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Mechanisms controlling tissue differentiation in embryonic development

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The research in our lab focuses on the regulation of tissue differentiation processes during embryonic development. We would like to understand how extracellular signals produced by the environment, control tissue formation and differentiation. We have been studied the molecular basis for the differentiation of the contractile tissue in the *Drosophila* embryo and have identified a set of molecular interactions that form the basis for a muscle-dependent differentiation of tendon cells (Volk, 1999). We are currently extending our studies to address whether similar molecular mechanisms operate in muscle and glia cell differentiation (A. Reuveny and H. Toledano-Katchalski).

A major focus in the lab is the functional and molecular analysis of the RNA-binding protein Held Out Wing (HOW). The *how* gene produces two protein isoforms, HOW(L) and HOW(S), whose expression is differentially regulated in the mesoderm, tendons, and glial cells. We showed that the two HOW isoforms act in opposing directions; while HOW(L) represses mRNA levels of its targets by inducing their fast degradation, HOW(S) stabilizes the same target mRNAs leading to their up-regulation (Nabel-Rosen et al, 1999). In tendon cells HOW(L) represses the mRNA levels of a key transcription factor, Stripe, responsible for tendon cell differentiation, leading to the arrest of the differentiation program. On the other hand, HOW(S) facilitates the levels of *stripe* mRNA levels driving tendon cells to resume differentiation (Nabel-Rosen, 2002).

Recent functional analysis of *how* mutant embryos lacking both maternal and zygotic HOW indicated an essential role for HOW(L) in the arrest of cell-cycle progression during mesoderm formation. We have detected extra cell divisions in *how* mutant embryos in early embryonic stages. Further analysis indicated that the basis for these extra cell divisions stems from the up-regulation of *cdc25*/*string* mRNA levels, as deduced from *in situ* hybridization and RT-PCR analyses. Protein-RNA precipitation experiments showed that the repressor isoform How(L) binds directly to *string* 3'UTR. Taken together, our results suggest a dual regulatory mechanism by which, Twist, a major inducer of mesoderm differentiation activates the transcription of the positive cell cycle regulator *cdc25*/*string*, together with a *string* negative regulator HOW(L). The end result is a temporal arrest of mesoderm cell divisions which enables the mesodermal cells to undergo the invagination process. An additional unknown signal presumably inhibits HOW(L) activity following mesoderm invagination to enable proper cell divisions in this tissue (Nabel-Rosen et al. Submitted).

We have mapped the binding site for HOW in *stripe* 3'UTR and are currently conducting a large screen to identify more HOW target mRNAs by microarray analysis, as well as by computer search

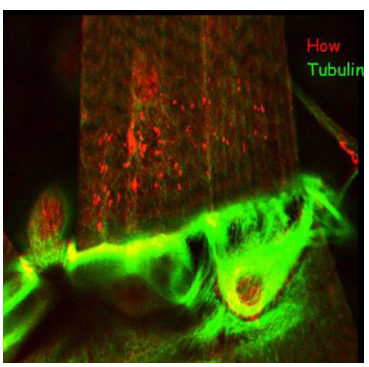

Fig. 1 *Drosophila larval tendon and muscle. Larvae flat opened, stained with anti HOW (Red) and anti Tubulin (Green), indicating the presence of small RNP particles rich with HOW protein. The tendon is highly enriched with Microtubules stained with anti tubulin. Microtubule organization in the tendon cells is regulated by Shortstop.*

for candidate genes containing the HOW binding sites (D. Israeli, H. Toledano-Katchalsi). In addition the role of Quaking, the mammalian HOW homolg is studied with regard to its inhibition of Krox20 mRNA levels in myelinating Schwann cells (A. Reuveny).

While the mechanism of HOW(L)-dependent degradation of mRNA is yet to be elucidated, we were able to elucidate the mechanism of HOW(S) facilitation of mRNA levels. Our recent studies suggest that HOW(S) is associated with the general splicing machinery and with factors involved in mRNA export, leading to the stabilization of HOW(S)-bound mRNAs. These interactions are currently functionally analyzed (Volohonsky and Volk, in preparation)

Another focus in the lab is the functional analysis of genes required for terminal differentiation of tendon cells. We have performed structure-function analysis of the large cytoskeletal protein Shortstop (previously called Kakapo, identified by our lab). Shortstop is a large, multi-domains protein involved in tendon cell maturation, as well as neuronal extensions and neuro-muscular junction formation (Strumpf and Volk, 1998). We found that Shot N-terminal domain containing the actin-binding and the plakin domains is recruited to the cytoplasmic faces of the integrin-mediated muscle-tendon hemi-adherence junction. Shot C-terminal domain containing the Gas2 and EF hands recruits EB1 to the junction domain, leading to the organization of a polarized network of microtubule arrays that connect the basal junction domain with the cuticle at the apical region. This network is essential for tendons to resist mechanical forces produced by muscle contraction (Subramanian et al., 2003). Furthermore, in collaboration with A. Prokop (Maniz University) we found that the Plakin N-terminal domain of Shot binds Paxillin, a focal adhesion phospho-protein involved in signaling and in actin polymerization. Phenotypic analysis of each mutation, and double *pax,shot* mutants suggests that Paxillin requires Shot activity to restrict neuronal extensions at the NMJ.

Additional research direction that we have taken recently is an attempt to elucidate the molecular basis for guided muscle migration, and tendon-specific adhesion. We have identified the entire set of genes that are regulated by the tendon-specific transcription factor *stripe*. These genes are currently tested for their involvement in guiding muscle migration and adhesion to tendon cells.

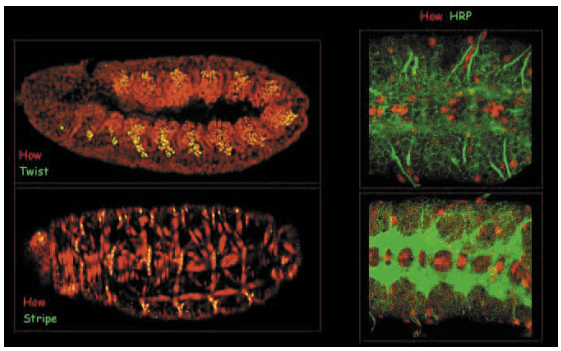

Fig. 2 *HOW is expressed in the mesoderm, tendon cells, midline and peripheral glia. The left two panels show embryos stained with anti HOW (Red) and anti Twist(Green, in the upper panel) and Stripe (Green in the lower panel). Both embryos show high levels of HOW expression in the mesoderm, muscles, and tendon cells. The right panels show two focuses of dissected CNS stained with HRP (Green) and HOW (Red).*

Selected Publications

Strumpf, D., and Volk, T (1998) Kakapo, a novel cytoskeletal-associated protein is essential for the restricted localization of the neuregulin-like factor, Vein, at the muscle-tendon junction site. *J. Cell Biol.* 143: 1259-1270.

Volk, T. (1999) Singling out *Drosophila* tendon cells: a dialogue between two distinct cell types *Trends in Genetics*, 15:448-453.

Nabel-Rosen, H, Dorevitch, N., Reuveny, A., and T. Volk. (1999) The balance between two isoforms of the *Drosophila* RNA-binding protein How controls tendon cell differentiation *Mol. Cell*, 573-584.

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.

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