
REVIEWS AND
THEORETICAL ARTICLES

Drosophila melanogaster as a Model for Studying the Function of Animal Viral Proteins

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Abstract—Studies in which *Drosophila melanogaster* individuals carrying transgenes of animal viruses were used to analyze the action of animal viral proteins on the cell are reviewed. The data presented suggest that host specificity of viruses is determined by their proteins responsible for the penetration of the virus into the cell, while viral proteins responsible for interactions with the host cell are much less host-specific. Due to this, the model of *Drosophila* with its developed system of searching for genetic interactions can be used to find intracellular targets for the action of viral proteins of the second group.

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INTRODUCTION

Drosophila melanogaster is a well studied model organism containing (according to the *Drosophila* genome project) 10000–12000 genes [1]. Many of these genes are similar to genes of other animals as well as to human genes. It was found that 70% of *Drosophila* genes have human homologs [2] and 75% of human disease-associated genes have homologs in *Drosophila* [3]. Irrespective of currently available large collections of mutant strains, it becomes more convenient to study the function of *Drosophila* genes with the use of rather elaborate transgenic constructs [4]. The system of ectopic expression of *Gal4-UAS* genes consists of two components: a driver (P element containing the yeast Gal4 protein coding region under the control of the genomic enhancer located close to the integrated element) and a target transgene under the control of the Gal4-specific promoter UAS (upstream activated system) [5]. *Drosophila* has a set of drivers (*hs-Gal4*) able to activate UAS transgenes in various tissues at different stages of development. There is also a possibility to activate the tissue-specific transgene conditionally using an external stimulus and even to form a feedback loop for autoactivation of the transgene (*UAS-Gal4*). These possibilities, initially developed for genetic analysis of *Drosophila* genes, can also be used to analyze the functions of foreign genes.

Viral genomes can maximally encode four to several hundred proteins (the genome of *Mimivirus* is 1.2 megabases in size and contains 1262 reading frames corresponding to 911 proteins [6]), although most typical is the influenza virus encoding 11 proteins [7]. Viral genomes encode viral envelope proteins

responsible for interactions of the virus with the host cell at the stage of penetration into the cell. At the same time, it is commonly known that in addition to these proteins serving the structural function viral genomes contain genes responsible for finer aspects of interaction with host cells: cell proliferation (in fact, isolation of oncogenes was made on the basis of this property) and cell apoptosis. In this work, we review the results and methodical approaches of studies in which *Drosophila* was used to analyze human viral infections.

PIONEER BACULOVIRUS STUDIES

Drosophila was successfully used to study viral genes and proteins inhibiting programmed death (apoptosis) of host cells. Animal viruses are known to carry genes able to prevent apoptosis of host cells [8–16]. On this basis, Hay et al. [17] cloned the gene *P35* of the *Autographa californica* baculovirus in the pGMR (glass multimer reporter) vector able to provide transgene overexpression in the eye. The effect of transgene overexpression in embryogenesis was examined in individuals containing an insertion of this vector and additionally carrying the *hs-glass* transgene capable of activating the pGMR transgene in all tissues under the action of heat shock. Heat shock induction in such individuals resulted in the lack of apoptosis in late embryos, while it is normally observed in wild-type individuals [18]. To study apoptosis of eye cells, activation of the pGMR transgene was used. Inhibition of apoptosis characteristic of normal development was demonstrated on the level of eye morphology of an adult individual and on the imaginal disc level. Inhibi-

tion of apoptosis induced by X-irradiation was also demonstrated. At present, constructs based on *P35* serve as a commonly accepted test for classifying cell death as apoptosis.

IMMUNODEFICIENCY VIRUS

Drosophila was successfully used to study HIV infection. The authors of the work [19] cloned the *HIV-tat* gene in the pCasPeR vector containing the hs promoter capable of heat shock activation of transgene expression in all tissues. Activation of the transgene in oogenesis resulted in an essential increase (from 1 to 10%) of the share of eggs with the phenotype of fused dorsal appendages. The authors found that the Tat protein affects the localization of the Gurken and Kinesin proteins, i. e., it alters both the dorsoventral and anteroposterior axes of embryos. Activation of transgene expression in spermatogenesis showed colocalization of Tat with alfa-tubulin. Furthermore, the authors established in an in vitro experiment that Tat inhibits the assembly of microtubules. The study of the viral protein in accordance to the commonly accepted scheme showed that it functions in the process of viral RNA translation [20]. The study with the use of the *Drosophila* model made it clear that in addition to this function the protein is also able to change the cell structure.

To study the role of the *HIV-nef* gene in HIV infection, Lee et al. [21] cloned DNA of this gene and DNA of its mutant form (G2A substitution altering the myristoylation site of the protein) into the pUAST vector and activated the transgene with the use of drivers *1096-Gal4* and *ap-Gal4* in wing imaginal discs. Expression of the wild-type gene led to abnormalities in the wing shape, while no abnormalities were observed in the mutant. Using BrdU incorporation and detection of mitoses with the help of antibodies against the phosphorylated H3 histone form, it was found that ectopic expression of *HIV-nef* itself and its mutant form had no influence on wing cell proliferation. Yet, ectopic expression increased the level of apoptosis, which was seen with the use of both acridin-orange detection and antibodies against *Drosophila* Drice caspase. The authors found that genes involved in the JNK (June N-terminal kinase) signaling pathway (*bsk*, *hep*) genetically interact with *HIV-nef* (both UAS constructs and point mutations) under conditions of overexpression, and the activity of the *puc* gene, which is a target for the action of *bsk*, is enhanced in the presence of an additional amount of the *HIV-nef* gene product. Since the activity of the JNK signaling pathway in *Drosophila* is characterized by a complicated spatial regulation (for example, dorsal and thorax closures regulated by JNK are complicated events of morphological alterations of an embryo or a growing pupa), the authors also checked cellular autonomy of the action of *HIV-nef* with the help of the mosaic analysis. Then the authors established that *HIV-nef*, but not its mutant form, inhibits expression of the antimicrobial peptide Dpt of *Drosophila*. The protein Relish (transcription factor NF- κ B) of *Drosophila*, which

controls Dpt transcription, is translocated from the cytoplasm into the nucleus in the case of microbial infections. *HIV-nef* expression inhibits this change in the localization of Relish. The authors also studied the question as to whether inhibition of *Drosophila* immunity is JNK-dependent and showed that the effects of *HIV-nef* on immunity and on JNK are independent of one another. Thus, despite a small length (206 a.a.), this viral protein has two different targets in animal cells.

Leulier et al. [22] cloned cDNA of the *HIV-vpu* gene and its mutant form (Ser52 and Ser56 substituted by Arg), which does not permit phosphorylation of the product, into the pUAST vector. The transgene was expressed with the use of the *yolk-GAL4* driver activating expression in the fat body. In human cells, HIV-vpu is found in the phosphorylated and nonphosphorylated forms. After treatment with alkaline phosphatase, the difference in the mobility of the proteins disappears. The authors showed that in *Drosophila* cells the protein behaves analogously, i. e., it can be phosphorylated. This fact by itself is a strong argument in favor of using *Drosophila* as a model object for studying viral infections. In *Drosophila*, responsible for resistance to fungal infections is the Toll signaling pathway controlling expression of the Drs and Mtk peptides [23]. Induction of these peptides is essentially disturbed in individuals with HIV-vpu expression, but is not disturbed in the nonphosphorylatable mutant. In support of this, the authors showed that the dominant *TI^{10B}* mutation of the Toll signaling pathway, which causes constitutive activation of Drs and Mtk, does not produce this effect against the background of Vpu overexpression. At the same time, the authors found that the resistance to gram-positive bacteria associated with the Imd signaling activity does not change in the case of HIV-vpu overexpression.

The data on the ability of the *Drosophila* model to reveal new aspects of HIV–host cells interactions were considered by Sprester and Carlson [24]. The authors proposed to study additionally genes *CCR3*, *Vpr*, *CA150*, *Pol-gamma*, *GL1*, *P-TEFb*, and *Spt-4*, -5, -6, since they have homologs in the *Drosophila* genome and may well be of interest for studying HIV infections.

CORONAVIRUS

Drosophila was also used as a model object for studying infections caused by other viruses. To study the functional role of the *3a* gene of the SARS (Severe Acute Respiratory Syndrome) coronavirus encoding a 274-a.a. protein (expression of the protein was confirmed for patients infected by this virus), a transgenic *Drosophila* strain containing a genomic insertion of the gene of this protein in the pUAST vector was generated in the work [25]. Ectopic expression of the 3a protein in *Drosophila* under the action of the eye driver *GMR-Gal4* caused the dominant phenotype of the eyes. It was found that ectopic expression of 3a influences the vesicular cell traffic and induces cell apoptosis that can be modulated by the level of cellular cyto-

chrome C and by the activity of caspases destroying cellular structures during apoptosis. As shown by genetic screening, the phenotype resulting from ectopic expression is modified by heterozygous mutations at 78 loci of *Drosophila*. Thus, the use of transgenic *Drosophila* individuals was proved to be efficient for studying the function of viral genes.

EPSTEIN–BARR VIRUS

The Epstein–Barr virus causes infectious mononucleosis, lymphomas, and nasopharyngeal carcinoma. The BZLF1 protein functions as a transcriptional activator of expression of early genes of the virus and controls the transition from the latent form to the lytic form. BZLF1 also interferes with the cell cycle regulation, intercellular signaling, and transcription. To study the function of BZLF1, Adamson et al. cloned cDNA of the gene into the pGMR vector [26]. The authors also cloned cDNA of the mutant form of the *A185L* gene, which alters the DNA-binding domain of BZLF1 and leads to an experimentally recorded binding defect. Several insertions differing in the level of expression of GMR-BZLF1 and UAS-BZLF1 transgenes were obtained.

The authors showed that insertions of the constructs obtained into the *Drosophila* genome bring about morphological defects in the *Drosophila* eye as a result of the disappearance of ommatidia. Morphological defects of the eye for normal and mutant BZLF1 did not differ, thus confirming that the viral protein acts not through transcription regulation, as was expected from the molecular function of the protein, but in some other manner [26]. The study of cell divisions in transgenic individuals showed a reduction in the number of dividing cells in the second mitotic wave in the eye imaginal disc and an intensification of cell apoptosis. This is in good agreement with the data obtained for human cells that demonstrate a cell cycle arrest under the action of BZLF1.

Then Adamson et al. [26] checked, using antibodies against the *Drosophila* eye protein Elav, whether BZLF1 acts on differentiating photoreceptors of the eye. It turned out that the protein had no influence on the cells of the photoreceptors at this, later, stage. In addition, the authors activated the UAS-BZLF1 transgene with the use of the *sev*-Gal4 driver (it acts later than *elav*) and confirmed the lack of the eye phenotype. It was further found that in the case of BZLF1 overexpression the eye conic cells lose Cut protein expression. The protein Shaven (encoded by the *sv* gene) normally activates the expression of *cut* in these cells by binding with its promoter. Using anti-Shaven antibodies, the authors demonstrated that BZLF1 expression affects the localization of Shaven and the level of its expression. The authors assumed that the virul protein influences the process of Cut transactivation with the help of Shaven. By comparing the eye morphology in *sv/+* and *BZLF1/+; sv/+* individuals,

the authors revealed genetic interactions between *sv* and *BZLF1*. On the basis of this finding, the authors proceeded to studying human homologs of Shaven: proteins Pax2, Pax5, and Pax8. Since the Epstein–Barr virus infects B cells of the human immune system, in which Pax5 is functional, rather than the Pax2 and Pax8 homologs, the authors tried to elucidate whether the proteins BZLF1 and Pax5 form a complex. It was found that these proteins are able to form a complex and that the formation of a complex causes binding of BZLF1 with mitotic chromosomes. Thus, the authors of this work succeeded in discovering and characterizing the human virul protein with the employment of the methods of molecular genetics of *Drosophila*. The results obtained and the existence of homology between the human and *Drosophila* proteins enabled the authors to return to studying the action of the human proteins.

OTHER VIRUSES

Embryonic infection by the human cytomegalovirus (HCMV) leads to microencephalia, microgyria, and hydrocephalus [27]. Transgenic constructs were obtained in the work [28], of which one contained the Major Immediate Early promoter (MIEP) of HCMV attached to the coding region of the YFP reporter. Another construct contained two viral genes, *IE72* and *IE86*, under the control of the *hs* promoter of the pCasPeR vector. The promoter activity was detectable only in early embryos at the stage of blastoderm. This fact agrees well with the data on the expression of this promoter in mouse embryos and is a strong argument in favor of using *Drosophila* as a model object for studying viral infections. Moreover, the viral proteins under study were found to be localized in the nucleus. Such a localization is expected for transcription factors. Overexpression of the construct containing viral genes leads to the death of 20–30% of embryos with a phenotype called by the authors “fragmented embryo.” Normally, DE-cadherin in *Drosophila* binds to the Arm protein. Both proteins are major components of intercellular contacts (adherence junctions). While the localization of DE-cadherin in transgenic embryos overexpressing a cassette of viral transgenes remains unchanged, the pattern of Arm expression representing a system of bands changes up to becoming uniform. The authors concluded that the viral transgenes alter the composition of adherence junctions in embryos.

CELLULAR TARGETS FOR THE ACTION OF VIRUL PROTEINS

The rabies virus, able to infect different mammals, is considered to have the widest range of hosts among animal viruses. At the same time, the action of many animal viruses is species-specific [29]. The question arises as to whether the range of hosts is limited by genes determining the penetration of the virus into the cell or whether viral genes responsible for virus–host interactions also

make a contribution to this limitation. The data considered in this review show that viral genes of the latter group can act on a broad diversity of organisms, including *Drosophila*. In particular, the Vpu protein of HIV can be phosphorylated in *Drosophila* cells, and the cytomegalovirus promoter (MIEP) functions only in embryonic tissues of *Drosophila*, which coincides with the phase of its action in mammals (see above). These facts cannot be accidental, and they indicate that the class of viral genes responsible for the interaction of the virus with host cells is similar to house-keeping genes that perform the same functions in all animals. This means that host specificity is mainly a result of species-specificity of proteins responsible for the penetration of the virus into the cell.

The range of intracellular targets for genes of the second type is rather wide: cell apoptosis, assembly of microtubules, JNK signaling, cellular immunity, intercellular contacts, and vesicular traffic. It is hard to imagine that this class of viral proteins influencing all this diversity of cell functions occurs in viruses de novo. It is most likely that genes of this type are adopted by a persisting virus from the host genome, as can be often seen from homology of the primary structures. On the other hand, the study of the functions of viral proteins in *Drosophila* can help in revealing the units of regulation of intracellular processes that have not yet been identified in direct studies.

Recall that Adamson et al. [26] succeeded in discovering and characterizing the human virul protein by the methods of molecular genetics of *Drosophila*, and, proceeding from the results obtained and on the basis of homology of the human and *Drosophila* proteins, they could return to studying the action of the virus on the human protein. Thus, *Drosophila* can be used as an intermediate object for studying human viral infections. What tasks can be solved at this stage? First, ectopic expression of transgenes in *Drosophila* is possible at any stage of development and in any tissue. This permits one to find a tissue and a developmental stage having targets for the action of viral proteins. Of course, there are other objects more similar to humans (e. g., mouse) that can be used in such studies, but the generation of a transgenic mouse is incomparable in costs with the generation of a transgenic *Drosophila* fly. Second, *Drosophila* has an elaborate system of genetic screenings for discovering genetic interactions. Cases of application of this approach can be found in the works [21, 25]. Genetic methods permit a more complete characterization of the spectrum of targets for the action of viral proteins. They also make it possible to discover weak interactions that can be revealed only by the modern methods detecting physical binding of macromolecules.

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