International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2015; 2(4): 24-28

Received: 05-10-2015
Accepted: 06-11-2015

Bernardo Pérez-Zamorano
Dirección de Infección e Inmunidad, Centro de Investigaciones sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Av. Universidad 655, Sta. Ma. Ahuacactitlán, Cuernavaca, Morelos, México 62100.

Verónica Valverde-Garduno
Dirección de Infección e Inmunidad, Centro de Investigaciones sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Av. Universidad 655, Sta. Ma. Ahuacactitlán, Cuernavaca, Morelos, México 62100.

Correspondence:
Verónica Valverde-Garduno
Dirección de Infección e Inmunidad, Centro de Investigaciones sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Av. Universidad 655, Sta. Ma. Ahuacactitlán, Cuernavaca, Morelos, México 62100.

Immune challenge induces DNA synthesis and nuclear fragmentation in Aedes aegypti fat body cells

Bernardo Pérez-Zamorano, Verónica Valverde-Garduno

Abstract
Some biological processess require a rapid increase in the production of specific proteins. A temporal or permanent increase in the number of copies of the relevant gene contributes to achive high levels of protein product in short periods of time. Polyploid cells may arise as a result of injury, stress, devolpmental and environmental signals. In insects, the innate immune response is a demanding process that requires the rapid synthesis of antimicrobial effectors. Here we investigated the potential involvement of DNA synthesis in *Aedes aegypti* response to immune challenge. An efficient way to selectively analyze genomic regions undergoing active nucleotide incorporation, is to label DNA *in vivo* while it is being synthetized. First, a reliable, practical technique to achieve *in vivo* BrdU-labelling of culicid DNA was developed. This BrdU feeding method allows *in vivo* incorporation that was detected by Southern blot and immunofluorescence. Both detection techniques confirmed BrdU incorporation into DNA from specific mosquito tissue. Labelling tools described in this work provide an integrative approach for the *in vivo* study of DNA replication and repair related phenomena in the culicid. Here we show that *Aedes aegypti* adult female fat body cells undergo *de novo* DNA synthesis upon injury and that immune challenge triggers DNA synthesis and subsequent formation of a pattern consistent with multiple nuclei in these cells. This multinuclear pattern suggest fat body cells may undergo endomitosis.

Keywords: Aedes aegypti, innate immunity, fat body, DNA replication, immune challenge, in vivo, DNA labelling

1. Introduction
Increasing cell´s DNA content in the absence of mitosis is involved in some key physiological and developmental procesess. In some cases only specific genetic loci are replicated (amplification) [1], in others the whole genome undergoes various rounds of replication in the absence of mitosis. Three mechanisms involving active DNA replication have been described for developmentally programmed polyploidy: acytokinetic mitosis, endomitosis and endoreplication. These mechanisms provide multiple gene copies available for a rapid increase in transcription and translation for specific protein products [1-5]. As a consequence, high protein concentrations are achieved for highly demanding biological procesess. Endoreplication, where a cell undergoes multiple rounds of S phase without entering mitosis, and gene amplification are well documented in *Drosophila melanogaster* [6-8].
Mosquitoes are vectors of a variety of infectious diseases; *Aedes aegypti* in particular, is competent for the transmission of viruses including Dengue virus, chikungunya virus, West Nile virus and Yellow Fever virus. This species is able to transmit these viruses owing to its blood feeding habits. Blood feeding is necessary for egg develoment and the fat body produces massive ammounts of vitellogenin for this process. It has been shown that in *Ae. aegypti* adult female mosquitoes, the fat body undergoes at least two cycles of DNA synthesis (with subsequent polyploidization) [9]. The first cycle of postemergence DNA synthesis occurs up to 72 hrs after emergence. Another cycle of *de novo* DNA synthesis in the adult fat body occurs after a blood meal and is dependent upon juvenile hormone levels [10,11].
In mosquitoes the fat body is also the main innate immune responsive tissue. It contributes to mounting a strong response against invading microorganisms. This response is characterized by induction and release of effector proteins, including antimicrobial peptides, into the hemolymph [12,13]. An efficient mechanism to achive high concentration of proteins is the generation of a large quantities of the corresponding mRNAs [14-17]. One possible way to

generate these mRNAs is to increase the number of copies for each relevant gene through *de novo* DNA synthesis. Therefore we set out to investigate the possible involvement of DNA synthesis in *Ae. agypti* adult fat body response to immune challenge.

To follow active synthesis of DNA, radioactively labelled nucleotides have been used before in *Ae. Aegypti* [9]. More recently the use of 5- bromo -2'- deoxyuridine (BrdU), a synthetic nucleoside analog of thymidine (Fig. 1), has substituted radiactively labelled nucleotides in a diversity of biological systems. It has been particularly useful for *in vivo* studies, extensively used in biology and medicine as a diagnostic tool [18-20]. Because of its chemical structure, BrdU is incorporated easily into DNA, substituting for thymidine during DNA replication or repair. Therefore BrdU can be incorporated into newly synthesized DNA in actively replicating cells. It is commonly used for detection of proliferating cells in living tissues [21]. BrdU-specific antibodies are then used to detect the incorporated chemical, revealing cells that are actively replicating their DNA. Binding of anti-BrdU antibodies to newly synthetized DNA, containg BrdU, requires DNA denaturation in order to expose nitrogenated bases, including BrdU. In *Drosophila* BrdU based experimental techniques for labelling of newly synthesized DNA have been used to analyze endorreplication and gene amplification.

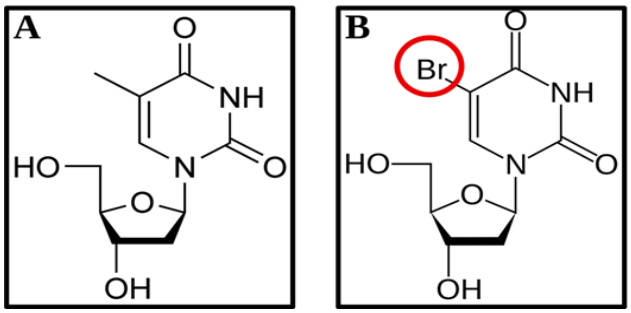

Fig 1: (A) Thymidine and (B) 5-bromo-2'-deoxyuridine (BrdU)

In order to analyse possible involvement of *de novo* DNA synthesis in *Ae. aegypti* fat body innate immune response, we devised a method for *in vivo* DNA labelling. To illustrate the potential utility of this method for other mosquito species, it was also tested on *Anopheles albimanus*. Therefore, for this species, only general BrdU incoporation on genomic DNA was demonstrated by southern blot. This labelling method is based on an *ad libitum* diet from a BrdU containig gel and is decribed in detail in the methods section (see bellow). Here, using such protocol, we show that injury, concommitant with injection of a sterile foreign substance induces *de novo* DNA synthesis in adult female *Ae. aegypti* fat body cells. Furter, we show that immune challenge stimulation leads not only to DNA synthesis, but also induces fat body cells nuclear fragmentation. DNA synthesis reported here is independent from the postemergence cycle previously reported, since we used 5 day old female mosquitoes [9]. It is also independent from the blood feeding induced DNA synthesis cycle, since female mosquitoes were not blood fed in our experiments. These results suggest that *Ae. aegypti* fat body may undergo two distinct types of polyploidization mechanisms in response to injury and immune challenge. We propose that these mechanisms may correspond to endoreplication and endomitosis, respectively.

2. Materials and Methods

2.1 Mosquitoes

Mosquitoes were obtained from already established insectary colonies. Male and female *Ae. aegypti* and *An. albimanus* mosquitoes were obtained from the insectary at the Instituto Nacional de Salud Pública, Cuernavaca, México. Five day-old mosquitoes were used in our assays. They were reared under a photoperiod cycle of 12 h light: 12 h dark, at 28°C and 70–80% relative humidity. They were fed *ad libitum* with 5% sucrose-soaked pad cottons.

2.2 Cell lines and reagents

Yeast and reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated. Cultures of *Aedes albopictus* derived C636 cell line were used as positive controls for active DNA replication.

2.3 In Vivo Bromodeoxyuridine (BrdU) Incorporation

Five day old *Ae. aegypti* and *An. Albimanus* mosquitoes were fed for 48 hours with 5-bromo -2- deoxyuridine (Boehringer Mannheim GmbH, Germany). This was performed using 15ml, 0.5% agarose gels in 60 x 15 mm disposable petri dishes (Corning). Mosquitoes were allowed to feed *ad libitum* from these gels (Fig. 2). Gels contained 8% sucrose, 100 ug/ml BrdU, 200u/ml penicillin, 200 µg/ml streptomycin sulphate and 0.5 µg/ml amphotericin B. Antibiotic and anti-fungal cocktail was added in order to avoid microorganism growth which could affect the DNA replication signal. Feeding was performed in the same conditions aforementioned in section 2.1. In addition, containers holding mosquitoes and BrdU gels were placed inside wet chambers, in order to avoid gel desiccation. Efficient wet chambers were made by placing containers on trays bearing 1 inch of water and setting everything up inside plastic bags. Feeding gels were replaced every 24 hours with freshly made ones. The employed BrdU concentration (100 micrograms/ml) was chosen because it produced the best detection signal *vs* survival rate ratio. BrdU toxicity became an issue at higher concentrations. BrdU incorporation mechanism can cause mutations, and its use is therefore a potential health hazard [22].

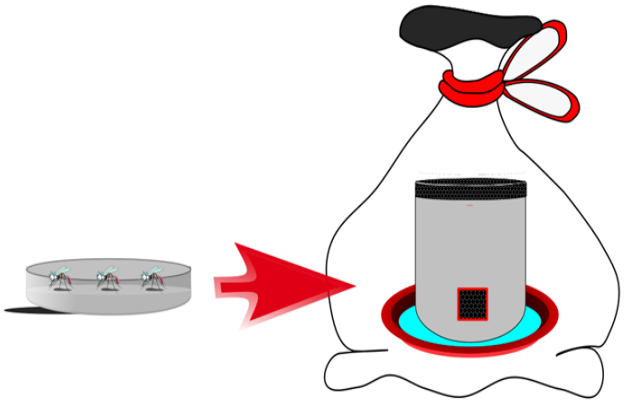

Fig 2. Feeding mosquitoes with BrdU containing gel

Mosquitoes were fed for 48 hours with 5-bromo-2-deoxyuridine (BrdU), using 0.5% agarose gels in 60 x 15 mm disposable petri dishes. Mosquitoes were allowed to feed *ad libitum. G*els' contents: 8% sucrose, 100 ug/ml BrdU, 200u/ml penicillin, 200 µg/ml streptomycin sulphate and 0.5 µg/ml amphotericin B. The containers holding the mosquitoes and

the BrdU gels were placed inside wet chambers in order to avoid gel desiccation.

2.4 Microbial inoculations

Inoculation needles were fabricated from glass capillaries; with an 1.1-1.2 mm internal diameter; by heating them on an open flame in the middle and pulling by the ends to obtain two needles from each capillary. The injection process was performed with the aid of a Drummond Captrol III Microdispenser and a stereoscopic microscope.

Mosquitoes were anesthetized by putting them at 4 ºC for 10 minutes and keeping them on ice during the inoculation process. Each mosquito was injected with 0.25 microlitres of a heat-inactivated *Saccaromyces cerevisiae* suspension, prepared in sterile RPMI 1640 culture medium (Gibco BRL, Grand Island, NY). The suspension had an approximate concentration of 4 x 10^6 cells/ml. Control mosquitoes were inoculated with 0.25 ml of sterile RPMI medium. Inoculations were made through the pleural membrane, between the fourth and fifth abdominal segments [23]. Mosquitoes were allowed to recuperate for 24 hours, period during which they were still fed BrdU.

2.5 DNA extraction and quantification

DNA was extracted from whole mosquitoes with a Zymo Reasearch Tissue & Insect DNA MiniPrep™ kit (Zymo Research, California, USA), and quantified at 230/260 nm in a nanodrop spectrophotometer (NanoDrop 2000, Thermo Scientific).

2.6 Basal BrdU incorporation detection by Southern Blotting

500 ng of each extracted DNA sample from BrdU-fed and non-inoculated mosquitoes (*An. albimanus* and *Ae. aegypti*) were electrophoresed in a 1% agarose gel and transferred to a nitrocellulose membrane. The membrane was incubated at room temperature for 1.5 h with a 1:1,000 dilution of anti-BrdU horseradish peroxidase conjugated antibody (Roche Mannheim GmbH, Germany) in PBS, followed by chemiluminescent detection.

2.7 Mosquito dissections and immunofluorescence analysis

Inoculated and non-inoculated *Ae. aegypti* mosquitoes were dissected, their viscera removed (intestines, ovaries, Malpighian tubules) and their abdomens collected. Each abdomen was split in half to expose each pleural membrane and abdominal fat body. Samples were then subjected to the following immunofluorescence protocol: samples were left overnight in a 3.65% paraformaldehyde solution. The paraformaldehyde was removed afterwards and the abdomens were placed in methanol for 10 minutes; this was followed by 6 washes in PBS Tween 1%. Samples were hydrolyzed in 2N HCl, 1 h at 37 ºC, and then neutralized by three changes of Hank's solution (0.137 M NaCl, 5.4 mM KCl, 0.25 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$, 4.2 mM $NaHCO_3$), each wash lasting 10 minutes. Specimens were subsequently washed three times with PBS- 1%Tween and blocked 1 h at 37 ºC with 2% bovine serum albumin in PBS. They were then incubated at 4 ºC overnight with a FITC-labeled-mouse anti-BrdU monoclonal antibody, 1:100 in PBS - Albumin (Roche, Indianapolis, IN). After removing the antibody solution samples were washed 4 times in PBS- 1% Tween and a 0.025 ug/ml propidium iodide solution was added and left at room temperature for 20 minutes. After rinsing 4 times in PBS- 1% Tween, samples were mounted on slides using 80% glycerol. Fluorescence was recorded using a photo-epifluorescent microscope (Nikon E-600, Tokyo, Japan).

3. Results and Discussion

3.1 Non-stimulated mosquitoes incorporate BrdU into their DNA

Southern blot assays in Fig. 3 show that 100micrograms/ml was the optimal BrdU concentration in order to obtain the best *in vivo* basal DNA labelling (no immune challenge). Both male and female mosquitoes of the *Ae. aegypti* (Fig. 3A) and *An. albimanus* (Fig. 3B) species incorporated BrdU in their DNA. This concentration produces a clean and reproducible signal in both male and female mosquitoes, without any saturation that could interfere with further analyses. As it can be appreciated in the images (Fig. 3), female mosquitoes show a distinct higher chemiluminescent signal, implying that there is a higher BrdU incorporation in their DNA. This comes as no surprise since BrdU could be incorporated in the ovaries. It is known that in some strains of *Ae. aegypti*, the autogeny phenomenon can occur, which is the ability to start the first gonadotropic cycle without ingesting blood [10]. Also, BrdU incorporation into, both, female and male DNA in the absence of any stimulli could be a consequence of genome maintence processess such as DNA repair mechanisms.

Our *in vivo* labelling protocol shows improvement over other published techniques. First, we found that feeding time with BrdU for proper labelling could be reduced to two days. The BrdU signal proved to be clear and reproducible after this time. In contrast, previously reported methods required feeding on BrdU for four days, hence reducing the time left to carry out further *in vivo* experimental treatments [24]. Also, the previously reported BrdU concentration of 1mg/ml for DNA labeling [24] created some difficulties. In our assays this concentration produced more than 80% mortality rate in BrdU fed, non-stimulated mosquitoes. In contrast, here, a concentration of 100 micrograms per millilitre of BrdU was used, sharply increasing mosquitoes survival. Therefore, the protocol developed in this work is efficient in the use of the main reagent, reduces time required for effective labelling, increases mosquito survival and is reproducible.

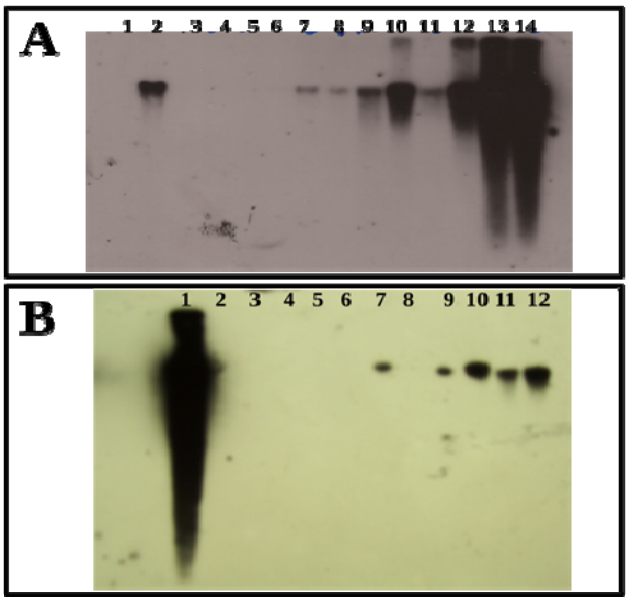

Fig 3: BrdU is incorporated into mosquito genomic DNA. (A)

Ae. aegypti genomic DNA, 1: control; 2: + control; 3: Male (M) 0 mg/ml BrdU ; 4: Female (F) 0 mg/ml BrdU; 5: M 0.001 mg/ml BrdU; 6: F 0.001 mg/ml BrdU; 7: M 0.01 mg/ml BrdU; 8: F 0.01 mg/ml BrdU; 9: M 0.1 mg/ml BrdU; 10: F 0.1 mg/ml BrdU; 11: M 0.2 mg/ml BrdU; 12: F 0.2 mg/ml BrdU; 13: M 0.5 mg/ml BrdU; 14: F 0.5 mg/ml BrdU. (B) *An. albimanus* genomic DNA, 1: + control; 2: - control; 3: Male (M) 0 mg/ml BrdU; 4: Female (F) 0 mg/ml BrdU; 5: M 0.001 mg/ml BrdU; 6: F 0.001 mg/ml BrdU; 7: M 0.01 mg/ml BrdU; 8: F 0.01 mg/ml BrdU; 9: M 0.1 mg/ml BrdU; 10: F 0.1 mg/ml BrdU; 11: M 0.2 mg/ml BrdU; 12: F 0.2 mg/ml BrdU.

3.2 Injury and immune challenge induce BrdU incorporation into fat body cells DNA

Our results show that *Ae. aegypti* mosquitoes inoculated with sterile RPMI incorporate BrdU in the nuclei of their fat body cells (Fig. 4 A). Staining fat body tissue DNA with propidium iodide showed that incorporated BrdU co-localizes with nuclear DNA. This confirms that BrdU is incorporated into nuclear DNA, indicating that there is active *in vivo* DNA synthesis in these cells. This activity could be induced by trauma (injection wound) or by the introduction of a foreign substance (RPMI). Incorporation of BrdU in fat body cells from BrdU fed, non stimulated mosquitoes (not injected) was not observed.

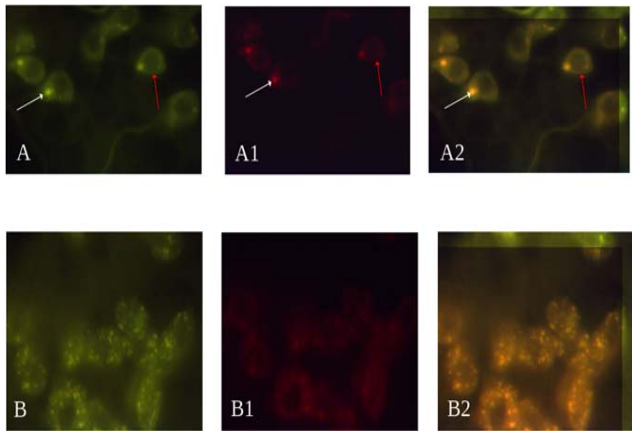

Fig 4: Immunofluorescence analysis of fat body cells from BrdU fed and immune challenged mosquitoes

Ae. aegypti mosquitoes inoculated with RPMI and yeast. In the two series of panels body fat cells from the abdomen are observed. Epifluorescence image: A and B, cell staining with anti-BrdU FITC antibody; A1 and B1, propidium iodide staining; A2 and B2, overlapping FITC and propidium iodide staining images. The A panels show cells of mosquitoes inoculated with RPMI, the B panels consist of cells from mosquitoes inoculated with a *S. cerevisiae*. White arrows indicate fat body cells nuclei and red arrows indicate central vacuoles.

Fat body cells from heat-killed yeast innoculated mosquitoes revealed a distinct pattern of multiple small BrdU containing bodies, scattered through cells´ cytoplasm (Fig. 4 B). Propidium iodide staining of the same tissue samples shows the presence of DNA in these bodies. These results confirm co-localization of incorporated BrdU with DNA in fat body cells of immune challenged mosquitoes. The asbsence of an apparent single nucleus and the presence of multiple small bodies containg DNA and BrdU suggest the possibility of nucler fragmentation in these cells. This pattern may suggest the kind of nuclear fragmentation which occurs in the final stages of endomitosis. Previous studies have reported *de novo* DNA synthesis in *An. Albimanus* upon immnue challenge in *ex-vivo* cultured organs [24,25]. In contrast, our study shows that there is *de novo* DNA synthesis *in vivo* in *Ae. aegypti* fat body cells, upon immune challenge. This *in vivo* synthesis results in a distinct pattern, consistent with a multinucleated fat body cell. This distinct pattern may potentially arise by nuclear fragmentation from an endomitosis process in this tissue. An additional key feature of this *de novo* DNA synthesis, occuring *in vivo* upon immune challenge, is that it occurrs independently of the postemergence and blood feeding induced DNA synthesis cycles [9].

4. Conclusion

The evident *in vivo* incorporatin of the nucleotide analog BrdU into *Ae. aegypti* fat body cells DNA, upon immune challenge, indicate that these cells enter active DNA synthesis. This shows that methods developed in this work allow for an efficient *in vivo* labelling of DNA molecules under active *de novo* synthesis. We anticipate that this set of tools would be useful for further studies on DNA replication and repair-related phenomena *in vivo* in culicides. The BrdU intake protocol described here represents a non-invasive, low-toxicity process, that improves mosquito survival with efficient DNA labelling. It also reduces potential physiological stress that could interfere with further experimental stimuli. Two distinct patterns of newly synthetised DNA were found in mosquito fat body cells. The first pattern, induced by injury and injection of strile RPMI into the hemocele, consisted of fat body cells with a single nucleus, where *de novo* DNA synthesis was evident by the presence of incorporated BrdU. The second pattern, induced specifically by immune challenge, consisted of the presence of multiple newly synthesiszed DNA containing bodies where BrdU was incorporated. These two patterns of newly synthetised DNA, discovered here in fat body cells, may correspond to at least two distinct polyploidization mechanisms. We suggest the first pattern may arise by endoreplication, and the second, triggered by immune challenge, may arise by endomitosis. Alternatively the first pattern may represent an intermediate phase of the endomitosis process, arising from a less stressful challenge. Results presented here warrant further studies to dilucidate molecular mechanisms involved in DNA synthesis triggered by immune challenge in *Ae. aegypti* abdominal fat body cells.

5. Acknowldgements

Funding was provided by CONACYT, México, grant 62389 and grant 84012.

6. References

1. Edgar BA, Orr-Weaver TL. Endoreplication cell cycles: more for less. Cell 2001; 105(3):297-306.
2. Zielke N, Edgar BA, DePamphilis ML. Endoreplication. Cold Spring Harb Perspect Biol, a012948, doi: 10.1101/cshperspect.a012948 2013; 5(1).
3. Buntrock L, Marec F, Krueger S, Traut W. Organ growth without cell division: somatic polyploidy in a moth, Ephestia kuehniella. Genome, doi: 10.1139/g2012-060 2012; 55(11):755-763.
4. Claycomb JM, Orr-Weaver TL. Developmental gene amplification: insights into DNA replication and gene expression. Trends Genet, doi: 10.1016/j.tig.2005.01.009 2005; 21(3):149-162.

5. Johnston JS, Ross LD, Beani L, Hughes DP, Kathirithamby J. Tiny genomes and endoreduplication in Strepsiptera. Insect Mol Biol, doi: 10.1111/j.0962-1075.2004.00514.x 2004; 13(6):581-585.
6. Park SY, Asano M. The origin recognition complex is dispensable for endoreplication in Drosophila. Proc Natl Acad Sci USA, doi:10.1073/pnas.0805189105 2008; 105(34):12343-12348.
7. Sun J, Smith L, Armento A, Deng WM. Regulation of the endocycle/gene amplification switch by Notch and ecdysone signaling. J Cell Biol doi: 10.1083/jcb.200802084. 2008; 182(5):885-896.
8. Orr-Weaver TL, Spradling AC. Drosophila chorion gene amplification requires an upstream region regulating s18 transcription. Mol Cell Biol 1986; 6(12):4624-4633.
9. Dittmann FK, PH, Hagedorn HH. Ploidy levels and DNA Synthesis in Fat Body Cells of the Adult Mosquito, Aedes aegypti: The Role of Juvenile Hormone. Archives of Insect Biochemistry and Physiology 1989; 12:133-143.
10. Attardo GM, Hansen IA, Raikhel AS. Nutritional regulation of vitellogenesis in mosquitoes: implications for anautogeny. Insect Biochem Mol Biol doi: 10.1016/j.ibmb.2005.02.013 2005; 35(7):661-675.
11. Raikhel AS, Kokoza VA, Zhu J, Martin D, Wang SF, Li C *et al*. Molecular biology of mosquito vitellogenesis: from basic studies to genetic engineering of antipathogen immunity. Insect Biochem Mol Biol 2002; 32(10):1275-1286
12. Bangham J, Jiggins F, Lemaitre B. Insect immunity: the post-genomic era. Immunity 2006; 25(1):1-5.
13. Osta MA, Christophides GK, Vlachou D, Kafatos FC. Innate immunity in the malaria vector Anopheles gambiae: comparative and functional genomics. J Exp Biol (Pt 15), doi: 10.1242/jeb.01066. 2004; 207:2551-2563.
14. Aggarwal K, Silverman N. Positive and negative regulation of the Drosophila immune response. BMB Rep 2008; 41(4):267-277.
15. Ferrandon D, Imler JL, Hetru C, Hoffmann JA. The Drosophila systemic immune response: sensing and signalling during bacterial and fungal infections. Nat Rev Immunol, doi: 10.1038/nri2194 2007; 7(11):862-874.
16. Lemaitre B, Hoffmann J. The host defense of Drosophila melanogaster, Annu Rev Immunol doi: 10.1146/annurev. immunol. 25.022106.141615 2007; 25:697-743.
17. Tzou P, De Gregorio E, Lemaitre B. How Drosophila combats microbial infection: a model to study innate immunity and host-pathogen interactions. Curr Opin Microbiol 2002; 5(1):102-110.
18. Dolbeare F. Bromodeoxyuridine: a diagnostic tool in biology and medicine, Part II: Oncology, chemotherapy and carcinogenesis. Histochem J. 1995; 27(12):923-964.
19. Dolbeare F. Bromodeoxyuridine: a diagnostic tool in biology and medicine, Part I: Historical perspectives, histochemical methods and cell kinetics. Histochem J. 1995; 27(5):339-369.
20. Dolbeare F. Bromodeoxyuridine: a diagnostic tool in biology and medicine, Part III. Proliferation in normal, injured and diseased tissue, growth factors, differentiation, DNA replication sites and in situ hybridization. Histochem J. 1996; 28(8):531-575.
21. Lehner B, Sandner B, Marschallinger J, Lehner C, Furtner T, Couillard-Despres S *et al*. The dark side of BrdU in neural stem cell biology: detrimental effects on cell cycle, differentiation and survival. Cell Tissue Res doi: 10.1007/s00441-011-1213-7 2011; 345(3):313-328.
22. Konishi T, Takeyasu A, Natsume T, Furusawa Y, Hieda K. Visualization of heavy ion tracks by labeling 3' -OH termini of induced DNA strand breaks. J Radiat Res. doi:10.1269/jrr.10097. 2011; 52(4):433-440.
23. Hernandez-Martinez S, Lanz H, Rodriguez MH, Gonzalez-Ceron L, Tsutsumi V. Cellular-mediated reactions to foreign organisms inoculated into the hemocoel of Anopheles albimanus (Diptera: Culicidae), J Med Entomol. 2002; 39(1):61-69.
24. Hernandez-Martinez S, Roman-Martinez U, Martinez-Barnetche J, Garrido E, Rodriguez MH, Lanz-Mendoza H. Induction of DNA synthesis in Anopheles albimanus tissue cultures in response to a Saccharomyces cerevisiae challenge. Arch Insect Biochem Physiol doi: 10.1002/arch.20150 2006; 63(4):147-158.
25. Hernandez-Martinez S, Barradas-Bautista D, Rodriguez MH. Diferential DNA synthesis in Anopheles albimanus tissues induced by immune challenge with different microorganisms. Arch Insect Biochem Physiol doi: 10.1002/arch.21108 2013; 84(1):1-14.