Journal of Cellular Biochemistry 91:170–184 (2004)

Molecular Aspects of Diagnostic Nucleolar and Nuclear Envelope Changes in Prostate Cancer

Andrew H. Fischer,* Svetoslav Bardarov Jr., and Zhong Jiang

Department of Pathology, University of Massachusetts UMMHC, Worcester, Massachusetts 01655

Abstract Prostate cancer is still diagnosed by pathologists based on subjective assessment of altered cell and tissue structure. The cellular-level structural changes diagnostic of some forms of cancer are known to be induced by cancer genes, but the relation between specific cellular-level structural features and cancer genes has not been explored in the prostate. Two important cell structural changes in prostate cancer—nucleolar enlargement and nuclear envelope (NE) irregularity—are discussed from the perspective that they should also relate to the function of the genes active in prostate cancer. Enlargement of the nucleolus is the key diagnostic feature of high-grade prostatic intraepithelial neoplasia (PIN), an early stage that appears to be the precursor to the majority of invasive prostate cancers. Nucleolar enlargement classically is associated with increased ribosome production, and production of new ribosomes appears essential for cell-cycle progression. Several cancer genes implicated in PIN are known (in other cell types) to augment ribosome production, including c-Myc, p27, retinoblastoma, p53, and growth factors that impact on ERK signaling. However, critical review of the available information suggests that increased ribosome production per se may be insufficient to explain nucleolar enlargement in PIN, and other newer functions of nucleoli may therefore need to be invoked. NE irregularity develops later in the clonal evolution of some prostate cancers, and it has adverse prognostic significance. Nuclear irregularity has recently been shown to develop dynamically during interphase following oncogene expression, without a requirement for post-mitotic NE reassembly. NE irregularity characteristic of some aggressive prostate cancers could reflect cytoskeletal forces exerted on the NE during active cell locomotion. NE irregularity could also promote chromosomal instability because it leads to chromosomal asymmetry in metaphase. Finally, NE irregularity could impact replication competence, transcriptional programming and nuclear pore function. J. Cell. Biochem. 91: 170–184, 2004.

Key words: nucleolus; nuclear envelope; molecular cytopathology; clonal evolution

WHY ARE CANCER CELLS STRUCTURED DIFFERENTLY FROM NORMAL CELLS?

The cell structural changes diagnostic of cancer have been proposed to be a consequence of the accumulation of numerous genetic and epigenetic disturbances during the acquisition of increased cell reproductive kinetics [Garcia-Schurmann and Coffey, 1997]. From this viewpoint, genetic instability is thought to generate diagnostic cellular pleomorphism, and increased proliferation could be envisaged to result in increased demands for more ribosomes and a consequent enlargement of nucleoli (see below). However, prostate cancer is not diagnosed on the basis of pleomorphism alone. Rather, there are specific diagnostic features that characterize certain steps in prostate cancer development, and the appearances of different cases of prostate cancer are different. Some prostate cancers show a fine euchromatic appearance compared to normal prostate cells, others show distinctly coarse heterochromatin. Some prostate cancers show predominantly round nuclei whereas others show predominantly irregular nuclear shapes. The irregularity in shape in some cases can be characterized as irregular folds and aneurismal outpouchings, in other cases nuclear envelope (NE) irregularity is characterized by poly-lobulation, and in still other cases (e.g., small cell carcinomas), the NE appears malleable and passively

*Correspondence to: Andrew H. Fischer, Department of Pathology, University of Massachusetts UMMHC, H2-466, 55 Lake Ave North, Worcester, MA 01655.
E-mail: fischa01@ummhc.org

Received 11 September 2003; Accepted 12 September 2003
DOI 10.1002/jcb.10735

distorted ("nuclear molding"). Furthermore, some aggressive, fast growing prostate cancers show small nucleoli as a diagnostic trait (e.g., "small cell" prostate cancers) while other more indolent early lesions (e.g., prostatic intraepithelial neoplasia, PIN) show large nucleoli. If the diagnostic features of prostate cancer cells were merely a result of pleomorphism or randomization of cell structure, then any two prostate cancers would be expected to look the same. A broader framework seems needed to explain the wide variety of specific diagnostic morphologic changes seen in the full range of cancers.

A BROAD EVOLUTIONARY FRAMEWORK FOR UNDERSTANDING CELL STRUCTURAL CHANGES ACCOMPANYING CANCER DEVELOPMENT

The development of a prostate cancer is believed to be a micro-evolutionary process, akin to "adaptive speciation" in Darwinian evolution, whereby mutations that promote the clonal expansion of a prostate cell are naturally selected for, while natural selection eliminates cells expressing genes that could inhibit their growth. Phenotypic changes provide essential clues to the mechanisms of adaptive speciation in Darwinian evolution. In fact, by careful analysis of structural features alone, Darwin could frequently deduce adaptive evolutionary mechanisms. Darwin recognized that the phenotypic structural differences between two related species generally pointed to the fitness alteration that allowed their functional divergence. Darwin also recognized that phenotypic/structural differences that distinguish two closely related organisms had a heritable basis. If a full evolutionary framework is applied to cancer, then the diagnostic cellular-level changes (not necessarily tissue architectural changes) that distinguish a step in the clonal evolution of a cancer should be mediated by the cancer genes active at that step, and these structural changes should reflect, in an essential manner, the functional changes that caused the clonal expansion [Fischer et al., 2003b].

Support for this model comes from studies of development of two morphologically and clinically distinctive types of thyroid tumors that arise from the same cell of origin [Rosai et al., 1992]. Distinctive sets of cancer genes are operative in these two types of tumors, and these genes appear to mediate the diagnostic structural features involving the NE and large scale chromatin organization that distinguish them [Fischer et al., 1998a]. The tumors themselves show a complete overlap of net growth, apoptosis rates, and MIB-1 staining [Basolo et al., 1997], and thus the physiologies that these cancer genes affect seem unlikely to be related to cell cycling kinetics per se [Fischer et al., 1998a,b]. In Darwinian evolution, alterations in fitness relate much more directly to structural/phenotypic changes than they do to alterations in reproductive kinetics. Thus, the full incorporation of the phenotypic changes diagnostic of cancer into the evolutionary model for cancer development suggests that cellular-level structural changes diagnostic of a particular step in clonal evolution can point to novel cell physiologies unrelated to cell growth kinetics [Fischer et al., 2003b].

It is important to note that prostate cancer is not a single disease. Thus (according to the model) any two samples of prostate cancer that differ in cellular-level morphology are likely to have different underlying genetic changes and functional alterations. Unfortunately few studies relate particular genetic findings back to the particular cellular-level phenotypic features to test this concept. Transgenic animals are not ideal to test these ideas since too many levels of organization and too many confounding influences could obscure any correlations. A large part of the difficulty in being able to relate genetic and functional changes with phenotypic changes is the inability to accurately provide morphologically characterized human prostate samples to researchers. Only recently, a technique is available to provide samples of viable cell populations that can be characterized morphologically at a tissue and cellular-level [Fischer et al., 2001a].

In this review, we describe two reproducible cellular-level changes of diagnostic importance—nucleolar enlargement and NE irregularity. We review how these diagnostic changes could relate to cell physiology and the activity of cancer genes active in prostate cancers. A description of the full diagnostic features of various neoplastic and reactive conditions of the prostate can be found in a number of textbooks [Bostwick and Eble, 1997]. Only the diagnostic features related to nucleolar and NE changes are discussed in this review. For reviews of molecular aspects of other larger-scale tissue-

organizational features of prostate cancer, see [Fornaro et al., 2001; Pihan et al., 2001; Sung and Chung, 2002].

NUCLEOLAR ENLARGEMENT IS CHARACTERISTIC OF PROSTATIC INTRAEPITHELIAL NEOPLASIA

The vast majority of prostate cancers appear to derive from the peripheral region of the prostate, from the epithelial cells that line medium sized secretory prostatic glands. The normal structure of these glands (Fig. 1A) includes a surrounding basal lamina produced through the cooperation of epithelial cells with the underlying stromal cells, basal cells that normally have replication competence and abut the basal lamina, and differentiated secretory cells located toward the lumen of the acini (reviewed in [De Marzo et al., 1998b]). The secretory cells appear to lack replication competence and are apparently derived from the basal cells by a process of asymmetric cell division. Basal cells tend to have bland chromatin and oval nuclei with that are smaller than secretory cells, and they have scant cytoplasm. Nuclei are usually inconspicuous in basal cells, but may be enlarged in some forms of basal cell hyperplasia. The secretory cells have generally spherical nuclei, relatively more "open" chromatin, and inconspicuous nucleoli. Nucleoli in secretory cells are generally difficult to distinguish from heterochromatin aggregates (so-called "chromocenters") on hematoxylin stained sections.

High grade PIN (Fig. 1B) is distinguished from normal prostate glands based on a relatively uniform ("clonal-appearing") population of prostatic secretory cells (also called acinar cells) which display a generally single [Bostwick and Brawer, 1987; Montironi et al., 1991] enlarged nucleolus. PIN cells also often show a uniformly slightly coarse chromatin, and often increased nuclear size (reviewed in [Montironi et al., 2002]) compared to normal secretory cells. The most characteristic change in the nucleolus is increase in size (up to several microns) compared to benign prostatic hyperplasia [Montironi et al., 1991]. A minority of PIN cells show multiple nucleoli [Montironi et al., 1991]. Nucleoli may be central or appear attached to the NE. Ultrastructural studies of PIN have also noted nucleolar enlargement [Bostwick et al., 1997], but not described whether the enlargement involved particular compartments (e.g., the granular component) that could help to pinpoint the stage at which ribosomal metabolism (see below) may be altered. It is important to note that mitotic figures are not easy to find in PIN. Furthermore, mitoses are expected in benign reparative conditions.

Several observations suggest that Pin is not a single disease. First, there is a sense of a

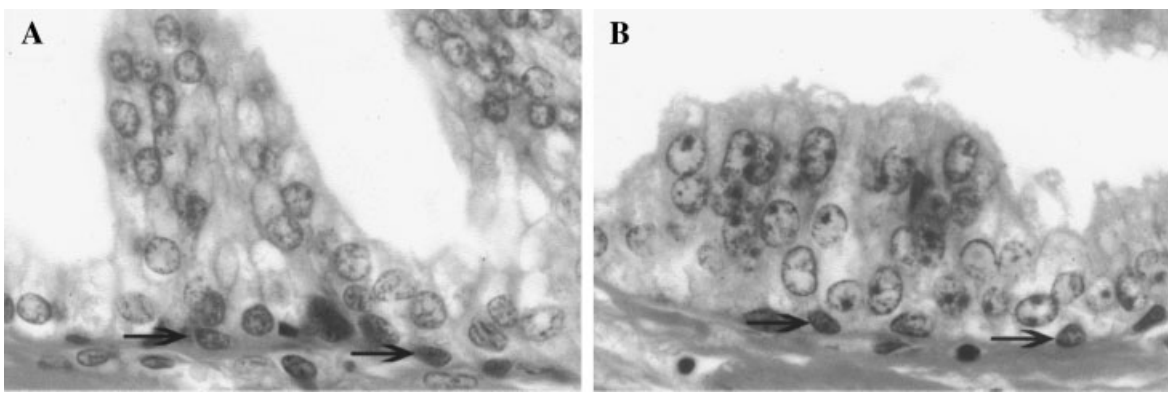


Fig. 1. Normal prostate tissue (**A**) consists of stromal elements (lower-most aspect) that grow in cooperation with the epithelial cells to produce a basal lamina. Inside the basal lamina are basal cells (two are marked with arrows). The next layer of cells above the basal cells in the figure consists of specialized secretory cells that normally have inconspicuous nucleoli. Secretory cells are normally terminally differentiated and appear to derive from replication-competent basal cells. **B**: Shows high grade prostatic intraepithelial neoplasia (PIN) characterized primarily by the presence of nucleoli in the secretory cells. Compared to normal secretory cells, the nuclei of PIN cells often show a mild nuclear size increase, and a slight ''chromatin coarsening'' (as seen in this example). Pin cells retain replication competence. The arrow shows residual basal cells beneath the layer of PIN cells. Note the inconspicuous nucleoli in basal cells in A and B. The inconspicuousness of nucleoli in basal cells is paradoxical since basal cells and PIN cells probably proliferate at nearly the same rate [Magi-Galluzzi et al., 1998b] (see text). A and B are both at 1,000X original magnification.

spectrum of morphologic changes with benign prostatic hyperplasia at one end, and invasive adenocarcinoma at the other. There is variability in the architectural arrangement of PIN cells from case to case, and even within one patient, suggesting that cell–cell and cell–matrix interactions can vary in different examples of PIN. Approximately half of PIN are aneuploid [Crissman et al., 1993; Qian et al., 1997], suggesting genetic instability starts early in prostate cancer, and that multiple genetic events drive the phenotypic features of at least some cases of PIN. However, it seems impossible to ascribe the nucleolar change in PIN to a stochastic variation resulting from genetic instability; the nucleolar appearance of PIN cells is actually quite uniform.

PHYSIOLOGIC CHANGES IN NUCLEOLAR MORPHOLOGY

The size of nucleoli in normal, untransformed cells can change in size depending on the cells' activity. Pathologists learn to recognize these changes in nucleolar size and correlate them with alterations in the cells' microenvironment to make inferences about the nature of pathologic processes. For example, if there is a local loss of cells in the prostate (or essentially any epithelium in the body), nucleoli enlarge in adjacent cells, within about 1–2 days, in a reaction termed "repair" (Fig. 2). The local loss of cells that provokes this response can be due to any number of causes. In the prostate, a common cause is prostatitis. The inflammation or bacteria presumably destroys some epithelial cells, and the residual cells respond and repopulate the basal lamina. Inflammation and infections are not the only causes for a repair reaction. Identical changes can occur next to a biopsy site, or next to an area of infarction (localized loss of blood supply) in the virtual absence of inflammatory cells. The nucleolar enlargement associated with repair has an important diagnostic trait. When there is nucleolar enlargement in an epithelial cell undergoing repair, there is a predictable increase in the amount of cytoplasm in the cell, and a predictable increase in cytoplasmic basophilia (i.e., hematoxylin staining that reflects the amount of polyribosomes) [Frost, 1986]. In many of the forms of cancer that show nucleolar enlargement, there is a tendency for an unpredictable relative paucity and pallor of the cytoplasm

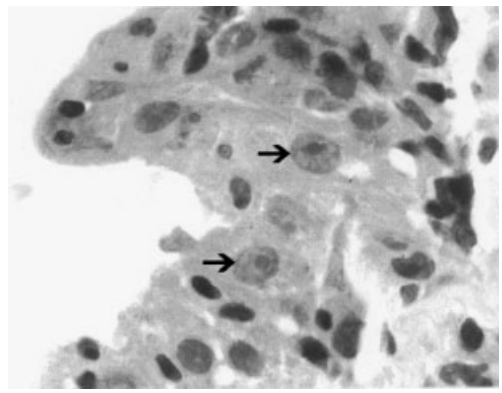


Fig. 2. Benign prostate tissue with granulomatous prostatitis is shown at the same 1,000X magnification as Figure 1. The granulomatous reaction was due to previous bacillus Calmette–Guerin treatment for bladder cancer. When prostatic secretory cells are destroyed by any pathologic process, the adjacent prostatic epithelial cells (arrows) respond in a ''repair'' reaction characterized by increased size of nucleoli, a corresponding increase in the amount and granularity of cytoplasm, and mitoses.

[Frost, 1986]. As an exception to this tendency, PIN cells may also show an increase in cytoplasmic basophilia. Invasive or metastatic prostate cancer tends to show pallor of the cytoplasm, and less cytoplasm compared to PIN, conforming to the trend seen in many other cancers. In an ultrastructural study, invasive prostate cancer cells with large nucleoli often show few organelles in their cytoplasm [Kastendieck and Altenahr, 1976]. Benign cells with large nucleoli generally have complexity of their cytoplasm, with abundant rough endoplasmic reticulum.

PIN IS A PRECURSOR TO SOME FORMS OF INVASIVE PROSTATE CARCINOMA

The evidence linking PIN to invasive prostate cancer is overwhelming (reviewed in [Bostwick and Eble, 1997]) and includes the cytologic similarity of the relatively uniform and distinctive cells in PIN with those of adjacent invasive cancer, their frequent spatial relationship, the similar immunohistochemical reactivities shared by PIN and invasive cancer, the sharing of genetic abnormalities by PIN and concomitant cancer [Alcaraz et al., 2001], and strong epidemiological associations. Some prostatic adenocarcinomas could conceivably arise independently from the clones of PIN, but a reasonable estimate is that half or more prostate adenocarcinomas evolve from clones of PIN.

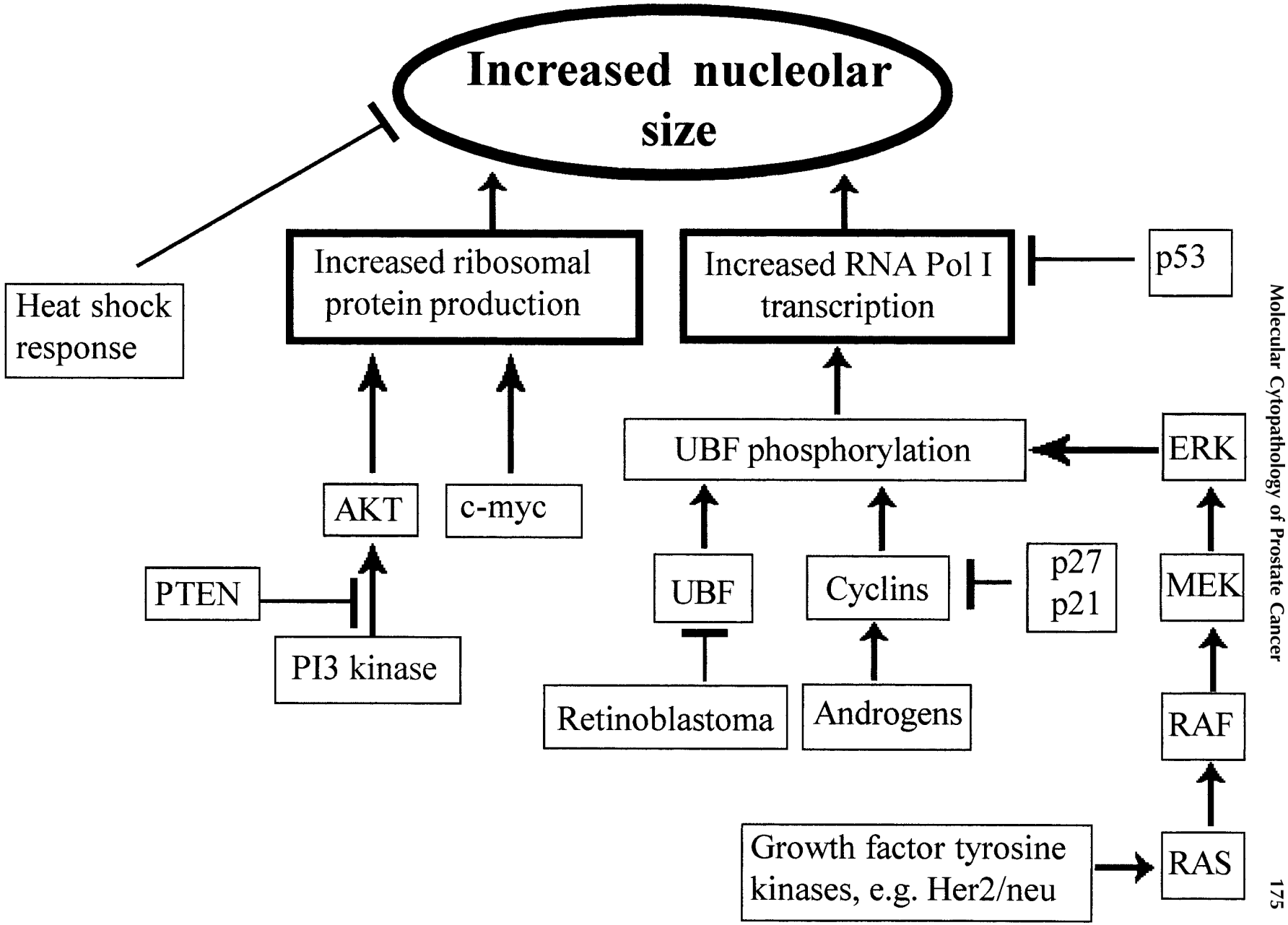
Not all invasive cancers arise from PIN. PIN is not seen in association with roughly 30% of invasive prostate cancers [McNeal and Bostwick, 1986; Helpap and Riede, 1995]. Another controversial potential precursor to some forms of invasive prostate cancer is termed atypical adenomatous hyperplasia (AAH). AAH is characterized by increased numbers of small secretory glands with minimal cellular-level structural atypia. Usually AAH is described as having nearly normal appearing nuclei, like secretory cells, without nucleoli, though some pathologists accept fairly prominent (>1.0 μm) nucleoli in AAH. Most cases of AAH are found in the central part of the prostate or "transition zone" where low-grade prostatic adenocarcinomas are relatively common. Since AAH and some low-grade prostatic adenocarcinomas exhibit similar cellular-level structural features (including relatively small nucleoli), there is speculation that AAH might be a precursor to some low grade prostate cancers in the transition zone, and this is supported by molecular and phenotypic studies [Doll et al., 1999; Yang et al., 2002].

Since there appears to be more than one way for a prostate cell to become a "PIN" cell, and since there are multiple different evolutionary pathways for a prostate epithelial cell to become fully malignant, the broad evolutionary model suggests there will be multiple spectra of cancer gene activations and functional alterations in different examples of prostate cancer. We predict that a subset of these genetic abnormalities relating to PIN will share the property of impacting nucleolar metabolism. It will be important for researchers to carefully describe the morphologic features of their experimental models in order to extract the expected important relationships between structure, function, and genotype to test this prediction.

FUNCTIONAL SIGNIFICANCE OF NUCLEOLAR ENLARGEMENT IN PIN

Approximately 80% of the energy expenditure of a proliferating eukaryotic cell can be spent in producing the protein synthesis machinery, of which ribosomes are the major element [Warner, 1999; Volarevic et al., 2000]. The nucleolus can be thought of as the organelle coordinating this expenditure [Warner, 1999]. The nucleolus is the site of ribosomal DNA transcription by RNA Polymerase I (RNA Pol I), processing of ribosomal RNA, maturation of the 79 ribosomal proteins (which are transcribed by RNA Pol II, translated in the cytoplasm, and imported back into the nucleus and nucleolus), and assembly of mature ribosomes prior to their export (reviewed in [Olson et al., 2000; Warner et al., 2001]. In yeast, 60% of total transcription is devoted to rRNA, 50% of RNA Pol II transcription is devoted to producing ribosomal protein message for the 79 ribosomal proteins, and 90% of mRNA splicing involves ribosomal protein transcripts [Warner et al., 2001]. One may wonder if DNA evolved merely to provide ribosomes with an efficient means (only a 20% cost) of making more ribosomes! The cell needs to match rRNA transcription with ribosomal protein production, and ribosomal proteins must themselves be produced at equimolar levels. Since ribosome production needs to match a level determined by the particular demands of a cell, it is not surprising that regulation of nucleolar function is complex and interdependent on many cellular processes [Warner et al., 2001].

As a classical and best available approximation, nucleolar size generally correlates with the rate of ribosome production [Kurata et al., 1978] (reviewed in [Derenzini et al., 2000; Frank et al., 2002]). The half life of ribosomes is at least several days [Liebhaber et al., 1978], relatively long compared to the growth rates of many cell lines. The regulation of ribosome degradation is apparently subject to complex and poorly understood regulation. SV40 can increase ribosome life span about 10-fold [Liebhaber et al., 1978]. At each cell division the number of ribosomes per cell is necessarily halved. Therefore cell division can be a major cause of increased need for ribosomes. In cell lines in which essentially all cells divide within a couple days, nucleolar size correlates strikingly with both cell doubling time and the rate of rDNA transcription [Derenzini et al., 2000]. At first glance, it would seem that the increased nucleolar size in PIN could reflect the need for more ribosomes to replace those lost through cell division. The details of the signaling pathways that can balance ribosome production with ribosome need are unclear. At least part of the balance is achieved through cross-talk between the cell proliferation signaling pathways and the ribosomal synthesis machinery.


Increased nucleolar size
Heat shock response
Increased ribosomal protein production
Increased RNA Pol I transcription
p53
UBF phosphorylation
ERK
AKT
c-myc
PTEN
PI3 kinase
UBF
Cyclins
p27
p21
MEK
Retinoblastoma
Androgens
RAF
Growth factor tyrosine kinases, e.g. Her2/neu
RAS

A SIGNALING PATHWAY FOR INCREASED NUCLEOLAR SIZE?

Two key control points in ribosomal synthesis have been identified (reviewed in [Ruggero and Pandolfi, 2003]), and several genes implicated in the early steps of prostate cancer development impact on one of these two control points. Figure 3 summarizes the signaling events that could regulate ribosome synthesis/nucleolar size. The first key control in ribosomal synthesis appears to be regulation of rDNA transcription by RNA Pol I. An apparently rate limiting step in RNA Pol I activity in turn is determined by the phosphorylation of the transcription factor up-stream binding factor (UBF). UBF is phosphorylated by several different kinases: CDK4-Cyclin D1 and CDK2-Cyclin E [Voit and Grummt, 2001], casein kinase II, and ERK1 and 2 [Stefanovsky et al., 2001] (reviewed in [Ruggero and Pandolfi, 2003]). Androgen stimulation has complex effects but appears capable of augmenting cyclin D levels (reviewed in [Fernandez et al., 2002]). P27 negatively regulates CDK2-cyclin E activity, and loss of immunohistochemical expression of p27 is seen in PIN and the majority of prostate carcinomas [De Marzo et al., 1998a]. ERK1 and 2 receive stimulation from the growth factor tyrosine kinase—ras signaling pathway. C-ErbB2 [Signoretti et al., 2000], RET [Dawson et al., 1998], and other tyrosine kinase growth factors up-stream of ERK 1 and 2 (reviewed in [Myers and Grizzle, 1996; Magi-Galluzzi et al., 1998a]) have been implicated in prostate cancer development. Tyrosine kinases appear able to activate androgen receptor in the absence of androgens (e.g., [Signoretti et al., 2000]). Protein phosphatases involved in downregulation of ERK signaling are also implicated at an early stage of prostate cancer development [Magi-Galluzzi et al., 1998a]. UBF activity is blocked by an interaction with the retinoblastoma (*Rb*) gene product, and loss of heterozygosity for Rb has been found in 60% of prostate cancers [Phillips et al., 1994]. p53 inhibits RNA Pol I transcription through binding to another RNA Pol I transcription factor, SL1. p53 mutations appear to be a late event in a subset of prostate cancers with frequent androgen independence [Heidenberg et al., 1995], but wildtype p53 has several other physiologic interactions with the nucleolus (reviewed in [Olson et al., 2000]).

A second level of control over ribosome biosynthesis is achieved through regulation of production of ribosomal proteins (Fig. 3). The lack of ribosomal proteins causes ribosome production and RNA Pol I activity to cease [Warner, 1999; Volarevic et al., 2000], and upregulation of RNA Pol I by increased amounts of ribosomal proteins also seems possible. Thus, these two levels of control appear somewhat interdependent. Ribosomal protein genes are a major transcriptional target of c-Myc [Ruggero and Pandolfi, 2003]. When c-Myc is overexpressed (in hepatocytes), protein synthesis increases, cell size increases, and nucleolar size increases [Kim et al., 2000]. *C-Myc* gene amplifications are seen in about 50% of PIN [Qian et al., 1997]. mRNA's for ribosomal proteins and other proteins involved in translation also share a terminal oligopyrimidine in their 5′ untranslated region (5′TOP mRNA's) that causes them to be preferentially translated by ribosomes bearing a phosphorylated S6 protein (reviewed in [Ruggero and Pandolfi, 2003]). In turn, S6 phosphorylation is regulated at several levels. Phosphatidyl inositol-3-kinase—AKT signaling causes S6 phosphorylation. This pathway is inhibited by the tumor suppressor phosphatase PTEN. PTEN mutations are present in roughly half of prostate cancers (reviewed in [Grunwald et al., 2002]), providing a link between PTEN inactivation and at least increased ribosomal protein production (if not increased ribosome synthesis). PTEN heterozygous mice which are homozygous for deletion of p27 develop prostate cancer with full penetrance by 3 months (see references within [Grunwald et al., 2002]).

Other levels of control of nucleolar size appear independent of the two classical pathways in Figure 3, for example heat shock response [Liu et al., 1996]. These data begin to provide plausible links between genetic events in prostate

Fig. 3. Is there a signaling pathway for nucleolar size increase? Nucleolar size has been classically considered to reflect the rate of ribosomal production. The factors that are currently known to affect ribosomal production are shown. Stimulatory effects are represented as arrows, and inhibitory effects are shown with blunt bars. Increased ribosomal protein production (the products of RNA Pol II transcription) and increased RNA Pol I transcription are currently the most likely stimuli for increased nucleolar size. Heat shock response also causes arrest of ribosomal synthesis and disassembly of nucleoli. This signaling scheme is derived from [Liu et al., 1996; Stefanovsky et al., 2001; Fernandez et al., 2002; Ruggero and Pandolfi, 2003].

cancer and the nucleolar changes. However, it is important to note that the relations predicted between nucleolar size and these genetic pathways, though widely assumed in the literature, are still largely unexplored, especially in prostate cells.

PROBLEMS RELATING NUCLEOLAR SIZE TO RIBOSOME PRODUCTION OR GROWTH RATE IN PIN

There appear to be at least several exceptions to the rule that nucleolar size and rate of ribosome production are linked. One reason for the exception is that ribosomes can be produced in the absence of a grossly organized nucleolus. This was shown by replacing the clustered ribosomal genes of yeast into an equivalent number of dispersed chromosomal copies [Nierras et al., 1997]. Ribosome synthesis could proceed and ribosomal production could be physiologically repressed even though there was no organized ultrastructural nucleolus [Nierras et al., 1997]. However, these yeast cells showed decreased maximal ribsome production rate, and their ability to physiologically up-regulate ribosome synthesis was not tested. *Drosophila* brain cells were observed to enlarge their nucleoli in response to loss of *BRAT* gene function, but this occurred without a change in rRNA content [Frank et al., 2002], suggesting that ribosome synthesis rate was not altered in spite of an enlarged nucleolus in this cell type. Serum starvation, which leads to dephosphorylated UBF and arrest of ribosomal production [Voit and Grummt, 2001], does not cause nucleoli to disappear (Fig. 4) (Fischer AH, unpublished observation). In fact, except that nucleoli become slightly more rounded, mitoses are not found, and cells appear slightly flatter, there is no perceptible change induced by serum starvation. Although activation of ERK signaling is known to up-regulate RNA Pol I activity in fibroblasts [Stefanovsky et al., 2001], a constitutively active MEK1, which activates ERK1 and 2 [Mansour et al., 1994], does not change fibroblast nucleolar morphology (Fig. 5) [Fischer et al., 1998b]. Thus, up-regulation of rDNA transcription per se does not appear to account for nucleolar size variation, at least in fibroblasts. This paradox is not a simple consequence of an inability of fibroblasts to alter their nucleoli since fibroblasts show dramatic changes in nucleolar size in vivo during wounding. The nucleolar size increase in fibroblasts following wounding in humans (e.g., as observed in a re-excision of a biopsy site) is observable within about 2 days. It becomes maximal after about 5–7 days and is associated with a grossly prominent golgi zone, and increased rough endoplasmic reticulum. Fibroblast nucleoli become less prominent over the ensuing 4–6 months. Loss of *Rb* gene product should up-regulate RNA Pol I (see above), but tumors characteristically lacking Rb, (for example small cell carcinomas, and HPV-associated

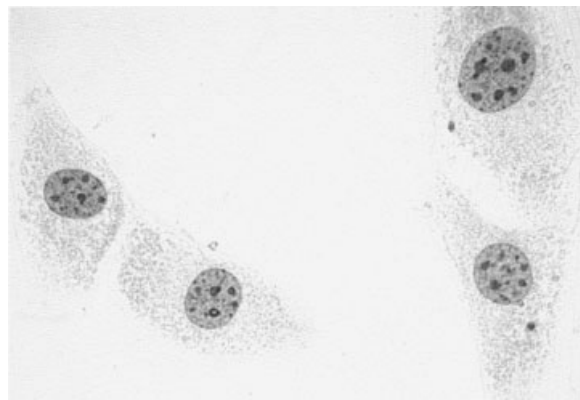


Fig. 4. Serum starved NIH3T3 cells (maintained for 10 days in 0.5% fetal calf serum) still show nucleoli that are about the same size as normal NIH3T3 cells, though they tend to have rounded contours. Since serum starvation is known to inhibit RNA Pol I transcription and new ribosomal synthesis [Voit and Grummt, 2001], there must be additional factors besides those shown in Figure 3 that have an impact on nucleolar size.

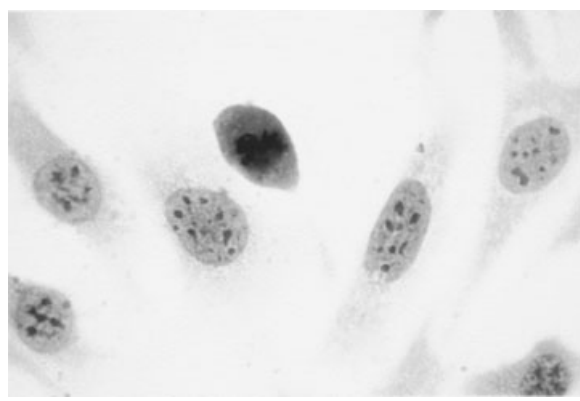


Fig. 5. NIH3T3 cells expressing a constitutively active mitogen activated kinase kinase (MEK 1 [Mansour et al., 1994]), which is 400 times more active than wild type MEK 1 at activating ERK 1/2 does not cause a significant change in nucleolar morphology compared to non-transfected cells [Fischer et al., 1998b]. Since ERK 1/2 is known to up-regulate UBF phosphorylation and RNA Pol I activity in mouse fibroblasts [Stefanovsky et al., 2001] there must be yet additional factors besides those shown in Figure 3 and discussed in Figure 4 that have an impact on nucleolar size.

cervical intraepithelial neoplasia) characteristically do not show conspicuous nucleoli. PTEN-null endometrial cells, which could be anticipated to up-regulate ribosomal protein synthesis, do not show nucleolar changes [Mutter et al., 2001].

Though cell lines with rapid growth rates demonstrate a striking relationship between nucleolar size and cell-cycle length [Derenzini et al., 2000], these results may be difficult to relate to spontaneous human tumors that likely develop over a period of years with a slower growth rate than tissue culture cells. PIN appears slow-growing by both clinical/epidemiologic and pathologic evidence. The growth rate of basal cells and putative prostate stem cells appears to overlap that of PIN [Helpap et al., 1995]. Mitotic figures are said to be more common in basal cells than the overlying PIN cells [Magi-Galluzzi et al., 1998b]. However, putative stem cells, and basal cells (which, like PIN, lack p27 expression) generally do not show as large nucleoli as the overlying PIN cells [De Marzo et al., 1998a], (and see Fig. 1B). In other organ types, intraepithelial neoplasia cells can proliferate orders of magnitude faster than PIN, yet nucleolar enlargement is characteristically not present. For example, in cervical intraepithelial neoplasia mitotic figures are readily found. Yet as a diagnostic trait, nucleoli are inconspicuous. The presence of nucleoli favors a benign reactive change over cervical intraepithelial neoplasia. Finally, it is obvious that cell division is not required for nucleolar enlargement. For example, post-mitotic adult human neurons have large nucleoli.

In fact, there is virtually no direct data to see if nucleolar enlargement in PIN or prostate cancer is directly related to either increased ribosome synthesis, or increased cell division rate! The finding of a nucleolar protein, p120, which appears specifically up-regulated in prostate cancer may provide important clues to the functional significance of nucleolar alterations in prostate cancer [Kallakury et al., 1999]. An excellent model for manipulating nucleolar size in the prostate appears to be the regression in nucleolar size in prostate cancer cells and in PIN following androgen blockade [Vailancourt et al., 1996] (See also references within). The time-course, ribosomal kinetics, and signaling pathway for this nucleolar shrinkage after androgen blockade apparently have not been studied.

In trying to anticipate what would seem to necessarily be important functional correlates of nucleolar size increase in prostate cancer, it is essential to note that the nucleolus has other functions besides ribosome production (reviewed in [Pederson, 1998; Olson et al., 2000]). These roles are beyond the scope of this review but include regulation of transport of specific mRNA's, maturation of the signal recognition particle required for protein translocation across the endoplasmic reticulum during translation, processing of some tRNA's, processing of mRNA splicing factors, and production of telomerase.

Several genes implicated in PIN cannot be easily related in any manner to nucleoli. Examples include glutathione-S transferase Pi down-regulation [Jimenez et al., 2000], and alpha-methyl CoA reductase up-regulation (P504S) [Jiang et al., 2001].

CELL PROLIFERATION AND RIBOSOMES: CAUSE OR EFFECT?

There is another interpretation to the association between cell proliferation and nucleolar size. Rather than cell proliferation driving the need for ribosome production and nucleolar enlargement, it appears that cell division is at least sometimes dependent on ribosome production. Inhibition of new ribosome production (through deletion of ribosomal protein S6) was shown to prevent cell-cycle progression, even when sufficient "old" ribosomes were present for adequate translation of proteins [Volarevic et al., 2000]. Two yeast proteins that affect ribosome production were picked up serendipitously in a screen for their ability to physically associate with the origin of replication complex: Noc3p, which was previously known to be required for pre-rRNA processing [Zhang et al., 2002], and yph1p, which was found to be required for 60S ribosome subunit assembly [Du and Stillman, 2002]. In fact, the interplay between ribosomal activity and cell cycling is so intimate, nucleolar and ribosomal metabolism can be reinterpreted to be a potential regulator of cell-cycle progression (reviewed in [Ruggero and Pandolfi, 2003]). Thus, if there is a relationship at all between cell proliferation and nucleolar enlargement in PIN, the nucleolar enlargement in PIN could well be the cause rather than the consequence of increased cell division.

NE IRREGULARITY IN PROSTATE CANCER

NE irregularity is a common diagnostic abnormality in a wide variety of human cancers (reviewed in [Fischer et al., 2001b, 2003a]). Normal cells may have NE irregularity at precise stages of differentiation, (e.g., centrocyte lymphocytes), and after terminal differentiation. For example, post-mitotic neutrophils and monocytes have irregular nuclei, and these irregularities may facilitate migration of these cells through endothelial cell junctions. Generally, the NE of most normal, replication-competent cells is approximately spherical.

Many forms of cancer show NE that deviate from spherical. The character of these diagnostic NE changes varies from one type of cancer to another, and even for different clinically distinctive cancers starting from the same cell of origin. Perhaps the most common NE abnormality, which is also the most common diagnostic abnormality of the NE of prostate cancers, can be described as deep infoldings in some areas, occasional aneurismal-like outpouchings in other areas, and often with fine ruffling in still other areas of the same nucleus. These differ in character from long nuclear grooves and intranuclear inclusions characteristic of other forms of cancer. NE changes tend to appear later than the stage of PIN. Small cell type of cancers (which may arise from prostatic epithelium) show fragile-appearing NE's as a key diagnostic trait. The nuclei of small cell carcinomas appear to passively conform to each other in a pattern called "nuclear molding" and they easily break when the tumors are biopsied, spilling chromatin in a pattern called "crush artifact." Interestingly, small cell carcinomas lack A-type lamins (see below) that could in part account for their lack of resiliency (though many other cell types can lack A-type lamins and not show such fragility) [Broers et al., 1993, 1997]. Rarely prostate cancers may show deep but smooth folds dividing the nucleus into discreet lobes.

The large-scale organization of the NE defines nuclear shape (reviewed in [Moir et al., 1995; Holaska et al., 2002; Fischer et al., 2003a]). The NE includes of two lipid bilayers with embedded nuclear pores, and the chromatin-associated nuclear lamina. The outer lipid bilayer is continuous with, and apparently functionally equivalent to the endoplasmic reticulum. There are no integral membrane proteins known to be unique to the outer NE; all are shared by endoplasmic reticulum. The inner lipid bilayer is continuous with the outer nuclear membrane at the nuclear pores. The inner NE contains a large and growing number of integral membrane proteins, as well as proteins specifically associated with the inner membrane by virtue of isoprenylation. In turn, these inner nuclear membrane proteins associate with a number of non-integral proteins and chromatin. This chromatin-bound cohort of proteins associated with the inner nuclear membrane forms a resilient, elastic structure called the nuclear lamina. Nuclear lamina proteins have a diversity of functions, indicating that NE abnormalities in cancer cells could have a variety of functional effects. The NE and lamina proteins function in defining replication competence [Newport et al., 1990; Hutchison et al., 1994; Goldberg et al., 1995; Spann et al., 1997; Yang et al., 1997; Gant et al., 1999; Moir et al., 2000]. Lamina proteins also organize transcription by binding to and segregating heterochromatin [Ye et al., 1997], (reviewed in [Cockell and Gasser, 1999]). In support of a role of the NE in regulating transcription, localization of genes near the NE is associated with their down-regulation [Kosak et al., 2002]. NE abnormalities are "conserved in evolution" (during tumor progression and in cells with genetic instability), suggesting that they are functionally significant. NE alterations need to be incorporated into any comprehensive model of prostate cancer since they are common in many advanced prostate cancers.

Several morphometric studies have shown a correlation between loss of round nuclear shape, and measures of invasiveness and adverse clinical outcome in prostate cancers [Epstein et al., 1984; Partin et al., 1992], (reviewed in [Mohler et al., 1994]). Correlations between irregular NE shape and prognosis are also found in cancers arising in many other organs including head and neck [Giardina et al., 1996], breast [Seker et al., 2002], kidney [Nativ et al., 1996], ovary [Gurley et al., 1994; Liu et al., 1999], and cervix [Ettler et al., 1999]. A key metric in the studies of prostate cancer is nuclear roundness factor, a dimensionless factor that is essentially the ratio of the perimeter to the area. The association of NE irregularity with prognosis in prostate cancer is not perfect, and the relation is complicated. Surprisingly, the best associations are not between the

magnitude of the nuclear roundness factor, but rather its variation [Epstein et al., 1984; Partin et al., 1992], as if tumors with mixtures of round and irregular nuclei in a tumor have the worst prognosis. Nuclear area and nuclear perimeter measurements by themselves appeared to be poor discriminators of clinical outcome [Partin et al., 1992]. Therefore, neither size per se, nor surface area per se appears as important as the apparent instability of NE shape. In these same studies, DNA content variation was not as prognostically significant as the NE changes. Thus, genetic instability or pleomorphism per se does not seem to describe this chaotic NE abnormality. [Mohler et al., 1992].

Relatively little is known about how or why cancer cells show NE irregularity. In a survey of seven of the major structural components of the NE, no gross alterations in the amounts or electrophoretic mobilities were observed in a thyroid model of NE irregularity [Fischer et al., 2001b]. Further, the immunofluorescent distribution of these NE proteins seemed to passively follow the irregular NE contour of the cells without a predictable relation to the various irregularities. These results suggest that these NE proteins may play only a passive role in the genesis of NE irregularity. Two possibilities for NE irregularity could be envisaged: post-mitotic NE reassembly could be abnormal such that a rounded contour is never achieved before the next cell division. Alternatively (but not mutually exclusive), post-mitotic NE reassembly could be normal, but dynamic forces could deform the NE contour during the subsequent interphase. As a direct test of whether post-mitotic NE reassembly is required for expression of the phenotype of NE irregularity in a cancer, microinjection was used to express an oncogene (RET/PTC, a constitutively active tyrosine kinase) known to be responsible for the irregular NE phenotype of papillary thyroid carcinomas [Fischer et al., 1998a]. Microinjection allowed the timing of expression, and the requirement for an intervening mitosis to be controlled. RET/PTC was able to induce NE irregularity within hours, without a requirement for a post-mitotic NE reassembly [Fischer et al., 2003a].

To see if the dynamic development of NE irregularity during interphase is seen in tumors outside the thyroid, we recently expressed green fluoresent protein conjugated to lamin A in DU-145 prostate cancer cells to visualize in time lapse the character of NE irregularity in this cell line that shows NE irregularity in fixed cell preparations (Fig. 6). We observed that the NE of many of the cells were highly dynamic during interphase (Fig. 7) ([Vanguri et al., 2003], manuscript in preparation).

Since the tyrosine kinase RET/PTC has only been tested for its effect on the NE in thyroid cells, we asked whether RET/PTC expression in DU-145 cells could augment the irregularity already present. Preliminary results suggest that RET/PTC expression following transfection leads to a hyper-convoluted nucleus in DU-145 cells (AH Fischer and JA Nickerson, unpublished observations). RET has been demonstrated by immunohistochemistry to be present in a subset of PIN and prostate cancers [Dawson et al., 1998], but it is not reported whether its expression is associated with a more convoluted NE. It seems likely that NE irregularity in prostate cancers will be induced by some of the cancer genes active in prostate cancer.

We hypothesize that the dynamic forces that deform the NE of interphase cancer cells reflect an imbalance of forces exerted on the NE from either the cytoskeleton or chromatin (see [Fischer et al., 2003a] for a full discussion).

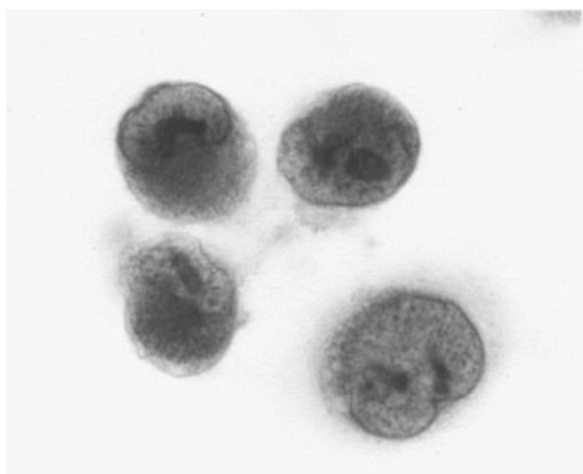


Fig. 6. DU145 prostate cancer cells provide an excellent model for studying diagnostic cell structural changes in prostate cancer. They show prominent nucleoli in spite of relatively scant pale cytoplasm, and they show stochastic nuclear contour abnormalities. It seems unlikely that either of these two structural features can be related simply to cell-cycle speed per se. Primary normal human prostate epithelial cell nuclei show a flattened but rounded contour, like the nuclei of the fibroblasts in Figure 4 or 5, and they tend to show only 2–3 small nucleoli [Fischer et al., 2001a].

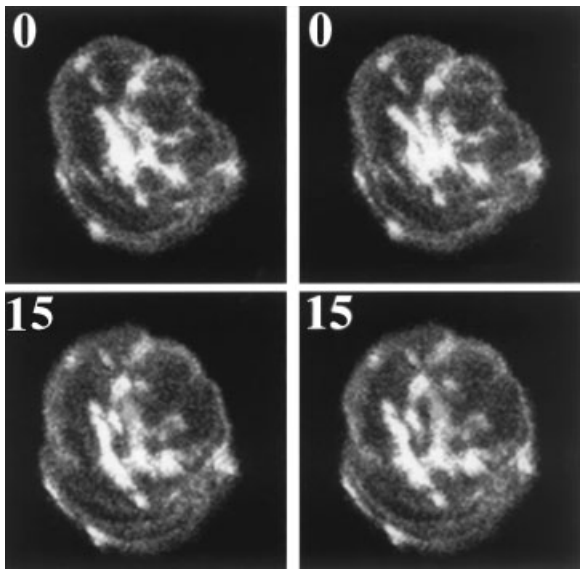


Fig. 7. Nuclear contour abnormalities in DU145 cells are dynamic in interphase ([Vanguri et al., 2003] manusciprt in preparation), as shown in two stereo pair views of the same nucleus 15 min apart. The cell is labeled with green fluorescent protein conjugated to lamin A. Since the nuclear envelope (NE) moves during interphase, forces must be exerted on it, probably from chromatin or cytoskeletal elements (discussed in [Fischer et al., 2003a]).

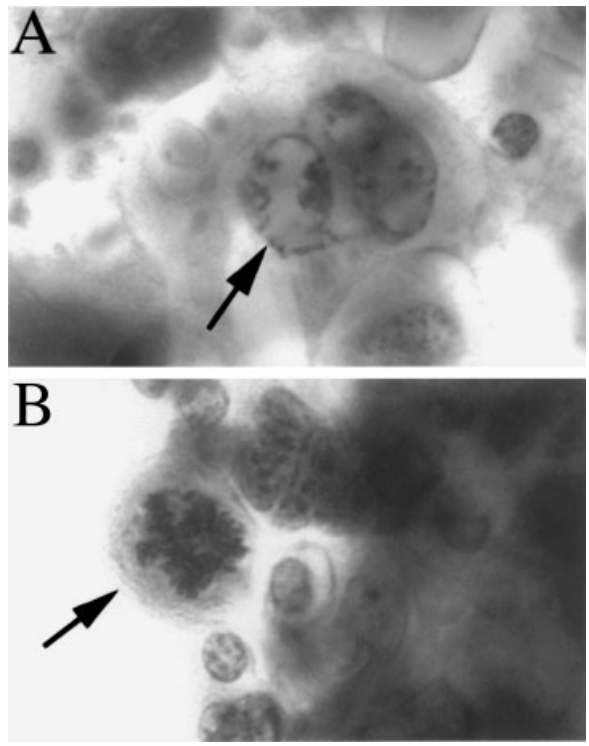


Fig. 8. Nuclear contour changes in cancer cells at prophase (**A**) cause chromosomes to be presented in an asymmetric manner to the mitotic spindle apparatus at metaphase (**B**). One may anticipate that this slows progression through the bi-orientation spindle checkpoint. Loss of a bi-orientation spindle checkpoint [Amon, 1999], and loss of a spindle checkpoint-associated apoptotic death [Li et al., 1998] would be favored in cells with NE asymmetries.

Chromatin-based forces that could mediate dynamic NE dynamic deflections may be related to the interphase large-scale dynamics of chromosomal domains [Belmont, 2001]. One could also imagine that the increased cell motility in some cancer cells could require cytoskeletal dynamics that deform the NE.

Another potential functional consequence of NE irregularity that has not received attention is as follows: NE irregularity at prophase (Fig. 8A) presents grossly asymmetric chromosomes to the mitotic spindle apparatus (Fig. 8B). The spindle checkpoint [Amon, 1999] would be anticipated to delay progression through metaphase until bi-orientation of the chromosomes is achieved [Tanaka, 2002]. It has been proposed that cells chronically exposed to mutagens should be under selection pressure to lose p53 cell-cycle checkpoints [Wynford-Thomas, 1996]. By analogy, there should be a selection pressure for cells with irregular NEs to lose components of the spindle checkpoint: If these components were lost, cells would progress more quickly through mitosis, and they would not be susceptible to a spindle checkpoint-associated apoptotic death [Li et al., 1998].

CONCLUSIONS

Nucleolar enlargement in PIN, and NE changes characteristic of tumor progression in prostate cancers, are akin to speciating characteristics in Darwinian adaptive evolution. Speciating characteristics in Darwinian evolution are generally mediated by the genetic changes responsible for evolution. Based on available evidence, it does appear that genetic changes implicated in prostate carcinogenesis could mediate the development of nucleolar and NE changes. Studies of the putative genetic events in prostate cancer will require a careful description of cell structure in the experimental materials to fully test this prediction. More importantly, phenotypic changes during evolution provide essential clues to the functional changes that allow evolution to occur. It is hard to imagine being able to make any useful deductions about the mechanism of adaptive Darwinian evolution without first considering

the associated phenotypic changes. Conservation of structure through evolution provides the strongest evidence that the structure is functionally important, and in fact nucleolar enlargement in PIN and subsequent NE irregularity are conserved in genetically unstable invasive prostatic adenocarcinomas. With such a large energy cost associated with increasing ribosome production and nucleolar size, and with so many levels of regulation imposed on nucleolar and NE function, these diagnostic alterations must represent major physiologic shifts for the evolving cancer cells. Prostate cancer research clearly should benefit by uncovering the structural and genetic basis of nucleolar and nuclear envelope changes.

REFERENCES

Alcaraz A, Barranco MA, Corral JM, Ribal MJ, Carrio A, Mallofre C, Llopis J, Cetina A, Alvarez-Vijande R. 2001. High-grade prostate intraepithelial neoplasia shares cytogenetic alterations with invasive prostate cancer. Prostate 47:29–35.

Amon A. 1999. The spindle checkpoint. Curr Opin Genet Dev 9:69–75.

Basolo F, Pollina L, Fontanini G, Fiore L, Pacini F, Baldanzi A. 1997. Apoptosis and proliferation in thyroid carcinoma: Correlation with bcl-2 and p53 protein expression. Br J Cancer 75:537–541.

Belmont AS. 2001. Visualizing chromosome dynamics with GFP. Trends Cell Biol 11:250–257.

Bostwick DG, Brawer MK. 1987. Prostatic intra-epithelial neoplasia and early invasion in prostate cancer. Cancer 59:788–794.

Bostwick DG, Eble JN. 1997. "Urologic Surgical Pathology." St. Louis: Mosby.

Bostwick DG, Pacelli A, Lopez-Beltran A. 1997. Ultrastructure of prostatic intraepithelial neoplasia. Prostate 33:32–37.

Broers JL, Raymond Y, Rot MK, Kuijpers H, Wagenaar SS, Ramaekers FC. 1993. Nuclear A-type lamins are differentially expressed in human lung cancer subtypes. Am J Pathol 143:211–220.

Broers JL, Machiels BM, Kuijpers HJ, Smedts F, van den Kieboom R, Raymond Y, Ramaekers FC. 1997. A- and B-type lamins are differentially expressed in normal human tissues. Histochem Cell Biol 107:505–517.

Cockell M, Gasser SM. 1999. Nuclear compartments and gene regulation. Curr Opin Genet Dev 9:199–205.

Crissman JD, Sakr WA, Hussein ME, Pontes JE. 1993. DNA quantitation of intraepithelial neoplasia and invasive carcinoma of the prostate. Prostate 22:155–162.

Dawson DM, Lawrence EG, MacLennan GT, Amini SB, Kung HJ, Robinson D, Resnick MI, Kursh ED, Pretlow TP, Pretlow TG. 1998. Altered expression of RET proto-oncogene product in prostatic intraepithelial neoplasia and prostate cancer. J Natl Cancer Inst 90:519–523.

De Marzo AM, Meeker AK, Epstein JI, Coffey DS. 1998a. Prostate stem cell compartments: Expression of the cell cycle inhibitor p27Kip1 in normal, hyperplastic, and neoplastic cells. Am J Pathol 153:911–919.

De Marzo AM, Nelson WG, Meeker AK, Coffey DS. 1998b. Stem cell features of benign and malignant prostate epithelial cells. J Urol 160:2381–2392.

Derenzini M, Trere D, Pession A, Govoni M, Sirri V, Chieco P. 2000. Nucleolar size indicates the rapidity of cell proliferation in cancer tissues. J Pathol 191:181–186.

Doll JA, Zhu X, Furman J, Kaleem Z, Torres C, Humphrey PA, Donis-Keller H. 1999. Genetic analysis of prostatic atypical adenomatous hyperplasia (adenosis). Am J Pathol 155:967–971.

Du YC, Stillman B. 2002. Yph1p, an ORC-interacting protein: Potential links between cell proliferation control, DNA replication, and ribosome biogenesis. Cell 109:835–848.

Epstein JI, Berry SJ, Eggleston JC. 1984. Nuclear roundness factor. A predictor of progression in untreated stage A2 prostate cancer. Cancer 54:1666–1671.

Ettler HC, Joseph MG, Downing PA, Suskin NG, Wright VC. 1999. Atypical squamous cells of undetermined significance: A cytohistological study in a colposcopy clinic. Diagn Cytopathol 21:211–216.

Fernandez PL, Hernandez L, Farre X, Campo E, Cardesa A. 2002. Alterations of cell cycle-regulatory genes in prostate cancer. Pathobiology 70:1–10.

Fischer AH, Bond JA, Taysavang P, Battles OE, Wynford-Thomas D. 1998a. Papillary thyroid carcinoma oncogene (*RET*/*PTC*) alters the nuclear envelope and chromatin structure. Am J Pathol 153:1443–1450.

Fischer AH, Chaddee DN, Wright JA, Gansler TS, Davie JR. 1998b. Ras-associated nuclear structural change appears functionally significant and independent of the mitotic signaling pathway. J Cell Biochem 70:130–140.

Fischer AH, Philips A, Taysavang P, McKenney JK, Amin MB. 2001a. Method for procuring specific populations of viable human prostate cells for research. Lab Invest 81:501–507.

Fischer AH, Taysavang P, Weber C, Wilson K. 2001b. Nuclear envelope organization in papillary thyroid carcinoma. Histol Histopathol 16:1–14.

Fischer AH, Taysavang P, Jhiang SM. 2003a. Nuclear envelope irregularity is induced by RET/PTC during interphase. Am J Pathol 163:1091–1100.

Fischer AH, Young KA, DeLellis RA. 2003b. Incorporating the criteria of malignancy into the evolutionary framework for cancer. Submitted for publication.

Fornaro M, Manes T, Languino LR. 2001. Integrins and prostate cancer metastases. Cancer Metastasis Rev 20:321–331.

Frank DJ, Edgar BA, Roth MB. 2002. The *Drosophila* melanogaster gene brain tumor negatively regulates cell growth and ribosomal RNA synthesis. Development 129: 399–407.

Frost JK. 1986. "The Cell in Health and Disease: An evaluation of cellular morphologic expression of biologic behavior." New York: Karger.

Gant TM, Harris CA, Wilson KL. 1999. Roles of LAP2 proteins in nuclear assembly and DNA replication: Truncated LAP2B proteins alter lamina assembly, envelope formation, nuclear size, and DNA replication efficiency in *Xenopus laevis* extracts. J Cell Biol 144: 1083–1096.

Garcia-Schurmann M, Coffey DS. 1997. The concept of evolution in the pathogenesis and treatment of prostate cancer. Br J Urol 79:9–17.

Giardina C, Caniglia DM, D'Aprile M, Lettini T, Serio G, Cipriani T, Ricco R, Pesce Delfino V. 1996. Nuclear morphometry in squamous cell carcinoma (SCC) of the tongue. Eur J Cancer. Part B, Oral Oncol 32B:91–96.

Goldberg M, Jenkins H, Allen T, Whitfield WG, Hutchison CJ. 1995. *Xenopus* lamin B3 has a direct role in the assembly of a replication competent nucleus: Evidence from cell-free egg extracts. J Cell Sci 108:3451–3461.

Grunwald V, DeGraffenried L, Russel D, Friedrichs WE, Ray RB, Hidalgo M. 2002. Inhibitors of mTOR reverse doxorubicin resistance conferred by PTEN status in prostate cancer cells. Cancer Res 62:6141–6145.

Gurley AM, Hidvegi DF, Cajulis RS, Bacus S. 1994. Morphologic and morphometric features of low grade serous tumours of the ovary. Diagn Cytopathol 11:220–225.

Heidenberg HB, Sesterhenn IA, Gaddipati JP, Weghorst CM, Buzard GS, Moul JW, Srivastava S. 1995. Alteration of the tumor suppressor gene *p53* in a high fraction of hormone refractory prostate cancer. J Urol 154:414–421.

Helpap B, Riede C. 1995. Nucleolar and AgNOR-analysis of prostatic intraepithelial neoplasia (PIN), atypical adenomatous hyperplasia (AAH) and prostatic carcinoma. Pathol Res Pract 191:381–390.

Helpap BG, Bostwick DG, Montironi R. 1995. The significance of atypical adenomatous hyperplasia and prostatic intraepithelial neoplasia for the development of prostate carcinoma, an update. Virchows Archiv 426:425–434.

Holaska JM, Wilson KL, Mansharamani M. 2002. The nuclear envelope, lamins and nuclear assembly. Curr Opin Cell Biol 14:357–364.

Hutchison CJ, Bridger JM, Cox LS, Kill IR. 1994. Weaving a pattern from disparate threads: Lamin function in nuclear assembly and DNA replication. J Cell Sci 107: 3259–3269.

Jiang Z, Woda BA, Rock KL, Xu Y, Savas L, Khan A, Pihan G, Cai F, Babcook JS, Rathanaswami P, Reed SG, Xu J, Fanger GR. 2001. P504S: A new molecular marker for the detection of prostate carcinoma. Am J Surg Pathol 25: 1397–1404.

Jimenez RE, Fischer AH, Petros JA, Amin MB. 2000. Glutathione S-transferase pi gene methylation: The search for a molecular marker of prostatic adenocarcinoma. Adv Anat Pathol 7:382–389.

Kallakury BV, Sheehan CE, Rhee SJ, Fisher HA, Kaufman RP Jr, Rifkin MD, Ross JS. 1999. The prognostic significance of proliferation-associated nucleolar protein p120 expression in prostate adenocarcinoma: A comparison with cyclins A and B1, Ki-67, proliferating cell nuclear antigen, and p34cdc2. Cancer 85:1569–1576.

Kastendieck H, Altenahr E. 1976. Cyto- and histomorphogenesis of the prostate carcinoma. A comparative light- and electron-microscopic study. Virchows Archiv. A, Pathol Anat Histol 370:207–224.

Kim S, Li Q, Dang CV, Lee LA. 2000. Induction of ribosomal genes and hepatocyte hypertrophy by adenovirus-mediated expression of c-Myc in vivo. Proc Nat Acad Sci USA 97:11198–12002.

Kosak ST, Skok JA, Medina KL, Riblet R, Le Beau MM, Fisher AG, Singh H. 2002. Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. Science 296:158–162.

Kurata S, Koga K, Sakaguchi B. 1978. Nucleolar size in parallel with ribosomal RNA synthesis at diapause termination in the eggs of Bombyx mori. Chromosoma 68:313–317.

Li F, Ambrosini G, Chu EY, Plescia J, Tognin S, Marchisio PC, Altieri DC. 1998. Control of apoptosis and mitotic spindle checkpoint by survivin. Nature 396:580–584.

Liebhaber SA, Wolf S, Schlessinger D. 1978. Differences in rRNA metabolism of primary and SV40-transformed human fibroblasts. Cell 13:121–127.

Liu Y, Liang S, Tartakoff AM. 1996. Heat shock disassembles the nucleolus and inhibits nuclear protein import and poly(A)+RNA export. EMBO J 15:6750–6757.

Liu CQ, Sasaki H, Fahey MT, Sakamoto A, Sato S, Tanaka T. 1999. Prognostic value of nuclear morphometry in patients with TNM stage T1 ovarian clear cell adenocarcinoma. Br J Cancer 79:1736–1741.

Magi-Galluzzi C, Montironi R, Cangi MG, Wishnow K, Loda M. 1998a. Mitogen-activated protein kinases and apoptosis in PIN. Virchows Archiv 432:407–413.

Magi-Galluzzi C, Murphy M, Cangi MG, Loda M. 1998b. Proliferation, apoptosis, and cell cycle regulation in prostatic carcinogenesis. Anal Quant Cytol Histol 20:343–350.

Mansour SJ, Matten WT, Hermann AS, Candia JM, Rong S, Fukasawa K, Vande Woude GF, Ahn NG. 1994. Transformation of mammalian cells by constitutively active MAP kinase kinase. Science 265:966–970.

McNeal JE, Bostwick DG. 1986. Intraductal dysplasia: A premalignant lesion of the prostate. Hum Pathol 17: 64–71.

Mohler JL, Partin AW, Epstein JI, Becker RL, Mikel UV, Sesterhenn IA, Mostofi FK, Gleason DF, Sharief Y, Coffey DS. 1992. Prediction of prognosis in untreated stage A2 prostatic carcinoma. Cancer 69:511–519.

Mohler JL, Figlesthaler WM, Zhang XZ, Partin AW, Maygarden SJ. 1994. Nuclear shape analysis for the assessment of local invasion and metastases in clinically localized prostate carcinoma. Cancer 74:2996–3001.

Moir RD, Spann TP, Goldman RD. 1995. The dynamic properties and possible functions of nuclear lamins. Int Rev Cytol 162B:141–182.

Moir RD, Spann TP, Herrmann H, Goldman RD. 2000. Disruption of nuclear lamin organization blocks the elongation phase of DNA replication. J Cell Biol 149: 1179–1192.

Montironi R, Braccischi A, Matera G, Scarpelli M, Pisani E. 1991. Quantitation of the prostatic intra-epithelial neoplasia. Analysis of the nucleolar size, number, and location. Pathol Res Pract 187:307–314.

Montironi R, Mazzucchelli R, Scarpelli M. 2002. Precancerous lesions and conditions of the prostate: From morphological and biological characterization to chemoprevention. Ann NY Acad Sci 963:169–184.

Mutter GL, Ince TA, Baak JP, Kust GA, Zhou XP, Eng C. 2001. Molecular identification of latent precancers in histologically normal endometrium. Cancer Res 61: 4311–4314.

Myers RB, Grizzle WE. 1996. Biomarker expression in prostatic intraepithelial neoplasia. Eur Urol 30:153–166.

Nativ O, Sabo E, Bejar J, Halachmi S, Moskovitz B, Miselevich I. 1996. A comparison between histological grade and nuclear morphometry for predicting the clinical outcome of localized renal cell carcinoma. Br J Urol 78:33–38.

Newport JW, Wilson KL, Dunphy WG. 1990. A lamin-independent pathway for nuclear envelope assembly. J Cell Biol 111:2247–2259.

Nierras CR, Liebman SW, Warner JR. 1997. Does Saccharomyces need an organized nucleolus? Chromosoma 105: 444–451.

Olson MO, Dundr M, Szebeni A. 2000. The nucleolus: An old factory with unexpected capabilities. Trends Cell Biol 10:189–196.

Partin AW, Steinberg GD, Pitcock RV, Wu L, Piantadosi S, Coffey DS, Epstein JI. 1992. Use of nuclear morphometry, gleason histologic scoring, clinical stage, and age to predict disease-free survival among patients with prostate cancer. Cancer 70:161–168.

Pederson T. 1998. Growth factors in the nucleolus? J Cell Biol 143:279–281.

Phillips SM, Barton CM, Lee SJ, Morton DG, Wallace DM, Lemoine NR, Neoptolemos JP. 1994. Loss of the retinoblastoma susceptibility gene (*RB1*) is a frequent and early event in prostatic tumorigenesis. Br J Cancer 70: 1252–1257.

Pihan GA, Purohit A, Wallace J, Malhotra R, Liotta L, Doxsey SJ. 2001. Centrosome defects can account for cellular and genetic changes that characterize prostate cancer progression. Cancer Res 61:2212–2219.

Qian J, Jenkins RB, Bostwick DG. 1997. Detection of chromosomal anomalies and *c-myc* gene amplification in the cribriform pattern of prostatic intraepithelial neoplasia and carcinoma by fluorescence in situ hybridization. Mod Pathol 10:1113–1119.

Rosai J, Carcangiu ML, Delellis RA. 1992. "Tumors of the thyroid gland." Washington: Armed Forces Institute of Pathology.

Ruggero D, Pandolfi PP. 2003. Does the ribosome translate cancer? Nature reviews. Cancer 3:179–192.

Seker H, Odetayo MO, Petrovic D, Naguib RN, Bartoli C, Alasio L, Lakshmi MS, Sherbet GV. 2002. Assessment of nodal involvement and survival analysis in breast cancer patients using image cytometric data: Statistical, neural network, and fuzzy approaches. Anticancer Res 22:433–438.

Signoretti S, Montironi R, Manola J, Altimari A, Tam C, Bubley G, Balk S, Thomas G, Kaplan I, Hlatky L, Hahnfeldt P, Kantoff P, Loda M. 2000. Her-2-neu expression and progression toward androgen independence in human prostate cancer. J Natl Cancer Inst 92: 1918–1925.

Spann TP, Moir RD, Goldman AE, Stick R, Goldman RD. 1997. Disruption of nuclear lamin organization alters the distribution of replication factors and inhibits DNA synthesis. J Cell Biol 136:1201–1212.

Stefanovsky VY, Pelletier G, Hannan R, Gagnon-Kugler T, Rothblum LI, Moss T. 2001. An immediate response of ribosomal transcription to growth factor stimulation in mammals is mediated by ERK phosphorylation of UBF. Mole Cell 8:1063–1073.

Sung SY, Chung LW. 2002. Prostate tumor–stroma interaction: Molecular mechanisms and opportunities for therapeutic targeting. Differentiation 70:506–521.

Tanaka TU. 2002. Bi-orienting chromosomes on the mitotic spindle. Curr Opin Cell Biol 14:365–371.

Vailancourt L, Ttu B, Fradet Y, Dupont A, Gomez J, Cusan L, Suburu ER, Diamond P, Candas B, Labrie F. 1996. Effect of neoadjuvant endocrine therapy (combined androgen blockade) on normal prostate and prostatic carcinoma. A randomized study. Am J Surg Pathol 20:86–93.

Vanguri V, Wang X, Wagner S, Patwardhan N, Khan A, Nickerson JA, Fischer AH. 2003. Nuclear envelope irregularity in cancer cells develops dynamically in interphase: A 3-D time lapse study of living cells. manuscript in preparation.

Voit R, Grummt I. 2001. Phosphorylation of UBF at serine 388 is required for interaction with RNA polymerase I and activation of rDNA transcription. Proc Nat Acad Sci USA 98:13631–13636.

Volarevic S, Stewart MJ, Ledermann B, Zilberman F, Terracciano L, Montini E, Grompe M, Kozma SC, Thomas G. 2000. Proliferation, but not growth, blocked by conditional deletion of 40S ribosomal protein S6. Science 288:2045–2047.

Warner JR. 1999. The economics of ribosome biosynthesis in yeast. Trends Biochem Sci 24:437–440.

Warner JR, Vilardell J, Sohn JH. 2001. Economics of ribosome biosynthesis. Cold Spring Harb Symp Quant Biol 66:567–574.

Wynford-Thomas D. 1996. p53: Guardian of cellular senescence. J Pathol 180:118–121.

Yang L, Guan T, Gerace L. 1997. Lamin-binding fragment of LAP2 inhibits increase in nuclear volume during the cell cycle and progression into S phase. J Cell Biol 139: 1077–1087.

Yang XJ, Wu CL, Woda BA, Dresser K, Tretiakova M, Fanger GR, Jiang Z. 2002. Expression of alpha-Methylacyl-CoA racemase (P504S) in atypical adenomatous hyperplasia of the prostate. Am J Surg Pathol 26:921–925.

Ye Q, Callebaut I, Pezhman A, Courvalin JC, Worman HJ. 1997. Domain-specific interactions of human HP1-type chromodomain proteins and inner nuclear membrane protein LBR. J Biol Chem 272:14983–14989.

Zhang Y, Yu Z, Fu X, Liang C. 2002. Noc3p, a bHLH protein, plays an integral role in the initiation of DNA replication in budding yeast. Cell 109:849–860.