**10**	>10,000
Carbacyclin	>10,000	>10,000	16,000	**31**	2,300	1,200	**110**	>10,000
17-Phenyl -PGE_2	>10,000	**14**	>10,000	**4**	1,000	**60**	>10,000	>10,000
Compounds developed after receptor cloning								
DP agonist								
L-644,698	**0.9**	>25,400	267	3,730	9,280	>25,400	>25,400	>25,400
EP1 antagonist								
ONO-8713	>10,000	**0.3**	3,000	1,000	>10,000	1,400	10,000	10,000
EP2 agonist								
ONO-AE1-259	>10,000	>10,000	**3**	>10,000	>10,000	>10,000	>10,000	>10,000
EP3 agonist								
ONO-AE-248	>10,000	>10,000	3,700	**8**	4,200	>10,000	>10,000	>10,000
EP4 agonist								
ONO-AE1-329	>10,000	>10,000	2,100	1,200	**10**	>10,000	>10,000	>10,000

like drugs with the actions of each prostanoid added exogenously. However, such studies did not clearly indicate which type of prostanoid and which type of prostanoid receptor mediate a given action or how important such prostanoid actions are. These issues were addressed by the generation of mice deficient in each of the prostanoid receptors as a result of disruption of the corresponding gene by homologous recombination. The phenotypes of these knockout mice are summarized in Table 2, and the physiological significance and clinical implications of some of these findings are addressed in the remainder of this review.

Prostanoid receptors and brain function. Systemic disease is associated with general and characteristic central nervous system symptoms and signs including fever, release of adrenocorticotropic hormone (ACTH), reduced locomotion and social contact, anorexia, and increased sleep.[33] These phenomena, collectively referred to as "sickness behavior," can be reproduced experimentally in animals by administration of noxious substances such as lipopolysaccharide (LPS) and inflammatory cytokines. Given that treatment with NSAIDs alleviates most of these behaviors as typically seen in fever generation, it had been thought, but not definitively proven, that prostanoids contribute to their generation. Studies of knockout mice have provided direct evidence for the participation of prostanoids in these phenomena and have increased our understanding of their molecular and neuronal mechanisms.

Fever. Fever is a prominent component of sickness behavior. Cellular components of infectious organisms, such as bacterial LPS, as well as cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α serve as exogenous and endogenous pyrogens, respectively, and stimulate the neural pathways responsible for increasing body temperature.[34] Fever is suppressed by NSAIDs, indicating that PGs are important mediators of fever generation. PGE_2 has been implicated as such a mediator on the basis of the observation that injection of PGE_2 into the brain induces fever in many species. Examination of mice lacking each subtype of PGE receptor (EP1, EP2, EP3, or EP4) revealed that the EP3 knockout animals failed to show a febrile response to PGE_2, IL-1β, or LPS (Fig. 4A).[35] This finding thus demonstrated that PGE_2 mediates fever generation in response to both exogenous and endogenous pyrogens by acting at EP3, a conclusion that was supported further by a study based on telemetry.[36] Fever induction occurs in the preoptic area of the organosum vasculatum lamina terminalis, a region of the brain with a relatively ineffective blood-brain barrier, and it is now known that LPS and IL-1 each induce expression of both COX-2 and mPGES-1 in brain micro-

Table 2. Phenotypes of mice deficient in prostanoid receptors.

Receptor	Phenotypes
DP	Decreased allergic responses in OVA-induced bronchial asthma[77]
	Impaired PGD_2-induced sleep[46]
	Facilitated mobilization of dendritic cells in skin immune responses[88),89]
CRTH2	Suppression of allergic inflammation in IgE-induced dermatitis[81]
	Augmentation of airway inflammation in OVA-induced asthma[82]
EP1	Decreased aberrant foci formation to azoxymethane[98]
	Deceased PGE_2-induced mechanical allodynia[61]
	Impaired ACTH response to systemic endotoxin administration[42]
	Impulsive behavior in response to environmental or social stress[43]
EP2	Impaired ovulation and fertilization[62)–64]
	Salt-sensitive hypertension[63]
	Vasopressor or impaired vasodepressor response to PGE_2[106),107]
	Loss of bronchodilation in response to PGE_2[108]
	Impaired osteoclastogenesis *in vitro*[104]
	Impaired amplification of COX and angiogenesis of intestinal polyps in $Apc^{\Delta 716}$ mice[97]
EP3	Impaired febrile response to pyrogens[35]
	Impaired duodenal bicarbonate secretion and mucosal integrity[109]
	Enhanced vasodepressor response to PGE_2[106]
	Loss of indomethacin-sensitive urine-diluting function[110]
	Decreased acetic acid–induced writhing after endotoxin treatment[54]
	Impaired PGE_2-induced potentiation of platelet activation[73),74]
	Impaired angiogenesis to transplanted cancer and chronic inflammation[111]
	Enhanced allergic response in OVA-induced bronchial asthma[85]
EP4	Patent ductus arteriosus[66),67]
	Impaired vasodepressor response to PGE_2[106]
	Decreased inflammatory bone resorption[102),103]
	Lack of PGE_2-induced bone formation *in vivo*[105]
	Exaggerated DSS-induced colitis[94]
	Impaired Langerhans cell migration in skin immune responses[87]
	Decreased aberrant foci formation in response to azoxymethane[99]
FP	Loss of parturition[65]
	Impaired generation of tachycardia in response to inflammatory stimuli[71]
IP	Thrombotic tendency[53]
	Decreased inflammatory swelling[53]
	Decreased acetic acid–induced writhing[53]
	Enhanced cardiac ischemia-reperfusion injury[112]
	Impaired adaptive gastric cytoprotection[113]
	Impaired capsaicin-induced gastric cytoprotection[114]
	Enhanced pulmonary hypertension and vascular remodeling under chronic hypoxic conditions[115]
	Enhanced atherosclerosis[75]
TP	Bleeding tendency and resistance to thromboembolism[68]
	Enhanced immune response due to facilitation of dendritic cell–T cell interaction[69]
	Suppression of initiation and progression of atherosclerosis[75]
	Impaired generation of tachycardia in response to inflammatory stimuli[71]
	Reduced endotoxin-induced microcirculatory dysfunction in the liver[116]

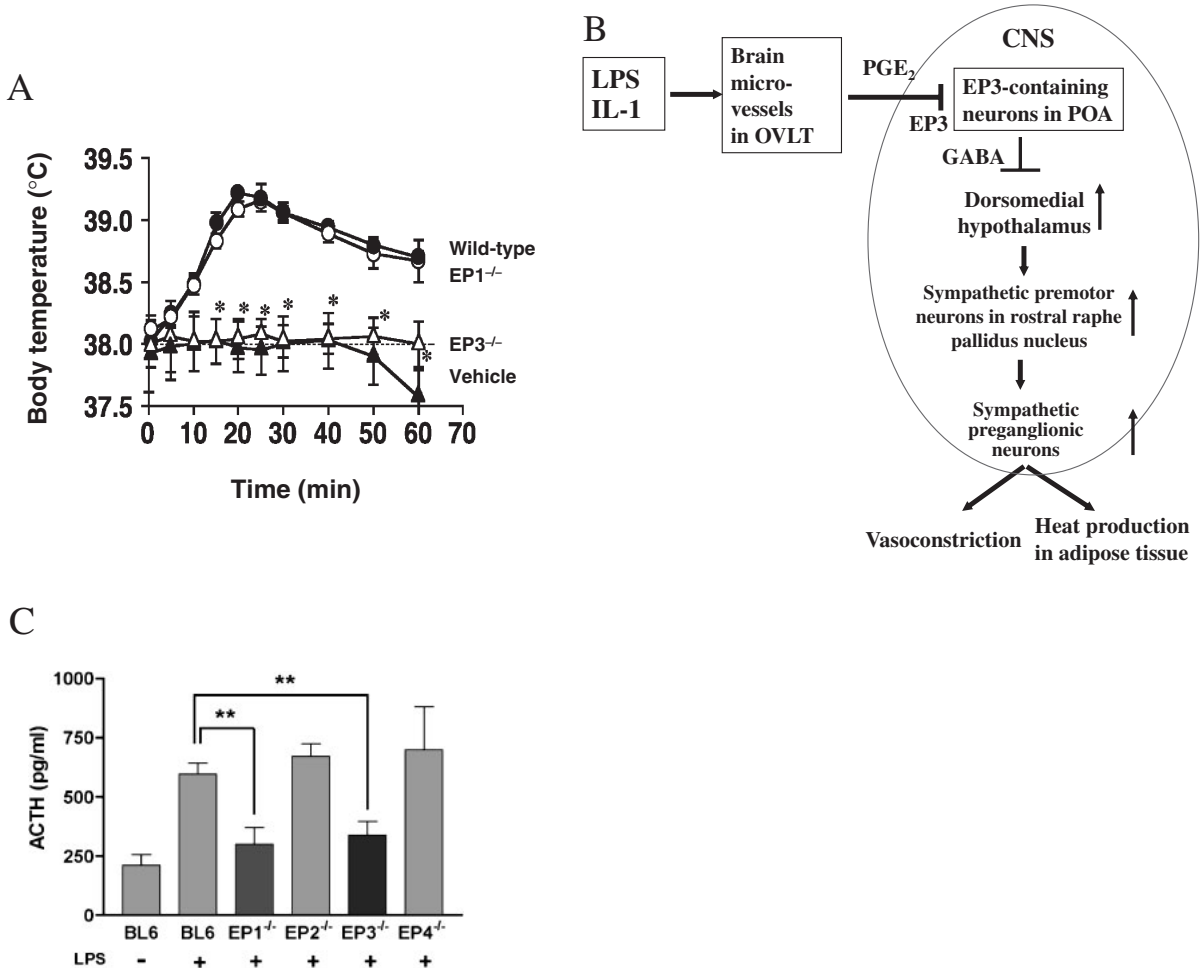

Fig. 4. (**A**) Impaired febrile response to LPS in mice deficient in EP3 (from ref. 35 with permission). Wild-type (closed circles), $EP1^{-/-}$ (open circles), or $EP3^{-/-}$ (open triangles) mice were injected with LPS at time zero; control wild-type mice were injected with vehicle (closed triangles). (**B**) Current model for the neural pathway that underlies fever generation. OVLT, organosum vasculatum lamina terminalis; POA, preoptic area. (**C**) Impaired ACTH response to LPS in mice deficient in EP1 or EP3 (from ref. 42 with permission). Wild-type (BL6) or the indicated EP knockout mice were injected with LPS or vehicle (−) and the plasma concentration of ACTH was measured 1 h thereafter. $^{**}P < 0.01$.

vessels and that both of these enzymes are essential for fever generation.[37)–39)] Identification of the role of EP3 was not only consistent with this afferent pathway but also helped to identify the efferent pathway in fever generation. Examination of EP3-expressing neurons in the preoptic area revealed that they contain the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and project to neurons in the dorsomedial hypothalamus that, in turn, send their projections to the medullary raphe region, where sympathetic premotor neurons responsible for febrile and thermoregulatory responses are localized.[40),41)] The pathway of pyrogen-induced fever generation deduced from these findings is shown in Fig. 4B.

ACTH release. Another prominent aspect of sickness behavior is activation of the hypothalamic-pituitary-adrenal axis. In this response, a population of neurons in the paraventricular nucleus of the hypothalamus is activated to secrete corticotropin-releasing factor (CRF), which then triggers the release of ACTH from the pituitary into the bloodstream and the consequent stimulation of corticosteroid secretion from the adrenal gland. A role for prostanoids in this response had been suggested but the evidence was inconclusive before the issue was

examined in EP knockout mice. Injection of wild-type mice with LPS revealed that the increase in the plasma concentration of ACTH consisted of two components, an NSAID-resistant transient response that peaked ~2 h after LPS administration and an NSAID-sensitive response that was sustained for 3 h.[42] Examination of this latter response in mice deficient in each EP subtype revealed it to be impaired in mice lacking either EP1 or EP3 (Fig. 4C). Further analysis revealed that the EP1-mediated pathway and the EP3-mediated pathway converge at activation of the CRF-containing neurons in the hypothalamus. Given that activation of EP1 induces a rise in intracellular Ca^{2+} concentration, the detection of EP1 immunoreactivity in the presynaptic terminals of neurons that form synapses on the somas and axons of CRF-containing neurons indicates that EP1 contributes directly to synaptic activation of the CRF neurons.

Stress behavior. Sickness behavior can be viewed as a stress response to stimuli associated with sickness. Phenomena such as fever generation and ACTH release that are components of sickness behavior thus also occur in other stress responses including those to psychological stimuli. However, prostanoids do not contribute to either fever generation or ACTH release in response to psychological stressors such as hyperthermia to restraint stress.[35] On the other hand, we recently showed that EP1 participates in the control of impulsive behavior of mice in response to environmental or social stress.[43] For example, EP1-deficient mice exhibited impaired cliff avoidance and jumped from the raised platform in this test. They also showed impulsive aggression to younger conspecifics in the resident-intruder test as well as a markedly shorter lag time to and more episodes of fighting induced by electric stimuli compared with wild-type animals. Conversely, injection of wild-type mice with an EP1-selective agonist attenuated their fighting episodes in the latter test. EP1-deficient mice exhibited increased dopamine turnover in several brain regions compared with wild-type mice, and their impulsive behavior was suppressed by injection with an antagonist of the D1 subtype of dopamine receptor, indicating that PGE_2-EP1 signaling modulates the activity of dopaminergic neurons to control behavior. Given that administration of NSAIDs to wild-type mice did not induce the behavioral phenotype observed in EP1-deficient mice, another PG-mediated pathway may oppose EP1 action in the brain. The balance between such opposing PG actions may set a point for impulsivity control during exposure to various types of stressful stimuli.

Sleep. PGD_2 is abundantly produced in the brain and is a potent endogenous promoter of sleep in rats and other mammals including humans.[44] The lipocalin-type PGD synthase is present in high amounts in the leptomeninges of the brain and is thought to be responsible for the production of PGD_2 in this organ. Consistently, when stimulation by tail clipping of transgenic mice that overexpress this type of PGD synthase caused increase in brain PGD_2 content and concomitantly induced a marked increase in slow-wave sleep.[45] This PGD_2-induced sleep is apparently mediated by the DP receptor also present in the leptomeninges. Infusion of PGD_2 into the lateral ventricle was thus found to increase slow-wave sleep in wild-type mice but not in DP-deficient mice,[46] showing that DP is required for PGD_2-induced slow-wave sleep.

PGD_2-induced sleep has been shown to be mediated by adenosine acting at the A_{2A} subtype of adenosine receptor.[47] Consistent with this finding, the extracellular adenosine content of the subarachnoid space was found to be increased in a DP-dependent manner after PGD_2 infusion.[46] Although baseline sleep-wake patterns were essentially identical between wild-type animals and either DP-deficient mice or mice deficient in the lipocalin-type PGD synthase, intracerebroventricular injection of selenium tetrachloride, an inhibitor of this PGD synthase, suppressed both non-rapid eye movement sleep and rapid eye movement sleep in wild-type mice but not in mice deficient in either DP or the PGD synthase,[48] indicating that selenium tetrachloride acts on the PGD synthase to suppress sleep. Furthermore, administration of a specific DP antagonist, ONO-4127Na, also markedly reduced the sleep period in rats.[48] These findings indicate that the PGD synthase–PGD_2-DP pathway regulates physiological sleep, and that the deficiency of a component of this pathway may be compensated for by other sleep-regulating systems during development of the knockout animals. On the other hand, this conclusion does not exclude the possibility that the PGD_2-DP system is also involved in generation of pathological sleep. Injection of IL-1β or TNF-α into the PGD_2-sensitive region of

the rat brain induces sleep in a COX-2–dependent manner.[49] Overproduction of PGD_2 has also been observed in certain sleep disorders, including systemic mastocytosis and African sleeping sickness, in humans.[44] Furthermore, the human malarial parasite *Plasmodium falciparum* produces PGs, including PGD_2, that may contribute to sleepiness and other symptoms of this disease.[50]

Prostanoid receptors and pain sensation. The role of PGs in inflammatory pain has been suggested by the antinociceptive effects of aspirin-like drugs. Consistently, studies in various model systems have shown that exogenous PGs induce hyperalgesia (an increased sensitivity to a painful stimulus) or allodynia (a pain response to a usually nonpainful stimulus). These studies also showed that PGE_2, PGE_1, and PGI_2 exert stronger effects than the other PGs, implicating EP and IP in the induction of inflammatory pain.[51]

Peripheral hyperalgesia. Although a spinal action is also suggested (see below), the main site of the hyperalgesic action of prostanoids lies in the periphery, where PGs are thought to sensitize the free ends of sensory neurons. The cell bodies of primary sensory afferents are located in dorsal root ganglia (DRG), and several prostanoid receptor mRNAs, including those for IP, EP1, EP3, and EP4, have been detected in DRG neurons.[27),52] Subjection of mice deficient in these receptors to various pain models revealed that they all contribute to mediation of pain sensation in a context-dependent manner. For example, we found that the pain response of IP-deficient mice in the acetic acid writhing test was reduced to a level similar to that observed in wild-type mice treated with the COX inhibitor indomethacin,[53] indicating that the hyperalgesic response in this model is evoked by endogenous PGI_2 acting at IP. On the other hand, treatment of $EP1^{-/-}$, $EP2^{-/-}$, $EP3^{-/-}$, $EP4^{-/-}$, $IP^{-/-}$, or wild-type mice with LPS before examination of acetic acid–induced writhing subsequently revealed that not only $IP^{-/-}$ mice but also $EP3^{-/-}$ mice showed a reduced response.[54] These findings thus indicated that nociception in the writhing response is mediated predominantly by IP in untreated mice, whereas it is mediated by both IP and EP3 in LPS-treated mice. The observation that intraperitoneal injection of either PGE_2 or PGI_2 induced moderate writhing responses in wild-type mice further suggested that, even in the same model, a hyperalgesic response can be induced by either of these PGs and that whether endogenous PGE_2 or PGI_2 (or both) mediates this response depends on which of these PGs is produced in a given context.

The TRPV1 cation channel is the receptor for capsaicin and detects heat and pH in pain sensation. Moriyama *et al.* examined how prostanoid signaling might be integrated with the action of this channel in pain sensation and found that both PGE_2 and PGI_2 augment thermal hyperalgesia mediated by TRPV1 and that such augmentation is attenuated by deficiency of EP1 and IP, respectively.[55] Further examination of the effects of prostanoid signaling on TRPV1-mediated currents in isolated DRG neurons as well as in cultured cell lines expressing the recombinant prostanoid receptors revealed that EP1 or IP signaling lowers the threshold for activation of the TRPV1 channel by temperature or pH in a manner dependent on protein kinase C, and that the channel can also be activated in a protein kinase A-dependent manner by IP or EP4 signaling. The involvement of EP4 seems consistent with the finding that a selective EP4 antagonist attenuated the thermal hyperalgesia apparent with peripheral inflammation induced by injection of Freund's complete adjuvant.[56] Signaling by prostanoids and other inflammatory molecules thus appears to be integrated at the level of DRG neurons. However, whereas the TRPV1 channel is a pain receptor for heat and pH, it does not mediate the pain response to mechanical stimuli[57]; given that prostanoids also augment mechanical pain sensation, a separate integration mechanism for PG-mediated hyperalgesia likely exists.

Central hyperalgesia. In addition to their hyperalgesic actions in the periphery, PGs augment the processing of pain information in the spinal cord. Administration of PGE_2 into the dorsal horn of the rat spinal cord was found to reduce in an EP2-dependent manner the extent of neurotransmission mediated by the inhibitory transmitter glycine during pain sensation.[58] This finding suggested that PGE_2 might facilitate by this mechanism the transmission of nociceptive input through the dorsal horn of the spinal cord to higher brain areas where pain becomes conscious. Consistent with this notion, EP2-deficient mice exhibited only short-lasting hyperalgesia after a peripheral inflammatory stimulus, failing to manifest a second

sustained hyperalgesic phase of spinal origin that is apparent in wild-type animals.[59] Together, the available evidence thus indicates that prostanoids acting at various receptor types or subtypes regulate pain sensation at multiple levels and in a redundant manner.

Allodynia. Intrathecal injection of PGE_2 induces allodynia in mice, as manifested by squeaking, biting, and scratching in response to low-threshold stimuli.[60] This response was also apparent in EP3-deficient mice but was not observed in EP1-deficient mice,[61] suggesting that EP1 mediates PGE_2-induced allodynia.

Prostanoid receptors and reproduction. Various reproductive effects of NSAIDs have long indicated that prostanoids function at multiple steps in pregnancy and parturition. Studies of knockout mice deficient in prostanoid receptors confirmed such functions and provided greater insight into their underlying mechanisms.

Three groups demonstrated failure of pregnancy at an early stage in EP2-deficient mice.[62–64] They found that EP2-deficient females consistently deliver fewer pups than do their wild-type counterparts irrespective of the genotypes of the mating males. Ovulation was slightly impaired and the rate of fertilization was greatly reduced in the EP2-deficient female mice. Hizaki *et al.*[62] further showed that this phenotype is due to impaired expansion of the cumulus oophorus. Given that EP2 and COX-2 are induced in the cumulus in response to gonadotropins, and that PGE_2 induces cumulus expansion by increasing the intracellular concentration of cAMP, these researchers proposed that PGE_2 and EP2 form a positive feedback loop to induce the oophorus maturation required for fertilization during and after ovulation (Fig. 5A). Indeed, the proportion of unovulated eggs in the ovary was found to higher for EP2-deficient mice than for wild-type controls.

$PGF_{2\alpha}$ is recognized as an inducer of luteolysis in domesticated animals such as sheep and cows, and it has been implicated in parturition through its action as a potent uterotonic agent. However, FP-deficient mice were found not to show any abnormalities in early pregnancy or in the estrous cycle.[65] This apparent discrepancy might be explained by the facts that luteolysis is not required for entrance into a new estrous cycle in mice, and that mouse ovaries contain corpora lutea from several previous estrous cycles. Sugimoto *et al.*[65] found that, despite the lack of apparent defects in the estrous cycle, FP-deficient mothers do not undergo parturition, apparently because of the absence of labor. They further showed that FP-deficient mice do not undergo parturition even when administered exogenous oxytocin and that such dams show no prepartum decline in progesterone levels. A reduction in progesterone levels induced by ovariectomy 24 h before term resulted in up-regulation of uterine receptors for oxytocin and normal parturition in FP-deficient mice. These observations indicate that the luteolytic action of $PGF_{2\alpha}$ is required in mice to reduce progesterone levels and thereby to allow the initiation of labor (Fig. 5B).

Prostanoid receptors and closure of the ductus arteriosus. At birth, with the commencement of respiration, humans and other mammals undergo a marked change in their circulation. The fetal pattern of circulation, in which blood is shunted from the main pulmonary artery directly to the aorta via the ductus arteriosus, is thus transformed into the pulmonary circulation system of the neonate. This adaptive change is achieved by closure of the ductus arteriosus. Maternal administration of NSAIDs induces contraction of the ductus in late-term fetuses, and administration of a vasodilatory PG such as PGE_1 maintains the patency of the ductus in neonates. The patency of the ductus during the fetal period has thus been thought to be maintained predominantly by the dilatory effect of a PG, with its closure being induced by withdrawal of the dilatory PG as well as resulting from active contraction elicited by an increased oxygen tension. Dilatory prostanoid receptors such as IP and EP4 are present in the ductus, suggesting that they function to maintain its patency. Disruption of the mouse IP gene, however, did not appear to result in any abnormality of the ductus.[53] On the other hand, most EP4-deficient mice die within 3 days after birth as a result of marked pulmonary congestion and heart failure.[66,67] Administration of indomethacin to maternal mice during late pregnancy did not induce closure of the ductus in EP4-deficient fetuses, indicating that the dilatory effect of PGE_2 on this vessel is mediated by EP4. These results thus indicate that EP4 plays an important role in the ductus arteriosus, and they suggest that, in the

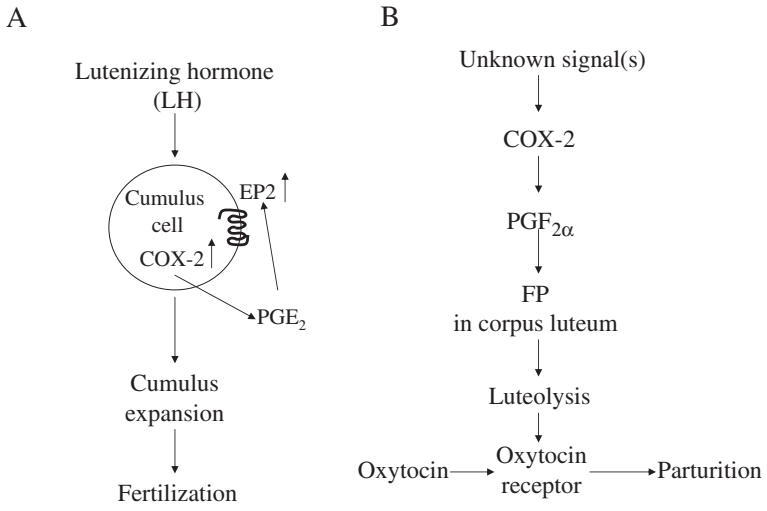

Fig. 5. Roles and mechanisms of action of the PGE_2-EP2 pathway in expansion of the cumulus oophorus and fertilization (**A**) and of the $PGF_{2\alpha}$-FP pathway in induction of parturition (**B**).

absence of EP4, a compensatory mechanism maintains ductus patency not only in the fetal period but also after birth.

Prostanoid receptors and cardiovascular homeostasis.

Blood pressure. Most PGs exert contractile or relaxant effects (or both) on vascular smooth muscle *in vitro* and *in vivo*. PGI_2 and TXA_2, which are produced in large amounts by vascular endothelial cells and platelets, respectively, exhibit potent vasodilatory and vasoconstricting actions, respectively. PGE_2 also induces either contraction or relaxation of blood vessels dependent on the EP subtype expressed. Although mice deficient in IP or TP do not show abnormalities of blood pressure under basal conditions,[53),68),69)] the vasodilatory and vasocontractile actions of PGE_2 become evident in mice deficient in EP subtypes. Kennedy *et al.*[63)] administered PGE_2 or PGE analogs intravenously to $EP2^{-/-}$ mice and found that PGE_2 or a mixed EP1/3 agonist, sulprostone, evoked pronounced hypertension, whereas an EP2 agonist, butaprost, failed to induce the transient hypotension seen in wild-type mice. The researchers suggested that the absence of EP2 abolishes the ability of the mouse vasculature to undergo vasodilation in response to PGE_2 and unmasks the contractile response mediated by the vasoconstrictor EP subtype (or subtypes). When fed a high-salt diet, the EP2-deficient mice developed marked hypertension with a concomitant increase in urinary excretion of PGE_2. These observations indicated that PGE_2 is produced in the body in response to a high-salt diet and functions to reduce blood pressure via the relaxant EP2 receptor, suggesting that dysfunction of this pathway may contribute to the development of salt-sensitive hypertension.

Hypertension is also induced by a reduction in renal perfusion, which is associated with an increase in the plasma concentration of renin and mobilization of the renin-angiotensin-aldosterone system. Fujino *et al.*[70)] examined the contribution of prostanoids to the development of this condition by subjecting mice deficient in either IP or each of the four EP subtypes to a two-kidney, one-clip model of renovascular hypertension. They found that hypertension in this model was ameliorated in the IP-deficient mice but not in any of the EP-deficient animals. Consistent with these observations, the plasma renin activity, the abundance of renin mRNA in the kidney, and the plasma concentration of aldosterone were all substantially reduced in the IP knockout animals compared with those in the wild type. Given that IP is expressed in the afferent glomerular arterioles, that expression of the renin gene is expanded to these arterioles in response to reduced perfusion of the kidney, and that PGI_2 induces renin release from cultured juxtaglomerular cells *in vitro*, these researchers suggested that PGI_2-IP signaling directly stimulates renin release. Al-

ternatively, such signaling may regulate the perfusion pressure of the juxtaglomerular apparatus locally and induce renin release indirectly.

Heart rate. Prostanoids have been thought not to contribute to the regulation of heart rate, given that ingestion of NSAIDs does not affect the heart rate of animals under basal conditions. However, animals subjected to systemic inflammation such as septic shock exhibit an increased heart rate, known as inflammatory tachycardia, that is often sensitive to NSAID treatment. Given that systemic inflammation is associated with many symptoms and signs of sickness behavior, including fever and ACTH release, in addition to tachycardia, and that fever is generated at least in part by elevated sympathetic discharge, inflammatory tachycardia has been thought to be induced by enhanced sympathetic activity at the sinoatrial node. However, by subjecting prostanoid receptor-deficient mice to a model of inflammatory tachycardia, Takayama *et al.*[71] found that EP3-deficient animals, which do not develop fever, manifested a level of inflammatory tachycardia similar to that observed in wild-type mice, thus ruling out a role for fever generation in this process. They further showed that prostanoids directly increased heart rate in a TP- and FP-dependent manner *in vitro*, and that the inflammatory tachycardia induced by either cytokines or LPS was suppressed in mice deficient in TP or FP and was almost completely abolished in mice deficient in both TP and FP. Moreover, injection of the β-blocker propranolol failed to prevent the LPS-induced increase in heart rate under the same conditions in wild-type mice. These results thus suggest that prostanoids are produced locally in the heart during systemic inflammation and are able to induce tachycardia directly.

Bleeding, hemostasis, and thrombosis. Given that PGI_2 and TXA_2 have opposing effects on platelets and blood vessels, a balance between these two prostanoids has been thought to be important for prevention of thrombosis and vasospasm and for hemostasis. Indeed, a study of $IP^{-/-}$ mice[53] showed that, whereas these animals develop and age normally, they manifest an increased thrombotic tendency in the presence of endothelial damage. These findings confirmed the long-held view of PGI_2 as an endogenous antithrombotic agent and suggested that this antithrombotic system is activated in response to vascular injury in order to attenuate the effects of such injury. In contrast to PGI_2, TXA_2 has been implicated in thrombosis and hemostasis on the basis of its ability to induce platelet aggregation and vasoconstriction. Indeed, TP-deficient mice showed increased bleeding tendencies and were resistant to cardiovascular shock induced by intravenous infusion of arachidonic acid or the TP agonist U-46619.[68),69)] Cheng *et al.*[72)] examined the interplay between IP and TP signaling in cardiovascular homeostasis. They subjected $IP^{-/-}$ mice and $TP^{-/-}$ mice to vascular injury by a balloon catheter and found that IP deficiency increased, whereas TP deficiency decreased, injury-induced vascular proliferation and platelet activation. They further showed that the augmented response apparent in $IP^{-/-}$ mice was abolished by ablation of TP. The researchers thus concluded that PGI_2, through its action at IP, modulates platelet-vasculature interactions *in vivo* and specifically limits the response to TXA_2.

In addition to PGI_2 and TXA_2, PGE_2 acts on blood platelets and promotes their aggregation. The identity of the receptor mediating this action of PGE_2 and its pathophysiological significance had remained unknown, however. By studying mice deficient in each EP subtype, Fabre *et al.*[73)] and Ma *et al.*[74)] identified EP3 as a receptor that mediates potentiation of platelet aggregation by PGE_2. $EP3^{-/-}$ mice thus exhibited a decreased response in an arachidonic acid–induced thrombosis model. In addition, Ma *et al.*[74)] found that bleeding time was markedly prolonged in EP3 knockout mice. These observations suggest that this action of PGE_2 may play a role in vascular homeostasis *in vivo*.

Atherosclerosis. Whereas PGI_2 and TXA_2, in addition to their roles in acute vascular accidents, have long been implicated in chronic vascular diseases such as atherosclerosis and diabetic vasculopathy, the contribution of these prostanoids was only recently assessed experimentally. We addressed this issue by mating atherosclerosis-prone ApoE-deficient mice with mice deficient in either IP or TP and then examining progression of atherosclerosis in the $ApoE^{-/-}IP^{-/-}$ or $ApoE^{-/-}TP^{-/-}$ double knockouts.[75)] We found that atherosclerosis was accelerated or delayed, respectively, in these mutant animals, despite the fact that they manifested similar plasma cholesterol and triglyceride concentrations. The lumen of the innominate artery was almost completely occluded

in 45-week-old ApoE$^{-/-}$IP$^{-/-}$ mice (Fig. 6). These observations suggest the requirement for preservation of vascular PGI_2-IP signaling in maintenance of vascular homeostasis, and they underline the importance of selective lifelong manipulation of vascular IP and TP signaling. This view has been clinically confirmed in patients treated with selective COX-2 inhibitors. Since COX-2 is rapidly induced by inflammatory cytokines and mitogens and accounts largely for PGs produced in inflammation and cancer, and COX-1 was speculated to function to produce protective PGs in gastric mucosa, selective COX-2 inhibitors have been developed as new types of NSAIDs with potent anti-inflammatory activity and minimal gastrointestinal toxicity. Indeed, these drugs have been widely used, for example, in arthritis patients, without gastrointestinal intolerance. However, a selective COX-2 inhibitor, rofecoxib, was recently withdrawn from the market because of an increased rate of cardiovascular accidents in patients taking this drug. This cardiovascular hazard has been suggested to be due to preferential inhibition by this type of drug of COX-2 that is induced by shear stress in the endothelium and mediates vascular PGI_2 production combined with preservation of platelet COX-1 that catalyzes TXA_2 production.[76)]

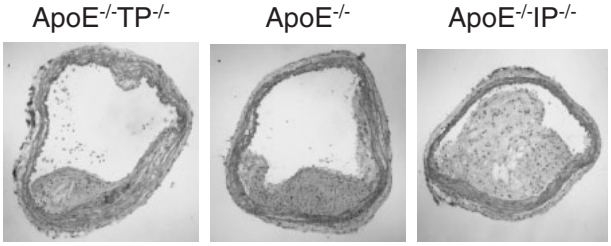

Fig. 6. Atherosclerosis in the innominate artery of ApoE$^{-/-}$, ApoE$^{-/-}$TP$^{-/-}$, and ApoE$^{-/-}$IP$^{-/-}$ mice (from ref. 75 with permission). Arterial cross-sections prepared from 45-week-old mice were stained with hematoxylin-eosin.

Prostanoid receptors, allergy, and immunity Given that NSAIDs are mostly without effects on allergy and immunity, it has been generally thought that prostanoids contribute little, if any, to these processes, although substantial amounts of prostanoids are produced during their course. Analysis of the receptor knockout mice has revealed, however, that prostanoids function at various steps in both allergy and immunity, and that different prostanoids often act antagonistically.

Allergy. The type I allergic reaction underlies the pathogenesis of bronchial asthma, atopic dermatitis, and anaphylactic shock. Affected individuals produce immunoglobulin (Ig) E antibodies to allergens such as those derived from house dust mites and plant pollen. Exposure to those allergens results in cross-linking of IgE receptors on the surface of mast cells, the consequent activation of these cells, and the development of an allergic reaction. The Th2 subset of T lymphocytes and their cytokines are important mediators of IgE production as well as the development of allergic disease. Various prostanoids are produced during the initial activation of mast cells and subsequent disease development. For example, PGD_2 is a major prostanoid produced by activated mast cells and is released in large amounts during asthmatic attacks in certain patients. The role of PGD_2 in allergic asthma long remained unclear, however. We examined this issue by applying the ovalbumin (OVA)-induced asthma model to DP-deficient mice.[77)] Sensitization and aerosol challenge of DP$^{-/-}$ mice with OVA induced increases in the serum concentration of IgE similar to those observed in wild-type mice. However, the DP-deficient animals developed substantially reduced asthmatic responses in this model; the concentrations of Th2 cytokines and the extents of lymphocyte accumulation and eosinophil infiltration in the lungs of the mutant animals after OVA challenge were greatly reduced compared with those apparent in the wild type. These observations thus indicated that PGD_2 is a mast cell–derived mediator that serves to mediate asthmatic responses. This conclusion was supported recently by single nucleotide polymorphism (SNP) analysis of the DP gene (*PTGDR*) in humans. This gene is located at chromosome 14q22.1, a region that has been associated with asthma and atopy. Oguma *et al.*[78)] examined SNPs of *PTGDR* in Caucasian and black individuals with asthma and control subjects. They identified three haplotypes that consisted of four SNPs in the promoter region of the gene and found that each haplotype manifested a different promoter activity. Furthermore, the promoter activity was shown to be significantly correlated with susceptibility to asthma.

PGD_2 may also function in allergy by acting at the receptor CRTH2. CRTH2 is expressed in cells important in allergy such as Th2 lymphocytes, eosinophils, and basophils. Given that stimulation

of CRTH2 induces chemotaxis of these cells *in vitro*, it has been suggested that CRTH2 facilitates allergic inflammation.[16] Indeed, administration of selective agonists for CRTH2 to the airway or painting of these substances on the skin of sensitized animals was found to augment infiltration of inflammatory cells into the lungs and skin, respectively.[79),80] The generation of CRTH2 knockout mice allowed further examination of the importance of the PGD_2-CRTH2 pathway in the natural course of allergy. Allergic inflammation associated with IgE-induced dermatitis was found to be suppressed in CRTH2-deficient mice,[81] whereas airway inflammation and eosinophil infiltration were augmented in the knockout mice subjected to the OVA-induced asthma model.[82] The latter study also showed that IL-5 production by activated T cells from CRTH2-deficient mice *in vitro* was increased compared with that observed with wild-type cells. These results indicated that CRTH2 indeed functions to facilitate allergy *in situ* at the site of inflammation, but that this receptor also regulates cytokine production in the early phase of allergy development, raising the question as to whether suppression of this pathway would result in an overall beneficial effect in patients.

These various observations suggest that PGD_2 signaling facilitates allergic responses not only in mice but also in humans. However, if PGD_2 is the only prostanoid that functions in allergic asthma, administration of NSAIDs would be expected to ameliorate asthmatic symptoms. Instead, NSAIDs are either without effect or induce severe attacks in certain asthmatic patients, a syndrome known as aspirin-induced asthma.[83] This discrepancy might be explained by the existence of a prostanoid other than PGD_2 that negatively modulates allergic reactions. The most likely candidate for such a prostanoid is PGE_2, given that PGE_2 has been known for some time to exert antiallergy effects in some contexts.[84] We subjected mice deficient in each EP subtype individually to the OVA-induced asthma model and examined their responses.[85] Among the four knockout mouse strains, only EP3-deficient mice exhibited substantially exaggerated airway inflammation compared with that observed in their wild-type counterparts while showing similar plasma concentrations of anti-OVA IgE. The EP3-deficient animals also manifested an enhanced passive cutaneous anaphylaxis reaction. These results thus implicated PGE_2-EP3 signaling in suppression of mast cell activation. Consistent with this conclusion, an EP3-selective agonist was found to inhibit the antigen-induced release of histamine and leukotrienes from sensitized lung tissue *in vitro* and to suppress airway inflammation in OVA-challenged mice *in vivo*. With regard to the latter observation, the EP3 agonist was most effective when administered subcutaneously 3 h after OVA challenge, indicating that the major site of EP3 action is at a step (or steps) downstream of mast cell activation. Further analysis revealed that administration of the EP3 agonist suppressed induction of the expression of various asthma-related genes, including those for chemokines and tissue remodeling factors, in the lung, and that EP3 is coexpressed with some of these molecules in the airway epithelium. On the basis of these findings, we suggested that PGE_2 produced during allergy acts at EP3 on both mast cells and airway epithelial cells, thereby blunting activation of mast cells and impeding progression of the allergic reaction by inducing down-regulation of the expression of relevant genes in the airway epithelium (Fig. 7). DP is also expressed in the airway epithelium, and, given its opposing mechanism of signal transduction relative to that of EP3, it may facilitate the asthmatic reaction by increasing expression of these latter genes.

Application of the OVA-induced asthma model to mice deficient in other prostanoid receptors revealed that airway inflammation was also augmented in IP-deficient mice. In contrast to EP3-deficient mice, however, $IP^{-/-}$ mice exhibited substantially higher plasma concentrations of antigen-specific and total IgE, indicating that PGI_2-IP signaling functions in sensitization to IgE production.[86]

Immunity. Whereas prostanoid receptors such as EP, IP, and TP are widely expressed in cells of the immune system and the immunomodulatory actions of PGE_2 *in vitro* were well established, little was known until recently of the *in vivo* roles of these receptors in immunity. Kabashima *et al.*[69),87] examined this issue with the contact hypersensitivity model, in which they painted dinitrofluorobenzene (DNFB) on the skin of the mouse abdomen on day 0 for immunization and applied the same hapten to the ear on day 5 for elicitation of immune inflammation, which was assayed by measurement

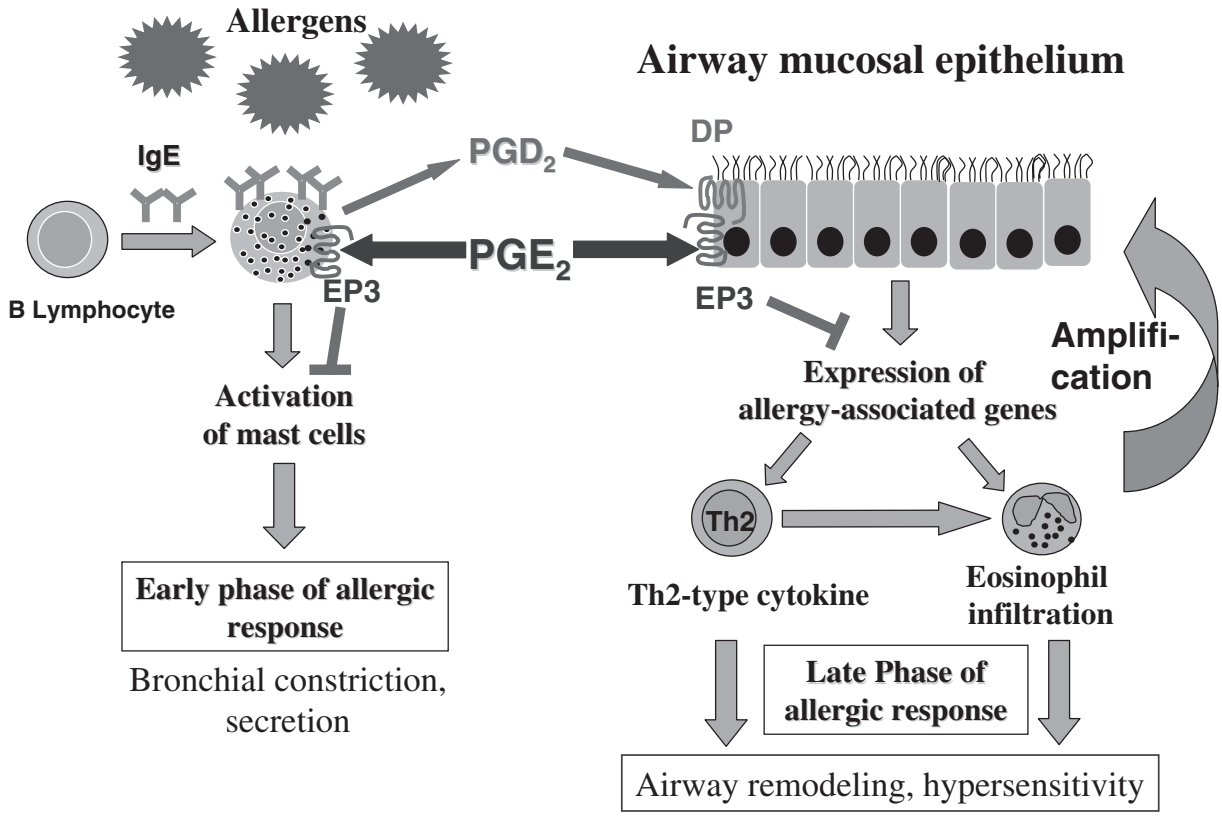

Fig. 7. A model for functional antagonism between the PGD_2-DP pathway and the PGE_2-EP3 pathway in development of allergic inflammation associated with asthma. PGD_2 is released in response to mast cell activation and facilitates allergic inflammation at least in part by acting at DP in the airway epithelium. On the other hand, PGE_2 is produced in the surrounding tissues during mast cell activation and suppresses development of allergic inflammation by inhibiting induction of various asthma-related genes in the epithelium.

of ear swelling the next day. They subjected mice deficient in TP or EP4 to this model and examined their phenotypes. Whereas the EP4-deficient mice exhibited impaired hypersensitivity,[87] the TP-deficient animals showed an exaggerated response.[69] Application of antagonists specific for TP or EP4 in a phase-specific manner revealed that both PGE_2-EP4 signaling and TXA_2-TP signaling function in the immunization phase of this model; that is, in the period between the initial hapten application and the generation of effector T cells specific for the applied hapten. Detailed analysis demonstrated that PGE_2-EP4 signaling facilitates mobilization, migration, and maturation of Langerhans cells after initial antigen application and that TXA_2-TP signaling modulates the interaction between naïve T cells and antigen-loaded dendritic cells in draining lymph nodes and thereby regulates the extent of T cell activation. PGE_2-EP4 and TXA_2-TP signaling thus contribute to different steps of immunization and functionally antagonize each other with regard to their effects on T cell activation. In addition, PGD_2-DP signaling likely directly antagonizes the effect of PGE_2-EP4 signaling in Langerhans cells. Trottein and collaborators studied *Schistosoma mansoni* infection in the skin and found that parasite-derived PGD_2 acts at DP in Langerhans cells to induce their retention in the epidermis.[88] This study was extended to show that activation of DP interferes with TNF-α–induced migration of Langerhans cells and migration of pulmonary dendritic cells in response to antigen challenge.[89] These prostanoid actions at different immunization steps are summarized in Fig. 8.

Prostanoid receptors and inflammation. Local reddening, heat generation, and swelling are classic signs of acute inflammation and are caused by increased blood flow and vascular permeability

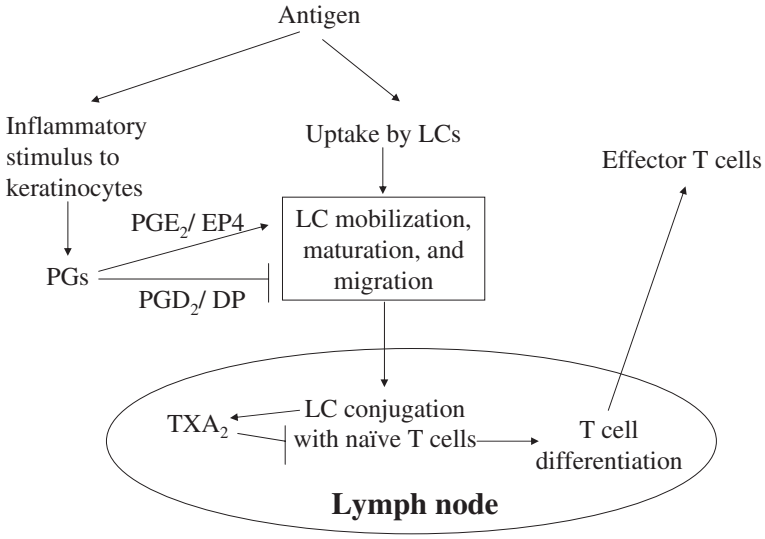

Fig. 8. Sites of prostanoid actions in skin immune responses. The PGE_2-EP4 pathway and the PGD_2-DP pathway regulate dendritic cell migration and maturation in mutually antagonistic manners, whereas the TXA_2-TP pathway regulates the interaction between dendritic cells and naïve T cells in draining lymph nodes. LC, Langerhans cell.

with resultant edema. The inhibitory effects of NSAIDs on these inflammatory signs and the vasodilatory action of exogenously administered prostanoids such as PGI_2 and PGE_2 implicated prostanoids as mediators of the vascular responses in acute inflammation. Indeed, a study of IP-deficient mice subjected to the carrageenin-induced paw swelling model showed that inflammatory swelling was reduced by ~50% in these animals, an effect similar in magnitude to that achieved by treatment of wild-type mice with NSAIDs, indicating that PGI_2 and IP constitute the principal PG system responsible for mediating vascular changes in this model of inflammation.[53] On the other hand, Yuhki *et al.*[90] showed that EP2 and EP3 as well as IP mediate exudate formation in carrageenin-induced mouse pleurisy, suggesting that PGE_2 and PGI_2 elicit inflammatory responses in a context-dependent manner, that is, one dependent on the type of stimulus and site in the body, and that their contribution may also change during the course of inflammation.

The above knockout mouse studies thus substantiated the role of prostanoids in acute inflammation. However, more novel and important findings obtained by studies using the knockout mice were that prostanoids exert both proinflammatory and anti-inflammatory actions and that these actions are often produced through regulation of gene expression in relevant tissues. Examples of these prostanoid actions include those observed in intestinal inflammation and collagen-induced arthritis. Human inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is a chronic, relapsing, and remitting condition of unknown origin that exhibits various features of immunological inflammation and affects at least one in 1000 people in Western countries.[91] IBD is characterized by inflammation in the large or small intestine associated with diarrhea, hemoccult, abdominal pain, weight loss, anemia, and leukocytosis. Studies in humans have implicated impaired mucosal barrier function, pronounced innate immunity, production of proinflammatory and immunoregulatory cytokines, and the activation of $CD4^+$ T cells in the pathogenesis of IBD. One of the major risk factors for triggering or worsening the disease is administration of NSAIDs,[92] as confirmed by experiments in which COX-deficient mice were subjected to dextran sodium sulfate (DSS)-induced intestinal inflammation, an animal model for IBD.[93] Whereas the evidence in humans and animals indicated that COX-derived prostanoids contribute to the defense against intestinal inflammation, the identity of the prostanoids and receptors involved in this process and their mode of actions remained obscure. We therefore examined the susceptibility of mice deficient in each of the eight types or subtypes

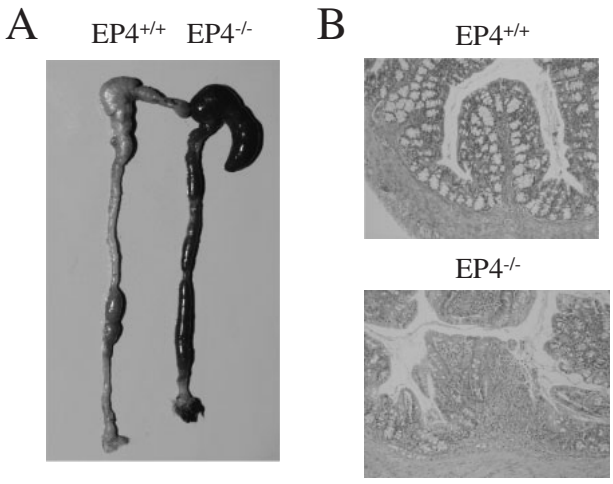

Fig. 9. Exacerbation of DSS-induced colitis in EP4-deficient mice (from ref. 94 with permission). $EP4^{-/-}$ mice exhibit severe hemorrhagic colitis in response to the administration of 3% DSS in drinking water, which induces only marginal colitis in wild-type mice. The gross appearance (**A**) and hematoxylin-eosin staining (**B**) of the intestine from wild-type ($EP4^{+/+}$) or $EP4^{-/-}$ mice are shown.

of prostanoid receptors to DSS treatment.[94] We found that only EP4-deficient mice developed severe colitis in response to treatment with 3% DSS (Fig. 9); wild-type animals require treatment with 7% DSS to develop this condition. We further confirmed this finding by reproducing the phenotype in wild-type mice by administration of an EP4-selective antagonist. EP4 deficiency was shown to result in impairment of mucosal barrier function that was associated with epithelial loss, crypt damage, and accumulation of neutrophils and $CD4^+$ T cells in the colon. DNA microarray analysis revealed increased expression of genes associated with immune responses and reduced expression of genes associated with mucosal repair and remodeling in the colon of EP4-deficient mice. On the basis of these findings, we concluded that EP4 maintains intestinal homeostasis by preserving mucosal integrity and down-regulating immune responses.

Rheumatoid arthritis is a disease of immunological inflammation that is widely treated with NSAIDs. Collagen-induced arthritis is an animal model of rheumatoid arthritis in which immunization with type II collagen induces polyarthritis within 2 to 4 weeks in susceptible animals such as DBA/1J mice. This model is sensitive to NSAID treatment, indicating that prostanoids participate in its pathogenesis. We backcrossed mice deficient in prostanoid receptors to the DBA/1J background and subjected the resulting progeny to collagen-induced arthritis.[95] Whereas the incidence of arthritis was unaffected, the extent and progression of this condition were markedly suppressed in IP-deficient mice as well as in mice both deficient in EP2 and treated with an EP4 antagonist. These findings thus indicated that PGI_2-IP signaling and PGE_2 signaling at EP2 and EP4 mediate joint inflammation in this model. Further analysis revealed that both PGI_2 and PGE_2 pathways regulate expression of arthritis-related genes, including those for IL-6, vascular endothelial growth factor–A, and RANKL (see below), in synovial fibroblasts and thereby contribute to arthritic inflammation, bone destruction, and pannus formation. Collagen-induced arthritis thus represents a condition in which pro-inflammatory actions of prostanoids are elicited through expression of pro-inflammatory genes. The same receptor, EP4, exerts an anti-inflammatory action in intestinal inflammation and a proinflammatory action in arthritis, emphasizing the context-dependent roles of prostanoid signaling.

Intestinal polyposis and colon cancer. A role for COX isoforms and their reaction products in the development of colon cancer was first suggested by epidemiological studies in humans showing a reduced incidence of colon cancer in users of aspirin and other NSAIDs. It was subsequently confirmed by pharmacological experiments with rodents administered with these drugs and finally verified by experiments with transgenic mice and two mouse models of human familial adenomatous polyposis. Both Min mice and $Apc^{\Delta 716}$ mice harbor truncation mutations in the APC gene and develop polyposis as a result of loss of heterozygosity in heterozygous mutants. Taketo and colleagues[96] showed that disruption of the COX-2 gene resulted in marked decreases in both the number and size of intestinal polyps that develop in $Apc^{\Delta 716}$ mice. Inhibition of polyp formation in $Apc^{\Delta 716}$ mice was also observed in response to treatment with a COX-2–selective inhibitor. This group further examined which type of prostanoid or prostanoid receptor contributes to polyp formation by generating $Apc^{\Delta 716}$ mice in which the EP1, EP2, or EP3 gene is ablated.[97] Homozygous deletion of the EP2 gene in $Apc^{\Delta 716}$ mice resulted in substantial decreases in the number and size of intestinal polyps, effects similar to those induced by COX-2 gene disruption.

Increased PGE_2-EP2 signaling was found to increase COX-2 expression through an increase in the intracellular level of cAMP as well as to increase the expression of vascular endothelial growth factor in the polyp stroma. It remains to be determined, however, whether these are the only prostanoid actions in colon carcinogenesis.

Wakabayashi and collaborators addressed this question more directly by examining azoxymethane-induced formation of aberrant cryptic foci in mice deficient in each prostanoid receptor. They found that the formation of such foci was inhibited in both $EP1^{-/-}$ mice and $EP4^{-/-}$ mice but not in those deficient in other receptor types or subtypes.[98),99)] In both instances, the number of foci was reduced to 50 to 60% of that apparent in wild-type mice. These researchers further found that administration of either an EP1-specific antagonist, ONO-8711, or an EP4 antagonist, ONO-AE2-227, in the diet reduced the number and size of polyps in Min mice. They also examined whether EP1 deficiency affects the development of colon cancer induced by azoxymethane.[100)] They found that colon cancer incidence and multiplicity as well as tumor volume were reduced and that tumor cell apoptosis was increased in EP1 knockout mice. These results thus indicate that EP1 plays a key role in colon cancer development and is a potential target for chemoprevention of this condition.

Bone metabolism. Bone undergoes continuous destruction and renewal, a process termed bone remodeling, with bone resorption and formation being mediated by osteoclasts and osteoblasts, respectively. Such remodeling is controlled by systemic humoral factors such as parathyroid hormone (PTH), estradiol, and vitamin D as well as by local cytokines such as IL-1β, IL-6, and insulin-like growth factor. Osteoclasts develop from precursor cells of the macrophage lineage in the bone microenvironment. Factors such as PTH, vitamin D, IL-1, and IL-6 act on osteoblasts to induce the synthesis of an osteoclast differentiation factor known as receptor activator of NF-κB ligand (RANKL), which in turn stimulates the formation of mature osteoclasts from hematopoietic precursors through cell-cell interaction. These factors induce COX-2 expression in osteoblasts, and their induction of osteoclast differentiation is inhibited, at least in part, by aspirin-like drugs; such inhibition was reversed by the addition of PGE_2, implicating both COX-2 and PGE_2 in this process.[101)] Sakuma *et al.*[102)] and Miyaura *et al.*[103)] examined the identity of the EP subtype responsible for mediating this action of PGE_2. Sakuma *et al.* found that PGE_2-induced osteoclast formation was impaired in cultures of osteoblasts from EP4-deficient mice and osteoclast precursors from the spleen of wild-type mice. IL-1β, TNF-α, and basic fibroblast growth factor also each failed to induce osteoclast formation in these cultures. Miyaura *et al.* added PGE_2 to cultures of parietal bone from mice deficient in each of the EP subtypes as well as from wild-type mice and examined bone resorption by measuring the release of Ca^{2+} into the medium. They found that the induction of bone resorption by PGE_2 was greatly impaired, whereas bone resorption in response to dibutyryl cAMP was unaffected, in bone from EP4-deficient mice. These studies unequivocally established a role for EP4 in the induction of osteoclast differentiation factor and in PGE_2-dependent bone resorption.

In contrast, Li *et al.*[104)] showed that the osteoclastogenic response to PGE_2, PTH, or 1,25-dihydroxyvitamin D *in vitro* was impaired in cultures of cells derived from EP2-deficient mice. This apparent discrepancy likely reflects redundant roles of the two relaxant PGE receptor subtypes. Sakuma *et al.*[102)] and Miyaura *et al.*[103)] detected a small but significant PGE_2-dependent osteoclastogenic response remaining in cells derived from EP4-deficient mice, and Li *et al.*[104)] observed a further decrease in osteoclastogenesis in response to the addition of an EP4-selective antagonist to EP2-deficient cells. Such redundant actions of EP2 and EP4 were confirmed by Suzawa *et al.*,[30)] who demonstrated both an additive effect of an EP2-selective agonist and an EP4-selective agonist in resorption of wild-type bone as well as bone-resorbing activity of an EP2 agonist in $EP4^{-/-}$ bone.

In addition to bone resorption, it has long been known that systemic administration of PGE_2 induces bone formation *in vivo*. The mechanism of this action and its relation to the bone-resorbing activity of this PG had remained obscure, however. Yoshida *et al.*[105)] infused PGE_2 into the periosteal region of the femur of wild-type mice or mice-deficient in each EP subtype with the use of a mini–osmotic pump. After 6 weeks, the femur was isolated and bone formation was examined. Radio-

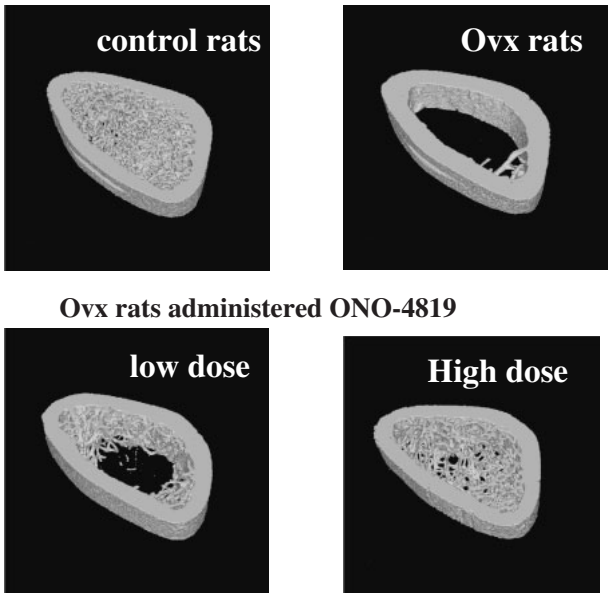

Fig. 10. Ovariectomy-induced bone loss and its prevention by administration of an EP4-selective agonist in rats (from ref. 105 with permission). Control rats as well as ovariectomized rats treated with vehicle (Ovx) or with a low or high dose of ONO-4819 before and after surgery were examined for bone loss by X-ray computed tomography.

graphic analysis revealed that PGE_2 induced extensive callus formation on the femur at the site of infusion in wild-type as well as EP1-, EP2-, and EP3-deficient mice. In contrast, no bone formation was detected radiographically in EP4-deficient mice. Consistent with these findings, bone formation in wild-type mice was induced by infusion of an EP4-selective agonist but not by that of agonists specific for other EP subtypes. The EP4-selective agonist was also shown to prevent bone loss and to restore bone mass in rats subjected to ovariectomy or immobilization (Fig. 10). Histomorphometric analysis further revealed that the density of osteoblasts lining the bone surface increased with the increase in bone mass in the rats treated with the EP4 agonist. The total number of osteoclasts was also increased with the increase in new bone surface, suggesting that EP4 is responsible for both bone resorption and bone formation induced by PGE_2 and that activation of EP4 *in situ* integrates these two actions for bone remodeling.

Conclusions

How prostanoids exert diverse functions in the body has been an enigma for decades. As reviewed in this article, the identification and cDNA cloning of prostanoid receptors and the generation of corresponding knockout mice have provided answers to this long-standing question and defined a physiological mechanism for each known prostanoid action. Furthermore, studies with mice deficient in each of these receptors have revealed previously unknown functions of prostanoids that had not been predicted from the effects of aspirin-like drugs. These newly discovered functions include actions in the brain, the immune system, and allergy. These studies have shown how this family of substances delicately regulates various processes in the body and its responses to environmental stimuli. Such knowledge will be exploited to provide insight into pathophysiology in humans and for the development of new therapeutics that selectively modulate each of these actions.

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Profile

Shuh Narumiya was born in 1949 and started his research career in 1971 with studies on poly-ADP ribosylation and poly-ADP-ribose glycohydrolase in the Department of Medical Chemistry of Professor Osamu Hayaishi, Kyoto University Faculty of Medicine. After studying tryptophan metabolism as a graduate student in the same Department, he spent two years as a postdoctoral fellow in the Wellcome Research Laboratories in the laboratory of Sir John Vane, studying lipoxygenases. He returned to Japan in 1981 and, since then, has been working in Kyoto University, being promoted to Professor in Pharmacology in 1992. During this period, he and his group first purified thromboxane A2 receptor from human blood platelets in 1989, then cloned cDNAs for the family of eight types and subtypes of protsanoid receptors, generated knockout mice deficient in each of these receptors individually and elucidated physiological and pathophysiological roles each receptor plays in the body. Their works stimulated a variety of research efforts to establish a research field for prostanoid receptors. Shuh Narumiya also discovered an ADP-ribosyl transferase in Clostridium botulinum now known as botulinum C3 exoenzyme, and identified small GTPase Rho as its target. C3 exoenzyme was then widely used to dissect functions of Rho protein and these studies revealed that Rho functions as a molecular switch in cell morphogenesis, adhesion and motility by inducing specific types of actin cytoskeleton. Shuh Narumiya then identified and cloned several effector proteins for Rho such as ROCK and mDia, and elucidated the pathway from Rho to the actin cytoskeleton. For these achievements, he was awarded the Osaka Science Prize in 1998, the Takeda Prize for Medicine in 1999, the Erwin von Baelz Prize in 1999, the Lorenzini Giovanni Gold Medal in 2000, the Uehara Prize in 2002, and the Imperial Prize and the Japan Academy Prize in 2006. Between 2005 and 2007, he was the Dean of Kyoto University Faculty of Medicine.