

Molecular principles directing tissue development during embryogenesis

The research in my lab focuses on signals promoting muscle, tendon and glia differentiation. In these tissues we discovered a common regulatory mechanism that is mediated by the RNA-binding protein Held Out Wing (HOW). Our data suggest that HOW coordinates the proper timing of developmental events by delaying expression, or altering the mRNA levels of essential genes whose transcription was previously activated by instructive signals. HOW regulates the levels of target mRNAs at various levels, including mRNA stability, and pre-mRNA splicing. It binds to specific RNA sequence located at the 3'UTR of its target mRNA, or within an intron close to the 5' splice site. The primary and secondary RNA sequence of the "HOW response element" has been characterized in our lab (Israeli et al, 2007).

In the developing mesoderm layer, we have identified specific mRNA targets whose levels are negatively regulated by HOW. In this tissue we showed that HOW-mediated repression is essential to enable proper invagination

of the cells during gastrulation and further spreading of the mesoderm over the ectoderm layer. When these mRNAs are not repressed properly in the mesoderm, as in *how* mutants or following their over expression in a wild type background, mesoderm invagination and spreading is defected (a summary scheme is shown in Figure 1). Importantly the aberrant morphogenesis of the mesoderm (detected in *how* mutant embryos) leads to defects in the development of mesoderm derivatives including the heart and somatic muscles. Thus, HOW in the mesoderm is required to repress the expression of genes that interfere with proper morphogenesis of this tissue (Nabel-Rosen et al., 2005, Toldeno-Katchalski et al., 2007).

The development of tendon cells is similarly regulated by HOW activity, however in this tissue the regulation is performed in two phases: in the first phase HOW represses the mRNA levels of a critical transcription factor (StripeB) that specifies tendon precursor cells. Low level of StripeB at that stage is

essential to arrest the differentiation of the future tendon cells at their premature state, so that they will not differentiate prior to their attachment to muscle cells. In a second phase HOW promotes the splicing of a distinct isoform *stripeA*, encoded by the same *stripe* gene. StripeA is essential for the maturation of tendon cells and is elevated only following muscle binding to tendon cells. Thus, in this tissue HOW-dependent repression/activation of the Stripe isoforms coordinate between the stages of tendon cell specification and their muscle-dependent maturation (Volohonsky et al., 2007) (see scheme in Figure 2).

HOW was also shown to be essential for glial cell development during embryogenesis. In the nervous system (both CNS and PNS) HOW is highly expressed in two types of glia cells, the midline glia, and the glia cells involved in nerve insulation. In the midline glia HOW forms a part of the apoptotic machinery that is essential to reduce the number of midline glia cells. This is achieved by reducing the mRNA levels of the inhibitor of apoptosis (Diap1) (Reuveny et al). In the insulating glia HOW is essential for the formation of the Blood Brain Barrier (BBB). We showed that at least part of the mechanism by which HOW mediates nerve insulation is through promoting a glia-specific splicing of the septate junction component NeurexinIV (see the scheme in Figure 3) (Edenfeld et al., 2006). We are currently performing additional studies to elucidate the exact mechanism by which HOW promotes nerve insulation and formation of the BBB in the adult fly brain.

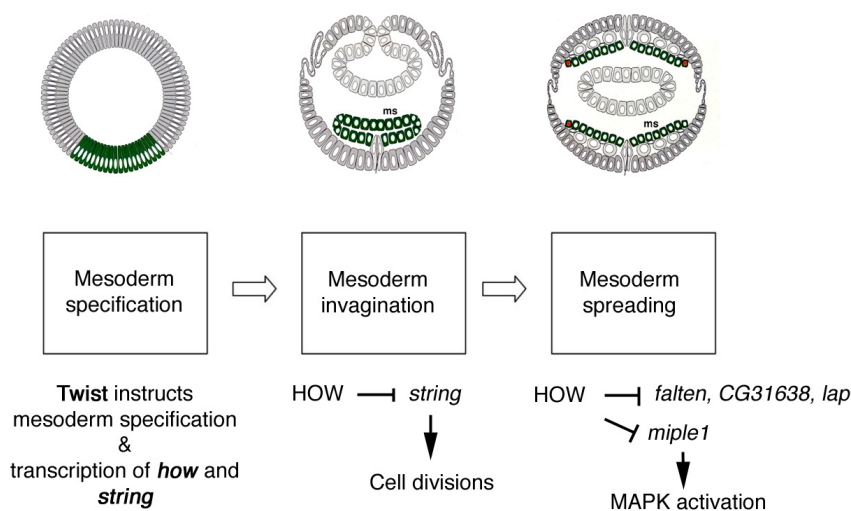


Fig. 1 HOW is essential for mesoderm invagination and spreading

Schematic representation of three stages in early mesoderm (green) development: mesoderm specification, mesoderm invagination, and mesoderm spreading; the corresponding involvement of HOW at each stage is shown. First, Twist instructs mesoderm specification as well as transcription of *how* and *string*. During mesoderm invagination, HOW represses *string* mRNA levels, arresting cell cycle progression. During mesoderm spreading, HOW represses the mRNA levels of several genes, including *falten*, *lap* and *miple1*. The repression of *miple1* is essential to maintain MAPK activation (Red) only in the most dorsal row of mesodermal cells.

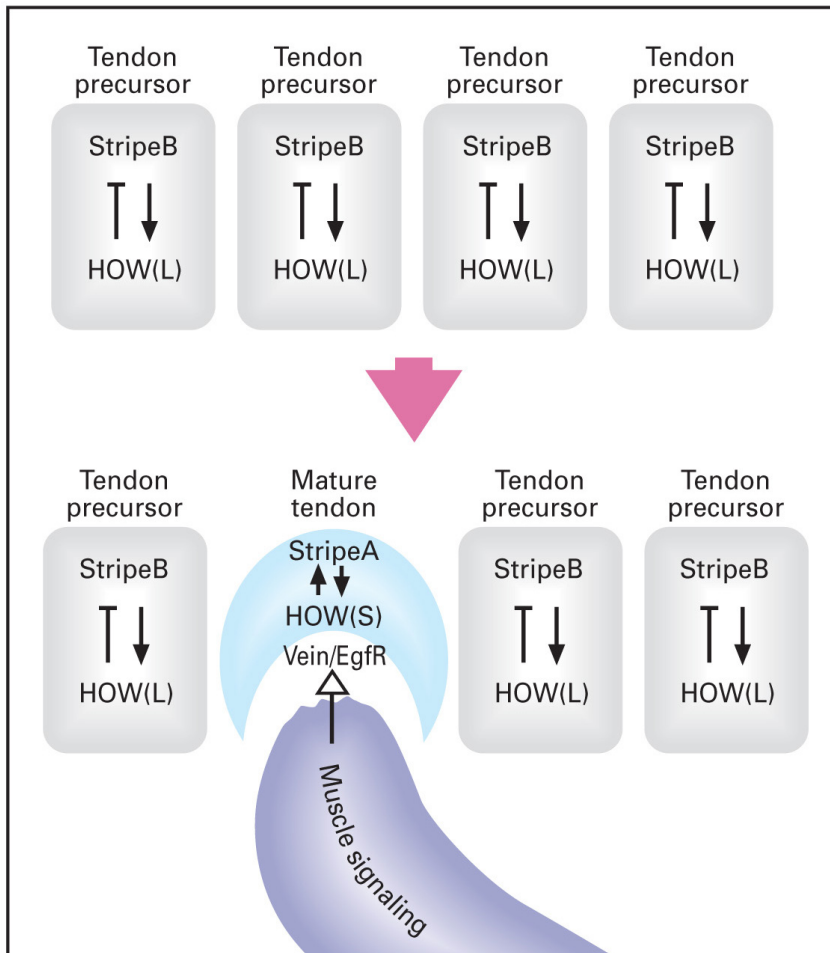


Fig. 2 HOW mediates a switch in tendon cell maturation

Tendon cells are specified in the ectoderm by the expression of the transcription factor, *StripeB*. A negative feedback loop between *StripeB* and *HOW(L)* maintains tendons at the precursor stage (upper panel). Somatic muscles migrate towards the tendon precursor cell and provide a differentiation signal, *Vein*, which binds and activates the *Egfr* signaling pathway in the tendon cell. This leads to elevation in *HOW(S)* levels, which then elevate the *StripeA* isoform characteristic of the mature tendon state.

Taken together, we suggest that HOW is part of a conserved mechanism that regulates distinct stages of tissue development by controlling the RNA levels of critical target mRNAs. We recently found that HOW is phosphorylated by MAPK and that this phosphorylation alters HOW activity (R. Nir). MAPK-dependent phosphorylation is capable of linking HOW activity with various signaling pathways affecting tissue differentiation and morphogenesis.

Our studies regarding the formation of the fly BBB together with recent studies suggesting that defects in the BBB of the adult fly led to aberrant

behavior, prompt us to address whether certain mental disorders are correlated with a temporal opening of the BBB in the human brain. Towards this end we are collaborating with scientists from the MRI unit in Sheba Medical Center (headed by Y. Mardor) to develop a method that monitors BBB alterations in the human brain (Dr. D. Israeli).

Separate line of studies in our lab focus on morphogenesis and the development of the musculoskeletal system. We found that muscle attachment to tendon cells is mediated by a group of gene products whose expression levels is elevated following the initial attachment of the muscles

to their corresponding tendon cells. For example, the expression of the secreted extracellular matrix protein Thrombospondin (Tsp) is highly elevated in tendon cells in response to muscle binding. We demonstrated that Tsp is required for the proper association of the muscle with its corresponding tendon cell. In its absence muscles tend to form connections with neighboring muscles, leading to disruption of the entire muscle system (Subramanian et al., 2007). Additional gene product required for correct muscle function is the *Drosophila* homologue of secreted factor *Egfr7* (DEgfr7). Our recent studies suggest that this tendon-specific protein binds to Tsp thereby modulating the site and mode of the integrin-mediated myotendinous junction. The activity of DEgfr7 is critical for proper muscle function and integrity (E. Gilsohn). An additional component that is essential for the arrest of muscle extension towards the tendon cell is a novel leucine-rich trans-membrane protein that we named Leucine-Rich Tendon protein (LRT). Our recent data suggest that LRT, expressed by tendon cells binds to the trans-membrane receptor Roundabout (Robo), and this leads to the arrest of muscle migration towards the tendon cells (B. Wayburn).

Muscle maturation is associated with the correct arrangement of large number of muscle nuclei included within each myotube following muscle fusion. We have identified the gene products required for the anchoring of muscle nuclei to the plasma membrane and show that this process is promoted by the formation of the myotendinous junction. In this context HOW is essential for the expression of the correct isoform of the KASH domain protein MSP300 (H. Elhanany).

Heart morphogenesis is studied via the functional analysis of the *Drosophila* extracellular protein Collagen XVIII (DCol-18). This protein is produced specifically by the heart cardioblasts during embryogenesis, and it is essential for correct morphogenesis of the heart tube (N. Woller). Lack of this gene leads to an extremely narrow heart tube that is not functional.

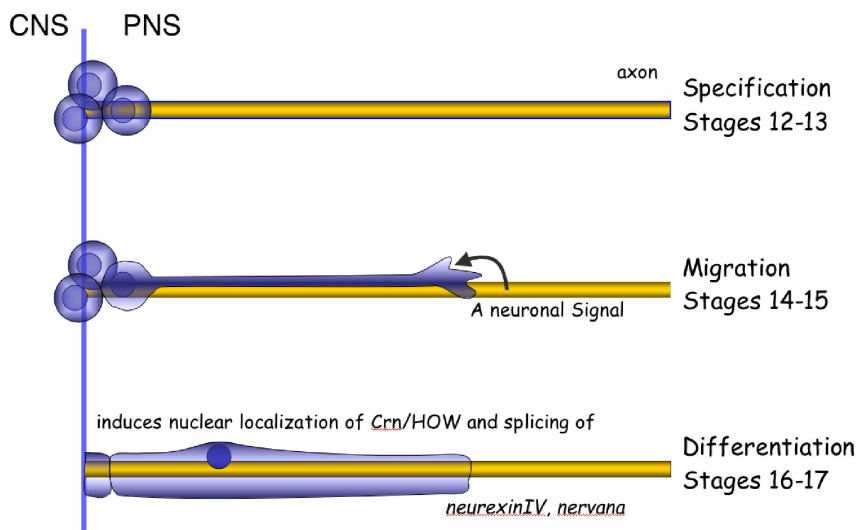


Fig 3 HOW mediates maturation of peripheral glial cells
Three stages of glial cell development are shown: glial specification (upper panel), glial migration (middle panel) and glial maturation (lower panel). HOW and Crn participate in the maturation of glial cells by promoting the alternative splicing of neurexinIV and nervana.

In summary, the research in our lab has elucidated the molecular basis for processes involved in tissue morphogenesis and development during embryogenesis, and will promote future treatment of human diseases in which aberrant tissue morphogenesis occurs.

Engelen, Adriana Reuveny, Talila Volk and Christian Klämbt (2006) The splicing factor Crooked neck associates with the RNA-binding protein HOW to control glial cell maturation in *Drosophila*. *Neuron* 52: 969-980.

Gloria Volohonsky, Gundula Edenfeld, Christian Klämbt, and Talila Volk (2007) Muscle-dependent maturation of tendon cells is induced by post-transcriptional regulation of stripeA. *Development* 134:347-356.

Subramanian A., Bess Wayburn, Thomas Bunch and Talila Volk. (2007) *Drosophila* Thrombospondin promotes muscle-specific interaction with tendon cells. *Development* 134: 1296-1278.

David Israeli Ronit Nir and Talila Volk. HOW binding to dpp mRNA reveals target specificity determined by both primary sequence and secondary structural features. (2007) *Development* 134: 2107-2114.

Hila Toledano-Katchalski, Gloria Volohonsky, Ronit Nir, and Talila Volk. Post-transcriptional repression of the *Drosophila* Midkine/Pleiotrophin homolog, miple1 by HOW is essential for correct mesoderm spreading. (2007) *Development* 134: 3473-348.

Talila Volk, David Israeli, Ronit Nir, and Hila Toledano-Katchalsky Tissue development and RNA control - "HOW" is it coordinated (2008), *Trends in Genetics* 2: 94-101.

Acknowledgements

Sir Ernest B. Chain Professional Chair ISF, GIF, MINERVA.

INTERNAL support

Benozio Institute for Molecular Medicine, Benozio Center for Neurological Diseases

Selected publications

Nabel-Rosen, H., Volohonsky, G, Reuveny, A., Zaidel-Bar, R., and T. Volk. (2002) Two isoforms of the *Drosophila* RNA-binding protein, How, act in opposing directions to regulate tendon cell differentiation. *Dev. Cell* 2:183-193.

Subramanian A., A.Prokop, M.Yamamoto, K.Sugimura, T.Uemura J.Betschinger, J.A.Knoblich and T.Volk (2003) Shortstop recruits EB1/APC1 and promotes microtubule assembly at the muscle-tendon junction. *Current Biol.* 13:1095.

Nabel-Rosen H., Toledano-Katchalsky H., Volohonsky G., and T. Volk. (2005) HOW represses cell divisions in *Drosophila* embryonic mesoderm by reducing string/cdc25 mRNA levels. *Current Biol.* 15:295-302

Gundula Edenfeld, Gloria Volohonsky, Karin Krukkert, Elke Naffin, Uwe Lammel, Alexandra Grimm, Daniel