Intranuclear localization of transcription factories and immunoglobulin heavy chain gene alleles during human B-cell maturation

A.V. Pichugin¹,²,³, O. V. Iarovaia¹,²,⁴, I. V. Sklyar¹,²,⁴, G. Lacombe⁵, S. V. Razin²,⁴,⁶, T. Fest⁵, M. Lipinski¹,², Y. S. Vassetzky¹,²,⁶

¹ CNRS UMR 8126, Université Paris-Sud 11, Institut Gustave Roussy
114, rue Edouard Vaillant, Villejuif, France, 94805
² LIA 1066 French-Russian Joint Cancer Research Laboratory
Villejuif, France–Moscow, Russian Federation
³ Peter the Great St. Petersburg Polytechnic University
29, Polytechnicheskaya Str, St.Petersburg, Russian Federation, 195251
⁴ Institute of Gene Biology, Russian Academy of Sciences
34/5, Vavilova Str., Moscow, Russian Federation, 119334
⁵ INSERM U917, Université de Rennes
2, avenue du Professeur Léon Bernard F - 35043 Rennes, France
⁶ Faculty of Biology, M. V. Lomonosov Moscow State University
Leninskie Gory, Moscow, Russian Federation, 119991
vassetzky@igr.fr

Immunoglobulin heavy chain (IGH) gene locus is expressed monoallelically in human B cells. **Aim.** To study the role of nuclear organization in regulation of the IGH expression during B-cell differentiation. **Methods.** Immuno-fluorescence in situ hybridization on 3D-preserved nuclei (3D immuno-FISH). **Results.** Active RNA polymerase II (Pol II) molecules and the IGH locus were detected in the periphery of the nucleoli at some stages of B-cell differentiation. **Conclusions.** We observed significant changes in the pattern of distribution of RNA polymerase II in the nucleus during B-cell differentiation, but no preferential co-localization of the productive IGH allele with the transcription factories in the vicinity of the nucleolus and in the nucleoplasm was observed.

**Keywords:** Immunoglobulin heavy chain gene, transcription, nucleolus, B-cell maturation

**Introduction**

Nucleus is divided into transcriptionally active and repressive compartments (reviewed in [1]). Transcriptional activation is associated with the euchromatic regions of the nucleus and transcriptional factories (reviewed in [2]). A functional immunoglobulin heavy chain is produced monoallelically (Rajewsky 1996); it undergoes regulated transcription during the B lymphocyte development. Transcription might be controlled by localized alterations in the chromatin structure. Whether such a mechanism is sufficient to account for the allele-specific transcription of the IGH locus is currently unknown. In mice, the localization of productive and non-productive IGH alleles changes during B-cell maturation. A non-productive IGH allele is located close to the nuclear periphery whereas the productive IGH allele is associated with euchromatin [5–7]; much less is known about the role of nuclear organization in the human IGH allelic transcription. We have recently found that the IGH locus was located...
close to the nucleolus in both naïve and activated B-cells [8] as well as in B-cell lymphoma [9]. However, the functional significance of this localization remains unknown.

Given the evidence that the eukaryotic gene activity can be regulated by subnuclear compartmentalization (reviewed in [10]), we investigated whether the perinucleolar localization of the IGH locus might play a role in controlling its transcription. Here we have used 3D-fluorescence in situ hybridization (3D-FISH) combined with immunofluorescence to co-detect the localization of the IGH alleles, nucleoli and transcription factories at different stages of B lymphocyte maturation.

Materials and Methods

In vitro B-cell differentiation

Peripheral blood cells from healthy volunteers were purchased from the French Blood Center. B-cells at different stages of differentiation were obtained essentially as described in [11]. Plasmocytes were isolated from peripheral blood using the CD138+ Plasma Cell Isolation Kit (Miltenyi Biotech).

Three-dimensional fluorescence in situ hybridization (3D-FISH) and immuno-detection

3D FISH experiments were essentially carried out as described elsewhere [9]. The stained probes used in this study (RP11-346120 that recognizes the constant region of the IGH locus and RP11-259B19 for the J/D region) were purchased from Blue Gnome (Cambridge, UK). Nucleoli were detected using mouse anti-B23 antibody (Sigma, St Louis, MO) and chicken anti-mouse Alexa 647 (Molecular Probes, Carlsbad, CA). The Pol II antibodies were from Active Motif, Carlsbad, CA. The images were processed and analyzed as described in [9].

Results and Discussion

In order to study whether the IGH localization vs. transcription factories undergoes dynamic changes linked to its functional status, we have followed the localization of the IGH alleles in human cells during B-cell maturation in vitro [11]; this system allows reproducing the major stages of B-cell differentiation in lymph nodes. We used B-cells at the following stages: naïve B-cells (Day0), activated B-cells (Day1), cells at the stage of plasmoblast differentiation (Day4), and cells at the stage of somatic hypermutation and class switch recombination (Day5/6).

The IGH locus being located close to a nucleolus at some stages of B-cell maturation [8], we wondered whether its transcription would actually take place in the perinucleolar region which, with some exceptions [9] is not known for being prone to [the] RNA polymerase II (Pol II)-dependent gene transcription [12]. To test this, B-cells were stained using the B23 antibody that decorates nucleoli and the antibody to the phosphorylated C-terminal domain of the transcriptionally-active form of Pol II. These active Pol II molecules are present in nuclear foci called transcription factories, where active transcription takes place (for review see [2]). Typical Pol II staining patterns are presented in Fig. 1. Uniform staining of active Pol II molecules was detected in the immediate vicinity of nucleoli in naïve B lymphocytes at Day0. Activation of B-cells at Day1 led to a decrease in Pol II staining with transcription factories being organized in clusters, some of them adjacent to the nucleolus. At Day4, Pol II was excluded from the perinucleolar regions; at Day5/6, this exclusion zone disappeared and intense Pol II staining surrounded the nucleolus. Finally, in plasmocytes, Pol II staining concentrated in the center of the nucleus, with some staining around the nucleolus (Fig. 1).

Next, the proximity between the IGH alleles and Pol II foci at different stages of B-cell differentiation was evaluated using an immuno-FISH approach whereby nucleoli, Pol II clusters, and the productive IGH alleles were all simultaneously visualized. [The] Signals from the productive IGH allele (green), and Pol II (white) were scored in relation to their proximity to the nucleolus (blue). The results are summarized in Fig. 2. These data indicate that the productive IGH allele is preferentially colocalized with the transcription factories at the nucleolus in naïve B-lymphocytes at Day0 and at the SHM and CSR stages at Day5/6.
B-cells are monospecific, i.e. they produce one antibody per cell. This is due to allelic exclusion of immunoglobulin (Ig) heavy (H) and light (L) chain genes established during V(D)J recombination (reviewed in [13]). While the somatic generation of functional Ig genes by V(D)J recombination is a subject to allelic exclusion, the expression of Ig loci per se does not appear to be monoallelic as Ig transcripts are expressed from both alleles whereas only one of the two Ig alleles is functional [14]. The molecular and cellular mechanisms regulating allelic exclusion during V(D)J recombination are well studied [15], much less is known on how these mechanisms operate at the final stages of B-cell differentiation. DNA methylation [16], chromatin organization [17] and subnuclear localization [5–8] might contribute to this process.

The positioning of one allele in the vicinity of a nucleolus, a statistically significant difference be-
tween the two alleles, may be linked to the IGH expression. Indeed, transcriptionally active Pol II clusters could be observed in the vicinity of the nucleolus in lymphoid cells [9]. It is also known that nuclear factors localized in and around the nucleolus, such as nucleolin, are essential for [the] IGH transcription [18]. Using immuno-FISH experiments, we analyzed the localization of the two alleles with clusters of transcriptionally active Pol II molecules (transcription factories, see [2] for review). We could not observe any preferential localization of the productive IGH allele with the transcription factories in the vicinity of the nucleolus and in the nucleoplasm. At the same time, the pattern of distribution of RNA polymerase II changed during B cell maturation, with the most striking feature being the exclusion of active RNA Pol II molecules from the perinucleolar region in activated B cells at Day4. We have also detected the presence of active Pol II clusters in the periphery of nucleoli that in normal cells is mostly occupied by heterochromatin [12] and therefore is not prone to the Pol II transcription. Further studies are necessary to establish a link between transcriptional activity and subnuclear organization of the IGH alleles.

Acknowledgements

This work was supported by the grant #14-24-00022 Russian Science Foundation (RSF).

REFERENCES

Intranuclear localization of transcription factories and $IGH$ gene alleles during human B-cell maturation

A. V. Pichugin, O. V. Ярова, I. V. Скляр, G. Лякомб, S. V. Разин, T. Фест, M. Липинский, E. С. Васецкий

Лишь один аллель гена тяжелой цепи иммуноглобулина (IGH) экспрессируется в Б-клетках человека. Цель. Изучить роль ядерной организации в регуляции экспрессии IGH в процессе дифференцировки B-клеток. Методы. Иммунофлюоресцентная гибридизация in situ (3D immuno-FISH). Результаты. Активная форма РНК-полимеразы II (Pol II) и ген IGH были обнаружены на периферии ядрышек на некоторых стадиях дифференцировки В-клеток. Выводы. Мы наблюдали значительные изменения в характере распределения РНК-полимеразы II в ядре во время дифференцировки В-клеток. При этом, не наблюдалось преимущественной локализации продуктивного аллеля IGH с транскрипционными фабриками ни в непосредственной близости от ядрышек, ни в нуклеоплазме.

Ключевые слова: гены тяжелых цепей иммуноглобулина, транскрипция, ядро, дозревание B-клеток

Received 14.02.2016