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Regulation of immune and tissue homeostasis by *Drosophila* POU factors

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ABSTRACT

The innate immune system of insects deploys both cellular and humoral reactions in immunocompetent tissues for protection of insects against a variety of infections, including bacteria, fungi, and viruses. Transcriptional regulation of genes encoding antimicrobial peptides (AMPs), cytokines, and other immune effectors plays a pivotal role in maintenance of immune homeostasis both prior to and after infections. The POU/Oct transcription factor family is a subclass of the homeodomain proteins present in all metazoans. POU factors are involved in regulation of development, metabolism and immunity. Their role in regulation of immune functions has recently become evident, and involves control of tissue-specific, constitutive expression of immune effectors in barrier epithelia as well as positive and negative control of immune responses in gut and fat body. In addition, they have been shown to affect the composition of gut microbiota and play a role in regulation of intestinal stem cell activities. In this review, we summarize the current knowledge of how POU transcription factors control *Drosophila* immune homeostasis in healthy and infected insects. The role of POU factor isoform specific regulation of stem cell activities in *Drosophila* and mammals is also discussed.

1. Characteristics of the POU transcription factor family

The nomenclature of the POU family was derived from three family members: the mammalian Pituitary-specific transcription factor 1 (**P**it-1) (Ingraham et al., 1988), the Octamer-binding protein 1 (**O**ct-1) (Sturm et al., 1988) and Oct-2 (Clerc et al., 1988), and the *C. elegans* gene ***U**nc-86* (Finney et al., 1988). All three members share a highly conserved DNA-binding domain called the POU domain consisting of a region of approximately 150–160 amino acids (reviewed in (Herr et al., 1988)). The POU domain encompasses two subdomains termed the POU-specific (POU_S) domain and the POU-homeodomain (POU_H), both of which are capable of binding to an 8-bp canonical Octamer sequence motif (5′-ATGCAAAT-3′) in a base-specific manner (Fig. 1). Structural and biochemical analyses show that these two POU subdomains are in direct contact with the major groove of DNA and that the contact sites lie on opposite sides of the DNA backbone (Assamunt et al., 1993; Cox et al., 1995; Dekker et al., 1993; Klemm et al., 1994). The POU_S domain (~75 amino acid residues) comprises four alpha-helices, while the POU_H domain (~60 amino acid residues) consists of three alpha-helices. Helices 2 and 3 of both POU_S and POU_H domains are folded so that they each form a Helix-turn-Helix (HTH) unit and bind to each half sites of the Octamer sequence independently (Fig. 1). The POU_S domain binds to the 5′-ATGC-3′ half site while the POU_H domain associates with another 5′-AAAT-3′ half site (Klemm et al., 1994; Verrijzer et al., 1992). The POU_S and POU_H subdomains bind to the Octamer sequence cooperatively and the entire POU_S domain is required for efficient affinity DNA binding (Ingraham et al., 1990; Klemm and Pabo, 1996; Verrijzer et al., 1990). Particularly, the bipartite POU domain is connected by a variable and non-conserved linker region (~15–30 residues) that holds the two POU subdomains in a structurally flexible status, allowing the POU proteins bind to the Octamer consensus sequence in different configurations (Herr and Cleary, 1995; Herr et al., 1988).

In addition to monomeric binding to the canonical Octamer sequence, POU proteins have the ability to assemble as homodimers and heterodimers that recognize additional sequence motifs. The More palindromic Oct factor Recognition Element (MORE) (Tomilin et al., 2000) and Palindromic Octamer Recognition Element (PORE) (Botquin et al., 1998; Tomilin et al., 2000) have been identified as dimeric binding sites by POU proteins (Fig. 1). Homo- and heterodimerisation of POU proteins (eg. Oct-1, Oct-2, Oct-4, Oct-6) on both consensus MORE (5′-ATGCATATGCAT-3′) and imperfect PORE (5′-ATTTGAAATGCAAAT-3′) DNA enhance transactivation activity of some target genes (Botquin et al., 1998; Tomilin et al., 2000). Moreover, PORE-mediated dimerization of POU proteins has the potential to recruit co-activators for synergistic transcriptional activation (Tomilin et al., 2000). In addition, the configuration of POU subdomains to the DNA backbone differs in the MORE and PORE dimer models. In the MORE dimer model, the POU subdomains bind to the same half site derived from two

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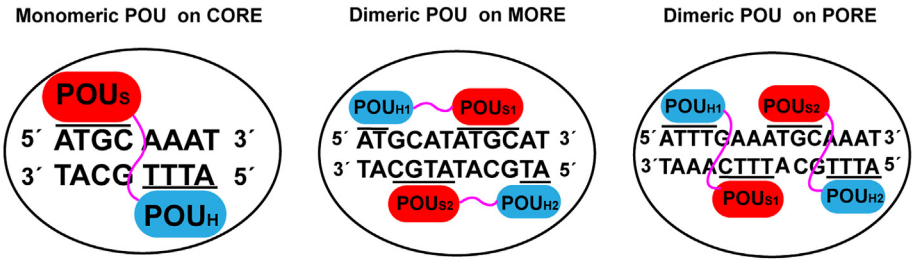

Fig. 1. Binding models of POU protein on different DNA sequence motifs.
Arrangement of monomeric POU protein on the canonical octamer recognition element (CORE) (left), dimeric POU proteins on the MORE (middle) and PORE (right). Generally, the POU_S subdomain (red) binds to the ATGC sequence, while the POU_H subdomain (blue) associates with the A/T-rich sequence. The POU subdomains derived from the same polypeptide chain have the same numbering and are separated by a hypervariable linker (purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

different protein molecules, leading to the binding of POU_S and POU_H subdomains on the same side of the DNA backbone. On the contrary, POU subdomains that bind to the same half site in the PORE dimer complex come from the same protein molecule, resulting in binding of POU_S and POU_H subdomains to the opposite sides of the DNA (Remenyi et al., 2001; Tomilin et al., 2000). Thus, the bipartite and versatile POU domain endows POU transcription factor family large diversity and complexity in target gene binding and regulation.

2. POU proteins in *Drosophila melanogaster*

The POU transcription factor family is typically classified into six different classes (from class I to class VI) based on the hypervariable linker length and similar amino acid sequence over the entire POU domain. Class II, III and V subfamilies belong to the group of Octamer binding proteins (POU/Oct), with high affinity DNA binding to the canonical Octamer sequence, while the class I, IV and VI subfamilies lack Octamer binding ability (reviewed in (Tantin, 2013)). Five different POU protein genes, belonging to four of the POU family classes, have been identified in the *Drosophila melanogaster* genome (Burglin and Affolter, 2016). The *Drosophila* POU domain protein 1 (Pdm1), also known as Nubbin (Nub) and dPOU-19, and Pdm2, also known as gene Miti-mere (Miti) and dPOU-28, are two Oct binding proteins belonging to the class II subfamily. Pdm1 and Pdm2 are paralogs, homologous to mammalian Oct1 and Oct2 (Burglin and Ruvkun, 2001). A putative role of Nub and Miti in development of the *Drosophila* embryonic central nervous system (CNS) was initially suggested based on their high expression levels in the embryonic CNS, and the phenotypes of the loss-of-function mutations *nub*^{1} and *miti* during embryogenesis (Billin et al., 1991; Dick et al., 1991; Lloyd and Sakonju, 1991). Subsequently, Pdm1 and Pdm2 have been implicated in regulating CNS development, neuronal precursor cell division, specification of neuroblast temporal identity, and cell fate lineage (Bahrampour et al., 2017; Bhat and Apsel, 2004; Isshiki et al., 2001; Yeo et al., 1995). In addition, Pdm1 plays a critical role in patterning and proximal-distal growth of the wing disc during larval development (Cifuentes and GarciaBellido, 1997; Neumann and Cohen, 1998; Ng et al., 1995). Pdm1 also acts as a downstream target gene of the Notch signaling pathway in leg joint formation (Rauskolb and Irvine, 1999), and work together with a subset of other transcription factors to control leg segmentation (Natori et al., 2012).

The POU/Oct factor Drifter (Dfr) or ventral veins lacking (Vvl) belongs to the class III subfamily and has been shown to participate in correct operation of wing vein patterning (Diazbenjumea and Garciabellido, 1990), tracheal cell differentiation and migration (Anderson et al., 1995), and steroid biosynthesis in the larval prothoracic gland (Danielsen et al., 2014). The two non-Oct binding POU factors, Abnormal chemosensory jump6 (Acj6) and POU domain protein 3 (Pdm3) belong to the class IV and VI subfamilies respectively. Both Acj6 and Pdm3 are important regulators of odor responses in the *Drosophila* olfactory sensory neurons (Ayer and Carlson, 1991; Tichy et al., 2008). Despite the numerous studies of the biological roles of POU proteins in *Drosophila* in the past decades, the immune function of POU proteins has just recently been uncovered.

3. Immune function of POU proteins in *Drosophila*

With the aim of isolating additional regulators of *Drosophila* innate immune defense gene expression, Engström's lab employed a double-interaction screen in yeast cells (Junell et al., 2007). In this approach, both DNA and protein baits are utilized simultaneously to isolate factors that either directly bind to the DNA bait sequence or are interacting with bait protein. A *cis*-regulatory region of one of the *Drosophila* antimicrobial peptide (AMP) genes, *CecA1,* was used as the DNA bait, together with a well-known NF-κB type immune activator, the Dorsal-related immunity factor (Dif) (Ip et al., 1993) as the second protein bait. Dif directly binds to κB sites in the regulatory region of the *CecA1* gene (Fig. 2) and regulates its expression (Petersen et al., 1995). This lethal yeast screen led to the re-isolation of cDNAs for the other NF-κB type immune activator, Relish (Dushay et al., 1996), serving as a proof-of-principle of the yeast screen, and of cDNAs for three *Drosophila* POU factors: Pdm1, Pdm2, and Dfr/Vvl (Junell et al., 2007). Further characterization demonstrated that all three factors were able to *trans*-activate *CecA1* expression in cell culture experiments. Moreover, the activation of *CecA1* expression in cells, mediated by these three POU proteins could be both Dif-dependent and independent, and exhibited much higher activation capacity than that by Relish or Dif (Dushay et al., 1996; Junell et al., 2007; Petersen et al., 1995). These primary findings indicated a potent role of POU proteins in regulating *Drosophila* immune defense gene expression. There have been no reports, however, suggesting an immune-regulatory role of the other two POU proteins, Acj6 and Pdm3, and we will focus the rest of this review on regulation of innate immune responses by Dfr/Vvl (hereafter referred to as Dfr) and Pdm1/Nubbin (hereafter referred to as Nub). Both constitutive epithelial immune reactions, as well as the intensity and duration of inducible immune reactions have now been shown to be regulated by members of the *Drosophila* POU transcription factor family, which will be further described below.

3.1. The role of POU proteins in protective immune function of barrier epithelia

The barrier epithelia provide the first line of immune defense in multicellular organisms by the action of both cellular and humoral immune responses that prevents further damage, invasion and growth of microorganisms. A group of small, immune-inducible and cationic AMPs has been found to be constitutively expressed in different epithelial tissues, like the epidermis and cuticular linings, the respiratory system, the digestive, renal and reproductive ducts, all which regularly come in direct contact with a variety of microorganisms (Tzou et al., 2000).

The POU factor Dfr (Table 1) has been shown to be highly expressed in adult immune-responsive tissues like fat body, epidermis, the epithelia of trachea, male reproductive organs, and of the digestive tract

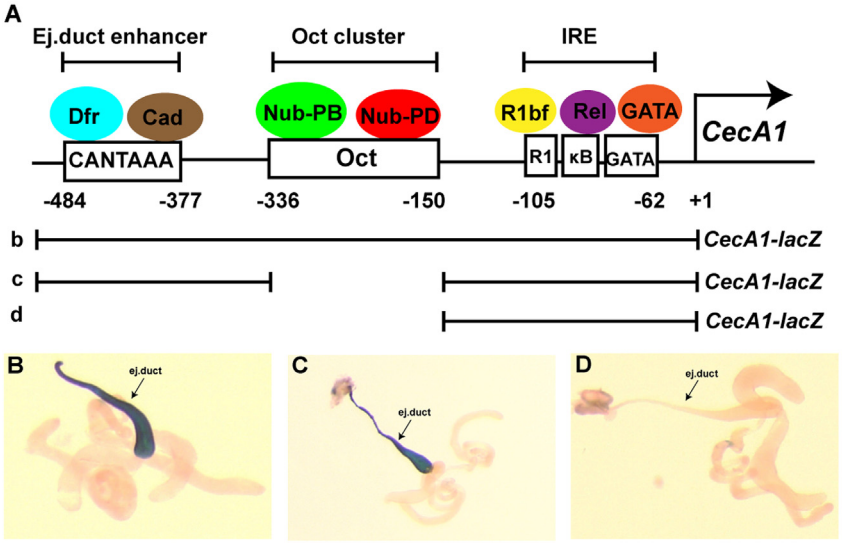

Fig. 2. Different modes of *CecA1* regulation and its *cis*-regulatory elements.
A. Schematic representation of the *CecA1* upstream regulatory region. In the proximal promoter region, an Infection-Inducible Regulatory Element (IRE) is located, which is composed of an R1 sequence motif, a κB site (binding site for NF-κB/Rel proteins Relish and Dif) and a GATA binding site, which is targeted by GATA factors like Serpent and dGATAe. The IRE is acting as an IMD and Toll pathway signal-dependent regulatory element in response to infection (reviewed in (Uvell and Engstrom, 2007)). Upstream of the IRE, an Oct cluster is the target of both negative regulation by Nub-PD and of positive regulation by Nub-PB (Dantoft et al., 2013; Lindberg et al., 2018). The distal ejaculatory duct enhancer contains five copies of the Dfr protein target sequence (CANTAAA) and also Cad binding motifs (CDREs), both of which are required for constitutive *CecA1* expression in the adult male ejaculatory duct (ej.duct) (Junell et al., 2010; Ryu et al., 2004). **b-d**: Outline of the *CecA1* upstream regions present in the *CecA1-lacZ* constructs used in (B–D). **B-D.** Dissected male reproductive organs stained for β-gal reporter activity, showing that the ej.duct enhancer (B), but not the Oct cluster (C), is necessary for constitutive *CecA1* expression. **D.** The IRE, which is required for infection-induced *CecA1* expression via the Toll or IMD pathways, is not sufficient for constitutive expression in the ej. duct.

Table 1
POU/Oct factors in *Drosophila*.

Gene	Transcripts	Length (nt)	Proteins	Molecular weight (kDa)
nub	nub-RB	4497	Nub-PB	103.9
	nub-RD	3267	Nub-PD	65.2
pdm2	pdm2-RA	2171	Pdm2-PA	55.5
	pdm2-RB	3627	Pdm2-PB	98.5
dfr/vvl[a]	dfr/vvl	4380	Dfr/Vvl	45.9
pdm3	pdm3-RA	4066	Pdm3-PA	106.3
	pdm3-RC	5153	Pdm3-PC	136.1
acj6[b]	acj6	2690	Acj6	44

[a] The dfr/*vvl* gene has been predicated to undergo stop codon readthrough, generating larger protein isoforms (Jungreis et al., 2011), which are not included in the table.
[b] The *acj6* gene undergoes multiple alternative splicing events, which give rise to up to 13 annotated transcripts and proteins, which are not listed here.

(Junell et al., 2010). In particular, constitutive *CecA1* expression in the male ejaculatory duct was found to require direct binding of Dfr to a consensus sequence 5′-CANTAAA-3′, of which five copies are located in a distal enhancer region of the *CecA1* gene called the ejaculatory duct enhancer (Fig. 2A). This sequence motif is also present in the regulatory region of most characterized *Drosophila* AMP genes, but not overlapping with the canonical Oct motif sites, which typically are located adjacent to infection-induced regulatory elements (IRE) carrying κB and GATA sites (Fig. 2A). These latter motifs have been shown to be necessary for AMP gene expression in fat body and hemocytes in response to infection, via the IMD and Toll pathways. (Dantoft et al., 2013; Junell et al., 2010; Lindberg et al., 2018). However, neither the IRE, nor the Oct sequence cluster, was necessary or sufficient for *CecA1* expression in the ejaculatory duct, indicating that Dfr do not interact with the consensus Octamer sequence for regulation of immune genes (Fig. 2B–D) and (Junell et al., 2010). Dfr over-expression activates several of these AMP genes in healthy flies, in an IMD and Toll pathway-independent manner, supporting that Dfr is not primarily a regulator of immune responses, but rather controls basal immune gene expression in barrier epithelia (Junell et al., 2010). Constitutive expression of *CecA1* in the male ejaculatory duct has also been found to involve the homeodomain transcription factor Caudal (Cad), binding to another motif called the Cad protein DNA recognition element (CDRE) (Ryu et al., 2004). Co-expression of Dfr and Cad in a cell transfection system showed strong synergistic activity that required intact Dfr and Cad target sequences (Junell et al., 2010), highlighting the existence of functional interactions between POU domain proteins and homeodomain proteins in the control of immune gene expression (Junell et al., 2010). Cad was also found to control constitutive expression of *Drosomycin (Drs)* and *CecA1* in adult salivary glands (Ryu et al., 2004), a tissue where Dfr also is highly expressed. However, the possible co-activation of these and other AMPs by Dfr and Cad in salivary glands and other tissues where their expression overlaps, awaits experimental validation. In summary, the POU transcription factor Dfr plays an important role in modulating *Drosophila* immune gene expression, especially for tissue-specific, constitutive AMP gene expression in barrier epithelia.

3.2. The role of POU protein isoforms in controlling immune gene homeostasis

The *nub* gene belongs to the class II POU/Oct subfamily. It has recently been shown to possess two functional promoters, separated by more than 30 kilo bases. Transcripts from these two promoters are subject to promoter-specific alternative splicing, and subsequent translation give rise to two independent transcriptional regulators, Nub-PB and Nub-PD (Dantoft et al., 2013; Lindberg et al., 2018) (Table 1). These two protein isoforms exhibit both overlapping and independent expression patterns in immunocompetent tissues, such as fat body, hemocytes, midgut epithelium and reproductive tracts (Dantoft et al., 2013; Lindberg et al., 2018). Despite the fact that Nub-PB and Nub-PD share the same POU/homeo DNA binding domain, the outcome of target gene regulation by the two isoforms differs, most likely due to differences in their N-termini and protein-protein interactions with other regulatory factors. Global transcriptional profiles of Nub-PD mutant flies and of Nub-PB overexpressing flies revealed that Nub-PD acts as a general repressor of immune gene expression, while Nub-PB acts as a general activator of the same genes in healthy flies (Dantoft et al., 2013; Lindberg et al., 2018). The short isoform Nub-PD, directly binds to the conserved Oct sites located in the regulatory regions of several AMP genes and represses their expression (Fig. 2). Moreover, Nub-PD-mediated negative regulation of AMP genes is NF-κB/Relish-dependent and it was suggested that the role of this negative regulation is to prevent aberrant and excessive immune activation (Dantoft et al.,

2013, 2016). In contrast, the long isoform Nub-PB, is a novel transcriptional activator that controls a large number of immune genes in immunoresponsive tissues. In the adult midgut, RNAi of Nub-PB led to loss of AMP gene expression, while overexpression of Nub-PB in fat body promoted very high expression levels of numerous immune genes. Interestingly, Nub-PB activated many of these target genes in an NF-κB/Relish-independent manner, while other genes required both Nub-PB and NF-κB/Relish for full activation, and the presence of both factors promoted synergistic activation of *CecA1* (Lindberg et al., 2018). It is likely that the different number and positions of Oct and κB sites in the regulatory regions of the target genes determine the Nub-PB/Relish-mediated activation patterns. Strikingly though, Nub-PB exhibits considerably stronger transcriptional activation of a larger set of *Drosophila* immune-regulated genes in overexpression bioassays compared with the core immune regulatory NF-κB factors Relish and Dif (Han and Ip, 1999; Lindberg et al., 2018). In addition, many JNK/JAK-STAT-dependent genes were found to be highly responsive to Nub-PB *trans*-activation both in a global transcriptome analysis and in targeted RT-qPCR experiments, the latter carried out both prior to and after infection (Lindberg et al., 2018). Thus, Nub-PB is a very potent activator of both immune- and stress-related gene expression. Additionally, overexpression of Nub-PB in gut enterocytes mimicked infection-induced pro-inflammatory responses, accompanied by a disorganized gut cellular epithelium arrangement, increased apoptotic and mitotic activities, and induction of down-stream stress signaling pathways (Lindberg et al., 2018).

Based on these studies it was hypothesized that the activator, Nub-PB, and the repressor, Nub-PD, act together to form a molecular rheostat, which controls the same immune target genes in an antagonistic manner, thereby maintaining immune homeostasis (Lindberg et al., 2018). Thus, the well-balanced expression levels of Nub isoforms seem to play a crucial role in immunocompetent tissues to retain immune homeostasis prior to infection and to regain immune homeostasis after an infection has been eradicated.

3.3. The role of POU proteins in control of host-microbe interaction in the gut

The *Drosophila* gut manifests a major function in food digestion and nutrient absorption, and also harbors a large number of commensal microbes. Early studies of the microbial gut flora, based on culture or cloning strategies, identified a low number of species and a narrow taxonomic diversity. Despite this, it was clear that the microbes have a broad impact on host physiological traits (reviewed in (Broderick and Lemaitre, 2012)). A fundamental question in the field of host-microbe interaction in the gut is how the host gut epithelium discriminates the potentially pathogenic bacteria and innocuous commensal flora. Given the fact that pathogenic bacteria and commensal flora exhibit different abilities to activate AMP gene expression, reactive oxygen species (ROS) generation, and intestinal stem cell (ISC) activities (reviewed in (Buchon et al., 2013)), the host genotype has been considered as one of the potential cues that influence gut microbial community. Thus, transcriptional regulation of immune gene expression, mediated by transcription factor families in the gut epithelium has gained a large interest in studying local host-microbe interaction both prior to and after infection.

As describe above, the POU transcription factor Nub regulates AMP gene expression in the gut epithelium in an isoform-specific manner. In nub^1 mutants, which specifically lack the repressive Nub-PD isoform but still expresses the activating form Nub-PB, transcriptional de-repression of AMP genes reduced the number of colony forming units (CFU) of cultivatable bacteria in the selected growth medium (Dantoft et al., 2013), suggesting that AMP expression led to a decrease in the growth rate of the microbial flora. However, deep bacterial 16S rDNA sequencing of the gut flora revealed that both the bacterial load and the taxonomic composition of gut bacteria was instead significantly *increased* in the nub^1 fly strain compared to control flies (Dantoft et al., 2016). Importantly, the nub^1 mutant had a very reduced life span, which appeared to be more linked to the bacterial load than to the immune gene activation, as rearing the flies in the presence of antibiotics prolonged life-span by more than 100%, while immune gene expression continued to be high in the presence of antibiotics. Many studies have shown that the fly gut is dominated by *Acetobacter* spp and *Lactobacillus* spp, especially in laboratory conditions (Adair et al., 2018; Broderick and Lemaitre, 2012; Staubach et al., 2013). This was also the case for the control flies in the study by Dantoft et al. (2016), although the microbial diversity as a whole was larger in the control flies than in many earlier studies, with typically 150 to 200 operational taxonomic units (OTUs) in each replicate. This difference most likely reflects the use of a deep sequencing strategy, as supported by other recent reports (Mistry et al., 2017; Wong et al., 2015). The comparative analysis of taxonomic bacterial distribution between Nub-PD mutant (nub^1) and control fly guts revealed that the microbial composition in nub^1 flies shifted during adult life, with an increase in many "new" *Acetobacter* spp, in several *Leuconostoc* spp, and other bacterial species that were not present in the control fly guts. This strongly indicates that host genotype and the ratio of Nub isoforms is important in controlling the composition of the gut microbial flora (Dantoft et al., 2016).

A recent study has shown that the expression level of Nub-PB in enterocytes (ECs), the major cell type in the gut epithelium, is negatively correlated with commensal bacterial load and host lifespan (Lindberg et al., 2018). Depletion of Nub-PB by RNAi in ECs increased the concentration of bacterial 16S rDNA in the gut and prolonged host longevity, indicating that higher bacterial loads in this genetic background is beneficial. It may be worth mentioning, that Nub-PB-depleted flies in the study by Lindberg et al. (2018) had low levels of immune gene activation and that this may be beneficial, at least in the short perspective. In line with this, overexpression of Nub-PB in ECs led to a decline in the number of gut bacteria and in fact shortened host lifespan, suggesting a negative effect on beneficial microbes by upregulation of AMPs and other immune effectors in the gut (Lindberg et al., 2018). In addition to these effects on the commensal flora, Nub-PB expression seemed to confer host resistance to pathogenic bacterial infection. Overexpression of Nub-PB promoted high expression levels of AMP genes and enhanced clearance of *Erwinia carotovora carotovara 15* (*Ecc15*) infection, while Nub-PB depletion caused low levels of AMP gene expression and reduced *Ecc15* clearance (Lindberg et al., 2018). Thus, Nub-PB-mediated activation of AMP genes is associated with pathogenic bacterial clearance capacity after infection (Lindberg et al., 2018).

It may seem somewhat contradictory that the de-repressed AMP expression levels in the Nub-PD mutant (nub^1) fly gut correlates with increased bacterial growth, as described above and in Dantoft et al. (2016). A possible explanation is that in the nub^1 mutant flies, which lacks the repressing Nub-PD form, the constantly over-active immune effector repertoire causes a selective pressure, driving the microbial gut flora to a composition of bacterial species that are resistant to the activity of AMPs and other immune effectors. The fact that nub^1 guts had a significantly shifted bacterial community and a short life span correlates with such a model. This may resemble the growth of antibiotic-resistant bacteria, which will escape further antibiotic treatment and grow in an uncontrolled fashion. Another possibility is that the strongly activated immune gene expression in the nub^1 mutant gut promotes inflammatory responses including aberrant ROS generation, which as a result leads to a shift in the microbial gut flora community. Taken together, the distinct host genotypes created by different expression levels of Nub transcription factor isoforms shows that host genotype has a strong effect on the growth and composition of both commensal and pathogenic microbes, and contributes to both host tolerance and resistance to the gut microbial flora.

3.4. Isoform-specific regulation of gut epithelium regeneration

The *Drosophila* intestinal immune responses consist of two major processes: regulation of AMP gene expression and generation of ROS. Excessive production of AMPs and ROS causes gut epithelium damage and trigger epithelium renewal mechanisms for tissue repair (Buchon et al., 2013). The multipotent ISCs located at the basal side of the adult midgut epithelium undergo both symmetric and asymmetric cell division to ensure both self-renewal and generation of differentiated cells that replace damaged or dying ECs. Both intrinsic and extrinsic signals are capable of triggering ISC activities for proper proliferation and differentiation (reviewed in (Jiang et al., 2016)). Notably, a series of well-characterized genetic markers for labeling *Drosophila* intestinal progenitor cells (ISC + enteroblasts (EBs)) and differentiated cells (ECs + enteroendocrine cells) enable studies of the mechanisms underlying how transcription factors regulate ISC activities to maintain gut epithelium homeostasis. The POU transcription factor Nub is highly expressed in differentiated ECs and has been utilized as a maker for mature ECs (Dantoft et al., 2013; Lee et al., 2009). However, the putative roles of Nub isoforms in regulation of ISC activities and of gut epithelium regeneration have only recently been investigated.

A recent report has shown that both Nub-PB and Nub-PD are highly expressed in differentiated ECs, using two independent promoter-specific report lines (Tang et al., 2018). Strikingly, these two Nub protein isoforms are also expressed in some EBs and regulate ISC proliferation in an antagonistic manner (Fig. 3). Depletion of Nub-PB in gut progenitor cells caused ISC hyperproliferation and loss of EB differentiation, at least in part by direct or indirect repression of *escargot (esg)* encoding a snail family transcription factor (Korzelius et al., 2014; Tang et al., 2018). In contrast, enforcement of Nub-PB expression in gut progenitor cells blocks ISC proliferation and promotes rapid EC differentiation (Tang et al., 2018). Thus, Nub-PB seems to repress ISC proliferation and simultaneously drive EB differentiation. On the contrary, loss of Nub-PD decreased ISC proliferation rate both prior to and after *Ecc15* infection. Thus, Nub-PD is required for ISC proliferation and maintenance in the contexts of both basal and immune-challenged conditions. Surprisingly, concomitant loss of Nub-PB and Nub-PD have no obvious effect on ISC proliferation and maintains normal epithelium regeneration (Tang et al., 2018). A likely explanation for this result can be redundant activity by the paralogous gene *pdm2* (Table 1). Such redundant activity between *nub* and *pdm2* has been deciphered in regulation of *Drosophila* embryonic neuroblast proliferation and cell fate specification (Bhat et al., 1995).

4. Isoform-specific activities of mammalian POU factors

The isoform-specific regulation of *Drosophila* ISC activities by Nub protein isoforms is in fact reminiscent of similar roles of mammalian POU protein variants, derived by alternative splicing, in regulation of embryonic stem cell (ESC) activities. The mouse Oct2 and human OCT4/POU5F1 have been shown to produce several isoforms that have different roles in regulating ESC functions. The Oct2 isoforms control mouse embryonic neuronal differentiation in opposite manners, as the short Oct-2.4 blocks neuronal differentiation, while the long Oct-2.2 activates the same (Theodorou et al., 2009). The OCT4 variants OCT4A and OCT4B share the same DNA binding domain but exhibit different abilities to control human ESCs self-renewal. The large OCT4A variant is specifically expressed in ESCs and is required for maintaining ESC pluripotency, while the short OCT4B variant is expressed in both pluripotent and somatic cells, and is unable in retaining ESCs in an undifferentiated, pluripotent state (Atlasi et al., 2008; Lee et al., 2006). In addition, the human OCT1/POU2F1 gene also generates several protein isoforms in human cell lines with different expression levels and with different capacity of regulating cell proliferation (Pankratova et al., 2016). Oct1 and Oct4 both target the same and different sets of genes in ESCs and their daughter cells for programming early developmental fate specification (Shen et al., 2017). Thus, POU protein isoform-specific regulatory mechanisms involved in stem cell identity seem to be prevalent in both *Drosophila* and mammals, possibly indicating the existence of an evolutionarily relationship, or of repeated evolvement of such regulatory modules.

5. Perspectives

A recent study showed that POU/Oct transcription factor DNA binding specificity is highly conserved between mammals and *Drosophila* (Nitta et al., 2015). This suggests that the POU/Oct transcription factor family may share evolutionarily conserved regulatory mechanisms between insects and mammals. In fact, the Octamer consensus sequence was initially identified in the regulatory regions of immunoglobulin genes, and mammalian POU/Oct factors have been shown to regulate immune-modulatory targets like immunoglobulin genes, cytokines and pro-inflammatory mediators. In addition, POU/Oct factors have been identified as core transcription factors in regulatory complexes that participate in both maintaining and inducing stem cell pluripotency, and programming of stem cell lineages (reviewed in (Malik et al., 2018; Tantin, 2013)). The roles of POU/Oct factors in regulating both immune gene expression and stem cell activities in *Drosophila* described in this review (Fig. 3), and their similar roles in mammals support the notion that such a dual mode of

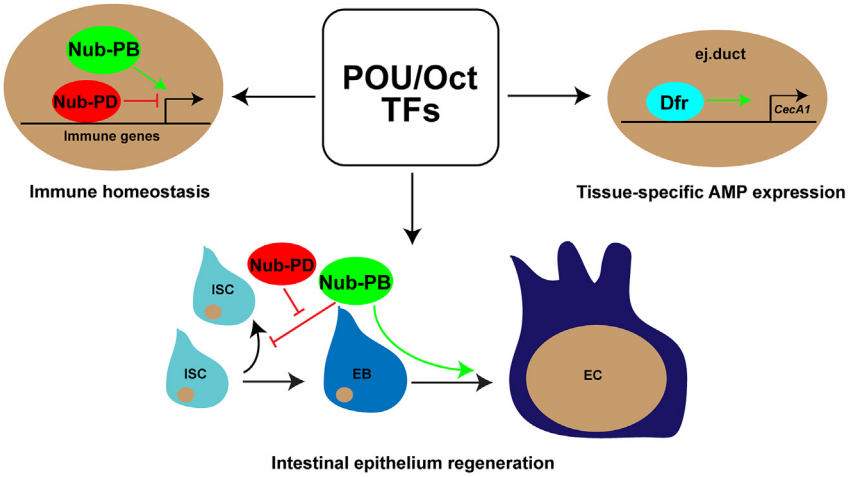

Fig. 3. Overview of the functions of *Drosophila* POU/Oct transcription factors in immune and tissue homeostasis. Well-tuned expression and activity of Nub isoforms are required to maintain immune homeostasis and intestinal epithelium regeneration. For normal gut epithelium regeneration, Nub-PB specifically acts in EBs to repress ISC proliferation, and drives EB differentiation into mature ECs. Simultaneously, Nub-PD represses Nub-PB activity to ensure proper ISC proliferation and ISC maintenance. In the case of immune homeostasis, Nub-PB acts as an activator, while Nub-PD serves as a repressor of the same immune genes to balance immune reactions. In basal, tissue-specific AMP expression, Dfr is required for constitutive *CecA1* expression in the male ejaculatory duct (ej.duct). ISC: intestinal stem cell; EB: enteroblast; EC: enterocyte; AMPs: antimicrobial peptides.

regulation might be evolutionarily ancient. This also raises the interesting question whether there is a functional link between POU/Oct regulation of immune responses and stem cell activities, *i.e.* between immune and tissue homeostasis? This seems rather likely, as the rate of ISC proliferation and maturation is increased upon infection and inflammation both in flies and in mammals. This is especially intriguing as the antagonistic action of *Drosophila* POU/Oct isoforms appears to be involved in tuning both the strength of immune responses and of the dynamic processes leading to epithelium re-generation. This further relates to that tissue inflammation is a risk factor for tumorigenesis in humans, and that mutations in human genes for POU/Oct factors have been linked to development of many types of cancer (Vazquez-Arreguin and Tantin, 2016).

Although transcriptional regulation of *Drosophila* immunity has been studied for long, the regulation of immune gene expression by POU factors has only recently become evident. One may ask why the POU factors/genes were not identified in the numerous transcriptional profiling and genetic screens carried out on *Drosophila* immune responses? One fact is that the POU factors are prominent regulators of developmental processes, and mutations in these genes are usually embryo lethal. This is in contrast with the NF-κB/Rel factors Relish and Dif, for which the corresponding mutants are viable and thereby prone to genetic analysis in a simple manner. Another reason can be that most transcription factors are not prominently upregulated upon infection, again with Relish as an exception, as the regulators should be present already at the time of infection to control their target genes. Thus, transcriptional profiling would often not identity transcription factors as differentially expressed genes upon infection even if they are crucial for the immune responses to occur. In addition, RNAi screens have typically been carried out so that the interfering dsRNA is directed to parts of the gene that are in common for several transcripts. As exemplified by the *nub* gene, such RNAi will downregulate both the Nub-PB activator and the Nub-PD repressor isoforms equally, and the effect on the immune target genes will be minor. It may be mentioned, that the *miti/pdm2* and *pdm3* genes have a similar organization producing a small and a large isoform (Table 1). This shows that it is not completely straightforward to identify all regulators of immune processes and we may still lack knowledge of additional ones, which will be needed to gain a fully comprehensive picture of immune gene regulation.

Transcriptional regulation of immune gene expression is also subject to regulation at the chromatin level, involving chromatin modeling complexes and histone modifications. There have been surprisingly few studies on chromatin regulation of *Drosophila* immunity. However, in both insects and mammals, members of the conserved Akirin family links NF-κB/Rel factors with the SWI/SNF chromatin-remodeling complex (Bonnay et al., 2014; Goto et al., 2014; Tartey and Takeuchi, 2015). Most interestingly for the scope of this review, is the finding that in *C. elegans,* which lacks NF-κB/Rel transcription factors, the Akirin homolog Akir-1, instead forms a complex with the POU transcription factor CEH-18. Together with the NuRD and MEC chromatin remodeling complexes, Akir-1 and CEH-18 upregulate AMP gene expression in the nematode following fungal infection (Polanowska et al., 2018). Future studies should shed more light on how the interaction of DNA-binding transcription factors of the POU family with different type of chromatin remodeling complexes may contribute to the opposite regulatory activities of POU factor isoforms identified in *Drosophila* and mammals.

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Appendix A. Supplementary data

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References

Adair, K.L., Wilson, M., Bost, A., Douglas, A.E., 2018. Microbial community assembly in wild populations of the fruit fly Drosophila melanogaster. ISME J. 12, 959–972.
Anderson, M.G., Perkins, G.L., Chittick, P., Shrigley, R.J., Johnson, W.A., 1995. Drifter, a Drosophila pou-domain transcription factor, is required for correct differentiation and migration of tracheal cells and midline glia. Genes Dev. 9, 123–137.
Assamunt, N., Mortishiresmith, R.J., Aurora, R., Herr, W., Wright, P.E., 1993. The solution structure of the oct-1 pou-specific domain reveals a striking similarity to the bacteriophage-lambda repressor DNA-binding domain. Cell 73, 193–205.
Atlasi, Y., Mowla, S.J., Ziaee, S.A.M., Gokhale, P.J., Andrews, P.W., 2008. OCT4 spliced variants are differentially expressed in human pluripotent and nonpluripotent cells. Stem Cell. 26, 3068–3074.
Ayer, R.K., Carlson, J., 1991. Acj6 - a gene affecting olfactory physiology and behavior in Drosophila. Proc. Natl. Acad. Sci. U.S.A. 88, 5467–5471.
Bahrampour, S., Gunnar, E., Jonsson, C., Ekman, H., Thor, S., 2017. Neural lineage progression controlled by a temporal proliferation program. Dev. Cell 43, 332–348 e334.
Bhat, K.M., Apsel, N., 2004. Upregulation of Mitimere and Nubbin acts through Cyclin E to confer self-renewing asymmetric division potential to neural precursor cells. Development 131, 1123–1134.
Bhat, K.M., Poole, S.J., Schedl, P., 1995. The miti-mere and Pdm1 genes collaborate during specification of the rp2/sib lineage in Drosophila neurogenesis. Mol. Cell Biol. 15, 4052–4063.
Billin, A.N., Cockerill, K.A., Poole, S.J., 1991. Isolation of a family of Drosophila pou domain genes expressed in early development. Mech. Dev. 34, 75–84.
Bonnay, F., Nguyen, X.H., Cohen-Berros, E., Troxler, L., Batsche, E., Camonis, J., Takeuchi, O., Reichhart, J.M., Matt, N., 2014. Akirin specifies NF-kappaB selectivity of Drosophila innate immune response via chromatin remodeling. EMBO J. 33, 2349–2362.
Botquin, V., Hess, H., Fuhrmann, G., Anastassiadis, C., Gross, M.K., Vriend, G., Scholer, H.R., 1998. New POU dimer configuration mediates antagonistic control of an osteopontin preimplantation enhancer by Oct-4 and Sox-2. Genes Dev. 12, 2073–2090.
Broderick, N.A., Lemaitre, B., 2012. Gut-associated microbes of Drosophila melanogaster. Gut Microb. 3, 307–321.
Buchon, N., Broderick, N.A., Lemaitre, B., 2013. Gut homeostasis in a microbial world: insights from Drosophila melanogaster. Nat. Rev. Microbiol. 11, 615–626.
Burglin, T.R., Affolter, M., 2016. Homeodomain proteins: an update. Chromosoma 125, 497–521.
Burglin, T.R., Ruvkun, G., 2001. Regulation of ectodermal and excretory function by the C. elegans POU homeobox gene ceh-6. Development 128, 779–790.
Cifuentes, F.J., GarciaBellido, A., 1997. Proximo-distal specification in the wing disc of Drosophila by the nubbin gene. Proc. Natl. Acad. Sci. U.S.A. 94, 11405–11410.
Clerc, R.G., Corcoran, L.M., Lebowitz, J.H., Baltimore, D., Sharp, P.A., 1988. The B-Cell-Specific oct-2 protein contains pou box-type and homeo box-type domains. Genes Dev. 2, 1570–1581.
Cox, M., Vantilborg, P.J.A., Delaat, W., Boelens, R., Vanleeuwen, H.C., Vandervliet, P.C., Kaptein, R., 1995. Solution structure of the oct-1 pou homeodomain determined by nmr and restrained molecular-dynamics. J. Biomol. NMR 6, 23–32.
Danielsen, E.T., Moeller, M.E., Dorry, E., Komura-Kawa, T., Fujimoto, Y., Troelsen, J.T., Herder, R., O'Connor, M.B., Niwa, R., Rewitz, K.F., 2014. Transcriptional control of steroid biosynthesis genes in the Drosophila prothoracic gland by ventral veins lacking and knirps. PLoS Genet. 10.
Dantoft, W., Davis, M.M., Lindvall, J.M., Tang, X.Z., Uvell, H., Junell, A., Beskow, A., Engstrom, Y., 2013. The Oct1 homolog Nubbin is a repressor of NF-kappa B-dependent immune gene expression that increases the tolerance to gut microbiota. BMC Biol. 11.
Dantoft, W., Lundin, D., Esfahani, S.S., Engstrom, Y., 2016. The POU/Oct transcription factor pdm1/nub is necessary for a beneficial gut microbiota and normal lifespan of Drosophila. J. Innate Immun. 8, 412–426.
Dekker, N., Cox, M., Boelens, R., Verrijzer, C.P., Vandervliet, P.C., Kaptein, R., 1993. Solution structure of the pou-specific DNA-binding domain of oct-1. Nature 362, 852–855.
Diazbenjumea, F.J., Garciabellido, A., 1990. Genetic-analysis of the wing vein pattern of Drosophila. Roux's Arch. Dev. Biol. 198, 336–354.
Dick, T., Yang, X.H., Yeo, S.L., Chia, W., 1991. 2 closely linked Drosophila pou domain genes are expressed in neuroblasts and sensory elements. Proc. Natl. Acad. Sci. U.S.A. 88, 7645–7649.
Dushay, M.S., Asling, B., Hultmark, D., 1996. Origins of immunity: Relish, a compound Rel-like gene in the antibacterial defense of Drosophila. Proc. Natl. Acad. Sci. U. S. A. 93, 10343–10347.
Finney, M., Ruvkun, G., Horvitz, H.R., 1988. The C-elegans cell lineage and differentiation gene unc-86 encodes a protein with a homeodomain and extended similarity to transcription factors. Cell 55, 757–769.
Goto, A., Fukuyama, H., Imler, J.L., Hoffmann, J.A., 2014. The chromatin regulator DMAP1 modulates activity of the nuclear factor B (NF-B) transcription factor Relish in the Drosophila innate immune response. J. Biol. Chem. 289, 20470–20476.
Han, Z.S., Ip, Y.T., 1999. Interaction and specificity of Rel-related proteins in regulating Drosophila immunity gene expression. J. Biol. Chem. 274, 21355–21361.

Herr, W., Cleary, M.A., 1995. The pou domain - versatility in transcriptional regulation by a flexible 2-in-One DNA-binding domain. Genes Dev. 9, 1679–1693.
Herr, W., Sturm, R.A., Clerc, R.G., Corcoran, L.M., Baltimore, D., Sharp, P.A., Ingraham, H.A., Rosenfeld, M.G., Finney, M., Ruvkun, G., Horvitz, H.R., 1988. The pou domain - a large conserved region in the mammalian pit-1, oct-1, oct-2, and caenorhabditis-elegans unc-86 gene-products. Genes Dev. 2, 1513–1516.
Ingraham, H.A., Chen, R., Mangalam, H.J., Elsholtz, H.P., Flynn, S.E., Lin, C.R., Simmons, D.M., Swanson, L., Rosenfeld, M.G., 1988. A tissue-specific transcription factor containing a homeodomain specifies a pituitary phenotype. Cell 55, 519–529.
Ingraham, H.A., Flynn, S.E., Voss, J.W., Albert, V.R., Kapiloff, M.S., Wilson, L., Rosenfeld, M.G., 1990. The pou-specific domain of pit-1 is essential for sequence-specific, high-affinity DNA-binding and DNA-dependent pit-1-pit-1 interactions. Cell 61, 1021–1033.
Ip, Y.T., Reach, M., Engstrom, Y., Kadalayil, L., Cai, H., Gonzalezcrespo, S., Tatei, K., Levine, M., 1993. Dif, a dorsal-related gene that mediates an immune-response in Drosophila. Cell 75, 753–763.
Isshiki, T., Pearson, B., Holbrook, S., Doe, C.Q., 2001. Drosophila neuroblasts sequentially express transcription factors which specify the temporal identity of their neuronal progeny. Cell 106, 511–521.
Jiang, H.Q., Tian, A.G., Jiang, J., 2016. Intestinal stem cell response to injury: lessons from Drosophila. Cell. Mol. Life Sci. 73, 3337–3349.
Junell, A., Uvell, H., Davis, M.M., Edlundh-Rose, E., Antonsson, A., Pick, L., Engstrom, Y., 2010. The POU transcription factor drifter/ventral veinless regulates expression of Drosophila immune defense genes. Mol. Cell Biol. 30, 3672–3684.
Junell, A., Uvell, H., Pick, L., Engstrom, Y., 2007. Isolation of regulators of Drosophila immune defense genes by a double interaction screen in yeast. Insect Biochem. Mol. Biol. 37, 202–212.
Jungreis, I., Lin, M.F., Spokony, R., Chan, C.S., Negre, N., Victorsen, A., White, K.P., Kellis, M., 2011. Evidence of abundant stop codon readthrough in Drosophila and other metazoa. Genome Res. 21, 2096–2113.
Klemm, J.D., Pabo, C.O., 1996. Oct-1 POU domain DNA interactions: cooperative binding of isolated subdomains and effects of covalent linkage. Genes Dev. 10, 27–36.
Klemm, J.D., Rould, M.A., Aurora, R., Herr, W., Pabo, C.O., 1994. Crystal-structure of the oct-1 pou domain bound to an octamer site - DNA recognition with tethered DNA-binding modules. Cell 77, 21–32.
Korzelius, J., Naumann, S.K., Loza-Coll, M.A., Chan, J.S., Dutta, D., Oberheim, J., Glasser, C., Southall, T.D., Brand, A.H., Jones, D.L., Edgar, B.A., 2014. Escargot maintains stemness and suppresses differentiation in Drosophila intestinal stem cells. EMBO J. 33, 2967–2982.
Lee, J., Kim, H.K., Rho, J.Y., Han, Y.M., Kim, J., 2006. The human OCT-4 isoforms differ in their ability to confer self-renewal. J. Biol. Chem. 281, 33554–33565.
Lee, W.C., Beebe, K., Sudmeier, L., Micchelli, C.A., 2009. Adenomatous polyposis coli regulates Drosophila intestinal stem cell proliferation. Development 136, 2255–2264.
Lindberg, B.G., Tang, X., Dantoft, W., Gohel, P., Seyedoleslami Esfahani, S., Lindvall, J.M., Engstrom, Y., 2018. Nubbin isoform antagonism governs Drosophila intestinal immune homeostasis. PLoS Pathog. 14, e1006936.
Lloyd, A., Sakonju, S., 1991. Characterization of 2 Drosophila pou domain genes, related to oct-1 and oct-2, and the regulation of their expression patterns. Mech. Dev. 36, 87–102.
Malik, V., Zimmer, D., Jauch, R., 2018. Diversity among POU transcription factors in chromatin recognition and cell fate reprogramming. Cell. Mol. Life Sci. 75, 1587–1612.
Mistry, R., Kounatidis, I., Ligoxygakis, P., 2017. Interaction between familial transmission and a constitutively active immune system shapes gut microbiota in Drosophila melanogaster. Genetics 206, 889–904.
Natori, K., Tajiri, R., Furukawa, S., Kojima, T., 2012. Progressive tarsal patterning in the Drosophila by temporally dynamic regulation of transcription factor genes. Dev. Biol. 361, 450–462.
Neumann, C.J., Cohen, S.M., 1998. Boundary formation in Drosophila wing: Notch activity attenuated by the POU protein Nubbin. Science 281, 409–413.
Ng, M., Diazbenjumea, F.J., Cohen, S.M., 1995. Nubbin encodes a pou-domain protein required for proximal-distal patterning in the Drosophila wing. Development 121, 589–599.
Nitta, K.R., Jolma, A., Yin, Y.M., Morgunova, E., Kivioja, T., Akhtar, J., Hens, K., Toivonen, J., Deplancke, B., Furlong, E.E.M., Taipale, J., 2015. Conservation of transcription factor binding specificities across 600 million years of bilateria evolution. Elife 4.
Pankratova, E.V., Stepchenko, A.G., Portseva, T., Mogila, V.A., Georgieva, S.G., 2016. Different N-terminal isoforms of Oct-1 control expression of distinct sets of genes and their high levels in Namalwa Burkitt's lymphoma cells affect a wide range of cellular processes. Nucleic Acids Res. 44, 9218–9230.
Petersen, U.M., Bjorklund, G., Ip, Y.T., Engstrom, Y., 1995. The dorsal-related immunity factor, Dif, is a sequence-specific trans-activator of Drosophila Cecropin gene expression. EMBO J. 14, 3146–3158.
Polanowska, J., Chen, J.X., Soule, J., Omi, S., Belougne, J., Taffoni, C., Pujol, N., Selbach, M., Zugasti, O., Ewbank, J.J., 2018. Evolutionary plasticity in the innate immune function of Akirin. PLoS Genet. 14, e1007494.
Rauskolb, C., Irvine, K.D., 1999. Notch-mediated segmentation and growth control of the Drosophila leg. Dev. Biol. 210, 339–350.
Remenyi, A., Tomilin, A., Pohl, E., Lins, K., Philippsen, A., Reinbold, R., Scholer, H.R., Wilmanns, M., 2001. Differential dimer activities of the transcription factor Oct-1 by DNA-Induced interface swapping. Mol. Cell 8, 569–580.
Ryu, J.H., Nam, K.B., Oh, C.T., Nam, H.J., Kim, S.H., Yoon, J.H., Seong, J.K., Yoo, M.A., Jang, I.H., Brey, P.T., Lee, W.J., 2004. The homeobox gene Caudal regulates constitutive local expression of antimicrobial peptide genes in Drosophila epithelia. Mol. Cell Biol. 24, 172–185.
Shen, Z.L., Kang, J.S., Shakya, A., Tabaka, M., Jarboe, E.A., Regev, A., Dean, T.T., 2017. Enforcement of developmental lineage specificity by transcription factor Oct1. Elife 6.
Staubach, F., Baines, J.F., Kunzel, S., Bik, E.M., Petrov, D.A., 2013. Host species and environmental effects on bacterial communities associated with Drosophila in the laboratory and in the natural environment. PLoS One 8, e70749.
Sturm, R.A., Das, G., Herr, W., 1988. The ubiquitous octamer-binding protein oct-1 contains a pou domain with a homeo box subdomain. Genes Dev. 2, 1582–1599.
Tang, X., Zhao, Y., Buchon, N., Engstrom, Y., 2018. The POU/Oct transcription factor nubbin controls the balance of intestinal stem cell maintenance and differentiation by isoform-specific regulation. Stem Cell Rep. 10, 1565–1578.
Tantin, D., 2013. Oct transcription factors in development and stem cells: insights and mechanisms. Development 140, 2857–2866.
Tartey, S., Takeuchi, O., 2015. Chromatin remodeling and transcriptional control in innate immunity: emergence of Akirin2 as a novel player. Biomolecules 5, 1618–1633.
Theodorou, E., Dalembert, G., Heffelfinger, C., White, E., Weissman, S., Corcoran, L., Snyder, M., 2009. A high throughput embryonic stem cell screen identifies Oct-2 as a bifunctional regulator of neuronal differentiation. Genes Dev. 23, 575–588.
Tichy, A.L., Ray, A., Carlson, J.R., 2008. A new Drosophila POU gene, pdm3, acts in odor receptor expression and axon targeting of olfactory neurons. J. Neurosci. 28, 7121–7129.
Tomilin, A., Remenyi, A., Lins, K., Bak, H., Leidel, S., Vriend, G., Wilmanns, M., Scholer, H.R., 2000. Synergism with the coactivator OBF-1 (OCA-B, BOB-1) is mediated by a specific POU dimer configuration. Cell 103, 853–864.
Tzou, P., Ohresser, S., Ferrandon, D., Capovilla, M., Reichhart, J.M., Lemaitre, B., Hoffmann, J.A., Imler, J.L., 2000. Tissue-specific inducible expression of antimicrobial peptide genes in Drosophila surface epithelia. Immunity 13, 737–748.
Uvell, H., Engstrom, Y., 2007. A multilayered defense against infection: combinatorial control of insect immune genes. Trends Genet. 23, 342–349.
Vazquez-Arreguin, K., Tantin, D., 2016. The Oct1 transcription factor and epithelial malignancies: old protein learns new tricks. Biochimica Et Biophys. Acta-Gene Regulat. Mechan. 1859, 792–804.
Verrijzer, C.P., Alkema, M.J., Vanweperen, W.W., Vanleeuwen, H.C., Strating, M.J.J., Vandervliet, P.C., 1992. The DNA-binding specificity of the bipartite pou-domain and its subdomains. EMBO J. 11, 4993–5003.
Verrijzer, C.P., Kal, A.J., Vandervliet, P.C., 1990. The oct-1 homeo domain contacts only part of the octamer sequence and full oct-1 DNA-binding activity requires the pou-specific domain. Genes Dev. 4, 1964–1974.
Wong, A.C., Luo, Y., Jing, X., Franzenburg, S., Bost, A., Douglas, A.E., 2015. The host as the driver of the microbiota in the gut and external environment of Drosophila melanogaster. Appl. Environ. Microbiol. 81, 6232–6240.
Yeo, S.L., Lloyd, A., Kozak, K., Dinh, A., Dick, T., Yang, X.H., Sakonju, R., Chia, W., 1995. On the functional overlap between 2 Drosophila pou homeo domain genes and the cell fate specification of a cns neural precursor. Genes Dev. 9, 1223–1236.