21 Control of Plant Development by Hydro-Electrochemical Signal Transduction: a Means for Understanding Photoperiodic Flower Induction

Edgar Wagner, Lars Lehner, Justyna Veit, Johannes Normann, Marco Vervliet-Scheebaum, Jolana T.P. Albrechtová

21.1 Introduction: photoperiodic flower induction

The hypothesis that flowering involves a specific stimulus is based upon the demonstration that (a) in photoperiodism the flowering response depends upon the day length conditions given to the leaves, whereas the response occurs in the apices, and that (b) a floral stimulus can be transmitted via a graft union from an induced partner (donor) to a non-induced one (receptor). Transmission of the floral stimulus by grafting has been demonstrated within various photoperiodic response types, as well as between different photoperiodic response types in interspecific and intergeneric grafts. The physiological evidence for a floral stimulus is clear-cut, but up to now the nature of the stimulus has remained obscure (Bernier 1988).

The specific kind of photoperiodic behavior depends very much on the exact environmental conditions, as was shown for four different North American ecotypes of *Chenopodium rubrum* (Tsuschiya and Ishiguri 1981). The southern ecotypes display an obligate short-day behavior under white (W), red (R) and blue (B) light. The most northern ecotype is day neutral in B and W and has an amphiphotoperiodic response in R light. Another northern ecotype has an amphiphotoperiodic response in B and a short-day response in W and R light. The amphiphotoperiodic response in B is modified to day neutral by changing the temperature from 20 to 12 °C. These data clearly indicate that photoperiodic behavior is extremely flexible in adapting to specific environmental conditions.

Irrespective of the flexibility of plants in modifying their photoperiodic behavior in adapting to specific environmental conditions as just mentioned, the following essentials of the photoperiodic reaction have to be kept in mind as a basis for further considerations:

(a) Short-day (SDP) and long-day plants (LDP) show opposite reactions to a given photoperiod.
(b) Reactions result from coincidence or non-coincidence of light and dark phases of the photoperiod with corresponding phases of an endogenous circadian rhythm and the main photoreceptors are the plant sensory

University of Freiburg, Institute of Biology II, Schänzlestr. 1, 79104 Freiburg, Germany

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pigment systems phytochrome and cryptochrome. Circadian rhythm and photoreceptors have the same properties in SDP and LDP.

(c) Critical photoperiodic induction produces irreversible changes in the leaves of SDP and LDP leading to a common state both in SDP and LDP, as proven by grafting experiments. There is no difference between SDP and LDP in their response towards a common inductor from a grafted leaf from an induced short- or long-day plant.

Analyzing the kinetics of change at the shoot apical meristem (SAM) during flower initiation can give hints on the mechanism(s) of signal transduction from leaves to SAM during photoperiodic flower induction.

21.2 Model system *Chenopodium*: induction of flowering from physiology to molecular biology

The model system *Chenopodium spec*. had been established to study photoperiodic control of flowering on the physiological, the biochemical and molecular level.

First *Chenopodium* was developed as a "Petri-dish plant" by Cumming (1959) for large scale screening of photoperiodic flower induction with several latitudinal ecotypes showing short-day, long-day and day-neutral responses (Cumming 1967; Tsuschiya and Ishiguri 1981).

Subsequent studies demonstrated that phytochrome photoreversibility could not act as an hour glass timer in photoperiodism, but was gated in its light sensitivity by an endogenous (circadian) rhythm presenting photophile and skotophile phases in daily 24-h light:dark cycles (Cumming et al. 1965). Following Bünning's (1942) and Hendricks' (1963) early concepts on metabolic control of timing in photoperiodism, *C. rubrum* has been used to establish an analysis of energy metabolism demonstrating a circadian rhythm in redox state and energy charge as macroparameters timing photoperiodic behavior (Wagner et al. 1975). Recently, *C. rubrum* was also used in molecular studies on signal transduction in photoperiodic flower induction. An ortholog of *LEAFY*, a transcription factor involved in a signaling cascade leading to flowering in *Arabidopsis* (Nilsson et al. 1998), was identified in *C. rubrum*. Expression kinetics of *LEAFY* ortholog *CrFL* at SAM is related to photoperiod (Veit et al. 2004). Transgenic plants were produced using RNA interference in order to analyse function of *CrFL* in signal transduction in *C. rubrum*.

With studies on the hydro-electrochemical integration of communication in *Chenopodium* plants, we could demonstrate that action potentials precede turgor mediated leaf movements and changes in stem extension rate (Wagner et al., 2005) (Fig. 21.1). Molecular and physiological studies at SAM of *C. rubrum* in transition to flowering presented evidence of changes in turgor and in aquaporin expression (Albrechtová and Wagner 2004;

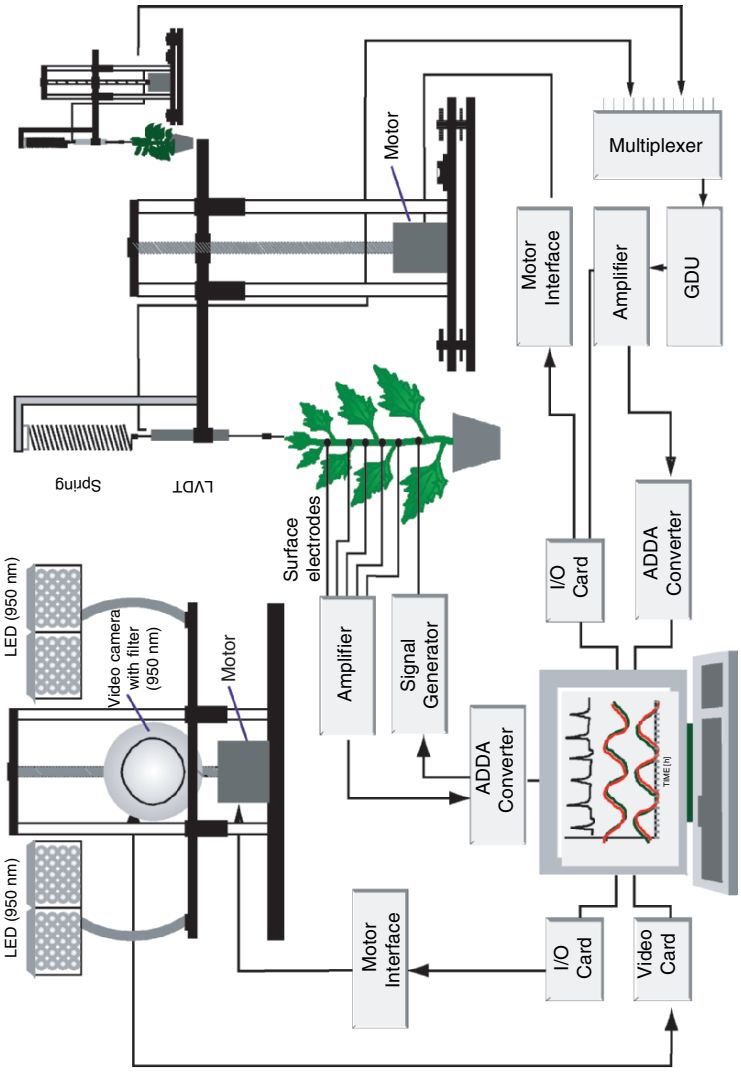

Fig. 21.1. Experimental setup. Measuring device for long term recording of changes in electric surface membrane potential, leaf movements (LM) and stem elongation rate (SER). Video imaging at 950 nm for continuous monitoring of leaf movements in light–dark cycles. Linear voltage differential transformers (LVDTs) hooked to the plant stem with a spring loaded constant pull of 1.5 g. Optimal measuring conditions for video imaging and SER registration are maintained via software controlled step motors positioning the devices according to the growth of the plants. Platinum electrodes are used together with a commercial contact gel for measuring and stimulation. Additional sensors monitor electromagnetic noise, temperature, light intensity and humidity. From Wagner et al. (2005)

Albrechtová et al. 2004) which are most likely triggered by APs traveling along the stem axis as a line of communication between leaves, roots and SAM. A rapid communication between roots and SAM is inferred from a reduction of O^{15}-water uptake by the roots after cutting off apical meristems (Ohya et al. 2005).

The recording of the frequency distribution of spontaneous APs moving basipetal and acropetal on the stem axis resulted in electrophysiograms (EPGs) which could be used to characterize the flowering and vegetative state in *C. rubrum* and *C. murale*. In addition to the characterization of such phase changes, the information from EPGs has been used for the electrogenic initiation of flowering (Lehner 2002) opening a new field for applications in horticulture, agriculture and silviculture (Wagner et al. 2004).

21.3 Electrophysiology and plant behavior

Chenopodium is very well characterized with respect to the kinetics of photoperiod controlled flower induction in a whole series of latitudinal ecotypes (Cumming 1959, 1967; King 1975). In *C. rubrum*, the circadian rhythmic organization of energy metabolism has been analyzed in detail (Wagner et al. 1975, 2004).

In view of demonstrated rhythmic and photoperiod mediated surface potential phenomena, we suggested that the flowering stimulus might be an electrical or electrochemical signal (Wagner et al. 1996, 1997). A similar concept was advanced in relation to systemic effects from wound responses; it was suggested that action potentials could be involved as signaling mechanisms in chilling injury, mechanical perturbation and invasion by pathogens (Davies 1987; Wildon et al. 1989, 1992), as well as for the influence of light and gravity (Davies et al. 1991). Application of direct current, i.e. electrical stimulation, induced proteinase inhibitor II Pin2 gene expression and modulation of photosynthetic activity in the leaves (Herde et al. 1995). Induction of proteinase inhibitor gene expression involved both action potentials and variation potentials in another set of experiments (Stankovic and Davies 1996). Systemic Pin2 gene expression in wild type and ABA-deficient tomato plants was triggered by mechanical wounding, current application and heat treatment (Herde et al. 1998a,b). Mechanical wounding of *Chenopodium rubrum* leaves induced changes in membrane potential, as seen by using a fluorescent probe as indicator (Albrechtová and Wagner 1998).

In sunflower plants, electrical activities could be evoked in response to various external stimuli or were generated spontaneously. Action potentials, graded potentials, changes in resting potential and rhythmic electrical activity were observed. Large graded potentials were paralleled by a decrease in

growth rate and in the rate of water uptake. Spontaneous action potentials along the stem axis were generated only at night in vegetative plants but they were generated during the day as well as during the night in flowering plants (Davies et al. 1991). Flower induction in *Spinacia* was correlated to the light-stimulated bioelectric response from the leaves (Greppin et al. 1973; Greppin and Horwitz 1975).

As suggested by some previous experiments on the control of flowering (Adamec and Krekule 1989a,b; Adamec et al. 1989), endogenous electrical activities of plants can be manipulated by electric current from outside; specifically in the long-day plant *Spinacia oleracea* (Montavon and Greppin 1983, 1986), as well as in the short-day plant *Chenopodium rubrum* (Adamec et al. 1989; Machackova et al. 1990; Machackova and Krekule 1991), photoperiodic flower induction could be inhibited by the application of direct electric current (DC) via felt-tipped electrodes. It was concluded that DC probably interfered with the translocation of a floral stimulus from induced leaves to SAM (Adamec and Krekule 1989a,b; Adamec et al. 1989).

There is controversial evidence concerning transport of the flower inducing stimulus in the phloem (see Bernier 1988 and references therein), which would be the transport path for the stimulus in florigenic and multicomponental theories. Phloem has been considered as a pathway for signal spreading: but a general symplast/apoplast interaction might be involved as well, as evidenced from studies on fast electric transients induced by electric stimulation in the apoplast of tomatoes (Herde et al. 1998a,b). Temporal changes in plasmodesmal patterning might be involved in modulating electric signal transduction (Wright and Oparka 1997; Liarzi and Epel 2005).

Bearing in mind the circadian rhythm in transcellular current in *Acetabularia* (Novak and Sironval 1976) and the diurnal rhythm in resting membrane potential in *Chenopodium* as shown previously (Wagner et al. 1998), it seems possible that a circadian rhythm in bioelectricity may be of great significance in the circadian rhythmic coordination of the whole plant responsible for sensitivity changes in membrane-bound activities (Vigh et al. 1998). The circadian rhythm in transcellular current probably arises from a circadian rhythm in energy metabolism and rhythmicity of transport processes at the plasma membrane (Mayer and Fischer 1994; Mills et al. 1994; Pickard 1994).

In *C. rubrum,* a detailed analysis of the occurrence of surface sum APs revealed electric activity in all organs (root, stem, leaves) of the plant. With a series of electrodes along the stem axis the basipetal or acropetal propagation of APs was recorded. Under quite different experimental condition, so-called reflected APs occasionally could be observed, i.e.APs were propagating basipetal and than seemed to be reflected acropetal (Fig. 21.2). They have not been studied in detail so far but might be very relevant for analyzing the importance of the communication between root meristems and SAM in control of growth and development.

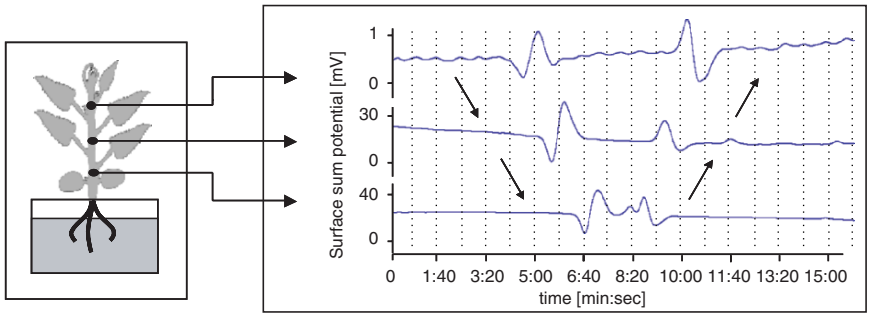

Fig. 21.2. "Reflection" of an action potential (AP) at the basal section of a stem axis of *C. rubrum*. The recordings of the surface sum potential are presented after a light to dark transition of a 5-week-old plant of *C. rubrum* at time 0. The AP is moving basipetal from the apical part of the stem axis, is successively passing three bipolar surface electrodes. Below the basal electrodes it is either reflected immediately and is moving acropetal, or, alternatively, the basipetal moving AP might trigger a new AP moving acropetal. Growing conditions: 20°C, 70% rh; photosynthetic active radiation (PAR): 120 mol/m^2s. The mirror image pattern of basipetal, acropetal propagating APs is due to the measuring principle which is using bipolar (+; –) electrodes and a differential amplifier (see also Zawadzki et al. 1991)

21.4 Circadian rhythms as metabolic bases for hydro-electrochemical signal transduction

Rhythmicity is one of the characteristics of life which expresses itself at all levels of organization from unicellular systems to humans. Rhythmic phenomena in physiology, development and behavior of all living systems show period lengths ranging from fractions of a second to hourly, daily and even annual cycles.

The most conspicuous rhythm is the so-called circadian oscillation. The length of exactly 24 h when the organisms are synchronized by the daily light-dark cycle of the earth. In constant conditions, however, its period length is only approximately 24 h, i.e. circadian. In contrast to biological rhythms showing other frequencies, circadian rhythms are temperature compensated and almost unsusceptible to chemical manipulation. It is this stability or homeo-dynamics of period length which qualifies the circadian rhythm as a precise physiological timer and thus is the essence of Bünning's (1973, 1977) theory of the physiological clock.

From an evolutionary point of view, circadian rhythmicity has been considered to be an adaptation of pro- and eukaryotic energy conservation and transformation to optimize energy harvesting by photosynthesis in the daily cycle of energy supply from the environment (Cumming and Wagner 1968; Wagner and Cumming 1970; Wagner 1977; Wagner et al. 1998). It was also assumed that this adaptation is dependent on the division of energy transformation within different compartments of the cell, such as chloroplasts,

mitochondria and the glycolytic space involving redox mediated transcriptional controls (Bauer et al. 1999). In photosynthetic prokaryotes, lacking cell organelles, a metabolic micro-compartmentation allows for a similarly sophisticated regulatory network as in eukaryotes.

High-frequency oscillations in energy-transducing metabolic sequences could give rise to low-frequency oscillations of energy flow in the metabolic network of the whole system, providing a basis for the evolution of a temperature-compensated circadian rhythm (Wagner and Cumming 1970). The clock's periodicity is genetically determined and provides the temporal frame for physiological and behavioral patterns that are necessary for adaptation of organisms and populations to environmental constraints. Thus the circadian rhythmic cell is a hydro-electrochemical oscillator driven or synchronized by the daily dark/light cycle with a temporal compartmentation of metabolism and a network of metabolic sequences to compensate for oxidative stress tin adapting to their light environment. This is best shown in the adaptation of photosynthetic machineries from bacteria (Joshi and Tabita 1996; Zeilstra-Ryalls et al. 1998; Bauer et al. 1999; Sippola and Aro 2000) to higher plants (Anderson et al. 1988; Asada 1999), which respond to changing light quality and quantity with coordinated changes of pigmentation, electron transport components, membrane composition, organization and function (Anderson et al. 1988). The acclimation of plants at the cellular level requires interaction between the nucleus, mitochondria and chloroplasts in a regulatory network involving several photoreceptors such as phytochromes, blue light receptors and chlorophyll.

The existence of circadian rhythmicity is dependent on the living cell and suggests that metabolic compartmentation spatial and/or temporal is of significance for its generation (Barbier-Brygoo et al. 1997; Flügge 2000). Furthermore, circadian rhythms in photosynthesis, respiration and chloroplast shape suggest that investigation of the metabolic controls may be fruitful for the elucidation of the mechanism of biological rhythms. Interrelations between cellular compartments have already been shown. For example, Könitz (1965) has shown reciprocal changes in the ultrastructure of chloroplasts and mitochondria in daily light–dark cycles. Murakami and Packer (1970) demonstrated that, within the same cell, mitochondria swell and chloroplasts contract upon illumination and the reverse occurs in the dark. The importance of the entire metabolic network for the display of circadian oscillations is underlined by the fact that, in contrast to the temperature-compensated circadian oscillations of the intact system, isolated organelles display high frequency oscillations (Gooch and Packer 1974; Gylkhandanyan et al. 1976) which are temperature dependent. Similarly in photosynthetic bacteria, cellular signal transduction integrates micro-compartmentation of photosynthesis, carbon dioxide assimilation and nitrogen fixation (Joshi and Tabita 1996). From a detailed analysis of rhythms in enzyme activities involved in compartmental energy metabolism with and without feeding of sugars (cf. Wagner et al. 1983; Jang and Sheen 1994) and on changes of nucleotide pool size levels

in the short-day plant *Chenopodium rubrum* L., we compiled evidence in favor of circadian rhythmicity in overall energy transduction. Our observations lead us to suggest that circadian rhythmicity, as the timer in photoperiodism, should be based on a circadian rhythm in energy metabolism. This rhythm would be the result of a compensatory control oscillation between glycolysis and oxidative phosphorylation, coupled to photophosphorylation in cyanobacteria (Huang et al. 1990), photosynthetic bacteria and green plants (Wagner and Cumming 1970; Wagner et al. 1974a,b; Wagner 1976a–c). This mechanism of circadian rhythmicity could involve energy control of ion transport processes at the membranes of cells and organelles. The membrane's physical state, e.g. modulated by temperature, could control transcription (Vigh et al. 1998) or via frequency coded calcium oscillations leads to differential gene activation and expression (Dolmetsch et al. 1998; Li et al. 1998).

Taking into account the symplastic organization of higher plants, the concept of compartmental feedback becomes even more attractive in view of bioelectric phenomena. The symplastic organization of higher plants might be the basis for translocation of electric, photoperiodic and morphogenetic stimuli (Genoud and Métraux 1999). The energetic integration of the entire system could be based on the same symplastic organization, so that proton translocation (Mitchell 1976) and concomitant ion movements would give rise to a circadian rhythm in electric potential paralleled by circadian leaf movements and stem extension rates (Aimi and Shibasaki 1975; Wagner et al. 1998).

The circadian rhythm in transcellular current of a single cell, as observed by Novak and Sironval (1976) in *Acetabularia*, probably arises from the compartmental feedback between mitochondria, chloroplasts and glycolysis, as suggested in our concept for a mechanism of circadian rhythmicity. The vacuole could be involved in this control net by acting as a reservoir for metabolites as in the case of oscillations in Crassulacean acid metabolism. A similar concept might hold true for photosynthetic active bacteria in general and their temporal organization of metabolism, but certainly for circadian rhythmic behavior of the cyanobacterium *Synechococcus* (Huang et al. 1990; Ishiura et al. 1998).

21.5 Hydraulic-electrochemical oscillations as integrators of cellular and organismic activity

The symplast of higher plants is probably not only the network for rapid electrical integration of metabolic activities but also the route for the translocation of sucrose and the transfer of the flowering stimulus. An observation that may have some bearing on the significance of changes in membranes during signal transduction is the detection of alterations in the distribution of the endoplasmic reticulum in cells of SAM of *Chenopodium album* after photoperiodic stimulation (Gifford and Stewart 1965). In spinach, the plasma membrane of

apical cells is modified during flower induction (Penel et al. 1988; Crèvecoeur et al. 1992). Changes in pH and Ca^{2+} patterning as measured with fluorescent dyes can be observed between the earliest events of photoperiodically inductive conditions in flower initiation in *C. rubrum* (Walczysko et al. 2000; Albrechtová et al. 2001, 2003). In the case of flower induction, the temporal organization of development at SAM might involve rhythmic symplastic transport of metabolites and the interaction of rhythmic (bioelectric) signals originating in the leaves (Novak and Greppin 1979) leading to a frequency-coded electrochemical communication between leaves and SAM (Wagner et al. 1998).

Communication by surface membrane action or variation potentials in higher plants has been observed for a series of systemic responses (Wagner et al. 1997).Changes in action potentials triggered by light to dark or dark to light transitions can be related to changes in photosynthetic electron transport (Trebacz and Sievers 1998). Observations on phytochrome action in the moss *Physcomitrella patens* are indicative of activation of plasma membrane anion channels (Ermolayeva et al. 1997), leading to membrane depolarization as a very first step in signal transduction.

Bearing in mind the circadian rhythm in transcellular current reported in *Acetabularia* (Novak and Sironval 1976), it seems possible that a circadian rhythm in proton flow, of action and variation potentials, may be of great significance in the circadian coordination of the whole plant, and the communication between plant organs such as the leaves and SAM in photoperiodic flower induction (Wagner et al. 1998; Elowitz and Leibler 2000; Smith and Morowitz 2004).

The display of circadian rhythms in energy charge and reduction charge favor the concept that the many non-linear oscillators of cell metabolism are coupled such as to evolve the circadian frequency of the system as a whole (Wagner et al. 2000; Smith and Morowitz 2004).

An interplay between oscillating enzymatic reactions and contractile elements of the structural proteins of the cell has even been used to design a mechanochemical model of the biological clock (Sorensen and Castillo 1980; Kung 2005), and most significant, with protoplasts from *Phaseolus* pulvinar motor cells a circadian rhythm in volume oscillations could be shown (Mayer and Fischer 1994). This "electrochemical" view of metabolic control could be very relevant in relation to the mechanism of growth and differentiation. Numerous experimental findings show the involvement of stable electric fields in growing and differentiating cells. The experimental evidence indicates the possibility that the chemical reaction network is controllable by electric fields. This could open up a way to visualize the synchronization of circadian rhythms by electric and magnetic fields and thus could allow for synchronizing inputs from so called subtle geophysical factors (Olcese 1990).

It is very likely that physiological rhythms are based on the same structural and functional principles underlying Mitchell's (1976) chemiosmotic hypothesis of energy transduction. Allosteric enzymes could function as molecular

high frequency oscillators as in glycolysis while compartmental feedback between organelles with vectorially organized metabolic reactions in their enclosing membranes could give rise to a circadian rhythm in energy transduction by proton flow through the entire cell. Thus the coupling between compartmented metabolic sequences is possibly achieved through cycles in nucleotide ratios and ionic balances via transport mechanisms and redox shuttles in the different energy-transducing biomembranes, which could act as coupling elements and frequency transformers. The membranes themselves could act as high-frequency oscillators (Morré and Morré 1998; Morré et al. 1999). Such high-frequency membrane oscillators could be the basis for perception and transduction of high-frequency signals from the environment (Novak and Greppin 1979).

The ratios of coupling nucleotides would be relatively temperature independent and the nucleotides themselves could thus, as rate effectors in compartmental feedback, fulfill the requirements for precise temperature-compensated time keeping. Proton flow and concomitant ion movement through the symplast could be the basis of rhythmic electrochemical integration of the whole plant (Wagner et al. 1997, 2000; Igamberdiev and Kleczkowski 2003; Stelling et al. 2004).

The circadian pacemaker oscillation expresses itself by temperature compensated phosphorylation/dephosphorylation or reduction/oxidation cycles as the time standard for the control of transcription and translation controlled ~24 h cycles of protein synthesis turnover (Elowitz and Leibler 2000; Richly et al. 2003; Tomita et al. 2005).

21.6 Local hydraulic signaling: the shoot apex in transition

Photoperiodic flower induction involves a reorganization of organogenesis at SAM from vegetative phylotaxis to floral development. Floral transition includes molecular signaling and physiological and physical changes. Each step in molecular signal transduction is influenced by feedback from sequences of events from other pathways. In this way, the "physiological state" of a plant controls signal transduction, and vice versa. Very little is yet known regarding relationships between the molecular and physiological level of the control of organogenesis. To analyze the change from the vegetative to the flowering apex, we studied kinetics of molecular and physiological changes at SAM of *C. rubrum* during floral transition in order to elucidate the kinetic relationships between the events.

Electric and hydraulic long-distance signals are anticipated to be involved in flower induction. The perception of a flower inducing dark span by phytochrome possibly leads to a change in the electrochemical signaling between leaves and SAM to allow flower initiation to occur (Wagner et al. 1998). Turgor dependent volume changes, stretch-activated membrane channels

(Kung 2005) and correlated changes in membrane potential might be an essential part of the "hardware" for signal transduction at the cellular and organismic level. The "software" could involve frequency-coded signals at the cellular, the tissue and organismic level.

Membrane potentials are ubiquitous in all living cells and provide the energy for the active transport of substances. The depolarization of the cellular membrane potential can generate APs which in higher plants can be propagated over long distances via the phloem and plasmodesmata. Localized strain of the plasma membrane can elicit electric signals via mechanosensitive ion channels. Such signals can stimulate turgor loss and may even activate genes (Banes et al. 1995; Lang and Waldegger 1997). Due to its mechanisms of action, hydraulic and electric signaling is always coupled.

Physical strain at the surface of SAM was previously suggested to play a key role in the patterning of organogenesis (Green 1994). The distribution of local forces is a result of the upward pressure given by the expanding inner cell layers (corpus) to the sheet on the surface (tunica), and from the local extensibility of cell walls at the surface. A signaling network regulates organogenesis, involving molecular, biophysical and biochemical pathways of signal transduction. Based on crosstalk between all pathways, the "physiological state" of a plant controls signal transduction, and vice versa.

Our studies aim at understanding local water transport and turgor changes as related to changing organogenesis at SAM of *Chenopodium* plants under photoperiodic flower induction. We could show that size of SAM increases during flower inducing treatment (Fig. 21.3) (Albrechtová et al. 2004). The expansion of the meristem results from cell enlargement rather than changes in cell division, and therefore is presumably based on water uptake. The phylotactic spiral changes to a circular pattern, visible as local differences in optical properties of cell walls. The results suggest that organogenesis changes long before flower induction is completed. We therefore conclude that the change of organogenesis at SAM during floral transition is initiated by an increased movement of water into SAM leading to its expansion and to the redistribution of the forces at its surface.

A change in water movement could involve aquaporins, which in consequence have been studied in SAM of *C. rubrum*. Aquaporins (AQPs) are highly selective water channels facilitating transport of water across the membrane (Baiges et al. 2002). We identified in *Chenopodium rubrum* a gene with high homology to aquaporins from other plants, CrAQP (Fig. 21.4). Its expression differs significantly in leaves and in SAM between vegetative and flower induced plants (Albrechtová and Wagner 2004). Involvement of aquaporins at SAM in flower initiation was proven using application of an inhibitor of aquaporin activity HgCl directly to SAM. HgCl partially inhibited flowering, if applied before or during the dark span.

A comparison of the kinetics of parameters studied revealed that the increase in SAM size is accompanied by an increase in calcium concentration and average pH value at SAM (Walczysko et al. 2000; Albrechtová et al. 2001,

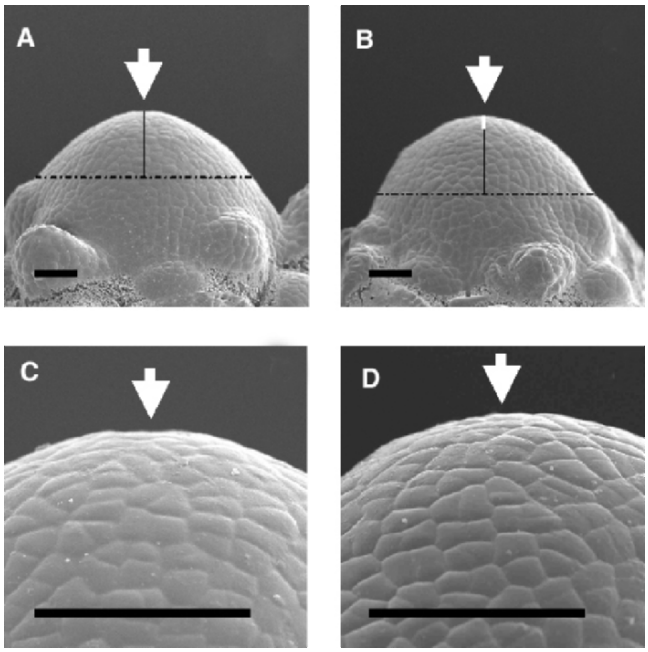

Fig. 21.3. Changes in geometry of SAM during flower inducing treatment. Cryo SEM. **A**, **C**, control plant, **B**, **D**, plant at the end of 12 h inductive dark span. The difference is mostly pronounced on the top (*arrows*): A subtle depression is visible at SAM of control plant (**A**, **C**), the SAM of the plant after inductive treatment is well rounded (**B**, **D**). Both apices have the same diameters of 250 Fm (*dotted lines*), but different heights (*full lines*, the difference is shown with a *thick white line* in **B**). The ratio height to diameter quantifies the typical difference between both treatments. *Bars* represent 50 Fm. From Albrechtová et al. (2004)

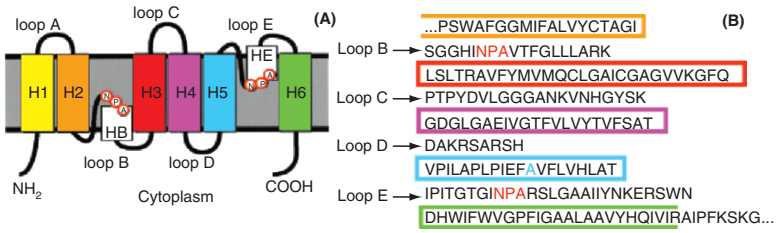

Fig. 21.4. The typical structure of aquaporins (**A**) and the putative protein sequence of a fragment of the aquaporin *CrAQP*, identified in *Chenopodium rubrum* (**B**). Helices are indicated by different colors identical in the putative sequence and on the scheme. The typical highly conserved motifs are indicated as red (NPA motif in loops B and E) and blue (alanine in helix H5, typical for plant aquaporins) letters in the sequence. Both C-terminus and N-terminus are missing in the identified fragment

2003). Further studies should reveal, if intracellular pH and calcium concentration can influence water transport by regulating activity of *CrAQP* (Tournaire-Roux et al. 2003). Our further observations confirmed a putative increase of free sucrose at SAM during floral transition, shown in other model plants (Albrechtová and Wagner 2004). The increase in sucrose concentration could lead to an increase in the osmotic pressure in the cells of SAM and thus produce together with a redistribution of ions a driving force for water transport.

A central role in the shift of organogenesis during floral transition is thus played by water status, local physical properties of cell walls and distribution of local forces at the surface of the meristem. Altogether, the results support the hypothesis about the involvement of hydraulic signals in organogenesis at SAM. It is anticipated that hydraulic changes at SAM leading to flower initiation are mediated by a specific hydro-electrochemical communication between (roots), leaves and SAM.

21.7 Summary and perspectives: electrophysiology and primary meristems

The hypothesis of a hydraulic electrochemical communication between plant organs prompted studies on the influence of local water transport and turgor changes on organogenesis at SAM of *Chenopodium* plants. Specific changes in shape and size of SAM were found to precede reorganization of organogenesis under photoperiodic flower induction. Optical properties of cell walls at the surface of SAM were found to precede reorganization of organogenesis under photoperiodic flower induction. Expression of the aquaporin *CrAQP* increased at SAM during an early phase of flower induction and the application of an inhibitor of aquaporin activity partially inhibited flowering. Changes in ion balance and carbohydrate levels in the cells seem also to be involved in the process. Altogether, the results support a hypothesis on the involvement of hydraulic signals in organogenesis at SAM. It is anticipated that hydraulic changes at SAM leading to flower initiation are mediated by a specific hydro-electrochemical communication between roots, leaves and SAM.

Studies on the uptake of O^{15} labeled water are indicative of a rapid communication between SAM and root meristems (Ohya et al. 2005). Water uptake was strongly reduced after removal of shoot apices, but was not affected, if roots were removed before removal of the apices. The root and shoot primary meristems might be centers of AP generation (Fig. 21.5) as a basis for coordination of physiological processes, such as water uptake and transpiration. Structure and function of both meristems is at present under intensive investigation (Schoof et al. 2000; Bäurle and Laux 2003; Baluska et al. 2004). Their hydro-electrochemical analysis should be most rewarding for an understanding of growth and differentiation.

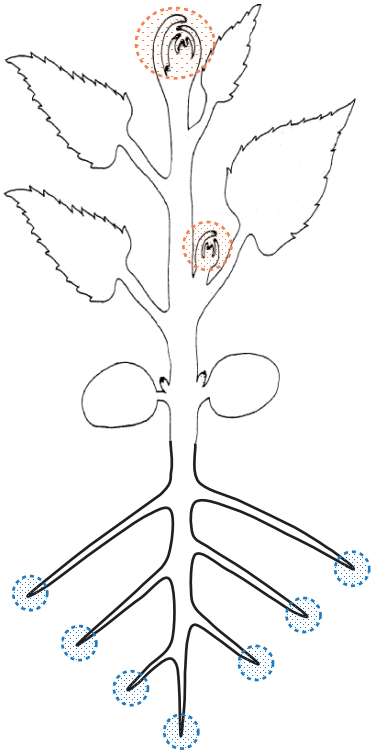

Fig. 21.5. Schematic drawing of a higher plant emphasizing elongating meristems as "centres" for root–shoot coordination. From the data reported so far it is concluded that in daily light dark cycles the rhythmic activity of the various plant organs [shoot apical meristem(s), leaves, stem (internodes), roots] is synchronized to 24 h with specific phase relationships between organ systems. Hydraulic and electric signals (APs, VPs) and their temporal structure are coordinating the developmental adaptation of the system to endogenous and environmental constraints. APs are predominantly generated at the root and shoot apical meristems. Leaf movements, rhythmic stem elongation (Wagner et al. 1996) and rhythmic root water transport (Lopez et al. 2003) are the main components of the hydraulic system. Changes in turgor are transduced via mechanotransductive ion channels into electric signals (APs). Plant systems can be considered as hydraulic–electrochemical oscillators. They display both circadian and higher frequency oscillations that are obviously taking part in the integration of the plant as a whole in its metabolic and developmental adaptation to the environmental conditions

Leaf movements, rhythmic stem elongation and rhythmic root exudation are components of the hydraulic system. Changes in turgor are transduced via mechanotransductive ion channels into electric signals (APs). Plant systems thus can be considered as hydraulic–electrochemical oscillators displaying both circadian and high frequency oscillations involved in the integration of the plant in its metabolic and developmental adaptation to the environmental conditions.

Further studies will monitor in detail the generation and propagation of APs in parallel to physiological and molecular studies to elucidate the patterns of cooperation between plant organs in response of the plant to seasonal changes in photoperiod as well as biotic and abiotic stress conditions.

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