Genetic screening of mechanisms for gene regulation and development

in mouse haploid embryonic stem cells

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Forward genetics continues to provide opportunities for fundamental discovery in higher organisms throughout today. In particular, long generation times and few offspring of mammals have in the past restricted the potential of classical genetic approaches. These limitations notwithstanding has the relevance for medicine spurred interest in approaches to advance the molecular mechanistic understanding of mammalian genomes. Recent derivation of embryonic stem (ES) cells from haploid mouse embryos can facilitate new strategies for functional genomics studies in mice. Haploid ES cells maintain a wide developmental potential in culture and suggest applications in genetic screens for pathways that are relevant to mammalian embryogenesis.

For applying this strategy to the systematic study of gene repression at the onset of X chromosome inactivation we have established haploid ES cell lines that contain a genetically encoded selection system. Using an inducible expression system for the mouse Xist gene controlled inactivation of the single X chromosome can be achieved in haploid ES cells. Through elimination of cells with an intact silencing pathway mutations can be purified that compromise *Xist* function. Insertional mutagenesis using viral gene trap vectors and high throughput analysis of insertion sites identifies several candidates that are required for initiating gene repression by Xist. Among these candidates are RNA and DNA binding factors with prior associations to chromatin and gene regulation that facilitate a view of a developmentally relevant mammalian gene repression system. The efficient identification of mutations opens up investigations into the into this gene silencing pathway and shows the usefulness of haploid ES cells for genetic screening of pathways for gene regulation.