Prostaglandins, together with thromboxanes (collectively termed prostanoids), are fatty acid derivatives of significant importance in many physiological processes. These compounds are formed following the action of cyclooxygenases (COX) and associated enzymes on C20 polyunsaturated fatty acid precursors released from phospholipids in membranes. Nearly all mammalian cell types have the biosynthetic machinery to produce at least one type of prostanoid. The same C20 fatty acid substrates can also be acted upon by lipoxygenases to produce mono- di- and tri-hydroxy derivatives such as leukotrienes, lipoxins and resolvins. The final route is the cytochrome P450 pathway that can convert C20 fatty acids to hydroxylated derivatives. Collectively these compounds are called eicosanoids; the term derived from the Greek eikosi that refers to the C20 backbone in the parent fatty acid.

Prostaglandins (PGs) were first discovered in the 1930s by von Euler and colleagues, who found a substance produced by the prostate gland that caused smooth muscle contraction. They christened the active substance 'prostaglandin', but it was over 30 years until the structure and mode of biosynthesis of these fatty acid derivatives became fully understood. PGs have many basic physiological functions where they act as 'local' hormones. For example, thromboxane (Tx) A2 and prostacyclin (PGI 2) generated by platelets and endothelial cells, respectively, regulate the aggregatory behaviour of blood platelets and endothelium.

Prostaglandins in invertebrates can be categorised into two main types; the classical forms, such as PGE 2 and PGD 2 that are found in mammals, and novel forms including clavulones, bromo- and iodo-vulones and various PGA 2 and PGE 2 esters. A significant number of reports of PG identification in invertebrates have relied upon methods such as enzyme immunoassay that do not have the necessary specificity to ensure the validity of the identification. For example, in the barnacle Balanus amphitrite, although there are PG-like compounds that bind to antibodies raised against PGE 2, mass spectrometric analysis failed to confirm the presence of these classical PGs. Therefore, care should be taken in drawing conclusions about what PGs are formed in invertebrates without employing appropriate analytical methods. Finally, the recent publication of the Ciona genome should facilitate studies on the nature and mode of biosynthesis of PGs in this advanced deuterostomate invertebrate.

Key words: barnacle, coral, cyclooxygenase, eicosanoid, leukotriene, prostaglandin, prostaglandin D synthase, tunicate, Ciona intestinalis, Balanus amphitrite.
platelets during haemostatic episodes (Moncada and Vane, 1979). Other PGs, including PGG2 and PGE2, are regulators of sleep–wake activity in mammals (Hayaishi 2000). For instance, in rat models, infusion of PGD2 specifically increases the duration of sleep in a dose-dependent way (Hayaishi et al., 1990). PGE2 also influences the central nervous system (CNS) in terms of temperature regulation, in which it acts as an endogenous pyrogen (see review by DuBois et al., 1998). Several PGs target smooth muscle cells, causing their contraction or relaxation. This is of particular importance in parturition where PGF2α is an activator of myometrial contraction and cervical ripening (Johnson and Everitt, 2000). In the kidney PGs, including PGE2 and PGH2, modulate haemodynamics as a result of their vasodilatory activity and also have an effect on both salt and water balance (DuBois et al., 1998; Frolich and Stichtenoth, 1998). Finally, PGs play a complex role in inflammation, not only in the early stages as pro-inflammatory mediators but also at a later stage in eliciting resolution (Colville-Nash and Gilroy, 2000).

Aquatic invertebrates have played significant roles in our understanding of the biological activities of PGs. In 1969, Weinheimer and Spraggins discovered that one species of coral (Plexaura homomalla) contains up to 8% of its dry mass as PG esters. For a short time, in the absence of other available routes to synthesize PGs, this coral provided a ready source of precursors for the synthesis of such compounds for use in studies with humans and other mammalian models. From the many studies that have followed over the last 30 years, it is apparent that PGs play important roles in reproduction, ion transport and defence across a wide range of invertebrates (reviewed in Stanley, 2000). For instance, in insects detailed research has revealed that PGs function in egg laying, immune defence mechanisms and chloride transport (see reviews by Stanley-Samuelson, 1990; Stanley and Miller, 1998; Stanley, 2000). Despite a growing understanding of the roles of PGs in invertebrates (reviewed by Stanley, 2000), the nature of the products formed and their mode of biosynthesis are still largely unknown, particularly in non-insectan forms. This account therefore focuses on these aspects of PG biology and reviews some recent findings from aquatic invertebrates including corals, barnacles and tunicates. It questions whether all of the reports of PG identification and presence in invertebrates are valid in light of these recent findings.

Prostanoid biosynthetic pathways in mammals

The great majority of our knowledge of PG and Tx generation comes from studies using mammals. Hence this section briefly reviews the mechanism of prostanoid biosynthesis in these animals with particular emphasis on enzymatic activities. The principal substrate for prostanoid synthesis in mammals is the C20 polyunsaturated fatty acid, arachidonic acid (20:4n-6) although other fatty acids can act as substrates, including eicosapentaenoic acid (20:5n-3) and eicosatrienoic acid (20:3n-6). PGs derived from arachidonic acid are termed 2-series PGs, while eicosatrienoic and eicosapentaenoic acids result in the formation of 1- and 3-series PGs, respectively. The enzyme at the heart of prostanoid biosynthesis is COX, also termed PGH synthase. This enzyme is responsible for the generation of PGH2 from arachidonate via the highly unstable endoperoxide, PGG2 (Fig. 1). There are several forms of COX. The first, termed COX-1, is usually constitutively expressed in nearly all cell types within mammals, while the second, COX-2, is mainly inducible and only expressed by a more limited range of cell types. COX-1 is often described as the ‘housekeeping’ form of the enzyme because it is responsible for the generation of PGs of importance in physiological and haemostatic events. COX-2, on the other hand, has been found to be rapidly expressed in inflammatory conditions and is the target for a new group of non-steroidal anti-inflammatory drugs such as celecoxib and rofecoxib that have negligible effects on the constitutive COX-1 (Hawkey, 1999). The recent controversial finding of a third type of COX, COX-3, derived from the COX-1 gene that is expressed in the cerebral cortex and heart, and is sensitive to analgesic/antipyretic drugs such as acetaminophen (Chandrasekharan et al., 2002), has given new insights into the mechanism of action of such agents (Chandrasekharan et al., 2002; Warner and Mitchell, 2002). It remains to be established if variants of COX-2 will be discovered (Chandrasekharan et al., 2002).

As can be seen from Fig. 1, the ultimate product of COX activity, PGH2, is subject to further conversion to give rise to the generation of ‘classical’ PGs including PGD2, PGE2, PGF2α, and PGI2 (prostacyclin) as well as TXA2. For such generation to occur, further enzyme activity is usually required. For example, PGD synthases, responsible for the generation of PGD2 from arachidonate, consist of at least two evolutionarily distinct enzymes: a haemopoietic form expressed in mast cells, Th2 lymphocytes and platelet precursors, and a lipocalin-type PGD synthase found in the brain, testes and heart (Urade and Eguchi, 2002). The haemopoietic form of PGD synthase is a member of the sigma-class glutathione S-transferase family that has widespread distribution in multicellular organisms (Thomson et al., 1998). PGE synthases also consist of both membrane-associated and cytosolic forms (Murakami et al., 2002). The dramatic increase in PGE2 generation in some inflammatory states appears to result from the induction of one of the membrane-associated PGE synthases (termed mPGES-1), and the stimuli responsible for the induction of COX-2 expression also induce the expression of this type of PGE synthase (Reddy and Hershman, 1997; Mancini et al., 2001; Umatsu et al., 2002). The recent addition of a second membrane-associated form of PGE synthase (mPGE synthase) that is linked to both COX-1 and COX-2 (Murakami et al., 2003) emphasises the potential complexity of the relationship between PGE synthases and COX-1 and COX-2. Various cytosolic glutathione S-transferases also have the ability to convert PGH2 to PGE2 and other PGs (Uijiraha et al., 1988). Finally, TXA2 and PGI synthases are distinct members of the diverse cytochrome P450 superfamily (Hara et al., 1994; Ullrich et al., 2001; Wang and Kulmacz, 2002).

As well as the ‘classical’ PGs, mention should be made of
several additional forms including PGA2, PGB2 and PGJ2. The J-type PGs are unusual in that they contain a cyclopentenone ring. PGD2 is the precursor for the non-enzymatic generation of PGJ2 and related forms such as $\Delta^{12}$-PGJ2 and 15-deoxy-$\Delta^{12,14}$-PGJ2 (Hirata et al., 1988). PGA2 (also called medullin) is a non-enzymatic dehydration product of PGE2, although the extent of its generation and biological activity in mammals remains unclear.

Following their biosynthesis, PGs are exported from cells across the cell membrane and bind to specific receptors on target cells. They can also be carried across membranes by a PG transporter (PGT; Kanai et al., 1995; Pucci et al., 1999). The finding that PGT is expressed in cell types that synthesize and release PGs may suggest that the transporter is involved in the re-uptake of PGs, either as a way of negating their leakage and/or facilitating the transport of such molecules to target nuclear receptors (Bao et al., 2002).

Our understanding of the nature and diversity of prostanoid receptors has increased dramatically in the last two decades. Each of the main type of prostanoid has its own specific G protein-coupled receptor. These are classified into five types termed EP, DP, FP, IP and TP, corresponding to the main prostanoids, PGE, PGD, PGF, PGI and TxA, respectively (Tsuboi et al., 2002). According to Tsuboi et al. (2002) with the exception of the EP receptors, all the others consist of a single type. The EP receptors for PGE consist of four main sub-types, EP1–EP4, in which each has a distinctive structure, signalling pathway and tissue distribution (Wright et al., 2001). The terminal product of PGD2 breakdown, namely 15-deoxy-$\Delta^{12,14}$-PGJ2, has its own specific nuclear receptor, the $\gamma$ form of the peroxisome proliferator-activated receptor (PPAR$\gamma$). This receptor is an important regulator of adipocyte differentiation (Negishi and Katoh, 2002).

**Evidence for prostanoid generation in non-insectan invertebrates**

One of the earliest descriptions of PG generation in invertebrates comes from the work of Christ and van Dorp (1972) who studied the ability of a wide range of invertebrates and vertebrates to synthesize PGs from radiolabelled eicosatrienoic acid. They found that conversion of this substrate to PGE1 occurs in tissue homogenates from Mytilus (mussel), Homarus (lobster), Lumbricus (earthworm) and Cyanea (jellyfish) but not Anthoplexaura (coral), although the levels of conversion were reported to be rather small. Since these initial findings, there have been many reports of PG biosynthesis in a wide range of invertebrates. The nature of the PGs generated in insects has received particular attention and, as the results of these studies have been recently reviewed elsewhere (Stanley and Miller, 1998; Stanley, 2000), this current account focuses on non-insectan invertebrates only (Table 1).
| Genus/species                                      | Tissue(s)                        | PG generated                      | Analytical method             | Functional significance                                                                 | Reference               |
|--------------------------------------------------|----------------------------------|-----------------------------------|--------------------------------|-----------------------------------------------------------------------------------------|-------------------------|
| **Sponges**                                      |                                  |                                   |                                |                                                                                        |                         |
| *Reniera mucosa*                                 | All                              | Mucosin                           | NMR, HPLC*                     | –                                                                                        | Casapullo et al. (1997) |
| **Cnidarians**                                   |                                  |                                   |                                |                                                                                        |                         |
| *Gersemia fruticosa*                             | Soft tissues                     | PGD₂, PGE₂, PGF₂₀₅, 15-keto-PGF₃₆₀⁺ | HPLC, GC-MS                    | –                                                                                        | Varvas et al. (1993, 1999) |
| *Clavularia viridis*                             | Whole organism                   | Chlorovulones I–IV                | NMR                            | –                                                                                        | Iguchi et al. (1985)    |
| *Clavularia viridis*                             | Whole organism                   | Bromovulones and iodovulones     | NMR                            | –                                                                                        | Iguchi et al. (1986); Watanabe et al. (2001) |
| *Clavularia viridis*                             | Whole organism                   | Clavulones, clavirins             | NMR                            | –                                                                                        | Kikuchi et al. (1982); Iwashima et al. (1999) |
| **Dendronephthya sp., Dendrophyllia sp., Tubipora muscida** | Whole organism                  | Bromovulones, bromopunaglandins   | HPLC, NMR                       | Antibacterial, defence against predation                                                 | Rezanka and Dembitsky (2003) |
| *Plexaura homomalla* (coral)                     | Soft tissues                     | Various PGA₂ and PGE₂ esters     | NMR                            | Defence against predation?                                                                | Baker et al. (1985); Baker and Scheuer (1994) |
| *Telessto riisei* (octocoral)                     | Whole organism                   | Punaglandins                      | NMR, MS                         | –                                                                                        |                         |
| **Nematodes**                                    |                                  |                                   |                                |                                                                                        |                         |
| *Bragia maliyi*                                  | Whole microfilariae              | 6-keto-PGF₁₀₅, PGE₂₀, PGD₂, but no PGF₂₀₅ or TxB₂ | Radio TLC and HPLC; EIA        | Assistance with invasive properties of parasite in host                                  | Liu et al. (1990, 1992) |
| *Wuchereria bancrofti*                           | Whole microfilariae              | PGE₂                              | EIA                            |                                                                                        | Liu et al. (1992)       |
| **Platyhelminthes**                              |                                  |                                   |                                |                                                                                        |                         |
| *Schistosoma mansoni*                            | Cercariae                        | PGE₁₀₂, PGD₂, PGA₂                | HPLC, RIA                       | Parasite penetration of host                                                              | Fusco et al. (1985, 1986, 1993) |
| **Mollusces**                                    |                                  |                                   |                                |                                                                                        |                         |
| *Argopecten purpuratus* (scallop)                | Gonad                            | PGE₂₀, PGF₂₀                      | RIA                            | Gonadal development                                                                     | Martínez et al. (1999) |
| *Mytilus edulis* (mussel)                        | Muscle, gill, mantle             | Various classical PGs and PG-like compounds | TLC                             |                                                                                        | Srivastava and Mustafa (1985) |
| *Ligumia subrostrata*                            | Gill homogenates                 | PGE₂₀, PGF₂₀                      | RIA                            | Ion balance                                                                             | Saintsing et al. (1983); Hagar et al. (1989) |
| *Lymnaea stagnalis*                              | Accessory sex glands             | PG-like compounds                 | HPLC                            |                                                                                        | Clare et al. (1986)    |
| *Octopus vulgaris*                               | Heart                            | PGE₂₀, PGD₂, PGF₂₀₅, PGI₁         | Radio TLC                       | Control of cardiac function                                                             | Agnisola et al. (1994) |
| *Patinopecten yessoensis* (scallop)              | Various                          | PGF₂₀₅, PGE₂₀, PGG₂₀₅, 6-keto-PGF₃₆₀⁺, TxB₂ | HPLC, GC-MS                    | Spawning behaviour                                                                      | Osada et al. (1989)    |
| *Tethys fimbria*                                 | Mantle, cerata and reproductive gland | PG 1,15-lactones of PGE₂₀ and PGF₂₀₅ | GC-MS, EIMS, NMR                | Chemical defence, control of oocyte fertilisation/production, smooth muscle contraction  | Cimino et al. (1989, 1991a,b); Di Marzo et al. (1991) |
| **Annelids**                                     |                                  |                                   |                                |                                                                                        |                         |
| *Hirudo medicinalis* (medicinal leech)           | Head region                      | 6-keto- PGF₁₀₅-like’               | RIA                            | Inhibition of host platelet aggregation?                                                 | Nikonov et al. (1999)  |
Table 1. Continued

| Genus/species                        | Tissue(s)                        | PG generated | Analytical method | Functional significance                            | Reference                        |
|--------------------------------------|----------------------------------|--------------|-------------------|---------------------------------------------------|----------------------------------|
| **Crustaceans**                      |                                  |              |                   |                                                   |                                  |
| *Balanus amphitrite* (barnacle)      | Whole cyprid larvae              | PGE          | EIA               | Inhibition of larval settlement                    | Knight et al. (2000)             |
| *Carcinus maenas* (shore crab)       | Blood cells                      | PGE, TxB, 6-keto-PGF₁α | RIA               | –                                                  | Hampson et al. (1992)            |
| *Penaeus japonicus* (kuruma prawn)  | Whole haemolymph and ovary       | PGF₂α, PGF₂β | HPLC/RIA          | Control of ovarian development                     | Tahara and Yano (2003)           |
| *Procambarus paeninsulaus* (Florida crayfish) | Ovary                        | PGF₂α, PGF₂β |                   | Ovulation (PGF₂α)                                 | Spaziani et al. (1993, 1995)     |
| **Acari**                            |                                  |              |                   |                                                   |                                  |
| *Amblyomma americanum* (lone star tick) | Whole salivary glands and saliva | PGE, PGF₂α,PGA₂, PGA₂/PGB₂ | RIA/GC-MS and bioassay; radio-TLC | Assistance in tick feeding                         | Bowman et al. (1996); Pedibhotla et al. (1997); Aljamali et al. (2002) |
| **Urochordates**                     |                                  |              |                   |                                                   |                                  |
| *Ciona intestinalis* (sea squirt)    | Tunic, basket, ovary, intestine and heart | PGE, PGF | EIA               | –                                                  | Knight et al. (1999); Pope and Rowley (2002) |

*EIA, enzyme immunoassay; EIMS, electron impact mass spectrometry; GC-MS, gas chromatography mass spectrometry; HPLC, high performance liquid chromatography; NMR, nuclear magnetic resonance; RIA, radioimmunoassay; TLC, thin layer chromatography.

As can be seen from Table 1, the PGs formed in invertebrates appear to fall into two categories, namely novel PGs only found in invertebrates, and the classical PGs (e.g. PGE₂, PGD₂ etc.) found in both invertebrates and vertebrates. The early studies of Weinheimer and Spraggins (1969) with the coral *P. homomalla* not only noted the unusual stereochemistry of the PGs formed (*R* rather than the *S* forms found in vertebrates) but also made the important finding that rather than the classical PGs, the main products synthesized were esters of PGA₂ and PGE₂ (Fig. 2). Subsequently, a number of other novel PGs have been reported from a diverse range of cnidarians and sponges including chloro-, bromo- and iodo-vulones, clavulones, punaglandins and mucosin (Table 1; Fig. 2). Several of these products have received much attention due to their potential antitumour activity (e.g. Iguchi et al., 1985, 1986; Honda et al., 1988; Iwashima et al., 1999). Their functional significance in the animals producing such compounds is unclear, but they may provide defence against predation by fish (Gerhart, 1991) as well as protecting against microbial attack (Rézanka and Dembitsky, 2003). Their potential as anti-predatory factors has, however, been questioned (Pawlik and Fenical, 1989) and further experimental work is required to confirm the original observations.

One of the most impressive series of studies on PG biosynthesis in invertebrates comes from the work on the opistobranch mollusc, *Tethys fimbria* (Cimino et al., 1989, 1991a,b; Di Marzo et al., 1991). These authors showed conclusively that this mollusc generates novel PG derivatives, the PG 1,15-lactones, apparently derived from PGE₂ and PGF₂α. The product profile in *T. fimbriae* also differs between the mantle, cerata and reproductive glands (Cimino et al., 1991b; Di Marzo et al., 1991), with PGs formed in the mantle exported to the cerata and reproductive glands where further structural modification occurs. This regional-specific generation of PGs may imply that these products perform different functions such as defence in the cerata, and control of the reproductive processes in the ovary/testis (Di Marzo et al., 1991). Because these studies have fully characterised the products formed and their mode of biosynthesis, *T. fimbria* would make a good model for detailed investigations aimed to determine the functional significance and mechanism of action of the PGs formed.

As can be seen from Table 1, there are many reports of the generation of classical PGs, particularly PGE₂, PGD₂ and PGF₂α, in invertebrates. A significant number of these have employed techniques such as enzyme immunoassay (EIA), radioimmunoassay (RIA) and thin layer chromatography (TLC) that alone do not provide the specificity to confidently report on the presence of absence of various PGs. For instance, Knight et al. (1999) used commercially available EIA kits to determine if PGF and PGF immunoreactivity was formed in ionophore-challenged tissues from the tunicate, *Ciona intestinalis*. Because this approach without HPLC or some other form of
high-resolution purification cannot differentiate between 2- and 3-series PGs (and other non-PG components), they expressed their results as ‘ng immunoreactive PGE’ rather than ng PGE2. Others, however, have taken it for granted that the product identified and quantified by EIA or RIA is only that defined by the assay (e.g. Hagar et al., 1989; Martínez et al., 1999; Tahara and Yano, 2003), despite the possibility of the presence of alternative fatty acid substrates in these animals. It must be remembered that the specificity of these assays totally depends on the antibodies used as well as the degree to which samples have been extracted prior to the assay. Most of the antibodies employed show low reactivity with other classical PGs so that in defined cell types/tissues in mammals this approach presents few problems. However, in invertebrates with the potential for novel PGs that have not been screened for cross-reactivity with the antibodies, such an approach has clear limitations. An additional problem arises because many aquatic invertebrates, unlike terrestrial mammals, have significant amounts of arachidonic and eicosapentaenoic acids in their phospholipids (Stanley, 2000). Both of these can act as substrates for PG generation and EIA and RIA do not differentiate between the products formed. For instance, both PGE2, formed from arachidonate, and PGE3, derived from eicosapentaenoate, react equally with the antibodies in some commercial PGE2 EIA kits. As discussed by Taylor and Wellings (1994), unless full structural analysis is achieved, there is little point in blindly using quantitative approaches, such as EIA, that lack specificity. Essentially, confidence in the accuracy of the identification and the quantification can only be achieved by a combination of approaches such as solid phase extraction prior to separation of analytes by high performance liquid chromatography (HPLC) or TLC, followed by mass spectrometry or nuclear magnetic resonance (NMR) spectroscopy to provide full structural identification and an appreciation of the stereochemistry of the products. In very few cases has such an approach been employed in the studies reported in Table 1 and therefore the results reported are equivocal.

Several authors have reported the presence of ‘PG-like’ compounds in some invertebrates. For example, in the blue mussel *Mytilus edulis* the products of incubating gill, mantle or muscle with [14C]arachidonic acid included one or more PG-like compounds that had an *rf* value on TLC similar to authentic PGE2, PGF2α and PGD2 (Srivastava and Mustafa 1985). Aspirin and indomethacin inhibited the generation of radiolabelled material, suggesting that they are products of COX activity. Overall, however, there was no convincing evidence that the compounds formed were identical to classical PGs. Similarly, leeches (*Hirudo medicinalis*) are said to produce a PGI2-like substance (Nikonov et al., 1999). Although the active substance inhibits human platelet aggregation and reacts with antiserum to 6-keto-PGF1α, the stable breakdown product of PGI2, no structural data were provided. It is entirely possible that the active factor is a PG, but not necessarily PGI2. In the snail *Lymnaea stagnalis*, the principal prostanoid synthesized following incubation of various tissues with radiolabelled arachidonate, did not correspond chromatographically to any authentic classical PG and was hence termed ‘PG-like’ (Clare et al., 1986). The COX inhibitor, aspirin, at 10 mmol l–1 reduced but did not completely eliminate the generation of this putative PG.

Finally, there are examples of studies with some invertebrates where authors have used known classical PGs and other eicosanoids in bioassays without first screening by any analytical method to see if the compound of interest is synthesised in the animal studied. Such an approach often results in the finding of biological activity without any indication that the animal or tissue under study can synthesize the appropriate substance. An example of this comes from work with sand dollars (*Echinaracnius parma*) where leukotriene B4 (LTB4), a 5-lipoxygenase product, has been found to regulate intracellular Ca2+ levels in eggs (Silver et al., 1994) when studies with eggs from other echinoderms (sea urchins, *Stronglyocentrotus purpuratus*) have shown categorically that the lipoxygenase products generated did not...
Recent insights from studies on barnacles

Barnacles have a complex life cycle involving free swimming larval stages that give rise to a sessile adult (Fig. 3). There are two main stages of this life cycle during which eicosanoids are thought to play key roles in the signalling pathways that may control barnacle development. The first is following fertilisation, when the fertilised eggs (embryos) are brooded in the mantle cavity of the adult. Upon hatching, the larvae are liberated into the surrounding water. This hatching process appears to be triggered by ‘barnacle hatching factor’ that is thought to consist of a ‘cocktail’ of different eicosanoids including the lipoxigenase products, hepoxilin A3 (Vogan et al., 2003) and various mono-, di- and tri-hydroxy fatty acid derivatives (Hill et al., 1993). Whether PGs have hatching activity is unclear, although Clare et al. (1982) found that crude barnacle hatching factor from Balanus balanoides prepared in the presence of the COX inhibitor, aspirin, lacked hatching activity, perhaps indicating COX involvement in its generation. They also noted that dried coral extract from P. homomalla has barnacle hatching factor activity, suggesting that a range of eicosanoids are likely to be involved and that the ligand specificity of the triggering process may be limited.

The second stage that may be influenced by eicosanoids is settlement when the cyprid larvae attach to the substratum, prior to a radical metamorphosis that ultimately gives rise to sessile adults (Fig. 3). Knight et al. (2000) demonstrated in Balanus amphitrite that PGE2, PGE3 and the stable synthetic analogue of PGE2, 15,15-dimethyl-PGE2, caused a dose-dependent inhibition of larval settlement, while indomethacin, a COX inhibitor, stimulated this process. They concluded from these preliminary findings that PGs might play key roles in controlling larval settlement. Studies using EIA alone found that the soft tissues of B. amphitrite generate significant amounts of PGE immunoreactive material (Knight et al., 2000), but taking into account the problems of using EIA alone for PG identification already discussed, such preliminary results required confirmation. Therefore, the potential biosynthesis of PGs by adult and larval barnacles was studied using a combination of solid phase extraction of analytes, separation by reverse phase-HPLC, followed by mass spectrometry (MS) of fractions found to have immunoreactivity in EIA. Such an approach was chosen to categorically identify all potential PGs generated. HPLC-negative ion electrospray MS of calcium ionophore-challenged barnacle tissues revealed two major peaks with PG-like masses and elution times. Firstly, a component with a retention time of ~14.23 min eluting ~0.7 min earlier than the authentic PGF3α, which generated a deprotonated (M-H –) ion at an m/z 353, and secondly, a component that eluted at ~16.43 min between authentic standards PGF2α and PGE2 (equivalent to peaks I and II, respectively, in Fig. 4), generating an M-H – at m/z 351. In order to boost product generation and overcome the problems of low sensitivity (ng levels) on HPLC-MS, B. amphitrite tissue samples were pre-incubated with the exogenous fatty acids EPA and AA. This generated two additional peaks with PG-like masses, an m/z 353 species with a retention time of ~18.54 min and an m/z 351 species, which eluted at ~20.66 min (peaks III and IV, respectively, in Fig. 4). However, when samples were pre-incubated with the COX inhibitor indomethacin (25 µmol l−1), all four peaks remained, suggesting that the peak identities were either non-prostanoid

Fig. 3. Life cycle of barnacles and the times when eicosanoids are thought to play a role in development (red boxes). Following fertilisation the eggs are brooded in the mantle cavity where hatching is under the control of hatching factors. The resulting planktonic larval stages undergo several molts until giving rise to the cyprid stage that uses its antennules to probe for suitable settlement sites. At settlement, these molt to give rise to juveniles (spat) that also grow and molt to give rise to a filter-feeding sedentary adult.
or that they were prostanoids derived via a non-COX route. HPLC fractions containing PG-like material were derivatised for electron impact GC-MS. This revealed the peak identities to be the lipoxygenase products, trioxilin A4 (peak II, Fig. 4) and trioxilin A3 (peak III, Fig. 4). The two remaining PG-like peaks (I and IV, Fig. 4) could not be identified on electron impact GC-MS. Thus, the presence of any classical prostanoids in *B. amphitrite* including PGE$_{2\alpha}$, PGF$_{2\alpha}$, PGD$_{2\alpha}$, TxA$_{2\alpha}$, PGJ$_{2\alpha}$, PGG$_{2\alpha}$ and PGI$_{2\alpha}$ could not be confirmed on either HPLC-MS or GC-MS. Hence it was concluded that they are either not produced or they are present in levels below the detection limit (ng) on HPLC/GC-MS. The latter hypothesis was further supported by the repeated detection of >100 pg mg$^{-1}$ protein of PG immunoreactivity on total PG, PGE and PGF EIA kits predominantly in HPLC fractions between 14–18 min, but particularly in the 16–17 min time fraction e.g. (Fig. 4). When samples were prepared in the presence of indomethacin (a COX inhibitor), immunoreactivity was completely suppressed, suggesting that this material was probably derived through a COX route (i.e. PG-like) and was not the result of antibody cross reactivity with trioxilin A$_4$ or other lipoxygenase-derived products.

Overall these barnacle studies highlight the fact that it is extremely easy to mis-identify other compounds (e.g. trioxilins) as PG-like compounds if no electron impact GC-MS work is conducted. It also indicates the problems encountered in gaining structural elucidation when material is generated in extremely low levels (i.e. sub-ng), as appears to be the case in *B. amphitrite*.

### Prostanoid biosynthetic pathways in invertebrates

Until recently there was a dearth of information about how PGs are generated in invertebrates. Initially, PG generation in corals, at least, was thought to proceed via a collaborative mechanism involving 8(R) lipoxygenase and allene oxide synthase activity (e.g. Corey et al., 1987; Song and Brash, 1991). However, it is clear from a number of reports that PG generation in a wide range of invertebrates is subject to inhibition by the presence of COX inhibitors such as indomethacin, aspirin and ibuprofen (e.g. Knight et al., 1999) suggesting the existence of a COX-derived mechanism for PG biosynthesis. More recent biochemical studies using the Arctic coral *Gersemia fruticosa* have demonstrated that radiolabelled arachidonate can be converted to the unstable intermediate PGG$_2$ (Varvas et al., 1999). The cDNA that codes for a COX isozyme was subsequently cloned from this coral (Koljak et al., 2001) and the deduced amino acid sequence of the *G. fruticosa* COX revealed the presence of Ile$^{523}$ that mainly confers the specificity of this enzyme towards COX inhibitors. In COX-2 the amino acid at this position is valine (Val$^{523}$), while in all known COX-1 isozymes it is isoleucine (Gierse et al., 1996; Garavito and DeWitt, 1999). As the coral COX contains isoleucine at this position, it is insensitive to COX-2 selective inhibitors such as nimesulide but subject to inhibition by the broad-spectrum COX inhibitor, indomethacin (Koljak et al., 2001). These and other key findings on the structure of coral COX (Valmsen et al., 2001) show that COX activity probably occurs widely within all multicellular invertebrates and is therefore likely to be central in PG generation in all protostomate and deuterostomate animals. Presumably the coral COX is the forerunner of the typical vertebrate COX-1 and COX-2 isozymes. What remains unanswered, however, is at what stage in metazoan evolution did the different forms of COX appear? As bony fish have been shown to have both a constitutive COX-1 as well as an inducible COX-2 with strong sequence homology to their mammalian counterparts (Zou et al., 1999; Roberts et al., 2000) this key event in the evolution of these two isozymes probably predates the emergence of bony fish some 350 Myr ago. Whether the more primitive jawed cartilaginous fish, such as sharks and rays, and the jawless lampreys and hagfishes, express one or two isoforms of COX is currently unknown but a recent report on the cloning of
of COX cDNA from shark Squalus acanthias rectal glands has revealed the existence of a single, constitutively expressed, isoform of COX sharing some features of both COX-1 and COX-2 (Yang et al., 2002). These findings could arguably support the hypothesis that sharks may only have a single COX isoform, but this tentative conclusion remains to be investigated.

Not only has the existence of COX been shown in some corals but also potential mechanisms for the biosynthesis of the unusual PG esters have been proposed (Valmsen et al., 2001). In this, the action of COX on arachidonate leads to the generation of an unstable PG endoperoxide similar to PGH2 found in mammals but with the R rather than the S configuration at C15 (Fig. 5). Following this COX-mediated stage, the 15(R)-PGE2 formed is converted to its methyl ester and acetylated to give rise ultimately to the large amounts of stable 15R-PGA2-methyl ester and 15R-acetate-PGA2-methyl ester stored in these animals.

Recent findings by Brash and colleagues on lipoxygenases in P. homomalla may explain how clavulones and related cyclopentenone eicosanoids are formed (Boutaud and Brash, 1999; Tijet and Brash, 2002). This coral contains an unusual allene oxide synthase – lipoxygenase fusion protein. Tijet and Brash (2002) have suggested that clavulones are formed by a pathway that commences with the action of 8(R)lipoxygenase on arachidonic acid to give rise to 8R hydroperoxyeicosatetraenoic acid that is subsequently converted to allene oxide by the allene oxide synthase activity. Subsequently, this gives rise to clavulones by a method analogous to that employed in plants in the formation of jasmonic acid from linolenic acid (Tijet and Brash, 2002). This provides a much needed explanation of how clavulones and related forms may be synthesized in marine invertebrates.

Little is known of the presence of any of the other enzymes involved in the generation of classical PGs in invertebrates with the exception of PGD synthase in parasites. Since the discovery of Fusco et al. (1985) that the penetration of the human host by cercariae of Schistosoma mansoni is apparently influenced by PGs, there has been heightened interest in the possibility that both protozoan and metazoan parasites may improve their success of survival either by generation of PGs themselves or by modifying the host’s ability to generate PGs. Haemopoietic PGD synthase is a member of the sigma-class glutathione S-transferase (GST) family (Kanaoka et al., 2000). GSTs in general are multifunctional enzymes found in both invertebrates and vertebrates, and it is unlikely that all of the sigma-class forms will have PG synthase activity because some lack the amino acid residues involved in substrate (PGH2) binding (Thomson et al., 1998). Recently, however, both the sigma class GSTs from the filarial parasite Onchocerca volvulus (Sommer et al., 2003) and Schistosoma (Johnson et al., 2003) have been shown to convert PGH2 to PGD2, while in Ascaridia galli a purified GST has PGD synthase activity (Meyer et al., 1996). In the case of the O. volvulus GST (OV-GST-1), this enzyme is located at the margins of the parasite, in the cuticle and hence in a prime location to influence the host responses. Similarly, in Brugia malayi and Wuchereria bancrofti, the parasite microfilariae become coated in PGE2 following in vitro culture as a result of its generation in the parasites (Liu et al., 1992). As PGs have been shown to be involved in immune regulation in mammals and some other vertebrates (e.g. Garrone et al., 1994; Knight and Rowley, 1995) as well as in inflammation (Colville-Nash and Gilroy, 2000), the synthesis of these compounds by parasites could affect the host immune response favouring parasite survival and host penetration (Daugschies and Joachim, 2000; Noverr et al., 2003).

Insights from the Ciona genome

Ciona intestinalis is a member of the Phylum Chordata that includes the vertebrates, urochordates (sea squirts, salps) and cephalochordates (amphioxus). This deuterostome invertebrate has probably retained many of the features of the ancient chordates prior to the emergence of the ancestors of the early vertebrates. Hence, it has been a popular model animal for comparative immunologists interested in tracing the roots of the vertebrate immune system (Cooper and Parrinello, 2001) and developmental biologists who have employed ascidians, including C. intestinalis, to study gene expression during development (Jeffery, 2002). Towards the end of 2002 the draft genome of C. intestinalis was published (Dehal et al., 2002) and the sequence database established (http://genome.jgi-psf.org/ciona4/ciona4.home.html). A recent cDNA/expressed sequence tag study has also identified a number of genes expressed in the haemocytes (blood cells) of C. intestinalis (Shida et al., 2003). Previous studies on eicosanoid generation in C. intestinalis mainly focussed on the putative lipoxygenase products rather than PGs (Knight et al., 1999; Pope and Rowley, 2002). Knight et al. (1999) did, however, report that PG generation as measured by EIA was selectively inhibited by COX-2 rather than COX-1 inhibitors. They deduced from this that the constitutive form of COX expressed in C. intestinalis is COX-2 like in terms of those amino acids that

![Fig. 5. The proposed mechanism of biosynthesis of prostaglandins in the coral, Plexaura homomalla (modified from Valmsen et al., 2001).](image-url)
confer such selectivity (Gierse et al., 1996). This conclusion is borne out by the Ciona genome project where >95% sequence homology with various piscine COX-2 was found. No gene encoding for a further COX-isofrom is apparent in the current annotated database, possibly implying that only a single form of COX exists in C. intestinalis. Other interesting findings include a gene coding for PGD synthase that shows >95% homology with other glutathione-dependent PGD synthases (haemopoietic PGD synthases) and known invertebrate and vertebrate glutathione S-transferases. Homologues of the mammalian PGT transporter and the EP4 receptor for PGE2 are also annotated in the Ciona database. The current annotations fail to identify any further PG/Tx synthase genes or any of the other receptor family for prostanoids. As the database is subject to further probing such genes may be still be found, but it has been concluded by Dehal et al. (2002) that the genes missing from the current assembly are probably absent from the genome itself. The study also noted the paucity of genes coding for rhodopsin-like heterotrimeric GTP-binding protein coupled receptor family of which the PG receptors are constitutive members. While it may be premature to speculate further, it appears as if Ciona may only have one receptor type for PGs with high homology to the EP4 receptor subtype. In the apparent absence of the enzymes required for TxA2 and PGI2 (prostacyclin) generation and their respective receptors, it is tempting to suggest that such molecules are absent from Ciona and perhaps all invertebrates. Their evolutionary origins may be linked to the emergence of haemostatic mechanisms based on fibrin generation and its interaction with platelets/thrombocytes that happen with the appearance of the first vertebrates (Rowley et al., 1997).

Concluding remarks

To our knowledge, no prostanoid receptors have been cloned from any invertebrate and only in a few selected cases (e.g. corals) outside the Insecta do we have even a basic understanding of the nature of the prostanoids formed and their modes of biosynthesis. This is clearly an unsatisfactory situation if an understanding of how such molecules influence physiological events in these animals is to be achieved. The recent publication of the Ciona genome, coupled with an extensive knowledge of this organism’s developmental biology, physiology and immunobiology, makes this a key model animal for future eicosanoid research in a deuterostomate invertebrate that will dissect both the pathways for eicosanoid biosynthesis and how such molecules are involved in signalling events at the molecular level.

List of abbreviations

GST glutathione S-transferase
CNS central nervous system
COX cyclooxygenase
EIA enzyme immunoassay
HPLC high performance liquid chromatography
mPGES membrane associated PGE synthase
MS mass spectrometry
NMR nuclear magnetic resonance spectroscopy
PG prostaglandin
PGI2 prostacyclin
PGT prostaglandin transporter
RIA radioimmunoassay
TLC thin layer chromatography
Tx thromboxane

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