with insecticidal activity. He dwelt on the identification of different stage-specific isoforms of actin present in insects which could be targeted to design highly specific novel insecticides. Since targeting stage-specific isoforms of actin causes global impact on insect embryos, larvae, pupae and adults, there was little scope for the insect to mutate itself to confer resistance to the insecticide. A phytochemical with selective target on insect actin has been reported, he indicated.

B. K. Tyagi (Center for Research in Medical Entomology, Madurai) presented a paper on arthropods and their biomedical importance. He summarized the biomedical and public-health importance of arthropods and stressed that the meticulous evaluation of the whole Arthropoda from the angle of biomedical significance and impact on public health would be an inevitable scientific necessity of the hour. Earlier in his presentation, Tyagi introduced a new concept of medical arthropodology, which encompasses all the disciplines, including medical entomology which deals with biomedical importance of insects, ticks, mites, spiders, centipedes, millipedes, scorpions and crabs. K. Narayanan (Multiplex Bio-Tech Pvt. Ltd, Bangalore) discussed the impact of host plant–insect and pathogen interactions on microbial control of insect pests. He highlighted the impact on the interaction between chemical composition of plants, insects and pathogens on the efficacy of various insect pathogens. He also indicated the change in insect behaviour due to viral infection in better utilization of insect viruses. N. K. Krishnakumar (Indian Institute of Horticultural Research, Bangalore) discussed the role of the predominant aphid vector species of Papaya Ring Spot Virus (PRSV), their influence of epidemiology, efficiency and few aspects of their management. According to him, three aphid species are involved, viz. *Aphis gossypii*, *Myzus persicae* and *Aphis craccivora*. Single-aphid inoculation studies indicated that *M. persicae* was the most significant vector compared to *Aphis craccivora* and *Aphis gossypii*. A simple and novel, leaf-disc assay was standardized to study the vector efficiency of PRSV, he indicated.

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RESEARCH NEWS

The florigen mystery: A potential solution for an ancient riddle

R. Jeyaraman and Susan Mary Varghese

Way back in 1865, Julius Sachs proved that leaves exposed to light produce a 'flower forming' substance, later christened 'florigen', which directs flower formation even in dark-adapted leaves. Much later, in 1934, Knott[1] proved that the day-length signal is perceived in the leaf, where it induces a signal that moves through the phloem sieve elements to the shoot apex, thus initiating the process of flowering. However, it was the classical experiment of Chailakhyan[2] in 1936 that proved beyond doubt, that the photoperiodic signal is indeed perceived by the leaf and translocated to the shoot apex where flowering is induced. In a set of simple and elegant experiments, he showed that this flowering signal could be transmitted from an induced leaf, via a graft union, to initiate the flowering process in a dark-adapted stock. However, though the existence of a flowering signal was firmly established, the exact nature of this signal has remained a riddle. Research into the nature of signal for the past eight decades has been inconclusive and has only kept the florigen mystery alive.

Recently, a seemingly significant breakthrough in unravelling the identity of florigen occurred when Huang *et al.*[3] reported that the mysterious flowering signal could after all be the mRNA of the flowering locus (*FT*) gene, one of the two genes that had been identified to be critical to photoperiod-induced flowering in *Arabidopsis thaliana*. Huang conjectured that upon light induction of leaves, the Constans (*CO*) gene, a transcriptional regulator, activates the transcription of the *FT* gene. The transcribed product (mRNA) is further believed to be transmitted from the induced leaf to the apical region through the phloem tissue[4]. Having come nearly 80 years after Chailakhyan's work, this report fuelled renewed interest into the story of florigen and the discovery made it to the list of 'Breakthroughs of the Year' in *Science*.

However, there was a twist in the tale when Corbesier *et al.*[5] reported the movement of the FT protein from the leaf to the shoot apex of *Arabidopsis*. They claimed that the FT protein itself, and not the mRNA, acts as the long distance, mobile flowering signal. *In situ* hybridization analysis by probing the chimeric DNA fragment spanning the junction between FT and the Green Fluorescent Protein (GFP) did not detect the presence of the FT mRNA in the shoot apex, thus excluding the possibility of the FT mRNA being the flowering signal. In another experiment, Corbesier *et al.*[5] fused the *FT* gene with the *GFP* reporter gene and reported the presence of GFP in the phloem and shoot apex. These results suggested that the FT protein per se, and not the mRNA, is the mobile flowering signal. Tamaki *et al.*[6] also corroborated these results in rice, where they further confirmed that the protein of the *FT* gene homologue is indeed the mobile flowering signal. Both papers failed to find evidence for movement of the FT mRNA[7].

Adding intrigue to the already colourful history of florigen, Nilsson, who led the team that reported the FT mRNA results, accused Huang, the first author of the original *Science* paper[3], of manipulating data[7]. Nilsson claimed that Huang selectively excluded some datapoints and statistically overweighted others[8]. Following these reports, the original paper that suggested the FT mRNA to be the mobile signal, has been retracted[8]. However, Huang, who left Nilsson's laboratory for the Xiamen University in China after publication of the paper, did not agree to the retraction, terming it premature.

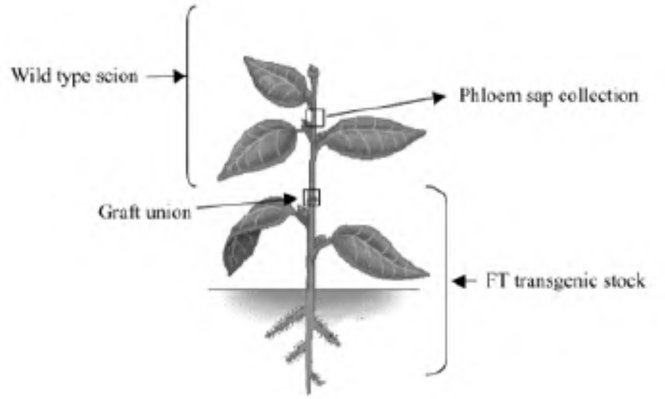

Figure 1. Schematic representation of graft union of FT transgenics.

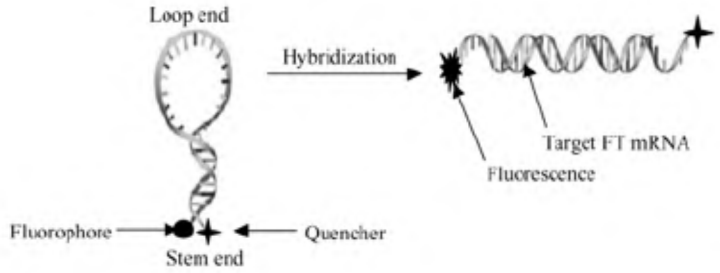

Figure 2. Schematic representation of FT mRNA hybridizing to molecular beacons.

But, such is the story of the jinxed florigen mystery that even the more recent hypothesis that the 'FT protein is the flowering signal', has not been spared from criticism. Reviewers have pointed out that the possibility of GFP movement on its own accord cannot be ruled out[8]. Besides, there are various convincing reports that establish the movement of both RNA and proteins of many different genes in the phloem[9,10]. Hence, the prospects of the FT mRNA indeed being the flowering signal need further investigation before being discarded entirely. The debate continues. But is there some way out of this muddle? Here we suggest an elegant approach to seek an answer to the vexing signal.

Starting with a photoresponsive species, where phloem sap collection is easy, one could develop a transgenic overexpressing the FT mRNA. Following Chailakhyan's original flowering experiment, the scion of the wild type of the same species can be grafted onto the stock of the FT transgenic (Figure 1) to prove the movement of the FT mRNA from an induced tissue, through the phloem cells. Since the flowering signal is known to travel along with photosynthates in the phloem, analysis of the phloem sap from above the grafted portion for the presence of FT mRNA and protein, could be an ideal technique to unravel the identity of florigen. Real-time PCR analysis using cDNA synthesized from the phloem-sap RNA, combined with the use of specifically synthesized molecular beacons (MBs) complementary to the target FT mRNA region, could be used to conclusively prove the nature of the phloem-transmitted flowering signal.

MBs, a relatively new tool for highly sensitive and specific real-time molecular recognition, are a class of DNA probes, which are single-stranded oligo-nucleotides with a stem and loop structure[11]. The loop portion of it reports the presence of a specific complementary nucleic acid, while the stem segment contains a fluorophore and a quencher linked to the two ends of the stem. The stem keeps these two moieties in close proximity, causing the fluorescence property of the fluorophore to be quenched by resonance energy transfer. When the MB probe encounters a target DNA/RNA, it undergoes a conformational restructuring that forces the stem to move apart, leading to the restoration of fluorescence. In closed state, the MB probe has minimal florescence. In the open state, when the fluorophore and quencher molecules are apart, it emits a strong fluorescence signal.

The primary advantage of the MB probe is that because of the low background signal, it displays an enhanced fluorescence upon hybridizing with the target sequence (Figure 2), which allows it to function as a highly selective probe for real-time monitoring. Another important advantage of the MBs are their molecular recognition specificity. MBs are extremely target-specific, ignoring target sequences that vary as little as a single nucleotide. MBs have also been reported to help visualizing mRNA in living systems[12,13], giving them a significant edge over many of the widely used molecular recognition techniques. This property could be used for detecting the presence of FT mRNA in the live shoot apex or the phloem, after photo-induction, in addition to the detection of the presence of FT::GFP protein. MBs, thus offer a potential technique to monitor the presence of FT mRNA in the leaves and the shoot apex, in real time, similar to the use of GFP. Looking for fluorescence of the FT mRNA-specific MBs in the leaf tissue and in shoot apical meristem would provide conclusive evidence for the identity of the flowering signal. One could also look for the presence of FT protein in the phloem sap of the same scion, thus unravelling the mystery that shrouds florigen.

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