MINIREVIEW

Regulation of cambial activity in relation to environmental conditions: understanding the role of temperature in wood formation of trees

Shahanara Begum\textsuperscript{a,b}, Satoshi Nakaba\textsuperscript{a}, Yusuke Yamagishi\textsuperscript{a}, Yuichiro Oribe\textsuperscript{c} and Ryo Funada\textsuperscript{a}

\textsuperscript{a}Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, 183-8509, Japan\textsuperscript{b}Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh, 2202, Bangladesh\textsuperscript{c}Tohoku Regional Breeding Office, Forest Tree Breeding Center, Takizawa, Iwate, 020-0173, Japan

Correspondence
*Corresponding author, e-mail: funada@cc.tuat.ac.jp

Received 19 February 2012; revised 4 May 2012

The timing of cambial reactivation plays an important role in determination of the amount and quality of wood and the environmental adaptivity of trees. Environmental factors, such as temperature, influence the growth and development of trees. Temperatures from late winter to early spring affect the physiological processes that are involved in the initiation of cambial cell division and xylem differentiation in trees. Cumulative elevated temperatures from late winter to early spring result in earlier initiation of cambial reactivation and xylem differentiation in tree stems and an extended growth period. However, earlier cambial reactivation increases the risk for frost damage because the cold tolerance of cambium decreases after cambial reactivation. The present review focuses on temperature regulation on the timing of cambial reactivation and xylem differentiation in trees, and also highlights recent advances in our understanding of seasonal changes in the cold stability of microtubules in trees. The review also summarizes the present understanding of the relationships between the timing of cambial reactivation, the start of xylem differentiation and changes in levels of storage materials in trees, as well as an attempt to identify the source of energy for cell division and differentiation. A better understanding of the mechanisms that regulate wood formation in trees and the influence of environmental conditions on such mechanisms should help in efforts to improve and enhance the exploitation of wood for commercial applications and to prepare for climatic change.

Introduction

Wood is the product of vascular cambium, and the formation of wood depends on the cambial activity of trees (Catesson 1994, Larson 1994, Chaffey 1999, Funada 2008). In temperate and cool zones, the vascular cambium of the stems of trees undergoes seasonal cycles of activity and dormancy, which are collectively known as annual periodicity. This periodicity plays an important role in the formation of wood and reflects the environmental adaptivity of trees, for example their tolerance to cold in winter in cool and temperate zones. The quantity and quality of wood depend on the division of cambial cells and the differentiation of cambial derivatives. Therefore, details of the cell biological and physiological aspects of the regulation of cambial activity in trees are of considerable interest.

Cambial activity in trees is regulated by both internal factors, such as plant hormones, and environmental factors, such as, temperature, rainfall and photoperiod.

Abbreviations – CRI, cambial reactivation index; IAA, indole-3-acetic acid; MAP, microtubule-associated protein.
However, with respect to plant hormones, no increase in the level of indole-3-acetic acid (IAA) was detected in the cambial region of *Pinus sylvestris*, *Pinus densiflora* and *Larix kaempferi* trees at the onset of cambial reactivation, suggesting the absence of a clear relationship between the timing of cambial reactivation and endogenous levels of IAA (Sundberg et al. 1991, Funada et al. 2001, 2002). Therefore, other factors appear to be necessary for cambial reactivation. Denne and Dodd (1981) highlighted the fact that temperature provides the appropriate physical conditions for the growth and development of trees in temperate and cool climates. More recent studies have demonstrated that the timing of cambial reactivation is controlled by temperature, which influences both the quantity and quality of wood (Oribe and Kubo 1997, Oribe et al. 2001, 2003, Gričar et al. 2006, Begum et al. 2007, 2008, 2010). In addition, Samuels et al. (2006) focused on the ultrastructural changes in cell organelles that occur during cambial reactivation in trees. Furthermore, cambium and its derivatives provide a good model system to study xylogenesis because it is possible to follow the process of cell division and xylem differentiation in single sections.

In this review, we focus on the cell biology of cambial growth as it relates to the control of the quality and quantity of wood and the environmental adaptivity of trees, with an emphasis on recent progress in characterization of the mechanisms that control the timing of cambial reactivation and xylem differentiation in trees. We shall also discuss recent advances in the understanding of seasonal changes in the cold stability of microtubules in trees, as well as relationships among the timing of cambial reactivation, the start of xylem differentiation and changes in levels of storage materials, such as starch and lipid droplets, in an attempt to identify the source of energy for cambial reactivation and xylem differentiation.

**Induction of cambial reactivation by localized heating**

The dormant cambium is located between the secondary phloem cells and the thick-walled secondary xylem cells that have formed during the previous growing season. During dormancy, the cambium consists of two or three radial layers of radially narrow and compactly arranged cells in the deciduous diffuse-porous hardwood hybrid poplar *Populus sieboldii X Populus grandidentata* (Fig. 1A). During the period from late winter to early spring, new cell plates are formed in the cambium and this springtime phenomenon is referred to as cambial reactivation (Fig. 1B; Catesson 1994, Larson 1994).

![Fig. 1](image.png)

**Fig. 1.** Light micrographs (A, B and D) and a confocal laser scanning microscopic image (C), showing transverse (A, B) and radial (C, D) views of the cambium and differentiating xylem in stems of hybrid poplar and Cryptomeria japonica. No new cell plates are evident and cells are arranged very compactly, indicating that the cambium is in a dormant state in (A), while active cambial cell division with differentiating xylem cells is evident in the locally heated stems of hybrid poplar (B). Depth-coding image in (C), which is a projection, shows starch granules in phloem ray parenchyma cells prior to cambial reactivation in locally heated stems of *C. japonica*. Different colors represent different depths of field. The image in (D) shows that the amount of storage starch (arrows) in phloem cells had decreased near the cambium during cambial reactivation in the locally heated stems of a hybrid poplar. Ca, cambium; Ph, phloem; Prp, phloem ray parenchyma; Xy, xylem; Nxy, new xylem. Bars = 100 μm.
Similar cambial reactivation has been observed in *Fraxinus excelsior* (Funada and Catesson 1991), *Aesculus hippocastanum* (Barnett 1992), *Robinia pseudacacia* (Farrar and Evert 1997a, 1997b), *Populus trichocarpa* (Arend and Fromm 2003), *Pinus contorta* (Rensing and Samuels 2004) and *Acer platanoides* (Frankenstein et al. 2005). Transmission electron microscopy has revealed details of the events that occur in cambial cells during natural reactivation in addition to structural differences between dormant and reactivated cambium. However, the cited studies failed to identify the factors that regulate the timing of cambial reactivation in trees.

Winter cambial dormancy in trees consists of two stages, namely, the resting and the quiescent stages (Little and Bonga 1974; Riding and Little 1984, 1986; Rensing and Samuels 2004; Sundberg et al. 1987, 2000). During the first 2–4 weeks of dormancy, the cambium is unable to produce new cells even when IAA is supplied under favorable environmental conditions (Little and Bonga 1974; Riding and Little 1984, 1986; Sundberg et al. 1987). This stage of dormancy, which is regulated by internal factors, is referred to as the resting stage (Little and Bonga 1974). After exposure to natural or artificial chilling, the cambium gradually regains the ability to produce new xylem cells in response to IAA under appropriate environmental conditions. When fully responsive to IAA in this way, the cambium is deemed to be in the quiescent stage of dormancy, which is imposed by adverse external factors (Little and Bonga 1974). The transition from rest to quiescence involves structural, histochemical and functional changes in cambial cells (Lachaud et al. 1999; Samuels et al. 2006).

Numerous researchers have noted that localized heating of stems over a range of temperatures from 22 to 26°C or from 25 to 30°C for 5 or 6 days in cold winter induces localized reactivation of the cambium in evergreen conifers, such as *P. contorta* (Savidge and Wareing 1981), *Picea sitchensis* (Barnett and Miller 1994), *Cryptomeria japonica* (Oribe and Kubo 1997), *Abies sachalinensis* (Oribe et al. 2001, 2003) and *Picea abies* (Gričar et al. 2006). These observations suggested that an increase in temperature might be a limiting factor in the onset of cambial reactivation during the quiescent dormancy of trees.

Localized heating of stems during dormancy also induced the division of phloem parenchyma cells and cambial cells in a deciduous diffuse-porous hardwood hybrid poplar, *P. sieboldii × P. grandidentata* (Begum et al. 2007). Localized heating for 4 weeks induced cambial reactivation that occurred earlier than natural cambial reactivation, and the difference in terms of timing between cambial reactivation in the heated and non-heated control portions of the stems was approximately 4 weeks. Moreover, 2 months of localized heating resulted in xylem differentiation in heated poplar stems (Begum et al. 2007). By contrast, 2 weeks of localized heating of stems of the deciduous conifer *Larix leptolepis* failed to activate the division of cambial cells (Oribe and Kubo 1997), while localized heating of stems of *C. japonica* induced cambial reactivation 6 days after the start of heating, with xylem differentiation starting after heating for 14 or 21 days (Begum et al. 2010a, 2012b). Longer localized heating of the stems of deciduous trees, as compared to those of evergreen conifers, might be required for conversion of cambium from a quiescent to an active state. In addition, when localized heating of stems of hybrid poplar and *C. japonica* trees was started in December, there was no accelerated reactivation of cambium. Thus, cambial cells in December appeared to be in a state of dormancy that could not be overcome by localized heating (Begum et al. 2007, 2010a).

The first cell division in the locally heated cambial zone occurred in the second layer of fusiform cambial cells in hybrid poplar and from the third or fourth layer of fusiform cambial cells in *C. japonica*, counted from the side of the previous year’s xylem. The patterns of cambial reactivation and secondary xylem differentiation that were induced by heating resembled those in the non-heated control stems of hybrid poplar and *C. japonica*. These observations suggested that cambial reactivation in response to heat treatment in winter might be a good model system to investigate the dynamics of cambial reactivation because it is easy to follow the process of activation from the division of phloem parenchyma cells and cambial cells to the development of secondary xylem over relatively short periods of time. The observations also indicated that an increase in temperature of the stem might be a direct trigger for cambial reactivation in trees.

It has been reported that bud burst and the development of new leaves are related to cambial reactivation and xylem differentiation (Aloni 1991). However, cambial reactivation in heated portions of the stems of hybrid poplar (*P. sieboldii × P. grandidentata*) was not associated with bud burst, indicating that a close relationship does not always exist between the timing of bud burst and cambial reactivation (Begum et al. 2007). In deciduous hardwood hybrid poplar, bud burst appears not to be a prerequisite for cambial reactivation from the quiescent state. By contrast, xylem differentiation in heated portions of stems started only after bud flushing, suggesting that some factor(s) from expanding new leaves might be required for the differentiation of xylem.

The timing of cambial reactivation is related to cambial age in *C. japonica* trees. In 55-year-old
Cambium, cambial reactivation and xylem differentiation occurred earlier than in 80-year-old cambium both under heated and under natural conditions (Begum et al. 2010a). Rossi et al. (2008a) reported that, in Larix, Pinus, Picea and Juniperus, the duration of cambial activity and xylem differentiation differed between adult and old trees even when the trees had been grown under the same climatic conditions. Thus, the duration of cambial activity appears to differ between adult and old trees. The difference in terms of the timing of cambial reactivation from late winter to early spring between two trees with cambium of different ages suggests that cambial sensitivity to temperature might be related to cambial age and the state of cambial dormancy in C. japonica stems.

Changes in levels and localization of storage materials during cambial activity

Sucrose is derived from photosynthates in leaves and needles and is transported basipetally through the phloem in stems. Low-level photosynthetic activity has been detected in evergreen conifers under mild temperate conditions in winter (Fry and Phillips 1977), while net photosynthesis is close to zero in evergreen conifers under cool temperate winter conditions, when the minimum temperature falls below zero (Troeng and Linder 1982). Thus, in temperate and cool zones, the low rates of photosynthesis in late winter and early spring might be expected to limit the supply of sucrose to stems. Under natural conditions, cambial reactivation in trees occurs from late winter to early spring, when photosynthesis is minimal or almost non-existent. An analysis of 13CO2 pulse-labeled photoassimilates demonstrated that compounds for the synthesis of cell walls of earlywood are derived from the previous year’s photoassimilates (Kagawa et al. 2006), suggesting that stored materials are crucial for radial growth. The utilization of reserve materials at various stages of growth might provide clues to a full understanding of the growth and development of trees (Harms and Sauter 1992).

During cambial dormancy, starch granules are present at low levels in ray and fusiform cambial cells of C. japonica (Itoh 1971) and they are rare in ray cambial cells of P. abies (Timell 1986). These observations suggest that, during cambial dormancy, levels of starch might be low as a consequence of the breakdown of starch that is associated with the generation of energy for the development of cold hardiness (Timell 1986).

After local heating of main stems of A. sachalinensis, Oribe et al. (2003) found that cell divisions in the heated and reactivated cambium ceased upon disappearance of starch from the storage tissue around the cambium. Therefore, they proposed that continuation of cambial activity might require a continuous supply of sucrose. Levels of starch around the cambium in heated poplar stems decreased during cambial reactivation (Fig. 1D) and starch granules disappeared completely from the cambium and phloem immediately after cambial reactivation and re-appeared with the start of xylem differentiation.

The level, number and size of starch granules in cambium and phloem tissues decreased during cambial reactivation in locally heated stems of C. japonica, suggesting that an elevated temperature might induce the enzymatic conversion of starch to sugars (Begum et al. 2010b; Fig. 1C, D). Druart et al. (2007) reported that, in Populus tremula, induction of genes for glycolytic enzymes activated the breakdown of starch to meet the increased energy demands of cambial activity, and they suggested that the breakdown of starch might play a key role in the generation of the necessary energy. In addition, prominent decreases in the levels of lipids and in the size of lipid droplets in the cambium, from cambial reactivation to the start of xylem differentiation, suggest that lipid droplets might be utilized as sources of energy for cell division and the biosynthesis of new cell walls in the cambium of locally heated stems of C. japonica (Begum et al. 2010b). Expression of the genes for malate synthase and isocitrate lyase in the glyoxysomal cycle was induced during cambial dormancy in P. tremula (Schrader et al. 2004), also supporting the generation of energy. Therefore, it seems likely that not only starch but also lipid droplets in the cambium might be used as sources of energy for cambial activity and the initiation of xylem differentiation in locally heated stems of C. japonica.

Cambial reactivation under natural conditions

Under natural conditions, temperatures in late winter and early spring are important external modulators of the induction of cambial reactivation and xylem differentiation in a hybrid poplar and C. japonica in temperate and cool zones. For example, in Tokyo, Japan, early spring in 2007 was warmer than in 2005 and 2008 and cambial reactivation occurred earlier in 2007 than in 2005 and 2008 in both hybrid poplar and C. japonica trees (Begum et al. 2008, 2010a). However, responses to increases in temperature differ among species even when trees are growing under the same climatic conditions.

A wide range of threshold temperatures has been used in efforts to predict the onset of bud burst, cambial activity and xylem differentiation (e.g. Schmitt et al. 2004). Rossi et al. (2007) observed that, in conifers such
as Larix decidua, Pinus cembra and P. abies, cambial activity and xylem differentiation occurred above a certain threshold value of mean daily temperature, which ranged from 5.6 to 8.5°C. Deslauriers et al. (2008) reported that, in Pinus leucodermis, the calculated threshold minimum, mean and maximum daily temperatures for wood formation were approximately 5.5, 8.2 and 11.5°C, respectively. Thus, threshold temperatures appear to differ among species.

The temporal integration of daily temperatures above a threshold value of 5°C, expressed in terms of degree-days, allowed the prediction of the effect of temperature on the date of onset of cambial activity in P. sylvestris (Seo et al. 2008). Thus, the sum of the number of degrees centigrade in excess of a threshold value for the daily maximum temperature from January 1 to the initiation of cambial cell division was calculated (Begum et al. 2008, 2010a) and this value was defined as the cambial reactivation index (CRI), as follows:

$$\text{CRI} = \sum (T_{md} - T_t)$$

where $T_{md}$ is the daily maximum temperature in excess of a given threshold temperature and $T_t$ is the given threshold temperature.

Cambial reactivation in stems of hybrid poplar and C. japonica occurred when the maximum daily temperature exceeded 15°C for 8–10 days and 10° or 11°C for 25–27 days, respectively. In the case of hybrid poplar, the cambial reactivation index (CRI), was 93°C in 2005 and 96°C in 2007. In the case of C. japonica, when 10°C was used as the threshold temperature, the CRIs of 94 and 97°C for 2007 and 2008 were closer together than other values of CRI. However, the threshold maximum temperature for induction of cambial reactivation and the response to increases in temperature differed among species. A more recently defined cambial reactivation index (CRI$_{md}$) based on the threshold maximum temperature might help us to predict the timing of cambial reactivation from analyses of meteorological data (Begum et al. 2008, 2010a).

Rossi et al. (2008b) observed that, in Abies balsamea, L. decidua, P. cembra, P. sylvestris, P. leucodermis, Pinus uncinata and P. abies, the critical average temperature for onset of cambial activity ranged between 8 and 9°C. By contrast, no clear similarities were found among members of pairs of calculated CRIs when the calculations based on threshold temperatures that reflected average temperatures. For example, there was no close relationship between the timing of cambial reactivation and a threshold average temperature in C. japonica trees (Begum et al. 2010a).

Once cambial reactivation has occurred, xylem differentiation starts within 3 or 4 weeks under natural conditions. Rossi et al. (2007) observed that high temperatures in spring induced an earlier resumption of cell production in the cambium and a consequent earlier onset of the differentiation of xylem cells in conifers. In hybrid poplar and C. japonica, the higher temperatures in the spring of 2007 and 2008 than those of 2005 induced the earlier onset of xylem differentiation after reactivation of the cambium (Begum et al. 2008, 2010a). The timing of xylem differentiation appeared to depend on the date of onset of cambial reactivation that might be controlled by temperature.

**Cold stability of microtubules and its relationship to cambial activity**

The structure and organelles of cambial cells exhibited seasonal changes that might be related to adaptation to...
changes in climatic conditions (Farrar and Evert 1997a, 1997b, Chaffey 2000). Microtubules, a major component of the cytoskeleton, play important roles in the division and differentiation of cells. They are sensitive to temperature, and low temperature tends to depolymerize or disassemble microtubules in plant cells (Nick 2008). Therefore, plant-science manuals generally suggest that low temperatures are inappropriate for the visualization of microtubules after chemical fixation (Funada 2002). Immunofluorescence microscopy reveals the presence of microtubules in cambium, xylem cells and phloem cells that have been fixed at room temperature. By contrast, low-temperature fixation (2–3°C) depolymerizes microtubules in fusiform and ray cambial cells and in differentiating cells when the cambium is active in *Abies firma*, *A. sachalinensis* and *L. leptolepis* (Fig. 2A; Begum et al. 2012a). Thus, low ambient temperatures above 0°C, namely, chilling temperatures, might be expected to influence and induce the disassembly of microtubules in cambium and cambial derivatives (Begum et al. 2012a).

Contrary to expectations, some researchers have observed microtubules, by transmission electron microscopy and immunofluorescence microscopy, in the dormant cambial cells of conifers in winter, when the temperatures are low (Itoh 1971, Funada et al. 2000). In the present studies, not only microtubules were detected in cambial cells, xylem cells and phloem cells of *A. firma*, *A. sachalinensis* and *L. leptolepis* in winter when cambium was dormant but also it was noted that the depolymerization of microtubules did not occur during low-temperature fixation (2–3°C) when cambium of *A. firma* was dormant (Fig. 2B, C). It is possible that, during cambial dormancy, microtubules might be resistant to the normally depolymerizing effect of low temperatures (Begum et al. 2012a). Clearly, the stability during low-temperature fixation of microtubules in cambial cells, xylem cells and phloem cells differs between seasons of dormant and active cambium. This phenomenon might be closely related to the seasonal changes in the cold tolerance of trees because microtubules play such important roles in cell division and differentiation (Begum et al. 2012a). It is noteworthy that low temperature has no effect on the stability of another major component of the cytoskeleton, namely,

![Fig. 3. A schematic diagram showing the annual periods of cambial dormancy and activity and the effects of an increase in temperature from late winter to early spring on wood formation in trees.](image-url)
actin filaments, in cambial cells and differentiating xylem cells during seasons of active cambium.

Microtubule-associated proteins (MAPs) play regulatory roles in the organization of microtubules (Lloyd and Hussey 2001), and modifications of MAPs might be intimately related to the stability of microtubules (Oda and Hasezawa 2006, Nick 2008). MAPs with a variety of functions have been identified in various plants (Sedbrook 2004), and recent microarray analysis of different tissues from hybrid aspen (P. tremuloides × P. tremula) revealed the enhanced expression of the MAP20 gene during the formation of secondary cell walls, as compared to the low-level expression of MAP20 in cambial cells with a primary wall (Rajangam et al. 2008). Certain MAPs might thus, also be involved in changes in the stability of microtubules in cambium and differentiating xylem cells.

**Conclusion**

A localized or environmentally induced increase in the temperature of the stem can directly induce the breaking of cambial dormancy in trees but the responses to such increases in temperature differ among species and depend on stage of dormancy. Prominent decreases in the levels of starch and lipid droplets in the cambium, from cambial reactivation to the start of xylem differentiation, indicate that starch and lipid droplets might be utilized as sources of energy for cell division and the biosynthesis of new cell walls in the cambium. In addition, differences in the behavior of microtubules during low-temperature fixation of active and dormant cambium suggest that microtubules might act as sensors of a change in growth conditions.

Future climate change, with increases in temperature from late winter to early spring, might induce earlier cambial reactivation and xylem differentiation, resulting in longer periods of cambial growth and increased production of wood biomass. In addition, such increases in temperature might promote the enzymatic conversion of starch to sugar to supply the energy required for the biosynthesis of new cell walls and for continuous cambial activity. By contrast, a sudden decrease in temperature after the onset of cambial reactivation, such as a late spring frost, when the cold tolerance of the cambium is low might induce the depolymerization of microtubules, with a consequent negative impact on tree growth and development (Fig. 3). Therefore, the effects of future climate change from late winter to early spring might have some positive impact on the production of wood biomass, but we cannot exclude the possibility that earlier onset of cambial activity might be associated with increased risk of frost damage to the cambium.

**Acknowledgements** – This work was supported, in part, by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (nos. 19580183, 20120009, 21380107, 205659, 2200104, 23380105 and 24380090).

**References**


Edited by M. Uemura