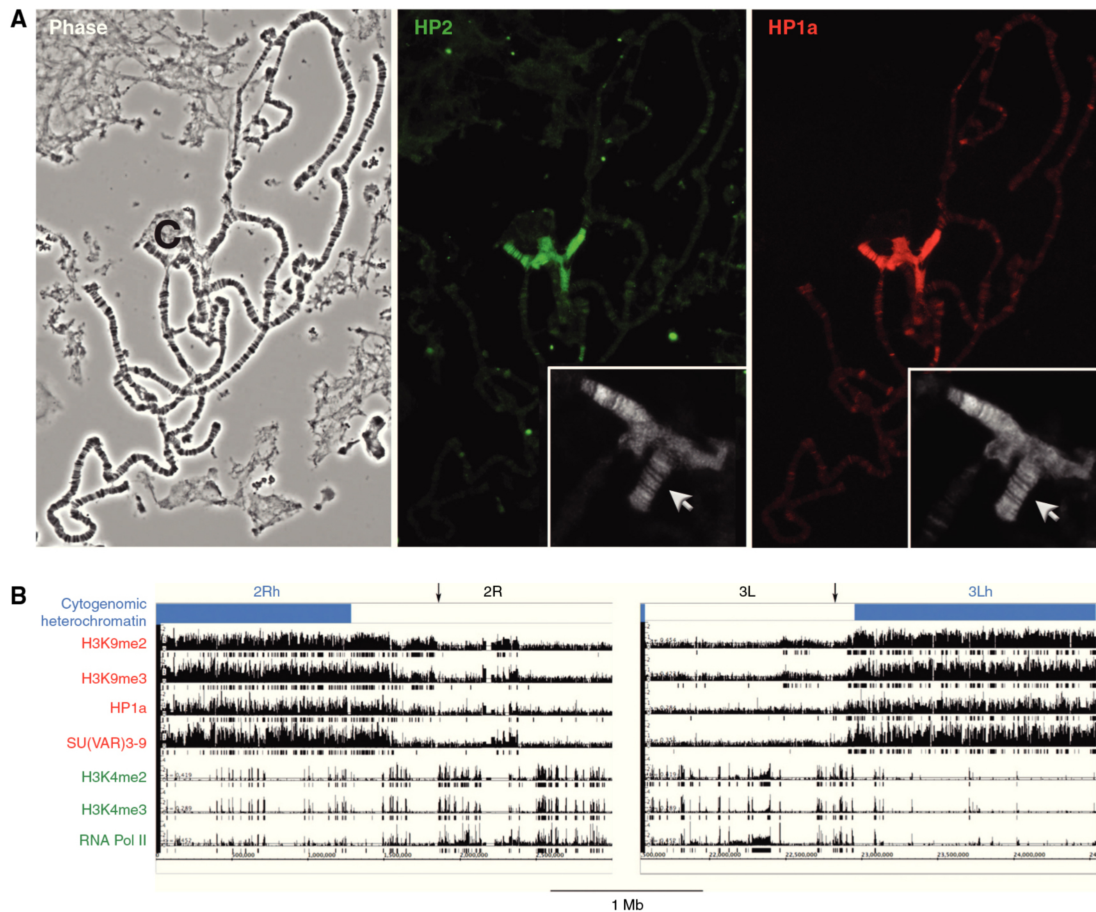

Figure 3. The distribution of chromosomal proteins and histone modifications defines different chromatin domains. (*A*) Immunofluorescent staining of the polytene chromosomes identifies proteins predominantly associated with heterochromatin. The polytene chromosomes, prepared by fixation and squashing of the larval salivary gland (shown by phase contrast microscopy, *left*; C = chromocenter) are "stained" by incubating first with antibodies specific for a given chromosomal protein, and then with a secondary antibody coupled to a fluorescent tag. HP1a (*right*) and HP2 (*center*) have similar distribution patterns, showing prominent association with the pericentric heterochromatin (found in the condensed chromocenter), small fourth chromosome (*inset*, arrow), and a small set of sites in the long euchromatin arms. (Adapted from Shaffer et al. 2002.) Note that the efficacy of any antibody can be affected by the choice of fixation protocol (see Stephens et al. 2003). (*B*) Chromatin marks define the epigenomic border between heterochromatin and euchromatin (indicated with an arrow). The border can be delineated based on chromatin immunoprecipitation (ChIP)-array data using antibodies to proteins known to be associated with heterochromatin (HP1a) or euchromatin (RNA polymerase II [RNA Pol II]), and to key histone modifications. Enrichment values are shown for the centromere-proximal 3 Mb of chromosome arms 2R and 3L (in BG3 cells). Boxes underneath the bar graphs indicate significant enrichment ($p < 0.001$). The cytologically defined heterochromatin is shown by the blue bar. The border, indicated here by the black arrow, is fairly well defined by the congruence of silencing marks. (*B*, Adapted from Riddle et al. 2011, © Cold Spring Harbor Laboratory Press.)

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