FEATURES

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A Novel Segmentation-Based Algorithm for the Quantification of Magnified Cells 1849

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Cell segmentation and counting is often required in disciplines such as biological research and medical diagnosis. Manual counting, although still employed, suffers from being time consuming and sometimes unreliable. As a result, several automated cell segmentation and counting methods have been developed. A main component of automated cell counting algorithms is the image segmentation technique employed. Several such techniques were investigated and implemented in the present study. The segmentation and counting was performed on antibody stained brain tissue sections that were magnified by a factor of 40. Commonly used methods such as the circular Hough transform and watershed segmentation were analysed. The tests were found to over-segment and therefore over-count samples. Consequently, a novel cell segmentation and counting algorithm was developed and employed. The algorithm was found to be in almost perfect agreement with the average of four manual counters, with an intraclass correlation coefficient (ICC) of 0.8.

Novel Cell Counting Algorithm

```
clc
clear all
%Read all files into MatLab environment.
Cellfiles = dir('*tif*');
for k = 1:(numel(Cellfiles));
    K = (Cellfiles(k).name);
    a = imread(K);
    %Obtain inverse of the image.
    b = imcomplement(a);
    %Apply local histogram equalization to the image.
    c = adapthisteq(b);
    %Convert grey-scale image to logical (binary) image,
keeping
    %intensities greater than 98% of the intensities in the
image.
    d = im2bw(c, 0.98);
    %Create structuring element of 12 connected pixels.
    se = ones(12);
    %Perform an opening - suppress bright details smaller than
the
    %structuring element.
    e = imopen(d, se);
    %Apply imoverlay function to
    %highlight cells.
    overlay = imoverlay(a, e, [.3 1 .3]);
    %Count 8-connected components within the image.
    [e, z] = bwlabel(e, 8);
    z;
    %Create 'Cell_Count.txt', a file used to display the
results of the
    %counting for each image.
    fid = fopen('Cell_Count.txt', 'a');
    fwrite(fid, K, 'char*1', 'native');
    fprintf(fid,'__has signal count %5.0f\n', z);
end
```


Activation of Muscle Enhancers by MyoD and Epigenetic Modifiers 1855

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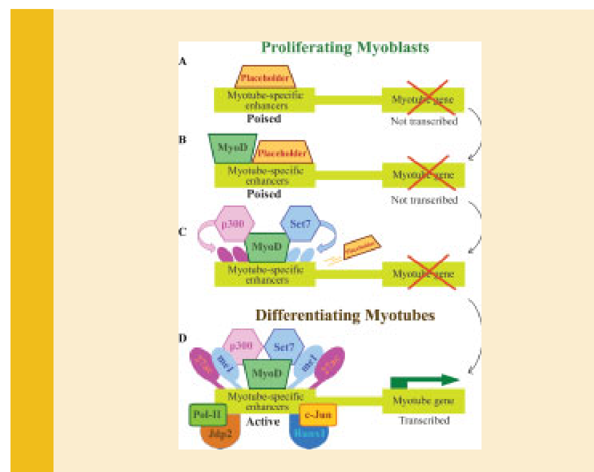
The early 1980s revelation of *cis*-acting genomic elements, known as transcriptional enhancers, is still regarded as one of the fundamental discoveries in the genomic field. However, only with the emergence of genome-wide techniques has the genuine biological scope of enhancers begun to be fully uncovered. Massive scientific efforts of multiple laboratories rapidly advanced the overall perception that enhancers are typified by common epigenetic characteristics that distinguish their activating potential. Broadly, chromatin modifiers and transcriptional regulators lay down the essential foundations necessary for constituting enhancers in their activated form. Modulation of muscle enhancers is suggested to be coordinated via transcription factors docking, including c-Jun and Jdp2 that bind to muscle enhancers in a MyoD-dependent manner. Distinct transcription factors may act as placeholders and mediate the assembly of newly formed myogenic enhancers.



Journal of Cellular Biochemistry

Changes in Chromatin Structure in NIH 3T3 Cells Induced by Valproic Acid and Trichostatin A 1937

Marina Barreto Felisbino, Maria Silvia Viccari Gatti, and Maria Luiza S. Mello

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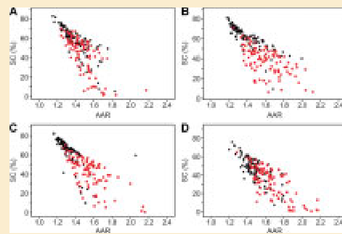

Valproic acid (VPA) and trichostatin A (TSA) are known histone deacetylase inhibitors (HDACIs) with epigenetic activity that affect chromatin supra-organization, nuclear architecture, and cellular proliferation, particularly in tumor cells. In the following study, chromatin remodeling with effects extending to heterochromatic areas was investigated by image analysis in non-transformed NIH 3T3 cells treated for different periods with different doses of VPA and TSA under conditions that indicated no loss of cell viability. Image analysis revealed chromatin decondensation that affected not only euchromatin but also heterochromatin, concomitant with a decreased activity of histone deacetylases and a general increase in histone H3 acetylation. Heterochromatin protein 1-α (HP1-α), identified immunocytochemically, was depleted from the pericentromeric heterochromatin following exposure to both HDACIs. Drastic changes affecting cell proliferation and micronucleation but not alteration in *CCND2* expression and in ratios of *Bcl-2/Bax* expression and cell death occurred following a 48-h exposure of the NIH 3T3 cells particularly in response to higher doses of VPA. The results demonstrated that even low doses of VPA (0.05 mM) and TSA (10 ng/ml) treatments for 1 h can affect chromatin structure, including that of the heterochromatin areas, in non-transformed cells. HP1-α depletion, probably related to histone demethylation at H3K9me3, in addition to the effect of VPA and TSA on histone H3 acetylation, is induced on NIH 3T3 cells.

Two Distinct Nuclear Localization Signals in Mammalian MSL1 Regulate Its Function 1967

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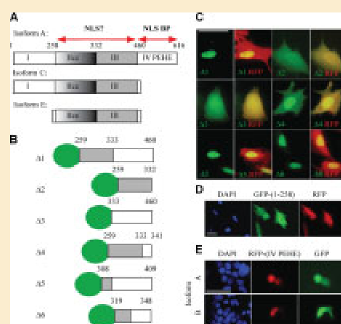

MSL1 protein regulates global histone H4 acetylation at residue K16 in stem and cancer cells, through interaction with KAT8. The functional significance of mammalian MSL1 isoforms, involved in various protein interactions, is poorly understood. The authors identify a novel nuclear localization signal (NLS), common to all MSL1 isoforms, in addition to previously known bipartite NLS, located in domain PEHE. Isoforms having both NLS localize to sub-nuclear foci where they can target co-chaperone protein TTC4. However, all MSL1 isoforms also have ability to affect H4K16 acetylation. Thus, presence of two NLS in MSL1 protein can mediate activity of KAT8 in vivo.