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The bristle pattern development in *Drosophila melanogaster*: the prepattern and *achaete-scute* genes

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The external drosophila mechanoreceptors, residing on the head and body of imago, are represented by bristles of different sizes (macrochaetes and microchaetes). Macrochaetes are arranged in the species-specific bristle pattern, where each of them is strictly positioned. The bristle pattern is formed starting from its prototype (prepattern) in the imaginal disc. The position specificity of future mechanoreceptors is determined by local expression of two proneural genes, *achaete* (*ac*) and *scute* (*sc*) belonging to the *AS-C* complex, in response to the action of certain factors, referred to as prepattern factors, nonuniformly distributed in the ectoderm of imaginal discs. The topography of their total distribution defines the bristle prepattern. Thus, the full-fledged adult bristle pattern is the result of interaction of two systems – the prepattern and the system responding to prepattern, i. e., the *achaete* and *scute* genes. A considerable volume of miscellaneous experimental data related to various aspects in development of the bristle pattern has been so far accumulated; however, any formalized and detailed representation of the molecular genetic interaction of the prepattern factors with both each other and the *achaete-scute* genes is yet absent. This review systematizes the available data on the regular patterns of this interaction and shows that local expression of these genes is determined by hierarchical two-level control system comprising both direct and indirect regulators of their activities. A generalized scheme of the system containing the functional interactions of its components is proposed. The structural organization and principles of operation of the hierarchical molecular genetic system enabling the local expression of *ASC* genes and the resulting formation of ordered bristle pattern are described.

Key words: *Drosophila melanogaster*; macrochaetes; proneural cluster; bristle pattern; prepattern; *achaete-scute* gene complex.

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Становление щетиночного узора у *Drosophila melanogaster*: предструктура и комплекс генов *achaete-scute*

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Внешние механорецепторы дрозофилы, локализованные на голове и теле имаго, представлены щетинками разного размера – макро- и микрохетами. Макрохеты образуют устойчивую структурную композицию, так называемый щетиночный узор, специфичный для каждого вида дрозофилы, в котором каждая из макрохет занимает строго определенное положение. Формирование щетиночного узора начинается с формирования его прообраза в имагинальном диске. Специфичность позиций будущих механорецепторов определяется локальной экспрессией двух пронейральных генов – *achaete* (*ac*) и *scute* (*sc*), входящих в комплекс *AS-C*, в ответ на действие неких факторов, за которыми закрепилось название «факторы предструктуры», гетерогенно распределенных в эктодерме имагинальных дисков. Топография их совокупного распределения и создает прообраз (предструктуру) щетиночного узора. Таким образом, полноценный щетиночный узор является результатом взаимодействия двух систем: предструктуры и системы ответа на предструктуру – генов *achaete* и *scute*. К настоящему времени накоплено значительное число разрозненных экспериментальных данных, касающихся различных аспектов формирования щетиночного узора, однако формализованное представление полного спектра молекулярно-генетических взаимодействий факторов предструктуры как между собой, так и с генами комплекса *AS-C*, в литературе отсутствует. В обзоре систематизированы данные о закономерностях этих взаимодействий. Показано, что экспрессия пронейральных генов *achaete-scute* детерминируется иерархически организованной двухуровневой системой управления, содержащей как прямые, так и непрямые регуляторы их активности. Предложена обобщенная схема системы, включающая функциональные взаимодействия ее компонентов.

Ключевые слова: *Drosophila melanogaster*; макрохеты; щетиночный узор; пронейральный кластер; предструктура; генный комплекс *achaete-scute*.

Development of ordered spatial structures of various degrees of complexity is one of the most important events in the development of multicellular organisms. The patterns of this process and underlying mechanisms are the subject of long-term study and discussion. The bristle pattern of *Drosophila melanogaster* is among the attractive model objects for studying this issue; this bristle pattern is formed of 20 pairs of external sensory organs, macrochaetes (large bristles), located at fixed positions on the fly head and body. The number and arrangement of bristles forming the bristle pattern are so constant and characteristic of individual Drosophila species that allows each bristle to be named according to its position and the bristle pattern to be used as a species-specific criterion in classification.

The adult sensory organ comprises four cells, namely, the shaft, socket, neuron, and glial cell. All these cells originate from a single cell, the sensory organ precursor (SOP) cell. Each SOP cell develops from cells of proneural clusters, that is, groups of 20–30 cells in the ectoderm of imaginal discs. The cells of the cluster differ from all the remaining cells of imaginal disc by the presence of the proneural proteins, Achaete (AC) and Scute (SC). Each sensory organ develops from its own proneural cluster. During development, the proneural clusters are formed and SOP cells are separated at the third instar larval and early prepupal stages. The bristle positions on the body of an adult fly are strictly determined by the positions of SOP cells (reviewed in Modolell, Campuzano, 1998; Gomez-Skarmeta et al., 2003; Furman, Bukharina, 2008, 2017; Bukharina, Furman, 2015; Troost et al., 2015).

At the very first stages of the research into the mechanisms underlying genetic determination of the bristle pattern, Aleksandr Serebrovsky, Nikolay Dubinin, and their colleagues clarified that the *achaete-scute* genes, represented by a set of alleles, played the key role in this process. Characteristic of the flies carrying different alleles is the absence of certain bristles from the standard set. The bristle development at strictly specified positions was supposed to be associated with a local gene activity (Serebrovsky, 1930; Dubinin, 1932). However, the mechanisms leading to local activation of the *achaete-scute* genes remained vague and for a long time were the subject of discussions. The most popular hypothesis among the proposed variants interpreting this phenomenon was the hypothesis proposed by Curt Stern in 1954 (Stern, 1954, 1968). This hypothesis postulates that the local activation of the *achaete-scute* genes is a response to induction with prepattern factors, distributed in the ectoderm of imaginal discs in a discrete manner. As a result of this induction, cells localized to certain regions of the imaginal disc acquire the ability to follow a neural developmental pathway and form proneural clusters (Reeves, Posakony, 2005). Thus, the bristle pattern emerges due to the interaction of two systems – the prepattern and the system responding to the prepattern, i. e., the *achaete-scute* genes.

In the current concept of macrochaete morphogenesis and the mechanisms of bristle pattern development, the Stern hypothesis has been confirmed at a molecular genetic level. In particular, the structure–function organization of the *achaete-scute* gene complex (*AS-C*) has been clarified and the transcription factors influencing its expression have been identified, including U-shaped (USH), Pannier (PNR), and the proteins encoded by the *iroquois* gene complex (*Iro-C*), such as, Araucan (ARA), Caupolican (COUP), and Mirror (MIRR). These are the prepattern factors in terms of the Stern hypothesis.

In turn, expression of the *u-shaped*, *pannier*, and *iroquois* complex genes is determined by their own set of factors – the segmentation proteins Decapentaplegic (DPP), Hedgehog (HH), Engrailed (EN), and Wingless (WG), which act at early stages of imaginal disc compartmentalization. Thus, the *AS-C* transcription activation comprises a hierarchy of developmental events provided for by a concerted action of genes and gene ensembles and ends with development of bristles at strictly determined positions (Dahmann, Basler, 2000; Calleja et al., 2002; Aldaz et al., 2003; Ikmi et al., 2008; Michel, Dahmann, 2016).

This review systematizes the published data on the factors that initiate a local expression of the *ac-sc* genes and their interactions at the stage of proneural cluster formation.

Abbreviations

ac, *achaete*; AC, Achaete
ARA, Araucan
AS-C, *achaete-scute* gene complex
bHLH, basic helix-loop-helix
BRK, Brinker
BX, Beadex
CHM, Chameau
CHN, Charlatan
ci, *cubitus interruptus*
COUP, Caupolican
DA, Daughterless
dCtBP (drosophila C-terminal binding protein)
DLL, Distal-less
DPP, Decapentaplegic
EN, Engrailed
E(spl), Enhancer of Split
EYG, Eyegone
HH, Hedgehog
Iro-C, *iroquois* gene complex
MIRR, Mirror
PNR, Pannier
SAL, Spalt
SAL-R, Spalt-related
sc, *scute*; SC, Scute
SENS, Senseless
SGG, Shaggy
SOP cell, sensory organ precursor cell
SPI, Spitz
SSDP, sequence-specific single-stranded DNA-binding protein
TOE, Twin of eyegone
TOU, Toutatis
TUP, Tailup
USH, U-shaped
VG, Vestigial
VN, Vein
WG, Wingless

Compartmentalization of the wing imaginal disc

The main morphogenetic events that determine development of the bristle pattern on the body of drosophila are associated with the pair of wing imaginal discs, each giving rise to half of an adult fly thorax.

The disc develops from 10–50 cells of an early embryo, which as early as the cellular blastoderm stage are predetermined to form the imago's wing structures and notum (Bate, Martinez-Arias, 1991; Potter, Xu, 2001; Aldaz, Escudero, 2010). At this stage, the cells differ in the amounts of some proteins, which later on determine the main stages in disc compartmentalization. These proteins include EN, DPP, Distal-less (DLL), Vestigial (VG), WG, and HH (Blair, 1995; Brook, 2000; Held, 2002; Hooper, Scott, 2005; Beira, Paro, 2016). Note that DPP, WG, and HH form a concentration gradient, while the EN protein is confined to a narrow band with a width of one cell.

As a mature morphological structure, the imaginal disc is identifiable at the first instar larval stage. Soon after the disc is formed, it divides into compartments with different developmental fates (Aegerter-Wilmsen et al., 2007; Restrepo et al., 2014) (Fig. 1).

Initially, the imaginal disc is divided into the anterior and posterior compartments with further separation of the dorsal and ventral part in each of them (Nienhaus et al., 2012). The compartmentalization is determined by differential expression of several genes. The gene *cubitus interruptus* (*ci*) is expressed in the anterior part of the disc and the gene *engrailed* (*en*), in the posterior part. The dorsal disc region is determined by coexpression of the *vg* and *ap* genes and the ventral region, by expression of the gene *wg*. The regions where the genes determining compartments are expressed do not overlap and the corresponding boundaries are indentified as conditional anterior–posterior and dorsal–ventral axes of the disc (Brook, 2000; Delanoue et al., 2002).

The further events in compartmentalization are controlled by a cascade of genes and the key initiator of the cascade is the morphogene Decapentaplegic (DPP) (Restrepo et al., 2014). Expression of the gene *dpp* and production of the corresponding protein, DPP, are observed in a narrow band of cells. This band, well evident after specific protein staining, lies along the anterior–posterior disc axis (Zecca et al., 1995; Nellen et al., 1996; Foronda et al., 2009; Beira, Paro, 2016). From this band, the morphogen spreads over the entire disc forming a concentration gradient. DPP, being involved in the corresponding signaling pathway, determines the further direction in development of different disc regions depending on the set of proteins they contain (Zecca et al., 1995; Gómez-Skarmeta et al., 2003; Garcia-Bellido, 2009). In particular, the region carrying the protein Brinker (BRK) will give rise to wing structures. The role of Brinker is to counteract Dpp signalling by repressing Dpp pathway target genes (Martín et al., 2004; Affolter, Basler, 2007; Schwank et al., 2008; Restrepo et al., 2014). The presumptive notum is determined by the expression of *Iro-C*, proteins Eyegone (EYG) and Twin of eyegone (TOE) continues further subdivision of the presumptive thorax (Diez del Corral et al., 1999; Aldaz et al., 2003; Barrios, Campuzano, 2015; Barrios et al., 2015).

The major developmental event in macrochaete morphogenesis is specification of the proneural clusters in the presumptive notum region; this event is initiated by the proteins of *Iro-C* and PNR (Ikmi et al., 2008). In this process, the presence of PNR is a necessary but not sufficient condition. It is known that the proneural cluster is formed of the cells carrying PNR but lacking the USH (Gómez-Skarmeta et al., 2003; Villa-Cuesta et al., 2007).

This is the general scheme of wing imaginal disc compartmentalization, which forms the background for development of the bristle pattern.

The *achaete-scute* genes as the key component in the molecular genetic system responsible for macrochaete development

The central players in the morphogenesis of individual macrochaetes and the overall bristle pattern are the genes *achaete* and *scute* (*ac-sc*), components of the similarly named gene complex (*AS-C*). This complex comprises four genes (*achaete*, *scute*, *lethal of scute*, *asense*), encoding basic Helix-Loop-Helix (bHLH) transcription factors. A local expression of *ac-sc* provides for emergence of the bristles at strictly specified positions (see Fig. 1, *b*), whereas inactivation of these genes results in the absence of some or all macrochaetes of the standard set on the body of an adult fly. Ectopic *achaete-scute* gene expression in the ectoderm of imaginal disc and the resulting switch of this developmental mechanism in the corresponding region to the neural pathway, gives additional or ectopic bristles (Rodríguez et al., 1990; Modolell, 1997).

The *achaete-scute* genes determine development of the complementary sets of the notum bristles (Campuzano, Modolell, 1992; Modolell, 1997; García-Bellido, de Celis, 2009).

In this process, the "area of responsibility" of the *achaete* gene is confined to development of the dorsocentral macrochaetes, while the *scute* gene expression is sufficient for development of the complete bristle set (Rodríguez et al., 1990).

The specificity in time and site of *achaete-scute* gene expression is determined by two types of enhancers. The enhancers of the first type, which are localized beyond *AS-C* at a distance of up to 100 kb, are necessary for *achaete-scute* gene expression in all cells of each proneural cluster (Gómez-Skarmeta et al., 1995). In particular, the dorsocentral enhancer drives *achaete-scute* gene expression in the proneural clusters for dorsocentral bristles. As has been shown, the protein PNR (Ramain et al., 1993; Gómez-Skarmeta et al., 1995; Garcia-Garcia et al., 1999) and some proteins of the EGFR signaling pathway (Culi et al., 2001) bind to this enhancer. The Iroquois complex proteins, namely, ARA, COUP, and MIRR bind to another enhancer of this type, the L3-TSM enhancer (Kehl et al., 1998; Ikmi et al., 2008).

Enhancers of the second type, SOPEs (sensory organ precursor enhancers), are responsible for *achaete-scute* expression in the SOP cell (Ayyar et al., 2010). Each of these genes has its own SOPE (Giagtzoglou et al., 2003; Jafar-Nejad et al., 2003). These enhancers carry sites for a number of transcription factors, namely, E boxes (CANNTG) for binding the proneural proteins AC and SC, α-boxes (ACTACAG) for binding transcription factors of the NF-κB/Rel family, AT-rich β-boxes with still unknown functions, N boxes for binding the proteins Hairy (CACGCG) and E(spl) (CACGAG and CACAAG), and S boxes for binding Senseless (SENS). It is known that Charlatan (CHN) also binds to certain still

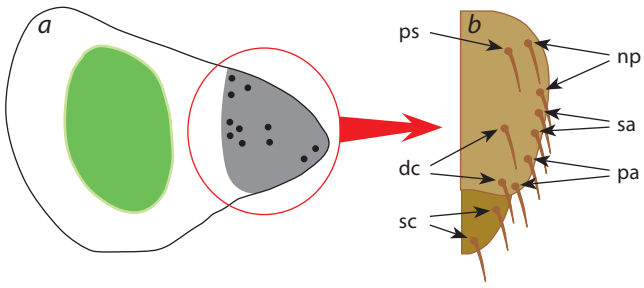

Fig. 1. Schemes of the wing imaginal disc (*a*) and the right half of adult fly notum (*b*).

The regions that give rise to the adult heminotum and wing blade, shown with gray and green, respectively. Black dots in scheme (*a*) denote the future proneural clusters and in scheme (*b*) localizations of macrochaetes (ps, presutural; dc, dorsocentral, np, notopleural; sa, supraalar; pa, postalar; and sc, scutellar).

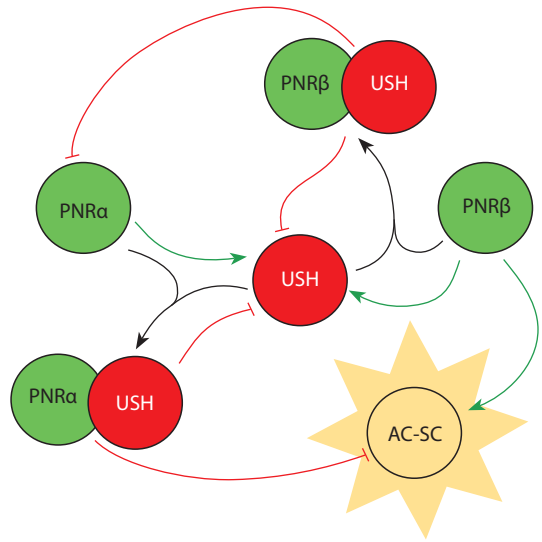

Fig. 2. Scheme of interactions between two PNR protein isoforms, USH and AS-C.

Black arrows indicate formation of PNR/USH heterodimers; green and blunt-end red arrows denote activating and repressive regulatory effects on genes coding PNR, USH and AC-SC proteins, respectively.

unidentified SOPE sites. The sets of specific sequences in the second type enhancers for the *achaete-scute* genes are different. In particular, the SOPE for *achaete* gene lacks α-boxes (Jafar-Nejad et al., 2003; Ayyar et al., 2007, 2010).

Direct regulators of *achaete-scute* expression: traditional prepattern factors

The spatial expression of the *achaete-scute* genes within the imaginal disc depends on combination of the transcription factors that specify development of macrochaetes at specific positions, thereby determining the bristle pattern geometry. These factors are currently regarded as the corresponding factors postulated by the Stern hypothesis (Stern, 1954, 1968; Gómez-Skarmeta et al., 2003). This set traditionally comprises the proteins of *Iro-c* (ARA, COUP, and MIRR) as well as PNR, USH, and Hairy, directly influencing the *achaete-scute* gene expression (Cubadda et al., 1997; Modolell, Campuzano, 1998). In particular, ARA, COUP, and MIRR drive development in the region that will give rise to the lateral notum and PNR, to the central notum (Tomoyasu et al., 1998; Garcia-Garcia et al., 1999; Calleja et al., 2002). Below, we will briefly consider the structure–function characteristics of the above listed regulators involved in *achaete-scute* expression.

The transcription factors Araucan, Caupolican, and Mirror contain homeodomains and directly bind to the first type enhancers, thereby activating *achaete-scute* expression (Kehl et al., 1998). These three proteins are encoded by the similarly named *Iro-C* genes. Phenotypically, mutations in these genes cause the absence of macrochaetes in the lateral notum. The bristles in the flies carrying such mutations form a characteristic comb, resembling the Iroquois hair dressing, after which they were named.

The *Iro-C* occupies about 130 kb in the genome (Cavodeassi et al., 2001). Expression of the genes *ara*, *coup*, and *mirr* commences at the end of the second instar and considerably increases in the third instar. The regions of *ara* and *coup* expression are completely identical but differ from the region of *mirr* gene expression. The presence of MIRR protein is characteristic of the imaginal disc regions where the proneural clusters will later appear as well as the SOP cells for notopleural and supraalar bristles, while the proteins ARA and COUP are detectable at the sites of the future proneural clusters for the anterior notopleural and posterior postalar bristles (Kehl et al., 1998; Ikmi et al., 2008).

The transcription factors Pannier and U-shaped both belong to the GATA-binding proteins (Ramain et al., 1993; Garcia-Garcia et al., 1999). As has been demonstrated, the protein PNR exists as two isoforms, PNRα and PNRβ. Expression of the corresponding mRNAs is controlled by two alternative promoters. The cells expressing PNR may contain either one or both isoforms, the ratio of which depends on USH, since the heterodimer PNRβ/USH has a negative effect on PNRα expression (Fromental-Ramain et al., 2008). The ratio of these isoforms also to a considerable degree determines the transcription activity of *achaete-scute* genes. It has been shown that PNRβ activates transcription, whereas PNRα/USH inhibits it (Fromental-Ramain et al., 2008, 2010). Figure 2 schematizes these interactions.

The regions of *pannier* and *u-shaped* gene expression in the imaginal disc partially overlap, that creates different conditions for the *achaete-scute* functional state and, consequently, for the macrochaete development within these regions, depending on the contents of the corresponding proteins (Modolell, Campuzano, 1998; Sato, Saigo, 2000).

Recent data provides more details for the role played by PNR in the regulation of *achaete-scute* gene expression. These data demonstrate that a certain protein complex containing several proteins along with PNR (in particular, SSDP (sequence-specific single-stranded DNA-binding protein) and Chip (Ramain et al., 2000; Bronstein et al., 2010) acts as the activator in question (find more details below).

The transcription factor Hairy contains a bHLH domain to bind to the N box CACGCG in the regulatory regions of its target genes, thereby prohibiting its transcription (Rushlow et al., 1989; Ohsako et al., 1994; Gómez-Skarmeta et al., 1995). Mutations in the gene *hairy* induce development of additional bristles (Ingham et al., 1985; Skeath, Carroll, 1991). As has

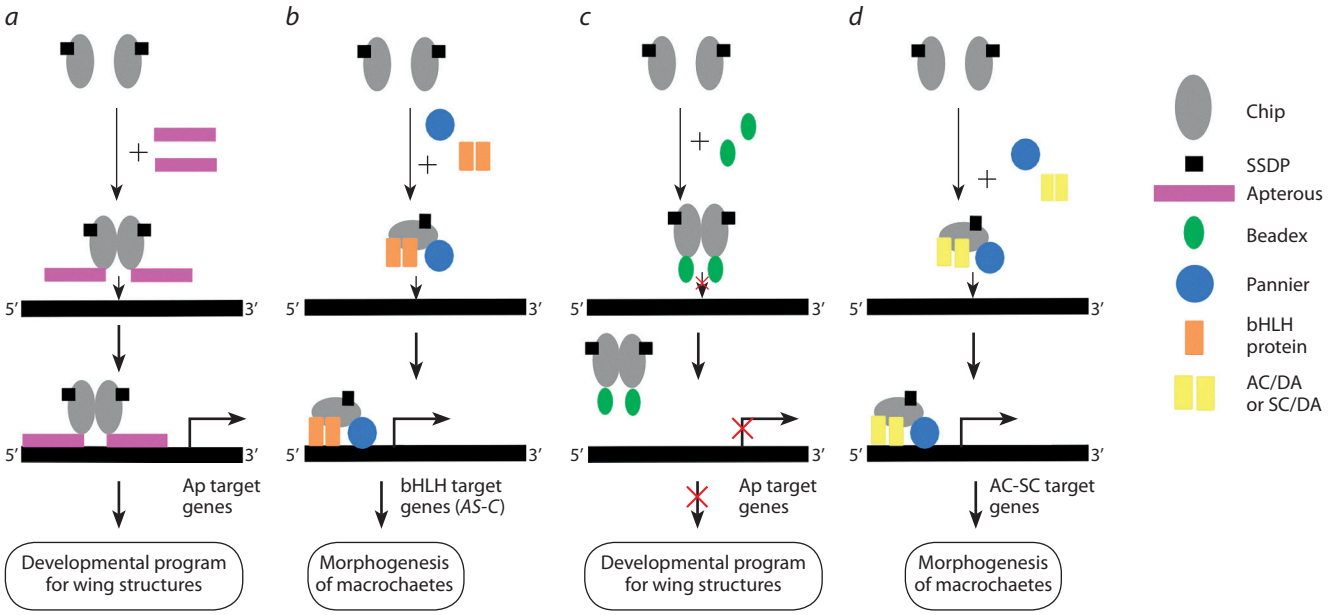

Fig. 3. Roles of the complexes containing SSDP, Chip, bHLH proteins, PNR, AP, and BX in determination of the cell fate (based on Bronstein et al., 2010). For details, see body text.

been experimentally shown, Hairy directly represses transcription of the *achaete-scute* genes; however, a binding site for this factor has been so far detected only in the regulatory region of the *achaete* gene (Wainwright, Ish-Horowicz, 1992; Ohsako et al., 1994; Gómez-Skarmeta et al., 1995, 2003; Costa et al., 2014).

Prepattern factors: new players

Recently, new data have been obtained on the proteins and protein complexes that bind to regulatory regions in the *achaete-scute* genes and influence their activity along with the traditional prepattern factors. These new factors include NFκB/Rel family proteins; dCtBP (drosophila C-terminal binding protein) cofactor; the complexes formed by Chip and SSDP; homeodomain-containing proteins Apterous (AP) and Tailup (TUP; synonym, Islet); and the zinc finger domain-containing protein Beadex (BX; synonym, dLMO, Drosophila LIM-only).

The proteins and protein complexes involved in a direct regulation of the *achaete-scute* gene expression

The NF-κB/Rel family proteins are considered to play an important role in the *achaete-scute* expression pattern. Three drosophila proteins belonging to this family have been identified, namely, Dorsal (DL), Dorsal related immunity factor (DIF), and Relish (Rel). They influence the *achaete-scute* expression both directly binding to the α-boxes in the *achaete-scute* regulatory regions responsible for transcription initiation and via posttranscriptional interactions with achaete-scute mRNA altering its stability and translation efficiency. There are the data demonstrating that a low content of the NF-κB/Rel family proteins in combination with a high level of Achaete-Scute (AC-SC) proteins triggers a neural fate of the cell, whereas a high level of NF-κB/Rel proteins at a low level of AC-SC proteins, on the contrary, excludes this developmental direction (Ayyar et al., 2007, 2010).

The corepressor dCtBP forms a complex with the heterodimer USH/PNR; this complex represses the *achaete-scute* gene transcription. The flies carrying a mutant *dCtBP* gene develop additional bristles, which correlates with the presence of additional SOP cells in proneural clusters (Stern et al., 2009).

The complexes obligatory containing Chip and SSDP play a special part in development of the stereotype bristle pattern; these complexes function in different imaginal disc compartments and at different stages of macrochaete development. These complexes are represented by three types: the first type comprises the complexes that contain bHLH proteins (including AC-SC and DA) and PNR along with Chip and SSDP; the second type complexes involve AP or TUP; and the third type contains BX (Ramain et al., 2000; Chen et al., 2002; Matthews, Visvader, 2003; de Navascués, Modolell, 2007; Zenvirt et al., 2008; Bronstein et al., 2010). Each component in these complexes fulfills its own function. According to the latest data, Chip acts as an adapter and forms the background for assembly of the complexes by recruiting proteins of various families; bHLH proteins, AP, and TUP provide for site specificity of these complexes in binding to DNA; PNR is responsible for reinforcing the interaction between enhancer and promoter; and SSDP acts as a transcription activator. The schemes for assembly of such complexes involving the listed proteins and their roles in determination of cell developmental fate are shown in Fig. 3.

The 2Chip/2AP/2SSDP heterohexamer initiates expression of the AP target genes with subsequent activation of the programs that provide for development of the wing structures (see Fig. 3, *a*). The Chip/SSDP/PNR/2bHLH pentamers are necessary for establishment of the presumptive notum in the imaginal disc (see Fig. 3, *b*). It is known that the regions of *apterous* and *pannier* gene expression in the disc partially

overlap, so that the cells localized to the overlapping region contain both types of complexes, 2Chip/2AP/2SSDP and Chip/SSDP/PNR/2bHLH, thereby being potentially able to form both wing and notum structures. The alternative developmental program is selected with involvement of the protein BX, playing the part of a kind of switch (Matthews, Visvader, 2003; Bronstein et al., 2010). In the cells containing Beadex, AP is displaced from 2Chip/2AP/2SSDP to give a new complex, 2Chip/2BX/2SSDP. Since BX is incapable of binding DNA, such a complex is unable to provide transcription of the AP target genes, thereby preventing formation of the wing structures (see Fig. 3, *c*). A finer structuring of the presumptive notum involves the complexes Chip/SSDP/PNR/2bHLH. By activating the *achaete-scute* genes, they determine the positions of proneural clusters in the central notum (see Fig. 3, *b*). In the cells of these proneural clusters, AC-SC proteins form the multimers Chip/SSDP/PNR/AC/DA or Chip/SSDP/PNR/SC/DA, which initiate transcription of the AC-SC target genes and create the conditions for these cells to follow a neural developmental pathway (see Fig. 3, *d*) (Bronstein et al., 2010).

The heterohexamer 2Chip/2TUP/2SSDP influences the *achaete-scute* transcriptional activity. In the cells of the future proneural clusters for dorsocentral macrochaetes, this complex binds to the *achaete-scute* DC enhancer and activates *achaete-scute* transcription (van Meyel et al., 1999; Biryukova, Heitzler, 2005; de Navascués et al., 2007). Thus, the effects of the complexes 2Chip/2TUP/2SSDP and Chip/SSDP/PNR/2bHLH in these regions of the imaginal disc are analogous. Since the TUP expression is observed in a narrower region as compared with PNR, it is assumed that TUP more finely specifies the positions of proneural clusters. As has been shown, the presence of the TUP protein at the sites for future proneural clusters for the remaining macrochaetes blocks emergence of additional SOP cells within the cluster. Two mechanisms underlying this effect are considered, namely, inhibition of *achaete-scute* expression via the TUP interaction with transcription activators or repression of the AC-SC target genes (de Navascués, Modolell, 2010).

The proteins indirectly influencing the *achaete-scute* gene activity

Along with the above listed transcription factors that have binding sites in the regulatory regions of *achaete-scute* genes, a set of proteins also influences the *achaete-scute* expression in an indirect manner. This set includes the proteins Toutatis (TOU) and Osa, transcription factors Bar (BarH1 and BarH2) and WG, histone acetyltransferase Chameau (CHM), kinase Shaggy (SGG), as well as the proteins of EGFR signaling pathway.

The proteins Toutatis and Osa modulate *achaete-scute* gene transcription by interacting with the complexes containing Chip and PNR. It is known that Toutatis increases transcription, whereas Osa decreases it. These proteins are believed to be involved in chromatin remodeling, entailing the changes in the efficiency of enhancer–promoter interaction (Heitzler et al., 2003; Vanolst et al., 2005).

The homeodomain-containing proteins BarH1 and BarH2 are necessary for development of the presutural macrochaetes (see Fig. 1). These proteins are encoded by similarly named adjacent genes of the small complex *Bar* (Higashijima et al., 1992). Their expression is controlled by the DPP and WG. Experiments have demonstrated that the Bar proteins are involved in *achaete-scute* activation (Sato et al., 1999); however, their direct interaction with the regulatory regions of *achaete-scute* genes has not been demonstrates so far.

WG is a negative regulator for the *achaete-scute* genes. The role of factor consists in expression activation of the gene *shaggy*. The produced Shaggy kinase phosphorylates PNR, which, being phosphorylated, is unable to bind to the enhancers of the first type and loses its function of a direct activator for *achaete-scute* gene transcription (Yang et al., 2012).

The acetyltransferase Chameau is another experimentally confirmed indirect negative regulator for the *achaete-scute* genes. As has been shown, *chm* genetically interacts with *ush*, *chip*, and *pnr*. Presumably, CHM may be involved in the activation of downstream targets of AC and SC in the formed proneural clusters (Hainaut et al., 2012).

The zinc finger transcription factors Spalt (SAL) and Spalt-related (SAL-R) are required in the presumptive notum when the future proneural clusters for the majority of macrochaetes are determined (including the dorsocentral, scutellar, and notopleural macrochaetes). The genes *sal* and *sal-r* are united together and have a complex regulatory region, one part of which controls *sal*/*sal-r* expression in the corresponding regions of the imaginal disc. Transcriptional activity of these genes is controlled by the proteins DPP and WG. The proteins SAL and SAL-R repress the *Iro-C* transcription, which entails prohibition of *achaete-scute* gene activation (de Celis et al., 1999; de Celis, Barrio, 2000; Sweetman, Münsterberg, 2006).

Proteins of the EGFR (MAP kinase) signaling pathway are involved in the establishment of presumptive bristle pattern; this pathway is initiated by two of the known ligands for this receptor, Vein (VN) and Spitz (SPI). In both cases, the result is transcription of the *achaete-scute* genes. The MAP kinase cascade triggered by the EGFR interaction with VN acts as an indirect regulator of the *achaete-scute* gene expression: first the *Iro-C* genes are transcribed, and then the proteins ARA, CAUP, and MIRR of this complex activate the *achaete-scute* genes (Wang et al., 2000; Zecca, Struhl, 2002; Letizia et al., 2007). The initiation of *achaete-scute* transcription when the signal is transmitted via the SPI ligand does not require any intermediate step, and EGFR acts as a direct regulator of the *achaete-scute* expression (Culi et al., 2001).

Conclusions

Development of the bristle pattern is a hierarchically organized process, where establishment of the prepattern, which determines positioning of adults bristles on the body of imago, is the most important and basic stage. According to the current concepts, prepattern is actually the combination of transcription factors characteristic of certain imaginal disc regions triggering and regulating expression of the *achaete-scute* genes. A developmentally final establishment of the prepattern takes place at the third instar larval stage. In turn, the main prerequisite for this is the difference in the cells forming the imaginal disc in the distributions of certain protein factors, which is determined by concentration gradients of the proteins encoded by segmentation genes and the morphogen DPP.

The general scheme illustrating the work of the system that determines the bristle pattern development is shown in Fig. 4.

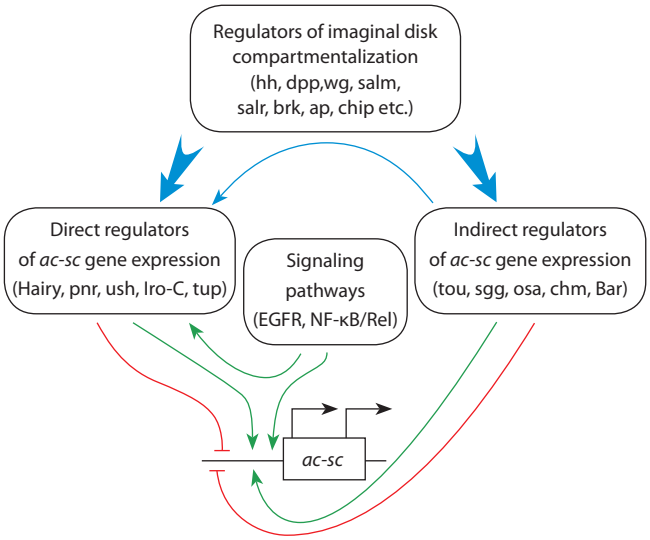

Fig. 4. The general scheme illustrating the work of the molecular genetic system that determines development of bristle pattern.

Designations: blue arrows indicate interactions between different regulator groups; green and blunt-end red arrows denote activating and repressive regulatory effects on the *ac-sc* expression, respectively.

A full-fledged adult bristle pattern is developed only in the case of coordinated functioning of the prepattern and the system responding to prepattern, the *achaete-scute* genes. The main factors of the prepattern directly regulating the *achaete-scute* expression are the proteins USH, PNR, ARA, COUP, MIRR, and Hairy as well as proteins belonging to the NF-κB/Rel family and EGFR signaling pathway.

Part of these proteins (HH, DPP, WG) act at early stages of imaginal disc compartmentalization, determined the expression of *brinker*, *apterous*, *chip*, *dCtBP*, *pannier*, *u-shaped*, *spalt* and *spalt-related* genes which proteins "specifies" compartmentalization of the imaginal disc. The other part (proteins of EGFR signaling pathway, ARA, COUP, MIRR, etc.) interacts with the corresponding enhancers to initiate expression of the *achaete-scute* genes, thereby determining the positions of proneural clusters.

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Conflict of interest

The authors declare no conflict of interest.

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