RESEARCH ARTICLE

Novel roles for GATAe in growth, maintenance and proliferation of cell populations in the *Drosophila* renal tubule

Guillermo Martínez-Corrales*†, Pablo Cabrero, Julian A. T. Dow, Selim Terhzaz and Shireen-A. Davies

ABSTRACT

The GATA family of transcription factors is implicated in numerous developmental and physiological processes in metazoans. In *Drosophila melanogaster*, five different GATA factor genes (*pannier, serpent, grain, GATAd* and *GATAe*) have been reported as essential in the development and identity of multiple tissues, including the midgut, heart and brain. Here, we present a novel role for GATAe in the function and homeostasis of the *Drosophila* renal (Malpighian) tubule. We demonstrate that reduced levels of GATAe gene expression in tubule principal cells induce uncontrolled cell proliferation, resulting in tumorous growth with associated altered expression of apoptotic and carcinogenic key genes. Furthermore, we uncover the involvement of GATAe in the maintenance of stellate cells and migration of renal and nephritic stem cells into the tubule. Our findings of GATAe as a potential master regulator in the events of growth control and cell survival required for the maintenance of the *Drosophila* renal tubule could provide new insights into the molecular pathways involved in the formation and maintenance of a functional tissue and kidney disease.

KEY WORDS: GATAe, *Drosophila*, Malpighian tubules, Principal and stellate cells, Renal stem cells, Proliferation, Maintenance, Migration

INTRODUCTION

The formation and development of an organ is a complex process involving several programmed events, including changes in cell shape and adhesion, proliferation and differentiation (Hatton-Ellis et al., 2007; Wan et al., 2000; Ainsworth et al., 2000). Understanding the genetic pathways involved is crucial, as defects in any of these aforementioned processes can lead to malformations and lethality (Jung et al., 2005; Denholm, 2013; Ainsworth et al., 2000). These events can be readily studied in *Drosophila melanogaster* Malpighian tubules (MTs), a powerful model system for investigating mechanisms of cell differentiation, proliferation and development, as well as a model of kidney disease (Millet-Boureima et al., 2018; Dow and Romero, 2010).

Insect MTs perform functions equivalent to vertebrate kidney and liver, and share similar characteristics in terms of classes of genes expressed and cellular origins. Development of the MTs occurs during embryogenesis and they are completely functional by the first instar larval stage. The development and physiology of the fly renal tubule have been extensively investigated and reviewed in previous publications (Beyenbach et al., 2010; Denholm, 2013; Skær, 1996; Dow, 2012; Beaven and Denholm, 2018; Bunt et al., 2010). Each tubule is composed of different segments (ureter, main, initial and transitional segments) that are functionally and genetically distinct (Chintapalli et al., 2012), and has two main epithelial cell types: the principal cell (PC) and the stellate cell (SC) (Dow, 2012; Denholm et al., 2003). In addition, the renal and nephritic stem cells (RNSCs, also known as tiny cells) may be observed in the ureters and lower tubules. It has been shown that these cells, which migrate from the midgut (MG) during metamorphosis, are able to act as stem cells in the MTs, and have a distinct gene expression profile from both the SC or PC (Takashima et al., 2013; Li et al., 2014, 2015; Singh et al., 2007; Sözen et al., 1997).

The GATA family of transcription factors (TFs) contain one or two zinc-finger domains that bind to the consensus DNA sequence A/T GATA A/G and is known to play crucial roles in the development and physiology of vertebrates, invertebrates, plants and fungi (Lentjes et al., 2016; Merika and Orkin, 1993). Aside from their developmental roles, vertebrate GATA factors are associated with diverse types of cancers, including intestinal tumors, leukemia, and breast cancer (Zheng and Blobel, 2010; Pihlajoki et al., 2016; Hellebrekers et al., 2009; Shaoxian et al., 2017). In humans, at least two GATA factors are expressed in the kidney (GATA3 and GATA5). Epigenetic alterations of these genes are associated with decreased survival in renal cell carcinomas (Peters et al., 2014a,b), and GATA3 itself is downregulated in this type of cancer (Yang et al., 2013). Moreover, GATA3 is required for the proper development of the human kidney, and haploinsufficiency of this gene induces hypoparathyroidism, sensorineural deafness and renal anomaly (HDR) syndrome (Van Esch et al., 2000; Ferraris et al., 2009). The GATA family is evolutionarily well known in both vertebrates and invertebrates, and although they perform similar functions across phyla, there are differences in the pattern of expression between all of them (Lentjes et al., 2016).

This study focused on the *Drosophila* TF GATAe, which is highly enriched in embryonic, larval and adult MTs (Wang et al., 2004; Okumura et al., 2005). Previous studies have demonstrated that GATAe is required for terminal differentiation of the embryonic MG (Okumura et al., 2005; Murakami et al., 2005; de Madrid and Casanova, 2018). In the adult MG, GATAe performs different functions depending on the cell type. First, it is necessary for the maintenance, differentiation and migration of the intestinal stem cells (Okumura et al., 2016; Zhai et al., 2017; Takashima et al., 2013; Dutta et al., 2015). In addition, GATAe is actively required to repress MG enterocyte shedding after bacterial infection (Zhai et al., 2018). More recently, GATAe has been shown to be involved in transcriptional regulatory changes associated with lifespan...
extension consequent to dietary restriction (Dobson et al., 2018). However, although GATAe expression in the MG has been well characterized, little is known about its possible MT functions, despite the abundance of GATAe in the MTs at all developmental stages.

Here, we demonstrate crucial roles for GATAe in determining and maintaining tubule morphology and functional capabilities by restrictively silencing GATAe expression to PC, SC and RNSC subpopulations in the tubule. Specific GATAe knockdown in PCs induced uncontrolled cell proliferation and significantly decreasing stress tolerance and lifespan. Silencing GATAe expression in SCs also affected cell survival, ultimately impacting on hormonal control of tubule osmoregulatory functions. Finally, GATAe is also required in RNSCs to ensure proper migration to the MT ureter. Taken together, these observations demonstrate novel requirements for GATAe, not only for the determination and maintenance of individual MT cell populations but as a crucial TF for tubule function and organismal viability.

RESULTS
GATAe is expressed in all cell types in the adult MTs

To characterize GATAe function in MTs, we employed the GAL4/UAS binary expression system (Brand and Perrimon, 1993; Duffy, 2002) to restrictively silence gene expression in the different MT cell populations. We first investigated the pattern of expression of GATAe larval and adult MTs, as FlyAtlas2 data indicated that GATAe is highly enriched in the larval and adult MTs (Leader et al., 2018). It has been shown that GATAe-Gal4 correlates the pattern of expression of GATAe in the adult MG (Zhai et al., 2018) and the embryonic MTs (Kvon et al., 2014). Using two different GATAe-Gal4 lines driving a GFP reporter, we found GATAe expression in larval stage 3 (L3) PCs but not in SCs (Fig. 1A), and that GATAe is present in all three types of cells (PCs, SCs and RNSCs, Fig. 1B,C) in the adult MTs.

We then silenced GATAe expression via RNAi using GATAe-Gal4. A flexible feature of the GAL4/UAS system is that the phenotypes induced are temperature sensitive (Duffy, 2002). At 29°C, GATAe>GATAe RNAi induced 100% lethality shortly after embryogenesis, phenocopying a GATAe null mutant (Okumura et al., 2016). Furthermore, qPCR experiments determined that GATAe>GATAe RNAi embryos exhibited significantly decreased expression levels of GATAe mRNA (~40%) compared with the control (Fig. S1A). At 26°C, GATAe>GATAe RNAi flies overcame embryonic lethality but died during metamorphosis. In addition, at this temperature, GATAe>GATAe RNAi pupae exhibited a smaller size compared with the controls (Fig. S1B). Only when GATAe>GATAe RNAi flies were allowed to develop at 18°C, did they survive until the adult stage, but none of them survived more than 7 days after adult eclosion.

We observed that the morphology and length of the GATAe>GATAe RNAi adult MGs (at 18°C) is drastically shorter compared with the parental controls (Fig. S1C,D), which correlates with previous studies that demonstrated the vital roles of GATAe for the terminal differentiation of the embryonic MG (Murakami et al., 2005; de Madrid and Casanova, 2018), and for its adult maintenance (Zhai et al., 2017, 2018; Okumura et al., 2016). Curiously, we found that the morphology of GATAe>GATAe RNAi adult MTs was also affected (Fig. 1D), an observation that has not been previously reported.

GATAe regulates PC morphology and is required for tubule function

We examined the effects of silencing GATAe in the MTs using the PC-specific Gal4 line CapaR-Gal4 (Terhzaz et al., 2015), and noticed that the CapaR>GATAe RNAi adult flies exhibited an inflated abdomen phenotype (Fig. 2A). We performed wet-dry weight measurements and confirmed that the increase in weight of CapaR>GATAe RNAi flies was indeed a consequence of increased water retention, presumably a consequence of impairment of fluid transport in the MTs (Fig. S1F). In addition, CapaR>GATAe RNAi flies exhibited significantly shorter lifespan compared with the controls (Fig. 2B). We then investigated whether the tubule-specific role of GATAe in fluid homeostasis could modulate desiccation and starvation stress responses, and survival of the whole organism. Indeed, their tolerance to both starvation and desiccation stresses were significantly reduced compared with the controls (Fig. S1E).

CapaR>GATAe RNAi MTs are shorter (less than half the length of wild-type parental MTs, Fig. S2B), thicker and with irregular shape compared with controls (Fig. 2D and Fig. S2C). Additionally, these MTs exhibited a significantly reduced number of cells (PCs

![Fig. 1. The localization of GATAe in renal (Malpighian) tubules of Drosophila.](image-url)
and SCs combined, Fig. S2A). This striking morphological phenotype was observed using three different UAS-GATAe RNAi lines (described in the Materials and Methods) crossed with PC-specific Gal4 drivers (ctB-Gal4 and CapaR-Gal4, Fig. S2A and C), and two different GATAe-Gal4 lines (Fig. 1D). Furthermore, CapaR> GATAe RNAi anterior MG regions did not show any detectable morphological defects (Fig. S3), indicating that the phenotype observed in their MT architecture is a consequence of GATAe knockdown specifically to the tubule PCs.

**Cell-autonomous role of GATAe in principal cells**

To determine a potential developmental role for GATAe, we silenced GATAe expression in the PCs from embryonic stage 9 using ctB-Gal4 (Saxena et al., 2014). No defects in morphology or migration of embryonic MTs were observed (Fig. 3B,B'). Interestingly, we obtained similar results using a null mutant line for GATAe (GATAe+/−; Okumura et al., 2016), reinforcing that GATAe is not required for the embryonic development of the MTs (Fig. S2D). In order to delineate precisely the developmental window for GATAe function, we employed a conditional system using the temperature-sensitive Gal80 (Gal80ts) construct (Pilauri et al., 2014), combined with the CapaR-Gal4 driver. CapaR> GATAe RNAi flies were maintained at 18°C (where Gal4 expression is suppressed by Gal80ts) until any of the larval stages (L1, L2 or L3 stage), and then switched to 29°C (where Gal4 is activated) until adult eclosion. The phenotype observed in these MTs phenocopied defects in MT architecture in GATAe−/−; CapaR-Gal4 flies raised at 29°C until L3 stage and then switched to 18°C until adult eclosion show similar MTs compared with controls (Fig. 3C). In addition, CapaR> GATAe RNAi flies exhibited normal MTs until L3 stage (Fig. S4A), after which stage MTs display strong morphological defects (Fig. S4B). These results demonstrate that GATAe is required from L3 stage onwards, and most probably during the pupal stage.

We then investigated whether reduced levels of GATAe in adult PCs could induce a tubule-specific phenotype over time in adult flies. As expected, CapaR> GATAe RNAi flies raised at 18°C until the adult stage did not present any detectable structural alterations in their MTs (Fig. S5A). These flies were then switched to 29°C and MT morphology was examined at 7-day intervals over 28 days. Cell shape defects and disruption of the MT architecture were observed from 14 days after switching to 29°C (Fig. S5A), including abnormal proliferation of RNSCs, as revealed by the expression of the marker Hindsight (Bohère et al., 2018) (Hnt, Fig. S5B). These results indicate that, while GATAe does not appear to be necessary during embryogenesis, its expression in PCs is essential for maintaining proper cellular morphology and integrity of the MTs during the adult stage.

We next determined the potential cell-autonomous roles of GATAe. A widely used technique to study clonal analysis is the mosaic analysis with a repressible cell marker (MARCM) (Lee and Luo, 1999) technique. However, MARCM relies on mitotic recombination and, although it has been used to study RNSC behavior (Bohère et al., 2018; Singh et al., 2007; Zeng et al., 2010), this technique cannot be used in the PCs as these are a post-mitotic cell population. To overcome this issue and analyze the effects of clonal downregulation of GATAe in the PCs, we used the Urate Oxidase Gal4; UAS-mCD8:GFP fly line, which drives expression of membrane-bound GFP in a subset number of PCs in the tubule main segment (Uro-GFP) (Terhzaz et al., 2010), in conjunction with the GATAe RNAi. Remarkably, Uro-GFP> GATAe RNAi MTs...
display an expansion of tubule diameter exclusively surrounding the GFP+ cells (Fig. 4C,D), suggesting that GATAe is required cell specifically. In addition, we observed several bi-nucleated cells (Fig. S5C), suggesting an alteration in cell division.

**Reduced levels of GATAe induce overproliferation of RNsCs**

We also noticed that CapaR>GATAe RNAi MTs contain significantly more cells with smaller nuclei not only in the ureters but also in the tubule main segment compared with the controls (see Fig. 5H for quantifications). Interestingly, these cells are positive for Armadillo (Arm), D-Cadherin (DCAD) and Hnt (Fig. 5D,G), which are known markers for RNsCs (Bohère et al., 2018; Singh et al., 2012). In addition, these cells were also positive for Delta (Dl+) (Fig. S5D), another known marker for RNsCs and intestinal stem cells (Li et al., 2014).

It is acknowledged that RNsCs of adult wild-type tubules are described as quiescent and rarely undergo division (Li et al., 2015; Zeng et al., 2010). However, using the cell division-specific marker phospho-histone H3 (PH3) (Micchelli and Perrimon, 2006), we confirmed that a number of potential RNsCs observed in adult CapaR>GATAe RNAi MTs were positive for PH3, indicating that these are proliferative cells (Fig. S5D). Furthermore, CapaR>GATAe RNAi MTs also contained cells that are DCAD−, Arm− and Dl−, and possess smaller nuclei than PCs, which might be immature renal blasts (RBs, white arrows in Fig. 5G and Fig. S5D; previously described in by Singh et al., 2007). This suggests that these proliferative population of cells may undergo a partial differentiation process.

**GATAe modulates cancer-related gene expression**

Several cancers are caused by the failure to control proliferation and differentiation of stem cell populations (Reya et al., 2001; Parvy et al., 2018). Given the apparent increase in RNsCs and RBs in CapaR>GATAe RNAi MTs, we investigated the level of expression of other cancer-related genes.
of genes involved in cell proliferation and tumorigenesis: Ras Oncogene at 85D (Ras85D) and warts (wts) (Ren et al., 2010); and downstream apoptosis-related effectors: death-associated Inhibitor of Apoptosis 1 (Diap1), Buffy and death executioner bel2 (Debel) (Terhzaz et al., 2010; Goyal et al., 2000). GATAe knockdown MTs exhibit a significant upregulation of Diap1 and the Ras85D expression (2.5- and 3-fold increase, respectively). By contrast, downregulation of Debel and wts (0.7- and 0.6-fold decrease, respectively) were observed (Fig. 5I). However, no significant difference in Buffy expression levels was measured in tubules with reduced GATAe levels. Additionally, these MTs also exhibited a strong upregulation of Adenosylhomocysteinase like 1 (AhcyL1) (∼5-fold increase, Fig. 5I), a gene involved with methionine metabolism and recently shown to be involved in the control of Drosophila lifespan (Parkhitko et al., 2016).

We also found that the morphological phenotypes observed in CapaR>GATAe RNAi MTs are strikingly similar to previously reported defects induced by constitutive activation of Ras signaling (Zeng et al., 2010). Given that CapaR>GATAe RNAi MTs induced upregulation of Ras85D, we investigated whether activating Ras signaling in the PCs would induce similar morphological abnormalities. Interestingly, inducing a constitutively activated form of Ras85D (Wu et al., 2010) in the PCs (CapaR>Ras[V12]) resulted in highly similar tubule defects to CapaR>GATAe RNAi flies (Fig. S6A). However, silencing both GATAe and Ras signaling (by the induction of Ras85D RNAi (Slack et al., 2015) or the dominant-negative form of Ras85D, Ras<sup>N17</sup> (Lee et al., 1996) did not rescue the tubule phenotype of GATAe knockdown (Fig. S6B), suggesting that other signaling pathways might be involved in the control of MT morphology. Altogether, these results indicate that GATAe directly or indirectly regulates the expression of several tumor-related genes in the MTs.

GATA<sub>e</sub> is required for stellate cell maintenance
To determine whether GATAe is necessary in the other secretory cells present in tubule main and initial segments, we silenced GATAe expression in SCs using a SC-specific GAL4 driver (ClC-a-Gal4; Cabrero et al., 2014). This resulted in a significant reduction in the number of SCs (∼11 cells in CIC-a>GATAe RNAi compared with ∼23 cells in control tubules, Fig. 6C). Surprisingly, these cells appeared localized to the initial segment of the MTs (Fig. 6B). We next investigated whether this reduction of SCs could impact on fluid secretion in isolated MTs (Dow et al., 1994). Data in Fig. 6D show that, although the basal secretion rates for ClC-a>GATAe RNAi MTs were not altered, fluid secretion rates were significantly reduced compared with the controls when MTs were stimulated with the diuretic neurohormone Kinin acting only in SCs (Terhzaz et al., 1999). These data demonstrate that the osmoregulatory MTs function is compromised in GATAe knockdown SCs.
showed a low but still significant reduction of ClC-a+ cells in the Tapadia, 2010; Denholm et al., 2003). Notably, the controls in the embryonic tubule (Campbell et al., 2010; Gautam and and do not mimic the phenotype of abnormal intercalation of SCs stage were located in a regular pattern along the tubule (Fig. S7E), ClC-a>GATAe (Fig. 6C). Furthermore, SCs in when compared with the number of SCs in the adult stage. ClC-a antibody also displayed a normal localization of RNSCs in their MTs (Fig. 7B’), but were significantly reduced in number compared with the controls (Fig. 7D). Furthermore, esg-GFPts>GATAe RNAi switched at 29°C 1 day post-eclosion did not result in any significant alteration of the pattern of RNSCs up to 20 days at the permissive temperature (Fig. 7C’). Strikingly, cell quantifications confirmed that the number of RNSCs in the ureters and lower tubules is only significantly reduced when GATAe is silenced in this cell type during metamorphosis (Fig. 7D). Thus, these data indicate that GATAe is required for the migration or survival of this population of RNSCs during metamorphosis, but not for their maintenance in the adult stage.

**DISCUSSION**

In this study, novel functions for GATAe in *Drosophila* MTs have been identified. First, GATAe is crucial in the PCs from metamorphosis to maintain the correct architecture and cell proliferation in the adult tubule. Second, GATAe is required in the SCs for their survival, with loss of GATAe resulting in a reduction in SC population and MT secretory function, presumably owing to a reduction in the overall SC population. Finally, GATAe is necessary for the early migration of the RNSCs from the MG to the MTs during metamorphosis.

**GATAe is required from the pupal stage in principal cells**

GATAe loss in the PCs promotes morphological defects and uncontrolled proliferation and migration of RNSCs to more distal regions of the MTs. This organ persists from embryonic development throughout metamorphosis. However, during metamorphosis they still undergo tissue remodeling, initially shrinking by half their length and then elongating before eclosion (Denholm, 2013; Wessing and Eichelberg, 1979). Several factors have been shown to modulate changes in MT structure and function during this period, like the tumor suppressors salvador and Scribbled, which have been implicated in the regulation of the RNSCs (Zeng et al., 2010). In line with the hypothesis of metamorphosis as a critical juncture in
the determination of the MT development, our data show that GATAe expression is required only from the pupal stage with no apparent requirement before this stage. In addition, the morphological defects of GATAe knockdown tubules phenocopied MTs with constitutive activation of Ras signaling either clonally (Zeng et al., 2010) or in the PCs (shown in this study). Upregulation of Ras85D gene expression in GATAe knockdown tubules indicates that GATAe could coordinate growth by regulating Ras pathway signaling. However, our data also suggest that GATAe could do so through alternative pathways, which deserve further investigation.

We demonstrated that GATAe requirement in the PCs is tissue-autonomous, as expression of GATAe RNAi driven by UrO-Gal4:GFP was enough to induce tumorous growth in the regions in and surrounding the GFP+ areas, indicating that GATAe could act autonomously and non-autonomously. Interestingly, these tubules did not show any obvious over-proliferation or presence of RNSCs, suggesting that tumorous growth is induced independently of any pathway that may produce RNSC overproliferation. Our data also show that GATAe appears necessary for regulation of factors such as Debcl or Diap1, which are known to regulate cell proliferation and homeostasis in a variety of tissues, including the MTs (Bohère et al., 2018; Ren et al., 2010; Terhzaz et al., 2010). A previous study has shown that Diap1 is expressed in the RNSCs to ensure their survival, a process that is regulated by the Hippo pathway and shavenbaby (ovo – FlyBase) (Bohère et al., 2018). We hypothesize that signals from the PCs could be required for the control of this cell population by regulating Diap1, and that GATAe could be involved in this signaling from the PCs to the RNSCs. Finally, GATAe regulates adult maintenance of the MTs.

**The implication of GATAe in the maintenance of SC and RNSC population**

The SC population undergoes mesenchymal-to-epithelial transition and integrates into the MTs during stage 13 of embryonic development; by stage 15, they are completely intercalated in specific regions of both anterior and posterior tubules (Denholm et al., 2003; Sözen et al., 1997). This process is dependent on multiple factors, such as hibris and serpent (orthologs of human nephrin and GATA1, respectively; Artero et al., 2006), the activity of the tiptop and teashirt (Denholm et al., 2013), and ecdysone signaling (Gautam and Tapadia, 2010). In addition, we show that the SCs also require GATAe for their survival through metamorphosis, as the number and localization of SCs are significantly decreased in tubules with reduced GATAe levels. Curiously, the shape of the remaining SCs is not altered when compared with wild-type tubules. These findings suggest that GATAe performs a different function from teashirt, which determines the proper shape and physiology of the SCs during pupariation (Denholm et al., 2013).

The RNSC population migrates from the MG to the MTs during metamorphosis, and several factors are required for its migration and survival, such as Rac1 or shavenbaby, or members of the Hippo pathway. However, our data suggest that GATAe could do so through alternative pathways, which deserve further investigation.
pathway (Bohère et al., 2018; Takashima et al., 2013; Zeng et al., 2010). We show here that GATAe has a potential role in RNSC migration, as depletion of GATAe in this cell population induced a migration defect, leading to a few or no RNSCs in adult MTs. Strikingly, a similar phenotype was observed employing a dominant-negative form of Rac1 (Takashima et al., 2013). This, together with our findings indicating no alteration in the RNSC population when GATAe was silenced from the adult stage, strongly suggest that GATAe could be required for RNSC migration rather than adult survival. The role of GATAe in the RNSC's contrasts with its function in the adult MG intestinal stem cells as GATAe is necessary for their maintenance (Okumura et al., 2016). This provides more evidence of the different functions of this TF, depending on its cellular context. However, unlike RNSCs, intestinal stem cells exhibit high division rates (Ohlstein and Spradling, 2007; Okumura et al., 2016), masking a possible subtle role for GATAe in regulating RNSC mitosis or proliferation. Therefore, it would be compelling for future investigations to explore whether GATAe could also be involved in regulating standard division rates and stemness of RNSCs.

**The evolutionary conservation of GATA factors in relation to cancer**

Our data indicate that knockdown of GATAe in PCs results in abnormal expression of different cancer and apoptosis-related genes in the adult Drosophila MTs. Our findings are in line with data from human GATA factors, which showed that GATA factors can act both as tumor suppressors or as oncogenes (Rodriguez-Bravo et al., 2017; Hellebrekers et al., 2009; Zheng and Blobel, 2010; Peters et al., 2014b).

We demonstrated that GATAe performs entirely different functions in each tubule cell-type, a behavior that has been previously reported for GATAe in the adult MG (as mentioned in the introduction). These divergent functions of one gene dependent on its cellular context also take place with human GATA factors; outside its hematopoietic functions, GATA3 has been linked to diverse types of human cancers. For example, GATA3 is a recognized indicator of breast cancer, and it has been shown that its expression is sufficient to stop tumor dissemination in breast cancers (Kourouss-Mehr et al., 2008). However, GATA3 can also induce carcinogenesis in lymphoid precursor cells and converts double-positive thymocytes into a premalignant state (Nawijn et al., 2011; Ohlstein and Blobel, 2010; Peters et al., 2014b).

Further potential evolutionary conserved interactions in the GATA family can be concluded from our study. Our data demonstrate that low levels of GATAe reduce the expression of the proapoptotic gene Debel, which is associated with the proliferation defects observed. Interestingly, interactions between GATA factors and members of the bcl-2 family have been reported. In vertebrates, GATA1 also interacts with Bcl-x to ensure the survival of erythroid cells (Gregory et al., 1999). In addition, GATA4 directly binds to Bcl-I (Kobayashi et al., 2006; Aries et al., 2004; Kobayashi et al., 2010), to induce cell survival due to drug-induced toxicity in the heart. We hypothesize that, in Drosophila, an interaction between GATA factors (GATAe) and bcl-2 members (Debel) also occurs for MT homeostasis. Further experimentation would be required to elucidate the precise relationship between GATAe and Debel in Drosophila.

Our findings demonstrate that MTs require expression of specific genes in all the three different cell types to ensure their correct architecture, maintenance and function in the adult stage. Our results reveal that GATAe plays a vital role in maintaining the adult homeostasis of the MTs, possibly acting as a tumor suppressor gene. They also show that the diverse roles of GATAe are highly dependent on the cellular context, in a similar way to its human counterparts. A model of the functions of GATAe in the three different cell types of the MTs is presented in Fig. 8. This model integrates the diverse roles of GATAe in all three MT cell types (PCs, SCs and RNSCs), although further investigation is required to identify those specific signaling pathways that interact with GATAe in these particular cellular contexts. To summarize, our findings provide a context for how abnormal cellular states may occur in different cell types of the same tissue and establish further homologies between insect and human GATA factors. Altogether, they provide evidence for the mechanisms of tissue homeostasis and tumor suppression that can be conserved throughout evolution.

**materials and methods**

**Fly stocks**

Flies were maintained on a standard medium at 22°C, 55% humidity with a 12:12 h light:dark photoperiod. Unless otherwise specified, wild-type Canton-S (CS) flies crossed with both parental transgenic lines were used as controls. GATAe-Gal4 lines were obtained from Vienna Drosophila RNAi Centre [VDRC, #209818 and #205372 (Zhai et al., 2018; Kvon et al., 2014)] and both GATAe-Gal4 lines exhibited identical pattern of expression in MG and MTs. GATAe RNAi lines were obtained from VDRC [#10420, #10418; Takashima et al., 2013; Okumura et al., 2016], and the Bloomington Drosophila Stock Centre (BDSC, #33748; Okumura et al., 2016; Dutta et al., 2015; Dobson et al., 2018). All experiments using GATAe RNAi lines were performed using the construct #10420, except in Fig. S2, in which #33748 was employed. Lines used were: CapaR-Gal4, described by Terhzaz et al. (2012), CapaR-Gal4, Tub-Gal80p5p(CapaR+) and UAS-mCD8:GFP; UrO-Gal4, described by Terhzaz et al. (2010); CIC-a-Gal4 (VDRC, #31124); and ctB-Gal4, a gift from Dr Barry Denholm (University of Edinburgh, UK; Saxena et al., 2014). Ess-Gal4.UAS:GFP/Cyo; TubGal80p5p/Tm6B (Ess-GFP+) (Micchelli and Perrimon, 2006) was a gift from Dr Julia Cordero (University of Glasgow, UK), and the mutant line GATAe/TA3, Ser (Okumura et al., 2016) was a gift from Prof. Takashi Adachi-Yamada (Gakushuin University, Tokyo, Japan).

**Fixation and immunostaining**

For embryo collection, adult females were allowed to lay eggs on grape juice agar plates for 9-24 h at 29°C. Embryos were collected and dechorionated in a fresh solution of 50:50 bleach and double-distilled H2O for exactly 2 min and then washed thoroughly with double-distilled H2O. Using a fine brush, the embryos were subsequently transferred to 2 ml of heptane and 2.25 ml of 4% formaldehyde in PBS (Thermo Fisher #12549079) was added. Embryos were left for 20 min on a shaker to allow fixation. The lower aqueous phase was then gently removed, and 2 ml of a fresh solution of methanol (100%) was added, and the sample gently shaken for 20 s. Devitellinized embryos, which appeared in the methanol phase, were collected and placed in methanol (100%) before being processed for immunocytochemistry.

Larval, pupal and adult (5-day-old adult female flies unless otherwise specified) tubes were dissected in PBS and fixed with 4% paraformaldehyde for 20 min at room temperature. The following primary antibodies and dilutions were used: mouse anti-Ct (DSHB, 1/100), mouse anti-Dig (DSHB, 1/500), mouse anti-DI (DSHB, 1/50), mouse anti-Arm (DSHB, 1/100), rat anti-DCAD (DSHB, 1/200), rabbit anti-CIC-a (Cabredo et al., 2014; 1/100), rabbit anti-GFP (Life Technologies, 1/1000) and rabbit anti-PH3 (Cell Signaling Technologies, Ser10, 1/1000). Incubations with the primary antibodies were performed overnight at 4°C. The following secondary antibodies were used: Alexa Fluor 546-conjugated goat anti-mouse (Thermo Fisher Scientific, 1/1000) and Alexa Fluor 488/633-conjugated goat anti-rabbit (Thermo Fisher Scientific, 1/1000). Other markers used included: DAPI (Sigma-Aldrich, 1 μg/ml) and rhodamine-coupled phalloidin (Thermo Fisher Scientific, 1/100). All samples were mounted in Vectashield (Vector Laboratories). Confocal images were captured using a Zeiss LSM 880 AxiowObserver microscope (Zeiss, Germany).
Quantitative RT-PCR
qRT-PCR amplifications were performed from adult female Malpighian tubules and embryos (12-14 h after egg laying). cDNA was synthesized from 500 ng total RNA using SuperScript II RT (Thermo Fisher Scientific), following the manufacturer’s instructions. qRT-PCR was performed on an ABI StepOnePlus Detection System (Applied Biosystems) using Brilliant III Ultra-Fast SYBR Green QPCR Master Mix (Agilent), with the following primers:

- **GATAe** forward, 5′-ACCGCTGTCGATGAAGAAGG-3′ and reverse, 5′-GGACTGGAATTCTGCTGGCT-3′;
- **Diap1** forward, 5′-CGTGGTGCGATAAGAGGTGA-3′ and reverse, 5′-TTGAATAGCTGGGTCGAGTTC-3′;
- **Ras85D** forward, 5′-GCAAGAGAGGTGGCCAAACA-3′ and reverse, 5′-TCGGCTTGTTCATTTTGCGG-3′;
- **wts** forward, 5′-AGCCGACAATAACTGGGTG-3′ and reverse, 5′-AGAGGTGATTGCCTTCTC-3′;
- **RpL32** forward, 5′-TGACCATCCGCCCAGCATAC-3′ and reverse, 5′-ATCTCGGCGGCTAACG-3′;
- **Buffy** forward, 5′-GCCACAC-TACATTCCGCATCAC-3′ and reverse, 5′-ATTCATCGCCCAGCACC-3′;
- **Ahcyl1** forward, 5′-GGCGAGACGGAAGAGGACT-3′ and reverse, 5′-AGAGAGCTGGAAGAGGGTG-3′;
- **Debcl** forward, 5′-TTTTTC-GCTCCAGCATCACC-3′ and reverse, 5′-CGTCAATCCCAAGAACG-3′;
- **GATAe** forward, 5′-ACCGCTGTCGATGAAGAAGG-3′ and reverse, 5′-GGACTGGAATTCTGCTGGCT-3′;
- **Ahcy1** forward, 5′-GGCGAGACGGAAGAGGACT-3′ and reverse, 5′-AGAGAGCTGGAAGAGGGTG-3′;
- **Buffy** forward, 5′-GCCACAC-TACATTCCGCATCAC-3′ and reverse, 5′-ATTCATCGCCCAGCACC-3′.

Data were normalized against the *rpl32* standard and expressed as fold change compared with controls±s.e.m. (*n=3*).

Lifespan and stress assays
For lifespan assays, adult female flies were kept on standard medium in groups of 30, transferred to fresh food every 2 days and were counted daily until no living flies remained. For starvation assays, 7-day-old female flies were anaesthetized on ice and placed in groups of 20 in 30 ml cotton-capped glass vials containing 1% aqueous agarose only. Vials were checked for dead flies every 4 h until no living flies remained. For desiccation experiments, the same protocol was followed, except that the vials were empty (no food or water), and the open end of the tube was sealed with parafilm (Bemis). Vials were checked every 2 h until no living flies remained. All experiments were run in triplicate with at least 30 flies in each run of specified genotype. All
vials were placed in an incubator at 22°C, 55% humidity with a 12:12 light:dark photoperiod. Survival data has been expressed as % survival±s.e.m.

**Gravimetric estimates of body water**

Body weight measurements of *Capar>GATAe* RNAi flies were performed as described previously (Cabreiro et al., 2014). Briefly, the weight of total body water was calculated by subtracting dry weight from wet weight. Experiments were run in triplicate with at least 30 flies of each genotype (90 flies for the three genotypes).

**Malpighian tubule secretion assays**

Secretion assays were performed as described previously (Dow et al., 1994). Basal and neuropeptide-stimulated secretion rates were measured every 10 min. After 30 min of baseline readings, the peptide Drosophila kinin was added at a concentration of 10^{-3} M, and secretion rates were measured every 10 min for 30 more min. At least seven tubules were used for each condition. Data were plotted as mean±s.e.m.

**Data analysis**

For mRNA level quantification or fluid secretion analysis, a two-tailed Student’s t-test, taking P=0.05 as the critical value (for each independent group) were used. For wet- and dry-weight measurements, two-way ANOVA was used to compare each condition. P values were adjusted using the Sidak multiple comparisons test. For survival curves obtained in ANOVA was used to compare each condition.

**Acknowledgements**

We thank Dr Anthony Dornan and Saurav Ghimire for discussions and comments on the manuscript; Dr Julia Cordero, Dr Barry Denholm, Prof. Takashi Adachi-Yamada, the Bloomington Drosophila Stock Centre and the Vienna Drosophila Resource Centre for providing fly stocks.

**Competing interests**

The authors declare no competing or financial interests.

**Author contributions**


This work was supported by funding from the European Union’s Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant (64293 RENALTRACT).

**Supplementary information**


---

**References**


