Survey of the Taxonomic and Tissue Distribution of Microsomal Binding Sites for the Non-Host Selective Fungal Phytotoxin, Fusicoccin

Christiane Meyer[a], Kerstin Waldkötter[a], Annegret Sprenger[a], Uwe G. Schlösser[b], Markus Luther[c], and Elmar W. Weiler[a]

[a] Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität, D-44780 Bochum, Bundesrepublik Deutschland
[b] Pflanzenphysiologisches Institut (SAG) der Universität, D-37073 Göttingen, Bundesrepublik Deutschland
[c] KFA Jülich, D-52428 Jülich, Bundesrepublik Deutschland

Z. Naturforsch. **48c**, 595–602 (1993); received June 4/July 13, 1993

Vicia faba L., Fusicoccin-Binding Sites, Taxonomic Distribution, Tissue Distribution, Guard Cell Protoplasts

The recent identification of the fusicoccin-binding protein (FCBP) in plasma membranes from monocotyledonous and dicotyledonous angiosperms has opened the basis for an elucidation of the toxin's mechanism(s) of action and indicated a widespread occurrence of the FCBP in plants. Results of a detailed taxonomic survey of fusicoccin-binding sites are reported. Binding sites were not found in prokaryotes, animal tissues, fungi and algae including the most direct extant ancestors of the land plants *(Coleochaete)*. From the *Psilotales (Psilophytatae)* to the monocotyledonous angiosperms, all taxa analyzed possessed high-affinity microsomal fusicoccin-binding sites. A heterogeneous picture emerged for the *Bryophyta. Anthoceros crispulus (Anthocerotae)*, the only hornwort available to study, lacked fusicoccin binding. Within the *Hepaticae* as well as the *Musci*, species lacking and species exhibiting toxin binding were found. The binding site thus seems to have emerged very early in the evolution of the land plants. The tissue distribution of fusicoccin-binding sites was studied in *Vicia faba* L. shoots. All tissues analyzed showed fusicoccin binding, although not to the same extent. On a per-cell basis, guard cells were found to contain, compared to mesophyll cells, a nine-fold higher number of binding sites. Based on cell surface area, the site density is by a factor of 32 higher in guard cells than in mesophyll cells. Tissue specific expression of the binding sites is suggested by these findings.

Introduction

The perception of signal molecules at the plasma membrane of higher plants has recently been studied with increased emphasis. Whereas the occurrence of hormone receptors at the plasma membrane is still a subject of debate, recent evidence strongly suggests that higher plants perceive certain signalling molecules at the plasma membrane (*e.g.* [1]). One of the best-characterized plasma membrane systems with receptor-like properties is the binding protein (FCBP) for the *Fusicoccum amygdaly* wilt-inducing toxin, fusicoccin (FC). The binding site is probably involved in the mediation of most, if not all, FC effects on plant cells, the most immediate being the stimulation of apoplastic acidification. The FCBP has been characterized in detail from oat root tissue [2], broadbean leaf [3, 4], *Arabidopsis thaliana* [5] and *Corydalis sempervirens* cell suspension cultures [6]. The purified FCBP of *Commelina communis* consists of two polypeptides of 30.5 and 31.5 kDa apparent molecular mass [7], and photoaffinity labelling experiments suggest a similar situation in broadbean and *Arabidopsis* [4, 5].

These studies have revealed remarkably constant properties of all the FCBPs characterized so far, suggesting a widespread occurrence and evolutionary conservation of FC-binding sites. However, a systematic study of FCBP distribution in the plant kingdom is not available and only a single report deals with a survey of FC-induced H^+/K^+ exchange in a limited number of species of higher and lower plants [8]. We therefore examined the taxonomic and tissue distribution of FC-binding sites. The results of this study are reported here.

Abbreviations: FC, fusicoccin; FCBP, fusicoccin-binding protein; GCP, guard cell protoplast; MCP, mesophyll cell protoplast.

Reprint requests to Prof. Dr. Elmar W. Weiler.

Verlag der Zeitschrift für Naturforschung, D-72072 Tübingen
0939–5075/93/0700–0595 $ 01.30/0

Materials and Methods

Higher plants

V. faba cv. "Osnabrücker Markt" was grown from May to September 1989 in standardized field plots at the Botanical Garden of the Ruhr-Universität Bochum. Plant development was classified according to the standard nomenclature [9]. The most relevant growth stages will be detailed in the experimental section (*cf.* Table V).

Leaf samples of angiosperms, gymnosperms and pteridophytes were, unless otherwise stated, collected during the growth season 1989 from the Botanical Garden of the Ruhr-Universität. Samples of healthy leaves were collected on ice and, unless processed immediately, were frozen in liquid nitrogen and stored at −80 °C for a maximum of two weeks. *Anemia phyllitidis* was a kind gift of Prof. Dr. R. Schraudolf, Ulm, and *Psilotum nudum* plants were generously made available by Prof. Dr. H.-J. Schneider-Pötsch, Cologne.

Bryophytes

Mosses were, unless stated otherwise, obtained as a gift from Prof. Dr. W. Hartung, Würzburg, and were grown on soil substrates. The thalli were thoroughly cleaned and rinsed with water prior to further processing. Sterile gametophytes of *A. crispulus* were generously provided by Prof. Dr. H. Binding, Kiel, and were maintained on agar-solidified B5 medium [10] containing 0.5% sucrose and no phytohormones. The thalli were grown in short days (8 h photoperiod, PAR 67 μE m^{-2} s^{-1}) at 20 °C ± 2 °C. *Marchantia polymorpha* thallus was grown sterile on agar containing 100 mg l^{-1} each of K_2HPO_4, $CaCl_2 \cdot 6\,H_2O$, $MgSO_4 \cdot 7\,H_2O$, 200 mg l^{-1} NH_4NO_3 and 5 mg l^{-1} $FeCl_2$, pH 3.5 (constant weak light, 4.6 μE m^{-2} s^{-1}, 24 °C ± 2 °C). *Ceratodon, Mnium* and *Leptobryum* species were provided as sterile flask cultures by Prof. Dr. E. Hartmann, Berlin. The *Scapania* and *Plagiochila* samples were obtained as sterile cultures from Prof. Dr. E. Becker, Saarbrücken, and *Polytrichum commune, Dicranium scoparium, Racomitrium canescens* and *Rhytidiadelphus squarrosus* were collected and made available by Prof. Dr. R. Mues, Saarbrücken.

Algae

Cladophora, Codium, Fucus, Laminaria, Ceramium and *Chondrus* species were locally collected by the Biologische Anstalt Helgoland and shipped in sea water. *Chlamydomonas* was grown as described [11]. *Chlorella, Chlorococcum, Scenedesmus* and *Monoraphidium* were grown in N8 Medium [12] with microelements according to Payer and Trültsch [13]. The brown and red algae as well as *Tribonema, Apatococcus, Coleochaete, Klebsormidium, Raphidonema* and *Stichococcus* were cultivated as described in [14]. The algae were grown to exponential phase and then harvested. *Chara corallina* was kindly provided by Prof. Dr. W. Hartung, Würzburg. Some algae cultures grown by one of us (U.S.) for our experiments were from the collection of algae strains at the University of Göttingen (SAG) carrying the following strain designations: *Raphidonema longiseta* (SAG 470-1), *Tribonema aequale* (SAG 200.80), *Dictyota dichotoma* (SAG 207.80), *Ectocarpus siliculosus* (SAG 63.81), *Antithamnion sp.* (SAG 95.79), *Chlamydomonas reinhardtii* (SAG 83.81), *Chlorococcum hypnosporum* (SAG 213-6), *Apatococcus lobatus* (SAG 34.83), *Coleochaete scutata* (SAG 50.90).

Fungi

All fungi tested were grown as pure cultures under standard conditions as specified in the ATCC manuals.

Bacteria

Escherichia coli JM83 was grown in bacto tryptone (10 g l^{-1}), yeast extract (5 g l^{-1}), and NaCl (10 g l^{-1}) at 37 °C and *Agrobacterium tumefaciens* C58, strain P1145, supplied by Prof. Dr. C. I. Kado, Davis, U.S.A., was grown in bacto peptone (10 g l^{-1}), NaCl (5 g l^{-1}) at 30 °C.

Animal tissues

All animal tissues analyzed were obtained fresh and using standard techniques from laboratories of the Departments of Biology and Medicine, Ruhr-Universität Bochum.

Protoplast preparation

Mesophyll cell protoplasts (MCP) and guard cell protoplasts (GCP) were prepared exactly as described [15]. When digestions were made in the presence of FC (3 μM), the following modifications were introduced: K^+ was omitted from all solutions; the mannitol concentration was increased to

0.4 M (step 1), 0.5 M (step 2) and 0.6 M (step 3) of the procedure of Key and Weiler [15]. Additionally, epidermal peels were pre-incubated in K^+-free, 0.4 M mannitol in order to lower the amount of apoplastic K^+, before being transferred to the enzyme solutions.

Preparation of microsomes

Microsomal preparations were obtained from the various tissues using standard techniques. For a detailed description of the method used for the higher plants and algae, see [3]. Unicellular algae were homogenized in a mortar in the presence of aluminum oxide; other tissues were homogenized in a Waring blendor. *Chlamydomonas* was processed according to Spalding and Jeffrey [16], *Phycomyces* according to [17], *Saccharomyces* as described by Gaber *et al.* [18], the other fungi according to [19]. Bacterial membranes were prepared according to Kaback [20]. Microsomes from animal tissues were prepared as described in [21].

Assays for the determination of FC-binding

The high affinity microsomal binding sites for the toxin were probed with the radioligand [^{3}H]-9′-norfusicoccin-8′-alcohol (spec. act. 1.05×10^{15} Bq mol^{-1}), used at 10 nM concentration [3]. All data were corrected for unspecific binding, and all analyses were performed in triplicate using 30 to 200 µg of membrane protein per assay. The procedure has been described in detail [3]. The separation of unbound from bound radioligand was either achieved by centrifugation [3] or by the polyethyleneimine filter assay [22]. The actual technique used is specified in the tables. Under the conditions described, the limit of detection of FC-binding sites is 0.05 pmol (mg of protein)$^{-1}$.

Results

While the host range of *F. amygdali* is narrow (almond and peach), the wilt-inducing toxin, FC, is also active in species not colonized by the fungus. The FCBP has been characterized in detail in only few species (for references, see introduction), and a survey of 105 species in tissue culture had revealed 33 species lacking FC-binding (E. Oelgemöller and E. W. Weiler, unpublished). This survey included *Gingko biloba*, a species which reportedly responds to the toxin with increased apoplastic acidification [8]. Plant tissue cultures may, however, loose certain cellular functions through degenerative mechanisms. On the other hand, hy-

Table I. Analysis of FC-binding in prokaryotic and lower eukaryotic organisms.

Taxon	Number of species analyzed	positive
Eubacteria	2[a]	0
Mycota		
Oomycetes	1[b]	0
Zygomycetes	1[c]	0
Ascomycetes	19[d]	0
Deuteromycetes	1[e]	0
Basidiomycetes	2[f]	0
Heterokontophyta		
Bacillariophyceae	2[g]	0
Xanthophyceae	1[h]	0
Phaeophyceae	4[i]	0
Rhodophyta	3[j]	0
Chlorophyta		
Chlorophyceae	10[k]	0
Charophyceae	1[l]	0

[a] *Agrobacterium tumefaciens* C58, *Escherichia coli* JM83.
[b] *Phytophthora megasperma* Drechsler.
[c] *Phycomyces blakesleeanus* Burgeff.
[d] *Alternaria alternata* (Fries) Keissler, *Aspergillus caespitosus* Raper et Thom, *A. flavus* Link, *A. fumigatus* Fresenius, *A. fresenii* Subram, *A. ochraceus* Wilhelm, *Fusarium graminearum* Schwabe, *Gibberella fujikuroi* (Sw.) Wr., *G. zeae* Schweinitz, *Neurospora crassa* Shear et Dodge, *Penicillium camemberti* Thom, *P. commune* Thom, *P. ochrachloron* Biourge, *P. palitans* Westling, *P. paxilli* Bainier, *P. puberulum* Bainier, *P. verrucosum cv. cyclopium* (Westling) Samson *et al.*, *P. verruculosum* Peyronel, *Saccharomyces cerevisiae* Meyen et Hansen.
[e] *Fusicoccum amygdali* Del.
[f] *Pleurotus* spec., *Polyporus ciliatus* Fries.
[g] *Monoraphidium minutum* (Nägeli) Korarkova-Legnerova, *Raphidonema longiseta* Visher.
[h] *Tribonema aequale* Pascher.
[i] *Dictyota dichotoma* (Hudson) Lamouroux, *Ectocarpus siliculosus* (Dillwyn) Lyngbye, *Fucus serratus* L., *Laminaria digitata* (L.) Lamouroux.
[j] *Antithamnion* spec. Nägeli, *Ceramium rubrum* (Hudson) C. Agardh., *Chrondrus crispus* (L.) Lyngbye.
[k] **Volvocales:** *Chlamydomonas reinhardtii* Degenhard; **Chlorococcales:** *Chlorella fusca* Shihira et Krauss, *Chlorococcum hypnosporum* Starr, *Scenedesmus obliquus* (Turpin) Kützing; **Ulotrichales:** *Stichococcus bacillaris* Nägeli; **Chaetophorales:** *Apatococcum lobatus* (Chodat) Boye-Peterson, *Coleochaete scutata* de Brebisson; **Cladophorales:** *Cladophora rupestris* (L.) Kützing; **Siphonales:** *Codium fragile* (Sur) Harriot, *Klebsormidium* spec.
[l] *Chara corallina* L.

Table II. Occurrence of FC-binding sites in *Bryophyta*. Remarks: 1, FC-binding determined by centrifugation assay; 2, FC-binding determined by PEI filter assay; 3, fresh material available; 4, frozen material available; 5, sterile thalli available.

Species	FC-binding [pmol (mg of protein)$^{-1}$]	Remarks
Anthoceropsida		
Anthoceros crispulus (Mont.) Duin	0	1, 2, 3, 5
Marchantiopsida		
Marchantiidae		
Conocephalum conicum (L.) Lindb.	0.21	2, 3
Exormotheca holstii Steph.	0	2, 3
Exormotheca megastomata Marquand.	0.14	2, 3
Marchantia berteroana L. et L.	0.07	2, 3
Marchantia polymorpha L.	0.62	1, 3, 5
Oxymitra paleacea Bisch.	1.50	2, 3
Plagiochasma rupestre (Forst.) Steph.	0.8	2, 3
Riccia fluitans L.	0.32	2, 3
Riccia stricta Lindenb.	0	2, 3
Riccia albornata Volk et Perold	0.21	2, 3
Ricciocarpus natans (L.) Corda	0.41	2, 3
Jungermanniidae		
Scapania nemorea (L.) Grolle	0	2, 3, 5
Plagiochila adiantoides Carl.	0.2	2, 3, 5
Targionia hypophylla L.	0.11	2, 3
Bryopsida		
Ceratodon purpureus (Hedw.) Brid.	0.74	2, 4
Leptobryum pyriforme (Hedw.) W. Wils.	0.19	2, 4
Mnium spinosum (Voit.) Schwaegr.	0.17	2, 4
Polytrichum commune Hedw.	0.11	2, 3
Dicranum scoparium Hedw.	0	2, 3
Racomitrium canescens (Hedw.) Brid.	0	2, 3
Rhytidiadelphus squarrosus (Hedw.) Warnst.	0	2, 3

potheses have been proposed for FC action which do not require the involvement of an FC receptor structure (*e.g.* [23]). Therefore, it was of considerable importance to analyze the occurrence of FC-binding sites in differentiated tissues of a representative number of species of different taxonomic position. Our study includes 99 species of angiosperms, 8 species of gymnosperms, 14 ferns, 18 mosses, 21 algae, 24 fungi, two bacteria and several animal tissues. The results are compiled in Tables I to IV. While the prokaryotes and algae were negative with respect to microsomal FC-binding (Table I), all of the 121 species of higher plants analyzed possessed high-affinity microsomal binding sites for FC (Table III). The situation within the *Bryophyta* was heterogeneous (Table II), and species lacking as well as species exhibiting high affinity FC-binding were found within the *Musci* and within the *Hepaticae*. The animal tissues tested lacked FC-binding sites (Table IV).

The tissue distribution of the FCBP was analyzed in detail in *V. faba* shoots, because tissues can readily be prepared in sufficient quantities, and the FCBP from this species has been characterized thoroughly and partially purified [3, 4]. An analysis of FC-binding in roots was only carried out at the seedling stage. It was found that the abundance of the binding sites in primary roots of 4 d old seedlings was *ca.* 0.5–0.8 pmol (mg of microsomal protein)$^{-1}$, *i.e.* less than half the level determined for shoot, and especially leaf, tissue. As can be seen from Table V, FC-binding sites were found in all shoot tissues analyzed and at all stages of development. Leaves and influorescences generally showed a higher abundance of FC-binding as compared to the stem. Two significant developmental patterns were noted (*cf.* Table V): (i) FCBP abundance markedly declined during fruit development, both in the pods and in the seeds. This decline was more pronounced in the seeds, which

Table III. Occurrence of FC-binding in higher plants.

Taxon	Number of species		FC-binding [pmol (mg of protein)$^{-1}$]	
	analyzed	positive	minimum observed	maximum observed
Pteridophyta				
Psilotopsida	1[a]	1	–	0.80
Lycopodiopsida	3[b]	3	0.10	1.36
Equisetopsida	1[c]	1	–	4.00
Pteridopsida	9[d]	9	0.09	0.77
Spermatophyta				
Gymnospermae				
Gingkoopsida	1[e]	1	–	0.14
Gnetopsida	1[f]	1	–	0.06
Pinopsida	5[g]	5	0.04	0.18
Cycadopsida	1[h]	1	–	0.14
Angiospermae				
Monocotyledonae				
Alismatidae	4	4	0.10	0.34
Liliidae	15[i]	15	0.09	1.80
Arecidae	2[j]	2	0.11	1.82
Dicotyledonae				
Magnoliidae	6	6	0.14	1.00
Ranunculidae	4	4	0.03	2.10
Caryophyllidae	7	7	0.04	1.08
Hamamelididae	13	13	0.16	0.93
Rosidae	20	20	0.07	1.50
Dilleniidae	16	16	0.06	0.84
Lamiidae	10	10	0.20	1.23
Asteridae	2	2	0.60	1.30

[a] *Psilotum nudum* L.
[b] *Lycopodium squarrosum* G. Forst, *Isoetes lacustris* L., *Selaginella vogelii* Spring.
[c] *Equisetum hyemale* L.
[d] *Anemia phyllitidis* L. Sw., *Azolla* spec., *Cyathea australis* (R. Br.) Domin, *Marsilea quadrifolia* L., *Ophioglossum pendulum* L., *Osmunda regalis* L., *Pilularia globulifera* L., *Polypodium aureum* L., *Polypodium australe* L.
[e] *Gingko biloba* L.
[f] *Gnetum gnemon* L.
[g] *Abies homolepis* Sieb et Zucc., *Calocedrus decurrens* (Torr.) Florin, *Metasequoia glyptostroboides* Hu et Cheng, *Tsuga canadensis* (L.) Carr., *Taxus baccata* L.
[h] *Cycas* spec.
[i] including *Tillandsia usneoides* (L.) L.
[j] including *Lemna gibba* L.

Table IV. Animal tissues analyzed for FC-binding.

Species	Tissue	FC-binding
Oryctolagus cuniculus f. *domestica* L.	brain	n.d.
Zoarces viviparus L.	heart	n.d
Mus musculus L.	liver	n.d.
Rattus norvegicus Berkenhout	liver	n.d.
	kidney	n.d.

n.d. = not detectable.

showed highest levels of FC-binding at a seed weight of 0.02–0.05 g (growth stage 74) and a steady decline during the phase of seed-filling up to stage 78 and further during the phase of seed dehydration and fruit ripening (not shown). At this stage, FC-binding became virtually undetectable. (ii) Concomitant with the phase of most intense fruit development and especially with the seed-filling period (stage 76–84), the level of FC-binding significantly increased in the leaves (stages 74 to

Table V. Distribution of FC-binding sites in microsomal membranes from different shoot tissues of *Vicia faba*. FC-binding is given as pmol (mg of protein)$^{-1}$. The growth stages were determined according to von Kittlitz *et al.* [9].

Growth stage[a] Tissue	57	64–66	Growth stage[a] Tissue	74	76	84
Sepals	3.2	2.8	Pods	2.3	1.7	1.1
Petals	1.1	2.3				
Stamina	3.1	2.0	Seeds	1.1	0.5	0.3
Pistils	1.7	1.7				
3rd Leaves	2.1	1.4	3rd Leaves	1.2	6.6	4.9
3rd Internodes	2.2	1.4	3rd Internodes	0.7	0.4	1.2
4th Leaves	3.1	1.3	4th Leaves	1.0	4.7	3.9
4th Internodes	2.9	1.3	4th Internodes	0.9	0.9	1.8

[a] Growth stage classification: 57, early flowering stage, petals just visible on most apical flowers; 64, full anthesis of three inflorescences per plant; 74, pods visible on three nodes per plant, pod length 3–5 cm; 76, pods on five nodes per plant, pod length 9–11 cm; 84, early fruit ripening stage, one third of all pods ripe.

76). The average increase measured was over five-fold in leaves from the third and fourth node at stage 76 as compared to the same set of leaves at stage 74, *i.e.*, it occurred just prior to the beginning of seed-filling. In the corresponding internodes, no changes in FC-binding were detected (*cf.* Table V).

To gain further insights into FC-binding in different leaf tissues, mesophyll *vs.* guard cells were analyzed. For this purpose, protoplasts were used which can be prepared in high purity. Even when up to 1% BSA was included in the digestion solutions as a scavenger protein, the FC-binding sites could not be recovered, or only to a small and variable extent (not shown). Treatment of plasma membrane vesicles from leaf tissues of several species with different commercial cellulases, likewise, resulted in rapid loss of those binding sites accessible to the enzymes (not shown), due to proteolytic degradation of the sites. It was found that the sites could be protected effectively by including 3 μM FC in the enzyme solutions during digestions. However, this required drastic readjustments of the experimental protocol in order to rescue the GCP which, when prepared in the presence of FC without precautions, burst immediately after release due to a rapid osmotic influx of water into the cells. This could be prevented by omitting potassium from all solutions and by simultaneously raising the level of mannitol in the incubation media (see Materials and Methods). The MCP could be prepared in the presence of FC without such adjustments. The abundance of the FC-binding sites was estimated after transfer of the protoplast preparations to FC-free media and exchange of bound FC with radiotracer, followed by preparation of the microsomes. As can be seen from Table VI, the

Table VI. FC-binding sites on guard cell and mesophyll cell protoplasts from leaves of three week old *V. faba* (growth stage 23–25, third to forth leaf fully developed).

Parameter	Guard cell protoplasts	Mesophyll cell protoplasts
Cell size		
diameter [μm]	17.7 ± 1.2 (n = 7)[a]	32.7 ± 2.1 (n = 9)
surface area [μm^2]	985	3360
FC-binding		
[pmol (mg of protein)$^{-1}$]	7.6 ± 2.1 (n = 7)	2.0 ± 0.6 (n = 9)
sites per cell	630,000 ± 38,700	70,000 ± 2,100
site density at plasmalemma	640 μm^{-2}	20 μm^{-2}

[a] n = number of independent experiments.

abundance of FC-binding sites is considerably higher, on a per cell and especially on an area basis, in GCP than in MCP showing that the apparent level of FCBP expression must be quite different for these tissues.

Discussion

The non-host specific fungal phytotoxin, fusicoccin, has proven a valuable tool in studies on membrane energetization and growth regulation in plants (for review [24]). The toxin receptor resides in the plasma membrane and has been identified by a range of techniques [2–7]. It is the first plasma membrane receptor of a plant that could be purified to apparent homogeneity [7]. The primary cellular target of FC-action is the H^+-translocating ATPase, and the toxin activates the ATPase by a mechanism involving the enzyme's C-terminal autoinhibitory domain [25; C. Oecking and E. W. Weiler, submitted]. However, the transduction chain between the FCBP and the proton pump is unknown. Furthermore, the physiological role of the FCBP is still not resolved.

The present study was carried out in order to understand the taxonomic and tissue distribution of the FCBP. FC stimulation of H^+/K^+-exchange has been reported to occur in both, angiosperms and gymnosperms, the pteridophyte *Marsilea quadrifolia* and two bryophytes (*Marchantia polymorpha* and *Plagiotecium* spec.), but was not found in bacteria, fungi and algae [8] while a marginal effect was observed in an undetermined species of *Chara* [8]. We found, in close agreement with this report (*cf.* Tables I to IV), microsomal binding sites for FC generally present in all taxa of tracheophytes analyzed while they were clearly absent in the algae including the two taxa generally placed closest to the ancestral precursors of the land plants *(Chara, Coleochaete)*. The significance of a marginal FC-effect on H^+/K^+-exchange in a *Chara* spec. [8] must thus be treated with caution. Interestingly, the groups of bryophytes gave a heterogenous picture, including, within the *Bryopsida* as well as the *Marchantiopsida,* both, FCBP negative and FCBP positive, members. The only available species of the *Anthoceropsida* was FCBP negative. This picture is compatible with recent cladistic models favouring a monophyletic origin of the land plants from an algal relative of *Chara* or *Coleochaete*. Evolution of the FCBP must then have occurred very early in the history of land plants, prior to the divergence of the bryophytes and the tracheophytes. Lack of FCBP activity in some, but not all, of the bryophytes, if not due to an as yet developmental regulation (considered unlikely, *vide infra*), thus can be taken as another evidence for the theory of regression through progressive and polyphyletic reduction of bryophytes from the ancestral propsilophyte progenitor of the bryophytes and the vascular plants [26, 27]. We propose that the FCBP evolved immediately after or during the colonization of the terrestrial habitat in conjunction with the development of other adaptations to terrestrial life (*e.g.* vascular system, stomata).

A key function of the FCBP for the vascular plants is also suggested by the finding that, (i) all organs and developmental stages of a plant except the ripe seed express the FCBP (*cf.* Table V). This finding agrees with the reported sensitivity of these plant organs towards FC [8]. (ii) Both a developmental and a tissue specific regulation of its abundance is suggested by the finding that (a) FCBP activity rises several-fold in leaf tissue of *V. faba* at the seed-filling stage, when leaves form the pre-dominant source tissues of the plant and (b) FCBP abundance in guard cells is considerably higher than in mesophyll cells (*cf.* Table VI). Since all functional (*i.e.* FC-binding) FCBP is associated with the plasmamembrane and the cell surface area of the protoplasts used in our study can be estimated with little error, we were able to calculate a ≥ 30 times higher surface density of the FCBP in a guard cell as compared to a mesophyll cell protoplast, proving tissue specific expression and/or functionality of the FCBP. Clearly, the guard cell lends itself especially to work towards the molecular details of FC action, inasmuch as guard cells can be prepared in relatively large quantities and high purity. (iii) Biochemical data from several laboratories have shown the FCBP to be highly conserved structurally and with respect to its binding characteristics among both, monocotyledonous and dicotyledonous plants [2–7]. This, together with its apparent universal occurrence in all tissues of the land plants, suggests an important function of the FCBP in the differentiated plant. This function is likely to be regulatory because we found ([6] and unpublished results) that plant cell suspension cultures may grow unimpaired without a function-

al (*i.e.* FC-binding) FCBP. Another conclusion from this finding would be that the FC-binding property is unrelated to the *in vivo* function of the FCBP. While endogenous, FC-like factors have been reported [28, 29], none of them has so far been purified or thoroughly characterized. Clearly, in order to properly assess the function(s) of this interesting membrane receptor, a conclusive answer to the question of an endogenous ligand as well as the study of the FCBP with the tools of molecular biology are required.

Acknowledgements

Financial support from the Deutsche Forschungsgemeinschaft, Bonn, and Fonds der Chemischen Industrie, Frankfurt (literature provision), is gratefully acknowledged. The authors are especially grateful to all colleagues who generously made plant material available for this study. Supplementary data material is available from the corresponding author upon request.

[1] E. G. Cosio, H. Pöpperl, W. E. Schmidt, and J. Ebel, FEBS Letters **175,** 309–315 (1988).
[2] A. H. de Boer, B. A. Watson, and R. E. Cleland, Plant Physiol. **89,** 250–259 (1989).
[3] M. Feyerabend and E. W. Weiler, Planta **174,** 115–122 (1988).
[4] M. Feyerabend and E. W. Weiler, Planta **178,** 282–290 (1989).
[5] C. Meyer, M. Feyerabend, and E. W. Weiler, Plant Physiol. **89,** 692–699 (1989).
[6] S. Schulz, E. Oelgemöller, and E. W. Weiler, Planta **183,** 83–91 (1990).
[7] C. Oecking and E. W. Weiler, Eur. J. Biochem. **199,** 685–689 (1991).
[8] E. Marré, Biol. Cellulaire **32,** 19–24 (1978).
[9] E. von Kittlitz, A. von Kries, U. Meier, R. Stülpnagel, and I.-M. Wittstock, Merkblatt Nr. 27/10 (Braunschweig) der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Aco Druck GmbH, Braunschweig 1984.
[10] O. L. Gamborg, R. A. Miller, and K. Ojima, Exp. Cell Res. **50,** 151–158 (1967).
[11] N. Amrhein and P. Filner, Proc. Nat. Acad. Sci. U.S.A. **70,** 1099–1103 (1973).
[12] C. J. Soeder, G. Schulze, and D. Thiele, Arch. Hydrobiol./Suppl. **23,** 127–171 (1967).
[13] H. D. Payer and U. Trültzsch, Arch. Microbiol. **84,** 43–59 (1972).
[14] U. G. Schlösser, Ber. Deutsch. Bot. Ges. **95,** 181–216 (1982).
[15] G. Key and E. W. Weiler, Planta **176,** 472–481 (1988).
[16] M. H. Spalding and M. Jeffrey, Plant Physiol. **66,** 353–359 (1980).
[17] T. Rausch, S. Stoffel, and W. Hilgenberg, Plant Physiol. **88,** 1163–1167 (1988).
[18] R. F. Gaber, C. Styles, and G. R. Fink, Molec. Cell. Biol. **8,** 2848–2859 (1988).
[19] J. L. Gianinni, J. S. Holt, and D. P. Briskin, Arch. Biochem. Biophys. **226,** 644–649 (1988).
[20] H. R. Kaback, Meth. Enzymol. **31,** 99–120 (1974).
[21] J. M. Armstrong and J. D. Newman, Arch. Biochem. Biophys. **238,** 619–628 (1985).
[22] R. F. Bruns, K. Lawson-Wendling, and T. A. Pugsley, Anal. Biochem. **132,** 74–81 (1983).
[23] A. Hager and I. Moser, Planta **163,** 391–400 (1985).
[24] E. Marré, Annu. Rev. Plant Physiol. **30,** 273–288 (1979).
[25] F. Johansson, M. Sommarin, and C. Larsson, Plant Cell **5,** 321–327 (1993).
[26] W. Frey, Jahrb. Syst. Bot. **111,** 433–456 (1990).
[27] R. Wyatt and A. Stoneburner, Biosystematics of bryophytes. An Overview, in: Plant Biosystematics (W. F. Grant, ed.), pp. 519–542, Academic Press Canada, Montreal 1984.
[28] P. Aducci, G. Crosetti, R. Federico, and A. Ballio, Planta **148,** 208–210 (1980).
[29] B. E. Sultanbaev, M. K. Gilmanoo, N. Y. Abramycheva, V. V. Stolpakova, and L. M. Ginodman, Plant Sci. **88,** 19–24 (1993).