prokaryotes (cyanobacteria) and eukaryotes (Rhodophyta (red algae) and Glaucocystophyta)). From these observations it has been believed that the ancestral chloroplast was rhodophyte- (and glaucocystophyte-) like in containing phycobilins, as do cyanobacteria, and that subsequently chlorophytes acquired chlorophyll *b* and lost phycobilins. Taking into consideration our finding that chlorophyll *b* in prochlorophytes and chlorophytes has a common evolutionary origin, it is more reasonable to assume that the origin of chloroplasts were oxygenic photosynthetic bacteria containing chlorophyll *b* and phycobilins, which would have been derived from the hypothetical common ancestor of prochlorophytes and cyanobacteria proposed here (Fig. 3). Therefore, the ancestral photosynthetic eukaryotes should have possessed both chlorophyll *b* and phycobilins (Fig. 3). This is consistent with the fact that all the algal groups that are believed to have primary-endosymbiotic chloroplasts (Chlorophyta, Rhodophyta and Glaucocystophyta)[1,2] contain either chlorophyll *b* or phycobilins as accessory pigments (Fig. 3). In the ancestral photosynthetic eukaryotes, chlorophyll *b* might have been bound to Pcbs, and then transferred to CABs, which arose soon after the primary endosymbiosis (Fig. 3). Subsequently chlorophyll *b* would have been lost in the lineages of Rhodophyta and Glaucocystophyta, while phycobilins were also lost in the Chlorophyta (Fig. 3). □

Methods

Gene isolation and sequencing. We isolated a full-length *CAO* cDNA clone of *A. thaliana* by screening an *Arabidopsis* cDNA library with an EST clone obtained from the Arabidopsis Biological Resource Center as a probe. A *CAO* cDNA clone of *O. sativa* was obtained from the Rice Genome Research Program. A *M. polymorpha* cDNA was a gift from H. Fukuzawa (Kyoto University, Japan). We extracted genomic DNA of *P. hollandica* from cultured cells and purified it on a CsCl gradient[23]. Genomic DNA of *P. didemni* was extracted[23] from cells that were collected from an ascidian *Lissoclinum patella* and were frozen. We carried out control PCR experiments with primers for eukaryotic and *Prochloron* rRNA and obtained amplified rRNA genes using primers for *Prochloron* but not for eukaryotes; accordingly, there was no contamination with chlorophyll *b*-containing eukaryotes in the *Prochloron* cells. Parts of *CAO* cDNAs from *M. polymorpha* and *D. salina* were amplified by PCR using cDNAs as templates with degenerate primers to the regions conserved in both *C. reinhardtii* and *A. thaliana CAO* sequences. We obtained a *CAO* cDNA clone of *D. salina* by screening a cDNA library using the PCR product for *Dunaliella CAO* as a probe. Parts of the *CAO* genes from *P. hollandica* and *P. didemni* were amplified by PCR using genomic DNAs as templates with the degenerate primers. The PCR products were cloned into a pBluescript plasmid vector (Stratagene). The nucleotide sequences were determined using the Dye Terminator DNA sequencing kit (Applied Biosystems) by a DNA sequencer (model 310, Applied Biosystems).

Phylogenetic analyses. The deduced amino-acid sequences of CAO and Tic55 (accession no. AJ000520) were aligned using CLUSTAL W[10] with manual refinement. Phylogenetic trees were generated using CLUSTAL W[10] for the distance matrix method[11] and using MOLPHY[15] for the maximum likelihood method[16]. All the amino-acid sites where gaps exist in the alignment were excluded from the calculation for the tree presented here. The same tree topology was obtained when those gap-located sites were included in the calculation (data not shown). We obtained the same tree topology when we used either slr1747 (ref. 13) or *lls*1 (ref. 14) sequence as an outgroup (data not shown).

Received 22 January; accepted 13 May 1999.

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Acknowledgements. We thank N. Iwabe for helpful discussions about phylogenetic analyses and comments on the manuscript; Y. Koshino for the use of unpublished sequences of *Arabidopsis thaliana*; A. Melis, M. Mimuro, and K. Shimizu for comments on the manuscript; R. Tanaka for technical assistance and comments on the manuscript; H. Fukuzawa for a *Marchantia polymorpha* cDNA; and T. Maruyama for helpful discussions.

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Improved auditory spatial tuning in blind humans

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Despite reports of improved auditory discrimination capabilities in blind humans[1–3] and visually deprived animals[4], there is no general agreement as to the nature or pervasiveness of such compensatory sensory enhancements[5]. Neuroimaging studies have pointed out differences in cerebral organization between blind and sighted humans[6–12], but the relationship between these altered cortical activation patterns and auditory sensory acuity remains unclear. Here we compare behavioural and electrophysiological indices of spatial tuning within central and peripheral auditory space in congenitally blind and normally sighted but blindfolded adults to test the hypothesis (raised by earlier studies of the effects of auditory deprivation on visual processing[13,14]) that the effects of visual deprivation might be more pronounced for processing peripheral sounds. We find that blind participants displayed localization abilities that were superior to those of sighted controls, but only when attending to sounds in peripheral

auditory space. Electrophysiological recordings obtained at the same time revealed sharper tuning of early spatial attention mechanisms in the blind subjects. Differences in the scalp distribution of brain electrical activity between the two groups suggest a compensatory reorganization of brain areas in the blind that may contribute to the improved spatial resolution for peripheral sound sources.

The ability to focus attention selectively on relevant external sound sources is essential for auditory perception. Behavioural studies have shown that auditory attention, like visual attention[15], is located in space in the form of a gradient, with the most efficient sound processing occurring at the attended locations and a progressive decline at increasingly distant locations[16]. The brain mechanisms of auditory spatial attention have been studied extensively in humans by means of non-invasive scalp recordings of event-related potentials (ERPs)[17,18]. In particular, focusing attention on a sound source in the environment is indexed by a negative ERP beginning 80–100 ms after the onset of the stimulus (the N1 component), which is greater in amplitude for sounds at attended than at unattended locations. A recent study using both behavioural and ERP measures found that the spatial gradient of auditory attention was less narrowly focused when subjects attended to peripheral as opposed to central sound locations[19], which is in agreement with previous behavioural studies showing decreases in auditory localization accuracy with increasing eccentricity of the sound source[20,21]. We have used similar behavioural and ERP methods to test whether mechanisms of auditory spatial attention display compensatory enhancements in blind individuals, and whether these effects are more pronounced for peripheral than for central auditory space.

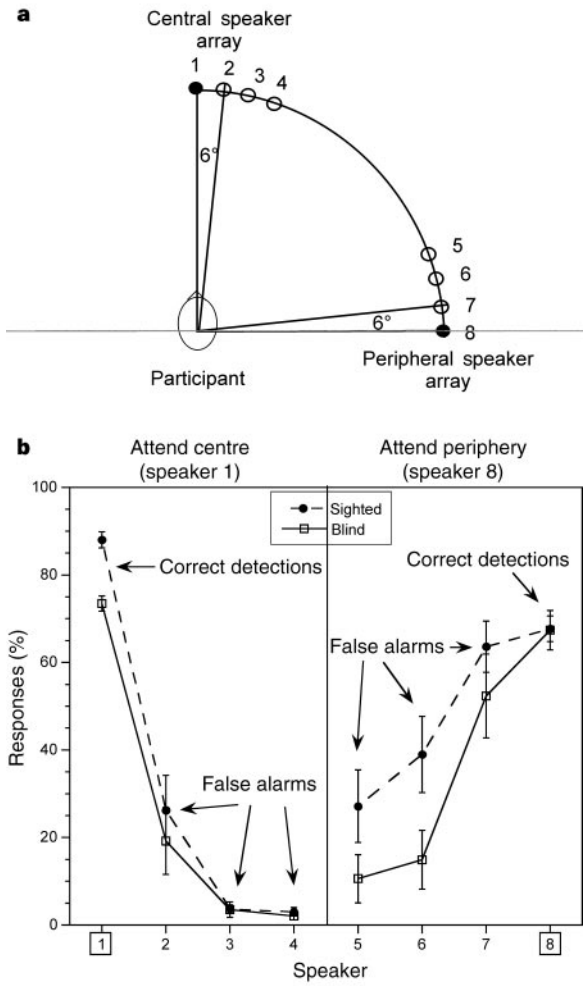

Figure 1 Speaker layout and response gradients. **a**, Central and peripheral speaker arrays. Participants had to detect rare deviants at speaker 1 (attend centre) or speaker 8 (attend periphery). **b**, Gradients of the percentage of detection responses (mean ± standard error) to deviants at the central speakers 1–4 and peripheral speakers 8–5 when the participant's task was to press a button to deviants at speaker 1 (attend centre) and speaker 8 (attend periphery), respectively. Responses to deviants at locations 1 and 8 were classified as correct responses, whereas responses to the remaining locations were considered false alarms. Response rates to deviants in the unattended speaker array were negligible and are not shown. Sighted and blind participants did not differ in their gradients of detection performance in the 'attend centre' condition, but blind participants showed a more sharply tuned gradient of attention than sighted subjects in the 'attend periphery' condition.

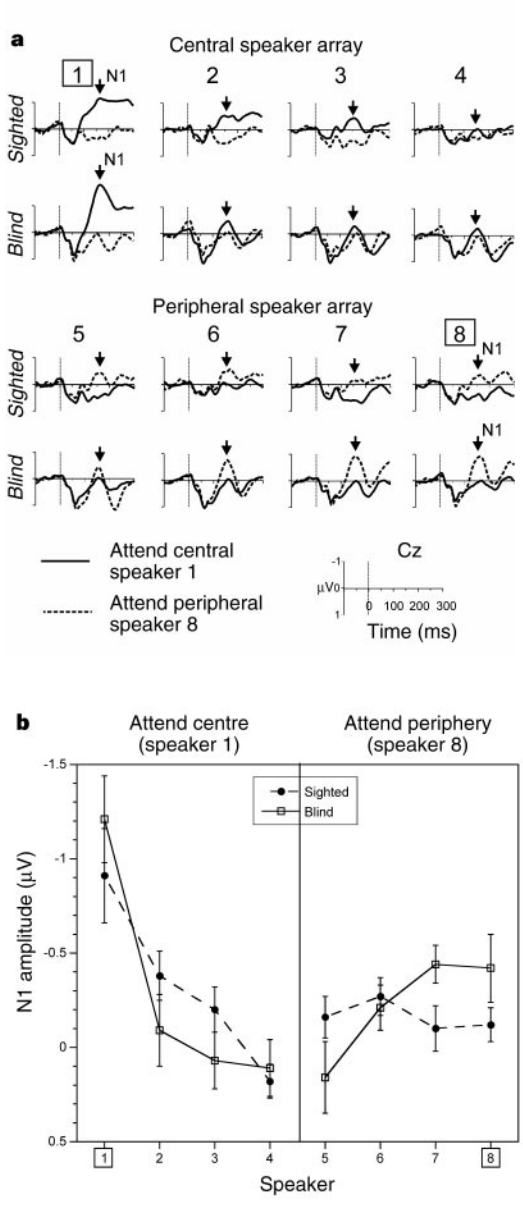

Figure 2 Event-related potentials to standard stimuli. **a**, ERPs recorded from the vertex (Cz) in response to standard stimuli when speaker 1 (solid line) and speaker 8 (dashed line) were attended. The N1 component is indicated by arrows above the waveforms. Because no behavioural responses were required to standards, the N1 amplitude reflects auditory spatial tuning without any contamination by motor activity. **b**, Gradients of mean N1 amplitude (±s.e.) to standard stimuli at speakers within the attended central and the attended peripheral arrays. N1 was measured from 100 to 200 ms poststimulus. The early attention mechanism indexed by N1 was more sharply tuned in the blind than in the sighted subjects when they were attending to targets at the peripheral speaker 8.

Table 1 Subjects' characteristics

Participant	Age (years)	Sex	Age and cause of blindness	Visual perception	Handedness
1	26	Female	Birth, glaucoma	Diffuse light	Right
2	43	Male	Birth, ROP	No	Right
3	36	Female	<1 year, retinoblastoma	No	Right
4	41	Female	Birth, ROP	No	Right
5	45	Female	Birth, ROP	No	Right
6	41	Female	Birth, ROP	No	Right
7	31	Male	Birth, retinoblastoma	No	Right
8	46	Female	Birth, congenital eye abnormalities, born with small eyes	No	Right

Blind subjects: mean age, 39 years (standard deviation, 7; range, 26–46 years). Control subjects: mean age, 41 years (s.d. 8; range, 28–52 years), 6 female and 2 male, 4 with normal and 4 with corrected-to-normal vision, all right handed. Sighted participants were blindfolded. All participants reported normal hearing, which was confirmed in a subsample by a hearing test.

Auditory spatial attention was assessed using brief noise bursts presented in random sequence with equal probability from four central and four peripheral speakers (see Fig. 1). In half the runs, the subject's task was to detect infrequent 'deviant' sounds (of a higher pitch) at the central speaker 1 (the 'attend centre' condition), and in the other half the subject had to detect deviants at the peripheral speaker 8 (the 'attend periphery' condition). Sounds from all other speakers were to be ignored. Behavioural measures of target detection accuracy and concurrently recorded ERPs were obtained from eight congenitally blind subjects and eight blindfolded, sighted control subjects matched on age, handedness and gender (Table 1). In each run, ERPs were recorded and averaged separately to the frequent ('standard') and rare deviant noise bursts at each speaker location.

As shown in Fig. 1, in the 'attend centre' condition, all subjects were highly accurate at detecting the deviant sounds at the designated location (speaker 1), and response rates declined progressively as a function of distance from the attended speaker. Although the correct detection rate at speaker 1 was higher in the sighted than the blind subjects ($t(1,14) = 5.7$, $P < 0.001$), the two groups did not differ significantly in the sharpness of attentional tuning, as reflected in the proportional decline of false alarm responses made to the adjacent speaker locations ($P > 0.2$ for all intergroup comparisons). In the 'attend periphery' condition, both groups were less accurate and made more false alarms to the adjacent speakers (analysis of variance (ANOVA) results: speaker, $F(3,42) = 112.56, P < 0.001$; condition, $F(1,14) = 31.59, P < 0.001$; speaker × condition, $F(3,42) = 21.15$, $P < 0.001$). Most importantly, however, the proportional decline in response rate between the attended and adjacent speakers was greater for the blind than for the sighted subjects, indicating a more narrow focusing of attention on the peripheral target location in the blind listeners. This steeper gradient of responding for the blind subjects across the peripheral but not the central array was reflected in a significant speaker × condition × group interaction ($F(3,42) = 3.45$, $P < 0.04$) and in a sharper decline in false alarm rates in the blind group going from speaker 8 to 6 ($t(14) = 1.90$, $P < 0.05$) and from speaker 7 to 6 ($t(14) = 1.78$, $P < 0.05$).

The effects of spatial attention on early auditory processing were indexed by the amplitude of the N1 component of the ERP elicited by sounds from each speaker. In both groups, the N1 amplitude (mean voltage over 100–200 ms) decreased progressively in response to sounds increasingly distant from the attended speaker and, like the behavioural data, this gradient was steeper

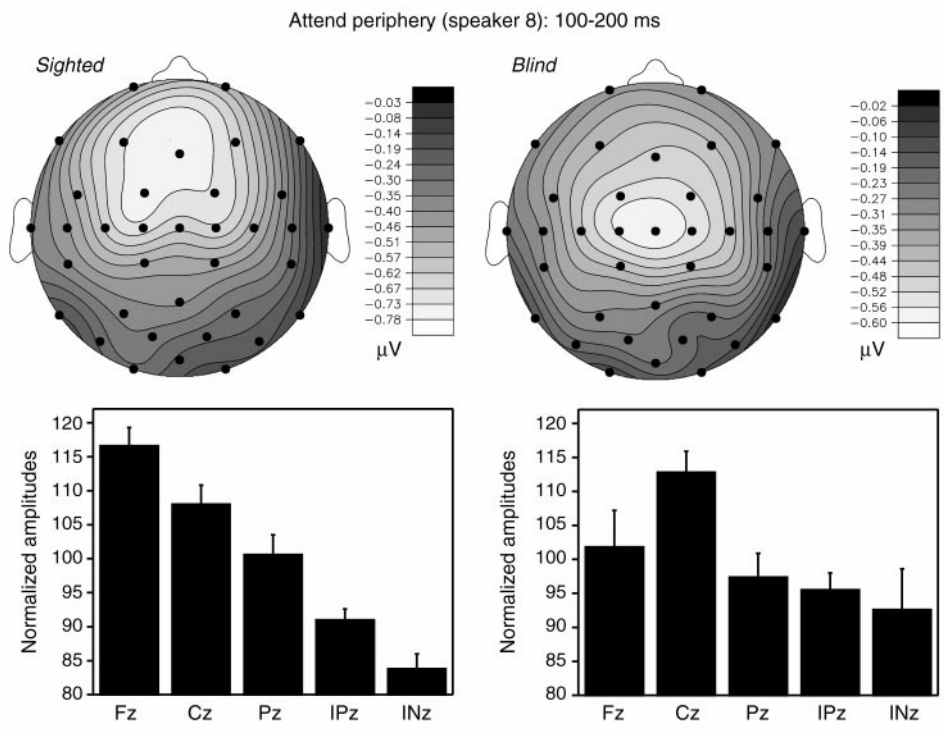

Figure 3 Topographic voltage maps of the N1 attention effect (attended minus unattended amplitudes) and the normalized anterior–posterior scalp distributions for the attended peripheral speaker. Left, sighted subjects; right, blind subjects. Lighter shading in the topographic maps indicates the greater amplitude of the N1 to attended relative to unattended standards. Bar graphs show standardized amplitudes of the N1 attention effect (mean, 100; s.d., 15) at frontal (Fz), central (Cz), parietal (Pz), parieto-occipital (IPz) and inferior-occipital (INz) electrode sites. The anterior–posterior distribution of the N1 attention effect differed between groups with a frontal maximum in the sighted subjects and a central maximum in the blind subjects.

when participants were attending to central compared with peripheral space (see Fig. 2; speaker, $F(3,42) = 23.19$, $P < 0.001$; condition × speaker, $F(3,42) = 12.38$, $P < 0.001$). However, whereas the gradient of N1 amplitude did not differ significantly between the two groups when attending to the centre, during attention to the periphery the N1 gradient was significantly steeper for the blind than for the sighted individuals (condition × speaker × group, $F(3,42) = 3.26$, $P < 0.05$; speaker × group interaction for 'attend periphery' condition, $F(3,42) = 4.69$, $P < 0.02$; for the 'attend centre' condition, $F(3,42) = 2.26$, $P > 0.1$).

To examine whether there is compensatory reorganization of the neural systems for early auditory selection in the blind, we compared the scalp distributions of the enhanced N1 component during auditory attention in the two groups. As shown in Fig. 3, in the sighted group the enhanced negativity was largest over the anterior scalp, whereas in the blind it was shifted posteriorly. This difference was only significant in the 'attend periphery' condition (condition × electrode (midline recordings) × group, $F(4,56) = 3.29$, $P < 0.04$; 'attend periphery' condition, $F(4,56) = 2.83$, $P < 0.05$; 'attend centre' condition, electrode × group, $F(4,56) = 1.61$, $P > 0.2$).

Our behavioural results are in agreement with a growing number of studies that report that blind individuals have equal or better auditory localization than sighted individuals[1–3,22]. Furthermore, our data show that this improved spatial acuity is not uniform but displays directional specificity: the advantage for blind subjects was found only at spatial positions where auditory localization is poorest in sighted humans, that is, at far lateral locations[21]. An improved focusing of attention in the periphery in the blind subjects was also evident in the spatial gradient of the N1 component, indicating that compensatory enhancement of early auditory spatial selection mechanisms may occur following visual deprivation from birth. Additionally, it is likely that the lack of the early neural stage of selectivity in the sighted (indexed by N1) contributed to the less finely tuned pattern of behavioural responses to peripheral sounds.

The posterior shift in the scalp topography of the enhanced N1 component in the blind subjects provides strong evidence for a reorganization of the neural substrates for early auditory selection. Previous research has reported a posterior shift of a later ERP response linked to target detection[7,12]. Studies in visually deprived animals indicate that such alterations in topography may arise from a recruitment of posterior multimodal brain areas in which visual space is represented in sighted individuals. For example, an increase in responsiveness to auditory and/or somatosensory stimuli after visual deprivation has been reported in multimodel areas, including the superior colliculus[23], parietal cortex[24] and the anterior ectosylvian sulcus[4]. In addition, neurons in this last area displayed sharper auditory spatial tuning in visually deprived than in sighted cats[4]. It has also been proposed that unimodal visual areas may be recruited for non-visual processing as well when visual input is absent[25].

Finally, we must consider how such a refined auditory spatial representation arises in the absence of visual guidance in the congenitally blind. Developmental studies have shown that human newborns orientate to sounds even in the dark and before the emergence of visual orientating[26], indicating that auditory spatial representations may develop initially on the basis of sensimotor feedback alone[4]. However, when visual input becomes available it appears to dominate and modulate the spatial representations of the remaining senses[2,27,28]. Our findings suggest that, when visual input is congenitally absent in human development, the early auditory spatial representations continue to develop and become increasingly refined.

Our combined behavioural and ERP data show that blind individuals have an enhanced capability for sound localization in peripheral space which appears to be mediated, at least in part, by an attentional tuning mechanism that operates within the first 100 ms after sound onset. These results are analogous to findings from studies of congenitally deaf people that report enhanced early processing of visual events in peripheral, but not central, space[13,14]. It seems that the brain reacts to the loss of one of the two main distance senses (vision or audition) by compensatory changes in the remaining modality in a parallel and specific manner. □

Methods

Participants. Eight congenitally blind adults and eight sighted but blindfolded control subjects, matched for age, gender and handedness, participated in the study (Table 1).

Stimuli and apparatus. Acoustic stimuli (noise bursts) were delivered from an array of eight matched speakers that were mounted on a horizontally orientated metal hoop at a distance of 1.2 m from the subject's head (Fig. 1). Speakers were placed at azimuthal positions 0°, 6°, 12° and 18° (central array) and at 72°, 78°, 84° and 90° (peripheral array). Bursts of broadband pink noise (76 dB, 83 ms duration) of two different bandwidths were used as 'standards' (500–5,000 Hz, $P = 0.84$) and 'deviants' (500–15,000 Hz, $P = 0.16$), respectively. Head movements were discouraged and were monitored by means of an infrared beam reflected from a mirror attached to the electrode cap[19].

Procedure. Subjects were seated in a sound-attenuated chamber throughout the experiment. Sighted participants were blindfolded. There were two separate attention conditions: subjects were instructed to attend to speaker 1 (at 0° azimuth) on half of the runs and to speaker 8 (90° azimuth) on the other half, with the task of pressing a button in response to deviants at the attended speaker (designated as 'targets') only. The responding hand was counterbalanced across subjects. Although participants were encouraged to press the button as quickly as possible, accuracy was stressed. A correct target detection was counted if the response occurred within a window of 200–800 ms after the target; a false alarm response to deviants from adjacent speakers was counted in a similar manner. Before the first experimental run, subjects were familiarized with the task and trained with one to two runs per condition. Each run (duration 2.5 min) consisted of 960 noise bursts that were presented with an average ISI of 180 ms (range 90–270 ms, rectangular distribution). A total of 10–12 runs per condition ('attend centre' or 'attend periphery') were presented.

Recording and analysis. ERPs were recorded from 41 scalp sites using tin electrodes mounted in an elastic cap (Electro-Cap International). ERPs were recorded from the following sites: frontal, Fp1, Fp2, F7, F3, Fz, F4, F8; frontocentral, FC5, FC1, FC2, FC6; temporal and central, T3, CT5, C5, C3, C1, Cz, C2, C4, C6, CT6, T4; central and parietal, P3, CP1, Pz, CP2, P4; temporo-occipital, IN5, T5, TO1, IPz, TO2, T6, IN6; parieto-occipital, PO1, PO2; and occipital, O1, O2, IN3, INz, IN4 (locations are specified in ref. 29). All recordings were referred to a right mastoid reference; an averaged left/right mastoid reference was calculated offline using an additional left mastoid recording. Vertical eye movements were monitored using an electrode below the left eye against the reference, and lateral eye movements were measured with electrodes placed at the outer canthi of the left and right eyes (bipolar recording). Electrophysiological recordings were amplified with a bandpass of 0.1–100 Hz. The electroencephalogram and electrooculogram were recorded continuously and digitized at 250 Hz. Experimental effects were analysed by analyses of variance with group (sighted versus blind) as a between-subjects factor; all other factors (condition, speaker and electrode site) were repeated-measure factors. P values were corrected according to the method of ref. 30.

Received 8 March; accepted 9 May 1999.

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Acknowledgements. We thank the San Diego Center for the Blind and Visually Impaired, the Braille Institute of America (San Diego), and the Blind Recreation Center in San Diego for help in recruiting blind participants; L. Anllo-Vento for helping to recruit matched sighted controls; C. Nava for help during data acquisition; and M. Marlow for computer assistance. This work was supported by the German Research Foundation (DFG) and grants from the National Institutes of Mental Health and National Institutes of Health.

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Distortion of proximodistal information causes JNK-dependent apoptosis in *Drosophila* wing

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Distinct and evolutionarily conserved signal-transduction cascades mediate the survival or death of cells during development. The c-Jun amino-terminal kinases (JNKs) of the mitogen-activated protein kinase superfamily are involved in apoptotic signalling in various cultured cells[1]. However, the role of the JNK pathway in development is less well understood. In *Drosophila*, Decapentaplegic (Dpp; a homologue of transforming growth factor-β) and Wingless (Wg; a Wnt homologue) proteins are secretory morphogens that act cooperatively to induce formation of the proximodistal axis of appendages[2–7]. Here we show that either decreased Dpp signalling in the distal wing cells or increased Dpp signalling in the proximal wing cells causes apoptosis. Inappropriate levels of Dpp signalling lead to aberrant morphogenesis in the respective wing zones, and these apoptotic zones are also determined by the strength of the Wg signal. Our results indicate that distortion of the positional information determined by Dpp and Wg signalling gradients leads to activation of the JNK apoptotic pathway, and the consequent induction of cell death thereby maintains normal morphogenesis.

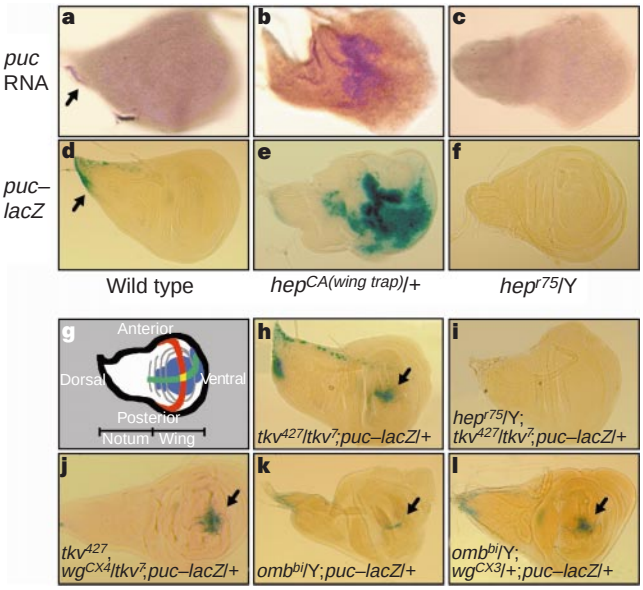

Figure 1 Interaction of the DJNK and Dpp/Wg-*omb* pathways in the wing disc. *puc* RNA expression is revealed by *in situ* hybridization (**a**–**c**) and enhancer trap *lacZ* reporter *E69* (ref. 15) (**d**–**f**, **h**–**l**). **a**, **d**, Wild type (**a**, *Canton-S*; **d**, puc^{E69}/+). Arrows indicate the scutellum anlage. **b**, **e**, Hep^{CA} producer (**b**, $hep^{CA(wing\ trap)}$/+; **e**, $hep^{CA(wing\ trap)}/puc^{E69}$). **c**, **f**, *hep* null mutant (**c**, hep^{r75}/Y; **f**, hep^{r75}/Y; puc^{E69}/+). **g**, Schematic representation of the wing-disc domains expressing *dpp* (green), *wg* (red) and *omb* (blue). Only the expressing domain in the wing primordia is shown for each. The area where the Dpp- and Wg-expressing domains intersect (yellow) corresponds to the distal-tip primordium. **h**, *tkv* hypomorph (tkv^7/tkv^{427}; puc^{E69}/+). **i**, *tkv* hypomorph in a *hep* null background (hep^{r75}/Y; tkv^7/tkv^{427}; puc^{E69}/+). **j**, *tkv* hypomorph heterozygous for *wg* (tkv^7/tkv^{427} wg^{CX4}; puc^{E69}/+). **k**, *omb* hypomorph (omb^{bi}/Y; puc^{E69}/+). **l**, *omb* hypomorph heterozygous for *wg* (omb^{bi}/Y; wg^{CX3}/+; puc^{E69}/+). Arrows indicate ectopic *puc* expression in the wing-tip primordium.

Drosophila has a JNK signalling cascade consisting of *Drosophila* JNK (DJNK) and the DJNK kinase Hep. Genetic studies have indicated that the Hep–DJNK pathway functions to promote embryonic dorsal closure by maintaining production of Dpp[1,8,9]. Disruption of the Dpp and DJNK pathways have similar phenotypic effects in both adult development and the embryonic dorsal closure. For example, viable loss-of-function mutant alleles of *hep*, *dpp* or *thick veins* (*tkv* encoding a type-I Dpp receptor), show a similar notum cleft phenotype[10–12]. Double mutants of the *hep* and *dpp* or *tkv* alleles resulted in a fully penetrant lethal phenotype (data not shown), indicating that they have a functional relationship extending beyond mere phenotypic similarity. To examine this relationship further, we first investigated *dpp* expression in *hep* mutants. *dpp* expression during embryonic dorsal closure is lower in *hep* mutants than in wild-type embryos[1,8,9]. In contrast, *dpp* expression in the wing imaginal disc of the *hep* null mutant was similar to that of the wild type (data not shown), indicating that the Hep–DJNK pathway does not regulate *dpp* expression in the wing disc. Similarly, loss of Hep did not affect the expression of *wg* (data not shown), which is expressed along the dorsoventral boundary.

Puckered (Puc) is a dual-specificity phosphatase, the expression of which is induced by the DJNK pathway and which inactivates