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Gene gating: A hypothesis

(discrete three-dimensional structures of genome/omnipotent three-dimensional structure of zygotic genome/nuclear pore complexes and lamina/development and differentiation/cell asymmetry and polarity)

GÜNTER BLOBEL

Laboratory of Cell Biology, The Rockefeller University, New York, NY 10021

Contributed by Günter Blobel, August 19, 1985

ABSTRACT **It is assumed that the genome of a higher eukaryotic organism is organized into a number of distinct three-dimensional (3-D) structures, each characteristic for a given differentiated state. These discrete 3-D structures are envisioned to develop in a hierarchical and largely irreversible manner from an omnipotent 3-D structure of the zygotic genome. The information for these processes is assumed to reside in the genome. The nuclear pore complexes, the peripheral nuclear lamina, and components of the nuclear core are proposed to be among the topologically most proximal organelles that interpret this information and thereby serve in the maintenance and the alteration of the 3-D structure of the genome during development, differentiation, and the cell cycle. The nuclear pore complexes are envisioned to serve as gene-gating organelles capable on interacting specifically with expanded (transcribable) portions of the genome. Their nonrandom distribution on the nuclear surface would reflect the underlying periodic organization of the genome into expanded and compacted domains, alternating with each other. All transcripts of a given gated gene would leave the nucleus by way of that pore complex to which the gene is gated. Implications for cell asymmetry and polarity are discussed and evolutionary considerations are presented.**

ASSUMPTIONS

Distinct Three-Dimensional (3-D) Structure of Genome. The DNA double helix (2 nm) is wound around histone cores to form an 11-nm "beads (nucleosomes) on a string" fiber, which, in turn, is arranged into a 30-nm chromatin fiber consisting of packed nucleosomes in a helical array. The 30-nm chromatin fiber is thought to be folded into higher-order structures of more (compacted) or less (expanded) condensed chromatin (1). Along a chromosomal DNA molecule in G_1 phase, expanded domains alternate with compacted domains, presumably representing transcribable euchromatin and nontranscribable heterochromatin, respectively. Within the global confines of the nucleus, the expanded and compacted domains of each chromosome are three-dimensionally coordinated with those of other chromosomes to yield a genomic 3-D structure. The extent of expansion and compaction as well as the location of the expanded and compacted domains along the chromosomal DNA can be expected to vary among differentiated cells of a complex multicellular organism. Therefore, a number of distinct 3-D structures, each characteristic for a given differentiated state (cell), are assumed to exist. All cells of a multicellular organism that are in an identical differentiated state and in an identical phase of the cell cycle are assumed here to possess an identical 3-D structure of their genome.

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3-D Structures Undergo Changes During Cell Cycle. The distinct 3-D structure of the genome in G_1 phase is likely to undergo changes as that cell passes through S and G_2 phases. In mitosis the chromatin fiber is supercompacted into the arms of the metaphase chromosome. On the microscopic level, the shape of a given chromosome from any of the differentiated cells of an organism appears to be identical. This poses a dilemma: the large number of *distinct* 3-D structures assumed to exist for the large number of differentiated states of a complex multicellular organism would have to be condensed into a *common* supercompacted 3-D structure and vice versa; the numerous distinct 3-D structures in G_1 phase would have to evolve from a common 3-D structure of metaphase chromosomes. To resolve this dilemma it is assumed that the distinct 3-D structure of a G_1-phase chromosome in a given differentiated cell is reflected in a distinct (submicroscopic) 3-D structure of the metaphase chromosomes of that cell. Moreover, the changes in the 3-D structure during the various phases of the cell cycle are assumed to be identical for all cells of the same differentiated state.

Development of Distinct 3-D Structure in Ontogeny. Upon fertilization, the genomic 3-D structures of the male and female pronuclei would accommodate each other to form a diploid 3-D structure. Because of genotypic differences among individuals of a given species, the genomic 3-D structures of the gametes and of the resulting zygotes will not be identical, though they can be expected to be similar. The distinct 3-D structure of a given zygote would represent an ontogenetically primordial and structurally omnipotent 3-D structure from which all of the other distinct 3-D structures of the *somatic* and *germ* cells of the mature individual would evolve in ontogeny in a hierarchical and largely irreversible manner. In the case of the germ cells these hierarchical changes would return the 3-D structure of the genome to a "ground state" haploid 3-D structure (different for male and female gametes) that is competent to form the primordial and omnipotent 3-D structure of the zygote. These zygotic 3-D structures would have evolved in phylogeny to be unique to each species and similar, albeit not identical, in all zygotes of a given species.

PROPOSALS

It is clear that, ultimately, the information for assuming distinct 3-D structures must reside in DNA, not only for the distinct 3-D structure of the gametic and zygotic genomes but also for the hierarchical development during ontogeny of the numerous distinct 3-D structures in the mature individual. Introns, pseudogenes, and "junk" DNA can be expected to contribute significantly to the overall information on which the 3-D structure depends.

However, it is also clear that, by itself, DNA could never assume the postulated distinct 3-D structures of the zygote nor could it, by itself, undergo the postulated hierarchical changes in ontogeny. Nuclear pore complexes, the peripheral

Abbreviation: 3-D, three-dimensional.

nuclear lamina, and yet undefined elements of the nuclear core are proposed to serve as organelles that would interpret information in the DNA and would thereby constitute topologically proximal organizers of the genome's 3-D structure.

Nuclear Pore Complexes Organize Expanded Genes. Nuclear pore complexes are large uniform organelles (estimated mass, $\approx 10^8$ daltons) that are associated with the nuclear envelope of all eukaryotic cells. They duplicate during S phase of the cell cycle (2). Macromolecular traffic in and out of the nucleus is likely to proceed entirely through the nuclear pore (3). It is proposed that expanded domains of the genome containing transcribable genes are attached to the pore complex. Attachment may be specified by a distinct DNA sequence (or domain) that is common to all genes (or sets of genes). However, only after this sequence is modified would it acquire competence for attachment, thereby distinguishing attachment-competent from attachment-incompetent genes (sets of genes). Attachment would be mediated by a DNA binding subunit of the nuclear pore complex that, in turn, is identical in—and common to—all nuclear pore complexes. Because of its 8-fold symmetry, each pore complex may contain eight such DNA binding subunits, accommodating eight genes (or sets of genes). A circumscribed space subjacent to the nuclear pore complex and extending into the interior of the nucleus in the form of channels is envisioned to serve as the locale where transcription and much of the co- and posttranscriptional processing would occur. An attachment of expanded (transcribable) genes to the nuclear pore complex would not only serve as an important 3-D coordination point for the genome but also would gate expanded genes to the cytoplasm, facilitating entry of cytoplasmically synthesized and assembled components and exit of all nuclearly synthesized and assembled products. Thus, that only transcribed genes are sensitive when isolated nuclei are subjected to DNase treatment (4) may be due not only to their expanded configuration but also to facilitated accessibility (by way of gating to nuclear pore complexes).

Another important characteristic of nuclear pore complexes is their nonrandom localization on the surface of the nucleus (5, 6). This nonrandom localization is proposed to mirror the underlying periodic organization of the genome: alternating expanded and compacted domains of the genome would be located beneath the pore and the interpore region, respectively.

Finally, nuclear pore complexes consist of morphologically distinguishable substructures (7, 8). In mitosis, the nuclear pore complex can no longer be detected as a morphological entity, presumably because it is disassembled into subunits. A DNA binding subunit(s) of the nuclear pore complex (see above) might dissociate from the organelle during mitotic compaction in pro- and metaphase and remain part of the compacted metaphase chromosome. By virtue of a location on the surface of metaphase chromosomes, these subunits would serve to tag those genes that are slated for expansion later on, in telophase and G_1 phase. Other subunits of the pore complex would remain associated with the vesiculated remnants of the nuclear envelope. Still others may become soluble components. In telophase and early G_1 phase, the differential expansion of the genome would position the chromatin binding subunits such that contact could be established with the other subunits, yielding, in a cooperative manner, reconstitution of the nuclear pore complex and the nuclear envelope. Reassembly of the pore complex would thereby ensure the gating of all expanded genes and would reestablish the 3-D structure of the genome. Thus, the nuclear pore complexes would serve as essential equipment in the maintenance and the propagation to daughter cells of a differentiated 3-D structure of the genome.

However, they would also serve in interpreting the information for the hierarchical alteration of the 3-D structure in ontogeny and differentiation. Obliteration of previous sites or generation of new sites in the DNA for attachment to DNA binding subunits of nuclear pore complexes (see above) would gate new sets of genes, at the expense of others or in addition to others. In addition to these *irreversible* changes in gene gating during differentiation, there might also be *reversible* changes that occur in differentiated cells.

It should be emphasized that gating of genes to a pore complex, although necessary, is not sufficient for transcription to occur. Soluble factors might associate with control regions of the gated genes to prevent transcription.

The Nuclear Lamina Participates in the Organization of the Compacted Genes. The nuclear lamina is intercalated between chromatin and the inner membrane of the nuclear envelope. It extends over the entire nuclear surface except for circular regions subjacent to the nuclear pore complexes (9, 10). In mammalian cells the lamina has been shown to be a polymeric structure consisting primarily of three proteins, termed lamins A, B, and C (11). It is proposed that the lamina participates in the organization of the compacted portion of the genome.

During mitosis the lamina disassembles (11–13). Lamins A and C become soluble monomers, whereas lamin B remains associated with the vesiculated remnants of the nuclear envelope (11). In telophase and early G_1 phase, the depolymerized lamins A and C would reassociate with a hypothetical marker on the surface of the compacted chromatin, on the one hand, and with membrane-bound lamin B, on the other hand. Polymerization of the lamins would then affix the compacted chromatin to the inner nuclear envelope membrane (14).

In addition to the peripheral nuclear lamina, other components located in the nuclear core (16, 17) are likely to play important roles in the maintenance and alteration of compacted and expanded genes.

Evolutionary Considerations. The prokaryotic genome (as well as that of mitochondria and chloroplasts) is attached to the prokaryotic plasma membrane (or to the inner mitochondrial and chloroplast membrane, respectively). The number of attachment sites is unknown. It has been suggested previously that in precellular evolution (15) membrane attachment sites of DNA played a role as gene-capturing devices.

It is proposed that these DNA attachment sites impose a nonrandom 3-D structure onto the prokaryotic genome as well. During evolution to eukaryotic cells, these DNA attachment sites would have been removed from the prokaryotic plasma membrane and delegated to the nuclear envelope, where they evolved into pore complexes and the lamina (15).

A nonrandom 3-D structure of the genome in prokaryotes (maintained by DNA attachment to the prokaryotic plasma membrane) might be responsible for establishing cellular asymmetry and polarity (18). Because translation is coupled to transcription in prokaryotes, a locally transcribed and translated gene might yield a high local concentration of a given protein in regions of either the cytoplasm or the membrane. In turn, such high regional concentrations may be determinants for cellular asymmetry.

Some lower eukaryotes undergo "closed" mitosis. Cell cycle-dependent compaction and expansion of the genome is not detectable. The nuclear envelope is not disassembled but undergoes fission. Nevertheless, the number of pore complexes needs to be duplicated, the lamina needs to be enlarged, and core components need to be added. A limited disassembly of the preexisting structures may be required to accommodate assembly of the new ones.

IMPLICATIONS

If, in fact, the nonrandom distribution of the pore complexes on the surface of the nuclear envelope reflects the nonrandom configuration of the underlying genome and if the 3-D

configuration of the genome is characteristic for each differentiated state, then the pattern of pore complexes on the nuclear surface should not only be unique to each differentiated state but also should be identical and superposable for all cells of the same differentiated state and the same phase of the cell cycle in a given individual. Therefore, the various constellations of nuclear pore complexes on the nuclear surface should be characteristic for specific underlying regions of a given genome.

Because of spatial constraints within the nucleus, it is likely that all transcripts of a gene that is gated to a given pore complex would exit the nucleus only by way of that pore complex. For a transcript to leave the nucleus, its corresponding gene has to be gated (or the gene has to be part of a gated set of genes), independently of whether it is coding for mRNA, rRNA, tRNA, or other small RNAs. In cases in which the transcript assembles with cytoplasmically synthesized proteins prior to exit of the resulting ribonucleoprotein from the nucleus, the corresponding proteins must somehow reach the assembly site. One possible way to do so could be by trial and error in and out of the nucleus (by way of nuclear pores) until the protein is retained by the proper assembly site. Another possibility is random entry and diffusion within the nucleus until capture by affinity is accomplished.

When expressed in "nucleographical" terms, one could arbitrarily assign the expanded paternal amylase gene in the G_1-phase nucleus of a human exocrine pancreas cell and its associated pore complex a specific reference coordinate on the surface of the nucleus analogous, in geographical terms, to the city of Eisenach on the surface of earth. All other compacted or expanded genes would then be three-dimensionally coordinated with respect to this reference point. For example, the expanded maternal amylase gene would be located beneath the city of Salzburg, whereas the maternal compacted globin genes would be located beneath the city of Bonn, etc. Such an arrangement would imply that the maternal and paternal amylase genes, although located on different chromosomes, are near each other in the 3-D structure of the genome. The mRNA transcribed from the paternal amylase gene would exit the nucleus by way of a pore complex situated at the city of Eisenach, whereas the mRNA from the maternal amylase gene would exit from a pore located at the city of Salzburg.

If one extrapolates these concepts on nuclear structure to the proposal (19, 20) that cytoplasmic mRNAs are bound to cytoskeletal elements (and thereby would be prevented from freely diffusing throughout the cytoplasm), one would expect that mRNAs leaving the nucleus by way of a defined pore complex are not randomly distributed in the cytoplasm but would be located in a cytoplasmic "sector" adjacent to its exit point from the nucleus. Such a nonrandom distribution of mRNA in the cytoplasm may contribute significantly to the generation of asymmetry in the structural and functional organization of a eukaryotic cell.

If, in fact, a hierarchical and irreversible development in ontogeny of distinct genomic 3-D structures from an omnipotent zygotic 3-D structure would occur, then it would be impossible to generate an individual by transplantation of a diploid nucleus of a differentiated cell into an enucleated zygote. Only diploid nuclei that retained an omnipotent zygotic 3-D structure of their genome or whose 3-D structure is similar enough and reversible to that of the zygote would be expected to have such a potential.

It should be noted that several aspects of the proposals made here have been considered previously by others. An ordered arrangement of chromatin in the interphase nucleus mediated by attachment of chromatin to the nuclear envelope has been proposed some time ago (21). Evidence for a nonrandom and cell type-specific 3-D organization of the interphase chromatin has been provided by several workers (22–28). As already mentioned, the nonrandom distribution of nuclear pore complexes has been noted (5, 6), and, more recently, experimental evidence for a nonrandom distribution of mRNA in the cytoplasm has been presented (29). The assumption here that the 3-D structure of the genome is unique for a given differentiated state and identical for all cells in that differentiated state takes the idea of a nonrandom 3-D configuration of the genome to its extreme. However, even a less extreme version of this idea, such as "preferred" (27) instead of identical 3-D structures for a given differentiated state, would still be compatible with at least the general aspects of the proposals made here.

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