Involvement of *winged eye* encoding a chromatin-associated bromo-adjacent homology domain protein in disc specification

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How organ identity is determined is a fundamental question in developmental biology. In *Drosophila*, field-specific selector genes, such as *eyeless* (*ey*) for eyes and *vestigial* (*vg*) for wings, participate in the determination of imaginal disc-specific identity. We performed gain-of-function screening and identified a gene named *winged eye* (*wge*), which encodes a bromo-adjacent homology domain protein that localizes at specific sites on chromosomes in a bromo-adjacent homology domain-dependent manner. Overexpression of *wge*-induced ectopic wings with antero-posterior and dorso-ventral axes in the eye field in a region-specific Hox gene-(*Antennapedia*) independent manner. Overexpression of *wge* was sufficient for ectopic expression of *vg* in eye discs. A context-dependent requirement of *wge* was demonstrated for *vg* expression in wing discs and for expression of *eyes absent* (*eya*), a control gene for eye development downstream of *ey*, in eye discs. In contrast to *vg*, however, overexpression of *wge* inhibited EY-mediated expression of *eya*. Consistent with colocalization on polytene chromosomes of WGE and Posterior sex combs (PSC), a Polycomb group gene product, we demonstrated an antagonistic genetic interaction between *wge* and *Psc*. These findings suggest that *wge* functions in the determination of disc-specific identity, downstream of Hox genes.

determination | imaginal disc | organ identity | epigenetic regulation

To generate complex organs such as compound eyes, wings, and antennae, the correct cell types must be formed and correctly organized in three-dimensional dimensions, i.e., the specific organ identity must be determined in some specific morphogenetic field during development. *Drosophila* imaginal discs, the primordia of adult appendages and trunk, are valuable for investigating how specific identity is determined and maintained during development. The disc-specific determination state is established during embryogenesis or at early larval stages and is maintained until the discs differentiate into adult structures during metamorphosis. Some genes, such as *eyeless* (*ey*), *vestigial* (*vg*), and *Distal-less* (*Dll*), so-called master control genes or field-specific selector genes, have crucial roles in these functions, together with a cohort of subordinate transcription factors (1–4). For example, *ey* is required for eye development, and ectopic eyes can be induced on wings, legs, and antennae together with downstream genes, *eyes absent* (*eya*), *sine oculis*, and *dachshund* (1, 5–8). The ectopic expression of *vg* with its cofactor Scalloped (SD) induces ectopic wing-like structures on parts of the body other than the thorax (2, 9). Therefore, investigation of the regulatory mechanisms of upstream control of gene expression will advance this field. The region-specific Hox selector genes, such as *Antennapedia* (*Antp*) and *Ultrabithorax*, and the intracellular signaling pathways generating positional information within a morphogenetic field, such as Notch, Wingless (WG), and Decapentaplegic (DPP) signaling, are thought to regulate the expression of field-specific selector genes (4, 10–13).

We previously reported that, under the control of the eye-specific enhancer of *ey*, forced activation of Notch signaling induces ectopic structures, such as eyes, wings, legs, and antennae, in the eye-antennal field of the head, and respective control genes, *ey*, *vg*, and *Dll*, in eye-antennal discs in a context-dependent manner (10). For example, activation of Notch signaling induces ectopic expression of *ey* in antennal discs and ectopic eyes at the rostral membrane of the head, which is derived from the antennal disc. In combination with the expression of *Antp*, which is a Hox selector gene for the second thoracic segment, activation of Notch signaling induces *vg* and *Dll* in eye discs and ectopic wings and legs on the head. Notch signaling is not required for *ey* expression in eye discs (14), therefore the overexpression experiments might not reflect normal development in some aspects. The system does, however, provide a unique genetic screen that is useful for identifying genes capable of changing disc-specific identity. In this paper, we used a gain-of-function screen with 9,710 Gene Search (GS) lines (15) and identified a gene named *winged eye* (*wge*) that encodes a bromo-adjacent homology (BAH) domain protein, the overexpression of which induced ectopic wings on the head in an *Antp*-independent manner.

The BAH domain is frequently associated with other domains in proteins that are suggested to be involved in epigenetic regulation of gene expression such as bromodomains, plant homeodomain (PHD) fingers, and Suppressor of variegation 3-9, Enhancer of zeste, Trithorax (SET) domains (16). For example, in *Drosophila*, the BAH domain is present in ASH1 (absent, small, or homeotic discs 1) protein, which contains a SET domain and a PHD finger and belongs to the trithorax group (trxG) of activators (17). ASH1 is required for maintaining transcription of region-specific Hox selector genes such as *Ultrabithorax* and is thought to function by modulating chromatin structure with its histone methyl-transferase activity (18, 19). Here, we describe that *wge* is a chromatin-associated protein that is involved in the determination of imaginal disc identity in a context-dependent manner.

Materials and Methods

Fly Stocks. We performed two gain-of-function screenings. For the prescreening, *ey*-GAL4 females were crossed with the GS strains (15). For the screening, *ey*-GAL4/*CyO* p{w^+, *Act*-GFP};UAS-N^{act}/TM3 p{w^+, *Act*-GFP} females were crossed

Conflict of interest statement: No conflicts declared.

Abbreviations: AED, after egg deposition; BAH, bromo-adjacent homology; GS, gene search; IR, inverted repeat; PSC, Posterior sex combs.

Data deposition: The sequence reported in this paper has been deposited in the DDBJ/EMBL/GenBank databases (accession no. AB190214).

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with the GS strains. UAS-*ey*, UAS-*vg*, *vg*BE-lacZ, *dpp*-GAL4, *ey*-lacZ, UAS-*Antp*, UAS-N^{act}, UAS-*eya*, *eya*-lacZ, *dpp*-lacZ, UAS-*p35*, and Psc^{1} were described in ref. 1, 2, 8, 10, and 20–23. The other transformants were generated by *P* element-mediated transformation. The wge^{40} strain, which had a 2,215-bp sequence deletion including the *wge* CDS, was established by retransposing the P element in the $wge^{GS15923}$ strain. Oregon R was used as the wild-type strain.

Clonal Analysis. Mutant clones for *wge* in a *Minute* background were generated by *flp*-mediated mitotic recombination (24) and marked by the absence of *Ubi*-GFP expression. To induce recombination, *yw hs-flp*/*yw*;FRT82B wge^{40}/FRT82B *Ubi*-GFP $Rps3^{Plac92}$ larvae were heat-shocked (37°C for 1 h) 48 or 72 h after egg deposition (AED). GAL4-expressing clones, based on the excision of the FRT cassette (25), were induced by heat shock (37°C for 1 h) 48 h AED to the following flies: *yw hs*FLP, *Ay*-GAL4 UAS-GFP/+ (or *eya*-lacZ or *ey*-lacZ), and UAS-*wge*/+.

The molecular cloning, RT-PCR, histochemistry procedures, and the primers used are published as *Supporting Text*, which is published as supporting information on the PNAS web site.

Results

Identification of Genes Capable of Inducing Ectopic Appendages in the Eye Field. We previously reported that artificial activation of Notch signaling induces various ectopic appendages, such as ectopic eyes, antennae, wings, and legs, in the eye field in a context-dependent manner (10). In this system, the *ey* enhancer-dependent activation of Notch signaling and gene expression was crucial for transdetermination within a limited time window and for shutting off the forced expression once the transdetermination was induced. To identify the genes capable of changing disc-specific identity, we used a system with a *P* element-based GS vector (15). For a pilot experiment, 106 lines harboring the GS vector (GS lines) were crossed with flies carrying the *ey* enhancer-GAL4 (*ey*-GAL4) and a construct for the constitutively active Notch receptor under an upstream-activating sequence for GAL4 (UAS-N^{act}). Ectopic structures were induced in the eye field in combination with Notch signaling activation in five lines. For example, ectopic wings were induced in GS 1068 in which *ey*-GAL4 drives the expression of *PGRP-LE*, *CG8509*, and *sd*, encoding a cofactor of VG (26). In the absence of Notch signaling activation, all five lines had reduced eye phenotypes. Therefore, to increase the screening efficiency, we introduced a prescreening step in which GS lines were crossed with the *ey*-GAL4 driver, and the resulting lines with reduced eye phenotypes were crossed with the *ey*-GAL4, UAS-N^{act} line. To confirm the effects of the prescreening, we crossed 74 negative GS lines with normal eyes in a prescreening with an *ey*-GAL4, UAS-N^{act} line. None of the lines had ectopic structures in the eye field, indicating that the prescreening was effective (data not shown). In the prescreening, 8,486 lines had normal eyes and 1,202 lines had reduced eye phenotypes. In 9 of 9,710 lines, ectopic structures were induced in the eye field, e.g., ectopic wings in the GS 15923, even in the absence of Notch signaling activation. We then crossed the resulting 729 lines with reduced eye phenotypes or ectopic structures in the eye field in the prescreening and 22 lines that were selected without prescreening with the *ey*-GAL4, UAS-N^{act} line, and ectopic structures were induced in 45 lines in combination with Notch signaling activation (wing, 3 lines; antenna, 26 lines; leg, 16 lines). The results of these screenings are summarized in Table 1, which is published as supporting information on the PNAS web site, and the data including the phenotype images are available on the *Drosophila* Gene Search Project web site (http://gsdb.biol.metro-u.ac.jp/%7Edclust).

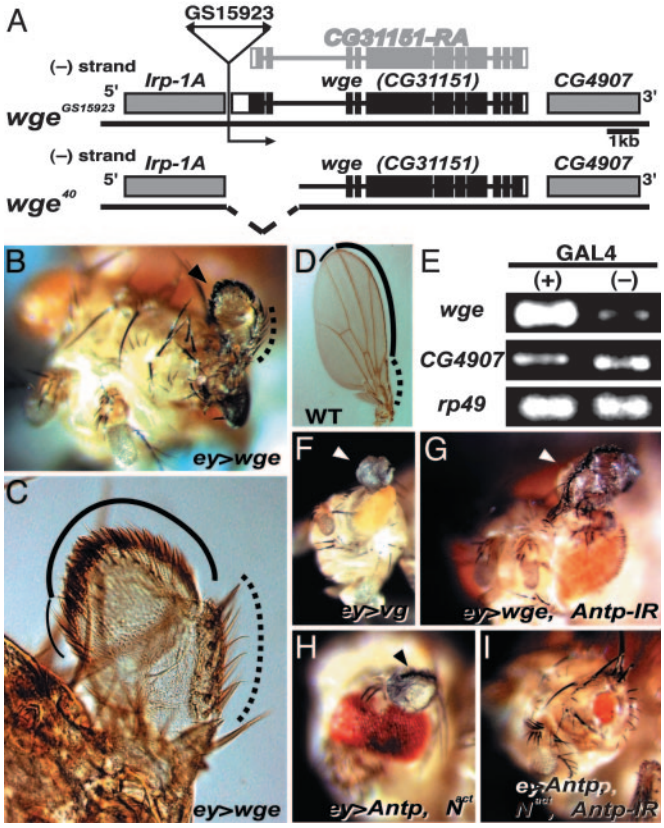

Fig. 1. Identification of *winged eye*, the overexpression of which induces ectopic wings in the eye field and its *Antp*-independent function on ectopic wing induction. (*A*) Schematic representation of the genomic regions of *wge*. The black boxes represent the *wge* ORF. The direction of GS vector-mediated transcription, the insertion site of the *P* element GS15923, and previously estimated transcript of CG31151 (RA) from EST clones are indicated. The broken line represents the deletion in wge^{40}. (*B*) WGE-mediated induction of ectopic wings in *ey*-GAL4, UAS-*wge* fly. Arrowhead indicates an ectopic wing. (*C*) Higher magnification of *B*. WGE-mediated ectopic wings have the costa with spine bristles (dotted lines), the triple row of bristles (bold line), the double row of bristles (narrow line), and the posterior rows of hairs (the remaining wing margin). (*D*) These structures are formed at the anterior-proximal part of wing (dotted line), anterior wing margin (bold line), distal wing margin (narrow line), and posterior wing margin in the wild-type wing, respectively. (*E*) GAL4-dependent induction of *wge*. RT-PCR was performed with hs-GAL4, GS15923 larvae (+), and GS15923 (−). *rp49* was used as an internal control. (*F*) VG-mediated wing-like outgrowth without margin bristles formed in the eye field of the *ey*-GAL4, UAS-*vg* fly. Arrowhead indicates ectopic outgrowth. (*G*) *Antp*-independent induction of ectopic wings in the *ey*-GAL4, UAS-*wge*, UAS-*Antp-IR* fly. Arrowhead indicates ectopic wing with margin bristles. (*H*) Induction of ectopic wing in the *ey*-GAL4, UAS-N^{act}, UAS-*Antp* fly. Arrowhead indicates an ectopic wing with margin bristles. (*I*) Eye field of the *ey*-GAL4, UAS-N^{act}, UAS-*Antp*, UAS-*Antp-IR* fly.

Overexpression of *winged eye* Induces Ectopic Wings with Antero-Posterior and Dorso-Ventral Axes in an *Antp*-Independent Manner. In this paper, we focus on the GS15923 line, because well organized wings with antero-posterior and dorso-ventral axes were induced in the eye field when GS15923 was crossed with *ey*-GAL4. Using the inverse PCR method, we determined the insertion site of the GS vector in GS15923 and found a predicted gene, *CG31151*, adjacent to the insertion site (Fig. 1*A*). *CG31151* was the only gene expressed in a GAL4-dependent manner in GS15923 (Fig. 1*E*). After cloning cDNAs corresponding to *CG31151*, we identified an ORF of a previously uncharacterized gene that we named *winged eye* (*wge*), which has a 345-bp extension at the 5′ end from the estimated translation initiation site of expressed

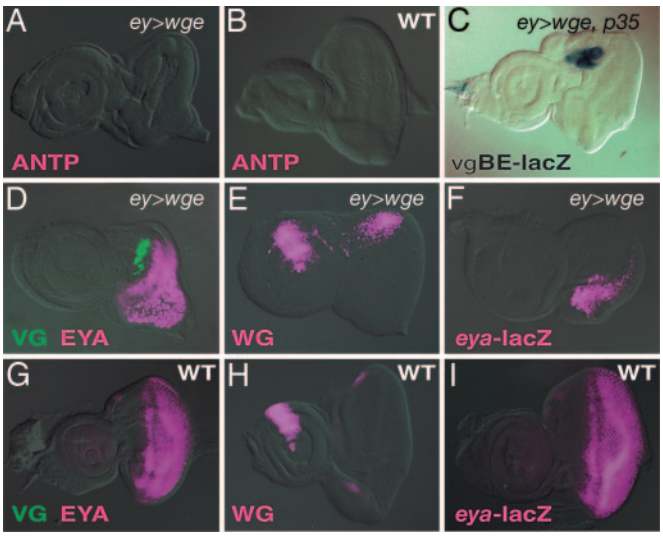

Fig. 2. WGE-mediated induction of VG and WG and WGE-mediated suppression of EYA in eye discs. (*A* and *D–F*) Immunostaining of eye discs of *ey*-GAL4, UAS-*wge* larvae with antibodies against ANTP (*A*), VG (green) and EYA (red purple) (*D*), WG (*E*), and β-galactosidase (*F*). (*F*) An eye disc of an *ey*-GAL4, UAS-*wge*, *eya*-lacZ larva. (*C*) X-gal staining (blue) of an eye disc of an *ey*-GAL4, UAS-*wge*, *vg*BE-lacZ, UAS-p35 larva. To avoid *vg*BE-lacZ-mediated cell death, p35, a cell death inhibitor (22), was coexpressed. (*B*, *G–I*) Immunostaining of eye discs of wild-type larvae with antibodies against ANTP (*B*), VG (green) and EYA (red purple) (*G*), WG (*H*), and β-galactosidase (*I*). (*I*) An eye disc of an *eya*-lacZ transgenic larva. Staining is merged with the bright field image (except *C*). In all images, posterior is to the right and dorsal is up.

sequence tag (EST) clones corresponding to *CG31151* (RA, Fig. 1*A*). The sequence of an EST clone, LP24488, overlapped by 693 bp with the 5′ end of the cloned cDNA and 20 bp of the 5′ end of the RA transcript, confirming the identified ORF. *Wge* encodes a 1,658-aa protein with two Gln-rich, one Ala-rich, and one Ser-rich domain at the N-terminal half and a bipartite nuclear localization signal and a BAH domain in the C-terminal half, implying that WGE is involved in epigenetic regulation of gene expression (Fig. 5*A*, which is published as supporting information on the PNAS web site). This prediction is consistent with the specific localization of WGE on polytene chromosomes as described below (Fig. 4*A*). Ectopic wing induction by forced *wge* expression was confirmed by using UAS-*wge* transgenic flies (Fig. 1 *B* and *C*; see also Table 2, which is published as supporting information on the PNAS web site). The WGE-mediated ectopic wings had the costa with spine bristles, the triple row of bristles, and the double row of bristles that are formed at the anterior-proximal part of the wing, at the anterior wing margin, and at the distal wing margin in wild-type wing, respectively (Fig. 1 *C* and *D*), indicating that WGE-mediated ectopic wings are correctly organized along the antero-posterior and dorso-ventral axes. In contrast, as reported before (2), VG-mediated ectopic wings in the eye field were outgrowths with wing hair that did not have any wing margins, suggesting that WGE is involved in the wing formation upstream of VG (Fig. 1*F*).

Previously, we have demonstrated that, in combination with activation of Notch signaling, expression of *Antp* by *ey*-GAL4 induces ectopic wings with antero-posterior and dorso-ventral axes, which are similar to WGE-mediated ectopic wings (Fig. 1*H* and ref. 10). To investigate the requirement of ANTP for the WGE-mediated induction of ectopic wings, we generated transgenic flies possessing an inverted repeat (IR) expression construct of *Antp* cDNA that specifically inhibits ANTP expression in a GAL4-dependent manner due to RNA interference. *Antp-IR* expression inhibited the formation of ectopic wings induced by the expression of *Antp* and Notch signaling activation, indicating that the construct works as a specific inhibitor of ANTP expression (Fig. 1*I* and Table 2). *Antp-IR* expression, however, did not inhibit the formation of ectopic wings when coexpressed with *wge* (Fig. 1*G* and Table 2). Consistent with this result, forced expression of *wge* did not induce ectopic expression of ANTP in eye discs of *ey*-GAL4;UAS-*wge* larvae (Fig. 2 *A* and *B*). These results indicated that *wge* overexpression induces ectopic wings in an *Antp*-independent manner. Moreover, *wge-IR* expression suppressed the formation of ectopic wings induced by the expression of *Antp* and Notch signaling activation (Table 2). These results indicate that WGE acts downstream of *Antp* and Notch signaling in the ectopic wing induction.

Overexpression of *wge* Induces Ectopic Expression of *vg* and *wg* and Represses EY-Mediated Expression of *eya*. Ectopic expression of *vg* in various imaginal discs induces ectopic wing-like outgrowth (2). In combination with WG signaling, however, it induces wings with wing margins (27). WG signaling is also suggested to participate in the determination of wing discs (13). We investigated whether *wge* overexpression induces ectopic expression of VG and WG in eye imaginal discs when it induces ectopic wings with wing margins. In wild type, VG is expressed in the wing discs but not in eye discs (Fig. 2*G*; see also Fig. 6*F*, which is published as supporting information on the PNAS web site), whereas, in *ey*-GAL4;UAS-*wge*, there was significant expression of VG in the eye discs (Fig. 2*D*). The forced expression of *wge* also activated *vg* D/V boundary enhancer-lacZ (*vg*BE-lacZ) (Fig. 2*C*). These results indicate that *wge* overexpression is sufficient to induce the ectopic expression of *vg,* a control gene for wing formation. In third-instar larvae, WG was expressed at the peripheral edge of the dorsal and ventral sides of wild-type eye discs (Fig. 2*H*). Overexpression of *wge* by *ey*-GAL4 enhanced the dorsal expression of WG and suppressed the ventral expression of WG in eye discs (Fig. 2*E*). These results were consistent with the findings that *wge* overexpression induced ectopic wings at the dorsal part of the eye field. Therefore, WGE is suggested to induce ectopic wings upstream of VG and WG.

We then investigated the effects of *wge* overexpression on the expression of genes involved in the determination of eye identity, such as *ey* and *eya*. Overexpression of *wge*-repressed EYA expression at the dorsal side of the eye discs, in contrast to WG expression, which was up-regulated at the dorsal side by WGE (Fig. 2 *D* and *G*). Similar results were obtained with *eya*-lacZ transgenic larvae (Fig. 2 *F* and *I*). The enhancer trap line of *eya*-lacZ reflects endogenous expression of EYA (Fig. 2 *G* and *I* and refs. 8 and 20). Double staining revealed that the ectopic induction of VG did not overlap with EYA expression when *wge* was overexpressed in eye discs (Fig. 2*D*). These results suggest that WGE-mediated down-regulation of *eya* is involved in the ectopic induction of *vg*. We could not examine the effects of *eya* overexpression on WGE-mediated ectopic *vg* expression, however, because *eya* overexpression in eye discs inhibits eye disc development. Further analysis is required to determine the relationship between the down-regulation of *eya* and the ectopic induction of *vg*.

Clonal activation of WG signaling represses *eya* expression in eye discs (28). To examine the effects of clonal induction of *wge* overexpression on *eya* and *wg* expression, we applied a cell lineage tracer technique by using a combination of the flp/FRT and GAL4/UAS recombinase systems (25). In this system, in which *wge*-overexpressing cells are labeled with GFP, *eya*-lacZ expression was repressed by clonal induction of *wge* overexpression in a cell-autonomous manner (Fig. 6*A*), whereas WG expression was not induced when the *wge*-expressing clone was induced, even on the dorsal side of the eye discs (Fig. 6*C*). These results indicate that overexpression of *wge* represses expression of *eya* in eye discs in a cell-autonomous manner, independent of

the up-regulation of *wg*. Overexpression of *wge*-inhibited expression of *eya*, a gene downstream of *ey*; however, *ey*-lacZ expression in eye discs was not repressed by clonal induction of *wge* overexpression (Fig. 6*B*). Consistent with these results, EY-mediated ectopic induction of *eya* was inhibited by coexpression of *wge*. Ectopic induction of *ey* under the control of *dpp*-GAL4 induced ectopic induction of *eya*-lacZ in the leg (Fig. 6*I*), wing, and antennal discs (8), but the ectopic expression of *eya*-lacZ was totally suppressed by the coexpression of *wge* (Fig. 6*J*). Coexpression of GFP did not suppress the EY-mediated induction of *eya*, indicating a specific effect of *wge* overexpression on *eya* expression (data not shown). These results suggest that overexpression of *wge* suppresses the eye development program downstream of *ey*. EYA repression occurs only on the dorsal side of the eye disc when *wge* is overexpressed with *ey*-GAL4, although clonally overexpressed *wge* also represses EYA on the ventral side. Further analysis is required to explain these phenomena.

Although *ey*-GAL4-dependent overexpression of *wge*-induced WG expression on the dorsal side of the eye discs, clonal induction of *wge* overexpression did not induce WG expression in eye discs. Consistent with these results, clonal induction of *wge* overexpression did not induce either ectopic expression of VG in eye discs (Fig. 6*D*) or ectopic formation of wings in the eye field on the head (data not shown), suggesting that overexpression of *wge* in a relatively large field is required for the transformation of eye to wing. Repression of *eya* but absence of *vg* expression in clonal overexpression of *wge* might represent an intermediate step toward wing transformation, which has to be analyzed further. In wing discs, clonal induction of *wge* overexpression did not affect expression of either VG or WG (Fig. 6 *E* and *G*), indicating a specific effect of clonal induction of *wge* overexpression on *eya* expression in eye discs.

Context-Dependent Requirement of *wge* for *vg* Expression in Wing Discs. To investigate the requirement of *wge* for wing development, we generated a *wge*-deficient mutant by mobilizing the *P* element. The deletion of *wge* was screened by using genomic PCR in 126 excision lines. In one line, wge^{40}, sequencing analysis after genomic PCR revealed that a 2,215-bp sequence, including the *wge* first exon, was deleted (Fig. 1*A*). In wge^{40}, there was no *wge* expression, and the expression of a gene neighboring *wge*, *Irp-1A*, was not affected, indicating that wge^{40} is a *wge* null mutant (Fig. 3*A*). The embryogenesis of wge^{40} was quite normal, but the development of wge^{40} gradually stopped after the first-instar larval stage, suggesting crucial roles of *wge* in larval development or growth (Fig. 3*B*). A rare rescue (3%) of larval lethality was observed by *wge* overexpression by using a heat-shock promoter, reflecting the context-dependent requirement of *wge* for development as described below. Rescue was never observed, however, in the absence of heat shock (29°C for 2 h every 24 h). We then introduced *wge* mutant clones by using the flp/FRT system with the *Minute* technique (24). When the *wge* mutant clones were introduced in wing discs 48 h AED by heat shock, no VG expression was observed in many cells within the clones, but some cells in the clones still expressed VG (Fig. 3*C*). The expression of *dpp*-lacZ was observed in the *wge* mutant clones, indicating a specific effect of the *wge* mutation on *vg* expression (Fig. 7*C*, which is published as supporting information on the PNAS web site). As described below, the size of these clones was relatively small, whereas VG expression was not affected in all but a few small-sized clones when the *wge* mutant clones were introduced 72 h AED (Fig. 3*D*). These results indicate that *wge* is required for *vg* induction in wing discs in a context-dependent manner. Compared with the *wge* mutant clones that were introduced 72 h AED, the *wge* mutant clones that were introduced 48 h AED were small in size, suggesting stage-specific involvement of *wge* on the growth of disc cells.

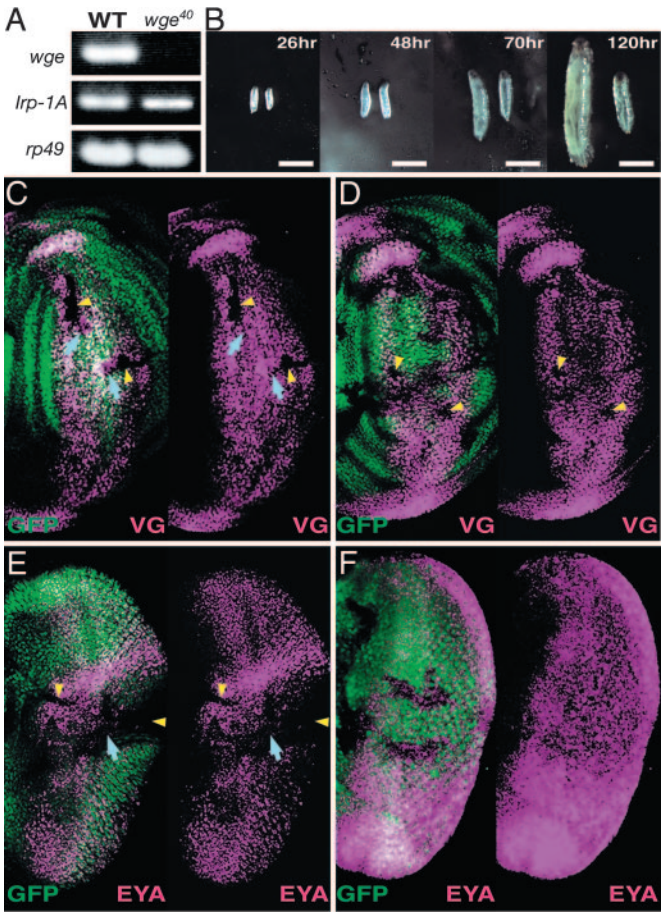

Fig. 3. A context-dependent requirement of *wge* for *vg* expression in wing discs and for *eya* expression in eye discs. (*A*) Lack of *wge* transcript in wge^{40} mutant. RT-PCR was performed with first- and second-larval stage wild-type (WT) and wge^{40}. *rp49* was used as an internal control. (*B*) Developmental defect of wge^{40}. Wild-type larvae (left side) and wge^{40} larvae (right side) at the indicated times AED are represented. (Scale bars: 1 mm.) (*C–F*) *wge* mutant clones were introduced in wing discs (*C* and *D*) and eye discs (*E* and *F*) at 48 h (*C* and *E*) and 72 h AED (*D* and *F*). Immunostaining (red purple) with antibody against VG (*C* and *D*) and EYA (*E* and *F*) is merged with GFP (green). *wge* mutant clones lack GFP signals. Yellow arrowheads indicate cells with no VG (*C*) or EYA expression (*E*) within the clones. Blue arrows indicate cells expressing VG (*C*) or EYA (*E*) within the clones. In *D*, yellow arrowheads indicate *wge* mutant clones with no VG expression. In *C* and *D*, dorsal is to the left and anterior is up. In *E* and F, posterior is to the right and dorsal is up.

***wge* Is Expressed Ubiquitously and Is Required for the Formation of Various Appendages.** Clonal induction of *wge* overexpression represses *eya*-lacZ expression in eye discs. We investigated derepression of *eya* in the *wge* mutant clones. Contrary to our prediction, there was no misexpression of EYA in *wge* mutant clones that were introduced at both 48 and 72 h AED in imaginal discs such as antennal, leg (data not shown), and wing discs (Fig. 7 *A* and *B*). In eye discs, EYA was not expressed in many cells within the *wge* mutant clones but was expressed in some cells when the clones were introduced 48 h AED (Fig. 3*E*), whereas the expression of EYA was not affected in the clones when the *wge* mutant clones were introduced 72 h AED (Fig. 3*F*). These results indicate that *wge* is required for *eya* expression in eye discs in a context-dependent manner, which is similar to the function of *wge* in the regulation of *vg* expression in wing discs. Consistent with the requirement of *wge* for the function of *vg* and *eya*, adult structures, such as eyes, wings, and legs, were malformed when *wge* mutant clones were introduced 72 h AED (Fig. 7 *D–H*). The

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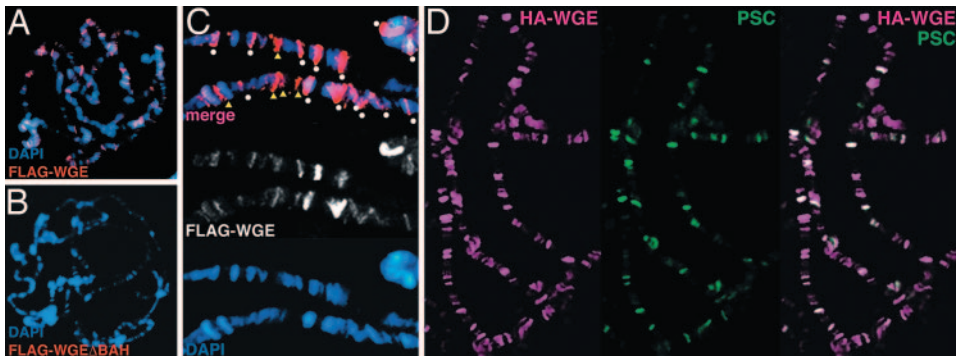

Fig. 4. BAH domain-dependent localization of WGE and colocalization with PSC at specific sites of polytene chromosomes. (*A* and *B*) FLAG-tagged wild-type WGE and FLAG-tagged mutant protein (ΔBAH) lacking the BAH domain were expressed in a salivary gland (nonspecific expression of target genes are induced in a salivary gland by the GAL4/UAS system). The polytene chromosomes of *ey*-GAL4, UAS-FLAG-*wge* larvae (*A*) and *ey*-GAL4, UAS-FLAG-*wge*ΔBAH larvae (*B*) were stained with an anti-FLAG antibody. Immunostaining (red) and DAPI staining (DNA, blue) is merged. (*C*) The binding sites of WGE (red color is changed to white) were analyzed with DAPI staining (blue) in higher magnifications. Some WGE signals are overlapped with DAPI staining (circles), and others do not overlap with DAPI staining (arrowheads) in a merged picture. (*D*) Colocalization of PSC with WGE on polytene chromosomes. The polytene chromosomes of *ey*-GAL4, UAS-HA-*wge* larvae were stained with anti-HA antibody (red) and anti-PSC monoclonal antibody (green). Almost all PSC binding sites are coincident with some of WGE binding sites.

mutant clones were distinguished by the absence of *Ubi*-GFP (Fig. 7*E*). Pupal lethality was induced when *wge* mutant clones were introduced 48 h AED. The participation of *wge* in the development and growth of various appendages confirmed the ubiquitous expression of *wge* (Fig. 8, which is published as supporting information on the PNAS web site). RT-PCR findings revealed constitutive expression of *wge* throughout the larval and pupal developmental stages and ubiquitous expression of *wge* in larval tissues and imaginal discs (Fig. 8 *A* and *B*). The ubiquitous expression of *wge* in various tissues was confirmed by *in situ* hybridization experiments (Fig. 8 *C–J*).

WGE Localizes at Specific Chromatin Sites in a BAH Domain-Dependent Manner. WGE has a BAH domain that is frequently found in proteins participating in the epigenetic regulation of gene expression. To investigate the nuclear localization and chromatin association of WGE, we expressed FLAG-tagged wild-type WGE and FLAG-tagged mutant protein (ΔBAH) lacking the BAH domain and stained salivary glands with anti-FLAG antibody (Figs. 4 and 5). Both wild-type WGE and ΔBAH were localized in the nuclei of the salivary glands (Fig. 5*C*), whereas only wild-type protein, and not ΔBAH, localized at specific sites on polytene chromosomes (Fig. 4 *A* and *B*). These results indicate that WGE associates with chromatin in a BAH domain-dependent manner. The association of WGE with chromatin seems to be crucial for WGE function, because ΔBAH did not induce ectopic wings in the eye field when it was expressed by *ey*-GAL4 (Table 2).

The binding sites of WGE were analyzed with DAPI staining (DNA) under higher magnification. Some signals overlapped with DAPI staining and others did not overlap with DAPI staining, suggesting that WGE localizes in both bands and interbands of polytene chromosomes (Fig. 4*C*). We then compared the WGE binding sites on polytene chromosomes to that of Posterior sex combs (PSC). Almost all PSC binding sites were coincident with some WGE binding sites, suggesting that some WGE function is related to PSC function (Fig. 4*D*). The genetic interaction between *wge* and *Psc* was investigated with wge^{40} and Psc^{1} (Table 3, which is published as supporting information on the PNAS web site). The extra sex comb phenotype of Psc^{1} was suppressed by the loss of one dose of *wge*, suggesting a *wge* function similar to that of trithorax-group (trxG) genes, which antagonize PcG genes. There was a maternal effect in wge^{40} single heterozygotes, as indicated by the appearance of an additional sex comb on the second tarsomere of the first leg. Such a transformation of the second to the first tarsomere of the leg occurs in some PcG mutants such as *multi sex combs* and *cramped* (29, 30). The additional sex comb phenotype of wge^{40} was suppressed by a partial loss-of-function of *Psc*. In both wge^{40} and Psc^{1} single heterozygotes, the number of sex comb teeth on the first tarsomere of the first legs was increased, whereas there was no significant modification of the number of sex comb teeth in double heterozygotes. These results indicate that *wge* and *Psc* have antagonistic roles in both transformation from the second thoracic legs to the first thoracic legs and transformation from the second tarsomere to the first tarsomere of the first leg but not in the increase in the number of sex comb teeth.

Database analysis revealed a genome sequence of *Anopheles gambiae*, ENSANGP00000005615, with striking similarity to *wge*; for example, there was a 77% amino acid identity in the BAH domain. Comparison of the two sequences led to the identification of a previously uncharacterized protein domain named highly corresponding region (HCR: 126 aa, amino acids 1130–1255) with similarity with the *A. gambiae* gene product (57% amino acid identity), KIAA1447 human protein (41%), CAGL79 human protein (33%), and BC060615 mouse protein (42%) (Fig. 5*B*). These results suggest that *wge* is evolutionarily conserved in insects and mammals.

Discussion

WGE localizes at specific sites on polytene chromosomes in a BAH domain-dependent manner, and *wge* overexpression induces a gain-of-function transformation of eyes to wings. The extra sex comb phenotype of Psc^{1} is suppressed by the loss of one dose of *wge*. The characteristics of *wge* are similar to that of trxG genes. The trxG genes were identified as suppressors of the Polycomb phenotype and are implicated in the activation of Hox selector genes (31). Therefore, similar to loss-of-function mutations in the PcG genes, gain-of-function mutations in trxG genes cause ectopic expression of Hox selector genes and homeotic transformations. TrxG proteins also localize at specific sites on polytene chromosomes, and one of the trxG proteins, ASH1, has a BAH domain (16). There are several functional differences, however, between *wge* and trxG genes. One major functional difference is Hox selector gene independence on homeotic transformation. Mutations of the trxG genes cause homeotic transformations through the modulation of transcriptional regulation of the Hox selector genes. On the other hand,

overexpression of *wge* induces ectopic wings in an *Antp*-independent manner. Although endogenous wing development is considered to be independent of *Antp* (32), *Antp* was the only Hox selector gene examined that induced eye-to-wing transformation in the system (F. Prince, T.K., S. Plaza, D. Resendez-Perez, M. Berry, S.K., and W.J.G., unpublished data). In addition, *wge* overexpression does not induce ectopic expression of *Antp* in eye discs. Therefore, *wge* induces eye-to-wing transformation in an independent Hox selector gene. Moreover, *wge* is required for the ectopic wing formation that is induced by the expression of *Antp* and the activation of Notch signaling. These results suggest that *wge* is involved in the regulation of field-specific selector gene expression but not in the regulation of region-specific Hox selector gene expression. There is probably a regulatory mechanism that determines the field-specific identity after determination of region-specific identity by Hox selector genes. Another difference between *wge* and trxG is that the trxG functions as activators, whereas *wge* overexpression also represses *eya*.

Wge is required for the expression of both *vg* in wing discs and *eya* in eye discs in a context-dependent manner. Overexpression of *wge*, however, induces ectopic expression of *vg* and represses *eya* expression in eye discs. In wing discs, *wge* overexpression does not induce either ectopic expression of *vg* or repression of *vg*. Therefore, *wge* regulates expression of *vg* and *eya* in a context-dependent manner. Consistent with the context-dependent function of *wge*, *wge* is expressed ubiquitously throughout larval to pupal development and in various tissues. The field-specific identity should be determined from an equivalent group of cells. This characteristic is observed not only in normal development but also in artificial situations of imaginal discs called transdetermination, in which, after regenerative cell growth, disc cells change their determined state to another determined state, e.g., a leg disc transdetermines to a wing disc (33, 34). Transdetermination is a polyclonal event and not the result of either differentiation of reserve cells or somatic mutations (34). Context-dependent regulation of gene expression by a ubiquitously expressed gene might explain how differences are created within a group of equivalent cells.

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1. Halder, G., Callaerts, P. & Gehring, W. J. (1995) *Science* **267,** 1788–1792.
2. Kim, J., Sebring, A., Esch, J. J., Kraus, M. E., Vorwerk, K., Magee, J. & Carroll, S. B. (1996) *Nature* **382,** 133–138.
3. Gorfinkiel, N., Morata, G. & Guerrero, I. (1997) *Genes Dev.* **11,** 2259–2271.
4. Affolter, M. & Mann, R. (2001) *Science* **292,** 1080–1081.
5. Bonini, N. M., Bui, Q. T., Gray-Board, G. L. & Warrick, J. M. (1997) *Development (Cambridge, U.K.)* **124,** 4819–4826.
6. Pignoni, F., Hu, B., Zavitz, K. H., Xiao, J., Garrity, P. A. & Zipursky, S. L. (1997) *Cell* **91,** 881–891.
7. Chen, R., Amoui, M., Zhang, Z. & Mardon, G. (1997) *Cell* **91,** 893–903.
8. Halder, G., Callaerts, P., Flister, S., Walldorf, U., Kloter, U. & Gehring, W. J. (1998) *Development (Cambridge, U.K.)* **125,** 2181–2191.
9. Simmonds, A. J., Liu, X., Soanes, K. H., Krause, H. M., Irvine, K. D. & Bell, J. B. (1998) *Genes Dev.* **12,** 3815–3820.
10. Kurata, S., Go, M. J., Artavanis-Tsakonas, S. & Gehring, W. J. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 2117–2122.
11. Kumar, J. P. & Moses, K. (2001) *Cell* **104,** 687–697.
12. Curtiss, J., Halder, G. & Mlodzik, M. (2002) *Nat. Cell Biol.* **4,** E48–E51.
13. Maves, L. & Schubiger, G. (2003) *Curr. Opin. Genet. Dev.* **13,** 472–479.
14. Kenyon, K. L., Ranade, S. S., Curtiss, J., Mlodzik, M. & Pignoni, F. (2003) *Dev. Cell* **5,** 403–414.
15. Toba, G., Ohsako, T., Miyata, N., Ohtsuka, T., Seong, K. H. & Aigaki, T. (1999) *Genetics* **151,** 725–737.
16. Callebaut, I., Courvalin, J. C. & Mornon, J. P. (1999) *FEBS Lett.* **446,** 189–193.
17. Tripoulas, N., LaJeunesse, D., Gildea, J. & Shearn, A. (1996) *Genetics* **143,** 913–928.
18. Beisel, C., Imhof, A., Greene, J., Kremmer, E. & Sauer, F. (2002) *Nature* **419,** 857–862.
19. Byrd, K. N. & Shearn, A. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 11535–11540.
20. Bonini, N. M., Leiserson, W. M. & Benzer, S. (1993) *Cell* **72,** 379–395.
21. Sanicola, M., Sekelsky, J., Elson, S. & Gelbart, W. M. (1995) *Genetics* **139,** 745–756.
22. Hawkins, C. J., Wang, S. L. & Hay, B. A. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 2885–2890.
23. Adler, P. N., Charlton, J. & Brunk, B. (1989) *Dev. Genet.* **10,** 249–260.
24. Xu, T. & Rubin, G. M. (1993) *Development (Cambridge, U.K.)* **117,** 1223–1237.
25. Ito, K., Awano, W., Suzuki, K., Hiromi, Y. & Yamamoto, D. (1997) *Development (Cambridge, U.K.)* **124,** 761–771.
26. Takehana, A., Katsuyama, T., Yano, T., Oshima, Y., Takada, H., Aigaki, T. & Kurata, S. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 13705–13710.
27. Baena-Lopez, L. A. & Garcia-Bellido, A. (2003) *Development (Cambridge, U.K.)* **130,** 197–208.
28. Baonza, A. & Freeman, M. (2002) *Development (Cambridge, U.K.)* **129,** 5313–5322.
29. Santamaría, P. & Randsholt, N. B. (1995) *Mol. Gen. Genet.* **246,** 282–290.
30. Yamamoto, Y. Girard, F. Bello, B. Affolter, M. & Gehring, W. J. (1997) *Development (Cambridge, U.K.)* **124,** 3385–3394.
31. Orlando, V. (2003) *Cell* **112,** 599–606.
32. Carroll, S. B., Weatherbee, S. D. & Langeland, J. A. (1995) *Nature* **375,** 58–61.
33. Hadorn, E. (1965) in *Genetic Control of Differentiation: Brookhaven Symposia in Biology No. 18* (Brookhaven Natl. Lab., Upton, NY) pp. 148–159.
34. Gehring, W. (1972) in *Results and Problems in Cell Differentiation, Vol.* 5, eds. Ursprung, H. & Nöthiger, R. (Springer, Berlin) pp. 36–58.