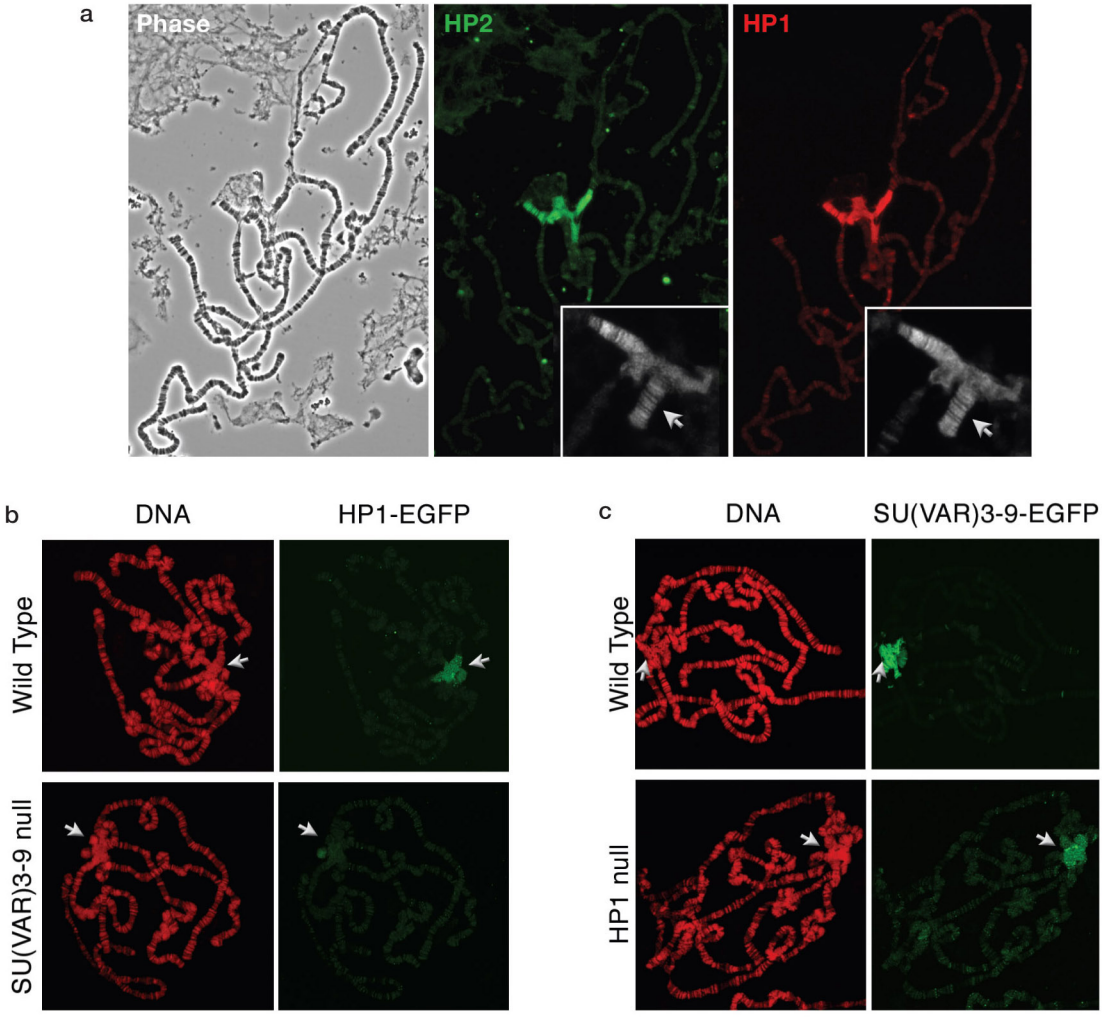

Figure 4. Immunofluorescent Staining of the Polytene Chromosomes Identifies Proteins Predominantly Associated with Heterochromatin

(*a*) The polytene chromosomes, prepared by fixation and squashing of the larval salivary gland (shown by phase contrast microscopy, *left*) are "stained" by incubating first with antibodies specific for a given chromosomal protein, and then with a secondary antibody coupled to a fluorescent tag. HP1 (*right*) and HP2 (*center*) have similar distribution patterns showing prominent association with the pericentromeric heterochromatin, small fourth chromosome (*inset*, *arrow*), and a small set of sites in the euchromatin arms. Note that the efficacy of any antibody can be affected by the choice of fixation protocol (see Stephens et al. 2003). (*b, c*) Association of HP1 and SU(VAR)3-9 with pericentromeric heterochromatin is interdependent. Mutations in *Su(var)3-9* result in a loss of HP1 from the pericentromeric heterochromatin (but not the fourth chromosome; see text) (*b*), whereas mutations in *Su(var)2-5* result in delocalization of SU(VAR)3-9 (*c*). (Adapted from Shaffer et al. 2002.)

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