FEATURES

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Emerging Mechanisms of Glutathione-Dependent Chemistry in Biology and Disease

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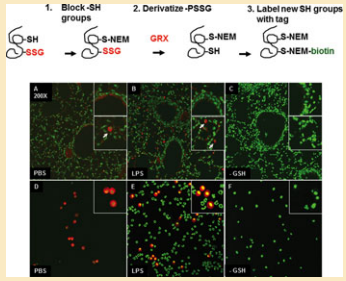
Glutathione has traditionally been considered as an antioxidant that protects cells against oxidative stress. Hence, the loss of reduced glutathione and formation of glutathione disulfide is considered a classical parameter of oxidative stress that is increased in diseases. Recent studies have emerged that demonstrate that glutathione plays a more direct role in biological and pathophysiological processes through covalent modification to reactive cysteines within proteins, a process known as S-glutathionylation. The formation of an S-glutathionylated moiety within the protein can lead to structural and functional modifications. Activation, inactivation, loss of function, and gain of function have all been attributed to S-glutathionylation. In pathophysiological settings, S-glutathionylation is tightly regulated. This perspective offers a concise overview of the emerging field of protein thiol redox modifications. Newly developed methodology to detect S-glutathionylation in situ, which will enable further discovery into the role of S-glutathionylation in biology and disease, will also be covered.



Cell-Cycle Specific Association of Transcription Factors and RNA Polymerase II With the Human β-Globin Gene Locus

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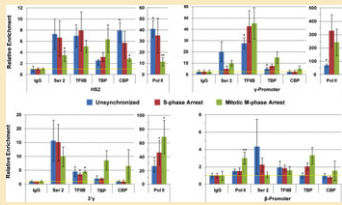
The human β-globin genes are regulated by a locus control region (LCR) and are expressed at extremely high levels in erythroid cells. How transcriptional fidelity of highly expressed genes is regulated and maintained during the cell cycle is not completely understood. Here, it is analyzed the association of transcription factor USF, the co-activator CBP, topoisomerase I (Topo I), basal transcription factor TFIIB, and RNA polymerase II (Pol II) with the β-globin gene locus at specific cell-cycle stages. The data demonstrate that while association of Pol II with globinlocus associated chromatin decreased in mitotically arrested cells, it remained bound at lower levels at the γ-globin gene promoter. During early S-phase, association of CBP, USF, and Pol II with the globin gene locus decreased. The re-association of CBP and USF2 with the LCR preceded reassociation of Pol II, suggesting that these proteins together mediate recruitment of Pol II to the β-globin gene locus during S-phase. Finally, it is analyzed the association of Topo I with the globin gene locus during late S-phase. In general, Topo I association correlated with the binding of Pol II. Inhibition of Topo I activity reduced Pol II binding at the LCR and intergenic regions but not at the γ-globin gene promoter. The data demonstrate dynamic associations of transcription factors with the globin gene locus during the cell cycle and support previous results showing that specific components of transcription complexes remain associated with highly transcribed genes during mitosis.



Normal Human Embryonic Stem Cell Lines Were Derived From Microsurgical Enucleated Tripronuclear Zygotes

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A normal fertilized human zygote contains two pronuclei, but zygotes may also display one, three, or even more pronuclei resulting from irregular insemination or meiotic division. Today diploid and triploid human embryonic stem cell (hESC) lines have been derived from tripronuclear (3PN) triploid zygotes, and an in-vitro fertilization (IVF) baby was born from a rescued diploid zygote by removing the extra male pronucleus of the 3PN zygote. However, whether hESCs can be derived from a rescued 3PN zygote is still unknown. Here, by microsurgical pronuclear removal, they restored 61 diploid zygotes from 3PN zygotes donated by 35 couples, and 11 blastocysts developed with a blastocyst rate of 18.0%, which seems higher than that of nonrescued 3PN zygotes according to previous reports. After the whole zona pellucida free embryos were plated onto feeder cells to grow and passage, 2 hESC lines (CCRM-hESC-22 and CCRM-hESC-23) were generated and both carried normal karyotype (46, XY). The hESC lines were then characterized by morphology, expansion in vitro, and expression of specific markers of alkaline phosphatase, OCT4, SSEA4, TRA-1-60 and TRA-1-81. Furthermore, the pluripotency of these 2 hESC lines was confirmed by in vitro embryoid body formation and in vivo teratoma production. This study indicates that depronucleared 3PN zygotes can improve the blastocysts formation rate, and normal hESC lines can be derived from those corrected 2PN embryos. Based on their multi-directional differentiation potential in vitro, the established hESC lines could be applied to the developmental risk assessment for IVF babies born from restored zygotes.

The TERE1 Protein Interacts With Mitochondrial TBL2: Regulation of Trans-membrane Potential, ROS/RNS and SXR Target Genes

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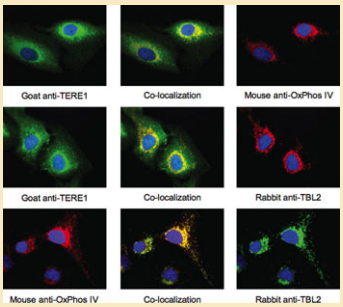

TERE1 was originally discovered as a potential tumor suppressor protein based upon reduced expression in bladder and prostate cancer specimens and growth inhibition of tumor cell lines/xenografts upon ectopic expression. Analysis of TERE1 (aka UBIAD1) has shown it is a prenyltransferase enzyme in the natural bio-synthetic pathways for both vitamin K-2 and COQ10 production and exhibits multiple subcellular localizations including mitochondria, endoplasmic reticulum, and golgi. Vitamin K-2 is involved in mitochondrial electron transport, SXR nuclear hormone receptor signaling and redox cycling: together these functions may form the basis for tumor suppressor function. To gain further insight into mechanisms of growth suppression and enzymatic regulation of TERE1 they isolated TERE1 associated proteins and identified the WD40 repeat, mitochondrial protein TBL2. They examined whether disease specific mutations in TERE1 affected interactions with TBL2 and the role of each protein in altering mitochondrial function, ROS/RNS production and SXR target gene regulation. Biochemical binding assays demonstrated a direct, high affinity interaction between TERE1 and TBL2 proteins; TERE1 was localized to both mitochondrial and non-mitochondrial membranes whereas TBL2 was predominantly mitochondrial; multiple independent single amino acid substitutions in TERE1 which cause a human hereditary corneal disease reduced binding to TBL2 strongly suggesting the relevance of this interaction. Ectopic TERE1 expression elevated mitochondrial trans-membrane potential, oxidative stress, NO production, and activated SXR targets. A TERE1-TBL2 complex likely functions in oxidative/nitrosative stress, lipid metabolism, and SXR signaling pathways in its role as a tumor suppressor.