A Disabled-1 (DAB1) Isoform is Differentially Expressed in Granulosa Cells of Healthy Antral Follicles and Contributes to Cytoplasmic Vesicular Transport.

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The cytosolic adaptor protein Disabled-1 (DAB1) plays a role in the Reelin signaling pathway and is important in the proper development of the brain and cerebellum cortices in vertebrates. Reelin, an extracellular protein, binds to its membrane receptor, the apolipoprotein E receptor 2 (ApoER2), to induce tyrosine phosphorylation of DAB1. We have previously shown that both Reelin and its receptor ApoER2 are differentially expressed in dominant or preovulatory follicles, with Reelin being expressed in theca cells (TC) while ApoER2 is expressed in granulosa cells (GC), suggesting that Reeling is involved in a paracrine signalling pathway during follicular development. Therefore, the objective of this study was to characterize the expression and function of DAB1 in GC of bovine follicles. For this study, an *in vivo* model composed of GC obtained at different follicular stages was used: small follicles (SF: 2-4 mm), growing dominant follicles (DF) at day 5 of a synchronized estrous cycle and ovulatory follicles (OF) obtained at 0, 6, 12, 18 and 24 hours post-hCG injection. We found that the DAB1 isoform expressed in GC is alternatively spliced, lacking exons 7, 8 and 16, and thus missing three phosphorylated tyrosine residues (Y^{200}, Y^{220} and Y^{232}) out of the five normally found compared to the canonical brain isoform. In addition the DAB1 isoform expressed in GC included exons 10, 11 and 18 which are not found in the brain isoform. RT-qPCR results show that this DAB1 GC isoform was significantly upregulated in DF compared to OF and decreased significantly 6 hr following hCG injection. Specific antibody raised against DAB1 exon 11 showed by western blotting a single protein band from GC extract, and the signal decreased significantly 6 hr following hCG in OF compared to DF. Immunohistochemistry observations localized DAB1 isoform in GC of healthy follicles, and in cumulus cells, with a stronger signal in the *corona radiata*. Moreover, the DAB1 signal was associated with endosomal and perinuclear vesicles. Vesicles were also observed under the zona pellucida in association with the cumulus cells transzonal projections. Post-translational modification analyses by immunoprecipitation followed by mass spectrometry of DAB1 has to date identified 7 phosphoryled sites (S^{64}, S^{90}, S^{114}, S^{468}, T^{88}, T^{101}, Y^{115}) and 4 glycosylated sites (S^{237}, S^{241}, S^{461}, T^{121}). Yeast two-hybrid screening identified potential DAB1 binding partners in GC that include: CLTA, CCT8, LAPTM4B and SRGN. Taken together, these results provide for the first time the characterization of a DAB1 isoform in bovine GC, and support a role in vesicular-transport in healthy growing follicles and a possible interaction between cumulus cells of the *corona radiata* and the oocyte.