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 defective (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.
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).

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.

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 CollagenXVIII (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.
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.

**Selected publications**


**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.