stabilizes chromatin topology around DSBs.

High-resolution microscopy and chromosome conformation capture techniques unveiled the 3D-organization of the genome and its regulatory function in crucial processes such as transcription [10] or replication [7]. The study by Ochs *et al.* [4] highlights a novel interplay between chromatin architecture and DNA damage response factors, which shape chromatin topology around DSBs, thus providing a new perspective on repair foci. Future studies, using imaging and orthogonal approaches, may provide further mechanistic insights into the formation and the resolution of these structures, their functional relevance for the regulation of DSB responses, and their possible long-term and long-range impact on chromatin architecture.

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Spotlight

Histone Lactylation: A New Role for Glucose Metabolism

Maria V. Liberti[1,2] and Jason W. Locasale[3,*]

Lactate is an end product of glucose metabolism, which serves metabolic and nonmetabolic functions. A new study by Zhang *et al.* establishes a novel function for lactate whereby it is utilized in a new histone modification, histone lysine lactylation, to regulate gene expression in macrophages.

Cellular metabolism consists of the uptake and utilization of diverse nutrients in the environment to sustain cellular energy demands and to promote growth and survival. Cells metabolize these nutrients using a network of biochemical reactions that are organized into metabolic pathways for catabolic or anabolic processes, which also confer signaling functionality, such as the regulation of post-translational modification of histone proteins.

Glucose, the major carbohydrate, is metabolized into pyruvate, which is canonically oxidized to acetyl-CoA for further metabolism in the citric acid cycle for energy production in the form of ATP. However, glucose can be incompletely oxidized to produce the metabolite lactate, even in the presence of oxygen, through a phenomenon known as the Warburg effect. The Warburg effect has been extensively studied, with numerous proposals for its function, including increased rates of ATP synthesis, macromolecule synthesis, restructuring of the tumor microenvironment due to lower pH in the environment from lactate, and cell signaling [1]. While the Warburg effect and its possible functions provide an advantage to cells for growth and survival, there is still uncertainty regarding its precise contribution to cellular function. Zhang *et al.* now establish a new signaling role for lactate, the end product of the Warburg effect, to confer specific gene expression signatures in M1 macrophages through its provision of substrate for a previously unknown histone modification, now termed lysine lactylation [2] (Figure 1).

Zhang *et al.* first showed that lysine lactylation is derived directly from either exogenous lactate, by conducting ^{13}C-lactate tracing experiments, or endogenous lactate, by conducting ^{13}C-glucose tracing using mass spectrometry. Importantly, ^{13}C-glucose tracing experiments and immunoblotting also confirmed that lysine lactylation exhibits different kinetics from lysine acetylation, indicating differential regulation by glucose metabolism. They then questioned whether lysine lactylation is regulated by altering the dynamics of glucose metabolism and thus lactate levels. They found that glycolysis inhibitors

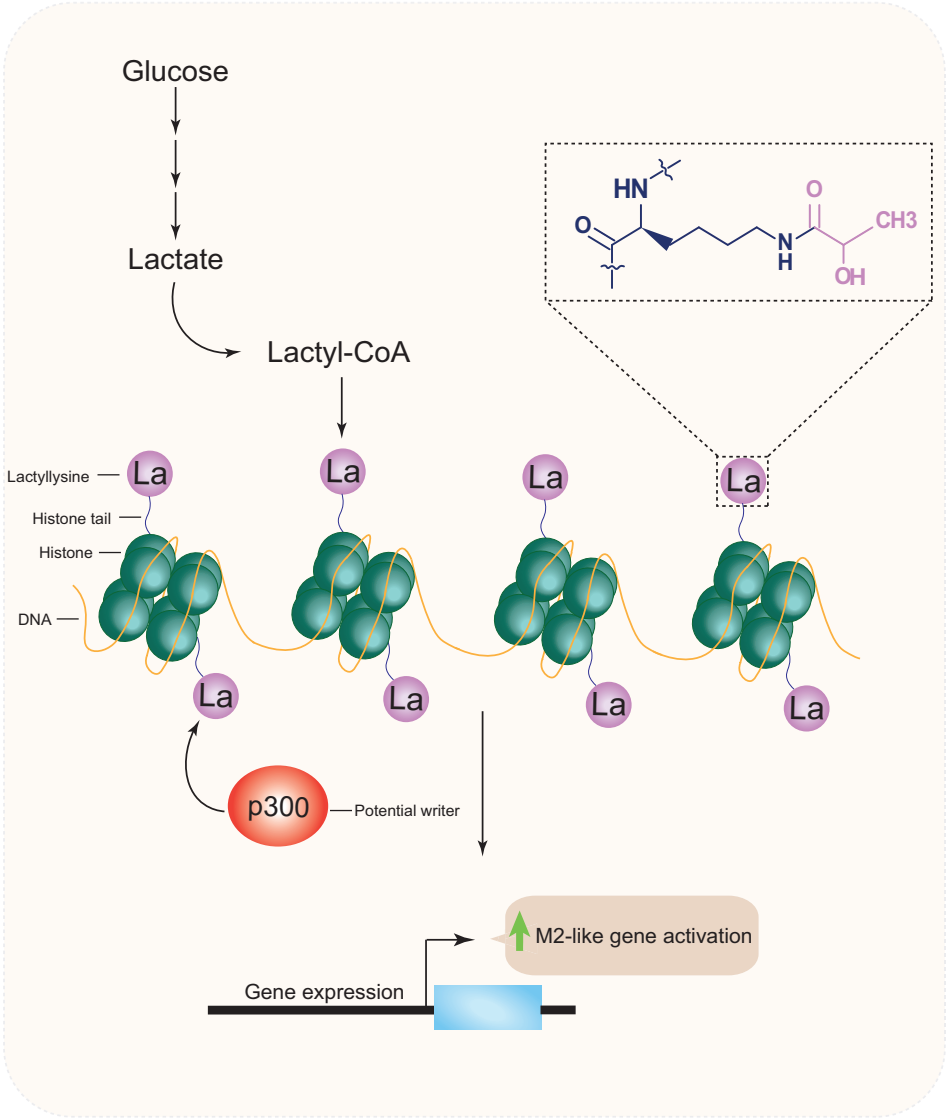

Figure 1. Histone Lactylation Mediates Gene Expression to Promote an M2-like Phenotype.
In M1 macrophages or under hypoxia, glucose is incompletely oxidized to produce the metabolite lactate. Lactate is then able to generate lactyl-CoA, which contributes a lactyl group to the lysine tails of histone proteins via the acetyltransferase enzyme p300 to produce a modification called lactyllysine. This post-translational modification allows for gene activation of genes belonging to wound-healing pathways, thus resulting in an M2-like phenotype.

that deplete lactate production decreased lysine lactylation and mitochondrial inhibitors or hypoxia that increase lactate production increased lysine lactylation. ^{13}C-glucose tracing upon glycolysis inhibition further confirmed that only glucose-derived carbon incorporation into lysine lactylation, but not into lysine acetylation, was decreased. To assert that the dynamics were directly influenced by lactate, the authors genetically deleted lactate dehydrogenase, which catalyzes the conversion of pyruvate to lactate, and found that lysine lactylation was fully abrogated. These findings establish a new histone modification that is specifically modulated by lactate.

Distinct histone modifications are important for regulating transcriptional processes of target genes in cellular physiology [3]. Further, multiple reports have highlighted the link between

metabolic regulation and histone modifications. For example, a number of histone lysine acylations have been characterized to have important metabolic functions [4,5]. Importantly, some lysine acylation modifications respond to changes in glycolysis rate, including histone butyrylation, histone propionylation, and histone 2-hydroxyisobutyrylation [6]. Another well-appreciated example is the role of glucose metabolism in regulating histone acetylation. A recent report has demonstrated that tuning the rate of glycolysis effectively decreases histone acetylation, specifically through modulation of the metabolic product acetyl-CoA, a substrate for acetyltransferases [6]. Others have shown that histone acetylation can be controlled by modulating enzymes that regulate acetyl-CoA synthesis, such as ATP citrate lyase and acetyl-CoA synthetase 2 [7,8]. Thus, the discovery of histone lactylation provides new knowledge to the role of glucose metabolism in regulating histone modifications independent of acetyl-CoA.

Zhang *et al.* next sought to establish whether lactate-derived lysine lactylation conferred a physiological relevance. They first identified that induction of intracellular lactate by hypoxia increases histone lactylation, but not histone acetylation levels. The authors next turned to M1 macrophage polarization as their model. Classically, M1 macrophage polarization, compared with M2 macrophage polarization, is associated with activation of inflammatory genes as well as increased glycolysis and production of lactate. Upon stimulation of bone marrow-derived macrophages with lipopolysaccharide and interferon-γ for M1 macrophage polarization, Zhang *et al.* observed increases in histone lactylation in a time-dependent manner. These increases were specific to M1 macrophages, and not M2 macrophages, highlighting the importance of active glycolysis in modulating histone lactylation.

Of considerable importance, the authors next determined whether the mark serves a functional purpose in downstream transcriptional processes. Using RNA-sequencing on M1 macrophages, they identified genes specifically enhanced by lysine lactylation. Notable enrichment was found in genes belonging to wound healing pathways, particularly homeostatic genes, which are involved in maintaining a biological steady state and associated with the M2-like phenotype. Corroborating these findings, previous studies have found that lactate is involved in driving an M2-like polarization of tumor-associated macrophages through expression of M2 genes such as arginase 1 (*Arg1)* [9]. Of note, Zhang *et al.* observed enriched lysine lactylation at the *Arg1* promoter as well as increased gene expression when M1 macrophages were treated with exogenous lactate. Similarly, deletion of lactate dehydrogenase A in M1 macrophages decreased histone lactylation and *Arg1* expression. Finally, a cell-free recombinant chromatin-templated histone modification and transcription assay revealed that histone lactylation can directly activate gene transcription in a p53-dependent p300-mediated manner. Altogether, the authors propose that histone lactylation directly stimulates gene expression to promote M2-like characteristics in the late phase of M1 macrophage polarization to ultimately achieve a homeostatic response.

The study by Zhang *et al.* introduces the intriguing possibility of a new function of the Warburg effect, which is to provide lactate as a substrate for the generation of lactyl-CoA for lysine lactylation on histones to promote gene expression. The findings from this study raise interesting questions for future efforts. Uncovering the biochemistry involved in the transfer of lactyl moieties to histones through p300 or other writer enzymes, as well as the removal of lactyl groups in cellular physiology, is an appealing possibility. Similarly, determining the reader proteins of lysine lactylation to result in the observed transcriptional signatures remains to be elucidated. Another compelling finding is the kinetic differences from glucose carbon contribution between lysine lactylation and lysine acetylation, which likely has functional consequences. In addition, future work aimed at determining the enzymes involved in generating lactyl-CoA in physiology will provide further insights on tuning mechanisms of histone lysine lactylation. One could speculate that by precisely controlling histone lactylation through modulation of enzymes directly involved in its biochemical process, downstream regulatory processes of homeostatic responses could be modulated in related disease contexts such as immune diseases and cancer, especially given the physiological implications of lactate in the environment [9,10]. Together, the discovery of histone lysine lactylation by Zhang *et al.* brings forth new biology and functionality to the role of lactate in regulating histone dynamics to confer downstream gene expression signatures.

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Forum

Do All Roads Lead to Rome in G-Protein Activation?

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High-resolution structural studies on G-protein-coupled receptors (GPCRs) have flourished recently, providing long-sought insights into the dynamic process of guanine nucleotide-binding protein (G-protein) activation. In parallel, analogous studies are starting to shed light on how the same G-proteins are activated by non-GPCR proteins. Can we learn about common themes and variations in G-protein activation from them?

Heterotrimeric G-proteins were identified in the late 1970s as transducers of signals emanating from GPCRs, but the structural basis for such coupling and activation has unraveled only within the last decade. The GPCR–G-protein signaling axis not only exemplifies evolutionary success that is conserved across eukaryotes, but also a paradigm of paramount biomedical importance representing the single largest class of targets in the 'druggable' human genome. The crux of this quintessential signaling mechanism is in how G-proteins become activated by GPCRs. GPCRs are guanine nucleotide exchange factors (GEFs) that loosen the grasp of G-proteins on GDP to permit subsequent binding of GTP to adopt an active state. Because GPCRs bind to G-proteins at a site that is far removed from the nucleotide-binding pocket, it has been long known that activation must be achieved through allosteric mechanisms, details of which have only begun to emerge in the recent years. Within the past decade, high-resolution structures have been leveraged and complemented by a variety of biophysical and computational approaches to collectively grasp the dynamic nature of the G-protein activation process [1–5]. In parallel, and also within the past decade, another evolutionarily conserved mechanism of G-protein activation has emerged; it too has important cellular functions and biomedical relevance [6,7]. Within this alternative paradigm, activation is triggered by cytoplasmic proteins with GEF activity analogous to that of GPCRs. Despite their relatively recent discovery, understanding the structural basis of how they bind/activate G-proteins has rapidly reached an advanced stage [8–10]. In this forum article, we summarize what has been independently learned about G-protein activation by GPCRs and by non-GPCRs; we highlight how the two classes of activators converge on a similar allosteric mechanism despite their divergent modes of physical engagement with G-proteins.

Activation of G-Protein by GPCR and Non-GPCR GEFs

From the first atomic resolution structure of a GPCR–G-protein complex (β2 adrenergic receptor with Gs) reported in 2011 [1] and other holocomplex structures reported later (e.g., [11]), the most striking conformational rearrangement observed is a large separation of the Ras-like and helical domains of Gα. Such interdomain separation does not seem to be the principal trigger for nucleotide release, but rather, a sequel to allosteric mechanisms that trigger the release [4]; it facilitates the escape of the released nucleotide by providing an escape route. Two main allosteric routes communicating GPCR contact sites with the nucleotide-binding pocket have been hypothesized (Figure 1A), both supported by computational, biophysical, and biochemical evidence in addition to high-resolution structures [1–5]. Both routes involve changes in structural elements of the core of the Ras-like domain and share a common stretch that converges onto the phosphate-binding loop (P-loop) and adjacent nucleotide-binding elements. The interaction of the P-loop with the β-phosphate of GDP contributes greatly to the overall nucleotide-binding energy; its disruption greatly favors nucleotide release.

The two proposed allosteric routes are initiated at opposite ends of the G-protein sequence that make direct contact with the receptor: the C-terminal region of the α5 helix and the αN/β1 loop [1–3,5]. For the first route, the N terminus