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Plasma-Membrane "Rosettes" are Present in the Lily Pollen Tube

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"Rosettes", putative cellulose synthesizing complexes in the PF of the plasma membrane, have now been described in a variety of objects from algae to higher plants (for a review see [1–3]), but have been claimed to be absent from pollen tubes [4].

We therefore re-examined pollen tubes of *Lilium longiflorum* for the occurrence of "rosettes". Tubes of ca. 50 μm length were cultivated and freeze-fractured as described [1, 5].

Avoiding cryoprotection, we found individual "rosettes" on the PF of the plasma membrane of lily pollen tubes (Fig. 1). They have the typical diameter of about 25 nm and consist of six particles (Fig. 1, arrows). The "rosette" frequency varies between 0 to 12 μm^{-2}, presumably depending on the distance of the membrane area from the growing tip as demonstrated for other tip growing systems [1, 6]. For moss protonema cells it could be shown that "rosette" frequency correlates well with growth rate: chloronema cells have less "rosettes" than the faster growing caulonema cells [7]. If the "rosette" frequency is related with the activity of cellulose synthesis, the pollen tubes can be expected to produce only low amounts of cellulose, the more because pollen tubes grow 10fold faster than moss caulonema cells. Correspondingly, Herth et al. [8] reported that cellulose is only a minor part of the lily pollen tube wall. The complete absence of "rosettes" in tobacco pollen tubes, described by Kroh and Knuiman [4], is presumably an artifact. Most of the pollen tubes burst during preparation, and freeze fracture studies of this object are very delicate.

The present study supports the assumption that "rosettes" are involved in cellulose synthesis, and that their frequency on the plasma membrane is an indicator for the quantity of cellulose produced.

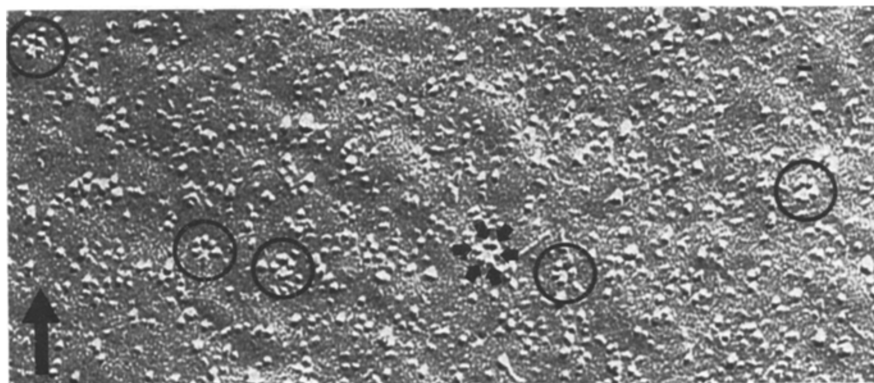

Fig. 1. Individual "rosettes" (encircled) on the PF of the plasma membrane of a lily pollen tube. The "rosette" consists of six single particles (small arrows). Big arrow: shadow direction. ×120000

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Leukotrienes in Teleost Fish Gills

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Leukotrienes (LT) are a class of polar lipids endowed with high biological activity [1]. Their formation from arachidonic acid is initiated by a 5-lipoxygenation to 5(S)-hydroperoxyeicosatetraenoic acid, which is further metabolized to the unstable LTA_4 [2]. Enzymatic hydrolysis of LTA_4 leads to LTB_4, whereas addition of glutathione at C-6 results in the formation of LTC_4 [3, 4]. This intermediate can be then transformed to LTD_4 and LTE_4 by stepwise enzymatic elimination of glutamic acid and glycine, respectively.

LTC_4, LTD_4 and LTE_4 constitute Slow-Reacting Substance of Anaphylaxis (SRS-A), an important mediator of immediate hypersensitivity. The ability to synthesize such mediators may represent the evolutionary outcome of interactions between the host and external noxious stimuli [7]; indeed, mammalian lungs, an important portal of entry to the body, are both a major source of leukotrienes and a target for their potent bronchoconstricting properties [1, 7].

Gills are a main site of gas and ion transfer in most groups of modern fish; they represent therefore a crucial contact surface between external and internal environment as well as a functional analogue of mammalian lungs.

This report describes the isolation of LTC_4, LTD_4 and LTE_4 from gill filaments of a teleost fish, *Anguilla rostrata*.

Gill filaments were incubated in teleost Ringer's solution [8] (ionic composition, g/l: NaCl 5.5; KCl 0.14; $CaCl_2$ 0.12), for 60 min at room temperature (25 °C), in the presence of arachidonic acid (30 μM), ^{14}C-arachidonic acid (10 μCi) and the Ca^{2+}ionophore

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A23187 (5 μ*M*). At the end of the incubation period the medium was removed, briefly centrifuged and analysed by reversed-phase liquid chromatography (RP-HPLC). A Nucleosil 5-C18 column was isocratically eluted at 1 ml/min with a mobile phase of methanol/water/acetic acid/ammonium hydroxide (67:33:0.08:0.04, pH 5.8). Eluted compounds were monitored with an UV detector at 280 nm, 30 s and 60 s fractions were collected and tested for radioactivity and biological activity, respectively. Leukotriene bioassay was performed on strips of guinea-pig lung parenchyma superfused with Krebs' solution (ionic composition, g/l: NaCl 6.9; KCl 0.35; KH_2PO_4 0.16; $MgSO_4 \cdot 7\ H_2O$ 0.29; glucose 1.00; $NaHCO_3$ 2.1; $CaCl_2$ 0.275; temperature: 37 °C) at a constant rate of 5 ml/min, as described by Piper and Samhoun [9]. The fractions purified by RP-HPLC were dried under a stream of Ar at 37 °C, reconstituted in warm Krebs' and 90% of each of them was injected into the superfusing solution. Quantitation of the material was carried out using synthetic LTC_4 as a standard.

Figure 1 shows UV absorbance profile at 280 nm, content of radioactivity and biological activity on lung strip of eluted products. Peaks I, II and III coeluted with synthetic LTC_4, LTD_4 and LTE_4, respectively, showed incorporation of labeled arachidonic acid and elicited contractions on the lung strip. These contractions were inhibited by compound FPL 55712, an SRS-A antagonist. It is therefore reasonable to conclude that peaks I, II and III represent LTC_4, LTD_4 and LTE_4, respectively. These findings support the hypothesis that the capacity to synthesize leukotrienes represents a common feature in the biochemical evolution of vertebrate respiratory organs.

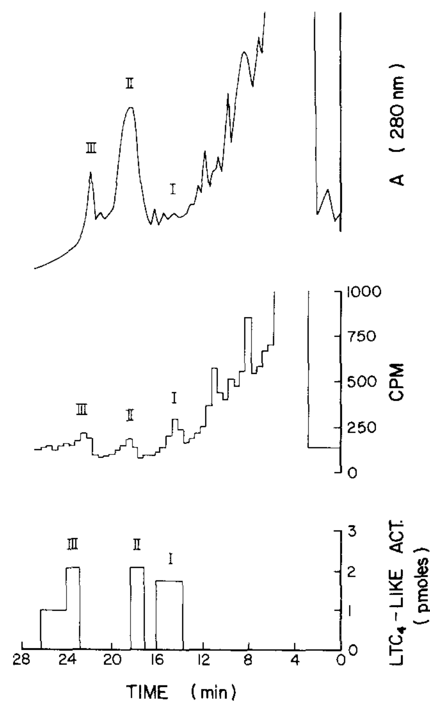

Fig. 1. The upper panel shows an RP-HPLC chromatogram obtained after injection of unextracted medium from incubation of gill filaments of *Anguilla* with labeled arachidonic acid and the Ca^{2+} ionophore A23187. The incorporation of label in the eluted products is given in the middle panel. The lower panel shows the biological activity of the eluted fractions on the guinea-pig lung strip. A drift in the retention times was consistently observed with injection of larger volumes of the unextracted incubation medium and can account for the slight misalignment of peak II

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Increased UDP-Glucuronyltransferase in Putative Preneoplastic Foci of Human Liver after Long-term Use of Oral Contraceptives

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Several different characteristic stages can be distinguished in chemical carcinogenesis of rat liver [1]. After initiation by the activated carcinogen focal preneoplastic lesions can be identified histochemically, for example by an increased content of glycogen [2], a reduction of cytoplasmic and canalicular ATPase activity [3] and a reappearance of the fetal enzyme γ-glutamyltranspeptidase (γ-GT) [4]. Focal lesions may develop into hyperplastic nodules, which appear to possess two options, remodeling or further development to hepatocellular carcinomas [5]. One of the characteristics of preneoplastic hepatocytes seems to be a relative resistance to the cytotoxicity of chemical carcinogens [6]. This resistance is probably due to an altered pattern of drug-metabolizing enzymes. Cytochrome P-450 dependent reactions, often involved in the activation of carcinogens to ultimate carcinogens, are decreased in hyperplastic liver nodules, whereas drug-metabolizing enzymes mostly involved in the inactivation of drug metabolites, are markedly increased [7]. In the presence of cytotoxic agents this resistance results in a preferential damage of normal liver tissue and a compensatory proliferation of preneoplastic foci [8].

Previously we could show an increased activity of the mostly inactivating enzyme uridine-diphosphate-glucuronyl-