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Technical note

Quantification of chromatin condensation level by image processing

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ABSTRACT

The level of chromatin condensation is related to the silencing/activation of chromosomal territories and therefore impacts on gene expression. Chromatin condensation changes during cell cycle, progression and differentiation, and is influenced by various physicochemical and epigenetic factors.

This study describes a validated experimental technique to quantify chromatin condensation. A novel image processing procedure is developed using Sobel edge detection to quantify the level of chromatin condensation from nuclei images taken by confocal microscopy. The algorithm was developed in MATLAB and used to quantify different levels of chromatin condensation in chondrocyte nuclei achieved through alteration in osmotic pressure. The resulting chromatin condensation parameter (CCP) is in good agreement with independent multi-observer qualitative visual assessment. This image processing technique thereby provides a validated unbiased parameter for rapid and highly reproducible quantification of the level of chromatin condensation.

1. Introduction

The cell nucleus is a double membrane bound organelle containing genetic information stored in form of deoxyribonucleic acid (DNA). Many essential functions take place in the nucleus, such as the initial steps of gene expression including messenger ribonucleic acid (mRNA) transcription, DNA replication, repair, and recombination, as well as the production of ribosomal RNA (rRNA). The DNA in a eukaryotic cell nucleus is grouped into chromosomes, each containing a linear DNA molecule associated with proteins, such as the histones, that fold and pack the DNA into a more compact structure called chromatin.

Chromatin can be classified into heterochromatin or euchromatin, based on the condensation level during interphase. Euchromatin consists of less condensed chromatin, which is typically transcriptionally active [1] and often located towards the interior of the nucleus. By contrast, heterochromatin is more condensed, generally associated with gene silencing [2,3] and often located at the nuclear periphery, around the nucleolus, and as patches throughout the nucleoplasm [4,5]. Chromatin condensation often correlates with the level of cell differentiation with a more decondensed chromatin organisation found in stem cells compared to the localised condensed regions found in the nuclei of differentiated cells [6]. Chromatin also condenses prior to cell division and apoptosis [7]. Additionally, chromatin condensation can be induced by physicochemical stimuli, such as the addition of multivalent cations [8,9], and alteration in osmolality [10–13].

It is therefore useful to be able to assess the degree of chromatin condensation using quantitative parameters. In most previous studies, chromatin condensation is typically assessed qualitatively by visual observation of microscopic images of the nucleus. Meanwhile, some studies assessed the level of chromatin condensation quantitatively via Fluorescence Recovery After Photobleaching (FRAP) analysis of H2B [14] or HP1 mobility [15], a protein present in condensed chromatin [16]. Here, we describe a novel procedure to quantify the level of chromatin condensation by processing of fluorescence or confocal microscopy images based on an edge detection algorithm.

2. Methods and results

2.1. Cell preparation

Primary bovine chondrocytes were isolated from full depth cartilage of the metacarpo-phalangeal joints of freshly sacrificed eighteen to twenty four months old bovine via a series of enzymatic digestions, as previously described [17]. Freshly isolated chondrocytes were seeded onto 175 cm^2 culture flasks (8×10^6 cells/flask) and cultured for 5 days in low glucose Dulbecco's modified Eagle's medium (DMEM) to ensure the adhesion of the cells. The media was supplemented with 20% foetal calf serum (FCS), 20 mM HEPES, 100 U ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin, 2 mM L-glutamine and 0.85 mM L-ascorbic acid (all from Sigma–Aldrich, Dorset, UK). For the H5 murine chondrocyte cell lines [18], the cells were also seeded at the same density onto 175 cm^2 flasks and cultured in

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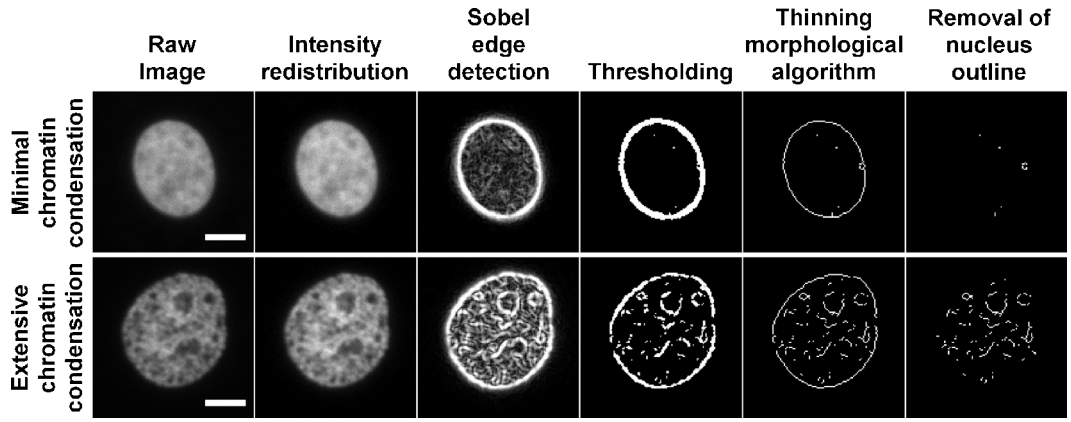

Fig. 1. Images of two separate nuclei with minimal (top) and extensive (bottom) chromatin condensation, showing the results of each image processing step leading to the calculation of the chromatin condensation parameter (CCP) (bar: 5 μm). From this quantification, the nucleus with minimal condensed chromatin has a CCP of 0.16%, compared to 4.86% for the extensively condensed chromatin.

DMEM/F12 media supplemented with 15 mM HEPES (Invitrogen, Paisley, UK) and 10% FCS. The cultured cells were trypsinised and seeded onto FCS coated coverslips, housed in 24 well plates, with a seeding density of 3×10^4 cells cm^{-2}.

In order to obtain nuclei with different degree of chromatin condensation, the cells were subjected to osmotic challenge by incubation medium with osmolalities in the range 100–800 mOsm kg^{-1} for 15 min to allow for chromatin reorganisation[10]. We have previously shown that this produces a rapid alteration in chromatin condensation [13]. The standard culture media had an osmolality of 320 mOsm/kg, which was increased by the addition of D-Mannitol (Sigma–Aldrich) and was reduced by the dilution with sterile distilled water.

2.2. Fluorescent staining and confocal microscopy

Cells were washed with osmotically balanced PBS and fixed by incubation in osmotically balanced fixative, 1% glutaraldehyde (Agar Scientific, Stansted, UK) buffered with 8 mM sodium cacodylate (Sigma–Aldrich), for 30 min. The specimens were subsequently washed with PBS and stained with 8 μM Hoechst 33342 (Sigma–Aldrich) for 15 min in 37 °C. The specimens were washed with sterile distilled water and mounted onto glass slides by using Prolong Gold (Invitrogen, Paisley, UK). The chondrocyte nuclei were imaged using confocal microscopy (Leica TCS SP2, Leica Microsystems GmbH, Wetzlar, Germany), using a 63×/1.4NA oil immersion objective at a resolution of 46.5 nm/pixel (512 × 512 pixels per image) and an imaging period of 7.68 s $image^{-1}$. Intensity levels for each pixel were recorded on an 8 bit scale (0–255).

2.3. Quantification of chromatin condensation parameter

The condensation of chromatin increases the number of distinct spaces within the nucleus. This is detected by the Sobel edge detection algorithm [19]. Thus, measuring the density of edges within the nucleus, normalised to its cross-section area, gives a measure of the level of chromatin condensation defined as the chromatin condensation parameter (CCP).

In order to calculate this parameter, images of nuclei obtained by confocal microscopy were subjected to a series of transformations as shown in Fig. 1 for two separate nuclei with different degrees of chromatin condensation. First, to standardise the image, the intensity profile was redistributed by dividing each pixel intensity by the maximum intensity of the image and multiplying by 255 (the maximum intensity for 8-bit image). This was followed by the edge detection procedure using Sobel edge detection, a gradient based algorithm where a gradient of consecutive pixels is approximated in X and Y direction. Sobel edge detection uses two 3×3 kernels to approximate the gradients between a pixel and the 8 surrounding pixels and its principle can be described as follow:

$$S_x = \begin{bmatrix} +1 & 0 & -1 \\ +2 & 0 & -2 \\ +1 & 0 & -1 \end{bmatrix} \quad \text{and} \quad S_y = \begin{bmatrix} -1 & -2 & -1 \\ 0 & 0 & 0 \\ +1 & +2 & +1 \end{bmatrix} \tag{1}$$

$$G_x = S_x \times I \tag{2}$$

$$G_y = S_y \times I \tag{3}$$

$$G = \sqrt{G_x^2 + G_y^2} \tag{4}$$

where S_x is Sobel kernel for the approximation of gradient in X direction, S_y is Sobel kernel for the approximation of gradient in Y direction, G_x is the approximated gradient in X direction, G_y is the approximated gradient in Y direction, I is the target image matrix and G is the approximated gradient magnitude. The resulting gradient magnitude is then used to represent the processed pixel of interest in the target image. This process is repeated for each pixel within the target image. The final step of the Sobel algorithm produces a new image showing the level of edges based on the gradient magnitude.

Chromatin condensation increases the number of distinct spaces within the nucleus, creating sudden dips of intensity across the nucleus image. These sudden dips of intensity are detected by the Sobel algorithm as strong edges. Hence, the increase of chromatin condensation level is directly correlated to the higher number of strong edges within the nucleus. The Sobel image produced was thresholded to acquire the strong edges.

In order to count the number of strong edges, the thresholded Sobel image underwent a thinning algorithm [20], which transforms an entity within the image into a single pixel thickness entity. Due to the marked difference in intensity between the imaged nucleus and the background, the Sobel filter generates an artificial edge at the boundary of the nucleus. This artificial edge should be removed, as they do not represent the level of chromatin condensation. With the removal of the nucleus outline, the number of edges was measured and dividing by the area of the nucleus gives the edge density, i.e. the CCP.