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Vitellogenin is an immunocompetent molecule for mother and offspring in fish

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ABSTRACT

Our understanding of the function of vitellogenin (Vg) in reproduction has undergone a transformation over the past decade in parallel with new insights into the role of Vg in immunity. Initially, Vg was regarded as a female-specific reproductive protein, which is cleaved into yolk proteins such as phosvitin (Pv) and lipovitellin (Lv), stored in egg, providing the nutrients for developing embryos. Recently, Vg is shown to be an immune-relevant molecule involved in the defense of the host against the microbes including bacterium and virus. Furthermore, Pv and Lv, that both are proteolytically cleaved products of Vg, play a defense role in developing embryos. Importantly, yolk protein-derived small peptides also display antimicrobial activity. These data together indicate that Vg, in addition to being involved in yolk protein formation, plays a non-reproductive role via functioning as an immune-relevant molecule in both parent fishes and their offspring. It also shows that yolk proteins and their degraded peptides are novel players in maternal immunity, opening a new avenue to study the functions of reproductive proteins.

1. Introduction

Vitellogenin (Vg, from latin *vitellus*, yolk, and *gener*, to produce) was initially proposed by Pan et al. [1] to describe female-specific insect hemolymph protein precursor of egg yolk, regardless of its amino acid sequence and structure. Vg is now known as a high molecular mass glycolipophosphoprotein usually circulating in the blood (vertebrates)/hemolymph (invertebrates) as a homodimer, which is usually encoded by multiple *vg* genes in several species including insects, fish and frog [2]. Vg displays a similar structural characteristic in vertebrates, such as fish, and invertebrates, particularly insects. In most cases, Vg contains three conserved domains, the LPD_N (also known as vitellogenin_N or LLT domain) which is identified at the N-terminus, the domain of unknown function (DUF) 1943, and the von Willebrand factor type D domain (vWD) which is located at the C-terminus and distributed over a wide range of proteins (Fig. 1). Occasionally, a domain of unknown function called DUF1944 is found to be present in between DUF1943 and vWD in some Vg proteins from vertebrates such as chicken and fish [3]. Beginning at the N-terminus, a complete fish Vg consists of a signal peptide, a lipovitellin heavy chain (LvH), a phosphorylated serine-rich phosvitin (Pv), a lipovitellin light chain (LvL), and a β-component (β-C) plus a C-terminal coding region (CT) comprising the vWD. Notably, Pv can be absent, as observed in zebrafish Vg3 and most invertebrate Vg [4,5].

Vg, as an egg yolk protein precursor, is present in the females of nearly all oviparous species including fish, amphibians, reptiles, birds, most invertebrates and the platypus. Vg is usually synthesized extra-ovarianly (in the liver of vertebrates, the hepatopancreas of crustaceans and the fat body of insects) and transported by the circulation system to the ovary, where it is internalized into growing oocytes via receptor-mediated endocytosis and proteolytically cleaved by the aspartic protease cathepsin D [6–8] to generate yolk proteins, such as Lv subunits, Pv and β-C. Lv subunits and Pv are stored in yolk globules or platelets, while β-C remains in cytoplasm as a soluble fraction [9–11]. These yolk proteins are later used as the nutrients by developing embryos [12,13].

Vg was once regarded as a female-specific protein; however, synthesis, albeit in smaller quantities, has been shown to occur in male and even sexually immature animals [14,15]. This suggests that Vg presumably fulfills a more general role independent of gender. In recent years, both Vg and its derived yolk proteins have been shown to be connected with the immune defense in fish, challenging the traditional view that Vg and its derived yolk

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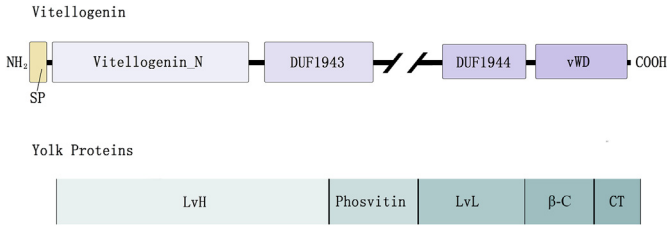

Fig. 1. Domain structure of teleost vitellogenin. Typical teloest vitellogenin contains a signal peptide (SP) at the N-terminal and several conserved domains in including (form N-terminal to C-terminal): Vitellogenin_N (also known as LPD_N), domain of unknown function (DUF) 1943, domain of unknown function (DUF) 1944 (which appears only in some vitellogenins of teleosts) and von Willebrand factor type D domain (vWD). Enzymatic cleavage products of Vg include a lipovitellin heavy chain (LvH), a phosvitin (Pv), a lipovitellin light chain (LvL) followed by a beta-component (β-C) and a C-terminal coding region (CT) cleaved from vWD.

proteins were simple source of nutrients for the developing embryos. Below we will discuss the immunological function of Vg, yolk proteins, and yolk protein-derived peptides, hoping to stimulate increased attention to reproductive proteins.

2. Defense roles of Vg in adult fish

Vg was previously considered to be the energy reserve for embryonic development and growth, but accumulating data demonstrated several non-nutritional roles for Vg. For example, in the advanced eusocial honeybee, Vg was shown to be linked to the social organization, temporal division of labor and foraging specialization, regulation of hormonal dynamics and change in gustatory responsiveness [16–19]. Besides, the honeybee Vg was demonstrated to be able to reduce oxidative stress by scavenging free radicals, thereby increasing the lifespan in the facultatively sterile worker castes and reproductive queen castes [20,21]. Similar antioxidant activity was also observed for the nematode (*Caenorhabditis elegans*) Vg and the eel (*Anguilla japonica*) Vg [22,23].

Another newly-identified function of Vg is associated with the host immune defense. Vg was first found to have hemagglutinating and antimicrobial activities in the amphioxus *Branchiostoma japonicum* by Zhang et al. [24]. Later, Vg of the rosy barb (*Puntius conchonius*) and the carp (*Cyprinus carpio*) was shown to possess a similar antimicrobial activity, and challenge with the Gram-negative bacterium *Escherichia coli* resulted in synthesis of Vg in male rosy barb [25,26]. Recently, Atlantic salmon (*Salmon salar* L.) serum Vg was shown to be able to neutralize the infectivity of infectious pancreatic necrosis virus (IPNV) [27], and mosquito Vg to interfere with the anti-*Plasmodium* response in *Anopheles gambiae* [28]. Further studies revealed that Vg was able to recognize the microbial conserved components, called pathogen-associated molecular patterns (PAMPs), including lipopolysaccharide (LPS), peptidoglycan (PGN), lipoteichoic acid (LTA) and glucan, to cause lysis of bacteria, and to enhance phagocytosis of bacteria by macrophages [29–31]. Additionally, DUF1943 and DUF1944 as well as VWD were found to contribute to the function of Vg as a pattern recognition receptor, and DUF1943 and DUF1944 to the function of Vg as an opsonin [32]. Collectively, these data indicate that Vg is an immunocomponent molecule important in the defense of fish against the microbes including bacterium and virus, via functioning as a pattern recognition receptor capable of identifying both Gram-negative and positive bacteria, an effector molecule capable of killing bacteria/neutralizing virus, and an opsonin capable of enhancing phagocytosis [33]. Interestingly, honey bee Vg has been shown to recognize endogenous damage-associated molecular patterns (DAMPs) such as phosphatidylserine [34], suggesting that Vg can be an anti-inflammatory factor similar to many other plasma proteins [35]. It is worth testing if fish Vg can also act as a receptor of DAMPs.

Recently, Tong et al. [36] showed that injection of LPS or LTA into male zebrafish (*Danio rerio*) induced a rapid and significant up-regulation of Vg at both transcriptional and translational levels; and the serum Vg produced was able to bind to both *E. coli* and *Staphylococcus aureus* (a Gram-positive bacterium) and to inhibit their growth in a dose-dependent manner. These results suggest that Vg may function as an acute phase protein *in vivo*, leading to the elimination of invading pathogens. Notably, several studies show that the liver is not the only site of Vg synthesis in fish. For example, expression of *vg* was reported in the heart and brain of the Chinese rare minnow *Gobiocypris rarus* [37], the ovary, gill and testis of the white cloud mountain minnow *Tanichthys albonubes* [38], and the gill, heart, white adipose tissue and skin of zebrafish *D. rereio* [39–42]. The wide extrahepatic expression of *vg* possibly suggests that Vg does not only function in the circulating blood, lymph and body fluid but also plays a role at the local sites after the onset of infection or injury. This is apparently supported by a recent observation showing that the expression of *vg1*, *vg2* and *vg4* in the skin of *D. rerio* was significantly increased following infection with the pathogen *Citrobacter freundii* [43].

3. Defense roles of Vg-cleaved yolk proteins in early embryos

Embryos of most mammalian species including humans develop in the uterus inside mother's body, and are thus well protected from external pathogenic attacks. However, eggs of most fish are released and fertilized externally, and the resulting embryos/larvae are therefore exposed to a hostile aquatic environment full of potential pathogens, which are capable of causing various types of diseases, eventually leading to death. For example, it was shown that exposure of salmon fry and juveniles to the Gram-negative bacterium *Yerinia ruckeri* caused occurrence of enteric redmouth disease, resulting in 60% mortality [44]. In addition, during the early stages of development, fish embryos have little or only limited ability to synthesize immune-relevant molecules endogenously, and their lymphoid organs are not yet fully formed [45,46]. How fish embryo/larvae survive the pathogenic attacks in such a hostile environment has received increasing attention in the past two decades. It is well known that fishes produce eggs endowed with all the nutrients and protective systems allowing the development of a fish embryo in an aquatic environment. Embryo protection is first ensured by a physical barrier, the chorion, the fertilized membrane, but also by a variety of maternally-transferred immune molecules including antibodies [47–51], complement component C3 [52–56], lectins [57–60], lysozymes [61,62] and chitinase [63].

Recently, Pv, a yolk protein derived from Vg, was proven to play a critical role in the immunity of zebrafish embryos via acting as a pattern recognition receptor and an antimicrobial effector molecule [64]. In line with this, hen egg yolk Pv was also shown to be able to inhibit the growth of the Gram-negative bacterium *E. coli* and the Gram-positive bacterium *S. aureus* under thermal stress [65,66]. Moreover, Pv from zebrafish was demonstrated to possess antiviral activity via inhibiting the formation of the cytopathic effect in lymphocystis disease virus-infected cells and reducing the virus quantities in the virus-infected cells and host, suggesting the possibility that Pv may be a maternal immune-relevant factor capable of protecting developing embryos from virus attack [32]. Like Pv, native Lv from rosy barb was also shown to be associated with the immune defense of early embryos and larvae [67]. All these data show that Pv and Lv are maternally-transferred proteins involved in both nutritional supply and immune defense in embryos and larvae in fishes.