



Figure 1-figure supplement 4. AAGAG RNA transcripts originate from 2R, X and 3R heterochromatin loci and are transcribed in embryos and larval brain. **a**, Unique regions adjacent to AAGAG(n), or AAGAG(n) within AG rich regions, were identified as potential sources of satellite transcripts, as described in Materials and Methods. (+) indicates transcript containing AAGAG(n) or AG(n) blocks, while (-) indicates transcript containing CUCUU(n) or CU(n) blocks. Note that this designation of (+) and (-) strand does not preclude the possibility of rearrangements, i.e. conversion or flipping of satellite units within an array. **b**, Northern blots of L3 RNA using probes to satellite (AAGAG or CUCUU), or adjacent unique regions. Unique regions shown are those containing at least one similar band size as AAGAG RNA. (-) strand regions for chrX 22,453,019-22,453,076 and the chr3R region did not exhibit any bands with a similar size as AAGAG RNA. **c—h**, Confocal sections of embryo ventral ganglia or L3 brain lobe nuclei stained with DAPI (blue), and RNA-FISH to AAGAG (yellow) and unique region locations (magenta). 'Unique Region' (single copy sequence) RNA-FISH required Tyramide Signal Amplification (TSA), and therefore displays poorer resolution compared to AAGAG RNA (detected without TSA). Images labelled (+) used probes complementary to the strand containing AAGAG(n) or AG(n) blocks, while those labelled (-) recognize the strand containing CUCUU(n) or CU(n) blocks. Note that 'unique region' probe binds to regions adjacent to AAGAG(n) or AG(n), and not AAGAG(n) or AG(n) sequences themselves. Also note that the chr3R region indicated in 'b' was not analyzed in larvae. Only regions that co-localized with AAGAG RNA are shown. **c**, Nuclei from late embryo ventral ganglia, RNA-FISH to AAGAG and probe from genomic coordinates chr2R:1,825,641-1,825,699 (top) or chrX:12,660,096-12,660,145 (bottom). **d-h**, Confocal sections from L3 brain lobe nuclei, RNA-FISH to AAGAG and the following: **d**, Unique region chr2R:1,825,641-1825699 **e**, chr2R:1,826,691-1,826,740 **f**, chrX:11,830,844-11,830,910 **g**, chrX:12,660,096-12,660,145 **h**, chrX:22,453,019-22,453,076