Although the genetic basis of circadian behavior is well established, the biochemical basis of the oscillatory function of core clock proteins is much less understood. Circadian clocks in mammals are based on a molecular negative feedback loop in which the bHLH-PAS transcriptional activator BMAL1 is an essential clock component. BMAL1 contains a highly conserved N-terminal core domain (NCD) encompassing bHLH, PAS-A and PAS-B domains, and a C-terminal regulatory domain (CRD). Although the NCD is functionally and structurally well characterized, the function of the CRD in establishing circadian timing remains elusive. Importantly, the CRD of BMAL1 is structurally disordered and has diverged significantly from other bHLH-PAS family proteins, including its close paralog BMAL2. Through cell-based genetic complementation assays, we show that, unlike BMAL1, BMAL2 is neither necessary nor sufficient to generate cell-autonomous circadian rhythms. Through domain swapping and mutagenesis, we show that the CRD of BMAL1 holds the key to maintaining normal rhythm amplitude and period length, functionally distinguishing BMAL1 from BMAL2. We further identified the critical sequence determinants (i.e., functionally distinctive domains, motifs and even specific amino acid residues within BMAL1), particularly two helical motifs within the CRD. Finally, data from NMR spectroscopy reveal how the distinctive residues in the transactivation domain of BMAL1 participate in dynamic interactions with p300/CBP coactivators and CRY repressors. Taken together, this study provides biochemical and structural basis of the oscillatory function of the BMAL1 CRD in the negative feedback mechanism and offers important new insights into the functional evolution of bHLH-PAS transcription factors.