experiment to ensure detection of many activated chromosomes in each case.
26. Immunostaining of polytene chromosomes with antibodies against tetra-acetylated histone H4 was carried out in the LW-1 line. This line contains the pU/l5 construct inserted at the 93B cytological location and hsGAL4$^{7-1}$ balanced over the CyO chromosome. Larvae raised from control embryos grown at 18°C or from embryos submitted to an embryonic GAL4 pulse showed the same pattern. In contrast, a transient hyperacetylation of histone H4 could be observed at 93B upon a 45-min 37°C heat shock at third instar larvae. The signal was lost 100 min after the end of the GAL4 pulse (*30*). Therefore, a line that does not show trxG-mediated inheritance of active chromatin states does not show stable H4 hyperacetylation.
27. Association of RNA polymerase II during transcriptional activation of the UAS-lacZ reporter in the *Fab-7* template of the FLFW-1 line was studied by immunostaining polytene chromosomes in a time course experiment of heat shock GAL4 induction. Strong recruitment of RNA polymerase II was observed 40 and 80 min after the end of a 45-min 37°C heat shock (*30*). This suggests that hyperacetylation at the same template could have been observed (note the absence of hyperacetylated H4 signal at *Fab-7* in Fig. 3, K and L) if it did occur to a substantial degree.
28. The line FLFW-1, carrying a construct with two *Fab-7* elements, is very inefficient in the meiotic transmission of the activated state. The FLW-1 line, with the *Fab-7* transgene p5F24 at cytological position 13F, showed effective meiotic transmission of active states. Flies of the FLW-1 line were activated by an embryonic pulse of GAL4. Twenty red-eyed F_0 females were crossed with thirty males of the 5F24 25,2 line (*9*), carrying the same *Fab-7* transgene but no GAL4-expressing construct. GAL4-less F_1 flies were selected for strong eye pigmentation (25 to 30% of the total progeny) and recrossed further to obtain F_2 and F_3. β-Gal staining of F_2 and F_3 embryos was performed as described by B. Zink, Y. Engström, W. J. Gehring, and R. Paro [*EMBO J.* **10**, 153 (1991)]. The staining pattern was compared with control FLW-1 embryos at 18°C or upon a 45-min heat shock followed by 2 hours of recovery or with embryos of the lines U/l5 1,1 and U/l5 2,1 grown at 18°C. Further proof of the absence of the hsp70-GAL4 transgene from activated FLW-1 flies was obtained by demonstrating that β-Gal staining of F_2 embryos from activated FLW-1 flies was now independent of a heat shock treatment. Furthermore, the absence of the GAL4 transgene was directly demonstrated by Southern (DNA) analysis of genomic DNA of activated FLW-1 flies (*30*).
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A Direct Estimate of the Human αβ T Cell Receptor Diversity

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Generation and maintenance of an effective repertoire of T cell antigen receptors are essential to the immune system, yet the number of distinct T cell receptors (TCRs) expressed by the estimated 10^{12} T cells in the human body is not known. In this study, TCR gene amplification and sequencing showed that there are about 10^6 different β chains in the blood, each pairing, on the average, with at least 25 different α chains. In the memory subset, the diversity decreased to 1×10^5 to 2×10^5 different β chains, each pairing with only a single α chain. Thus, the naïve repertoire is highly diverse, whereas the memory compartment, here one-third of the T cell population, contributes less than 1 percent of the total diversity.

Adaptive immunity is dependent on a genetic recombination machinery that assembles a diverse set of functional immunoglobulin or TCR genes from a pool of discontinuous gene segments. The available pool, for the human αβ TCR, consists of 42 variable (V) and 61 joining (J) segments in the α locus and 47 V, two diversity (D), and 13 J segments in the β locus. During the V_α-J_α or V_β-D_β-J_β rearrangement, nucleotide additions or deletions at the junctions add to the diversity (*1, 2*). As a result, most of the variation in each chain lies in the complementarity-determining region 3 (CDR3), which is encoded by the V(D)J junction and interacts with the antigenic peptide presented by the major histocompatibility complex molecule (*3*). A further diversifying factor is the pairing of an α chain to a β chain to form the TCR heterodimer. The potential diversity thus created has been calculated to be perhaps 10^{15} (*2*). Obviously, not all of it is used, but the T cell repertoire remains complex enough to have precluded any attempts to directly measure it. Here, we have analyzed a fraction of the repertoire in a way that allowed us to extrapolate the results to the whole repertoire (*4*).

To determine the diversity of the TCR β chain, we selected a V-gene family with a single member, $V_\beta18$, and studied its rearrangement to the $J_\beta1.4$ segment. Complementary DNA from 10^8 peripheral blood T cells from a healthy donor (male, between 20 and 30 years old) was amplified with $V_\beta18$- and $J_\beta1.4$-specific primers and separated on an acrylamide gel, producing a pattern of eight bands spaced by three nucleotides that correspond to in-frame TCR transcripts with different CDR3 lengths (Fig. 1A). The band corresponding to a CDR3 with a length of 12 amino acids was purified and cloned, and all of the different sequences within it were then identified by sequencing (Fig. 1B) (*5*). The number of different sequences, 17, was used to calculate the β-chain diversity in the sample (Table 1). The intensity of the bands in a V-J profile is proportional to the diversity within them (*6*), so the 12–amino acid band contained 9.3% of the total $V_\beta18$-$J_\beta1.4$ diversity. The $J_\beta1.4$ gene is used, on the average, by 3.0% of T cells (*7*), and the frequency of $V_\beta18^+$ cells in our donor was 0.8%. Together, these give an average frequency of $(0.093 \times 0.03 \times 0.008)/17 = 1.3 \times 10^{-6}$ for any one β sequence; that is, the sample contains 0.8×10^6 different β chains. This was confirmed by an analysis of the $V_\beta16$-$J_\beta2.2$ rearrangement in the same sample. The $V_\beta16$ family also consists of only one member, but unlike the $J_\beta1.4$ gene, the $J_\beta2.2$ gene may be rearranged to both D_β genes and thus has, in principle, more potential to generate diversity. We found 15 different sequences with a 13–amino acid CDR3 and a total diversity of 0.8×10^6 different β chains. In a second donor (female, 26 years old), the $V_\beta16$-$J_\beta2.2$, 13–amino acid band contained 14 different sequences, with the total diversity in this sample of 10^8 T cells being 1.2×10^6. Thus, analysis of three rearrangements, from two donors, produces estimates that are in close agreement and indicates that the repertoire in the blood consists of at least 10^6 different β chains.

To determine how well our sample reflected the whole diversity in blood, we analyzed the $V_\beta18$-$J_\beta1.4$ 12–amino acid rearrangement in two more samples of 10^8 T cells from the first donor. Overall, 69% of the distinct sequences were shared between the three samples. The second sample contained four previously unidentified sequences, and the third sample contained only one. This suggests that together these three samples contain most of the total β-chain diversity in the blood. Thus, there are probably less than

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30 different 12–amino acid $V_\beta 18$-$J_\beta 1.4$ sequences present in the blood of this donor, or, calculated as above, no more than 1.3×10^6 different β chains.

Nevertheless, it could still be argued that by random sequencing we might, despite the apparent plateau in the appearance of new sequences, miss less frequent clones. Indeed, a recent estimate of the human β-chain diversity in the $CD4^+$ population, based on limiting dilution analysis of the frequency of arbitrarily selected β-chain sequences, suggests a diversity that is an order of magnitude higher than that observed by sequencing (*8*). To address this possibility, we analyzed the second sample from the first donor by colony hybridization. We designed a panel of oligonucleotides, specific to the $V_\beta 18$-$J_\beta 1.4$ 12–amino acid CDR3 sequences already found by random sequencing, to look for colonies that did not give a positive signal and might thus contain new CDR3 sequences. Among >3000 colonies, 150 did not give a signal and were sequenced, but no previously unidentified sequences were found (*9*). Another panel of oligonucleotides was used, specific to CDR3 sequences apparently absent from the second sample but present in the other samples from the same donor. By using this panel, we tried to determine whether these sequences were truly absent or simply not yet found. Among >2500 colonies, 16 were positive and were sequenced, but none contained new CDR3 sequences. Thus, even by increasing the number of analyzed clones by 10-fold, no further distinct sequences were found, including those present in other samples from the same donor. A further indication that our method detected practically all of the sequences present in the sample was that, within a sample of 750,000 cells, we measured a diversity of 500,000 different β chains (*10*).

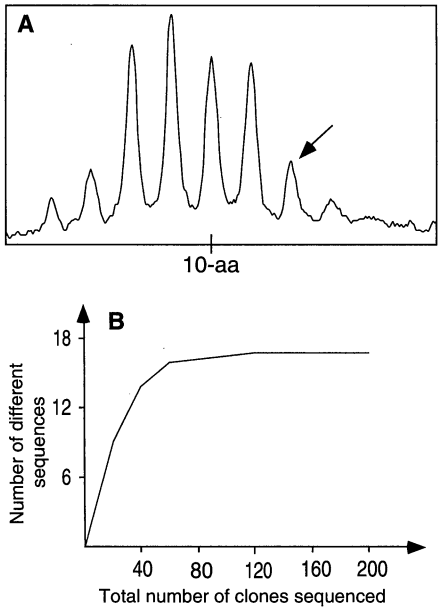

Fig. 1. Analysis of the $V_\beta 18$-$J_\beta 1.4$ rearrangement. Complementary DNA from 10^8 T cells was amplified by $V_\beta 18$- and C_β-specific primers for 40 cycles, followed by a run-off reaction with a fluorescent $J_\beta 1.4$-specific primer. (**A**) Electrophoresis of the PCR products. The denatured amplification products were separated on a 6% acrylamide gel, and the bands are shown as converted to peaks (*4*). The band corresponding to CDR3 with a length of 12 amino acids (aa) (arrow) was excised after silver staining and cloned, after 20 PCR cycles, to pCR2.1 vector with the TOPO-TA cloning system (Invitrogen). (**B**) Sequencing of the clones. The inserts were amplified with M13-40 and reverse primer, and the product was treated with exonuclease I and shrimp alkaline phosphatase. The sequencing reaction was done with M13-20 primer and Big Dye Terminator mix and analyzed with an Applied Biosystems 377 sequencer. Sequencing was continued until it was obvious that no new distinct sequences would be found. The sequences are plotted in the order of appearance.

Another layer of diversity is introduced by the heterodimerization of TCR chains. To estimate how freely a given β chain may associate with different α chains, we isolated 4×10^6 $V_\alpha 12^+$ T cells (*11*) and determined the β-chain diversity within this sample. Two β rearrangements were sequenced, indicating a diversity of 0.5×10^6 to 0.7×10^6 different β chains in the $V_\alpha 12^+$ sample (Table 1). The frequency of the $V_\alpha 12$ gene in this donor was 2.5%, or one-fortieth of the total α-chain repertoire. Therefore, the total αβ TCR diversity must be at least 40 times the β-chain diversity observed in the $V_\alpha 12^+$ fraction alone, that is, 25×10^6. Comparison with total β-chain diversity, 10^6, shows that each β chain must, on the average, pair with at least 25 different α chains. This is probably an underestimate, if only because it assumes that every β chain within the $V_\alpha 12^+$ population represents a clone, which is unlikely to be the case. We also determined the α-chain diversity itself, although it is not needed for the estimate of αβ TCR diversity. Analysis of two CDR3 lengths of the $V_\alpha 12$-$J_\alpha 20$ rearrangement, performed as described for the β chain, indicated a repertoire of $\sim 0.5 \times 10^6$ different α chains (Table 1).

Finally, we analyzed 15×10^6 $CD45RO^+$ memory T cells (*11*) from a healthy 38-year-old female in whom this subset comprised 37% of T cells. Sequencing of two rearrangements (*12*) indicated a β-chain diversity of 0.1×10^6 to 0.2×10^6, or 10 to 20% of that in the total population (Table 1). Moreover, unlike in the total population, the α to β pairing was highly restricted. Within the $CD45RO^+$ population, sorting of $V_\alpha 12^+$ cells drastically decreased the β-chain diversity. In this donor, the $V_\alpha 12$ was expressed by 12%, or one-eighth of the memory cells. At least 75% of V_β-J_β rearrangements observed in the $V_\alpha 12^-$ population were absent in the $V_\alpha 12^+$ cells (Fig. 2), and sequencing of the nine–amino acid peak of the $V_\beta 17$-$J_\beta 2.7$ rearrangement showed that, within a given rearrangement, the diversity was further decreased fourfold. Thus, within the memory compartment, each β sequence is probably paired with only one α chain, and the memory αβ TCR repertoire of this donor is not larger than 2×10^5. Although factors such as age and antigenic exposure may cause individual variation, they are unlikely to change this difference between naïve and memory repertoire.

Our findings firmly place a lower limit to the total αβ TCR diversity in the blood at 25×10^6 different TCRs. The upper limit is set by the number of different α chains that each of the 10^6 β chains can pair with. In both humans and mice, during thymic development, the β chain is rearranged first, after which the cells proliferate until the α rearrangement (*13*). In mice, this proliferation results in a 20- to 50-fold expansion in thymocyte numbers, with an interval between β and α rearrangement of ~2 days (*14*). Not all of the potential pairs will be functional or survive selection, and in the mature reper-

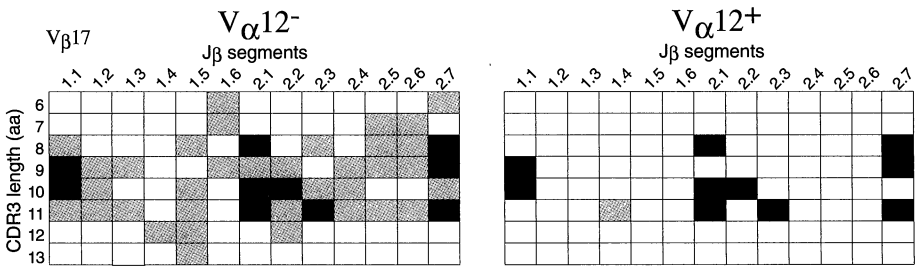

Fig. 2. The β-chain diversity in $V_\alpha 12^+$ and $V_\alpha 12^-$ memory T cells. Peripheral blood lymphocytes were enriched for $CD45RO^+$ cells by immunoaffinity chromatography, and then the $CD45RO^+$ $V_\alpha 12^+$ population was isolated to a purity of 95% by cell sorting. Complementary DNA from 150,000 sorted $V_\alpha 12^+$ or $V_\alpha 12^-$ memory cells was amplified with $V_\beta 17$- and C_β-specific primers, followed by a run-off reaction with fluorescent primers that were specific for each of the 13 J_β genes. Detectable rearrangements are shown as black squares if shared by the two populations and as gray squares if otherwise. Three-quarters of the rearrangements found in the $V_\alpha 12^-$ population are absent from the $V_\alpha 12^+$ population. The $V_\beta 16$-J_β and $V_\beta 18$-J_β rearrangements were also analyzed, with results similar to those shown here.

Table 1. Determination of TCR diversity. The average frequency of a given β or α sequence was calculated from these values and the number of different sequences found, as described in the text.

Rearrangement	CDR3 length	Frequency of V gene (%)	Frequency of J gene (%)	Size of CDR3 peak* (%)	Total number of sequences analyzed	Number of different sequences	Total size of repertoire ($\times 10^6$)
			β chain				
$V_\beta 18$-$J_\beta 1.4$	12 aa	0.8†	3.0‡	9.3	190	17	0.8
$V_\beta 16$-$J_\beta 2.2$	13 aa	0.4†	8.9‡	5.3	201	15	0.8
$V_\beta 16$-$J_\beta 2.2$	13 aa	0.5	8.9‡	2.6	479	14	1.2
			α chain				
$V_\alpha 12$-$J_\alpha 20$	11 aa	2.5†	2.2	2.5	22	5	0.4
$V_\alpha 12$-$J_\alpha 20$	10 aa	2.5†	2.2	13.5	150	34	0.5
			β chain in $V_\alpha 12^+$ subset				
$V_\beta 16$-$J_\beta 2.2$	13 aa	0.5	8.9‡	2.5	231	7	0.7
$V_\beta 23$-$J_\beta 2.4$	11 aa	0.8	1.6‡	9.6	107	6	0.5
			β chain in memory subset				
$V_\beta 17$-$J_\beta 1.3$	10 aa	3.0†	1.0	13.3	38	6	0.2
$V_\beta 17$-$J_\beta 1.3$	11 aa	3.0†	1.0	26.5	88	10	0.1

*Size was calculated by comparing the area of the corresponding peak to the combined area of all peaks. †These frequencies were based on immunofluorescence staining; other V-gene frequencies were based on a published average value for healthy donors. ‡These frequencies were based on random sequencing of TRC V_β-C_β sequences; other J-gene frequencies were measured by semiquantitative PCR.

toire, each β chain pairs, on the average, with only three or four different α chains (*15*). The scanty existing data for humans suggest a longer period of perhaps 5 days between the β and α rearrangement (*16*), which could allow an ~1000-fold expansion. In the mature repertoire, the upper limit of average pairing may therefore be ~100 different α chains per each β chain. Other mechanisms that might modify the α to β pairing and contribute to the difference between humans and mice include less stringent allelic exclusion of the α chain or more extensive editing of the α chain in humans than in mice (*17*).

The naïve repertoire consists of cells that have survived the negative and positive selection in the thymus, perhaps only 5% of the thymocyte population (*18*). From this pool, some cells are selected by antigen to proliferate. At the height of the response, the antigen-specific T cells may account for 25% of the population in the spleen and even more locally (*19*). Although most have a life-span of only a few days, some will survive as long-lived memory cells. Another feature of the response is its relative oligoclonality, shown, for example, for influenza and Epstein-Barr viruses (*20*). Our results reflect these facts in that although in our donor the memory compartment comprised one-third of the total T cell population, it contributed <1% to the total diversity. The overall diversity may be preserved by independent homeostatic regulation of the naïve and memory populations (*21*) and, perhaps, by preferred survival of recent thymic migrants (*22*). Indeed, it has been reported that the thymus retains its function as a primary lymphoid organ well into adulthood, albeit with a decreasing output (*23*). The maintenance of a stable level of total diversity is also indirectly supported by the convergent values we obtained from two different donors. Thus, by maintaining two pools of T cells with a very different repertoire, the immune system combines two conflicting needs: a recognition of a wide array of antigens and an efficient and timely response.

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3. M. M. Davis *et al.*, *Annu. Rev. Immunol.* **16**, 523 (1998).
4. Our approach was based on the immunoscope methodology [C. Pannetier *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4319 (1993); C. Pannetier, J. Even, P. Kourilsky, *Immunol. Today* **16**, 176 (1995)]. Complementary DNA is amplified with primers in the TCR V and C segments, followed by a run-off reaction with a nested fluorescent C gene– or J gene–specific primer. The products are analyzed with an Applied Biosystems PE373 automatic sequencer. The bands are expressed in graphic form as peaks, the area of each peak corresponding to the intensity of the band. A normal, polyclonal repertoire produces a Gaussian distribution of different CDR3 lengths, centering around 9 to 10 amino acids. An immune response can be detected as a deviation from the Gaussian distribution, the proliferating cells causing some peaks to expand in relation to others.
5. The CDR3 sequences were extracted from the raw sequence data with software developed by E. Beaudoing and others at Information Genetique et Structurale, CNRS, Marseille, France. Because of the infidelity of the Taq DNA polymerase, we excluded from the analysis sequences that were likely to represent polymerase chain reaction (PCR) mistakes, defined as sequences differing from another sequence by a single nucleotide. Analysis of conserved areas in the V gene showed that the actual rate of PCR mistakes was 1.6 per 1000 base pairs, that is, a mistake in 5% of a set of 10–amino acid segments. Our criterium resulted in the exclusion of 6% of the CDR3 sequences, a rate slightly in excess of the predicted amount. Thus, the data reported may be a slight underestimate.
6. The relative intensity of a band was calculated by comparing it to the combined intensity of all bands in the V-J rearrangement. To test the correlation between the intensity of the band and the sequence diversity within it, we isolated two bands from the same V-J rearrangement. In bands with relative intensities of 2.5 and 13.5%, we found 5 and 34 different sequences, respectively. Thus, the differences in the intensity and in the diversity were 5.4- and 6.8-fold, respectively. In another experiment, peaks of 4 and 11% contained 45 and 150 different sequences, respectively (*15*), that is, a 2.8-fold difference in intensity and a 3.3-fold difference in diversity.
7. If not otherwise indicated, the frequency of J_β genes was based on the sequencing of 427 random β chains [from W. M. C. Rosenberg, P. A. H. Moss, J. I. Bell, *Eur. J. Immunol.* **22**, 541 (1992); L. Ferradini and J. Even, unpublished material]. These estimates correlated well with values we obtained by measuring the J-gene expression by semiquantitative PCR and also with other published estimates [for example, M. A. Hall and J. S. Lanchbury, *Hum. Immunol.* **43**, 207 (1995); T. Nanki, H. Kohsaka, N. Miyasaka, *J. Immunol.* **161**, 228 (1998)].
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9. Bacterial colonies were lifted on nylon filters (Hybond), lysed, denatured, fixed, and then hybridized overnight at 63°C in 6× concentrated standard saline citrate, 1 mM EDTA, 0.5% SDS, 0.1% bovine serum albumin, preboiled salmon sperm DNA (100 μg/ml), with 10 pmol [γ-^{32}P]-adenosine-5′-triphosphate–labeled oligonucleotides, as recommended by Amersham Pharmacia Biotech and by J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2, 1989). The oligonucleotides were 20 to 22 mers, each with melting temperature of 64°C. The first oligonucleotide mix consisted of 12 oligonucleotides and, in control experiments, hybridized to 87% of a mix of bacterial clones known to bear a relevant insert. We also tested two relevant clones separately, and in both cases, the mix hybridized to >90% of colonies, although the strength of the signal was not uniform. Of 28 different clones with unrelated CDR3 sequences, none gave a positive signal. The second mix consisted of five oligonucleotides. In random sequencing, 19% of the clones in the first sample bore relevant inserts; the second oligonucleotide mix gave a detectable signal in 10% of the clones. We identified one relevant clone not detected by the mix, and none of 16 irrelevant clones were positive.
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11. We used specific monoclonal antibody and goat antimouse Dynabeads, with a ratio of four beads per target cell, for positive selection. For $V_\alpha 12^+$ cells, the purity was 99%; for CD45RO$^+$ cells, the purity was 95%.
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Arabidopsis NPH3: A NPH1 Photoreceptor–Interacting Protein Essential for Phototropism

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Phototropism of *Arabidopsis thaliana* seedlings in response to a blue light source is initiated by nonphototropic hypocotyl 1 (NPH1), a light-activated serine-threonine protein kinase. Mutations in three loci [*NPH2*, root phototropism 2 (*RPT2*), and *NPH3*] disrupt early signaling occurring downstream of the NPH1 photoreceptor. The *NPH3* gene, now cloned, encodes a NPH1-interacting protein. NPH3 is a member of a large protein family, apparently specific to higher plants, and may function as an adapter or scaffold protein to bring together the enzymatic components of a NPH1-activated phosphorelay.

Plants are able to sense and respond to changes in light quality, quantity, and direction through the action of a number of photoreceptors and associated signal-response systems. A variety of photoreceptor molecules, such as the red or far-red light–absorbing phytochromes (phy) and blue light–absorbing cryptochromes (cry) (*1*, *2*) have been characterized. Components functioning downstream of such photoreceptors include two photoreceptor-interacting proteins PIF3 and PKS1, which are both phytochrome-interacting proteins (*3*). Potential postperception signaling components identified through mutational analyses (*1*, *2*) include a set of genes in *Arabidopsis* (*NPH2*, *RPT2*, and *NPH3*) that is required for phototropism, or the bending response of plant organs toward or away from directional light stimuli (*4*, *5*).

Null mutations in the *NPH3* locus abolish phototropic responses of etiolated seedlings to blue light at a low fluence rate (*4*) (Fig. 1A). A similar aphototropic phenotype is observed with seedlings carrying null mutations at the *NPH1* locus (*4*) (Fig. 1A), which encodes NPH1, a primary photoreceptor for phototropism (*6*–*8*). However, mutations in neither *NPH1* nor *NPH3* affect other light-dependent responses, such as blue light– and red light–dependent hypocotyl growth inhibition (Fig. 1, B and C). Moreover, despite efforts to identify additional alterations in development, the only clear phenotypic changes observed in *nph1* and *nph3* mutants are those associated with phototropism (*9*). The phototropism-specific phenotypes of the *nph1* and *nph3* mutants imply that NPH1 and NPH3 act in the same genetic pathway and suggest that NPH3 may function biochemically close to the photoperception event mediated by NPH1.

We have cloned the *NPH3* gene by positional cloning (Fig. 2A). Several predicted genes mapping to the region containing the *NPH3* locus (Fig. 2A) were sequenced in mutant backgrounds to identify the *NPH3* gene (Fig. 2B). A full-length *NPH3* cDNA was isolated (*10*) and found to encode a protein of 745 amino acids (*11*) (Fig. 2C). NPH3 is, however, part of a family of proteins in *Arabidopsis* (*12*). Although *NPH3*-related sequences have also been found in other plant species, no paralogous sequences have been found outside the plant kingdom (*9*).

Four regions of sequence conservation have been identified within the NPH3 family (*12*) (Fig. 2C). Region IV exhibits the highest level of sequence identity within the family (≥52.5%, relative to NPH3), with two motifs being most prominent: LYRAID and HAAQNERLPL (*13*) (Fig. 2C). The functional importance of these conserved sequence motifs is currently unknown. However, Tyr^{545} within the LYRAID motif is part of a consensus phosphorylation site ([RK]-x(2,3)-[DE]-x(2,3)-Y; where x(2,3) can be any two or three amino acids) (*14*), in which the Arg, Asp, and Tyr are invariant across the entire NPH3 family (*12*), and deletion of this residue in the *nph3-2* mu-

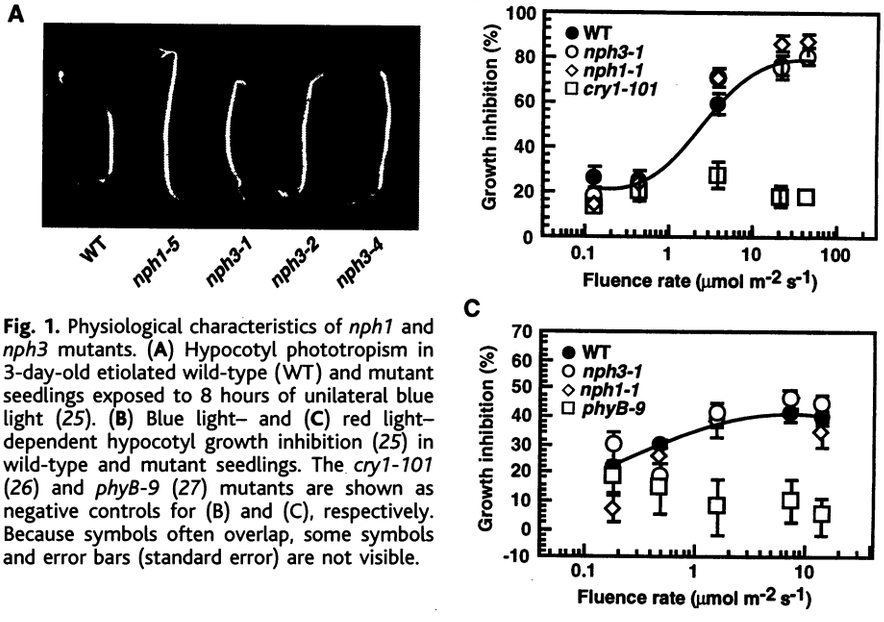

Fig. 1. Physiological characteristics of *nph1* and *nph3* mutants. (**A**) Hypocotyl phototropism in 3-day-old etiolated wild-type (WT) and mutant seedlings exposed to 8 hours of unilateral blue light (*25*). (**B**) Blue light– and (**C**) red light–dependent hypocotyl growth inhibition (*25*) in wild-type and mutant seedlings. The *cry1-101* (*26*) and *phyB-9* (*27*) mutants are shown as negative controls for (B) and (C), respectively. Because symbols often overlap, some symbols and error bars (standard error) are not visible.

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