The stability of the nuclear lamina polymer changes with the composition of lamin subtypes according to their individual binding strengths

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The nuclear lamina, which provides a structural scaffolding for the nuclear envelope, consists largely of a polymer of the intermediate filament lamin proteins. Although different cell types contain distinctive relative amounts of the major lamin subtypes (A, C, B1, and B2), the functions of this variation are not understood. We have investigated the possibility that subtype variation affects lamina stability. We find that homotypic and heterotypic binding interactions of lamin B2 are substantially less resistant to chemical dissociation in vitro than those between the other lamin subtypes, whereas lamin A interactions are the most stable. Surprisingly, removal of the central four-fifths of the rod domain did not substantially weaken the interactions of lamins A and B2, suggesting that other regions also strongly contribute to their binding interactions. In contrast, this rod deletion strongly destabilizes the binding interactions of lamins B1 and C. Consistent with the binding studies, lamins are more readily solubilized by chemical extraction from cells enriched for lamin B2 than from cells enriched for lamin A. This suggests that the distinctive ensemble of heterotypic lamin interactions in a particular cell type affects the stability of the lamina polymer, and, correspondingly, could be relevant to tissue-specific properties of the lamina including its involvement in disease.

The nuclear lamina is a filamentous protein meshwork that lines the inner nuclear membrane. Its core consists of a polymer of the intermediate filament (IF) lamin proteins, which binds to chromatin and connects to the inner nuclear membrane via integral membrane proteins (reviewed in Refs. 1 and 2). Mutations in lamins and associated membrane proteins cause a variety of debilitating human diseases (laminopathies) including muscular dystrophy, neuropathy, and developmental disorders (reviewed in Refs. 3 and 4).

Differences are observed in the relative amounts of the major products of the three lamin genes (encoding lamins A/C, B1, and B2) in different cell types and at different stages of development. Lamins B1 and B2 are expressed throughout development, whereas lamins A and C, which are splice variants differing in their C termini, usually appear only near the time of or following differentiation in specific tissues of chicken and mammals (5–7). These different subtypes are presumed to interact in vivo, since binding of A/C lamins to lamin B1 is observed in blot overlays (8), column binding assays (9), and two-hybrid analysis (10). However, the binding between other pairs of lamin subtypes has not yet been examined. The observation that different nuclear envelope (NE) diseases preferentially affect certain tissues (3, 4) may relate to the distinctive expression patterns of lamins in different tissues and the specific biochemical properties of each subtype.

Similar to other IF proteins, lamins contain a central α-helical “rod” domain composed of ∼48 heptad repeats, flanked by N- and C-terminal “head” and “tail” domains. The rod domain assembles into a parallel, unstacked coiled-coil homodimer that is stable in 8 M urea. In vitro, dimers of each major lamin subtype can assemble laterally into homotypic filaments by staggered antiparallel interactions (11–13). The five heptads at each end of the rod, particularly those at the tail end, have been suggested to be critical for initiating the coiled-coil (14, 15). Fragments containing these terminal heptads together with either the head or tail can assemble into dimers and tetramers in vitro but not into filaments (14). Both charged and hydrophobic residues on the outer surface of the dimer rod are thought to contribute to stabilizing filaments. Although there are few details on how different subtypes assemble into the lamina in vivo, their resistance to chemical extraction from cells and the low rates of exchange for lamins A and B1 fused to GFP (16) argue that they are stably integrated into a polymeric structure.

In this study, we have used biochemical approaches to compare the properties of all four principal mammalian lamin subtypes. We found that each pairing yielded a different binding “strength” as defined by its resistance to chemical dissociation. Interestingly, we found that lamin B2 had much weaker homotypic and heterotypic interactions than the interactions seen among the other lamin subtypes, and, correspondingly, all lamin subtypes were more readily extracted from cultured cells that were induced to express relatively high levels of lamin B2. Paralleling this at the opposite extreme, lamin A interactions were the strongest among the different lamin subtypes, and lamins were less readily extracted from cells expressing high levels of lamin A. Analysis of lamin mutants lacking the middle four-fifths of the rod domain suggested that this region contributes significantly to the homotypic binding interactions of lamins B1, A, and C and that lamin A contains additional binding sites unique to its C terminus that are important for its unusually strong interactions. These results suggest that
changes in the ratio of lamins A and B2 during development or in different cell types could directly influence the dynamic properties of the lamina and its functions as a structural scaffolding for the NE and chromatin.

EXPERIMENTAL PROCEDURES

Plasmid Construction—Primers that added 5' BamHI/Ndel and 3' NotI sites were used to amplify by PCR the coding sequences of human lamins A, C, and B1 and mouse lamin B2. To produce deletion mutants lacking the middle four-fifths of the rod domain (referred to throughout as Δ), these primers were used in conjunction with internal primers containing HindIII sites that fused nucleotides 207 and 1017 for lamin B1 and the corresponding residues of the other lamins via an added aline codon. All genes were moved to pET28a (Novagen) for protein expression and pHHS10B (which bears an HA epitope tag) for mammalian transfection.

Protein Purification—WT lamins and deletion mutants were purified from inclusion bodies. The proteins were expressed in BL21-(DE3) cells by induction with 0.3 mM isopropyl-1-thio-D-galactopyranoside at 30°C for 3 h at 37°C, collected by centrifugation, and lysed by sonication in 25 mM HEPES, pH 8.0, 0.1 mM MgCl₂, 3 mM β-mercaptoethanol containing 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μM leupeptin, and 1 μM pepstatin. The pellets from a 20-min centrifugation at 27,000 x g were washed with 1% Triton X-100 and resuspended in 20 mM HEPES, pH 8.0, 8 M urea, 3 mM β-mercaptoethanol. For further purification, this was incubated with nickel resin (Qiagen) for interaction of the His₆ tag from the pET28a vector, and proteins were eluted with the same buffer containing 200 mM imidazole. Proteins were then dialyzed into 20 mM Tris-HCl, pH 8.0, 8 M urea, 2 mM dithiothreitol, 1 mM EDTA with protease inhibitors for storage.

Lamin Binding Assays—Purified WT lamins and deletion mutants were covalently coupled individually to Affi-gel 10 or 15 (based on their PI values) (Bio-Rad) for a solid support as described in Refs. 9 and 17, except that ~3 mg was bound per ml of matrix. Matrices were pre-washed with the buffers used in subsequent binding assays to avoid confusion of coupled versus bound protein when testing homotypic interactions. Uncoupled lamins were diluted to 20 μg/ml and dialyzed out of urea into binding buffer: 25 mM HEPES, pH 7.4, 300 mM NaCl, 5 mM β-mercaptoethanol with protease inhibitors. This was centrifuged (16,000 x g, 15 min) to remove significantly polymerized lamins and incubated with the matrix overnight at 4°C. The columns were washed in the same buffer and eluted with increasing concentrations of urea in 25 mM HEPES, pH 8.0, 500 mM NaCl, 5 mM β-mercaptoethanol (4 column volumes each fraction). Alternatively, matrices were eluted with 5 mM NaCl followed by 8 M urea. Fractions were analyzed by Western blotting with antibodies specific to each lamin subtype (17). Lamins in each fraction were quantified using a PhosphorImager densitometer with ImageQuant software and graphed as the percentage of the total input eluted material. Although lamins were eluted in steps with increasing urea concentrations, the median urea concentration (ureaMED) was calculated as if a gradient had been used (i.e. if the median occurred three-fourths of the way between 6 and 8 M, a ureaMED value of 7.5 M was calculated).

Solubility Assays—The solubility of lamins was tested by similarly diluting and dialyzing purified lamins into binding buffer and then incubating overnight at 4°C. Samples were then subjected to centrifugation at 16,000 x g for 15 min to pellet significantly polymerized material. Supernatants and pellets were equivalently loaded onto SDS-PAGE, resolved, and analyzed by Western blotting with lamin subtype-specific antibodies, and densitometry to determine the effects of drug treatments on relative lamin levels.

Salt and Detergent Extraction of Lamins—293T cells were transfected with either lamin A or lamin B2 constructs in the pHHS10B vector using Polyfect (Qiagen) according to the manufacturer’s instructions. Transfection efficiencies were measured at 30–40%. At 54 h post-transfection, cells were recovered by pipetting, washed in PBS, and divided into separate tubes for extractions. Cells were resuspended and incubated on ice for 5 min in 25 mM HEPES, pH 7.4, 450 mM NaCl, 3 mM MgCl₂, 0.5 mM EDTA, 1 mM dithiothreitol, 0.4% Triton X-100. Insoluble material was then separated by centrifugation at 9000 x g in a micro-centrifuge for 2 min, and both fractions were resolved by Western blotting and densitometry.

Alternatively, HL-60 cells were treated with 1 μM trans-retinoic acid (Calbiochem) from a 1 mM stock dissolved in ethanol or 16 mM O-tetradecanoylphorbol-13-mustard acetate (TPA) (Calbiochem) from a 160 μM stock dissolved in acetone. After 4 days, attached differentiating cells were treated with trypsin-EDTA, and all cells were counted and collected by centrifugation. Equal numbers of cells lysed in SDS-sample buffer were analyzed by SDS-polyacrylamide gel electrophoresis, Western blotting with lamin subtype-specific antibodies, and densitometry to determine the effects of drug treatments on relative lamin levels.

RESULTS

Assay for Measuring Lamin Dimer-Dimer Interactions—To directly compare the binding properties of the four major lamin subtypes, recombinant WT lamins and deletion mutants that lacked approximately the middle four-fifths of the rod (Fig. IA, Δ) were prepared from bacteria. Each of the rod deletion mutants retained five heptads from both ends of the rod (indicated by slashes) that were fused in register. These were predicted by computer algorithm to form a single coiled-coil of 10 heptads, and experimental support for this comes from biophysical analysis of the lamin B1Δ mutant that retained a strong α-helical signal in solution by circular dichroism and assembled into

FIG. 1. Design and purification of mutants. A, the domain structure of lamins (drawn to scale for lamin A). Lamins contain a short amino-terminal head domain (~33 amino acids) followed by a coiled-coil rod domain (354 amino acids) and a carboxy-terminal tail domain (~200–300 amino acids). The rod is predicted to contain four separate α-helices: coil 1A, 1B, 2A, and 2B of 6, 20, 16, and 16 heptads, respectively (a slash delineates each heptad), that are separated by short, flexible linker regions (filled boxes). The lamin Δ mutants lack the central four-fifths of the rod, retaining only five heptads at each end that are fused in register to form a single coiled-coil of 10 heptads. B, the percentage identity/percentage homology for the head, first five rod heptads, central rod, last five rod heptads, and tail is indicated with AV, VLI, ST, DE, QN, RK, or FY counted as homologous residues. Because lamins A and C are identical except for the last 6 residues of lamin C and the last 98 residues of lamin A, the calculations for lamin C are given separately in parentheses. C, the quality of the protein preparations used in this study was examined by Coomassie staining of a 10% SDS-polyacrylamide gel. Molecular weights of protein standards are designated on the right.
filaments that had a similar Fourier transform infrared spectroscopy pattern as WT filaments (17). In contrast to the short Δ coiled-coil, the WT lamin dimers are predicted to have four distinct coiled-coil helices formed by sets of 6–20 heptads each, separated by short linkers. The ends of the rod are the most conserved regions among lamins (~77–100% homologous), but the central portions of the rod, as well as the heads and tails, are more highly conserved (~53–71% homologous) (Fig. 1B).

We directly compared the homotypic and heterotypic binding of recombinant lamins A, C, B1, and B2 and the homotypic binding of their respective Δ mutants (Fig. 1C) using an in vitro assay that preferentially measured the strength of dimer-dimer interactions. For this assay (modified from a previous method) (9, 17), each purified recombinant lamina was covalently coupled to an agarose bead support in 7 M urea (a condition favoring homotypic dimers) at a relatively high lamin concentration (~3 mg/ml). Separately, each lamin was diluted and dialyzed out of urea into a “binding buffer” at a protein concentration that was too low to support self-assembly into polymers (20 μg/ml) and then incubated with the immobilized lamin beads. Thus, the assay would strongly favor the interaction of the soluble lamins (present at a low concentration) with the immobilized lamins (present in excess). A large amount (~1 ml) of matrix was used for each assay to reduce potential sampling errors associated with small sample sizes. Matrices were washed, and then noncovalently bound lamins were eluted stepwise in the binding buffer containing increasing concentrations of urea. Fractions were analyzed by Western blotting with subtype-specific lamin antibodies followed by densitometry. Each column assay was repeated two or more times, yielding similar results. To facilitate comparison of the different lamin subtypes, a calculation of the median urea molarity at which 50% of the total bound lamin had been eluted (urea50) was used as an index of the binding “strength.”

**Comparison of the Binding Strength between Various Lamin Dimers**—We first used this approach to examine homotypic dimer-dimer interactions (Fig. 2). We found that the majority of the lamin B2 homotypic linkages were disrupted at relatively low concentrations of urea (urea50 = 2.9 M) (Fig. 2A). In contrast, ~2.5-fold higher urea concentrations were required to separate the lamin A (urea50 = 8.0 M), lamin C (urea50 = 6.9 M), and lamin B1 (urea50 = 7.6 M) homotypic interactions (Fig. 2A). If 5 M NaCl was used to elute the matrices instead of urea, only a small fraction of homotypically bound lamins was released. However, nearly 10-fold more lamin B2 was eluted (~10% of the total bound) as compared with lamin B1 (Fig. 2B). Thus, in relation to lamin B1, lamin B2 homotypic interactions are substantially more susceptible to disruption by chemical conditions that perturb hydrophobic associations (urea) as well as electrostatic interactions (high NaCl). Since the coiled-coil dimer formed by the lamin rod is not disrupted by high urea or salt, we speculate that the greater sensitivity of lamin B2 to elution by these conditions results from disruption of binding interfaces between dimers, although we cannot rule out the possibility that the tail domain of recombinant lamin B2 is misfolded or that the folding of the tail domain of lamin B2, rather than its binding interfaces per se, is selectively disrupted by the urea elution. However, we note in this regard that the predicted immunoglobulin fold domain of the lamin B2 tail (18, 19) is as similar in sequence to the homologous regions of lamin B1 and lamin A as the homologous region of lamin B1 is similar to lamin A (residues 436–552, percentage identity/percentage homology as follows: A-B1, 49/64; A-B2, 54/66; B1-B2, 47/63).

To confirm that the soluble lamins were not themselves significantly polymerizing or aggregating during the course of the assays, the lamins were tested for their solubility under assay conditions. Proteins were diluted into the assay buffer and incubated overnight as in the column binding studies. Samples were then subjected to centrifugation, and the percentage of pelletable material was determined. We found that the vast majority of all of the lamin constructs analyzed remained soluble (Fig. 3A), thus validating the binding assay by demonstrating that it was measuring neither polymerization nor aggregation and arguing that the proteins are properly folded.

Heterotypic interactions of lamin B1 with a mixture of A/C lamins were previously shown to be stronger than the lamin B1 homotypic interactions (9), an observation we confirmed in our assay (Fig. 3B). However, by analyzing lamins A and C separately, we further learned that the lamin B1 heterotypic interaction with lamin A (urea50 = 8.0 M) is stronger than its interaction with lamin C (urea50 = 6.8 M). When we examined the binding interactions of lamin B2 by this method, we found that the heterotypic interactions of lamin B2-A (urea50 = 3.4 M), lamin B2-C (urea50 = 3.3 M), and lamin B2-B1 (urea50 = 3.8 M) were substantially weaker than the homotypic lamin A and lamin B1 associations (Fig. 3C). Nonetheless, they were slightly stronger than the homotypic lamin B2 interactions (urea50 = 2.9 M).

**Contributions of the Peripheral and Central Regions to Lamin Interactions**—The Δ lamin mutants are expected to maintain the same orientation of the heads and tails that occurs in the WT dimers and thus could be used to analyze the relative contributions of the central four-fifths of the rod versus more peripheral lamin regions for lamin-lamin interactions (e.g. central rod-central rod and head/tail-central rod interactions compared with head-tail and rod end interactions. We found that the ΔΔ construct retained a strong homotypic interaction (Fig. 4; ΔΔ, urea50 = 7.1 M) that was quite similar to the homotypic interaction of WT lamin A (urea50 = 8.0 M; Fig. 2A). Even interactions between WT
Effects of Lamin Subtype Composition on the Solubility of the Lamin Polymer in Cells—The in vitro binding assays predict that a lamina enriched in lamin B2 in situ would be less stable than one enriched in lamin A. Furthermore, if different lamin subtypes co-assemble into the same filaments, then increasing the relative level of lamin B2 in the nuclear lamina of cells would increase the sensitivity to chemical extraction of all endogenous lamin subtypes, whereas increasing the relative amount of lamin A would reduce their extraction. To test this model, lamin B2 or lamin A was transfected into 293T mammalian cultured cells, and the cells were then analyzed by Western blotting for changes in the percentage of each lamin subtype extracted using a buffer containing high salt and non-ionic detergent, which disrupts both hydrophobic and electrostatic interactions. The percentage of all lamin subtypes that were extracted was decreased in cultures of cells transfected with lamin A as compared with controls, whereas the percentage of extracted lamin B1 and B2 was increased in cells transfected with lamin B2, as compared with control cells (Fig. 5A). Our values significantly underestimate the effects of exogenously expressed lamin on the solubility of the endogenous lamins, since only ~30% of the cells in the populations analyzed were transfected. Since cells were lysed at times when all transfected lamins were concentrated at the nuclear rim as seen by immunofluorescence staining (Fig. 5B), the changes in the solubility of the transfected lamin are not due to its improper targeting or aggregation. Similarly, all endogenous lamins retained their normal distribution, yet their solubility was also altered. Untransfected cells yielded somewhat variable levels of solubilized lamins from experiment to experiment, so after quantification by densitometry, the percentage of solubilized lamins from the transfected samples was normalized to the percentage solubilized in a nontransfected control.
and salt. Pressed in 293T cells, and cells were extracted at 54 h with detergent endogenous lamin polymer in opposite ways. Lamins were overexpressed in A/C lamins. Western blotting with antibodies specific for each lamin subtype. The experiment is presented as a ratio to that of the nontransfected control for each experiment. The averaged results from four experiments calculated after densitometry increase solubility is not merely due to excess lamin. Immunofluorescence staining for its epitope tag. This indicates that the exogenously expressed lamin targeted to the NE as determined by immunofluorescence staining for its epitope tag. This indicates that the increased solubility is not merely due to excess lamin. The percentage of soluble material from four experiments calculated after densitometry is presented as a ratio to that of the nontransfected control for each experiment. The bars indicate the calculated S.D.

As an alternative to changing the relative levels of lamins in the NE by overexpression, we used a cell system where endogenous lamin polymer in opposite ways. Lamins were overexpressed in 293T cells, and cells were extracted at 54 h with detergent and salt. A, solubilized (s) and pelletable (p) material was analyzed by Western blotting with antibodies specific for each lamin subtype. B, the exogenously expressed lamin targeted to the NE as determined by immunofluorescence staining for its epitope tag. This indicates that the increased solubility is not merely due to excess lamin. C, the percentage of soluble material from four experiments calculated after densitometry is presented as a ratio to that of the nontransfected control for each experiment. The bars indicate the calculated S.D.

Fig. 6. Effect of altering lamin ratios on the stability of the lamina in cells. A, manipulation of the lamin composition in a model differentiation system alters the solubility properties of the endogenous lamin polymer. A, HL-60 cells were treated with RA or TPA. The lamin protein levels from equal numbers of cells were analyzed by densitometry, revealing a 50% increase in lamin B2 with RA and a ~3-fold increase in A/C lamins with TPA. B, after 4 days of induction, cells were collected and extracted with 450 mM NaCl and 0.4% Triton X-100. Solubilized (s) and pelletable (p) lamins were analyzed with subtype-specific antibodies. The full range of immunoreactive species is shown; multiple post-translationally modified species or degradation products appeared, which were analyzed together for C. Pelletable A/C lamins were loaded at one-fourth the level for TPA-induced cells because of their high levels. C, densitometric analysis of the results of four experiments. The percentage of soluble material is presented as a ratio to that of the untreated HL-60 cells for each experiment. The bars indicate the calculated S.D.

Lamin modifications induced by the drug treatments could contribute to the lamin solubility differences in this experiment. However, we propose that changes in the relative lamin levels are likely to underlie the solubility changes, since both systems that we used for altering lamin levels in vivo correlated lamin B2-enriched polymers with decreased resistance to chemical extraction compared with lamin A-enriched polymers.

DISCUSSION

Many IF protein families have undergone amplification and diversification in higher organisms. Nearly 50 keratin subtypes form specific heterodimer pairs in different epithelial cell types, each with a different stability to disruption by urea in vitro (22, 23). Neurofilament H, M, and L subtypes combine in different ratios that are suggested to determine axon diameter (24). Lamins have no particular obligate pairing and can occur in any ratio. However, the distinctive patterns of lamin composition in differentiated cell types and at various stages of development are largely conserved between mice (5, 7), cows (25), and chickens (6), suggesting that each cell type gains a functional advantage from its particular lamin ratio. We are investigating the hypothesis that the unique biochemical properties of each lamin subtype and its heterotypic associations are responsible for functional changes in the lamina. We find that each of the possible pairings of the four principal mammalian lamin subtypes has a different interaction strength, as defined by our column binding/urea elution assays, and that different regions of different proteins contribute to this variation (Fig. 7). In particular, the homotypic and heterotypic interactions of lamin B2 (the least studied mammalian subtype)
are much less stable than those of other lamin subtypes, which may explain why distinctive conditions were required for in vitro assembly of chicken lamin B2 into filaments (26). The relative “weakness” of lamin B2 correlated with an increased sensitivity to extraction from cultured cells using salt and detergent. The reports that lamin B2 is expressed in most or all cell types of adult mice (27, 28) and that lamin B2 is essential in cultured cells (29) support the hypothesis that unique biochemical properties of lamin B2 related to this “weakness” may be important for the functions of some cells.

By using recombinant proteins and being able to analyze lamin A and lamin C separately, our results provide a potential explanation for why one earlier study found the lamin A/C-lamin B1 interactions to be stronger than lamin A/C-lamin A/C interactions (9), whereas another (8) found no difference. We observed differences between the binding interactions of lamin A and lamin C, attributed to the unique carboxyl-terminal extensions of these two proteins. Therefore, differences in the ratio of lamins A and C between the preparations analyzed in the earlier studies could produce differences in the average measured strength of binding. Our findings add to other studies showing differences between lamins A and C subtypes: lamin C appears to follow a different pathway from lamin A for integrating into the lamina (30) and may differ from lamin A in its binding affinity for emerin (31).

Post-translational modifications clearly also contribute to lamina stability as phosphorylation drives lamina disassembly in mitosis (32–34), and lamins are also phosphorylated during interphase (35, 36). The binding studies presented here using recombinant proteins measured the basal interaction without phosphorylation. Once a greater understanding exists of which residues are modified and the timing of that modification in the cell cycle, it will be possible to test this additional layer of complexity in these binding studies. However, since S-phase phosphorylation of lamins should have occurred in our in vitro assays, we do not expect our general conclusions to be altered.

In our in vitro binding assays, we used the disruption of binding by the chaotrope urea as an operational definition of interaction “strength.” This is a commonly used approach for studies of IF proteins that are inherently insoluble (22, 23). The strong α-helical signal from the rod domain, which even is seen in the Δ mutants that contain only 10 heptad repeats in circular dichroism studies (17), indicates that circular dichroism is unlikely to be useful for determining whether unfolding of protein domains by the chaotrope contributes to the disruption of binding. Nonetheless, we think the latter is unlikely because of the considerable homology between the different subtypes in the core of the tail, which has been shown to form a stable all-β-immunoglobulin-like fold in A/C lamins (18, 19). Also, the validity of the in vitro binding data is supported by its correlation with our observations on the chemical extractability of lamins from cultured cells; in two different cell models, an increase in expression of the “weaker” binding lamin B2 increased the percentage of all lamin subtypes that are chemically extracted, whereas an increase in levels of the “stronger” binding lamin A led to a decrease in the extractability of all lamin subtypes. Although it is possible that differences in integral membrane proteins that bind lamins in the HL-60 model (21) also influence lamina solubility, this effect is probably minor due to the much greater excess of lamins, and the integral membrane proteins are not likely to be altered in the second model system (transient transfections).

We predict that the aggregate binding strengths for each unique ratio of lamin subtypes in different cells will correlate to the mechanical stability of the lamina in each of those cell types. Such a correlation has been made for the intermediate filament keratin proteins. Certain keratin subtypes (K5/K14) that are the least soluble in urea are expressed in stratified epithelia, whereas the most readily dissociated (K8/K18) occur in the internal epithelia; the stratified epithelium is believed to require greater mechanical stability than internal epithelia, a hypothesis borne out by keratin mutations that cause blistering skin diseases (23).

The notion that lamin A promotes lamina stability is consistent with the observations that lamin A disruption in cells results in altered nuclear morphology (37), nuclear herniations (38), and greater susceptibility to mechanical stress (39). Loss of lamina stability is probably relevant to laminopathies, since lamins exhibited increased solubility in fibroblasts from Emery-Dreifuss muscular dystrophy patients (40). The functional advantage that lamin B2 confers could be a relative reduction in the mechanical stability of the lamina that would be important for rapid nuclear morphogenesis in proliferating cell types. Consistent with this suggestion, proliferating basal epithelial cells and lymphocytes are rich in lamin B2 and poor in lamin A (27, 28). Reduced lamina stability may also be advantageous for tumorigenic cells that frequently undergo a reduction in levels of lamin A (41, 42). Although still providing structural support, such relative “instability” compared with lamin A-enriched systems may additionally facilitate migration of tumorigenic or immune cells by enabling nuclear shape changes. This is consistent with the lamin B2-rich neutrophils that undergo extensive lobulation during terminal differentiation (which is blocked in the laminopathy, Polger-Huet anomaly) (43). Decreased lamina “stability” in neutrophils would also facilitate the “extrusion” of their nuclear contents that occurs during pathogen invasion and is thought to be part of the immune defense mechanism (44).

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FIG. 7. Schematic summary of lamin binding interactions. Binding strength of each lamin pairing as measured by stability to extraction with urea is presented to scale by the urea50 value. Lamin B2 interactions were clearly “weaker” than those of all other lamin subtypes.
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