MEDICINE

Next-generation epigenetic inhibitors

Selective bromodomain inhibitors provide new opportunities to modulate transcription

By **Panagis Filippakopoulos**[1] *and* **Stefan Knapp**[2]

Ten years ago, the discovery of potent inhibitors targeting the bromodomains of the BET (bromo- and extraterminal) family caused considerable excitement (*1*, *2*). The surprising efficacy of these pan-BET inhibitors in mouse models of various diseases, including cancer, led to rapid translation into the clinic. However, pleiotropic effects of pan-BET inhibitors have limited clinical applications to oncology. Moreover, their equipotent activity toward all BET bromodomains complicated mechanistic studies aiming to delineate the functions of the first (BD1) and second (BD2) bromodomains present in each of the four human BET proteins. On page 387 of this issue, Gilan *et al*. (*3*) developed BD1- and BD2-selective inhibitors with unprecedented selectivity. These will enable future research on the role of BD1 and BD2 in transcriptional control and the development of more specific BET-targeting therapies.

Histones contain a large number of posttranslational modifications that constitute the epigenetic code, a complex language that is interpreted by a large diversity of protein interaction domains that "read" these modifications. Acetylation of lysines is a frequent modification in histones that is recognized (read) by BET proteins. These comprise a family of bromodomains consisting of four members [bromodomain-containing 2 (BRD2), BRD3, BRD4, and bromodomain testis–specific protein (BRDT)]. Each BET protein harbors two highly homologous bromodomains (BD1 and BD2). The roles of BET proteins in transcription were initially thought to be limited to the recruitment of P-TEFb (positive transcription elongation factor-b), a factor required for transcriptional elongation, by BRD4 and BRDT. However, pan-BET inhibitors (*1*, *2*) revealed specific effects on gene expression that often affected lineage-specific genes. This unexpected finding was rationalized by the presence of BET proteins bound to acetylated chromatin at cell type–specific enhancers and superenhancers, which are transcriptional regulatory regions that increase gene expression (*4*). The specific down-regulation of expression of cell type–specific regulatory genes by pan-BET inhibitors led to numerous potential applications in seemingly unrelated diseases. In oncology, the strong down-regulation of expression of the *MYC* proto-oncogene provided a key biomarker for pan-BET inhibitors and made a compelling case for targeting BET proteins in MYC-driven cancers (*5*).

The excitement of this discovery resulted in countless preclinical studies and the initiation of 39 clinical trials. However, the general role of BET proteins in regulating tissue-specific gene expression resulted in pleiotropic effects in vivo and toxicity in some clinical studies, albeit with encouraging but often short-lived efficacy (*6*). Moreover, the rapid translation of these targets into the clinic, and their poorly understood mechanism of action, raised concerns that "clinical trials run ahead of science" (*7*), making a case for more basic research. An important tool for such studies would be inhibitors with improved selectivity for one of the four BET family members or for BD1 or BD2.

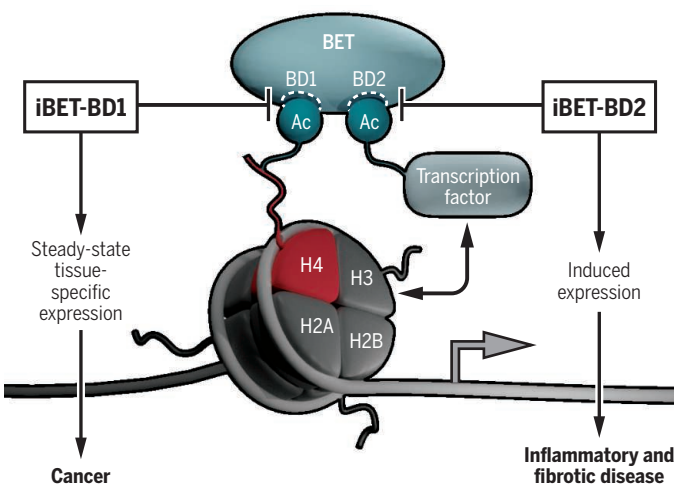
Owing to sequence conservation within the acetyl-lysine binding site of BD1 and BD2, the development of protein- or domain-specific inhibitors represents a considerable challenge (*8*). Early indications that domain selectivity might be achievable came from relatively weak BET inhibitors such as RVX-208 that exhibits ~8- to 20-fold selectivity for BD2 over BD1 (*9*). This inhibitor interacted with a histidine located at the rim of the acetyl-lysine–binding site, which is conserved in all BD2s, but not in BD1s (which harbor an aspartate at this position). This residue variation has been exploited in the development of the BD2-selective inhibitor ABBV-744 (*10*).

In contrast to pan-BET inhibitors, ABBV-744 showed a narrow antiproliferative activity restricted to hormone-dependent breast and prostate cancers and some types of leukemia. Similar to the effect of RVX-208, BD2 inhibition by ABBV-744 yielded limited transcriptional changes. In prostate cancer, ABBV-744 displaced BRD4 from androgen receptor–containing superenhancers, whereas P-TEFb recruitment to promoters was not affected, demonstrating a more restricted mode of action. ABBV-744 has now entered clinical testing in patients with relapsed and refractory acute myeloid leukemia (NCT03360006).

The study by Gilan *et al*. expands the chemical toolbox for examining BD1 and BD2 selectivity. The authors report the development of GSK046 (iBET-BD2), a potent BD2-selective inhibitor with >1000-fold selectivity over BD1. They also report the development of GSK620, an orally bioavailable BD2-selective inhibitor, and GSK778 (iBET-BD1), a BD1-selective inhibitor (see the figure). The authors found that in mouse models of various cancers, BD1 inhibition is reminiscent of pan-BET inhibition. This was explained by displacement of BET proteins from promoter and enhancer regions that control *MYC* expression, suggesting that BD1 anchors BET proteins to acety-

Diverse roles of bromodomains in transcription

BET (bromo- and extraterminal) proteins contain two bromodomains, BD1 and BD2, that bind acetylated (Ac) lysine on histone H4. Selective inhibitors of BD1 (iBET-BD1) and BD2 (iBET-BD2) delineate the role of BET proteins in modulating gene expression and identify bromodomain-specific areas for drug development.



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lated lysine residues in histones through its affinity to diacetylated histone H4 (*11*).

By contrast, BD2 inhibition did not yield strong antiproliferative effects in cancer cell lines that are sensitive to BD1 inhibitors and did not displace BET proteins from chromatin. Thus, BD2 mediates interactions with nonhistone proteins, such as transcription factors. In support of this idea, BD2 inhibition altered gene expression signatures triggered by extracellular stimuli such as interferon-γ and phorbol-myristate, which activate specific transcription factors. The requirement of BD2 for induced gene expression was also evident in stimulated primary $CD4^+$ T cells, in which strong suppression of proinflammatory cytokine expression suggested applications of BD2-specific inhibitors in inflammatory disease. Indeed, selective BD2 inhibition showed efficacy in mouse models of arthritis and psoriasis, which are characterized by pathogenic inflammation. In addition, encouraging activity was also observed in mouse models of nonalcoholic fatty liver disease, in which GSK620 reduced deposition of fat in the liver (steatosis) and scarring of liver tissue (fibrosis).

Taken together, the development of BD1- and BD2-selective inhibitors will help to delineate the functions of these conserved proteins. The role of BD2 in induced transcription programs predestines BD2-selective inhibitors for treatment of inflammatory disease and fibrosis, potentially bypassing the rewiring of BET-protein interactions observed with pan-BET inhibitors (*12*). The effect of BD2 inhibition on hematopoiesis, a differentiation program that is also regulated by a myriad of transcription factors, remains to be investigated. In clinical studies, side effects of pan-BET inhibitors have been associated with defects in blood cell differentiation such as low platelet counts causing abnormal blood clotting. However, this new generation of domain-selective inhibitors will provide exciting research tools for studying transcriptional regulation by epigenetic readers. ■

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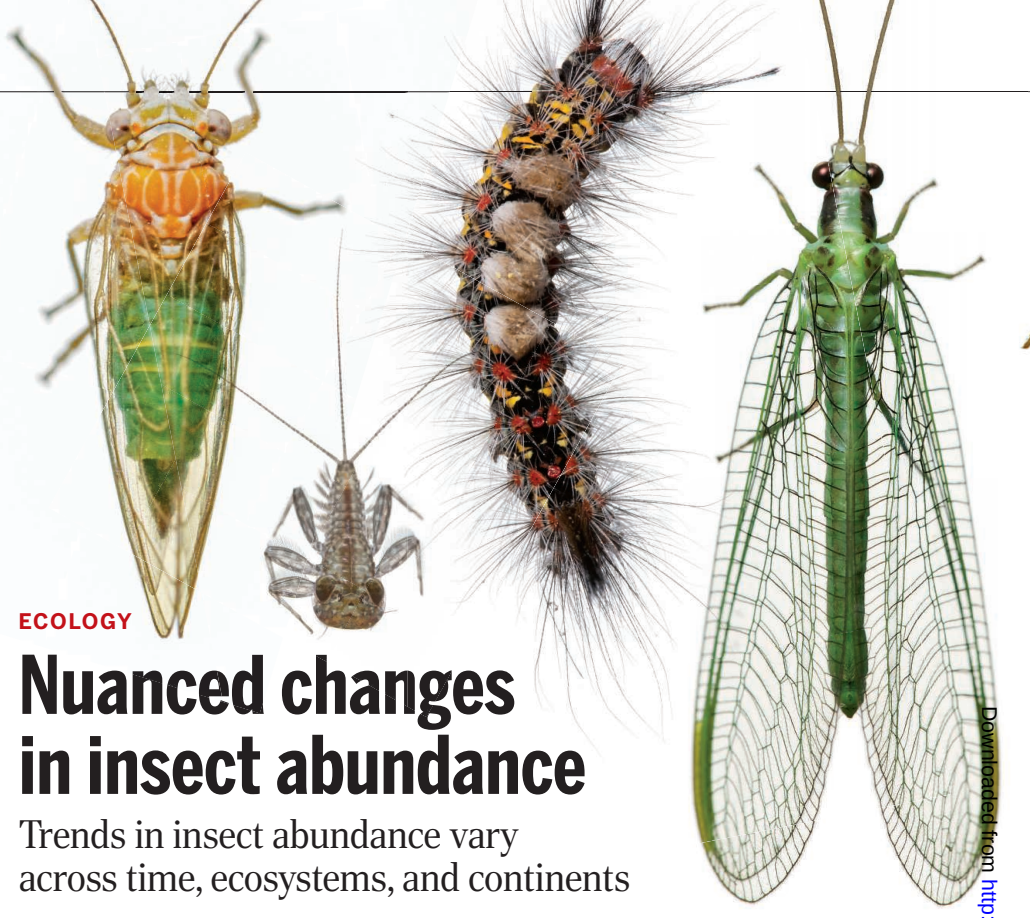
ACKNOWLEDGMENTS

The authors are supported by the Structure Genomics Consortium. S.K. acknowledges support by the German Cancer network DKTK and the Frankfurt Cancer Centre (FCI).

10.1126/science.abb5060



ECOLOGY

Nuanced changes in insect abundance

Trends in insect abundance vary across time, ecosystems, and continents

By **Maria Dornelas**[1] *and* **Gergana N. Daskalova**[2]

Drastic declines in insect biomass, abundance, and diversity reported in the literature have raised concerns among scientists and the public (*1*–*3*). If extrapolated across Earth, biomass losses of ~25% per decade (*1*) project a potential catastrophe developing unnoticed under our noses. The phrase "insect Armageddon" has captured the collective attention and shined a spotlight on one of the most numerous and diverse groups of organisms on the planet. Yet, insects are critically understudied. For example, the BioTIME database (*4*)—a compilation of biodiversity time series—contains records for 22% of known bird species but only 3% of arthropods (the phylum that includes insects and spiders). On page 417 of this issue, van Klink *et al.* conduct a thorough global assessment of insect abundance and biomass trends and paint a more nuanced picture than that predicted by extrapolations (*5*).

Given the critical environmental functions of insects, the consequences of their declines could propagate across ecosystems and affect the services they provide (for example, pollination of crops such as almonds, apples, and cherries). The prospect of widespread insect decline has prompted calls for rigorous scientific study and monitoring (*6*–*8*). The drivers of biodiversity changes are almost never simple, and their discovery requires context. Thus, simple extrapolation from a handful of locations is unlikely to reveal the layers of complexity that underpin real-world biodiversity change (*6*, *9*). To unpick insect-decline events, scientists must decipher whether site- and region-specific declines are representative of the state of insects around the world. This requires a systematic assessment of insect-abundance trends.

In what is the largest and most complete meta-analysis to date, van Klink *et al.* revealed substantial variation—surges and declines—in abundance and biomass trends. Similar to what is found across other taxa (*10*), the meta-analysis in the new study detected no net directional trend among 166 studies of 1676 geographical sites in 41 countries. Yet, van Klink *et al.* found that terrestrial insects declined in abundance by 9% per decade on average, whereas freshwater insects increased by 15%. The authors also noted variation across continents, with North America and some European regions emerging as hotspots of decline in insect abundance.

The findings of heterogeneity in insect abundance and biomass trends over time reinforce the need to consider spatial variation in biodiversity change (*11*). Other

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Science **368** (6489), 367-368.
DOI: 10.1126/science.abb5060