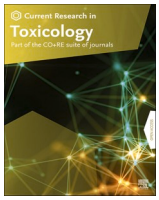
Current Research in Toxicology 7 (2024) 100191

Contents lists available at ScienceDirect

Current Research in Toxicology

journal homepage: www.journals.elsevier.com/current-research-in-toxicology



Research Paper

Crowdsourcing AOP development: Leveraging the thesis literature review to identify knowledge gaps and facilitate research translation

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ARTICLE INFO

Keywords:
Adverse outcome pathways (AOP)
New approach methodologies (NAMs)
Research translation
Regulatory toxicology
Graduate education

ABSTRACT

Chemical risk assessment still primarily relies on extrapolation of data from high-confidence *in vivo* studies. Emerging 21st Century Toxicology tools and approaches have potential to figure more prominently in chemical risk assessment, but many challenges in translating this research into assessments remain. One of these tools, the Adverse Outcome Pathway (AOP) Wiki provides a framework to map and evaluate adverse chemical dynamics, that is the biochemical and physiological effects that occur after chemical exposure. The AOP-guided targeted review of relevant literature, described here, shares similarities with a doctoral thesis or literature review but forces critical evaluation of each step in a pathway including those of central dogma. Additionally, it provides valuable translational regulatory relevance. Data gaps identified through this process can be targeted areas of study in the thesis itself to increase translational relevance. One of the challenges with this tool is that many AOPs are under- or undeveloped. To help fill this need, a concerted effort by subject matter experts to speed the development of AOPs supported under the Organization for Economic Cooperation and Development (OECD) framework would benefit this translational problem. As a case study, we present our experience developing AOP 460: Antagonism of Smoothened receptor leading to orofacial clefting (OECD AOP workplan project 1.101) as part of a graduate literature review. AOP development offers clear benefits to the regulatory and academic communities and increased dissemination of AOPs replete with the most current state of scientific knowledge will promote research translation and increased risk assessment capabilities.

1. Introduction

In the field of toxicology, an Adverse Outcome Pathway (AOP) is an analytical concept that describes how exposure to a stressor (e.g. environmental toxicant) can lead to an adverse outcome (AO) (e.g. orofacial cleft) through a series of causally linked events organized at different biological levels (Ankley, 2010). AOPs are an essential component of a toxicological knowledge framework being built to support hazard assessment of a wide variety of chemicals based on mechanistic reasoning of current scientific knowledge (Maxwell, 2014; Aleksic, 2024). AOP development creates a living repository of expert-curated knowledge that is easily accessible to a spectrum of researchers from the curious public to world-renowned experts in their field as well as nonacademic stakeholders including industry (Carusi, 2018). Like other data commons, the responsibility to create and update AOPs falls on a willing few and not necessarily those with the time, breadth of expertise, and resources to properly perform this increasingly critical task. The Organization for Economic Cooperation and Development's (OECD) AOP development program, launched in 2012, has been providing guidance and tools for the scientists and academic community to enable and assist the creation of AOPs (OECD. Adverse Outcome Pathways, 2024).

Organizing the state-of-the-art knowledge into an AOP identifies a clear list of data gaps and inconsistencies that can help guide future investigations. The question of who is to create these AOPs and AOP networks with reliable data and information is not always clear as authors may be voluntarily developing AOPs without any additional incentives. This would often lead to delayed completion of the AOPs or even incomplete AOPs due to lack of time and/or resources from the authors causing the majority of AOPs in the wiki to be incomplete. However, recently, the OECD has partnered with several journals under a memorandum of understanding (MOU) to review and publish AOPs

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https://doi.org/10.1016/j.crtox.2024.100191
Received 20 May 2024; Received in revised form 26 July 2024; Accepted 6 August 2024
Available online 8 August 2024

providing a clear route to publication, thus providing some benefits for authors (especially graduate students) to work towards the completion of the AOPs (Knapen, 2021).

As a case study, we present our experience with development of an AOP network linking disruption of the Sonic Hedgehog (SHH) signaling pathway with the birth defect, orofacial clefting (OFC). In particular, the development of the first AOP in the network, AOP 460, was included as part of a doctoral student's research project as the literature review (Reynolds, 2024). We showcase how this development not only fills the role of a traditional literature review but also adds additional training and research translation as part of the graduate program. In parallel with our AOP development, we are also refining our microphysiological model (MPM) of orofacial development to facilitate increased screening and experimental setups to address identified data gaps (Reynolds et al., 2022; Johnson, 2021). As calls for the reduction of traditional animal testing continues, the development of robust New Approach Methodologies (NAMs) to refine toxicity testing is paramount. To accomplish this, there needs to be dissemination of AOPs replete with the most current state of scientific knowledge to enable better understanding of the regulatory needs and define both the physiology of interest and the endpoints of interest to engineer the assay for. We hope that the developmental and reproductive toxicity (DART) community can employ similar strategies as we continue to work towards assays that will allow for the detection of DART without the need for animal testing.

2. Discussion

The main objective of this paper is to highlight the need for well-developed AOPs and to propose that traditional thesis literature reviews are a unique avenue for students to publish their review, learn to apply systematic evaluation criteria to published data, gain translational knowledge of regulatory needs, and identify key research gaps they can address. We highlight the development of AOP 460 and showcase how it has filled the role of graduate thesis literature review while providing additional training and research translation. The components of an AOP are only described briefly here and are not the focus of this publication; readers are advised to go to the OECD's AOP Development Program to learn more about the individual elements of an AOP (OECD. Adverse Outcome Pathways, 2024).

With the number of AOPs being developed and/or completed (i. e. reviewed and endorsed by OECD) steadily increasing over time, regulators are relying more on scientifically sound AOPs to facilitate more informed conclusions on the hazard assessment of chemicals. One toxicological area in which regulators frequently use AOPs is endocrine disruption (ED) (Regulation (EC), 2009; Regulation (EU), 2012). For example, even though there are extensive data requirements to be fulfilled in the approval process of active substances to be used as pesticides (Niemann, 2023), there can still be data gaps regarding potential endocrine modes of action, which might lead to the need for additional animal testing. However, with the publications of well-developed AOPs on endocrine-related mechanisms (Ankley, 2023; Zilliacus, 2024), it is becoming more feasible for regulators to identify ED potential of chemicals without additional *in vivo* data (Svingen, 2022). Even with this steady increase in development, many AOPs remain under or undeveloped. Subject matter experts including the academic community are needed to continue the push to develop the AOP knowledge base.

The AOP development process provides a clear framework that can be used by authors to structure scientific data and perform a literature review similar to that performed in a graduate thesis or dissertation. Fig. 1 details some of the benefits of AOP development and shows the large overlap that it has with a traditional literature review commonly performed in a graduate student's research project. Important aspects of the graduate literature review including identifying data gaps and areas for further investigation, recognition of the main techniques used, improvement of vocabulary and critical writing, and organization of the current state of art, are clear overlaps between AOP development and a

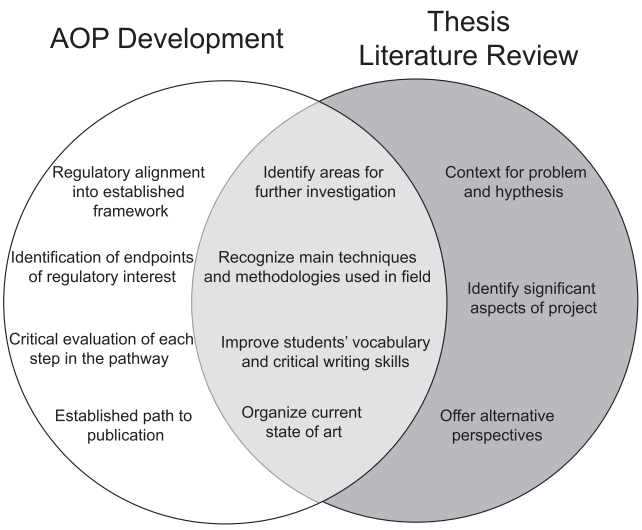

Fig. 1. Venn diagram of AOP development (white), the traditional thesis literature review (dark gray) and the overlap between the two (light gray).

traditional graduate literature review (Hart, 1998; Leite et al., 2019). The graduate literature review sets the context of the problem and hypothesis that frames the graduate thesis. Through the review the student is introduced to the significant aspects of their project and the field of their work while also being introduced to alternative perspectives that can help shape future directions. The development of an AOP allows for the critical evaluation of the pathway at each step. One key feature that AOP development teaches that is not often found in a traditional literature review is the critical weight-of-evidence (WoE) evaluation using Bradford-Hill criteria to evaluate the causality of each set of KEs in a pathway (Hill, 1965). This allows for a deep understanding of the pathway and what is known and not known about it. Data gaps and inconsistencies are identified as part of this evaluation and can direction for further experiments. AOP development aligns the review into an established regulatory framework allowing the developer to gain a better understanding of the endpoint(s) of regulatory interest. The established path to publication is another difference from the traditional review for while the traditional review can be published there is usually not a clear or streamlined route to do so. While there are clear differences between the traditional review and AOP development, many similarities exist. They both allow the student to organize the state of the art and gain a sense of understanding of their field and project. This promotes the identification of areas in need for further investigation as well as the ability to recognize the techniques and methodologies employed in the field. Organizing and writing either a traditional review or AOP will help the student improve their vocabulary and technical writing skills.

As for our AOP network project on orofacial clefting, the goal of predicting developmental toxicants without *in vivo* testing is extremely ambitious. At the moment, apical developmental effects, such as orofacial clefting, are evaluated and investigated in validated test guidelines (TG) such as OECD TG No. 414 (Prenatal Developmental Toxicity Study), No. 416 (Two-Generation Reproduction Toxicity), or No. 443 (Extended One-Generation Reproductive Toxicity Study); mechanistic data (e.g. upstream events) leading to adverse developmental effects are rarely available for regulatory purposes (OECD, Test No. 414: Prenatal Developmental Toxicity Study, 2018; OECD, Test No. 443: Extended One-Generation Reproductive Toxicity Study, 2018; OECD, Test No. 416: Two-Generation Reproduction Toxicity, 2001). Our AOP network could provide some scientific understanding of the mechanisms leading to orofacial clefting and demonstrate key events in the AOPs where NAMs could be developed such that developmental toxicants could be identified before conducting an *in vivo* reproductive/developmental

toxicity study.

To those looking to begin the development of an AOP, we highly recommend perusing the OECD's AOP development program, which is overseen by the OECD Advisory Group on Emerging Science in Chemicals Assessment (ESCA) (OECD. Adverse Outcome Pathways, 2024). The program offers, among others, a Guidance Document on Developing and Assessing Adverse Outcome Pathways as well as a Developers' Handbook for using the AOP-Wiki, a central repository for AOPs (OECD, 2013; Daniel Villeneuve et al., 2023; AOPs, 2024). Through this program, we submitted a proposal for the AOP network on SHH signaling pathway as mentioned above. The proposal consisted of a brief overview of the proposed work including a project description, flow diagram, regulatory relevance, and a project planning section. The proposal was reviewed and discussed by the ESCA for scientific merit and regulatory relevance and was subsequently accepted into the OECD AOP workplan (Project 1.101) (OECD. The Adverse Outcome Pathways development programme workplan, 2024). Acceptance of the proposal into the OECD AOP Workplan is an attractive option for the AOP authors because it not only provides the authors with support from the OECD to facilitate the AOP development process but it can also increase the visibility and potential regulatory use of their AOPs. In particular, developed AOPs in the OECD AOP Workplan undergo scientific review by experts in accordance with the Guidance Document for the scientific review of AOPs, and those AOPs accepted after the scientific review can be considered for endorsement by the OECD (OECD, No. 344: Guidance Document for the scientific review of Adverse Outcome Pathways, in Series on Testing and Assessment, 2021). OECD-endorsed AOPs are officially published in the OECD Series on Adverse Outcome Pathways, which risk assessors and regulators consider a as reliable source of scientific information for regulatory assessments and decision-making (OECD, OECD Series on Adverse Outcome Pathways, 2024).

We found communication of the initial acceptance with the OECD coordinators to be prompt and within a month of submitting the proposal, we were included in the AOP workplan, and a coach had been assigned to aid in development. The role of the coach is to help with ensuring development conforms to OECD guidance document and to act as a resource as questions about development occur. It is important to understand that the role of the role of the coach is to advise on compliance with the AOP Guidance Document, not to assist with the scientific development. Our experience with having a coach has been extremely positive. It was very helpful to have someone experienced with AOP development provide regular feedback on our progress, answer questions, and assist in planning an effective AOP development.

The overall time spent on development of an AOP will vary with the complexity of the pathway and the state of the events already in the AOP-Wiki. From our experience, the development took longer than a traditional literature review would have due to time spent learning the AOP development framework and getting the data and writeup organized for the Wiki. Since our proposal is focused on a network of AOPs, we first had to select an appropriate starting point to narrow the initial scope. With feedback from the OECD and our coach, we decided to select a well-known molecular initiating event (MIE) for the pathway, antagonism of the Smoothened (SMO) receptor (Reynolds, 2023). To select the key events for AOP 460, we compiled existing knowledge of the pathway through a systematic literature review of the Sonic Hedgehog (SHH) pathway to assemble a path that was physiologically plausible. Care was taken to select events that would be of direct regulatory relevance (e.g. a quantitative method exists). After the pathway and relationships were mapped out, we then searched the Wiki to determine which KEs would need to be added or created. Creating the KEs did take a strong effort to become proficient in the AOP development framework, gather the needed information for each event, and write and transfer it into the Wiki.

We employed a systematic search of PubMed using MeSH terms associated with either of the events in the KER. Initial results were screened for relevance by title/abstract and any of suspected relevance were reviewed in full to determine their applicability for the KER. After this was all organized and evaluated, we went through the data to make our evidence calls for each KER and the AOP overall. The WoE of the data was assessed using Bradford-Hill criteria and the AOP development handbook (Daniel Villeneuve et al., 2023). In particular, the strength and reliability of the AOPs are assessed for several criteria including biological plausibility, essentiality of the key events, empirical support, and the quantitative understanding of the pathway. We found the process of digging through and critically evaluating the evidence useful for gaining a better understanding of the pathway and the state of the field with regards to its understanding. This process also identifies data gaps, inconsistencies, and uncertainties that can be used to drive further investigation. For AOP 460, the largest data gap found was a lack of dose–response or time-course data. We hope to address these gaps using our microphysiological model. For example, one of the large data gaps identified is in proliferation. We plan to use our MPM to generate dose–response data for known prototypical stressors of SMO and assess proliferation in both the epithelial and mesenchyme using fluorescent staining for markers such as Ki-67.

Our experience with AOP development has been largely positive, but we did run into some obstacles in our development process that should be noted. One aspect we struggled with is the presence of duplicate events in the AOP-Wiki for common events (e.g. cellular proliferation, apoptosis). It was challenging to know which KE to link to our AOPs and ultimately required communication with our coach and OECD to get guidance on which were the main KEs. However, the coordinators of the AOP-Wiki are aware of this issue and are actively working on merging and removing duplicate events in the wiki. To continue to do so and work on other technical challenges in the wiki, it is highly encouraged for AOP users and developers to inform the coordinators of the wiki of duplicate events or similar challenges (aopwiki@googlegroups.com). Additionally, the presence of these duplicate events led to confusion on how to treat cell specificity as some of the duplicate events do specify cell type in the KE. For example, for our AOP network, the pathway involves two cell types, epithelial and mesenchymal cells, which are believed to each play a different role in signal transduction (Kurosaka, 2015; Lan and Jiang, 2009). Via communication with the coach as well as other AOP developers/experts, it was agreed that the cell-specific mechanisms for a specific KE be described in the overall AOP itself rather than the KE itself. Where cell specificity is needed, it was recommended to specify cell type within the KER.

Another challenge we have noticed is the inability of the AOP-Wiki to display the assessed WoE levels easily and visually. The ability to quickly look at a pathway and get a sense for the level of evidence and quantitative understanding is something we feel would benefit all users of the AOP-Wiki. Currently, the Wiki is set up to show the evidence level of the KERs by arrow weight in the network view (Fig. 2A). This does not include information on the quantitative understanding and is not represented in the main AOP schematic (Fig. 2B). One way to possibly implement this would be a combination of colors and symbols as we depict in Fig. 2**C** for AOP 460. We have used this approach to present the evidence and quantitative understanding of the AOP more easily to those unfamiliar with the AOP framework.

The advancement of NAMs plays a crucial role in transitioning from animal testing towards human-based models for evaluating drugs and chemicals. To develop robust assays that may be used as a test guideline in the future, we first need to understand the existing data and the key physiology/endpoints of interest as well as the knowledge gaps requiring further investigation. AOPs have been proposed to guide selection of endpoint for NAMs development in multiple spaces including DART and cancer therapeutics (Johnson et al., 2024; Morgan, 2016). A clear illustration of the success that AOP development alongside NAM development is showcased by the *in vitro* skin sensitization and the AOP network that helped guide assay development and selection (Maxwell, 2014; Aleksic, 2024). As more successfully integrated NAMs and AOPs are developed, other challenges will likely be identified but the

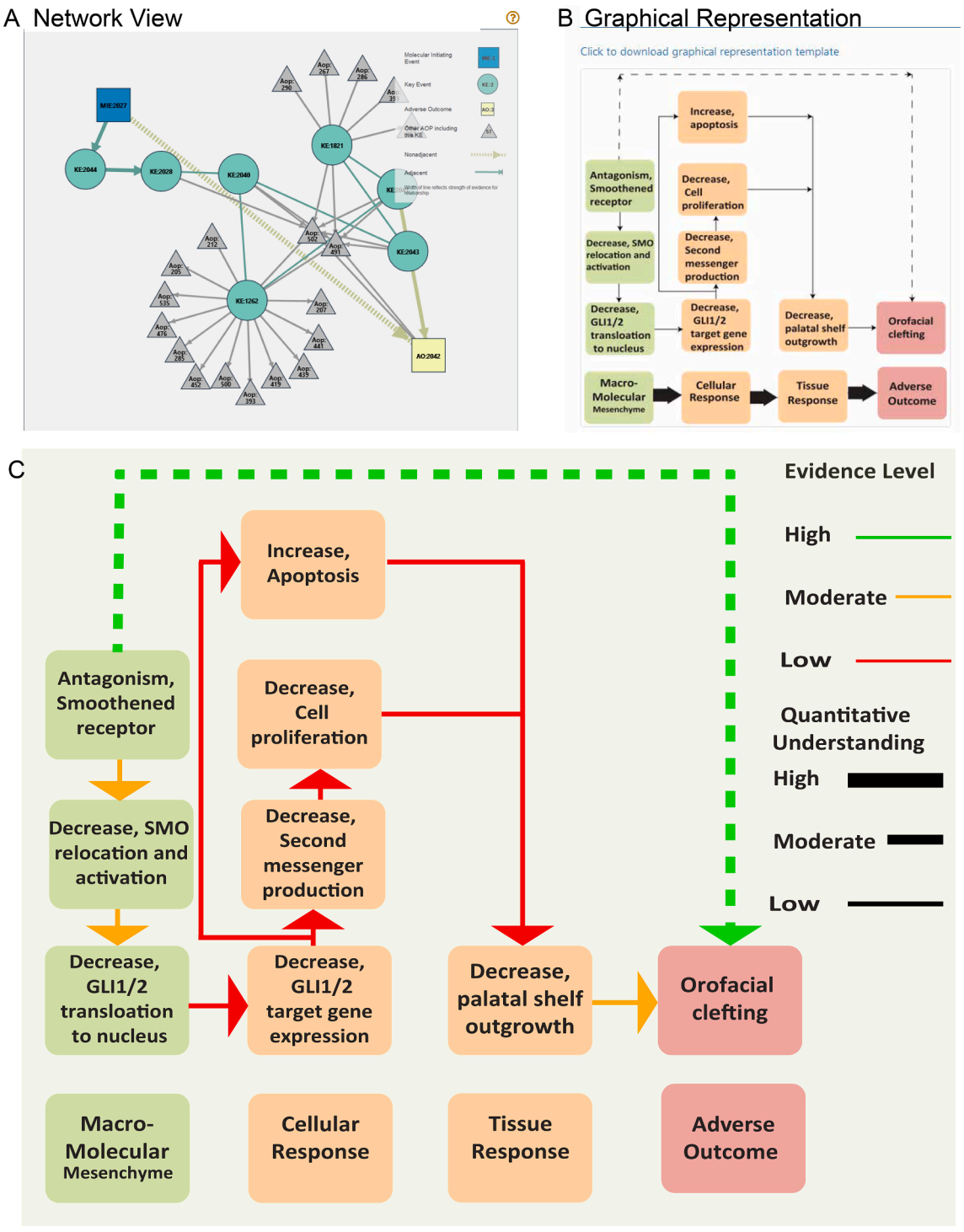

Fig. 2. Schematic of AOP 460. Adjacent relationships are shown as solid arrows, nonadjacent as dashed. WoE is shown by the color of the arrow (high green, moderate orange, low red) and the level of quantitative understanding is shown by the thickness of the line.

editability of the AOP wiki makes it adaptable to updated formats.

3. Conclusion

AOPs are one tool that 21st century risk assessors have at their disposal to help understand the pathway through which a chemical exposure might act. The continued development of these AOPs is needed and can be aided by the academic community by combining AOP development with the literature review. AOP development shares many similarities of the traditional academic literature review while also adding in regulatory alignment and increased skills in critical analysis of each step in a pathway.

AOP development has improved the translational potential of our academic lab's efforts, introducing students to a regulatory framework and the risk assessment community. Partnering in these translational projects benefits the scientific community as a whole but requires communication between the regulatory community and the academic labs with the expert knowledge to develop these AOPs. We see the AOP framework as a tool that can readily facilitate this interaction with clear benefits to both parties. The regulatory community benefits from

knowledge experts involvement in developing AOPs (e.g. on ED) that can be applied to risk assessment of chemicals. Authors from academia, especially graduate students, developing AOPs can easily use it to fulfill a literature review requirement with a deliverable that is both translatable and publishable. Using the AOP framework as a literature review will identify data gaps and inconsistencies, which can help guide further investigation in an academic setting. Consequently, researchers also can apply the knowledge from the AOP to select key design parameters, such as physiology of interest and endpoints of regulatory relevance, for their development of NAMs with increased regulatory relevance.

CRediT authorship contribution statement

Jacob I. Reynolds: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Visualization. **Judy Choi:** Methodology, Writing – original draft, Writing – review & editing. **Brian P. Johnson:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Visualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Brian Johnson owns equity in Onexio Biosystems, LLC.

Data availability

No data was used for the research described in the article.

Acknowledgements

We thank the OECD Advisory Group on Emerging Science in Chemicals Assessment (ESCA) for their inclusion of this project in their workplan (ESCA workplan project 1.101).

This work was supported by the National Institutes of Health R00-ES028744 to BPJ and the National Institute of Environmental Health Sciences P42ES004911 to BPJ.

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