

Voices

What differentiates a stress response from responsiveness in general?



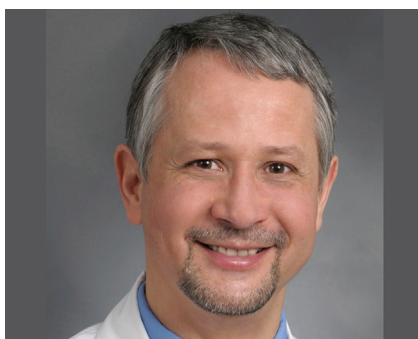
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No one-size-fits-all solution

Cellular stressors are abundant and diverse, and they can be acute or chronic and mild or intense. Accordingly, the stress response is complex, involving transcription, RNA stability, translation, protein turnover, and changes in interactions, as well as modifications and re-localization of molecules. Different response types have their specific triggers, regulators, and dynamics, with the common goal of reestablishing cellular health.

The resulting lack of a one-size-fits-all solution is a good thing. First, a stress response network, assembled from the entirety of the different pathways and their connections among each other, provides the flexibility to adjust to the type, intensity, and dynamics of the stressor. Second, it allows cellular protection at multiple levels, i.e., ensuring the correct abundance and folding of proteins as well as the protection of the genetic material. Third, and importantly, a response network provides robustness: if one pathway fails, other pathways can still provide protection. Robustness is as critical as flexibility and uncertainty, and they are deeply intertwined.

The challenge for research is to fully embrace this complexity. For example, in addition to deciphering the details of specific pathways, fully understanding the complexity would require simultaneous analysis of multiple processes over time and for many genes, e.g., profiling of translation changes, along with changes in transcript and protein stability. It would require accepting that exceptions are the rule, and that no single pathway will explain everything. And finally, it would require an integration of the resulting multi-dimensional datasets in a way that incorporates the biological relationships between them to extract new insights and to foster a view of the highly integrated stress response as a system that is bigger than the sum of its parts.



Gábor Balázsi
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Multiscale stress response

Stress response is the structural or compositional change of a biological system following events that reduce fitness. Generally, responsiveness includes processes related to fitness improvement, such as bacteria finding a preferred sugar, epithelial cells detecting hormones, or immune cells encountering antigens. Yet, none of these are stress responses. In contrast, a sudden temperature increase, mutation, or antibiotic treatment can cause protein misfolding, so cell division slows or stops, or some cells die, while other nondividing, stress-tolerant persister cells may be unaffected.

Nonlethal stress manifests as a decline of cell division frequency or cell population growth rate, which are fitness proxies at two different scales. Stress responses then ensue at different length and timescales. At the intracellular-molecular scale, stress-affected cells upregulate chaperone levels to promote correct protein folding and eventually resume cell division. At the population scale, short-term stress response manifests as regrowth or stasis upon transient growth rate reduction or net death as some cells resume division or vulnerable cells die off. Cell populations may respond to stress even if no single cell responds, i.e., if each cell either dies or persists. Additionally, stress can unmask or induce cell-cell variability, and cell populations can evolve over longer timescales as adaptive mutations or stable epigenetic changes spread so the evolved population copes better with stress. These examples illustrate the need to examine how stress responses at distinct space and timescales are related from a statistical physics perspective.

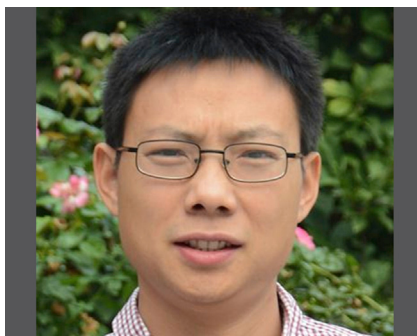


Alexander Löwer
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Always on guard

For most of us, stress is part of everyday life, and the same holds true for the cells in our bodies. Among their most stress-plagued components is the genome, which is threatened by lesions resulting from internal sources, such as replication and transcription, as well as external insults, such as radiation and toxins. Stress response pathways have to recognize the damage and decide if it is revertible or if terminal fates such as apoptosis should be induced. The DNA damage response thus needs to keep a delicate balance between high sensitivity, allowing it to recognize even single lesions, and sufficient tolerance to let dedicated DNA repair pathways do their job.

In addition to the amount of genotoxic stress, other criteria, such as its duration, factor into this decision—acute damage generates different signals than persistent stress. Moreover, information about the state of a cell and the activity of other signal pathways are integrated to align cell-autonomous decisions with the needs of the surrounding tissue. Taken together, one of the defining features of stress response pathways is that they are able to continuously evaluate the health of a cell. Therefore, the dynamics of the underlying molecular networks often play a decisive role. For p53, the guardian of the genome, time-resolved single-cell measurements revealed how feedback and feed-forward interactions shape these dynamics, implement filtering systems to distinguish transient from sustained inputs, and help to diversify cellular responses.



Caifu Jiang
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Complex stress response

Any plant in nature grows at a particular rate and yields a certain number of offspring. Nevertheless, lack of water, light, or essential minerals, salted soil, extreme temperatures, or a variety of biotic attacks could all reduce the growth vigor of plants and even kill them. I consider all the factors lessening a plant's capacity to produce an optimal number of healthy offspring to be “stress.”

The natural environment is full of all kinds of stresses, and the plant's response to any of them is complex. I address plant stress response and its complexity using salt-stress as an example. Several processes occur successively under salt-stress: salt sensing, sensor-mediated activation of downstream signaling, and salt transport. These processes to a certain extent distinguish salt-stress response from other responses. However, salt-stress also activates the responses to osmotic stress and oxidative stress, which are physiologically important but not unique to the salt-stress response. Moreover, several groups, including ours, observed that salt-stress responses in different organelles, cell types, or tissues are distinctive and hinge on their location, function, morphology, etc. Although the complexity of response to stress (e.g., salt) has been recognized, we are far from clarifying the networks underlying it. The time has come to investigate the specificity and complexity of plant stress response more precisely in time and space dimensions, for which single-cell omics techniques will be of great help.



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Beyond the outer limits

Microbial extremophiles are remarkable examples of life's resilience, thriving in hot springs at boiling temperatures, in brine lakes saturated with salt, and in deserts once thought to be sterile. Although some bacterial species hold some records for growth under extreme conditions, organisms of the domain Archaea tend to dominate microbial communities at the limits of life. We have chosen to study stress response mechanisms in archaeal extremophiles because they require a complete reframing of the question of stress. What are the mechanisms that enable such resilience? How is “stress” defined for an organism that thrives constantly in a condition that would kill most others?

Extreme stress adaptation occurs on two temporal scales. On the evolutionary time-scale, specialized macromolecular adaptations enable vitality in an extreme environment. For example, we study hypersaline-adapted species living in 10× the salt concentration of seawater. They have evolved a salt-in strategy, accumulating up to 4M potassium to maintain osmotic balance. An enrichment in acidic residues solvates

protein surfaces under these denaturing cytoplasmic conditions. Although these adaptations support proliferation in extreme stress, moderate conditions are toxic: cells burst below 1.5M NaCl due to osmotic imbalance.

On a more fleeting temporal scale, dynamic adaptation on the order of minutes to hours requires highly interconnected gene regulatory networks. We hypothesize that, like tightly knit social networks, interlocking feedback loops within regulatory networks enable more frequent communication between master regulators to coordinate timely deployment of stress response systems. This crosstalk may result in a wider range of tolerance to setbacks or perturbations than species adapted to moderate conditions. Variable environments may have selected for such broad tolerance, for example, in hypersaline ecosystems where cells are subjected to evaporative cycles in ephemeral salterns and lakes. Perhaps what makes stress response of such biological outliers remarkable is not the extreme limits they withstand, but instead their broad range of dynamic stress tolerance under variable conditions.



Morten Sommer

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The stresses of fermentation

In a world that relies increasingly on green manufacturing of chemicals, fuels, and foods, we are dependent on large-scale biomanufacturing. Microbial biomanufacturing typically uses microorganisms engineered to satisfy a defined production objective. To supply enough product, these engineered microorganisms are grown up to large densities in large volumes. As an example, Brazilian sugar cane ethanol is produced in fermentation tanks containing $\sim 10^{16}$ yeast cells. These conditions result in both physiological and evolutionary stresses.

In large scale fermentations, cell densities and metabolic activities are high, leading to depletion of both oxygen and growth substrates along with increases in byproducts that may inhibit growth. These nutrient and inhibitor gradients vary with time and space in a fermenter, resulting in a dynamic stress response, which is poorly understood. These stresses likely lead to differential responses depending on the state of the cell. Indeed, engineered cells overexpressing a production pathway that depletes a cellular substrate may be more vulnerable to these stresses in a fermentation.

These physiological impacts are further compounded by evolutionary pressures resulting from the large population sizes in industrial fermentations. In such a competitive environment, engineered cells must compete with emergent non-producer cells that may have mutated to lose a costly production pathway.

These challenges must be addressed to enable a greener biobased future.



Laurence Yang

Queen's University

Systems-level balancing act

Stress responses can seem to be paradoxical. We breathe oxygen to power thousands of vital metabolic reactions through aerobic respiration. Yet, when oxygen first became abundant in the Earth's atmosphere, countless organisms went extinct due to toxic reactive oxygen species (ROS). Aerobic organisms maintain a delicate balance between reaping the energetic benefits of respiring oxygen and detoxifying the resulting ROS.

Stress is the state of imbalance between production and mitigation of toxicity. Many cells "breathe" oxygen and constantly produce ROS; however, in unstressed states, the ROS is sufficiently detoxified. With thermal stress, reactions proceed faster at high temperatures, but protein stability also decreases. An elaborate protein homeostasis network maintains enzyme folding and prevents harmful protein aggregation.

Stress responses against ROS or temperature change are characteristically systemic. ROS can damage DNA and possibly dozens of metalloenzymes, while heat can destabilize potentially hundreds of enzymes that catalyze vital cellular functions. Thus, stress responses characteristically involve elaborate proteome shifts such as switching metabolic pathways and upregulating protein homeostasis machinery.

Trade-offs are a hallmark of stress responses. *E. coli* can be laboratory-evolved to survive under extreme oxidative stress but at the cost of lowered fitness under stress-free environments. Such lab evolution experiments, along with genome

re-sequencing and transcriptomic profiling, are crucial for discovering stress response mechanisms. These datasets are interpreted effectively using genome-scale models—reconstructed networks of a cell's metabolic and macromolecule expression reactions.



Christian Münch
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The nature of cellular stress responses

On a cellular level, we often distinguish between responses to benign conditions, including changing hormone or nutrient levels, versus potentially harmful stress conditions, such as starvation, infection, heat, or poisoning. Cells are constantly exposed to a wide range of stress conditions of fluctuating severity. To respond rapidly, stress responses appear to have evolved toward two main features—integration and adaptation. (1) Most stress responses activate upon diverse (primary) stresses and initiate extensive cellular rearrangements. These focus on increasing cellular robustness, emphasizing housekeeping and quality control at the cost of anabolic functions. (2) Considering the gradual nature and continuous occurrence of stresses, cellular stress responses are often better thought of as adaption mechanisms that fine-tune core cellular functions and quality mechanisms rather than simple on-off responses. They activate to varying degrees in order to adjust quality control machineries to the level currently required to maintain cellular homeostasis.

These features increase the necessity for additional studies at the systems level to better understand the role of different stress responses (under a wider range of cellular conditions), how they change cellular function in different contexts, and how stress response output is modulated by additional layers of regulation. Answering these open questions will be crucial to gain a more nuanced understanding of cellular stress responses and bring us closer to the elusive potential for therapeutic intervention.



Andrew Wang and Kavita Israni-Winger
Yale University

A measure of response-“ibility”

Cells have evolved to respond to unpredictable external environments in order to maintain their function (and hence tissue and organismal function). Most environmental characteristics (temperature, oxygen tension, etc.) are continuous variables, and dedicated programs have evolved to enable cells to respond to fluctuations within these variables and return them to ideal set-points. When they are within certain dynamic ranges, the responses engaged are normal, and homeostatic physiology is maintained. Distinct stress responses occur when these environmental variables are outside of homeostatic ranges. We conceptualize this using a U-shaped curve, where the x axis is the variable and the y axis is the response. The curve captures the range of normal responses, whereas stress responses are represented by the arms of the U.

Some environmental variables, like pathogens, predators, or poisons, are binary variables. These are on/off systems, where “on” is the stress state. On the organismal level, the net result of engaging these stress pathways is stress physiology, which enables survival often at the cost of normal function. When environmental conditions persist outside of ideal set-points, both types of responses can be chronically engaged and become maladaptive. Inflammation—cells, signals, and pathways historically associated with infection or injury responses—appears to be a common downstream response to stress. It will be interesting to systematically understand the unique stress responses provoked by specific environmental perturbations and if there are ways of recapitulating their beneficial aspects on host resiliency while minimizing loss of normal function, both in the acute and chronic setting.



Timo Mühlhaus
University of Kaiserslautern

STRESSistance in plants

Stress is a concept that describes a state of a biological system in which it is unable to cope with specific demands or events. In plant research, stress is typically classified according to the kind of stressor, the strength of the effect on the system, and its duration. However, for classification, it might be more helpful to focus on the specifics of the response to stress to gain a deep mechanistic understanding. The term “stress response” summarizes a complex dynamic interplay between genes, proteins, and metabolites that appears to consist of general and specific sub-responses relieving the constraints imposed by the stressor.

Plants have developed an impressive variety of mechanisms for stress resistance or avoidance that can be categorized as acclimation, adaptation, and response to damage. Acclimation responses are reversible and immediate adjustments of the molecular phenotype and physiology regarding enzyme activity, protection, metabolic fluxes, photosynthetic capacity, or transpiration. In contrast, plants can also respond to stressors by long-term adaptations that are inheritable coping mechanisms implemented for future protection. Nevertheless, the effect size of a stressor can exceed the capacity of the respective resistance mechanisms, resulting in damage with specific responses of repair, protection, or even cell death.

Therefore, optimization of plants aiming to increase tolerance to environmental influences requires a deep understanding of specific and generic elements of stress responses.



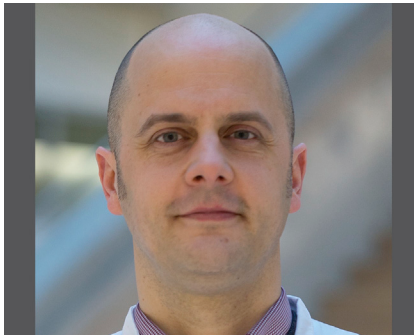
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Massachusetts Institute of Technology

Stress, strain, and response

“Stress” is one of the most poorly defined concepts in plant biology. Plants live in a constantly varying environment in which growth conditions may transition from optimal to harmful and back over timescales ranging from hours to seasons. Most temperate ecosystems and croplands experience periods of soil drying interspersed with rainfall events, extreme temperatures between periods of moderation, and episodic pathogen and herbivory challenges. Each cue must be accurately interpreted by the plant amidst its own circadian rhythms, developmental transitions, and daily balance of carbon assimilation and respiration.

With this perspective, I argue that for sessile organisms there is no clear distinction between “response” and “stress response” with respect to abiotic environmental factors. As one example, plant cells alter their physiology at the earliest signs of reduced water potentials—for many plants, these alterations occur under the midday sun every day of their growing season. As soil water potentials become progressively more negative, cascades of responses from cells to tissues to whole plant follow with the collective aim to prevent *strain* on the system as a whole. A key observation from recent systems-level analysis of plant-environment interaction is the strong integration of environmentally induced signaling cascades with primary metabolism and other “housekeeping” functions of cells; it has become increasingly difficult to delineate stress response as a distinct organismal trait.

A central challenge for plant biology, then, is to study environmental stress from the perspective of the measurable strain exerted on the plant itself and the attendant system-level response while recognizing many “responses” represent daily cycles or resource deficits well within plant tolerances. The onset of strain will vary by genotype, by species, by developmental stage, and by earlier stresses experienced by the plant. Such a nuanced view of response fits well with our growing appreciation of organisms as highly integrated, dynamic systems.



Henrik Oster
University of Lübeck

It is (just) a question of time

Biological timing systems such as the circadian clock depend on the proper integration of multiple external stimuli—so-called *zeitgebers*—to convey reliable temporal information to downstream biological processes. In essence, any *zeitgeber* signal is a temporally confined stressor of the circadian clock, and its precise temporal structure defines how it impacts internal timekeeping and rhythm coordination across tissues and physiological functions.

Independent of any environmental input, the circadian system employs stress effector hormones such as catecholamines and glucocorticoids as internal timing signals to coordinate endogenous clock phase across tissues. External stimulation of stress axis activity at circadian peak times (such as the morning in diurnal species like humans) has little effect on or may even strengthen circadian clock rhythms and rhythm coherence across tissues. Conversely, out-of-phase glucocorticoid upregulation promotes circadian disruption at molecular, tissue, and systemic levels.

Another form of chrono-stress is the chronic disruption of natural environmental rhythms such as the light-dark cycle, experienced by many in our 24-hour globalized society. Internal clocks fail to adapt in a unified way to such conditions, resulting in phase incoherence within and across tissues. It remains a challenge to dissect the impact of single stressors in this context and define at which point—and at which organizational level—chronodisruption translates into pathology.



Merav Socolovsky
University of Massachusetts Medical School

Erythropoietic PID controller

How do homeostatic mechanisms maintain a stable internal environment in the face of constant external perturbations (“stress”)?

We study this question by looking at mammalian erythropoiesis, the process of red blood cell (RBC) production, a key homeostatic mechanism in the maintenance of stable tissue oxygen tension. The most abundant cell in the body, RBCs transport oxygen from lungs to tissues. In a healthy human at sea level, 2 billion RBCs are generated daily. This rate may increase by a staggering 10-fold in response to stresses that threaten oxygen availability, such as blood loss, anemia, lung disease, or high altitude.

Erythropoietic rate is responsive to oxygen levels through a negative feedback loop. Tissue oxygen shortfall induces the hormone erythropoietin (Epo), which binds its receptor on erythroblasts, accelerating erythropoiesis. The increase in RBCs resolves the hypoxia, returning Epo to basal levels. It is not clear, however, whether this single negative feedback loop explains all of the remarkable properties of the erythropoietic stress response, including its rapidity (within hours), precision in matching demand over a wide stress range, absence of overshoot or oscillations, and robustness to internal random noise. How is this achieved?

Newer data suggest that the erythroblast compartment carries out the computational task of a Proportional-Integral-Derivative (PID) controller of the type used in a car’s cruise control. Some erythroblast survival pathways are activated by the *rate of change* (derivative, D) of Epo levels, accelerating response to acute stress; other pathways are activated *proportionally* (P) to Epo levels, responding during chronic stress. Erythroblast cycling increases erythropoietic rate in response to the cumulative recent history, or *integral* (I), of Epo. Negative autoregulation between erythroblasts within their niche suppresses noise fluctuations.

Together, these features comprise a powerful control system that is both robust and exceptionally responsive to real-time demands. Important open questions include the roles of newly discovered erythroid regulators and whether different types of hypoxic stress, for example, malaria or lung disease, elicit distinct responses.