

*Invited Review***Facts and paradoxes in current notions of nuclear organization and function****M.G. Manfredi Romanini and A. Fraschini**

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Summary. Invisible compartments, identified rather by their activities than by their morphology, seem to operate in the nucleus. These compartments interrelate somehow, including mediation by the nuclear matrix. As our knowledge about the nucleus increases, more paradoxes become evident. We here consider some of them: 1) the well-known C-paradox of Cavalier-Smith, concerning the disproportionate amount of nuclear DNA content in comparison with the amount of DNA potentially able to transcribe; 2) the DNA folding in the chromatin fibre and its superorganization within the nucleus, which seems to be in opposition with the transcribing and self-replicating activities; 3) the elusive role of the DNA sequences with different degrees of repetitivity; and 4) the compartmentalization in the nucleus and how it relates to transcription, processing and transport of transcripts, and to DNA reduplication. We conclude by introducing the concept of species specific, minimal, but essential genome components, i.e. the elusive few thousand DNA bases that, in our hypothesis, act as a functional bridge between the nuclear matrix and chromatin.

Key words: Chromatin organization, DNA replication, Transcription, C-heterochromatin, Nuclear matrix

Introduction

One of the most interesting aspects of all cell biology is to try to understand in functional terms the morphology of the nucleus, which is rather elusive. The machinery in the nucleus has to be very complex since it is able to absolve many functions contemporaneously, or with certain precise time intervals, in an apparent absence of any structural support. These functions are: 1) DNA replication, starting from numerous «foci», every one of which contains more than 20 «early» or «late» initiation sites along the S phase of the cell cycle

(Tomilin et al., 1995); 2) transcription of RNAs: a) heterogeneous RNA, the «large transcripts», which are then processed within the nucleus with the use of different splicing factors (Spector et al., 1993; Visa et al., 1993; Zirbel et al., 1993), b) pre-ribosomal RNA (for a review see Schwarzacher and Wachtler, 1993), and c) pre RNA transfer; and 3) selective transcript transport outside the nucleus through gene-gated nuclear pores (Blobel, 1985; Lawrence et al., 1993).

Thus, the nucleus may be thought of as a complex structure as to provide for ordered concatenation of these functions. Many functions are performed in spaces not morphologically compartmentalized within the nucleus and are mediated by the nuclear matrix to which chromatin is bound (Gasser and Laemmli, 1986, 1987). The nuclear matrix itself has a complex, structural and enzymatic protein composition (Comings, 1980; Lebkowski and Laemmli, 1982a,b) and particular AT-rich DNA sequences, bound to the nuclear matrix have been recognized (MARS or SARS: Berezney and Coffey, 1974, 1975, 1976, 1977; Berezney, 1979, 1991; Laemmli et al., 1992). Most of identified proteins are common to all cell types, but increasing evidence shows that some proteins are tissue specific and change with the cell state (Getzenberg, 1994). The nuclear matrix may act, first of all, to maintain the shape and size of the nucleus (see the presence of actin, according to Verheijen et al., 1988), apart from changes in the cytoplasm or in the extracellular environment (Neves et al., 1993). But are also much more complex functions ascribed to the nuclear matrix, i.e. the regulatory roles in reduplication through MARS and probably other flanking DNA sequences (Jackson and Cook, 1985, 1986; Kaufmann and Fields, 1986; Kalamdadze et al., 1990; Vaughn et al., 1990; He et al., 1991; Bode et al., 1992) and in transcription, through the action of certain enzymes (topoisomerase II, histone deacetylase, primase, DNA and RNA polymerases, RNA elicase, etc.) (Hendzel et al., 1991; Kaufmann and Shaper, 1991; Hendzel and Davie, 1992) and of transcription factors and hormone receptors (Getzenberg, 1994) now known to be present in the nuclear matrix. It has been shown that almost newly synthesized RNA is associated with

the nuclear matrix (Jackson, 1991), as well as the snRNPs and the SC-35 splicing factor (Sahlas et al., 1993).

The nuclear matrix also contributes to the canalization of certain RNA transcripts which are routed to the cytoplasm via specific pores (gated pores, according to Blobel, 1985), and recent data indicate that some early signs of entry of the cell into apoptosis occur in the nuclear matrix (i.e. solubilization and release of some proteins of the nuclear matrix; Miller et al., 1993). Thus, the matrix appears to be involved in all chromatin functions throughout all phases of the life cycle of the nucleus.

More knowledge about the interactions among nuclear matrix, chromatin and interchromatin structures would help us to better understand the organization of the nucleus, helping us to clarify certain paradoxical and, for the present, inexplicable situations.

The C-paradox of Cavalier-Smith

In 1978 Cavalier-Smith described as a paradox the finding that the nuclear DNA content, although it is in constant quantity - a characteristic of all cells in all individuals of a species - has the genetic capacity to transcribe what amounts to only a small percentage (about 3% in man). What does the non-transcribing 97% of the DNA mean («junk», according to Doolittle and Sapienza, 1980 or «nucleotypic», according to Bennet et al., 1976)? The nature of this phenomenon, termed the «C - paradox» (C= nuclear DNA content) by Cavalier-Smith has been put partially in perspective by the introduction of the concept of «introns» (Gilbert, 1978, 1985): that is, by the demonstration that genetically inert zones (which do not, however, entirely correspond to that 97%) may be present inside a gene, even if, to date, the true role of the introns has not been clarified (Hurst, 1994).

There are several other characteristics of the nucleus that can be defined as paradoxical in that they seem to make its functioning too difficult to be understood.

The three-dimensional arrangement of DNA in the chromatin fibre and its organization in the nucleus

The nucleus of a human cell, with an average size of $5 \times 5 \times 7 \mu\text{m}^3$, contains about 7 picograms of DNA composed of 6.72×10^9 base pairs. Its total length extends out about 2 metres. This DNA compaction in a small volume is allowed by the well-known organization of the chromatin fibre (Olins and Olins, 1974; Olins et al., 1977) and by its progressive degrees of condensation. The DNA strands wind around histone cores to form the nucleosome fibre (with an average diameter of 11 nm). This fibre folds on itself to form the «solenoid», which has a diameter of 30 nm (Finch and Klug, 1976). The solenoid interacts with the nuclear matrix at specific sites, giving rise to the loop organization (Wolffe, 1994). Interaction between tail

domains in lamin molecule of the nuclear matrix and core histones seems particularly important in determining higher order organization of the chromatin in the interphase nucleus (Taniura et al., 1995). The loops have an average size of between 5 and 200 Kb, with 20% of the loops being around 7.5 Kb and 80% being between 50 and 175Kb. It has been speculated (Jackson et al., 1990) that the large loops constitute the inactive chromatin fraction, and the small ones the active chromatin. This complex arrangement of the chromatin must be related to the problem of the availability of the DNA, since it is the repository of all genes, active, potentially active or repressed, in every nucleus of a species; these genes, which in the human genome occupy about 3% of the DNA, as stated above, will have to be expressible in a certain order and with extreme rapidity. According to Lawrence et al. (1993) a genome can express 50-100,000 genes at the same time.

The nucleosomal structure of the chromatin fibre is thus seen as the means by which the primary task of assuring order in the unwinding during transcription and replication of the double-helix DNA wound around the histone cores, is maintained. Each core, composed of two molecules of each histone H2a, H2b, H3 and H4, engages about 160 base pairs. DNA can thus be catalogued as nucleosomal and internucleosomal (linker or spacer): the two together amount to slightly more than 200 base pairs and constitute the basic element of the fibre, which is the unit composing the solenoid fibre, with 6 nucleosomes per turn and a pitch of 11 nm. The histone H1, which is not included in this core, is involved, mainly through its phosphorylation/de-phosphorylation (Roth and Allis, 1992) and acetylation (Loidl, 1988, 1994; Davie and Hendzel, 1994) in the folding and unfolding of the chromatin fibre, especially during the transcription and replication of genes (Kamakaka and Thomas, 1990; Croston et al., 1991).

Since the 30 nm solenoid model was suggested (Finch and Klug, 1976), it has been hypothesized that the H1 histone is situated inside the solenoidal fibre. This location has recently been confirmed by measurements of neutron scattering: in the 30 nm chromatin fibres, the H1 histone has a radial location at the inner face of the nucleosome. This location agrees with the fact that the H1 interacts with the nucleosome at the entry and at the exit points of nucleosomal DNA and can improve the unfolding of the solenoidal DNA (Graziano et al., 1994).

The nucleosome model of the chromatin fibre may seem «paradoxical» if thought of as a support for the DNA when transcribing or self-replicating, or in any cause undergoing a very precise divarication of the helices. There is extensive literature on whether or not the nucleosomes persist at the level of the active genes (van Holde, 1988; Felsenfeld, 1992).

Paradoxically, in fact, the device which gives the chromatin fibre a structure of a certain consistency situates the greater part of the DNA, the nucleosomal DNA, in a repressed condition due to the electrostatic

forces which act to model it around the histone core (Crothers, 1994). It is not clear whether, and if so, to what extent, the length of the linkers justifies the hypothesis that gene activity takes place at their level only (Richmond et al., 1993).

Among the transcription models more recently proposed (Kronberg and Lorch, 1995), the most acceptable today are the ones proposed by Clark et al. (1993) and by Wolffe (1994), according to whom the nucleosomal organization is disrupted in the neighbourhood of promoters and enhancers to allow the initiation of the transcription, but the coding regions of transcribed genes lack H1 histone and continue to be covered with nucleosome core particles, at least partly depleted of histone H3.

The repeated sequences of DNA

Another possible «paradox» was described more than two decades ago when Britten and co-workers (1968, 1971) showed that the DNA sequences are, for the most part, repeated, from a few to a great number of times. The amounts of sequences defined as «highly» or «medially», repeated, or as a «unique» sequence have been used to describe a genome as characteristic of one taxonomic group, or to differentiate it from another (Britten, 1994; Charlesworth et al., 1994).

The concept of isochores based on such differences was used by Bernardi's group as a broadly comparative character among different genomes in living organisms (Bernardi et al., 1985; Bernardi and Bernardi, 1990; Bernardi, 1993). These authors maintain, in fact, that «vertebrate genomes are a mosaic of isochores, namely, of long, compositionally homogenous DNA segments that can be subdivided into a small number of families characterized by different GC levels».

Most of the highly repeated DNA is satellite DNA and, for the most part, makes constitutive heterochromatin. C-heterochromatin is localized, as is known, in specific zones of the metaphasic chromosomes (centromeres, interstitial zones of the arms, telomeres; Sumner, 1994) and is distributed, in the interphasic nucleus, in clumps of condensed chromatin, together with the so-called «facultative heterochromatin» which contains the genes characteristically silent in a cell type.

Various roles are attributed to constitutive heterochromatin. Haaf and Schmid (1991) ascribe a structural significance to it, as if the clumps of C-heterochromatin were supporting points in the spatial arrangement of the chromatin. Vogt (1990, 1992) suggests that the different degrees of repetitivity of the DNA could play a role in making chromatin available to functional activation. The problem, however, of integration between the two phenomena (DNA repetitivity and chromatin condensation) is not clear, even if the chromatin architecture can be interpreted as essential for genetic activity (Felsenfeld, 1992).

The functional compartmentalization of the nucleus

The most recent reviews on the structure and functions of the nucleus (Manuelidis and Chen, 1990; De Boni, 1994; Walters, 1995) support the idea that different functions can be recognized using different technical approaches, i.e. ultrastructural histoautoradiography, immunolabelling of specific molecules, or the recognition of different features of the DNA. Apart from the larger division between eu- and heterochromatin, the evidence is becoming even more striking that there exist poorly defined nuclear regions where complex and coordinate functions are performed through the phases of the cell cycle: the transcription and the processing of the transcripts and the DNA replication. Regarding transcription, recent literature (Spector, 1990, 1993; Fu and Maniatis, 1990; Spector et al., 1991; Zirbel et al., 1993; Visa et al., 1993; Fakan, 1994; Wansink et al., 1994; Hendzel and Bazzett-Jones, 1995; Walters, 1995) has begun to compare the electron microscopical pictures (perichromatin fibrils, interchromatin granules, coiled bodies) with those obtained by fluorescence microscopy («speckles», «spots»), but this comparison does not appear so easy. At the ultrastructural level, some areas have been recognized in the nucleus which are characterized by the presence of well defined molecular components (Visa et al., 1993; Fakan, 1994; Thiry, 1995). At the borders of condensed chromatin, the perichromatin fibrils (PF) are considered as nascent pre-mRNA (hnRNA) since they incorporate ³H uridine and contain poly(A) RNA. However, their functional meaning is more complex since the snRNPs and the SC-35 splicing factor, are bound to the PF suggesting that they can also be the sites of splicing of the pre-mRNA. Splicing proteins (snRNPs and SC-35 splicing factor) are highly present also in the clusters of interchromatin granules (IG), distributed throughout the euchromatin. They are poor in hnRNPs and weakly labelled with ³H uridine. It has been suggested that they are involved in the assembly of mature spliceosomes and/or in the regulation of RNA transport. Comparison with the immunofluorescence pictures suggests that clusters of IG represent the main components of the «speckles». On the contrary, it is more difficult to relate the PF to the fluorescence patterns.

Coiled bodies represent further nuclear structures enriched in snRNPs, but not in SC-35 splicing factor. They represent occasional nuclear components, easily identified also by fluorescence microscopy, since they contain a «marker» protein, the p80 coilin (Fakan, 1994; Wansink et al., 1994; Gall et al., 1995). It has been suggested that they could be involved in processing of specific transcripts and/or in post-splicing events (i.e. recycling of snRNPs or degradation of introns; Lamond and Carmo-Fonseca, 1993).

As mentioned above, according to Blobel (1985), the transcript of a gene leaves the nucleus via the pore complex to which the gene is gated.

As previously mentioned, the nuclear matrix is

believed to play an active role in the regulation of the different functional steps through the specific enzymes and transcription factors (Adachi et al., 1989; Kas et al., 1989). It thus may be conceived that the activation of a gene, followed by the elaboration of the transcript, processing and the emission of the gene product through an active site in the nuclear pore may all be regulated (controlled) by the nuclear matrix (Carter et al., 1991; 1993; Xing et al., 1993).

Recent data signal the coincidence of phospholipid components in the nuclear areas linked to transcription and processing (Fraschini et al., 1992; Frasnini and Fuhrman Conti, 1995) and strongly suggests that nuclear phospholipids can play a role in transcription process, in addition to their involvement in cell proliferation (inositides turnover; Irvine, 1992; Irvine and Divecha, 1992; Divecha et al., 1993). Their part in these processes is also supported by their presence in the nuclear matrix (Zini et al., 1993).

Taken together, these findings lead us to accept the notion of Haaf and Schmid (1991) that «multicomponent functional structures» exist in the nucleus whose task is to perform highly differentiated and sophisticated functions in the same, very restricted space and, sometimes, contemporaneously.

It is curious, in this context, to mention the potential involvement of the nucleolus in pre-mRNA processing, as hypothesized by Carter et al. (1993), since such data make all the too rigidly «compartmentalized» models of the nucleus more problematical, and re-introduce the paradox that even very well morphologically identified structures, such as the nucleolus, could participate in different functions.

Finally, with regard to the DNA reduplication and its relationships with the nuclear matrix, worthy of consideration are the data of Jackson and Cook (1986) and Cook (1988), according to whom the sites where replication starts in a nucleus, i.e. the sites where the replicons, whether early or late, begin their activity, do not change when a nuclear matrix deprived of 90% of chromatin is analyzed. At these replication sites, cytochemical approaches have demonstrated the presence of enzymes (i.e. helicase, DNA polymerase) and PCNA (proliferating cell nuclear antigen), essential for the DNA reduplication and known to be associated with the nuclear matrix (Hozak et al., 1993). The fact that replication is initiated by a stimulus coming from the nuclear matrix was confirmed first by light microscopy (Nakayasu and Berezney, 1989), and then by electron microscopy (Hozak et al., 1993). Moreover, incubation with biotin 11 dUTP provides evidence that «the polymerization factories (i.e. the sites of DNA synthesis) are fixed to a skeleton, with replication occurring as the template moves through them» (Hozak et al., 1993). Jackson (1995) suggests that the temporally coordinated activation of different sets of replicons, through the S phase, is influenced by the nuclear structure, reinforcing the idea of Felsenfeld (1992) that structure and function might be intimately linked in the

chromatin.

The early replication sites, distributed mainly in the decondensed chromatin at the beginning of the S phase, coincide spatially, but not temporally, with the domains of transcript elaboration and processing, discussed above, evidenced during the G1 phase.

According to Jackson et al. (1990), the presence of a DNA polymerizing activity, bound to the nuclear matrix and replicating in a cell cycle-dependent manner, prompt us to believe that the quantity of chromatin DNA which remains bound to the matrix (about 10% in human and mouse cells) does not, however, appear to be casual: its amount corresponds to the chromatin component most resistant not only to enzymatic digestion and to elution during the preparation of the nuclear matrices, but also to the so-called C-pretreatments, used according to Sumner (1972) to evidence the constitutive heterochromatin (C-heterochromatin).

We have shown by quantitative cytochemical studies performed after the C-pretreatments, that this chromatin fraction is the only one to which a species-specific value can be attributed, at least among the Primates (Manfredi Romanini et al., 1991, 1994). In comparing the gorilla, the chimpanzee and man, the total DNA content characteristic of the species is, respectively, 8.67, 7.86 and 7.30 pg. After Sumner's pretreatments, the resistant DNA amounts are respectively 2.45, 1.68 and 1.9 pg, so maintaining the differences found in the total DNA contents.

It is «paradoxical» that this residual 10%, which seems responsible for the species-specificity of a genome, may coincide with the C-heterochromatin, known to be for the most part constituted by highly repeated satellite DNA, poor in genes. This lead us to believe that within this 10%, in addition to the satellite fraction, there may exist another fraction of DNA responsible for species-specific characters.

This selected small quantity of chromatin could contain, for instance, the 2-3% attributed by Cook (1988) to the DNA sequences characteristic of the matrix, MARs or SARs and/or the telomeres, of which the essential role in the life of the cell has been discussed recently by Marx (1994).

We can hypothesize a «paradoxical», inversely proportional relationship between total DNA and this small, probably, species-specific fraction. Such a hypothesis impels us towards an analytical investigation, aimed at «dissecting» in functional terms this privileged zone of the genome, of which the existence is increasingly evidenced, but which is still very mysterious. It has to be supposed that this component of the genome acts in close contact with, and partly belongs to the nuclear matrix (De Lange, 1992). Its role is in initiating important functions, whether of replication or of transcription, so controlling the cell cycle rhythm and the differentiative fate of the cell.

The right question to ask is: what are the characteristics which can help us to describe this minimal but essential component of the genome? If we

accept that the way in which the DNA is organized in the chromatin (the tri-dimensional high ordered structure) is designed to control reduplication and transcription (Felsenfeld, 1992), then we must agree to attribute to the nuclear matrix, not only a mechanical but also a functional importance, indivisible, in our opinion, from the chromatin.

We are probably asking the right question: what is it that renders essential and species-specific that minimal quantity of genomic sequences that makes, for example, man, something totally different from the chimpanzee?

According to O'Neill et al. (1994), «Genetically, that is, in terms of information content, humans are 99% identical to chimpanzees. Where is the difference?»

The differences probably lies precisely in the role played by those elusive few thousands of kilobases of DNA that act as a bridge between the nuclear matrix and the chromatin and make them act together in the nucleus.

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