Antibakteriální aktivita hmyzí hemolymfy

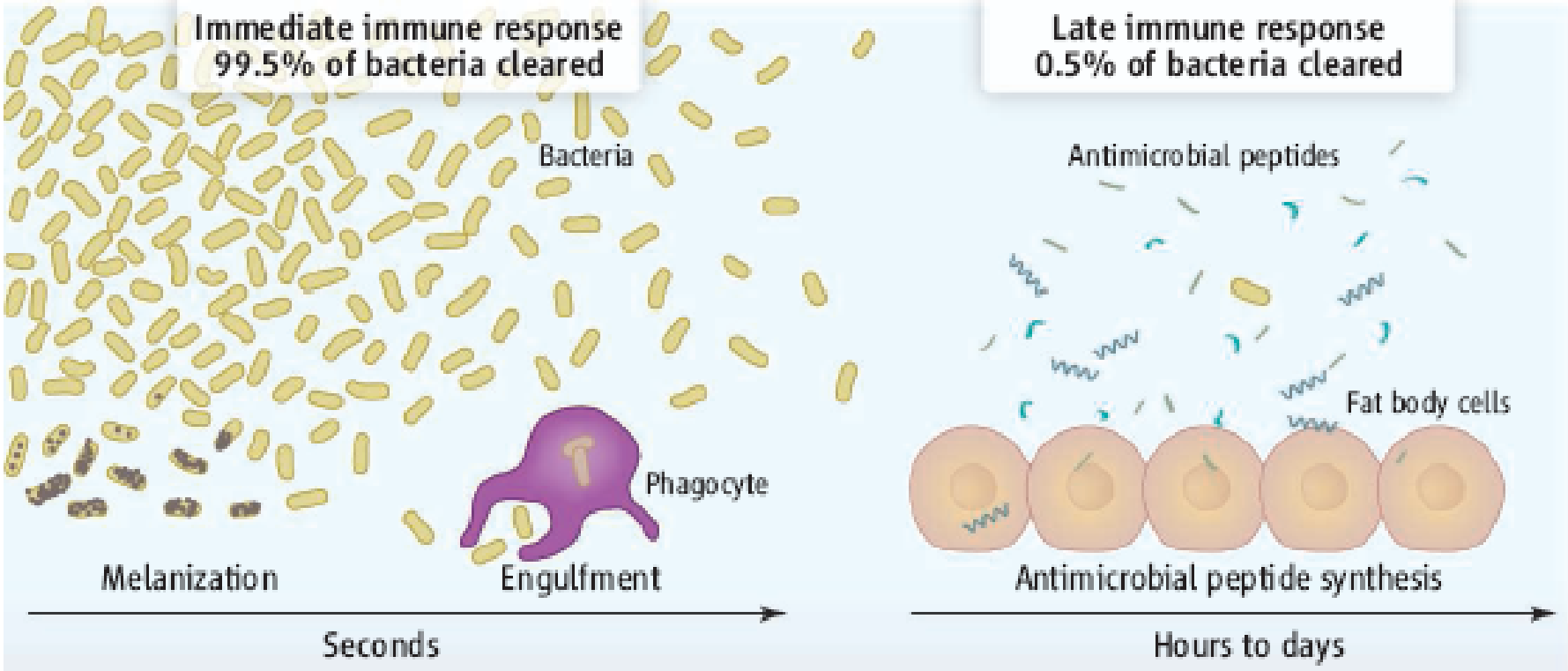
1. Stručný úvod – antibakteriální agens hmyzu + (bioluminiscenční) bakterie.

2. Zónová difúze v agarózovém gelu – nalití předem připravených 1,25% agarózových gelů obsahujících kulturu bakterií *Escherichia coli* (gram negativní) a *Micrococcus luteus* (gram pozitivní), vysekání jamek po zatuhnutí gelu a napipetování vzorků + příprava a pipetování kalibračních roztoků lysozymu. Inkubace ve vlhké komůrce při laboratorní teplotě. Fotodokumentace vzorků následující den.

3. Bioluminiscenční stanovení antimikrobiální aktivity hemolymfy bource morušového (*Bombyx mori*) pomocí bioluminiscenčních bakterií *E. coli* K12, stanovení koncentrační závislosti (0,5 – 40 % hemolymfa) baktericidních účinků hemolymfy pomocí luminometru Hidex Chameleon, vyhodnocení bioluminiscenčního měření. Diskuse o inducibilitě antimikrobiálních peptidů hemolymfy, ukázka starších měření.

4. Na starších výsledkcích ukázka povrzení bioluminiscenčního měření (viability jednotlivých bakterií) metodou platingu na agarové misky .

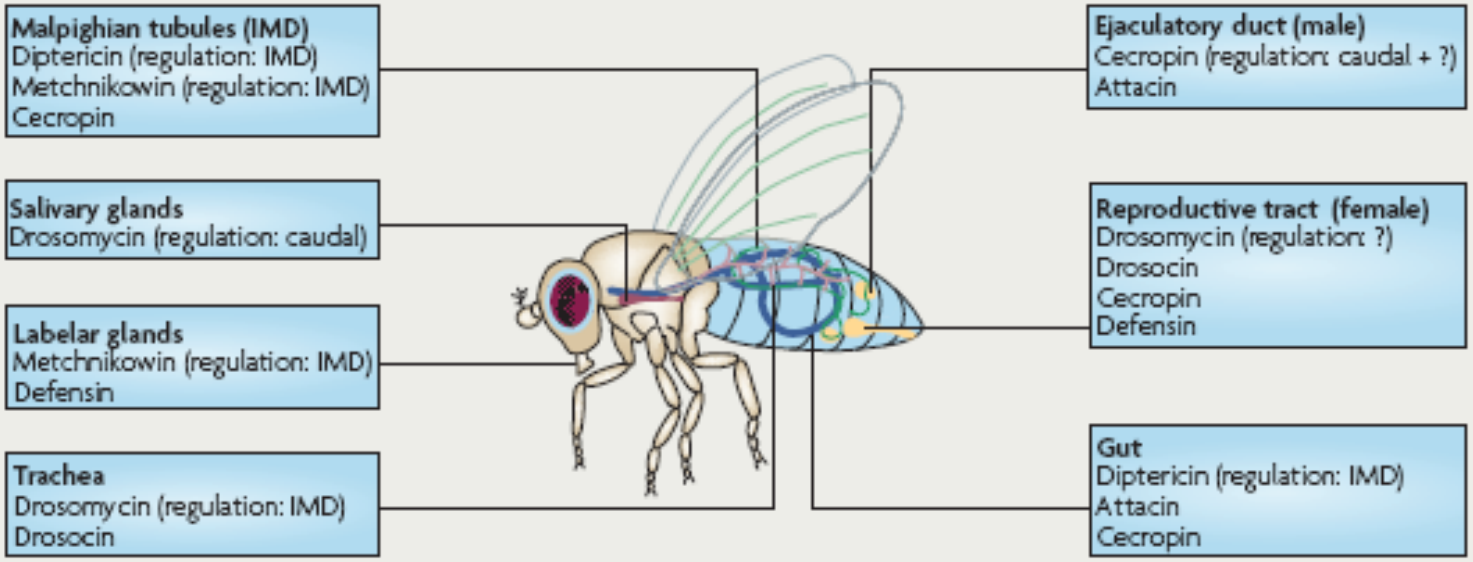
5. Diskuse stanovení antimikrobiálních účinků hemolymfy pomocí turbidimetrických metod a jejich srovnání se zónovou difúzí.



Clearing and mopping up. The majority of bacteria that infect an insect are cleared by immediate-acting immune responses, whereas the late-acting antimicrobial peptide immune response targets the few bacteria that are resistant to the early response.

Schneider and Chambers 2008

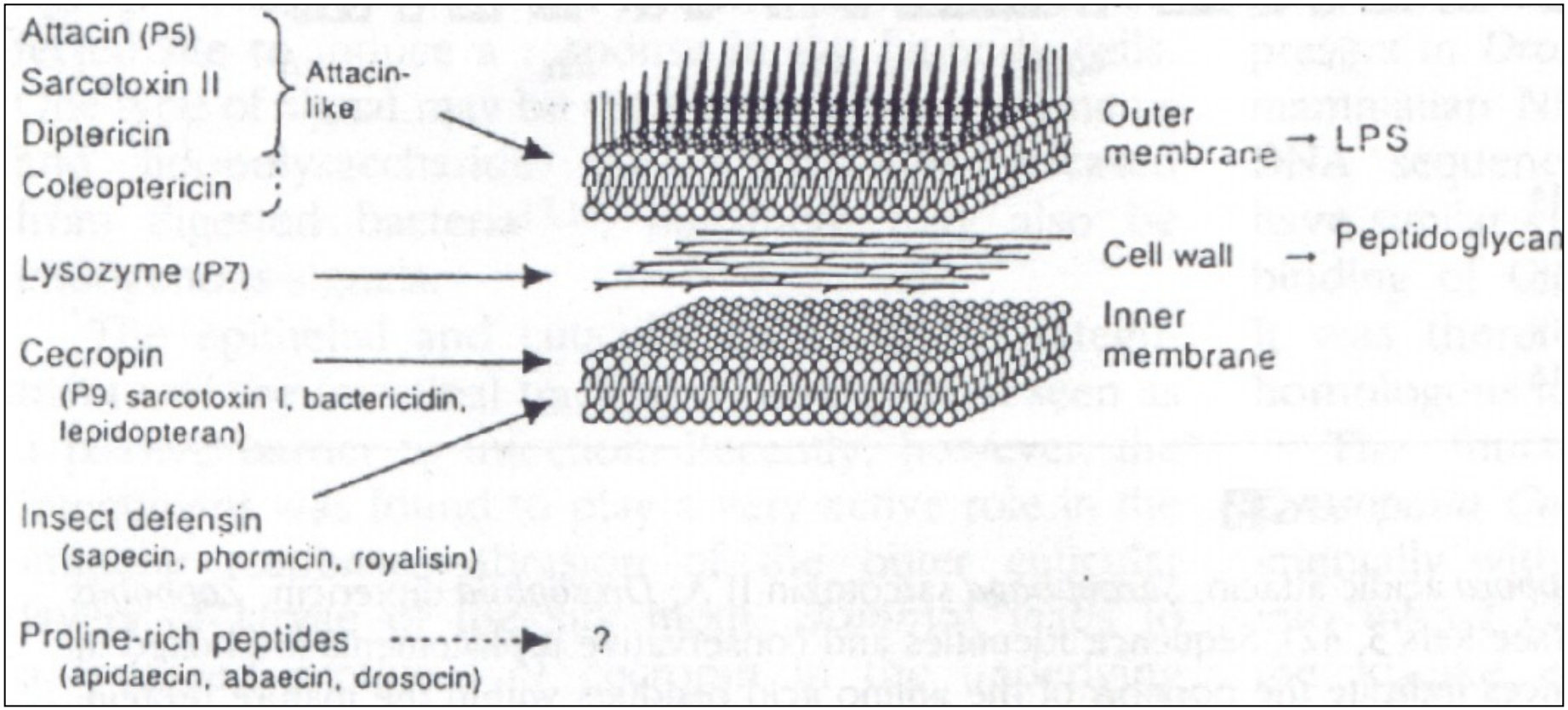
Box 2 | **Immunity in barrier epithelia**



Drosophila melanogaster interacts continuously with microbes in its environment through the interfaces provided by contact epithelia in the digestive, respiratory and reproductive systems. LacZ and green fluorescent protein (GFP) reporter genes have revealed that several antimicrobial peptides (AMPs) are expressed in various tissues of laboratory-reared flies[120–122] as illustrated in the figure. Whereas some AMPs are constitutively expressed in the reproductive tract or salivary glands, others are induced through the immune deficiency (IMD) pathway by microbes in direct contact with the epithelium.

Multiple physical and immunological barriers of the digestive tract

The digestive tract is the primary source of contact between *D. melanogaster* and microbes. The midgut absorbs nutrients while preventing the entry of microorganisms by several mechanisms. The first layer of protection is a physical barrier, the chitinous peritrophic matrix that lines the midgut and is secreted by the proventriculus (cardia). Most microorganisms are blocked by this matrix, except for *Serratia marcescens*, which can traverse the intestinal epithelium[124]. The second level of protection relies on the local production of AMPs in the proventriculus and in the midgut. A third level of protection is provided by the production of reactive oxygen species (ROS) in response to the ingestion of large quantities of dead or live bacteria or yeasts[123]. The fly is protected from this oxidative shock by a catalase: in its absence, the flies succumb to an oral challenge with dead bacteria[107]. The enzyme responsible for the production of intestinal ROS is Duox, an NADPH oxidase that also contains a myeloperoxidase domain[123]. The mechanism of activation of this oxidative response is currently unknown and does not require either the Toll or IMD pathways. This response is less effective, however, against microorganisms that produce ROS protective enzymes such as catalases[99]. These resistant microorganisms are controlled by a local activation of the IMD pathway in the cardium and midgut[95,99,124]



Obr. 1 : Navržené schema působení indukovaných antibakteriálních proteinů a peptidů na buněčnou stěnu bakterií (Hultmark 1993)

Fig. 1. Evolutionary plasticity of insect immunity displayed by extreme differences in constitutive antibacterial activity in the hemolymph of three coccinelid species: the native and non-invasive ladybird *Adalia bipunctata* (left), the native and invasive (in northern America) seven-spotted ladybird *Coccinella septempunctata* (middle), and the introduced and invasive harlequin ladybird *Harmonia axyridis* (right). Three microliters of hemolymph collected upon reflex-bleeding of each beetle were put into a whole punched in Petri-dishes containing agar which was inoculated with *Micrococcus luteus* bacteria. 24 h upon cultivation at 30 °C zones of bacterial growth inhibition indicate the presence of a strong constitutive antibacterial activity in the hemolymph of *H. axyridis* which is absent from *A. bipunctata* and *C. septempunctata*. Beetles were immobilized by keeping them for 30 min. in the refrigerator, and then put on the wholes to illustrate the corresponding size of inhibition zones. The antibacterial activity in the hemolymph of *H. axyridis* has been attributed to harmonine, a secondary metabolite, which displayed broad-spectrum antibacterial and antimalaria activity. The production and release within the hemolymph of harmonine putatively saves fitness costs associated with the synthesis of AMPs and may, therefore, contribute to the invasive success of *H. axyridis* (Röhrich et al., 2012).