**Vague hypothesizing about introns**

It is not known whether Groucho peaks arising inside genes are the result of direct recruitment of Groucho to those sites by interaction with sequence-specific transcription factors, or if Groucho is recruited to intergenic regulatory regions which are brought into contact with these introns through looping or a similar rearrangement of the local topology, resulting in immunoprecipation of these regions during ChIP-seq. However, it does appear that the interaction of Groucho with these introns is specific due to the restricted size of the intronic binding sites. Therefore, some property of the intron is directing Groucho to associate with specific points within. Whether that property is a protein interaction with another intron-associated factor, or a topological property of the surround chromatin, is unknown.

**Groucho and Dorsal-activated genes**

In contrast, recruitment of Groucho to the *twist* locus is relatively weak. Dorsal binds within the ventral activation region (VAR) directly upstream of *twist,* where it serves to activate gene expression via the cooperation of the co-activator dCBP. A small yet significant Gro peak is present within this region during the first time window, but disappears by later stages (Fig. 2-5B). While Groucho may be involved in repressing *snail* in dorsal and dorsolateral regions of the embryo, it appears *twist* repression is initiated or maintained by another, unknown, mechanism.

Dorsal is also necessary for the activation of a number of genes in ventrolateral regions of the embryo, a process that is thought to be Groucho-independent. These ventrally-activated genes include *rhomboid* (*rho*), *single-minded* (*sim*), and *short gastrulation* (*sog*) {Ip, 1992 #3042;Gonzalez-Crespo, 1993 #3043}. Loss of Gro activity was shown to result in restricted expression of these genes in 1.5 – 2 hour embryos, but did not result in significant change in expression pattern along the dorsoventral axis, so it is hypothesized that Gro is not involved in Dorsal-mediated activation of these genes {Dubnicoff, 1997 #2366}.

ChIP-seq data reveals, however, that Groucho potentially plays a role in regulating expression of these genes in some portions of the embryo (Fig. 2-6). A significant Gro peak overlaps a regulatory region termed the neuroectoderm element (NEE) upstream of *rho* in 1.5 – 4 hour embryos. The area contains multiple Dorsal, Twist, and Snail binding sites, which are required for restriction of rhomboid expression to the presumptive neuroectoderm {Ip, 1992 #3042}. The Gro peak shifts towards an adjacent CRM termed the midline element (MLE) during 4 – 6.5 hours post fertilization. It is unknown which factors bind to the MLE and would be responsible for recruitment. At both timepoints, recruitment of Groucho to the regulatory region is associated with additional binding at the TSS of *rho*. This may represent a looping of the enhancer region over the 1.5 – 2 kb intervening sequence, which is depleted for Gro, again indicative of a repressive mechanism whereby Gro interacts with or blocks assembly of the primary transcriptional machinery.