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Gene Note

Natural variation of polyglutamine repeats of a circadian clock gene *ELF3* in *Arabidopsis*

Takeomi Tajima, Atsushi Oda, Mayu Nakagawa, Hiroshi Kamada, Tsuyoshi Mizoguchi*

Institute of Biological Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572
* E-mail: mizoguchi@gene.tsukuba.ac.jp Tel: +81-298-53-6005 Fax: +81-298-53-7723

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Abstract EARLY FLOWERING 3 (ELF3) plays key roles in the control of circadian rhythms, photoperiodic flowering, hypocotyls length and response to light in *Arabidopsis*. *ELF3*, however, encodes a protein without any motifs or domains of known function. Biochemical function of ELF3 has been largely unknown. Here we show the first demonstration of diversity of numbers of polyglutamines in the ELF3 among 60 *Arabidopsis* wild-type accessions. Significant correlation of the numbers of polyglutamines in ELF3 with two parameters of circadian rhythms, period and phase, suggest that the length of polyglutamine tract may affect the functions of ELF3 in the control of circadian rhythms.

Key words: *Arabidopsis*, circadian rhythms, ELF3, natural variation, polyglutamine.

Natural plant populations offer many advantages for the discovery of genes affecting the circadian rhythms of leaf movements, flowering time, and light responses (Koornneef et al. 2004; Maloof et al. 2001). In the present work, we identified genes that affect circadian rhythms in *Arabidopsis* by examining the *Arabidopsis* clock genes for expressed variations in sequence. In the course of our study, we found that the L*er* and Col *Arabidopsis* accessions differ in the number of polyglutamine-repeat tracts (Q-repeats) present in the C-terminal region of one of their circadian clock proteins, EARLY FLOWERING 3 (ELF3) (Figure 1). A similar difference between the Col and Ws accessions has been previously noted, but the other regions of the clock proteins are highly conserved (Hicks et al. 2001).

By comparing the lengths of the Q-repeats in sixty wild-type *Arabidopsis* accessions using PCR analysis (Figure 2), we found that the numbers of Q-repeats are remarkably divergent. This result was surprising because *ELF3* plays key roles in circadian clock function and is highly conserved in plants (Hicks et al. 2001; Miwa et al. 2006). Sequencing of the Q-repeat regions revealed that the genes contain anywhere from seven (found in Col-0, C24 and Lan-0) to 29 (found in Kas-1) Q-repeats (Table 1). Our results are novel in that they demonstrate for the first time that the amino acid sequences of plant clock proteins can vary to such a wide extent.

The proteins CLOCK and dClock play key roles in circadian clock function in vertebrates and *Drosophila*, respectively (Mizoguchi et al. 2006). These proteins have PAS and bHLH domains, as well as Q-repeat sequences, and the number of Q-repeats varies among different *Drosophila* wild-type accessions (Saleem et al. 2001). The *Neurospora* WHITE COLLAR 1 (WC-1) protein contains both PAS and zinc-finger domains and has Q-repeat sequences similar to those of CLOCK and ELF3. The WC-1 *rhy*-2 mutant, which lacks these Q-repeat sequences, exhibits an arrhythmic conidiation phenotype in constant dark (Toyota et al. 2002), indicating that the WC-1 Q-repeats are essential for clock function.

Plants with the *elf3* mutation exhibit a variety of defects at different growth stages. They have long

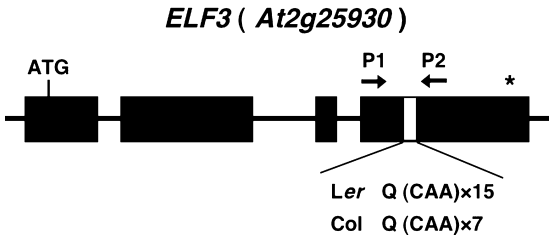

Figure 1. Polyglutamine-repeat tracts (Q-repeats) of ELF3 in the L*er* and Col wild-type *Arabidopsis thaliana* accessions. Black boxes indicate exons, and horizontal lines indicate promoters and introns. The initiation and stop codons are denoted by ATG and an asterisk (*), respectively. P1 (5′-ATGAT GCCCA CCATA ATGAA CCCA-3′) and P2 (5′-AAAGG ACTTG CTACC AGAGA TTCC-3′) are primers used to PCR-amplify DNA sequences, including the Q-repeats shown as an open box. Col and L*er* have 7 and 15 Q-repeats, respectively.

Abbreviations: ELF3, EARLY FLOWERING 3; LD, long days; LL, continuous light; SD, short days.
This article can be found at http://www.jspcmb.jp/

hypocotyls, long petioles, and pale-green leaves, and they flower early (Zagotta et al. 1996). They also exhibit arrhythmias in clock-controlled outputs such as stem elongation (Dowson-Day et al. 1999), leaf movement, and gene expression (Hicks et al. 1996), suggesting that ELF3 is a multi-functional protein. Furthermore, the transcriptional cascade "*GIGANTEA* (*GI*)–*CONSTANS* (*CO*)–*FLOWERING LOCUS T* (*FT*)" plays an important role in photoperiodic flowering in *Arabidopsis* (Mizoguchi et al. 2002, 2005). Although the floral activator genes *GI*, *CO*, and *FT* are expressed at higher levels in *elf3* mutants than in wild-type plants (Súarez-López et al. 2001; Kim et al. 2005), the *co* mutation does not significantly affect the early-flowering phenotype in the *elf* background (Kim et al. 2005), indicating that ELF3 regulates flowering, even in the absence of the CO-dependent pathway.

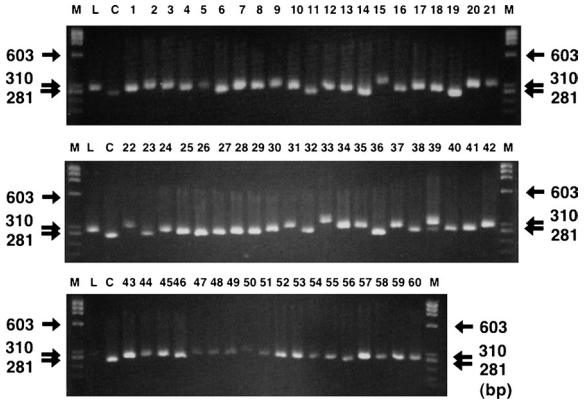

Figure 2. Different lengths of Q-repeats among sixty *Arabidopsis* accessions. L*er* (L) and Col (C) were used as controls. M denotes the molecular size markers (603, 310, and 281 bp; indicated by arrows). Numbers 1–60 indicate the wild-type accessions listed in Table 1. *Arabidopsis* wild-type accessions, AUA, Ben, Col, C24, DIG, Est, L*er*, Muh, Nie, No-0, RLD, RLD1, S96, and Ws were obtained from the Lehle Seeds, USA. Other accessions listed in Table 1 were obtained from the Arabidopsis Biological Resource Center (ABRC). Sequences containing Q-repeats in ELF3 were amplified by PCR with primers P1 and P2 shown in Figure 1. The number of Q-repeats in each gene was determined by DNA sequencing.

The N-terminal region of ELF3 interacts with phyB, a plant photoreceptor protein, which may partially explain the phenotypes of the *elf3* and *ELF3-ox* mutants with regard to light signaling (Liu et al. 2001; Reed et al. 2000). Both phyB and ELF3 play key roles in entrainment of circadian rhythms and red-light signaling (Carré 2002). The quantitative trait loci (QTLs) for these parameters were identified based on the phenotypes of the recombinant inbred lines (RILs) between Col and Kas-1 (Wolyn et al. 2004), and between Col and L*er* (Michael et al. 2003). Some of the QTLs, such as *RED2* (Wolyn et al. 2004) and *PHI2A* (Michael et al. 2003), map near the *phyB-ELF3* locus on chromosome 2. Natural variations of sequences in the *phyB* gene have been shown to be candidates for the *RED2* QTL (Wolyn et al. 2004). A candidate gene for the *PHI2A* QTL has not yet been identified, but natural variation in the number of Q-repeats in ELF3 may be partially responsible for the QTLs *PHI2A* and *RED2*.

Q-repeat modules are found in various proteins, including transcription factors and co-factors and the products of triplet-repeat disease genes (Imafuku et al. 1998), and have been suggested as forming polar zipper structures. Expanded polyglutamine tracts in triplet-repeat disease proteins self-aggregate to form neurotoxic nuclear inclusion bodies. The Q-repeat sequences of Brain2 (Brn-2), a Class III POU transcription factor, interact with proteins that are rich in polar residues such as Arg, Lys, His, Asp, Glu, and Ser (Imafuku et al. 1998). In ELF3, the C-terminal Q-repeats may play a key role in the interaction with other partner proteins containing Q-repeats or clusters of polar amino-acid

Table 1. Summary of numbers of the polyglutamine repeats.

Numbers of polyglutamines	Numbers of accessions	Names of wild-type accessions (lane no. in Gig. 2)
7	3	Col-0 (C), C24 (11), Lan-0 (36)
9	3	Cvi-0 (26), Bla-1 (19), Co-1 (23)
10	1	Te-0 (56)
11	4	No-0 (6), Ct-1 (25), Da-0 (27), Mh-0 (38)
12	3	Bs-5 (21), Dra-0 (29), JL-1 (32)
13	5	Dr-0 (28), Mrk-0 (40), Np-0 (43), Pi-0 (46), Van-0 (55)
14	6	AUA (13), Condara (16), Stw-0 (54), Litva925 (58), CS927 (59), CS929 (60)
15	4	L*er* (L), Est-0 (24), Ms-0 (41), St-0 (53)
16	11	Ws (1), Ben (4), Nie (5), Ken (12), Muh (10), Bl-1 (18), Edi-0 (30), Nok-0 (44), Oy-0 (45), Sci-0 (52), Tsu-0 (57)
17	2	DIG (7), S96 (8)
19	10	RLD (2), Est (3), RLD1 (9), An-1 (17), Ka-0 (34), Kin-0 (35), Lm-2 (37), Pog-0 (47), Rd-0 (48), Ri-0 (51)
20	1	Rak-2 (49)
21	2	Gr-1 (31), Mt-0 (42)
23	2	Br-0 (20), Bur-0 (22)
24	1	Mr-0 (39)
26	1	Rsch-0 (50)
27	1	Kyoto (15)
29	1	Kas-1 (33)

residues. ELF3 may regulate multiple signaling pathways through these interactions with different partners, including those factors involved in flowering and circadian rhythms.

The PERIOD (PER) protein plays a key role in the negative-feedback system that controls circadian rhythms in *Drosophila*. Although it does not have Q-repeat sequences, it does have Thr and Gly repeats (TG repeats) (Peixoto et al. 1998; Sawyer et al. 1997). Similar repeats are found in the FREQUENCY (FRQ) and WHITE COLOR 2 (WC-2) proteins of *Neurospora crassa* and their mammalian homologues (Peixoto et al. 1998). *Drosophila* PER displays a robust latitudinal cline in the length of the TG repeat; the southern variant more effectively maintains a 24-hour period at high temperatures (Sawyer et al. 1997).

Although mammals/insects, plants, and fungi employ different sets of molecules in maintaining their circadian clocks, some of the key molecules in each system have repeat sequences in common, and the lengths of these repeats can differ among wild-type variants of the same protein. Our work demonstrates a significant correlation between the numbers of Q-repeats in ELF3 and two circadian markers, period and phase in *Arabidopsis* (Figure 3). The *ELF3* related genes have been identified from rice, *Lemna* and soybean (Miwa et al. 2006), but these appear to lack the Q-repeat region. These results suggest that the length of polyglutamine tract may affect the functions of ELF3 in the control of circadian rhythms in *Arabidopsis*, but other plants may have different systems. So far, there has been no report on correlation between length of the Q-repeats and flowering time, hypocotyls length, petiole length and red-light response. Identification of molecules that interact with the ELF3 Q-repeats is the next important goal for increasing our understanding of the role of the circadian clock in fitness over a broad geographic range.

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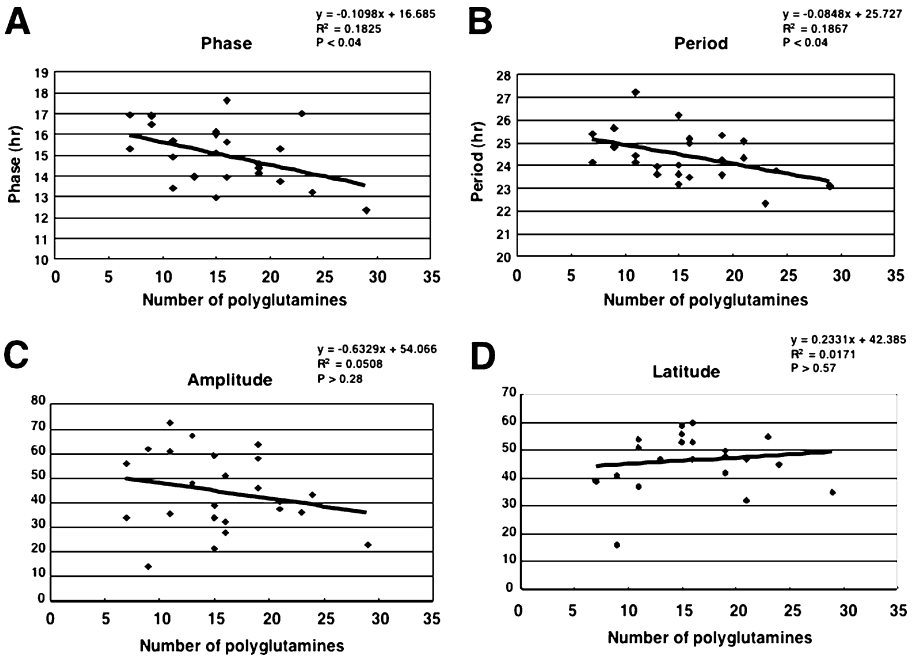

Figure 3. Correlation of the numbers of Q-repeats with the circadian clock parameters phase (A), period (B), and amplitude (C) in *Arabidopsis* wild-type accessions. The number of Q-repeats negatively correlates with phase (A) and period (B) ($P<0.04$) but does not correlate with amplitude (C), or latitude (D) ($P>0.28$ and $P>0.57$, respectively). The period, phase, amplitude, and latitude of 24 *Arabidopsis* wild-type variants reported by Michael et al. (2003) were used for statistical analysis.

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