Identification of genetic modifiers of CagA-induced epithelial disruption in Drosophila

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INTRODUCTION

Helicobacter pylori strains containing the CagA protein are associated with high risk of gastric diseases including atrophic gastritis, peptic ulcers, and gastric cancer. CagA is injected into host cells via a Type IV secretion system where it activates growth-factor-like signaling, disrupts cell-cell junctions, and perturbs host cell polarity. Using a transgenic Drosophila model, we have shown that CagA expression disrupts the morphogenesis of epithelial tissues such as the adult eye. Here we describe a genetic screen to identify modifiers of CagA-induced eye defects. We determined that reducing the copy number of genes encoding components of signaling pathways known to be targeted by CagA, such as the epidermal growth factor receptor (EGFR), modified the CagA-induced eye phenotypes. In our screen of just over half the Drosophila genome, we discovered 12 genes that either suppressed or enhanced CagA disruption of the eye epithelium. Included in this list are genes involved in epithelial integrity, intracellular trafficking, and signal transduction. We investigated the mechanism of one suppressor, encoding the epithelial polarity determinant and junction protein Coracle, which is homologous to the mammalian Protein 4.1. We found that loss of a single copy of coracle improved the organization and integrity of larval retinal epithelia expressing CagA, but did not alter CagA localization to cell junctions. Loss of a single copy of the coracle antagonist crumbs enhanced CagA-associated disruption of the larval retinal epithelium, whereas overexpression of crumbs suppressed this phenotype. Collectively, these results point to new cellular pathways whose disruption by CagA are likely to contribute to H. pylori-associated disease pathology.

Keywords: CagA, Helicobacter pylori, Drosophila, genetic modifier, epithelia, coracle, crumbs

Abbreviations: Moc, Modifier of CagA; ESEM, Environmental scanning electron microscopy.
over-activation of myosin light chain (Muyskens and Guillemin, 2011), which has been implicated in disruption of gastrointestinal epithelial barriers (Shen et al., 2009) and H. pylori pathogenesis (Wroblewski et al., 2009). In this study we describe a forward genetic screen to uncover additional host genes that influence CagA’s activity in the retinal epithelium.

The Drosophila eye has been a fertile genetic system for discovering genes involved in cellular signaling pathways, including many RTK signaling pathway members (Voas and Rebay, 2004). Because of the Drosophila eye’s repeating pattern of facets or ommatidia, even subtle perturbations in signaling pathways that regulate eye morphogenesis can be distinguished by the severity of the rough eye phenotype of the adults, making possible rapid, high throughput screens for dominant enhancer and suppressor mutations (St Johnston, 2002). These genetic screens have proven to be extremely fruitful because of the high degree of conservation in molecular signaling pathways in eukaryotic cells. For example, the important CagA interactor SHP-2 was originally identified in a genetic screen in the Drosophila eye (Simon et al., 1991), and subsequently identified in mammals (Freeman et al., 1992). The functional conservation between human and Drosophila SHP-2 is illustrated by the fact that expression of the human protein can rescue the eye defects of a csw mutant lacking the Drosophila SHP-2 (Oishi et al., 2006). The high degree of molecular conservation in cellular processes targeted by bacterial pathogens has allowed researchers to screen for host factors that interact genetically with bacterial effector proteins in genetically tractable systems such as fruit flies and yeast (Siggers and Lesser, 2008; Boyer et al., 2012).

Here we exploited the CagA-induced rough eye phenotype to identify host genes that are important for pathogenic mechanisms of CagA. We used molecularly defined chromosomal deficiencies to screen over half of the Drosophila genome for dominant suppressors or enhancers of CagA-induced epithelial disruption. Our deficiency screen identified 12 novel genetic interactors, capable of modulating the severity of CagA-induced disruption of the adult retinal epithelium. We refer to these genetic interactors collectively as the modifier of CagA (Moc) genes. Moc genes have been shown to function in numerous cellular pathways including those involved in maintenance of epithelial integrity, intracellular trafficking, and signal transduction. We further investigated CagA’s genetic interactions with one Moc suppressor, the epithelial polarity determinant coracle that is the homolog of the mammalian 4.1 protein. In addition, we extended our genetic interaction network to show that other polarity determinants with antagonistic functions to coracle behave as dominant enhancers of CagA-associated epithelial phenotypes. The Moc genes provide new avenues of investigation toward understanding CagA’s pathogenicity in humans.

MATERIALS AND METHODS

Drosophila STRAINS

All flies were raised on standard Drosophila media at 22°C unless otherwise noted. The P[w/ UAS-CagA] transgenic line was generated as described (Botham et al., 2008). Transgenes were expressed in the eye using P[w/+; Mc] = GAL4-ninaE.GMR]12 [GMR, Bloomington Stock Center (BSC # 1104)]. Deficiency lines used for the initial identification of genomic regions were generated by Exelixis (Parks et al., 2004). The genetic null allele of csw (csw(w1118)) was obtained from Michael Simon (Stanford University). All other alleles used are described on FlyBase (Tweedie et al., 2009), including EGR(F1) (FBst0002079), par1k06323 (FBal0064446), rh01722 (FBst0007326), and the Moc genes listed in Table 1.

Moc GENETIC SCREEN

Males carrying a genetic deletion (generally spanning between 5 and 30 genes) on one chromosome and a visual marker such as CyO on the other were crossed to female virgins homozygous for the GMR-GAL4 driver and CagA. Moc mutants were identified by comparing the overall eye roughness of adult flies carrying a deficiency to siblings that carried the visual marker by light microscopy. We screened 237 deficiency stocks covering 7451 genes, or 53% of Drosophila genes. We found a surprisingly high proportion—49%—of the deficiencies resulted in suppression, while only a few enhancers were identified (Figure 1). Particularly severe Moc mutants were chosen for further investigation in an attempt to identify a single gene responsible for the modification. Additional deficiencies overlapping the genetic region of interest were used to narrow the number of potentially responsible genes. Assuming that a single gene were responsible for the modification of the rough-eyed phenotype, genes within an overlapping deficiency that did not act as a Moc could be eliminated as candidates, while genes not included within deficiencies that acted as a Moc could also be eliminated. Once the number of candidate genes was sufficiently low, males carrying null alleles for candidate genes were crossed to GMR-GAL4/GMR-GAL4; UAS-CagA/UAS-CagA female virgins and the eyes of adult progeny were screened for modification of the rough-eyed phenotype. This method allowed for identification of a single Moc gene in 17 of the 22 initial Moc deficiencies that were chosen for analysis.

ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY

To evaluate the CagA-induced eye phenotypes at higher resolution, we used environmental scanning electron microscopy (ESEM). Flies were anesthetized with FlyNap (Carolina Biological Supply Company) and imaged using an FEI Quanta 200 environmental scanning electron microscope. Images of at least 10 flies of each genotype were recorded and scored in a blinded fashion by five investigators. Scoring classes were defined as follows: (0) Geometric organization intact. (1) Loss of geometric organization, fewer than 25% of ommatidia fused or malformed. (2) Loss of geometric organization, greater than 25% of ommatidia fused or malformed. (3) Loss of geometric organization, greater than 25% of ommatidia fused, malformed, and greater than 1% but less than 25% of the eye lacks a recognizable morphology. (4) Loss of geometric organization, greater than 25% of ommatidia fused or malformed, and greater than 5% of the eye lacks a recognizable morphology. (5) Loss of geometric organization, greater than 25% of ommatidia fused and malformed, greater than 25% of the eye lacks a recognizable morphology, and pronounced invaginations on the eye surface.

IMMUNOHISTOCHEMISTRY

Eye discs were dissected from third instar larvae and fixed for 30 min (4% formaldehyde, 0.1 M PIPES (pH 6.9), 0.3% Triton X-100).
### Table 1 | Moc genes identified in Drosophila adult eyes.

| Gene          | Moc class | Mutant allele                                                                 | Function                                                                                     | Human homolog                  | References                  |
|---------------|-----------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-------------------------------|----------------------------|
| **EPITHELIAL INTEGRITY**                                                                                                                      |
| Coracle       | S         | cora<sup>EY07588</sup> (FBst0016848)                                         | Septate junction polarity protein; epithelial polarity determinant                           | Protein 4.1                   | Laprise et al., 2009      |
| Lasp          | S         | Lasp<sup>DG14805</sup> (FBst0020424)                                         | Actin binding protein; cell migration; RNA localization to cytoskeleton                      | Lasp1                         | Suyama et al., 2009      |
| outspread     | S         | osp<sup>1</sup> (FBst001023)                                                 | Binds actin, RhoA, and myosin phoshatase                                                   | Myosin phosphatase-RhoA interacting protein | Surks et al., 2003; Mulder et al., 2004 |
| moladietz     | S         | mol20270 (FBst0018073)                                                      | Numb binding; asymmetric cell division                                                      | Numb-Interacting Protein/Dual oxidase maturation factor | Qin et al., 2004 |
| gartenzwer<sup>6</sup>     | S         | garz<sup>EP2028</sup> (FBst0017017)                                          | GEF for Arf; protein trafficking and epithelial morphogenesis                              | Arf1GEF                        | Szul et al., 2011 |
| Epac          | S         | Epac<sup>70338</sup> (FBst0019033)                                           | GEF for Rap1; E-cadherin mediated cell adhesion and eye development                        | RapGEF                         | Dupuy et al., 2005      |
| **INTRACELLULAR TRAFFICKING**                                                                                                               |
| ranGAP        | S         | RanGap<sup>EP1173</sup> (FBst0016995)                                         | GAP for Ran; nuclear import and eye development                                           | RanGAP1                        | Minakhina et al., 2005 |
| unc104        | E         | unc-104<sup>R757</sup> (FBst0024631)                                         | Kinesin; organelle trafficking                                                            | Kinesin family member 1A      | Klopfenstein et al., 2002 |
| **RECEPTOR TYROSINE KINASE SIGNALING**                                                                                                       |
| disabled<sup>7</sup> | E       | Dab<sup>10190</sup> (FBst0016974)                                            | Able kinase signaling antagonist; epithelial morphogenesis and vesicle trafficking          | Disabled-1                     | Song et al., 2010; Kawasaki et al., 2011 |
| Spitz         | S         | sp<sup>3547</sup> (FBst0010462)                                              | Agonist of EGFR signaling; eye development                                                | Neuregulin 1                   | Tio and Moses, 1997    |
| **NUCLEAR SIGNALING AND EYE DEVELOPMENT**                                                                                                     |
| spalt major   | S         | salm<sup>1</sup> (FBst0003274)                                               | Transcription factor; eye development                                                      | Spalt-like zinc finger transcription factor | Domingos et al., 2004 |
| CG5790        | S         | CG5790<sup>04536</sup> (FBst0018803)                                         | Cell cycle kinase that promotes G1/S transition                                           | CDC7 kinase                    | Grishina and Lattes, 2005 |
| Rotund        | S         | m<sup>ne-1</sup> (FBst0000572)                                               | Transcription factor; eye development                                                      | Zinc finger transcription factor | St Pierre et al., 2002  |
| String        | S         | stg<sup>4614b</sup> (FBst001382)                                             | Cell cycle phosphatase that promoting G2/M transition; eye development                    | CDC25B phosphatase             | Thomas et al., 1994    |
| **OTHER AND UNKNOWN FUNCTION**                                                                                                              |
| chitinase-like | S         | CG30463<sup>KG07702</sup> (FBst0014380)                                     | Putative chitinase                                                                       | Chitinase 1                     | Zhu et al., 2004         |
| CG13272       | S         | CG13272<sup>DG29412</sup> (FBst0020510)                                     | Unknown                                                                                     | None                           |                           |
| CG17141       | S         | CG17141<sup>103838</sup> (FBst0018700)                                      | GTPase involved in mitochondrial translation                                               | mitochondrial GTPase 1          | Barrientos et al., 2003 |

1 Moc genes that were confirmed in the secondary ESEM screen are indicated in bold.
2 Moc classes are Suppressors (S) and Enhancers (E).
3 Drosophila mutant allele name (FlyBase strain number).
4 Whenever possible, the biochemical and relevant cell or tissue function of the gene product are indicated.
5 Most relevant references to the gene’s function, and whenever possible to the gene’s function in the Drosophila eye.
6 Also belongs to intracellular trafficking class.
7 Also belongs to epithelial integrity and intracellular trafficking classes.
The relative integrity of the epithelium, with higher values representing a more disrupted tissue. Intensities were normalized to the maximum density in the Z-stack to generate the final metric.

4.8 microns below the peak intensity was taken to represent the 1 m step size and compiled in ImageJ. The areas chosen for Z-stacks were ~1 mm² in area and devoid of ectopic furrows. The intensity of fluorescence was therefore asked whether reducing RTK signaling by removing a single copy of the egfr gene would reduce the severity of the CagA-induced rough eye phenotype. As predicted, the severity of eye disruption was significantly reduced in egfr⁻/⁻ flies expressing CagA as compared to CagA-expressing control flies (Figures 1B,C). This demonstrated that it is possible to genetically suppress CagA’s disruption of the Drosophila adult eye, thus motivating us to use this system for an unbiased genetic screen for Moc genes.

For our genetic screen, we crossed homozygous GMR-GAL4; UAS-CagA females to males carrying molecularly defined chromosomal deletions maintained over a balancer chromosome with a dominant marker, such as the CyO balancer on the second chromosome (Figure 1A). Half of the resulting progeny would contain the deficiency and could be compared to the other half with the marker to look for enhancement or suppression of the CagA-induced rough eye phenotype.

To assess the feasibility of this genetic screening strategy, we tested whether deletion of single copies of genes encoding known genetic interactors of CagA would modify the CagA-associated phenotype. CagA is a potent activator of RTK pathway signaling in tissue culture cells (Backert et al., 2010). In the Drosophila eye, EGF receptor is a critical RTK required for multiple steps during development (Dominguez et al., 1998). We, therefore, asked whether reducing RTK signaling by removing a single copy of the egfr gene would reduce the severity of the CagA-induced rough eye phenotype. As predicted, the severity of eye disruption was significantly reduced in egfr⁻/⁻ flies expressing CagA as compared to CagA-expressing control flies (Figures 1B,C). This demonstrated that it is possible to genetically suppress CagA’s disruption of the Drosophila adult eye, thus motivating us to use this system for an unbiased genetic screen for Moc genes.

We took advantage of a publicly available collection of Drosophila stocks containing deficiencies in defined genomic regions (Parks et al., 2004) to systematically search for chromosomal regions that modify CagA’s disruption of the epithelium. Using this collection, we tested 237 stocks with genomic deletions for their ability to modify the CagA-induced rough eye phenotype. Combined, this collection covered 7451 genes, or approximately 53% of all Drosophila genes (Figure 1D).

From this initial panel of deficiency stocks, 22 chromosomal regions were identified that modified CagA’s disruption of the eye epithelium with high expressivity and penetrance. To identify the individual genes responsible for the modification of CagA’s activity, CagA-expressing flies were subsequently crossed to fly stocks containing smaller deficiencies located within the 22 chromosomal regions identified in the initial screen to narrow the number of candidate Moc genes. Once we whittled the number of

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candidates down to 5–10 genes, we obtained all available strains with mutations in the candidate genes within the interval to test for their ability to modify the rough-eyed phenotype. This method allowed us to identify a single Moc gene in 17 of the 22 initial Moc intervals. These 17 genes are listed in Table 1. Moc genes fit broadly into the functional classes of epithelial integrity, intracellular trafficking, signal transduction, and nuclear signaling, with three additional genes of miscellaneous or unknown function.

A SECONDARY SCREEN FOR STRONG MODIFIERS OF THE CagA-INDUCED PHENOTYPE

To assess the degree of modification caused by the identified modifiers, we used ESEM to obtain high-resolution images of adult eyes from multiple individuals expressing each of the Moc genes in the GMR>CagA background. At this high resolution, we saw that CagA expression induced mispolarized and supernumerary bristles, fusion of ommatidia, and in the most severe instances, loss of apparent ommatidial patterning and the development of large invaginations in the epithelium. From our large data set of images, we were able to discern a continuum of severity and develop a scoring system that enabled us to quantify the rough eye phenotype (Figures 2A–F). Eyes scoring 0 resembled wild-type flies and had no apparent sign of disruption. In contrast, eyes scoring 5 were the most severely disrupted. Eyes scoring 1–4 had intermediate levels of disruption. For a complete description of the scoring system, see Materials and Methods.

For each of the Moc genes, at least 10 ESEM images of adult eyes from different flies were scored in a blinded fashion by five investigators, and the average score was tallied. From this analysis, we verified that 12 of the 17 genes were bona fide modifiers of CagA-induced eye disruption (Figure 2G and Table 1). Two of these behaved as enhancers and 10 were suppressors. The most abundant functional group among these 12 genes was the epithelial integrity class.

To calibrate the effects of the validated Moc genes, we quantified the ability of four known CagA signaling modulators to modify the CagA-induced eye phenotype: egfr (Keates et al., 2007; Bauer et al., 2009), csw (the homologue of SHP-2) (Higashi et al., 2002), rhoA (Muyskens and Guillemin, 2011), and par1 (Saadat et al., 2007). Of these, egfr proved to be the most potent suppressor of CagA-induced disruption. Loss of single copies of both csw and rhoA caused suppression, whereas loss of a single copy of par1 did not cause a significant modification of the CagA rough eye phenotype (Figure 2G). This analysis demonstrated that the 12 newly identified Moc genes had similar or stronger effects on the CagA-induced phenotype than known CagA signaling modulators.
MODIFICATION OF CagA-INDUCED DISRUPTION OF THE LARVAL RETINAL EPITHELIUM

Our Moc screen identified genetic modifiers of the CagA phenotype in the adult eye. We had previously reported that CagA expression with the GMR driver induces profound disruption of the morphogenesis of the larval retinal epithelium shortly after initiation of CagA expression (Muyskens and Guillemin, 2011). We showed that by overactivating Rho and non-muscle myosin in the larval epithelium, CagA causes ectopic furrowing of the epithelial sheet. Because so many of the Moc genes were implicated in epithelial integrity, we wished to determine whether any of them might modify CagA’s effects at these early stages of epithelial disruption. We decided to focus on Coracle (Cora), because it had the best characterized function as an epithelial polarity determinant (Laprise et al., 2009, 2010).

To measure the integrity of the larval retinal epithelium, we stained the tissue with an antibody against Discs large (Dlg), a component of the septate junction, the invertebrate cell junction that is structurally homologous to the chordate tight junction. We used laser scanning confocal microscopy to image from the apical to basal poles a region of epithelium devoid of obvious ectopic furrows. We quantified the intensity of the Dlg signal as a function of depth from the apical surface. Maximal Dlg signal was just below the apical epithelial surface at the septate junction.

When we compared the relative intensity of Dlg signal below this maximal point, we found that the CagA-expressing discs had significantly more Dlg signal at deeper positions relative to the GMR control discs (Figure 3A). For our further analysis, we quantified the relative Dlg intensity 4.8 microns below the point of peak Dlg intensity (arrow in Figure 3A), the point at which we observed the maximum difference between CagA-expressing and control larval retinal epithelia.

We used this method to analyze the Dlg distribution in CagA-expressing larval eye discs lacking a single copy of a Moc gene. We first tested the consequence of depleting the CagA interactor and junctional protein Par1. Loss of a single copy of par1 caused a slight expansion of Dlg staining (Figure 3B). We also tested the consequence of depleting Cora, which is normally localized to the septate junctions. Loss of a single copy of cora had no effect on the distribution of Dlg at the septate junctions (Figure 3B). We then analyzed the Dlg distribution when these genes were deleted in the presence of CagA. In the larval epithelium, par1 behaved as a dominant enhancer of the CagA-associated disruption in Dlg protein distribution (Figure 3B), despite having no effect on the adult eye phenotype caused by CagA. In contrast, cora behaved as a dominant suppressor of the CagA phenotype in the larval epithelium, as it had done in the adult eye (Figures 2G,3B). The dominant enhancement of the CagA-induced epithelial disruption by par1 could be explained as the further impairment of a compromised tissue through the depletion of a junctional component. Less obvious was the mechanism by which cora deletion suppressed the CagA phenotype, which we sought to understand with further experiments.

CORA REDUCTION SUPPRESSES CagA-INDUCED EPITHELIAL DISORGANIZATION BUT NOT CagA PROTEIN LOCALIZATION TO SEPTATE JUNCTIONS

Our finding that Dlg protein extended deeper from the apical surface in the CagA-expressing epithelium as compared to wild-type tissue could arise from multiple mechanisms. Two possible mechanisms are illustrated in Figures 4A–C. In the first model, CagA expression could cause a loss of junctional integrity and expansion of Dlg protein toward the basal end of the cell (Figure 4B). Alternatively, CagA could cause disorganization of the epithelial sheet, resulting in a broader zone of Dlg expression when averaged across multiple cells (Figure 4C). To distinguish these possibilities, we examined the organization of the larval epithelium and cell junctions at high resolution. We co-stained larval retinal epithelia for both the septate junction marker Dlg and the adherens junction marker E-cadherin (E-cad). In Drosophila epithelia, the adherens junction is apical to the septate junction, and in the larval retinal epithelium these junctions are located at the apices of photoreceptors and supporting cells that surround the ommatidia (Figure 4D). In CagA-expressing eye imaginal disc epithelia, the zone of Dlg expression was frequently found to extend deeper from the apical surface. In some instances this
appeared to be due to more disorganized junctions (arrowhead in Figure 4E), but frequently the integrity of the junctions looked normal and the Dlg staining was displaced deeper into the tissue due to irregularities in the epithelial sheet (arrow in Figure 4E). Because the integrity of Dlg and E-cad staining looked mostly normal in the CagA-expressing larval retinal epithelia, we concluded that loss of Cora suppresses CagA-associated phenotypes in this tissue by reducing the overall disorganization of the epithelial sheet.

We had previously shown that CagA is localized to the apical junctional structures in the larval retinal epithelium and that a CagA mutant that fails to localize in this manner is a less potent disruptor of epithelial integrity (Muyskens and Guillemin, 2011). We wondered if loss of a single copy of cora could disrupt the localization of CagA to the apical cell junctions. We visualized CagA distribution using an HA epitope tag we had engineered into the protein. As we previously reported, we found that CagA was enriched in apical foci of CagA-expressing eye discs (Figure 4F). We found that this expression pattern was not different in eye discs lacking a single copy of cora, (Figure 4G). Therefore, cora’s ability to suppress CagA-induced eye morphology does not appear to be due to failure of CagA protein to localize to apical cell junctions in the absence of one copy of cora.

FIGURE 4 | cora reduction suppresses CagA-induced epithelial disorganization but not CagA protein localization to septate junctions. (A–C) Model for the basal displacement of Dlg. Panel A represents the wild-type distribution of Dlg (represented as red structures on the lateral membranes of the epithelial cells). Panel B represents basally expanded Dlg expression due to expansion within individual cells. Panel C shows how epithelial disruption can cause basal mispositioning of Dlg expression by positioning cells deeper within the epithelium. (D) Control larval retinal epithelium (GMR-Gal4) stained with Dlg (red) and E-cad (green). YZ and XZ orthogonal planes are shown on the side and top, respectively, in D and E. Scale bar is 30 microns for all panels. (E) CagA-expressing larval retinal epithelium (GMR-Gal4; UAS-CagA) also stained with Dlg (red) and E-cad (green). Arrowhead in the upper orthogonal section shows basally mispositioned Dlg staining. Arrow indicates Dlg staining that is deep within the epithelium due to irregularities in the epithelial sheet. (F) cora+/− larval retinal epithelium expressing CagA (GMR-Gal4; UAS-CagA) showing CagA localization as labeled with anti-HA. Apical HA puncta are present. (G) A larval retinal disc expressing CagA (GMR-Gal4; UAS-CagA) labeled with HA antibody.
EPITHELIAL POLARITY DETERMINANTS MODIFY CagA-INDUCED DISRUPTION OF THE LARVAL RETINAL EPITHELIUM

To further explore the basis for cora suppression of CagA-induced larval retinal epithelial disorganization, we tested other epithelial polarity determinants for their ability to modify the CagA-induced larval retinal epithelium phenotype. Polarity in many epithelial tissues of both Drosophila and mammals is established and maintained by four conserved groups of polarity determinants: the apically localized Crumbs (Crb) group and three functionally distinct basolaterally distributed groups with defining members Cora, Scribble (Scrib), and Par1 (Laprise and Tepass, 2011). In contrast to cora, and similar to par1, crb, and scrib behaved as dominant enhancers of the CagA-induced larval epithelial disruption (Figure 5A), whereas loss of a single copy of these genes caused no epithelial disruption on their own (data not shown). To ask whether all Cora group members behaved as suppressors of CagA, we tested another Cora group member, Na, K-ATPase (encoded by the atpa gene). Unlike cora, atpa behaved as a dominant enhancer of CagA in the retinal epithelium (Figure 5A), and had no effect when depleted in the absence of CagA (data not shown). Cora and Crb mutually inhibit each other’s activities in many epithelial structures (Laprise et al., 2009, 2010; Laprise and Tepass, 2011). We therefore asked whether overexpression of Crb would have the same effect as loss of Cora. Using a UAS-crb construct, we were able to achieve a significant suppression of CagA-induced epithelial disorganization, as measured by the basal distribution of Dlg (Figure 5A).

DISCUSSION

We have demonstrated that a transgenic Drosophila model can be used to identify conserved genes that modulate the effects of a virulence factor from a human pathogen. We show that CagA-induced perturbation of the Drosophila adult eye is a sensitive read-out for identification of genes that can alter CagA’s ability to disrupt this tissue. Our approach is reductionist in that it characterizes the bacterial effector in isolation from other aspects of the infection process, such as immune responses to the bacteria and cellular interaction with the type IV secretion system that normally delivers CagA. The potential utility of the screen is limited by the extent of conservation between Drosophila and human genes and by the functional similarity between retinal and gastric epithelia. Nonetheless, we found that when depleted by one copy, genes encoding three known effectors of CagA, EGFR, Csw (SHP-2), and RhoA, significantly suppressed the eye morphological defects caused by CagA expression. This validated our approach to screen for dominant modifiers of CagA in this tissue. In our Fl screen we surveyed over half the Drosophila genome and identified 17 Moc genes, 12 of which we confirmed by high resolution ESEM.

Across the list of Moc genes, several themes of cellular and biochemical functions emerge. Eight of the 17 genes have known roles in epithelial integrity, including interactions with polarity determinants (coracle and moladizet) and the actin cytoskeleton (lasp and outspread). Three function in protein or organelle trafficking (gartenzwerz, ranGAP, and unc104) with known or suspected roles in epithelial organization. Indeed, the ArfGEF, gartenzwerz, which is required for normal protein trafficking and morphogenesis of the Drosophila salivary gland epithelium (Szul et al., 2011), exemplified a growing appreciation of the connection between epithelial polarity and intracellular trafficking (Shivas et al., 2010). A frequent biochemical function among the Moc genes is interaction with GTPases or GTPase activity (outspread, gartenzwerz, epac, ranGAP, CG17141), which is interesting in light of the fact that we have shown that CagA’s disruption of the larval retinal epithelium is due in part to excessive RhoA signaling (Myskens and Guillemin, 2011). Another theme among the Moc genes is signal transduction and nuclear signaling, including two zinc finger transcription factors (spalt major and rotund) and two cell cycle regulators (homologs of CDC25B and CDC7). An additional signaling Moc, disabled, is an antagonist of Abl kinase (Song et al., 2010). In gastric epithelial cells, CagA has been shown to activate Abl and subsequently be phosphorylated by this kinase, resulting in enhanced CagA-mediated signaling, including RTK-dependent cell elongation (Tammer et al., 2007). Consistent with its molecular function as an inhibitor of Abl, loss of one copy of disabled results in enhancement of the CagA-mediated rough eye phenotype, the opposite effect of reduction of egfr, or the EGFR ligand, spitz. disabled has also been shown to be required for normal epithelial morphogenesis in Drosophila (Song et al., 2010) and to function in vesicle trafficking (Kawasaki et al., 2011), thereby linking the functions of RTK signaling, epithelial morphogenesis, and intracellular trafficking that run throughout the Moc list.

Within this group of modifiers, we focused our attention on cora because of its previously characterized role in epithelial polarity and septate junction regulation. The septate junction and its mammalian equivalent, the tight junction, regulate paracellular flux across epithelia. In the gastrointestinal tract, tight junctions are often targeted by enteric pathogens for invasion of deeper tissues or access to nutrients (Vogelmann et al., 2004). CagA has been shown to alter the distribution of the tight junction component ZO-1 and, over extended periods of time, impair tight junction integrity in H. pylori-infected cultured epithelial
cells (Amieva et al., 2003). However, even under conditions when tight junctions remain intact, CagA confers on H. pylori the ability to replicate in the nutrient-poor environment of the epithelial apical surface (Tan et al., 2009). This CagA-mediated adaptation involves disruption of apical-basal polarity and expansion of basolateral markers to the apical surface (Tan et al., 2011). We found that depletion of cora suppressed CagA-induced disruption of the larval retinal epithelium, but not by a perceptible change to the organization of the cell junctions or the localization of CagA to these structures. Intriguingly we found that over-expression of crb resulted in the same phenotypic suppression achieved by depletion of cora. Cora and Crb have mutually antagonistic activities, and in the absence of Cora, Crb will promote expansion of apical cell surfaces within the epithelium (Laprise et al., 2006, 2009). We hypothesize that this activity of Crb counteracts CagA’s ability to promote more basolateral cell surface identities (Figure 5B). Thus, over-expressing Crb, or depleting its inhibitor, Cora, achieves a more balanced pull between apical promoting forces from Crb and basolateral promoting forces from CagA that is manifest as more normal epithelial organization in the CagA-expressing retinal epithelium in these genetic backgrounds.

In summary, our genetic screen has identified a number of host signaling pathways that modulate CagA’s potency in disrupting host tissue. Further analysis of these Moc genes should lead to new insights into CagA’s mechanism of action in host tissue and may yield new strategies for pharmaceutical modulation of these pathways to treat H. pylori-associated pathologies.

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