

# The granulocyte nucleus and lamin B receptor: avoiding the ovoid

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Received: 21 November 2006 / Revised: 27 December 2006 / Accepted: 28 December 2006 / Published online: 24 January 2007  
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**Abstract** The major human blood granulocyte, the neutrophil, is an essential component of the innate immunity system, emigrating from blood vessels and migrating through tight tissue spaces to the site of bacterial or fungal infection where they kill and phagocytose invading microbes. Since the late nineteenth century, it has been recognized that the human neutrophil nucleus is distinctly not ovoid as in other cell types, but possesses a lobulated (segmented) shape. This deformable nucleus enhances rapid migration. Recent studies have demonstrated that lamin B receptor (LBR) is necessary for the non-ovoid shape. LBR is an integral membrane protein of the nuclear envelope. A single dominant mutation in humans leads to neutrophils with hypolobulated nuclei (Pelger–Huet anomaly); homozygosity leads to ovoid granulocyte nuclei. Interestingly, LBR is also an enzyme involved in cholesterol metabolism. Homozygosity for null mutations is frequently lethal and associated with severe skeletal deformities. In addition to the necessity for LBR, formation of the mature granulocyte nucleus also depends upon lamin composition and microtubule integrity. These observations are part of a larger question on the relationships between nuclear shape and cellular function.

## Introduction

Every milliliter of normal adult human blood contains ~3 to 5 million neutrophils. These cells derive from adult bone marrow by a process (granulopoiesis) that takes approximately 2 weeks: a mitotic phase (7.5 days), a post-mitotic phase (6.5 days) involving significant nuclear lobulation, and chromatin condensation (Baehner 2000; Bainton et al. 1971). Different sets of cytoplasmic granules containing a variety of bactericidal agents (e.g., myeloperoxidase, acid and alkaline phosphatases, lactoferrin) develop during the 2-week period (Baehner 2000). Normal circulating neutrophils die by apoptosis with a half-life of ~7 h, surviving longer after encounter with inflammatory factors. At localized sites of infection, the vascular endothelium provokes attachment of the circulating neutrophils that flatten, squeeze through the endothelial lining, erode the underlying basement membrane, and migrate through adjacent tissue spaces (Baehner 2000; Edens and Parkos 2003; Feng et al. 1998; Johnson-Leger et al. 2000; Middleton et al. 2002). The serial-section electron microscopy of neutrophils emigrating from venules demonstrates the elongation of the nucleus during transendothelial migration (Feng et al. 1998).

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Communicated by E.A. Nigg

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## Description of the lobulated granulocyte nucleus

Early microscopists of the seventeenth century (Leeuwenhoek, Swammerdam, Malpighi) examined blood and observed the red “corpuscles” (Hughes 1959). However, due to their lower titer and lack of distinguishing color, it is very unlikely that granulocytes were ever identified. In the eighteenth century, microscopists (Lieutaud, de Senac, Hewson) described white cells in pus and the lymphatic

system (Piller 2001). The introduction of tissue staining with synthetic dyes during the latter half of the nineteenth century by Waldeyer, Ehrlich, Frey, and other histologists (Bracegirdle 1978), combined with improved microscope optics, derived from the collaborative efforts of Zeiss, Abbé, and Schott, permitted detailed descriptions and identifications of subcellular structures (Hughes 1959). Paul Ehrlich (1854–1915) was probably the first scientist to stain leukocytes (white cells) to distinguish acidophilic, basophilic, and neutrophilic cells, and to describe the lobulated nucleus (Bracegirdle 1978; Hallett 1989; Himmelweit 1956). Ehrlich also developed the blood smearing method that is still used today. Some of Ehrlich's drawings of "polynuclear neutrophil leucocytes" stained with "triacid solution" are presented (Fig. 1), illustrating the lobulated nuclear shape. The idea that neutrophil cells employed phagocytosis to battle microbial infections can be attributed to Elie Metchnikoff (1845–1916; Metchnikoff 1905). In the twentieth century, the development of the transmission electron microscope, combined with improved fixation, sectioning and staining methods provided clear ultrastructural information about the human granulocyte nucleus (Zucker-Franklin 1968, 1975). The normal human neutrophil nucleus possesses an abundance of peripheral heterochromatin with only remnants of nucleoli. Despite the extensive amount of heterochromatin, this terminally differentiated cell is capable of significant changes in genetic expression in response to activation by inflammatory stimuli (Zhang et al. 2004). The lobulated character of neutrophil nuclei, when examined in thin sections, gives the false impression of multiple nuclei in a single cell. Only serial thin-section electron microscopy unequivocally demonstrates the single lobulated granulocyte nuclei. These advances in ultrastructural methods permitted accurate descriptions of the various stages of granulopoiesis, encompassing both nuclear and cytoplasmic differentiation (Bainton et al. 1971).

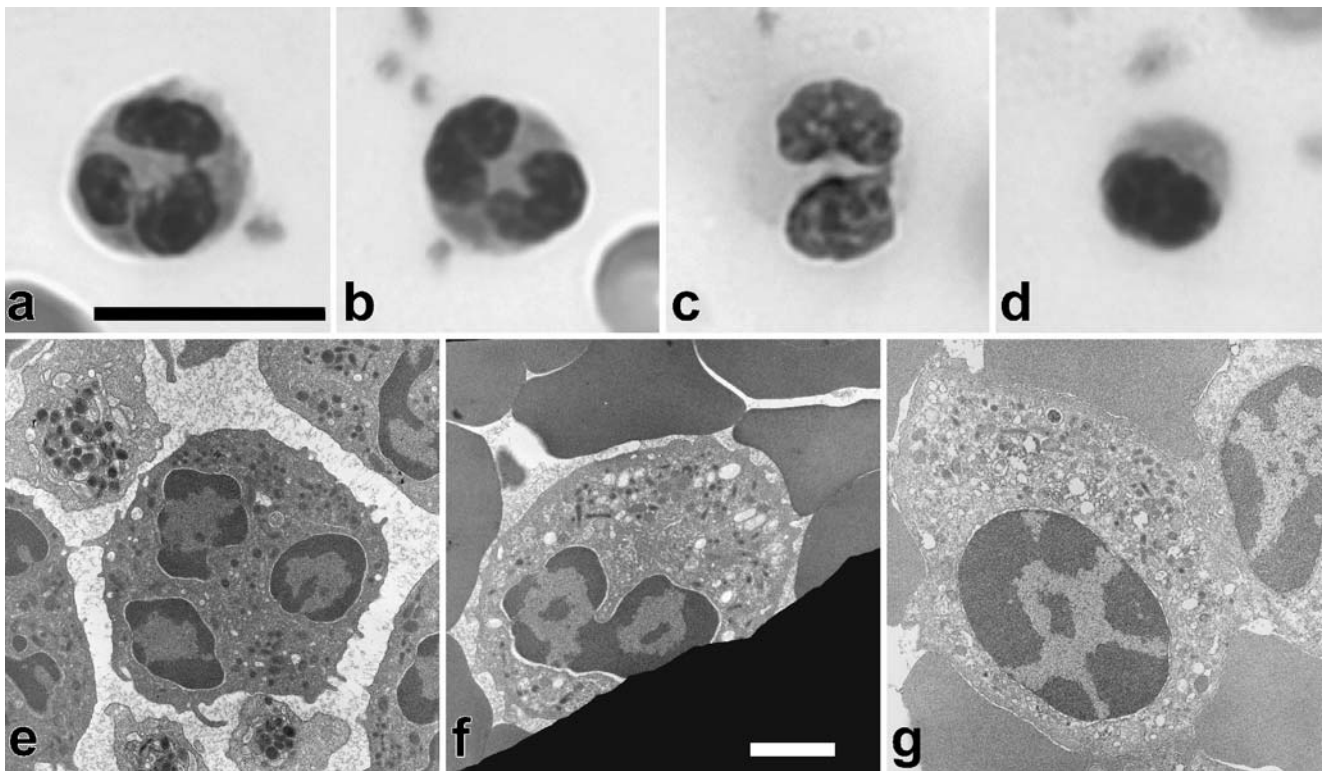
### Pelger–Huet anomaly

In 1928, a Dutch hematologist (K. Pelger) described "a rarely occurring type of leukocyte" with unsegmented, short, and stubby nuclei possessing a coarse chromatin texture (Pelger 1928). Subsequently, a pediatrician (G. Huët) demonstrated that this leukocyte nuclear trait is heritable (Huët 1931). As monitored using blood smears, Pelger–Huet anomaly (PHA) exhibits a characteristic granulocyte nuclear morphology: Heterozygous individuals reveal a rod-like or bilobed (spectacle or "pince-nez") appearance, demonstrating a "dominant" genetic disorder; homozygous individuals reveal ovoid nuclei with coarse chromatin (Fig. 2). For earlier published micrographs, see Begemann and van Lookeren Campagne (1952) and Stobbe (1959). PHA has been observed in diverse populations around the world with prevalence varying from 0.01–0.1%. Two exceptions in prevalence have been noted: Västerbotten (northeast Sweden), 0.6%; Gelenau (south-east Germany), 1.01%. A plausible historical connection between these two communities may be a founder Swedish soldier from the Thirty Years' War (1618–1648). In the heterozygous state, PHA is benign, with the only apparent phenotype being the altered neutrophil nucleus. The few (11) reported homozygous human individuals possess a variety of clinical phenotypes, some with mental retardation, a few with skeletal defects (Hoffmann et al. 2002; Oosterwijk et al. 2003). PHA is usually found by the examination of blood smears when hypolobulated granulocyte nuclei are detected. Similar "immature" granulocyte nuclei are observed in infections, toxic drug effects, and myeloid leukemias, collectively described as "pseudo-Pelger" (Curnutte and Coates 2000). When PHA granulocytes have been tested *in vitro*, they show insignificant differences from normal granulocytes, comparing cytoplasmic granular enzymes, NBT reduction,



**Fig. 1** Paul Ehrlich's drawing of "polynuclear neutrophil leucocytes" stained with "triacid solution" taken from Plate IV (Himmelweit 1956). P. Ehrlich and A. Lazarus (1900), quoted in (Himmelweit 1956), write "These cells are...distinguished by a characteristic,

polymorphous nucleus; this is represented by a relatively long, irregularly bulged and indented nuclear rod having the appearance, at first, of an S, Y, E, or Z."



**Fig. 2** Granulocyte nuclear hypobolubation and heterochromatin redistribution in human Pelger–Huet anomaly. **a–d** Wright–Giemsa stained light micrographs of blood smears showing: **a** normal neutrophil with lobulated nucleus; **b** heterozygous PHA neutrophil with a bilobed nucleus, taken from the mother of the homozygote; **c** heterozygous PHA neutrophil with a bilobed nucleus, taken from the father of the homozygote; **d** homozygous PHA neutrophil showing an ovoid nucleus with chromatin clumping. Scale bar for light micro-

graphs, 10  $\mu$ m. **e–g** Thin-section electron micrographs showing: **e** normal human neutrophil nucleus with three apparent lobes and extensive peripheral heterochromatin; **f** heterozygous PHA neutrophil with a bilobed nucleus taken from the father of the homozygote; **g** ovoid nucleus from a homozygous PHA granulocyte exhibiting extensive heterochromatin redistribution. Scale bar for electron micrographs, 1  $\mu$ m

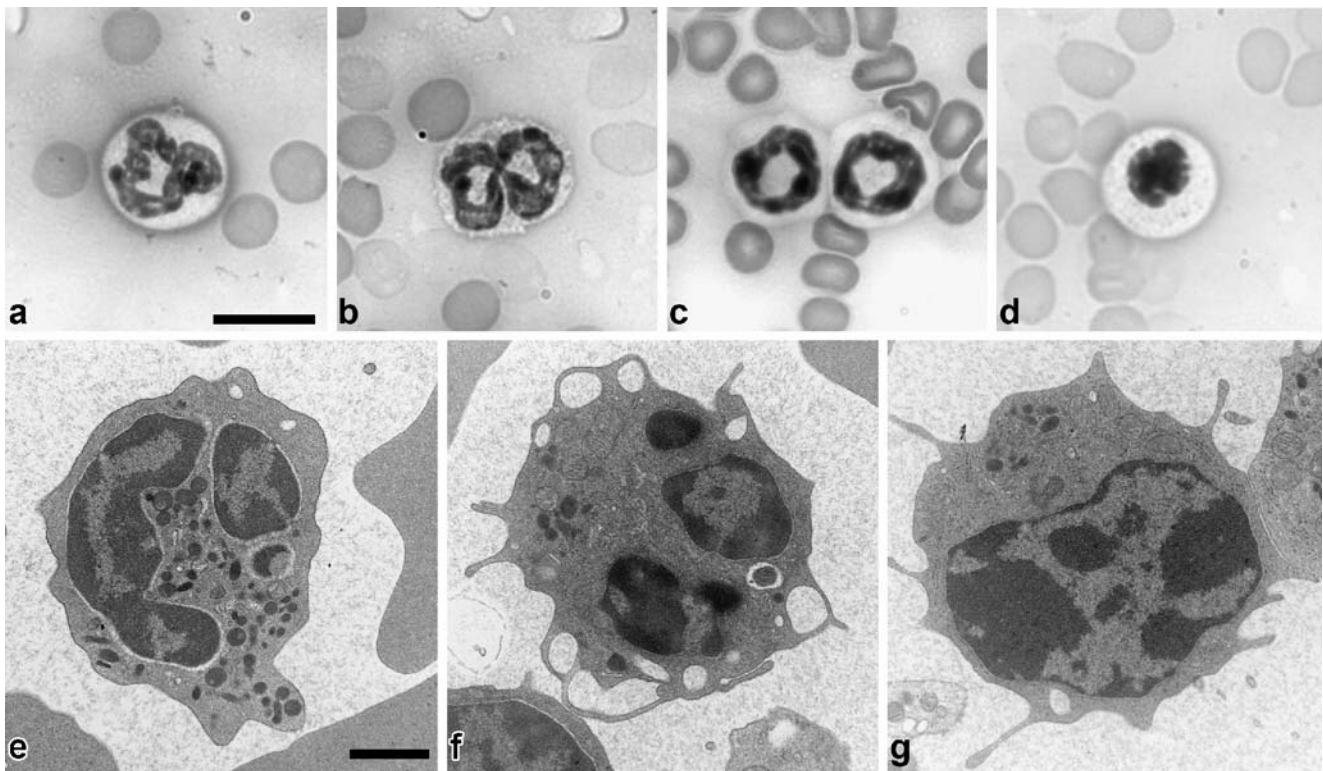
superoxide production, phagocytosis, and chemotaxis (Johnson et al. 1980; Matsumoto et al. 1984; Skendzel and Hoffman 1962; Stobbe and Jorke 1965), with one notable exception (Park et al. 1977). Granulocytes from five heterozygous PHA individuals (from one family) were tested for chemotaxis function in vitro through 3-, 5-, and 8- $\mu$ m diameter pore Millipore filters and in vivo through “skin windows.” PHA granulocytes migrated significantly more slowly through these structural barriers than did control granulocytes. Comparable studies have not been performed with homozygous PHA granulocytes, and information is unavailable about the impact of ovoid nuclei on natural immunity.

PHA has been reported and examined in a variety of animal species. In 1943, PHA was reported in rabbits (Nachtsheim 1943; Undritz 1939, 1943). The homozygous condition in rabbits is characterized by ovoid granulocyte nuclei; severe skeletal, developmental, and skin anomalies; and increased pre- and postnatal mortality. The PHA nuclear phenotype has also been observed in dogs (Bowles et al. 1979; Kiss 1967; Latimer et al. 1987), cats (Latimer et al.

1985), and mice (Green et al. 1975). In the case of mice, the authors were searching “among...existing mutant mice showing skin and hair deficiencies for effects on the immune system.” Ichthyosis (*ic*) had been described in 1950 (Carter and Phillips 1950). Upon investigation, the authors (Green et al. 1975) noted chromatin clumping in lymphocytes, neutrophils, and other cells with round or oval neutrophil nuclei, resembling PHA in humans and rabbits, but they did not report any compromise of the immune system. Heterochromatin redistribution can be clearly observed in granulocytes derived from *ic* homozygous animals (Fig. 3).

### Defining the Pelger–Huet gene

In 2002, fine mapping and sequencing studies identified lamin B receptor (LBR), an integral membrane protein of the nuclear envelope (Gruenbaum et al. 2005), as the mutated gene underlying PHA (Hoffmann et al. 2002). Continuing an earlier study in the village Gelenau, where 4,700 individuals were screened for PHA by blood smears,



**Fig. 3** Granulocyte nuclear shape change and heterochromatin redistribution in mouse *ic*. **a–d** Wright–Giemsa stained light micrographs of blood smears showing **a, b** normal neutrophils with ring-shaped nuclei, **c** heterozygous *ic* neutrophils with ring-shaped nuclei, and **d** homozygous *ic* neutrophil showing an ovoid nucleus with chromatin clumping. Scale bar for light micrographs, 10  $\mu$ m. **e–g**

Thin-section electron micrographs showing **e** normal neutrophil nucleus (possibly ring-shaped) with extensive peripheral heterochromatin, **f** heterozygous *ic* neutrophil with a lobulated nucleus, **g** ovoid nucleus from a homozygous *ic* granulocyte exhibiting extensive heterochromatin redistribution. Scale bar for electron micrographs, 1  $\mu$ m

a genome-wide scan with microsatellites in nine families with 29 affected members localized the Pelger gene to 1q41–43. Pelger individuals from different parts of the world encompass a variety of splice acceptor, splice donor, frameshift, and nonsense gene mutations residing within the *LBR* gene. The best-studied mutation, the Gelenau founder mutation, contained a 6-bp deletion within intron 12, resulting in defective splicing and the loss of exon 13 from the processed mutant transcript. Interestingly, the homozygous patient with this mutation showed trace amounts of normally spliced *LBR* messenger RNA (mRNA). In his lymphoid cells, there was no evidence for a mutant *LBR* protein, but a small amount of wild-type protein was detectable (Hoffmann et al. 2002).

By performing synteny comparisons of the mouse *ic* locus (chromosome 1, between 97 and 98 cM) with the human *LBR* locus, it became obvious that the mouse *ic* locus was likely to contain the gene for mouse *Lbr*. Two extinct and one remaining cryopreserved *ic* mutations were sequenced, exhibiting frameshift and nonsense mutations in the *LBR* gene. A substantial number of homozygous *ic* mice perish in utero; survivors generally look less healthy at birth, possess a scaly skin, and show variable degrees of

syndactyly and hydrocephalus with no immunologically detectable *LBR* (Shultz et al. 2003). From both of these studies (Hoffmann et al. 2002; Shultz et al. 2003), it appeared that the severity of PHA or *ic* depended upon the dosage of *LBR*.

The striking contrast between the severe, semi-lethal phenotype of homozygous mice, rabbits, and dogs, and the rather mild phenotype of the affected humans presented a dilemma. Moreover, it was difficult to understand why, despite the worldwide occurrence of PHA heterozygote individuals, only 11 homozygotes were reported so far. An explanation for these seemingly discrepant findings became obvious when a second homozygous Pelger individual could be studied (unpublished data). Again, there was no evidence for mutant *LBR*, but a minor amount of wild-type protein was detected. Thus, we surmise that the severely affected mice might be due to null mutations, whereas the mildly affected homozygous PHA humans are due to hypomorphic alleles. As a consequence, it was predicted that, also in man, homozygote null-mutant individuals could exist, characterized by severe skeletal deformities and early death. Recent findings support this assumption with the demonstration that the lethal autosomal-recessive HEM/Greenberg skeletal dys-

plasia is due to null mutations of the *LBR* gene (Waterham et al. 2003). Thus, most of the “missing” homozygotes might be represented by undiagnosed fetuses with HEM/Greenberg skeletal dysplasia. This diagnosis can be made postmortem because the heterozygous parents should exhibit the PHA phenotype on blood smears.

### Lamin B receptor

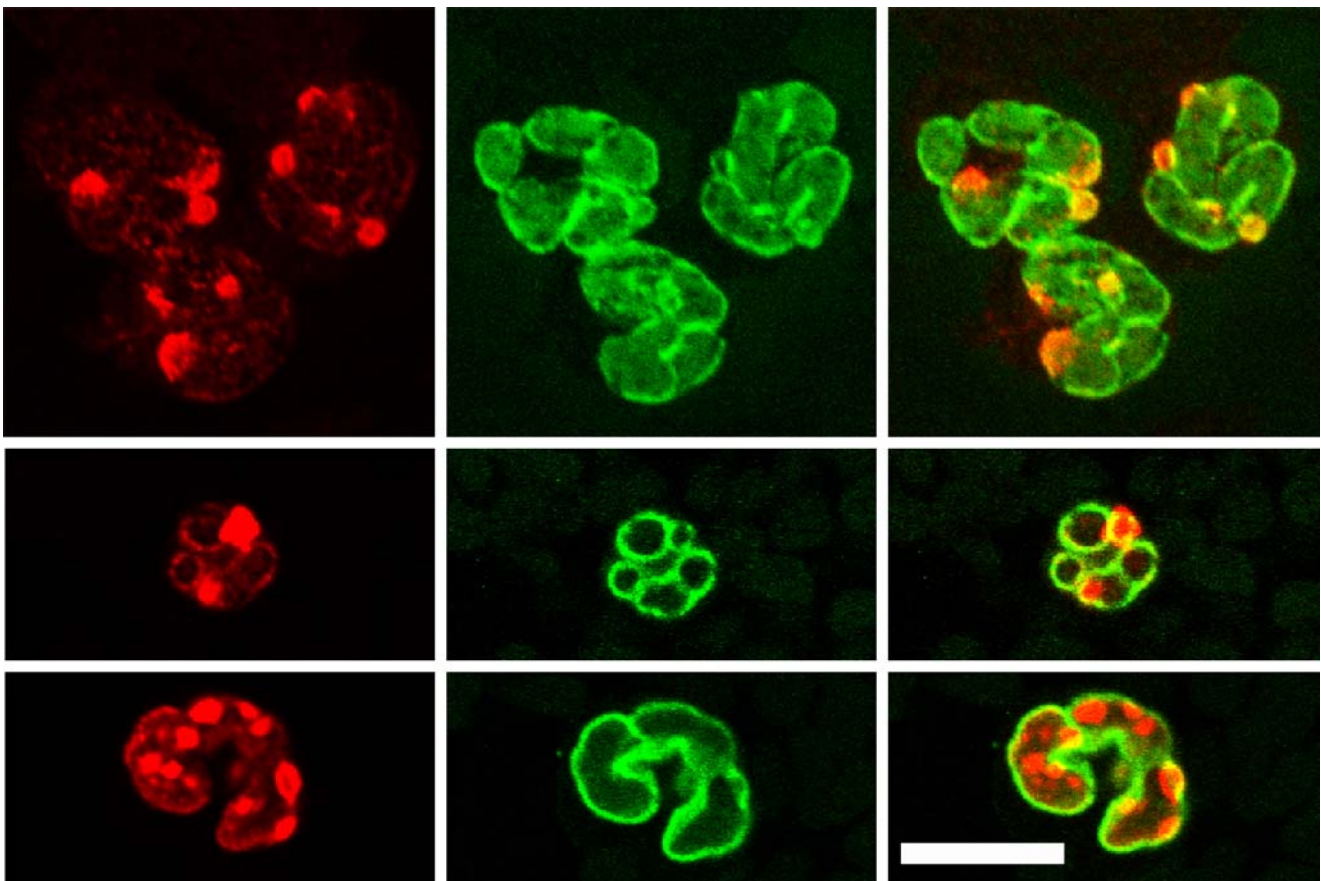
LBR was originally described by Worman et al. (1988), with properties summarized in a number of reviews (Gruenbaum et al. 2005; Holmer and Worman 2001; Ye et al. 1998). LBR is normally embedded within the inner nuclear membrane. Human LBR is 615 aa, consisting of two principal domains: 1–208 aa basic globular region, believed to interact with lamin B, heterochromatin protein 1 (HP1), and heterochromatin in the interphase nucleus; 209–615 aa region with eight putative transmembrane segments with homology to genes of the sterol reductase family. Thus, LBR has a remarkably divergent internal structure. Live cell microscopy has indicated that LBR laterally diffuses from its site of synthesis in the ER through the membranes of the nuclear pore being “trapped” by binding to the lamina (Ellenberg et al. 1997). Candidate binding partners for this trapping of LBR have been suggested by a number of in vitro binding experiments and yeast two-hybrid assays and include lamin B, HP1, DNA, histones, chromatin, and H95 (Gruenbaum et al. 2005; Holmer and Worman 2001; Makatsori et al. 2004; Martins et al. 2000; Takano et al. 2002, 2004; Ye et al. 1998). There is a paucity of in vivo information about the binding partners of LBR, but it is demonstrated that lamin A/C is not essential for LBR trapping in the nuclear envelope (Ostlund et al. 2006).

The protein sequence of LBR is conserved among vertebrates. For example, the basic local alignment search tool comparisons of the human LBR against various vertebrate genomes yield the following: mouse, ~80%; chicken, ~70%; frogs, ~50%; fish, ~50%. The C terminus (~400 aa) appears to be the most conserved portion of LBR, with two orthologs of the sterol reductase family, SR1 (TM7SF2) and SR2 (DHCR7; Ostlund et al. 2006). Indeed, this portion of the LBR possesses ~38% sequence identity with the FACKEL sterol reductase of the plant *Arabidopsis* (Schrack et al. 2000). The analysis of *Drosophila* LBR revealed an absence of sterol reductase activity, suggesting the possibility of a loss of function during evolution (Wagner et al. 2004). The amino ~200 aa of the human LBR reveals very few sequence similarities within the human genome. One of the most interesting regions is an SR domain (~65 to ~100 aa) that is differently phosphorylated, comparing interphase and mitotic cell states affecting in vitro binding interactions to chromatin (Takano et al. 2002, 2004).

### Influences on granulocyte nuclear shape

The molecular genetic studies of human PHA and mouse *ic* demonstrate that LBR is necessary for normal granulocyte nuclear differentiation during granulopoiesis (Hoffmann et al. 2002; Shultz et al. 2003). Studies with tissue culture models for granulopoiesis indicate that other factors, namely nuclear envelope lamin composition and microtubule integrity, appear to influence granulocyte nuclear shape (Olins et al. 1998, 2001; Olins and Olins 2004). HL-60 cells are a human myeloid leukemic tissue culture line capable of being differentiated by simple chemicals in vitro into either granulocytic forms (using retinoic acid, RA) or monocytic forms (using phorbol ester, TPA). Immunoblotting experiments employing antibodies to various nuclear envelope proteins examined the changes that occur during HL-60 differentiation into granulocytes or monocytes (Olins et al. 2001). The undifferentiated and granulocytic forms exhibited a paucity of lamins A/C and B1; the monocytic forms revealed increasing accumulation of these lamins. Lamin B2 was present under all conditions. LBR was low in undifferentiated forms, but quickly increased in amount after RA or TPA treatment. These experiments indicate that although LBR is necessary for granulocyte nuclear shape differentiation, the elevation of LBR content is not sufficient to promote nuclear lobulation. Other factors (such as a paucity of lamins A/C and B1) may support or promote the nuclear shape change. Given the fact that many nuclear envelope proteins remain to be adequately described and quantified (Schirmer et al. 2003), other unknown proteins may play pivotal roles in granulocyte nuclear differentiation. At least one other influencing factor for granulocyte nuclear lobulation is the requirement of microtubule integrity during in vitro granulopoiesis with HL-60 cells (Olins and Olins 2004). The exposure of RA-differentiating HL-60 cells to nocodazole (which disrupts microtubules) prevents nuclear lobulation; the disruption of actin microfilaments with cytochalasin D does not prevent lobulation. In accordance with these experiments, it was already demonstrated in 1953 that the bilobed granulocyte nuclei of Pelger rabbits became ovoid after injection of colchicine, another microtubule-disrupting chemical (Harm 1953). The mechanistic basis for this requirement of microtubule integrity is not yet clear. As an additional consideration, current investigations of the perturbation of nuclear shape in human laminopathies and in model organisms underscore the importance of proper “management” of farnesylated nuclear envelope proteins, reviewed in Melcer and Gruenbaum (2006). This issue has not yet been explored in cells undergoing granulopoiesis.

An important question is: To what extent does granulocyte heterochromatin influence granulocyte nuclear shape?



**Fig. 4** Immunostaining of mouse blood neutrophils with anti-histone antibodies demonstrating the localization of constitutive heterochromatin markers within nuclear envelope drumsticks. *Top row* Anti-trimethyl H3K9 (*left, red*), anti-lamin B (*middle, green*), included to outline the nuclear envelope), merged images (*right*). *Two bottom rows* Anti-trimethyl H4K20 (*left, red*), anti-lamin B (*middle, green*),

and merged images (*right*). In mouse cells, these methylated histone markers are concentrated at the pericentric heterochromatin. The remainder of the peripheral heterochromatin (compare to Fig. 3) does not stain with comparable intensity. Confocal images taken from Olins and Olins (2005b). Scale bar, 10  $\mu$ m

In vitro binding evidence has been presented that demonstrates that (in nucleated avian erythrocytes, HeLa and mouse embryonic fibroblasts) LBR binds to nucleosomes with various heterochromatic (but not euchromatic) epigenetic histone marks (Makatsori et al. 2004). More recently, we (Olins and Olins 2005b) have observed that the frequent “drumsticks” of mouse granulocytes are enriched with the repressive markers (Schotta et al. 2004) trimethyl histone 3 lysine 9 (H3K9) and trimethyl histone 4 lysine 20 (H4K20) that bind to the AT-rich pericentromeric constitutive heterochromatin (Fig. 4). In the human female granulocyte, in situ hybridization evidence has been presented that “drumsticks” contain the inactive heterochromatic X chromosome (Karni et al. 2001). It appears possible that the granulocyte nuclear envelope can be severely distorted by the underlying heterochromatin, possibly mediated by LBR bridges. It would be of interest to investigate by immunofluorescence and immunoelectron microscopy whether LBR oligomers or “microdomains” (Makatsori et al. 2004) are concentrated in the nuclear envelope regions surrounding “drumsticks.”

#### A role for cholesterol

As mentioned earlier, homozygous LBR deficiency leads not only to ovoid granulocyte nuclei but also to severe skeletal dysplasia, most readily observable in rabbits and mice. The likely explanation for this complex phenotype comes from the observation that LBR is an essential enzyme of cholesterol biosynthesis (Waterham et al. 2003). The C-14 sterol reductase activity of LBR has been known for many years (Holmer and Worman 2001). Waterham et al. presented evidence that LBR catalyzes the conversion of cholesta-8, 14-dien-3 $\beta$ -ol to cholesta-8-en-3 $\beta$ -ol, an intermediate in cholesterol biosynthesis. The authors postulate that this enzyme activity forms the basis of human HEM/Greenberg skeletal dysplasia, a condition of homozygous LBR deficiency characterized by fetal developmental and skeletal abnormalities and early death. In Greenberg dysplasia, it is not clear whether fetal death is a consequence of altered nuclear and chromatin structure, changes in expression patterns of essential genes, a deficit of cholesterol, and its byproducts or accumulation of the

cholesta-dien intermediate. Certainly, the developmental and skeletal malformations place Greenberg dysplasia in the family of other cholesterol biosynthetic defects, including the Smith–Lemli–Opitz syndrome, desmosterolosis, lanthosterolosis, and other syndromes (Porter 2003). An underlying common mechanism for these human malformation syndromes, based upon genetic defects in cholesterol synthesis, might be cholesterol addition to the cleavage product of sonic hedgehog, a critical morphogen during tissue development (Goetz et al. 2002). The opinion of the authors of this review is that the most severe clinical consequences of homozygous PHA or *ic* are the effects of the developmental malformations arising from unknown critical requirements for cholesterol rather than from defects in granulocyte nuclear shape. Interestingly, the human tissue with the highest expression level of LBR mRNA appears to be bone marrow (Bennati et al. 2006).

Still mysterious is whether there is any functional and evolutionary significance to the divergent capabilities of vertebrate LBR, i.e., nuclear envelope organization and cholesterol biosynthesis. We may ask (without present answers): Does cholesterol biosynthesis affect nuclear envelope–heterochromatin interactions, or vice versa? Does LBR participate in cholesterol biosynthesis only when residing in the ER, or only when in the nuclear envelope? Does LBR participate in cholesterol biosynthesis in all tissues, or only in selected tissues during development?

### Phylogeny of granulocyte nuclear shape

The nuclear shape of “neutrophil-like” granulocytes changes along the evolutionary line (Ragan 1999). Most non-vertebrates and a few vertebrates (reptiles: turtles and snakes) have mature round nuclei. Hyposegmented granulocyte nuclei are observed in many fish and birds (e.g., eel and chicken) and in elephants. Most mammals and the frog (*Bufo vulgaris*) possess granulocytes with lobulation comparable to humans. Some amphibians (fire salamander) and some mammals (camel, hyena, guinea pig, rabbit) exhibit hypersegmented blood granulocytes. Rats and mice possess ring-shaped neutrophil nuclei (Biermann et al. 1999; Olins and Olins 2005a). It is worth noting that ring-shaped granulocyte nuclei have been observed in human myeloproliferative disorders and in plasma cell dysplasia (Kanoh 1991), suggesting some plasticity in nuclear shape differentiation within any particular species. Although the evolutionary scale of granulocyte nuclear complexity roughly parallels changes in LBR peptide structure, there is insufficient morphologic and protein data to test the fidelity of the correlation. Hopefully, this review will stimulate more detailed investigations of the comparative structure of granulocyte nuclei.

### A plethora of shapes and questions

Admittedly, our current knowledge of the structure of the granulocyte nucleus and how the ovoid shape is avoided is still in its infancy. Nonetheless, it is valuable to state current assumptions that lead to a testable hypothesis. Our model (Olins and Olins 2004) is based upon four assumptions: (1) Due to the paucity of lamins A/C and B1, the granulocyte nuclear envelope is more deformable than ovoid nuclei possessing lamins A/C and B1. (2) Elevated levels of LBR bridge the deformable nuclear envelope to the underlying peripheral heterochromatin. (3) Intact microtubules emanating from the juxtannuclear centrosomal region are required for the nuclear shape change during granulopoiesis, possibly involving microtubule-associated motors (e.g., dynein). We assume that during granulopoiesis, the ovoid myeloid precursor nuclear envelope is deformed by motors attached to the envelope and pulling towards the centrosomal region to create folds around the centrosome (Olins and Olins 2005a). (4) Membrane lipids and integral membrane proteins (e.g., LBR) migrate into the nuclear envelope from the ER by lateral diffusion.

The model is testable in several ways. One can ask: (1) Does the elevation of lamins A and B1 by transfection of HL-60 cells inhibit RA-induced nuclear lobulation? (2) Can normally ovoid nuclei (e.g., HeLa cells) be made to appear lobulated by elevating LBR and knocking down lamins A and B1? (3) Will inhibition of dynein function inhibit RA-induced nuclear lobulation? (4) Do particular DNA sequences and/or chromatin modifications preferentially localize at the junction of lobes, proximal to centrosomal regions or adjacent to deformed nuclear envelope? (5) Would manipulation of cellular cholesterol levels influence nuclear envelope growth and deformation?

There are a number of related clinical hematological mysteries that await examination from the point of view of this review. Cobalamin (Vitamin B12) and folic acid deficiency in humans produces megaloblastic anemia that, among other findings, results in significant hyperlobulation of neutrophil nuclei and the appearance of “nuclear blebs” (Antony 2000; Carmel et al. 2003). Is there a corresponding elevation of LBR? The hyperlobulation of neutrophil nuclei and the appearance of nuclear blebs (denoted by us as “envelope-limited chromatin sheets” or “ELCS”) are observed when HL-60 cells are differentiated with RA (Olins et al. 1998; Olins and Olins 2004). Cobalamin is believed to be important for deoxynucleotide synthesis; the deficiency may result in uracil misincorporation and DNA fragmentation (Antony 2000; Carmel et al. 2003). But, the connection to granulocyte nuclear shape remains obscure. Hypolobulation in conditions of pseudo-Pelger also awaits further investigation. Is there a repression (by epigenetic mechanisms) of LBR in the affected bone marrow? The

study of granulocyte nuclear differentiation in PHA and *in vitro* model cell systems has contributed to our understanding of nuclear envelope–heterochromatin interactions (Gruenbaum et al. 2005). It is likely that this synergistic relationship between hematology and basic cell biology will continue to be mutually productive and informative.

## Summary

The expression of LBR protein affects nuclear shape and chromatin structure in a dose-dependent manner. LBR is necessary but not sufficient to promote the normal segmentation of neutrophil nuclei. Nuclear envelope deformability appears to play an important role. The additional factors implicated in determining granulocyte nuclear shape include the integrity of the microtubule system and the existence of heterochromatin and its epigenetic modifications. Yet to be adequately defined are the roles of LBR-binding proteins and the possible influences of cholesterol metabolism upon nuclear shape and nuclear envelope interactions.

**Acknowledgment** We dedicate this paper to Prof. H. Stobbe (Charité, Berlin) on the occasion of his 85th birthday. Prof. Stobbe, together with K. Kaps, correctly predicted the increased prevalence of PHA in the village Gelenau from the existence of a single PHA homozygous individual. This facilitated the mapping and identification of the PHA gene in later decades. We would also like to thank L. Shultz (Jackson Laboratory, Bar Harbor, ME, USA), the Max Planck Institute for Molecular Genetics in Berlin, T. Lindner, and H. Herrmann for their ongoing creative and pleasant collaborations. K. H. is a recipient of the Rahel Hirsch scholarship provided by the medical faculty at the Charité Humboldt University, Berlin. K.H. and K.S. are supported by DFG grants SP 144/18 and SFB 577/A9. A.L.O. and D.E.O. wish to thank Bowdoin College for providing laboratory facilities and stimulating colleagues. A.L.O. and D.E.O. are supported by NIH/NHLBI R15 HL075809.

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