In focus in HCB

Douglas J. Taatjes¹ · Jürgen Roth²

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In this July issue of the journal, we will highlight three original articles describing (1) the regulatory role of phosphorylation of the scaffold protein gephyrin in its binding to microtubules; (2) the effect of the addition of the gastrointestinal hormone ghrelin to conditioned media on the neural differentiation of human adipose mesenchymal cells; and (3) the determination of a possible germline differentiation factor in the bivalve Manila clam *Ruditapes philippinarum*. As always, we wish you good reading!

Phosphorylation of gephyrin regulates binding to microtubules

Gephyrin is an evolutionary highly conserved multifunctional scaffolding protein attaching glycine- and subtypes of GABA type A-receptors at inhibitory postsynaptic membrane specializations by binding to the microtubule (MT) and/or the actin cytoskeleton (Maric et al. 2011; Nawrotzki et al. 2012; Tyagarajan and Fritschy 2014). The activity of gephyrin may be modulated by various posttranslational modifications including phosphorylation (Ghosh et al. 2016; Groeneweg et al. 2018), and as shown earlier, the phosphorylation at gephyrin Ser270 depends on collybistin (Kuhse et al. 2012). In continuation of earlier studies, Kuhse and colleagues (Zhou et al. 2021) have now investigated how gephyrin phosphorylation is involved in its interaction with tubulin. For this, the phospho-specific anti-gephyrin antibody mAb7a, which detects phosphorylation at Ser270 (Kuhse et al. 2012), was of critical importance. Although gephyrin binding occurred to polymerized

microtubules sedimented by high-speed centrifugation from rat brain, it was not the phosphorylated form recognized by mAb7a. Actually, Ser270-phospho gephyrin was found in the soluble fraction. Further experimental evidence that gephyrin phosphorylation prevented its interaction with microtubules was obtained in binding assays with recombinant collybistin-phosphorylated gephyrin. Conversely, substitution of Ser270, Ser198 and Ser200 by alanine permitted gephyrin binding to microtubules. By high-resolution immunofluorescence applying the anti-gephyrin Ab-175, small gephyrin spots were seen along filamentous microtubules (Fig. 1).

Fig. 1 Double immunofluorescence for endogenous gephyrin (green, Ab-175) and tubulin (red) in U2OS cells reveals puncta of gephyrin codistributed with microtubules. From Zhou et al. (2021)

✉ Douglas J. Taatjes
douglas.taatjes@med.uvm.edu

¹ Department of Pathology and Laboratory Medicine, Larner College of Medicine, University of Vermont, Burlington, VT 05405, USA

² University of Zurich, CH-8091 Zurich, Switzerland

The main conclusion drawn by the authors was that a specific fraction of gephyrin, which is not Ser270-phosphorylated, interacts with microtubules in brain and cultured cells (HEK293T and U2OS) and, hence, that the transient binding of gephyrin to microtubules is regulated by its phosphorylation.

No time to “clam up” about bivalve germ granule assembly

Germlines are critical determinants for passing genetic hereditary information to the next generation. Within this complex developmental process, cytoplasmic factors are involved in forming supramolecular structures known as “germ plasm-related structures” which are critical for germ cell differentiation (Reunov et al. 2020). Although much information regarding this process has been uncovered in a variety of species, to date, a general and ubiquitous factor involved in the assembly of these supramolecular structures has not been identified. Filanti et al. (2021) have now undertaken a search for a potential germline factor in the Manila clam *Ruditapes philippinarum* (*R. philippinarum*). This clam species is of particular interest in this regard, as it undergoes an annual renewal of gonads at the beginning of the mating season. During this phase, germ cells differentiate into gonadal tissue inside a connective tissue layer located near the intestines. Following the spawning period, the clams enter a “rest” phase during which the gonads are degraded. Since germ plasm factors have been uncovered in other model species, the authors chose the very well-studied Oskar (present in polar granules in *Drosophila*), which consists of two functional domains (RNA-binding OSKAR and LOTUS). While OSKAR has to date only been identified in insects and bacteria, LOTUS is found in other germline-related proteins, including the homologues of TDRD7. This led Filanti et al. (2021) to begin their search with a LOTUS domain protein, since it might lead to identification of an Oskar-like germ granule assembly factor in a species such as *R. philippinarum* with no known master regulator factor. They used a BLASTing approach of the *Drosophila* Oskar protein sequence, which yielded a TDRD7A-like protein, eventually uncovering an orthologue transcript of this protein in *R. philippinarum*. From the determined protein sequence, two antibodies were raised against two synthetic peptides representing different critical portions of the TDRD7 protein. With these antibodies in hand, the authors performed immunolocalizations, with both chromogenic dyes and confocal immunofluorescence, as well as Western blot analyses on male and female *R. philippinarum* samples from both the gametogenic stage and “rest” (reproductive spent stage). Their immunostaining results showed that in both male

and female specimens, (1) in the gametogenic phase, the anti-TDRD7 antibodies stained only some cells near the basal lamina of the intestinal structures and in the connective tissues (Fig. 2); (2) in the gonadic acinus periphery, staining was observed in early germ cells; and (3) in the “spent” phase, immunostaining was very faintly expressed in the same cells as observed in the gametogenic stage.

Fig. 2 Double immunofluorescence for TDRD7 (red) and dsDNA (green) in the intestine of female *Ruditapes philippinarum*. Many TDRD7-positive putative primordial stem cells (arrows) are present throughout the intestinal epithelium. From Filanti et al. (2021)

The Western blotting results were more variable, displaying several bands with both of the antibodies; the authors speculate that these ambiguous results may be due to the premise that the individual specimens may not always be in perfect synchrony within the gametogenic phase. However, based upon their results, the authors conclude that the TUDOR domain-containing protein TDRD7 may be acting in the early germline differentiation in *R. philippinarum*.

What's all the yellin' about ghrelin?

Interest in human adipose tissue-derived mesenchymal stem cells (ASCs) has been high for many years due to their ability to differentiate into numerous cell types during cell culture (Dai et al. 2016). Various types of matrices and scaffolds for cell culture have been tested to examine the potential for ASCs to differentiate into different cell lineages (Gomarasca et al. 2020). Similarly, the effects of the composition of the conditioned culture media on lineage differentiation of ASCs have been well studied. For instance, Lo Furno and colleagues have investigated the type of conditioned media required to drive ASCs to a neural type of differentiation (Lo Furno et al. 2018a,b). This same group has now (Russo et al. 2021) further expanded on these studies to analyze the potential effect of adding the gastrointestinal hormone ghrelin to specialized ASC-conditioned media due to its known multiple influences on neuronal cells and tissues (Fothergill and Furness 2018; Gong et al. 2020). ASCs were cultured in either (1) a basal control medium; (2) basal control medium with added ghrelin (ASC + Ghre); (3) culture medium derived from olfactory ensheathing cells (OEC); (4) OEC medium + Ghre; (5) culture medium derived from Schwann cells (SC); and SC medium + Ghre. Following 1 or 7 days of growth in these culture media, the cells were fixed and subjected to flow cytometry and immunofluorescence to stain for the presence of neural cell markers (PGP9.5, MAP2), a glial cell marker (GFAP), and the ghrelin receptor (GHSR-1a). Overall, their results confirmed their earlier work indicating that culture of ASCs in OEC- or SC-conditioned media resulted in differentiation into neuronal-phenotypic cells. Moreover, they importantly found that although addition of ghrelin to the basal culture medium had a relatively mild effect on neural cell differentiation, when added to the OEC- or SC-conditioned medium, increases in the neuronal cell markers were observed, especially after 7 days of culture; this effect was particularly enhanced in the SC-conditioned medium (Fig. 3). Likewise, the expression of GHSR-1a was enhanced when ghrelin had been added to the OEC- or SC-conditioned medium compared to addition to control medium alone. Interestingly, only very moderate effects on GFAP immuno-expression were found when ghrelin was added to the conditioned media. Their results suggest that the addition of ghrelin to the conditioned medium of ASCs helps promote a neuronal as opposed to a glial phenotypic differentiation.

Fig. 3 Immunofluorescence for protein gene product 9.5 (green) in human adipose tissue-derived mesenchymal stem cells (ASC) cultured for 7 days in Schwann cell-derived conditioned medium (SC-CM) without (upper panel) or with ghrelin added (middle panel) is increased by ghrelin. The ghrelin effect was substantiated by flow cytometry (lower panel). From Russo et al. (2021)

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