by 2D gel analysis to have weak origin activity, whereas other proORis that functioned as ARSs lacked origin function.A group of intermediate branching yeasts seem to be in the transition of losing RNAi (e.g., C. albicans236; Figure 4), since they still carry some but not all genes that encode RNAi proteins (or non-canonical Dicer gene).The S. cerevisiae and K. lactis (Figure 4) clade of budding yeasts have lost centromere- associated repeated sequences and have replaced RNAi with Silent Information Regulator (SIR) proteins that silence gene transcription in a DNA sequence-specific manner at the silent mating type loci, as well as maintaining rDNA and telomere repeats by preventing recombination238.5.1 Co-evolutionary transitions of origin specificity, gene silencing mechanisms and centromeres In species that lack DNA sequence-specific origins of replication, inherited transcriptional gene silencing can occur by RNA interference (RNAi)141.DNA sequences associated with ARS activity have been shown to either bind the initiator protein ORC, exclude nucleosomes or bind to other proteins that mediate ARS plasmid stability, such as proteins that tether plasmids to the nuclear periphery234.RNAi contributes to the silencing and stability of repetitive DNA sequences like heterochromatin satellite repeated sequences at centromeres and remnants of transposable elements, as well as organization of rDNA repeats237.In addition, the loss of RNAi and the acquisition of sequence-specific point centromeres were suggested to co-evolve with maintenance of the circular 2-micron plasmid, which is known as a selfish DNA element that uses the host segregation apparatus components for plasmid stability235.These species also have .epigenetically defined centromeres (CEN)235