Cell mechanics and stress: from molecular details to the 'universal cell reaction' and hormesis

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Summary

The 'universal cell reaction' (UCR), a coordinated biphasic response to external (noxious and other) stimuli observed in all living cells, was described by Nasonov and his colleagues in the mid-20th century. This work has received no attention from cell biologists in the West, but the UCR merits serious consideration. Although it is non-specific, it is likely to be underpinned by precise mechanisms and, if these mechanisms were characterized and their relationship to the UCR elucidated, then our understanding of the integration of cellular function could be improved. As a step towards identifying such mechanisms, I review some recent advances in understanding cell mechanics and the stress response and I suggest potentially testable hypotheses. There is a particular need for time-course studies of cellular responses to different stimulus doses or intensities. I also suggest a correspondence with hormesis; re-investigation of the UCR using modern biophysical and molecular-biological techniques might throw light on this much-discussed phenomenon. ***BioEssays*** **29:324–333, 2007.** **© 2007 Wiley Periodicals, Inc.**

Introduction: the 'universal cell reaction' (UCR)

Modern cell biology is dominated by molecular-level accounts of gene expression, signal transduction, transport and assembly/disassembly processes, which have been established by an ever-growing array of molecular-biological, immunological, microscopic, rheological and other techniques. They have revolutionized our understanding of cell structure and function during the past three decades. Concomitantly, however, interest in certain broad, qualitative, nearly ubiquitous aspects of cell behaviour has declined. These general properties are encapsulated in the 'universal cell reaction' (UCR) or 'protoreaction', described by Dmitrii Nasonov and his colleagues in the early/mid-20th century and recently reviewed by Matveev.[1] Does our new understanding of cell structure and function enable us to interpret them? In other words, can we—and should we—try to explain the UCR in terms of the mechanisms elucidated by modern mainstream studies?

Nasonov and co-workers showed[2] that cells respond to a wide variety of external stressors with a standard array of structural and functional changes. Their focus was on these *changes* in cell properties, not steady-state descriptions. The noxious stimuli used were heat, mechanical stress, hydrostatic pressure, electric currents, general anaesthetics, altered pH and tonicity of the medium, heavy metal ions, hypoxia and sound irradiation (200–7000 Hz, 94 dB). The cells examined included epithelial, nerve, muscle and connective tissue cells from ectothermic vertebrates, germ cells from many invertebrate taxa, protozoa and some plant cells. The responses embraced changes in the turbidity, viscosity and biopotentials of cytoplasm and nucleoplasm, and resistance to the damaging actions of the agents deployed. A simple but efficient surrogate for this constellation of changes was vital dye binding, which became the staple method of the Nasonov School.

Irrespective of stimulus and cell type, the UCR is *biphasic*. Low-intensity stimuli (phase I) evoke decreases in turbidity and viscosity, with concomitant increases in membrane potential and resistance to the harmful agent. Higher-intensity stimuli (phase II) increase the cell's turbidity and viscosity, depolarize the membrane and decrease resistance to the stimulus (Fig. 1). During phase II, the cytoplasm scatters light, appearing pale blue because of the Tindall effect, and vital-dye binding is strongly enhanced. The intracellular pH falls and there is increased uptake of Na^+ and Cl^- Interestingly, these changes can occur *locally* in a defined subcellular region as well as throughout the cell. Whether they are local or global, all the changes in each phase of the UCR occur *simultaneously.* This simultaneity was emphasized by Nasonov.

Although the changes encapsulated in the UCR are very general, they may be underpinned by mechanisms that involve specific, possibly unidentified, cell components. However, the phenomenon was explored when cell biology in both the

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Abbreviations: UCR, universal cell reaction; ROS, reactive oxygen species; HSP, heat-shock protein; NSK, nucleoskeleton.

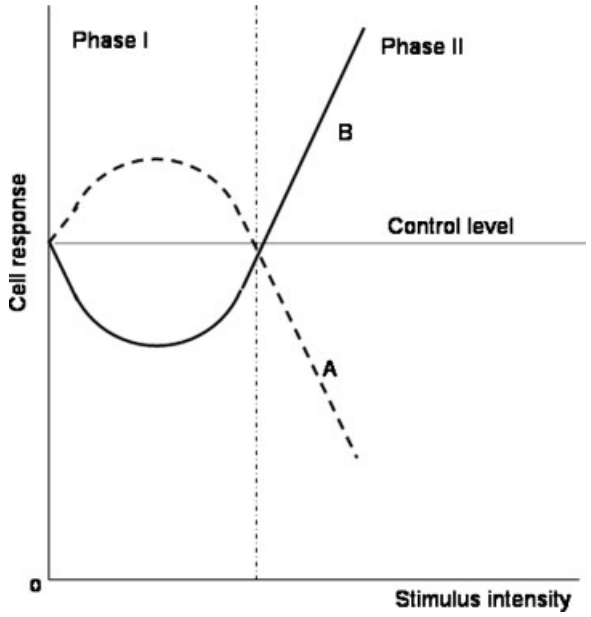

Figure 1. The 'universal cell reaction' (UCR). Curve **A:** As the external stimulus intensity increases from zero, membrane potential and resistance to the stimulus agent first increase (phase I) and then decrease to below their resting or control values (phase II). Curve **B:** As the external stimulus intensity increases from zero, intracellular viscosity and turbidity and vital dye binding capacity first decrease (phase I) and then increase to above their resting or control values (phase II). All these changes occur simultaneously. Figure adapted from Fig. 1 in Matveev[1] following discussions with the author.

western and eastern worlds was in its infancy. In this article, I shall consider (a) how the UCR may be related to data from recent cell biological research, (b) possible connections with the phenomenon of hormesis. 'Hormesis' is a dose–response phenomenon characterised by stimulatory responses at low doses and inhibitory responses at high doses, or vice versa. Like the UCR, it denotes a class of biphasic responses to external stimuli. I suggest that hormesis and the UCR may be different manifestations of the same underlying intracellular processes, and I outline possible experimental approaches to these processes.

Does the UCR merit re-investigation?

Nasonov proposed the same mechanism for the UCR no matter whether the response is local or cell-wide: cellular proteins serve as receptors for the noxious stimuli; they denature and then aggregate within the cell under stress, and vital dyes are adsorbed to them electrostatically. Protein chemistry was scarcely developed when Nasonov proposed this mechanistic sketch, so it cannot be related (except trivially) to modern understanding.

Is a more satisfactory explanation worth seeking? Old data, obtained using techniques that are crude by modern standards, might not be worth reconsidering. In addition, for political and economic reasons, the quality of some of the research conducted within the Soviet bloc during the mid-20th century is considered dubious. However, data from earlier times should not be ignored if they are sound, though they may demand retrospective refinement and reinterpretation, and by no means all scientific work in Soviet countries was poor. Findings similar to the UCR were reported by other laboratories, mostly outside the Soviet world, although only the Nasonov School pursued the phenomenon tenaciously. For example, Heilbrunn[3] cited a number of studies describing biphasic responses, albeit critically; Vasliliev and Gelfand[4] investigated the connection between UCR-related changes and cell proliferation; the early studies by King and co-workers on cell death processes[5] indicated biphasic responses; the wide-ranging protective effects of low-dose noxious stimuli were investigated by Penttila et al.,[6] and recent work in Russian laboratories has shown that Nasonov's account applies to aspects of mammalian brain metabolism under stress conditions.[7,8] In short, results from many different laboratories over a number of years have proved remarkably consistent, notwithstanding the variety of cell types and stimuli studied. Therefore, the coherent set of "general cell properties" constituting the UCR seems to be established beyond reasonable doubt.

The UCR merits our attention for two inversely related reasons. First, a set of simultaneous changes in *whole-cell* properties tests the explanatory capacity of our molecular-level descriptions. Second, a better understanding of the generality may elucidate the biological significance of specifics. The phases of the UCR are the *contexts* in which molecular-level changes occur in cells exposed to external stimuli.

Hydrophobic phase volume

The challenge, therefore, is to relate the specific to the general. The foregoing summary suggests three approaches to meeting this challenge. First, recent microrheological studies of cells may add details about the UCR-related changes in viscosity and turbidity and suggest a molecular basis. Second, some features of phase II imply the stress response. Third, the UCR[2] and several related findings[3,4,6] recall various examples of hormesis, though intracellular or transcellular rather than cell-surface events are probably involved.

Matveev[1] proposed that the UCR is underpinned by changes in the 'hydrophobic phase volume'. The basis of this concept is that virtually all proteins contain substantial hydrophobic domains. In the resting cell, the total volume of these hydrophobic domains is small; the volume of the lipid phase is insignificant. However, during phase II of the UCR, soluble proteins undergo structural changes and aggregate,

and the net volume of their hydrophobic domains rises dramatically, though the volume of the lipid phase is unchanged. This overall increase in 'hydrophobic phase volume' drastically alters the physicochemical properties of the cell internum: there is a marked redistribution of lipophilic compounds, both internally and between the cell and the medium, and this can have significant consequences for cell structure, metabolism and signalling processes.

Several modern publications are compatible with this account of the UCR, though none of them cites Nasonov or his School. For example, Fazly Bazaz and Salt[9] showed that lipophilic local anaesthetics increase the turbidity of stationary-phase *E. coli* cells. At high concentration they precipitate the cell contents; less lipophilic anaesthetics are not effective at the same concentrations. The turbidity increase is modulated by the ionic composition of the environment. Goloubinoff et al.[10] showed that two *E. coli* chaperone systems acting in sequence efficiently solubilize a variety of protein aggregates and refold them into active proteins. The first chaperone, ClpB, binds directly to the aggregates. ATP-induced changes in the structure of ClpB then appear to increase the hydrophobic exposure of the aggregates, allowing the second chaperone, the prototypical heat-shock protein complex DnaK–DnaJ–GrpE, to bind and solubilize the proteins, and mediate their dissociation and refolding into active molecules. The coordinated action of these two chaperones is associated with changes in the binding of Congo Red (which is hydrophobic) and turbidity, consistent with the changes in 'hydrophobic phase volume' proposed by Matveev.[1] Also, *Saccharomyces cerevisiae* responds to oxidative stress in a UCR-like manner. The yeast cells adapt to low doses of reactive oxygen species (ROS) and become more resistant, but at higher doses cell division is delayed and antioxidant and repair systems are induced, with indications of intracellular protein aggregation; very high doses kill the cell.[11,12] However, such studies—although illuminating—might not reflect the mechanisms underlying the UCR in all cell types. Recent investigations of the kind suggested earlier in this section may be more informative.

The mechanics of the cytoplasm

An obvious suggestion is that many of the UCR-linked changes in viscosity, turbidity and birefringence in eukaryotes may be explained by the dynamics of the actin cytoskeleton. Several studies appear to support this view. For example, Marion et al.[13] showed that *Entamoeba histolytica* cytoplasm is a soft viscoelastic medium; the viscosity shows a power-law dependence on shear, with an exponent of −0.65. This behaviour depends on the presence of an intact actin cytoskeleton stiffened by myosin II. Feneberg et al.[14] measured cytoplasmic dynamics in *Dictyostelium discoideum*. The motion of single nanoparticles comprised rapid linear steps of about 1 μm interspersed with slower random walks of about 0.1 μm, reflecting marked local differences in yield stress in the cytoskeleton and cisternae. When the external force applied to the cell was increased over an 8-fold range (pulse amplitudes 50–400 pN), the cytoplasmic viscosity increased 35-fold to 350 Pa s. Feneberg and colleagues interpreted the apparent viscosity as an inverse measure of bond stability within the cytoplasmic network. Bond breaking in this system is determined by the Arrhenius-Kramer law; the apparent viscosity depends sensitively on the amplitude of applied force, because the work done by this force reduces the activation energy. Myosin II-deficient *Dictyostelium* cells exhibited higher yield stresses. Thus, both phases of the UCR seem to be reflected in recent rheological studies of the actin cytoskeleton in protists.

Alternatively, these results might reflect dynamic events in the 'cytomatrix', i.e. the parts of the cytoplasm outside the cytoskeleton proper, rather than (or as well as) the actin cytoskeleton. Elsewhere,[15] the cytomatrix has been dubbed the 'microtrabecular lattice', but it is probably a metastable structure with no fixed or definite composition.[16,17]

Experiments on various mammalian cells, notably neutrophils,[18–20] chondrocytes[21] and 3T3 fibroblasts,[22,23] have yielded similar results: the cytoplasm is a non-Newtonian, essentially thixotropic medium (its viscosity decreases as the shear stress increases). However, the range of protein–protein interactions responsible for changes in cytoplasmic viscosity has not been fully characterized. The (actin) cytoskeleton probably has an important role, but the viscosity changes may indicate marked rearrangements in *general* ('cytomatrix') protein–protein linkages throughout the cell. In neutrophils subjected to identical aspiration pressures, cytochalasin B reduced the cortical tension by 19% at 3 μM and by 49% at 30 μM, and reduced the cytoplasmic viscosity by approximately 25% at 3 μM and by approximately 65% at 30 μM. The apparent viscosity decreased linearly with the applied shear rate.[20] Although once again these effects probably reflect disruption of the actin cytoskeleton, cytochalasin B is not absolutely specific; non-cytoskeletal parts of the cytoplasm might (also) be involved. In any event, the findings are qualitatively reminiscent of the UCR: cytoplasmic viscosity decreases in response to a low-intensity external stimulus and increases in response to a high-intensity stimulus. Do other UCR variables change concomitantly? For instance, does vital dye uptake decrease when a mild shear force is applied externally and there are concomitant rheological changes in the cytoplasm? Would hydrophobically coated microbeads show greater mobility when a low-intensity stress was applied? In principle, such experiments could be conducted.

Biophysical differences among subregions of the cytoplasm can be quantified. Drury and Dembo[18] showed that a mechanical model of the neutrophil must incorporate both shear thinning and surface viscosity, but even this may not

capture all the rheological properties of the cell. At low shear rates, the surface dilatation viscosity was around 100 poise-cm but the bulk cytoplasmic viscosity was about 1000 poise. Both variables decreased similarly when the shear rate exceeded 0.05 s^{-1}. Hochmuth et al.[19] found a cytoplasmic viscosity of around 600 poise and a cortical tension of 0.024 dyn/cm at zero shear, and inferred that the neutrophil must be modelled as a series of shells of fluids with different rheological properties. Recent studies revealing the contribution of membrane lipid rafts to cell mechanics are compatible with this view.[24] Ragsdale et al.[22] monitored cytoplasmic deformation in Swiss 3T3 fibroblasts by observing the displacement of microinjected 200 nm beads. The time-dependence of cytoplasmic strain, calculated from the bead displacements, fitted a Kelvin–Voight model for a viscoelastic solid with a mean limiting strain of 0.58 and a mean strain rate of 4.3×10^{-3} s^{-1}. Such results can be seen as placing phase I of the UCR on a more quantitative basis. The time course seems particularly informative.

As for phase II of the UCR, the changes induced by strong external stimuli may result from increased calcium uptake. It was recognized half a century ago that cell injury is usually accompanied by an increase in cytoplasmic Ca^{2+}.[3] The turbidity/birefringence of *Amoeba proteus* extracts is increased by adding micromolar concentrations of calcium along with Mg-ATP,[25] and there are concomitant increases in the number of actin filaments and extent of myosin-dependent cross-linking. The turbidity of eye lens cells increases with increasing calcium concentration; the scattering particles are tens of nm in diameter.[26] Other metal ions might also be involved: cytoplasmic concentrations of sodium and potassium change in response to ethylene glycol exposure,[27] and the potassium content of mouse zygotes changes in response to microsurgical manipulation,[28] again recalling phase II of the UCR. Drury and Dembo[18] observed very high rates of aspiration of neutrophils into micropipettes during the initial moments after ramping of pressure, and they were unable to explain this in terms of their two-phase mechanical model (see above). Rapid calcium influx under these conditions seems a possible explanation, but the slight delay in the increase in apparent cytoplasmic viscosity after application of a strong noxious stimulus again suggests that phase II of the UCR could be elucidated by detailed time-course studies.

Another possibility, also difficult to relate to the high-shear response of neutrophils, is that phase II of the UCR entails modification or oxidative cross-linking of protein sulphydryl groups. The actin cytoskeleton is likely to be involved in some cells[29] but not in all. Extracellular nickel ions cause microtubule polymerization in some cell types, and this is associated with a decreased concentration of protein sulphydryl groups and lower GSH levels.[30] Intravenous iodoacetate causes turbidity in cells of the eye lens, first in the nucleus and then in the cortex.[31] α-B-crystallin, a lens cell chaperone[32] functionally homologous to a small heat-shock protein,[33] protects against turbidity increase by controlling tubulin polymerization. A further possible interpretation of phase II of the UCR is strain hardening of the actin cytoskeleton.[34,35]

There is a risk of overgeneralizing these conclusions. Some 'UCR-like' responses could be underpinned by quite different processes. For example, some turbidity changes might be cell-type specific; adrenal medullary cells become more turbid when granule fusion is induced by specific intracellular signals.[36] Overall, however, there seems to be a prima facie case for associating phase I of the UCR—in part—with reversible depolymerization of the actin cytoskeleton, and phase II with associations among microtubular and other proteins effected by uptake of calcium and other ions, sulphydryl group oxidation, stress hardening of the actin cytoskeleton and/or other mechanisms (Fig. 2).

These possible correspondences merit investigation, but extracytoskeletal ('cytomatrix') proteins should also be considered. It could be especially rewarding to obtain detailed time courses of changes in the cytoskeleton and cytomatrix when cells are exposed to noxious and other stimuli at low as well as moderate or high intensities.

The mechanics of the nucleoplasm

Tseng et al.[37] used particle nanotracking to investigate the micro-organization and viscoelastic properties of interphase Swiss 3T3 fibroblast nuclei. The nucleoplasm is much stiffer than the cytoplasm (mean shear viscosity 520 poise) and highly elastic (elasticity 180 dyn cm^{-2}), values consistent with other findings.[38,39] Therefore, transport of organelles such as promyelocytic-leukaemia bodies requires propulsive forces of 3–15 pN to overcome the intranuclear viscosity. Spontaneous movements of the microinjected nanospheres suggest that the nucleus contains relatively fluid microdomains of mean diameter 290 ± 50 nm, which may be relevant to the more rapid movement of smaller particles. Domains of this size are mostly absent from the cytoplasm. In general, however, microrheological studies show that the nucleoplasm, like the cytoplasm, comprises microdomains of densely packed structures separated by very soft, liquid-like compartments.

Changes in the rheological properties of the nucleus in response to external noxious stimuli seem not to have been described. They could be studied using modern biophysical techniques; Nasonov and his colleagues were restricted to cruder methods, but were able to describe consistent patterns of change. For example, it would be interesting to know whether changes in the intranuclear 'hydrophobic phase volume' occur when stresses are applied. Once again, dye-binding studies, or injection of microspheres with hydrophobic surfaces, might answer this question. In the meantime, existing information about the stress response may be suggestive.

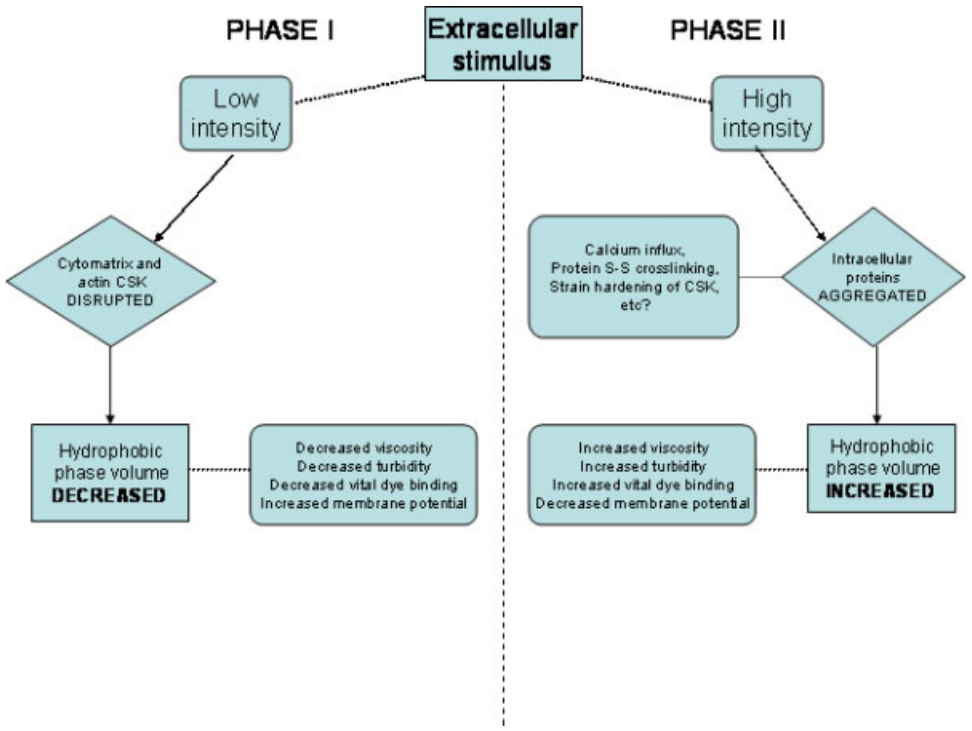

Figure 2. Possible mechanisms linking mechanical changes in the cytoplasm to the UCR. When the intensity of the external stimulus is low, disruption of the actin cytoskeleton (CSK) and/or the 'cytomatrix' may lead to an overall decrease in 'hydrophobic phase volume', potentially accounting for the decreases in viscosity and turbidity associated with phase I of the UCR. A higher stimulus intensity may cause calcium influx to the cell and/or promote oxidative cross-lining among cytoplasmic proteins and/or lead to strain hardening of the actin cytoskeleton. As a result, the 'hydrophobic phase volume' may be markedly increased and the changes associated with phase II of the UCR (increased viscosity, turbidity and vital dye binding) would ensue. A similar scheme can be supposed to account for UCR-related changes in the nucleoplasm.

Heat-shock proteins and cell mechanics

Phase II of the UCR is readily identified with the stress response, which is also non-specific but underpinned by the actions of specific proteins. Several authors[40,41] have noted that stresses of all kinds—thermal, mechanical, electrical or chemical—induce qualitatively similar responses in many different cell types, though they do not cite Nasonov. Broadly, stresses such as heat denature some cellular proteins and rapidly induce the synthesis of heat-shock proteins (HSPs). HSPs sequester the unfolded proteins by binding to exposed hydrophobic regions and can help in refolding. These actions usually, but not always, depend on ATP binding and hydrolysis.

Some HSP functions may be subtler and more complex. Kampinga[42] points out that much of our knowledge about individual HSPs is based on in vitro experiments using denatured purified proteins. However, cells contain many chaperones and chaperone cofactors that shuttle among subcompartments, and numerous protein substrates in different folding/unfolding states, so the range of possible interactions is very large. Moreover, folding in vivo competes with proteasome-dependent degradation, which is not simulated by cell-free studies.

Most researchers are interested in molecular-biological details of the effects of (usually single) HSPs, particularly the suppression of certain genes and the expression of others. For instance, oxidative stress downregulates 35 genes in the anaerobe *Desulfovibrio vulgaris* and upregulates 19 others.[43] Most protein biosynthesis is repressed when *Drosophila* cells are heated from 25 to 37°C, but HSP synthesis is rapidly induced.[44] The effect is reversed when the cells are cooled again to 25°C. HSPs are involved in transcriptional regulation and in altering the stabilities of cytoplasmic mRNAs. More pertinently, however, HSPs appear when *many* normal cellular proteins have been denatured, and the 'hydrophobic phase volume'[1] has presumably increased in consequence. The details of particular HSP functions might deflect attention from this general feature of cellular stress, and *general* features rather than molecular minutiae are pertinent to the UCR.

Disruption of the cytoskeleton is a regular concomitant of the stress response.[45–48] In general, microtubules are reorganized, intermediate filaments are compacted around

the nucleus, microfilaments are at least partially disassembled and mitochondria are redistributed and show altered morphology. These changes are associated with increased levels of one or more HSPs; they do not appear to mediate the HSP-induced alterations in transcription, translation and protein translocation associated with thermal stress,[49] though thermal stress inhibits mRNA export from yeast nuclei.[50]

Some of the G-actin released during stress-induced disassembly of cytoplasmic microfilaments probably remains in the cytoplasm, unevenly redistributed, along with myosin and other associated proteins.[51] The cytoplasm increases in density,[52] probably because G-actin becomes increasingly involved in the loose protein–protein associations constituting the 'cytomatrix'. Myosin, too, might be a component of the 'cytomatrix', even under non-stress conditions.[22] Increased levels of cyclic AMP may have a similar effect on cytoplasmic protein distribution.[53]

Some of the dissociated actin enters the nucleus, where it repolymerizes, presumably together with endogenous intranuclear actin; nuclear F-actin rods are assembled when fibroblasts respond to thermal stress.[47] These nuclear actin filaments may be components of the nucleoskeleton (NSK) or 'nuclear matrix', but they do not appear to be causally related to HSP induction.[47] They serve as binding sites for the proline-rich sequences of the tumour suppressor protein p53, particularly in association with sites of DNA damage.[54,55] The increase in nuclear F-actin implies an increase in the already high intranuclear viscosity, and this may be equivalent to one aspect of phase II of the UCR. Other studies have corroborated the viscosity increase. Although NSK proteins are particularly thermolabile,[56] changes in the NSK induced by thermal stress, like the concomitant changes in the cytoplasm, include increased aggregation of a heterogeneous set of proteins, increased protein mass, higher electron density and a more heterogeneous fibril morphology.[57,58] These changes take place up to 24 hours after the exposure to thermal stress, so they may indicate chronic alterations in the nucleus after phase II of the UCR is completed.

HSPs associate with the nuclear envelope lamins and possibly with the internal NSK,[59] especially with the larger HnRNP units.[57,60] Thermal stress causes a markedly increased association of protein kinase CK2,[61] delayed DNA replication at NSK-associated sites,[62] and disruption of transcription and RNA processing.[56] Interestingly, heating *activates* the NSK-associated replication sites (MARs) involved in HSP gene transcription. Roti Roti et al.[56] wrote: "We have found a heat-inducible MAR covering the promoter region of murine hsp70.3, implying that changes in matrix association are needed for hsp70 expression. However, the hsp70.1, hsp70.3, and hsc70t gene family is organized as an active gene with respect to the nuclear matrix. Thus, it may be that heat-inducible genes have a unique matrix-dependent organization." In other words, there appear to be specific stress-induced changes in nuclear organization and function, but there also appear to be general, non-specific ones that could underpin the nuclear facet of phase II of the UCR.

Associations between hsp70 isoforms and the NSK might also support the suggested relationship between the UCR and 'hydrophobic phase volume'. Heating elicits a reversible conformation change in Hsc70, resulting in increased exposure of hydrophobic residues and increased protease susceptibility.[63] Increased hydrophobic exposure could underpin at least some of the protein—protein associations described above. Once again, dye-binding studies, or measurement of the mobility of microspheres with hydrophobic surfaces, could elucidate this.

Time-course studies using low as well as high-intensity stressor stimuli would be particularly informative. The apparent correspondence between phase II of the UCR and the stress response (Fig. 3) implies that a low-intensity stressor should have effects qualitatively opposite to those elicited by the same stressor at moderate or high intensity. This prediction is clearly testable.

A wider perspective: hormesis

More than 100 years ago, Schultz[64] showed that very low doses of mercuric chloride stimulated yeast fermentation; higher doses were inhibitory. Similar biphasic effects of external stimuli have proved widespread. Several workers have expressed misgivings about the evidence for the 'paradoxical' effects of low-dose stimuli,[3,65] but *hormesis* is now generally accepted as a real phenomenon, thanks in part to improved statistical methods that confirm the significance of low-dose effects.[66,67] A recent analysis of almost 57,000 dose-response studies of some 2100 anticancer drugs on 13 yeast strains in the National Cancer Institute database showed that the conventional threshold model fails to explain low-dose responses.[68] Biological responses exhibiting hormesis include cell proliferation, transformation and differentiation. Suggestions that hormesis be used as a basis for health and environmental protection decisions are controversial,[69] but its significance for many aspects of biology and medicine is increasingly appreciated.[70,71] The generality and biphasic character of both hormesis and the UCR suggest that the two phenomena may be related. They may be underpinned by the same intracellular mechanisms.

Hormesis gives rise to U-shaped or J-shaped dose–response curves[72,73] (Fig. 4). It is typical of the biological effects of physical, chemical and physiological stressors and appears to be independent of the system investigated, the endpoint measured and the stressor agent.[74] It has been described as 'adaptive'; low doses are alleged to activate defence mechanisms, leading to 'overcompensation'.[72] Nasonov and other members of his School characterized the UCR in almost the same words 50–60 years ago. For example, large doses of ionizing radiation have harmful biological

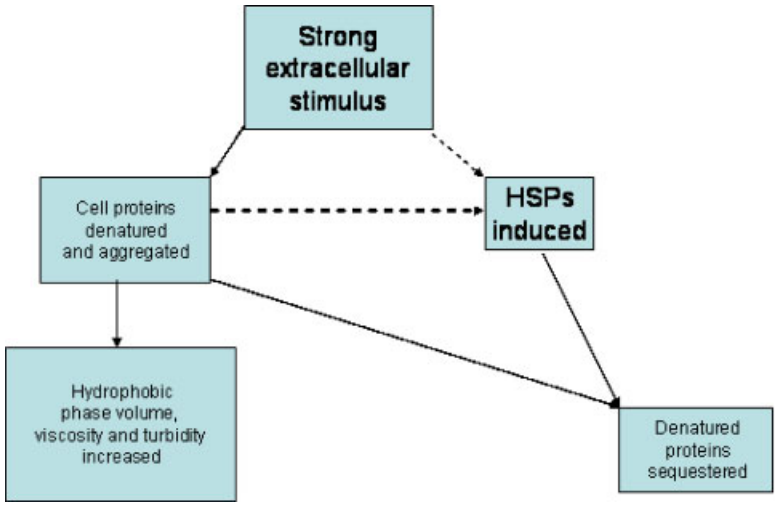

Figure 3. Possible relationship between stress response and phase II of the UCR. This scheme might be seen as either complementary or supplementary to that shown on the right hand side of Fig. 2.

effects, but low to intermediate doses enhance growth and survival, augment the immune response and make plants, bacteria, insects and mammals more resistant to further radiation. Such 'adaptive' responses could have far-reaching implications for (e.g.) radiation protection.[75]

The potential importance of hormesis in cancer biology has been noted.[68,76] Brandes[77] reported that antidepressants, hormones and hormone antagonists exert hormetic effects on cancer cell proliferation in vitro and in vivo. Prehn[78,79] showed that the mammalian immune response elicits biphasic behaviour in tumour cells. It therefore seems clear that hormesis has considerable practical (including clinical) significance. One reason why it is commonly overlooked may be the lack of a satisfactory mechanistic explanation.[70,71] If a mechanistic understanding of the UCR were secured, perhaps by experimental studies of the kind suggested in this article, the mechanism underpinning hormesis might be revealed a fortiori. The possibility that new insights into aspects of cancer biology, ageing and toxicology could (indirectly) be obtained by investigating the UCR with modern methods is a strong argument in favour of such investigations.

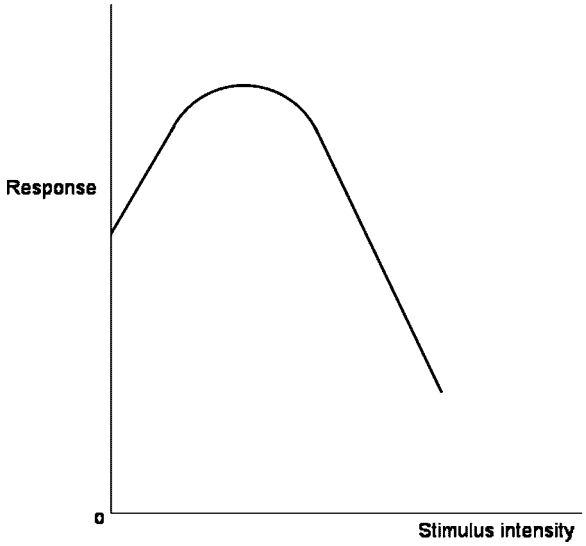

Figure 4. Hormesis. Typically, responses vary biphasically with stimulus intensity. The general qualitative similarity to the UCR (Fig. 1) is immediately apparent.

Some recent publications suggest that this possibility is not remote. Rattan and co-workers have shown that mild heat-stress delays ageing and promotes longevity[80–82] and other studies support this finding.[83] At the cell level, mild heat shock has hormetic effects on protein glycation and oxidation[84] and sustains fibroblast function in vitro, at least partly through the production of HSPs.[85] Hormesis is also apparent at the cell level in the response of the proteome to external stimuli.[86] Day and Suzuki[87] found hormesis in cellular GSH levels and cell proliferation in response to external ROS, recalling the yeast studies[11,12] mentioned earlier. The phenomenon has also been described in bacteria.[88] All these reports seem compatible with the account of the UCR proposed in the present article.

To date, however, most attempts to identify the mechanistic basis of hormesis have taken different approaches. For example, Conolly and Lutz[89] suggested four possible cell-level mechanisms for hormesis: (1) two membrane receptor

subtypes with different ligand affinities and contrasting downstream effects, (2) two intracellular steroid (e.g. androgen) receptor subtypes that activate responsive genes in homodimeric but not heterodimeric form, (3) DNA repair by enzymes that are activated by forming adducts with the damaging xenobiotic, and (4) mutation rate being the product of the rates of DNA damage and of cell division (the latter is decreased by low-level DNA damage, and increased by cytotoxic doses of the damaging agent owing to regenerative hyperplasia). These proposals identify mechanisms that are intrinsically plausible but limited in scope, belying the generality of hormesis. I suggest that better understanding will be gained if hormesis is regarded as an aspect, or alternative manifestation, of the UCR. In cell population dynamics, hormetic effects can be simulated mathematically by a feedback model that assumes a known target cell population.[90] This approach might also prove informative, but successful mathematical simulation does not necessarily imply a mechanism.

Conclusions

Recent studies on the rheology of the cytoplasm and nucleoplasm are generally consistent with the Nasonov School's accounts. They suggest possible explanations for both phases of the UCR in terms of contemporary mainstream cell biology. The comparisons outlined in this article lead to testable hypotheses. For example, since intense noxious stimuli of all kinds in a wide variety of cell types tend to increase calcium uptake, or protein sulphydryl oxidation, or stress hardening of the actin cytoskeleton, the prediction is that low-intensity stimuli of the same type will have the opposite effects. Experimental testing of this prediction might elucidate the general responses of cells to stress, and the hitherto unexplained phenomenon of hormesis. Such tests might be difficult in practice, since phase I of the UCR may be too weak and/or short-lived for reliable measurement;[1] but the statistical procedures that are now used by students of hormesis[66,67] could prove useful.

It is now clear that the cytoplasm and nucleoplasm are rheologically highly heterogeneous, so it is tempting to infer that further investigation of the molecular differences among the dynamic microcompartments of the cell will be more rewarding than attempts to elucidate general cell-wide responses. Such focused and specific studies will certainly be informative, but the ineluctable fact remains that cells show *general* responses to noxious stimuli. The UCR probably results from a number of specific interactions, but the significance of these interactions can only be appreciated from the standpoint of the general response; the importance of a single street in the dynamics of city traffic can only be appreciated when a map of the whole city is studied. Local and molecular details of the mechanical and other changes that occur when cells are exposed to external stimuli will only reveal their true biological significance when they are seen in the context of the whole.

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