attributed to an excited-state dipole moment, induced by the presence of local electric fields. These fields, which vary over time and produce spontaneous spectral diffusion, are thought to result, in part, from the presence of charge carriers on or near the QD surface.

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23. Data were collected from 47 single QDs for the 37.5 Å overcoated sample and from 57, 83, 74, and 16 single QDs for the 37.5 Å, 29 Å, 26 Å, and 22 Å standard samples, respectively. Calculations included screening by the CdSe core and ZnS shell as necessary.

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Natural Variation in a *Drosophila* Clock Gene and Temperature Compensation

Lesley A. Sawyer,* J. Michael Hennessy,* Alexandre A. Peixoto,† Ezio Rosato, Helen Parkinson, Rodolfo Costa, Charalambos P. Kyriacou‡

The threonine-glycine (Thr-Gly) encoding repeat within the clock gene *period* of *Drosophila melanogaster* is polymorphic in length. The two major variants *(Thr-Gly)17* and *(Thr-Gly)20* are distributed as a highly significant latitudinal cline in Europe and North Africa. Thr-Gly length variation from both wild-caught and transgenic individuals is related to the flies' ability to maintain a circadian period at different temperatures. This phenomenon provides a selective explanation for the geographical distribution of Thr-Gly lengths and gives a rare glimpse of the interplay between molecular polymorphism, behavior, population biology, and natural selection.

The clock gene *period* (*per*) in *Drosophila melanogaster* is an essential component of circadian rhythmicity, and its product is involved in a negative autoregulatory feedback loop with the Timeless protein [reviewed in (*1*)]. The *per* gene has a repetitive region, which encodes alternating pairs of predominantly threonine-glycine, but also serine-glycine dipeptide pairs (*2*). This repetitive region is conserved in the mammalian *per* homolog, suggesting that it may play an important functional role in circadian phenotypes (*3*). However, the only role assigned for the Thr-Gly region is to convey the species-specific characteristics of the ultradian male courtship song cycle (*4*).

Within natural populations of *D. melanogaster* and *D. simulans*, the Thr-Gly repeat is polymorphic in length (*5*). In *D. melanogaster*, Thr-Gly alleles that encode 14, 17, 20, and 23 dipeptide pairs [termed *(Thr-Gly)14*, *(Thr-Gly)17*, and so on] make up about 99% of European variants (*6*). The *(Thr-Gly)17* and *(Thr-Gly)20* alleles are distributed as a highly significant latitudinal cline, with high occurrences of the former observed in the southern Mediterranean and the latter predominating in northern Europe (*6*). In both *D. melanogaster* and *D. simulans*, analyses of intraspecific Thr-Gly haplotypes aimed at testing neutral models suggest that the repetitive regions are under selection (*7*, *8*). Furthermore, several studies revealed that Thr-Gly repeat length coevolves with the immediate flanking amino acids (*9*, *10*). If selection is shaping variation in the repetitive region, then the Thr-Gly cline in Europe implicates temperature as a possible selective agent.

Therefore, we studied the temperature responses of natural *Thr-Gly* length variants, which have the sequences shown in Fig. 1 (*11*). For simplicity, the *(Thr-Gly)17c* allele, which has the downstream (Thr-Gly)2 deletion, is referred to as *(Thr-Gly)15*. The Ser-to-Phe replacement is the only amino acid polymorphism that has been encountered in the immediate flanking regions surrounding the repeat in European (*11*) and other populations (*12*).

Free-running circadian locomotor activity rhythms of males from 37 different attached-X lines, whose *per*-carrying X chromosomes originated from eight European and North African localities, were examined at 18° and 29°C (Table 1) (*13*). A further attached-X line whose original male carried the *(Thr-Gly)23* allele from the American Canton-S laboratory strain was also added. This Thr-Gly haplotype is also found in European populations (*11*). The results based on spectral analysis (*14*) are presented in Table 1 and Fig. 2, A and B. Similar results were obtained with autocorrelation (Table 1) (*15*), but are not presented in detail. Two-way analysis of variance (ANOVA), performed with the 38 lines and temperature as the variables, gave significant Line and Temperature × Line interactions ($P < 0.01$), and further ANOVA of the data pooled into genotypes also gave significant Genotype and Temperature × Genotype interaction (both $P < 0.001$). Planned comparisons revealed that six of the 38 lines showed significant period differences between the two temperatures, whereas nine were significantly different when periods were determined with autocorrelation (Ta-

L. A. Sawyer, J. M. Hennessy, A. A. Peixoto, E. Rosato, H. Parkinson, C. P. Kyriacou, Department of Genetics, University of Leicester, LE1 7RH, UK.
R. Costa, Dipartimento di Biologia, Universitá di Padova, Via Trieste 75, 35121 Padova, Italy.

*These authors contributed equally to this work.
†Present address: Departamento de Bioquimica e Biologia Molecular Fundacao Oswaldo Cruz, Avenue Brasil, 4365-Manquinhos, CEP 21045-900, Rio de Janeiro, Brazil.
‡To whom correspondence should be addressed. E-mail: cpk@leicester.ac.uk

ble 1). These significant differences tended to fall in the comparisons involving the shorter (Thr-Gly) variants (Table 1). However, these results should be treated with some caution, because the critical P value of 0.05 was not adjusted for the 38 planned comparisons.

(Thr-Gly)20 variants showed the most efficient circadian temperature compensation, with no overall significant difference between the periods at the two temperatures (Fig. 2A; $P = 0.47$). However, the mean periods at both temperatures were somewhat shorter than 24 hours. The *(Thr-Gly)17* lines, on the other hand, produced a period closer to 24 hours at the warmer temperature, but the period shortened significantly at the colder temperature ($P = 0.029$). Nevertheless, the *(Thr-Gly)17* and *(Thr-Gly)20* variants, which make up 90% of natural alleles (6), are better compensated than the others. In addition, the periods of the *(Thr-Gly)14* and *(Thr-Gly)17* flies became longer as temperature increased (the direction of the arrow in Fig. 2A shows the change in period at warmer temperatures), whereas the converse was seen with *(Thr-Gly)23* (Fig. 2A). The *(Thr-Gly)15-21-24* variants, which are structurally "out of phase" with the (Thr-Gly)3 interval of the *(Thr-Gly)14-17-20-23* allelic series, appear less predictable in this respect as can be seen from Fig. 2B, which illustrates temperature differences in period as computed from the pooled means for each genotype. The "in-phase" *(Thr-Gly)14-17-20-23* series of variants, which differ by units of (Thr-Gly)3, fall close to the regression line ($r = -0.98$, $P < 0.02$; for these four variants only), whereas the out-of-phase variants fall further from the illustrated regression line (overall $r = -0.57$, not significant; for all seven variants). However, because of the unavoidably small sample sizes for the rare variants, we also weighted the correlation, using the period differences shown for each strain (Table 1). A significant correlation was obtained ($r = -0.328$, $P = 0.044$, $n = 38$). Removing the five lines with out-of-phase 15-21-24 lengths again strengthened the correlation ($r = -0.365$, $P = 0.037$, $n = 33$), but not significantly. Thus, it appears that an approximately linear relationship exists between Thr-Gly length and temperature compensation; this is particularly evident with the *(Thr-Gly)14-17-20-23* series, which make up the vast majority of natural variants (6). Structural studies of Thr-Gly peptides show that a (Thr-Gly)3 peptide represents a conformational monomer, generating a β turn (16). Perhaps then, the relationship between Thr-Gly length and temperature compensation has a structural component related to the dynamic properties of the (Thr-Gly)3 motif (16).

The phenotypic effects associated with these very small changes in natural Thr-Gly length are marginal. To test their validity, we generated *per* transgenes in which internal deletions of the repetitive tract from a cloned *(Thr-Gly)20 per* gene were made. *(Thr-Gly)17* and *(Thr-Gly)1* transgenes were constructed, and a Δ*(Thr-Gly)* transgene was included (Fig. 1) (17, 18). The free-running circadian locomotor rhythms were examined in two to four independent-

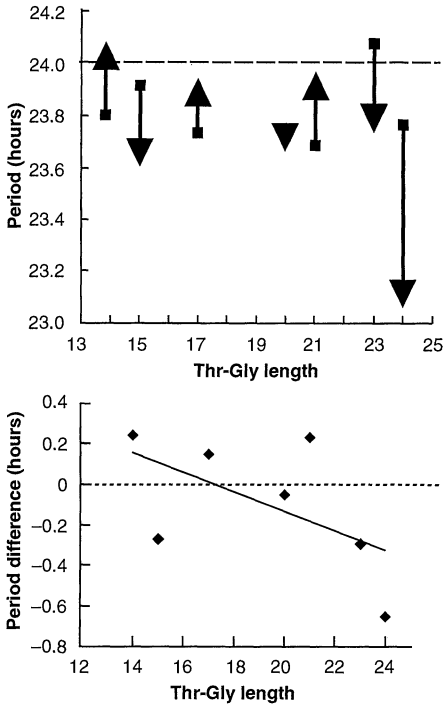

Fig. 2. (**Top**) Mean-free running periods at 18° (box) and 29°C (arrowhead) of males carrying different Thr-Gly length alleles. The means represent the pooled period averaged across the number of individuals within each Thr-Gly length (Table 1). The arrow reflects the change in direction of the period at 29°C. The *(Thr-Gly)20* variants show almost identical periods at the two temperatures. (**Bottom**) The same data as (A) is used, but is plotted as the mean period obtained at 18°C subtracted from that obtained at 29°C. The regression line is plotted.

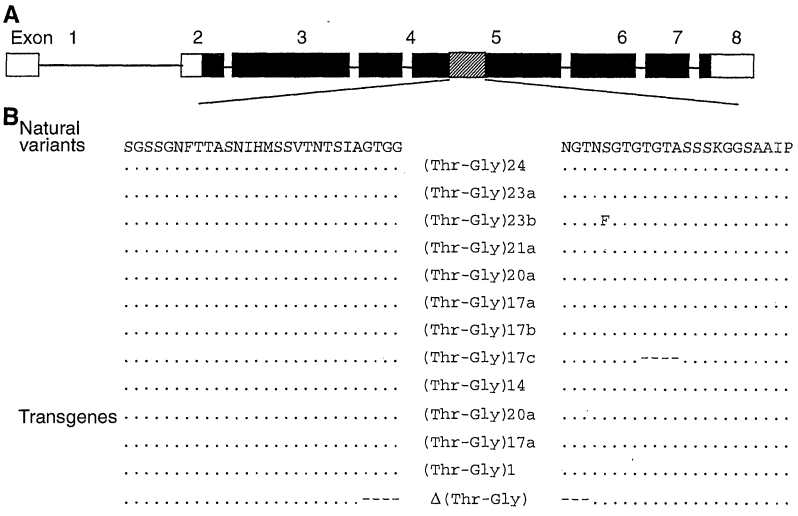

Fig. 1. (**A**) Intron and exon structure of *per* showing the position of the Thr-Gly repeat. Filled boxes represent translated exons, and the hatched box represents the Thr-Gly region. (**B**) Amino acid sequences of the Thr-Gly region from the natural strains and from the Thr-Gly transgenes (27). The uninterrupted Thr-Gly repeat length is given, and a, b, or c identify different isolength DNA sequences (5, 11). Dots denote identical amino acids; dashes show deletions. All European *(Thr-Gly)23b* variants show a fixed substitution (Ser to Phe) in the 3′ flanking region. The *(Thr-Gly)17c* variant has a downstream deletion of two Thr-Gly pairs and is therefore referred to as *(Thr-Gly)15* in the text. Fly populations were sampled from Europe and North Africa (5, 6), and isofemale lines were established immediately. One male from each isofemale line was crossed to attached-*X* females, generating a stable line in which the males carry the original paternal *X* chromosome. The length of the Thr-Gly encoding minisatellite within *per* was examined in the males of each attached-*X* line by PCR, by heteroduplex formation, and by subsequent DNA sequencing.

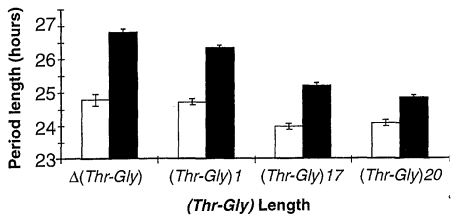

Fig. 3. Mean (and SEM) for free-running periods of per^{01} transformants at 18° (open bars) and 29°C (filled bars), which carry a single copy of a *Thr-Gly* transgene. The spectrally derived data from the different independently transformed lines within each *Thr-Gly* genotype have been pooled (19).

ly transformed lines for each Thr-Gly transgene, on a per^{01} background (Fig. 3). Two-way ANOVA on spectrally derived data gave significant Line, Temperature, and Line×Temperature interaction effects (all $P < 0.001$). A posteriori tests revealed that all lines for the Δ*(Thr-Gly)*, *(Thr-Gly)1*, and *(Thr-Gly)17* transgenes gave significantly longer periods at 29°C ($P \ll 0.01$ for each case). In contrast, only two of the four *(Thr-Gly)20* lines gave significant lengthening of the period at 29°C, and the temperature differences were smaller than for the other genotypes (*19*). These results convincingly support those based on the natural variants, even to the extent that the *(Thr-Gly)20* transformants show overall better temperature compensation than do the *(Thr-Gly)17* variants. Furthermore, the design of the transgenes (*17*) means that the associated temperature compensation differences cannot be due to any linkage disequilibrium with the different repeat arrays (*7*), but are caused by changes in the number of Thr-Gly pairs, with similar implications for the natural Thr-Gly variants.

A free-running circadian period of 24 hours may be optimal in *Drosophila*, reducing the physiological "cost" of a daily resetting of the circadian clock (*20*). Thus, at warmer temperatures, the *(Thr-Gly)17* variant has a period very close to 24 hours (Fig. 2A) and may enjoy an advantage, whereas at colder temperatures, its period shortens significantly. The more robust temperature compensation of the *(Thr-Gly)20* allele might therefore be at a premium in colder, more thermally variable environments, such as in northern Europe (*21*). Consequently, a balancing selection scenario can be envisaged, whereby the *(Thr-Gly)17* circadian periods are particularly adapted to warmer environments and the *(Thr-Gly)20* to colder climates. In fact, in Europe (*6*) and Australia (*22*), the *(Thr-Gly)17* allele generally predominates over the *(Thr-Gly)20* and only starts to fall in frequency at the more extreme, cooler regions within these continents. The behavioral differences we see in these variants in the laboratory represent only a limited snapshot of the true variation in circadian period that would be observed in the wild, where far greater extremes of temperature will challenge the *Drosophila* clock, both on a daily and seasonal basis (*21*). Consequently, the differences observed with natural length variants in the laboratory are likely to be considerably amplified in the wild.

There are considerable difficulties associated with measuring putative fitness characters for an organism such as *D. melanogaster* with an effective population size (n_e) of about 10^5 to 10^6 (*23*). Because the smallest selection coefficient visible to natural selection is the reciprocal of n_e, laboratory experiments are usually orders of magnitude too insensitive to detect tiny, but evolutionarily significant adaptive differentials (*24*). Nevertheless, in spite of this, we detected subtle behavioral differences among the naturally occuring Thr-Gly genotypes, which may illuminate our understanding of the European spatial patterning of the two major length alleles. Furthermore, our conclusions are buttressed by studies of linkage disequilibria involving the Thr-Gly repeat, which have revealed patterns of associations that are consistent with the major *Thr-Gly* alleles as being under selection (*7*). Finally, the differences in temperature compensation associated with the different Thr-Gly lengths are consistent with the coevolutionary dynamics that have been shown to act in this region (*9*, *10*). Within *D. melanogaster*, the differences in repeat length are not compensated by changes in flanking haplotypes, so small but detectable phenotypic changes are observed.

It is rare that natural variation in a behavioral phenotype can be shown to be caused by a molecular polymorphism at a single locus. The Thr-Gly array appears to provide an additional dimension to the fly's circadian temperature compensation system. This association between behavior and Thr-Gly polymorphism may "fine-tune" the circadian clock to different thermal environments and leads us to propose a simple selective explanation for the clinal pattern of Thr-Gly length variation seen in Europe (*6*).

Table 1. Spectrally-derived (*14*) free-running periods in constant darkness (DD) of males from attached-*X* lines carrying various Thr-Gly length alleles at different temperatures. The origins of the different lines are Cognac (CO), France; Conselve (CON), Pietrastornina (PI), and Lecce (LEC), Italy; Leiden (LE), Netherlands; Casablanca (CAS), Morocco; Rethimnon (RET), Greece; Canton-S (Cant.s), United States; and North Wooton (NW), United Kingdom. Significant differences for a priori comparisons based on ANOVA are highlighted (*$P < 0.05$,**$P < 0.01$; see text). These spectrally derived results are more conservative than those obtained from autocorrelation (*28*), in which significant period differences at the two temperatures were obtained in nine of the comparisons, six of which involved *(Thr-Gly)14* and *(Thr-Gly)17* variants.

Line number	Origin	(Thr-Gly) length	*n*		Period (in hours) ± SEM	
			18°C	29°C	18°C	29°C
1	CON	14	33	29	23.97 ± 0.15	24.12 ± 0.12
2	CON	14	28	28	23.43 ± 0.16	24.00 ± 0.14**
3	CO	14	29	28	23.92 ± 0.13	24.16 ± 0.13
4	LEC	14	13	16	23.85 ± 0.17	23.69 ± 0.17
5	CO	15	12	19	23.92 ± 0.20	23.65 ± 0.16
6	CON	17	31	32	23.94 ± 0.13	24.55 ± 0.15**
7	CON	17	15	29	23.31 ± 0.19	23.71 ± 0.15
8	CON	17	19	24	23.66 ± 0.29	23.91 ± 0.20
9	LE	17	21	9	23.49 ± 0.15	23.78 ± 0.36
10	PI1	17	12	14	23.75 ± 0.10	23.91 ± 0.25
11	PI9	17	15	14	23.99 ± 0.16	23.81 ± 0.22
12	CAS	17	59	40	23.21 ± 0.08	23.09 ± 0.17
13	RET9	17	27	12	23.84 ± 0.19	23.94 ± 0.15
14	RET2	17	7	10	24.20 ± 0.32	24.37 ± 0.20
15	LEC60	17	19	20	24.18 ± 0.17	24.31 ± 0.17
16	LEC12	17	28	22	24.13 ± 0.12	24.14 ± 0.17
17	NW6	17	13	20	24.48 ± 0.14	23.84 ± 0.14*
18	CO	20	25	29	23.70 ± 0.11	23.39 ± 0.18
19	LE	20	20	12	23.81 ± 0.11	23.91 ± 0.30
20	PI5	20	4	8	23.37 ± 0.42	23.61 ± 0.22
21	PI8	20	10	8	24.01 ± 0.18	24.28 ± 0.16
22	CAS	20	38	13	23.69 ± 0.08	23.12 ± 0.15*
23	RET4	20	16	11	23.81 ± 0.18	24.20 ± 0.29
24	CON	20	25	30	23.47 ± 0.13	23.28 ± 0.14
25	CON	20	22	26	23.72 ± 0.17	23.79 ± 0.14
26	LEC6	20	15	14	23.90 ± 0.13	24.40 ± 0.19
27	LEC3	20	12	14	23.88 ± 0.14	24.27 ± 0.12
28	NW1	20	10	18	23.73 ± 0.23	23.20 ± 0.17
29	CO	21	16	17	23.47 ± 0.21	23.85 ± 0.20
30	CON	21	26	25	23.81 ± 0.16	23.93 ± 0.13
31	CON	21	27	22	23.71 ± 0.10	23.96 ± 0.16
32	CO	23	19	11	24.38 ± 0.19	24.78 ± 0.15
33	CON	23	30	24	23.99 ± 0.15	23.75 ± 0.14
34	CON	23	21	23	24.03 ± 0.14	24.03 ± 0.14
35	Cant.S	23	16	19	24.00 ± 0.19	23.19 ± 0.21**
36	LEC79	23	12	13	23.95 ± 0.17	23.49 ± 0.19
37	LEC30	23	8	10	24.18 ± 0.17	23.70 ± 0.34
38	NW5	24	15	16	23.77 ± 0.15	23.11 ± 0.14*

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17. Two *(Thr-Gly)20* and two Δ*(Thr-Gly)* transformant lines in which the *per* transgenes were inserted within the cp20.1 vector marked with the *rosy*$^+$ gene were used (*18*). In addition, the *(Thr-Gly)20 per* transgene was also ligated into the pW8 vector marked with *white*$^+$ to further produce two transformant lines. The *(Thr-Gly)*1 construct was made by amplifying a 716–base pair (bp) fragment from the *(Thr-Gly)*20 clone and incorporating a deletion encoding 19 Thr-Gly pairs. This was done with 5′ primer (A), 5′-AACTATAACGAGAACCTGCT-3′ (4874 to 4893); with 3′ primer (B), 5′-ATTGCCGGTACCAC-CAGTGCCGGCAATGCT-3′ (5094 to 5113); with 5′ primer (C), 5′-GCACTGGTGGTACCGGCAATG-GAACAAATTCCGG-3′ (5231 to 5250); and with 3′ primer (D), 5′-GCTACGCCTGTTCCGGATCC-3′ (5627 to 5646), using hybrid polymerase chain reaction (PCR) methods (*25*). Numbers in brackets denote the nucleotide positions corresponding to the *per* sequence described (*26*). Primer B and C create a Kpn I site (GGTACC) using the two underlined mismatch bases. Primer A ends 37 bp upstream of an Sst I site, and primer D incorporates a Bam HI site (underlined); these sites were used to substitute a 607-bp Thr-Gly deleted fragment into a 7-kb Xba fragment, which encodes the 3′ half of the *per* gene. This Xba fragment was then ligated to a 5′ Bam HI–Xba fragment in several cloning steps, thereby reconstituting the 13.2-kb *per* transcription unit within the pW8 transformation vector. The *(Thr-Gly)17* construct was generated by amplifying a 364-bp Thr-Gly fragment using a natural *(Thr-Gly)17a* variant as template and 5′ primer (A), in conjunction with 3′ primer 5′-CATTGCCGGTACCAGTGCCT-3′ (5199 to 5215 and 5233 to 5236), which carried two mismatch bases (underlined) forming a Kpn I site. The amplified product was digested with Sst I (cuts at position 4930) and Kpn I, and was used to replace the Sst I–Kpn I fragment in the *(Thr-Gly)*1 construct.
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27. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
28. L. A. Sawyer *et al.*, unpublished results.

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Arabidopsis NPH1: A Protein Kinase with a Putative Redox-Sensing Domain

Eva Huala, Paul W. Oeller,* Emmanuel Liscum,† In-Seob Han,‡ Elise Larsen,§ Winslow R. Briggs‖

The *NPH1* (nonphototropic hypocotyl 1) gene encodes an essential component acting very early in the signal-transduction chain for phototropism. *Arabidopsis NPH1* contains a serine-threonine kinase domain and LOV1 and LOV2 repeats that share similarity (36 to 56 percent) with *Halobacterium salinarium* Bat, *Azotobacter vinelandii* NIFL, *Neurospora crassa* White Collar–1, *Escherichia coli* Aer, and the Eag family of potassium-channel proteins from *Drosophila* and mammals. Sequence similarity with a known (NIFL) and a suspected (Aer) flavoprotein suggests that NPH1 LOV1 and LOV2 may be flavin-binding domains that regulate kinase activity in response to blue light–induced redox changes.

Plants irradiated with unilateral blue light grow toward the light source, a phenomenon known as phototropism. We studied a 120-kD plant plasma membrane–associated protein that becomes heavily phosphorylated on irradiation with blue light both in vivo and in vitro. Physiological and genetic evidence implicates this protein in an early step in the signal-transduction pathway for phototropism (*1*, *2*). We previously isolated phototropism mutants at four loci in *Arabidopsis thaliana* (L.) Heynh., designated *NPH1* through *NPH4* for nonphototropic hypocotyl (*2*). Three *nph1* alleles generated by fast neutron irradiation (*nph1-1*, *nph1-4*, and *nph1-5*) lack all known phototropic responses, demonstrating that the NPH1 protein is essential for all phototropic signal-transduction pathways in *Arabidopsis*. In addition, these mutants lack the 120-kD phosphoprotein, suggesting that *NPH1* might encode the phosphoprotein itself (*2*).

The *NPH1* locus is located on chromosome III, within 26 centimorgans (cM) of *GL1* (*2*). Using described methods (*3*), we employed amplified fragment length polymorphism (AFLP) (*4*) to identify DNA markers flanking the *NPH1* gene at distances of 0.3 and 0.4 cM (Fig. 1A). These markers were used to screen an *Arabidopsis* yeast artificial chromosome (YAC) library (*5*) by polymerase chain reaction (PCR) (*6*). Of three YACs identified, two (yUP1C7 and yUP21F3) overlap each other and map to chromosome III near the *NPH1* gene (*2*). Using sequences from yUP1C7 as a probe, we detected an altered restriction fragment pattern in *nph1-5* and cloned an 18-kb Eco RI fragment that appeared in *nph1-5* but not in the wild type. From this fragment, we subcloned a 7-kb Bgl II fragment that contains an end point of the DNA rearrangement in this mutant (Fig. 1B). Because we had biochemical evidence that the *NPH1* gene product was

Department of Plant Biology, Carnegie Institution of Washington, 260 Panama Street, Stanford, CA 94305, USA.

*Present address: DNA Plant Technology, 6701 San Pablo Avenue, Oakland, CA 94508, USA.
†Present address: Biology Department, University of Missouri, Columbia, MO 65211, USA.
‡Present address: Department of Biology, University of Ulsan, Kyungnam, Ulsan 680-749, Korea.
§Present address: Department of Botany and Plant Science, University of California, Riverside, CA 92521, USA.
‖To whom correspondence should be addressed. E-mail: briggs@andrew.stanford.edu