A crossroad of epigenetic/omic control: insights into the KAP1/ZFP57 network of factors

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Epigenetic marks shape the epigenome and are of importance in development, cell differentiation and health state of individuals. They are reprogrammed following fertilization, built during embryo development and epigenetic memory relies in their faithful inheritance. Central to these processes is the opposing action of maintenance/de novo DNA methylation and demethylation. DNMTs, Tets, modified histones tails and chromatin-associated proteins shape the epigenome of the diverse cell types playing pivotal roles in gene regulation programs, including genomic imprinting, X-chromosome inactivation, as well as genome homeostasis.

A chromatin modifying network in mammals is made by KAP1, histone methyltransferase SETDB1 (and other factors) that lay repressive histone H3 modification (H3K9me3) and heterochromatin proteins Hp1. Their DNA targeting is driven by the several hundred DNA binding zinc finger proteins endowed with the KAP1 interacting module KRAB (KRAB-ZFPs). ZFP57, a KRAB-ZFP, maintains, in mouse embryos and embryonal stem cells (ESCs), the parent-of-origin-dependent methyl allelic asymmetry of the Imprinting Control Regions (ICRs) driving imprinted genes expression. ICRs are CpG islands (CGIs) marked by both allele-specific DNA methyl imprints and “opposing” histone marks, e.g. repressive H3K9me3 and permissive H3K4me3 at methylated and unmethylated ICRs alleles, respectively.

Multiple DNA binding profiles of ZFP57, KAP1, Hp1γ and H3K9me3 were established in serum/LIF-primed ESCs showing that, apart from ICRs, unique loci, intra-genic CGIs and, among repeats, ERVK-IAPs retrotransposons (notably their various LTRs) are preeminent targets of the network. Cross-analysis showed that targets epi-marks are in part endowed with germ line methylation memory, heterogeneous, contradictory, and differentially sensitive to the methylome transitions marking distinct pluripotent ESCs states, surrogates of the early pluripotent naïve and primed epiblast, and reveal diverse DNA methyl response/maintenance at ZFP57 targeted regions. Chip-Bs-seq showed that, in vivo, ZFP57 selects DNA methylated alleles at ICRs as well as at additional targets whose methyl-CpG profiles are resistant to “primed” ESCs hypermethylation. This globally provides direct proof that DNA methylation of the copious potential targets is selective for ZFP57 association in vivo; further, while required for in vivo binding, BS methylation appears insufficient and recruitment occurs in a context and ESCs pluripotent state(s)-dependent dynamics which guides the repertoires of targeted loci. ZFP57 binding in vitro was previously shown to be dependent on asymmetric strand methylation of the TGCCGC BS and, in vivo, on loss of DNMTs activity. Targeted regions by the ZFP57/KAP1 network are endowed with extended BS (eptameric, octameric); comparative in vitro binding experiments on these highlights further and extends both the asymmetric methyl CpG contribution and the impact of DNA-methyl CH3 groups on ZF-target interaction, as supported by BS-ZF interaction modeling.

Thus, the dynamics and diversity of DNA methylation of ESCs pluripotent states combine with the potential ZFP57 BS content, driving different combinations of epigenomic signatures at distinctly methylated loci (allele asymmetric (ICRs), symmetric, heterogeneous, unmethylated) that might contribute to diversity of pluripotent ESCs state(s) and heterogeneity of ESCs populations, and highlight how they affect selection of potential targets repertoires within and between cells states/types.

References: Quenneville et al. Mol Cell. 44, 361, 2011; Baglivo et al. FEBS Lett. 587, 1474, 2013; Anvar et al., NAR 44, 1118, 2016; Riso et al. NAR. 44, 8165, 2016; Lad et al., to be submitted.
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