A FEEDBACK APPROACH TO BIFURCATION ANALYSIS IN BIOCHEMICAL NETWORKS WITH MANY PARAMETERS

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Abstract: Feedback circuits in biochemical networks which underly cellular signaling pathways are important elements in creating complex behavior. A specific aspect thereof is how stability of equilibrium points depends on model parameters. For biochemical networks, which are modelled using many parameters, it is typically very difficult to estimate the influence of parameters on stability. Finding parameters which result in a change in stability is a key step for a meaningful bifurcation analysis. We describe a method based on well known approaches from control theory, which can locate parameters leading to a change in stability. The method considers a feedback circuit in the biochemical network and relates stability properties to the control system obtained by loop–breaking. The method is applied to a model of a MAPK cascade as an illustrative example.

Keywords: Feedback circuit, stability, bifurcation analysis, MAPK cascade

1. INTRODUCTION

Feedback circuits are an important structural feature of biochemical networks (Tyson and Othmer, 1978). The presence of complex behavior such as bistability, i.e. the existence of several stable equilibria, and sustained oscillations can be attributed to the presence of feedback circuits (Cinquin and Demongeot, 2002; Kaufman and Thomas, 2003). These types of complex behavior are directly related to how feedback circuits influence stability properties of equilibria. In consequence, stability analysis of biochemical networks involving feedback is a recurring field of interest, and several theoretical results have been obtained (Dibrov *et al.*, 1982; Thron, 1991; Angeli and Sontag, 2004).

Models for biochemical networks in cellular signaling typically contain a large number of parameters whose values are not exactly known and which can even vary due to differential gene expression (e.g. the concentration of an enzyme) or external influences (e.g. cofactors). These parameters often have a considerable influence on stability, which needs to be evaluated in order to understand the function of a network (Eißing *et al.*, 2007; Kim *et al.*, 2006).

A classical tool to study the influence of parameter variations on stability is bifurcation analysis. It has been applied to many cellular signalling systems, such as the lac operon (Yildirim and Mackey, 2003) and the MAPK cascade (Markevich *et al.*, 2004; Chickarmane *et al.*, 2007), to name but a few. When considering models with many parameters, one faces the difficulty that in classical bifurcation analysis, only one parameter at a time can be varied. Thus the effect of simultaneous variations in several parameters can not be evaluated properly.

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In this paper, we present a new approach to locate bifurcations in systems with feedback loops containing many parameters which may be varied simultaneously. To this end, we make use of an appropriate frequency domain description of the system and of mathematical conditions representing necessary conditions for a bifurcation. The paper is structured as follows. In Section 2, we present the theoretical results required for our approach and suggest an optimization–based method to actually find interesting parameter values. In Section 3, we apply these results to a model of the MAPK cascade with a negative feedback circuit (Kholodenko, 2000). The relevance of our results is discussed in Section 4. The mathematical proofs of the theoretical results are not presented in this paper but will be provided elsewhere (Waldherr and Allgöwer, in preparation).

2. THEORETICAL BACKGROUND

2.1 The loop–breaking approach

We consider a nonlinear differential equation which may describe the biochemical network constituting a cellular signaling pathway,

$$\Sigma: \quad \dot{x} = F(x, p), \tag{1}$$

with $x \in \mathbb{R}^n$ and $p \in \mathcal{P}$, where $\mathcal{P}$ is a connected subset of $\mathbb{R}^m$. Typically, x will represent the concentrations of the signaling molecules, and p collects parameters like reaction constants or enzyme concentrations. We assume that an equilibrium point $\bar{x}(p)$ exists for all parameter values and can be computed at least numerically, such that $F(\bar{x}(p), p) = 0$ for all $p \in \mathbb{R}^m$.

Mathematically, the system (1) is said to contain a feedback circuit if the influence graph of its Jacobian $\frac{\partial F}{\partial x}$ contains a nontrivial loop (Cinquin and Demongeot, 2002). We want to study the influence of such a feedback circuit on the dynamical properties of the system. Control theory provides efficient tools to study this problem. A useful approach in our setup is to consider the system (1) as a closed loop control system. It is then possible to study the corresponding open loop system, and one can resort to the rich stability theory developped for control systems.

An open loop control system corresponding to the closed loop system (1) is obtained by loop breaking, as defined in the following.

Definition 1. A *loop breaking* for the system (1) is a pair (f, h), where $f : \mathbb{R}^n \times \mathbb{R} \times \mathbb{R}^m \to \mathbb{R}^n$ is a smooth vector field and $h : \mathbb{R}^n \to \mathbb{R}$ is a smooth function, such that

$$F(x, p) = f(x, h(x), p). \tag{2}$$

The corresponding open loop system is then given by the equation

$$\sigma: \begin{cases} \dot{x} = f(x, u, p) \\ y = h(x). \end{cases} \tag{3}$$

The closed loop system can again be obtained by "closing the loop", i.e. setting $u = y$. Notice that by the assumption that an equilibrium exists for the closed loop system, the open loop system also has the equilibrium $\bar{x}(p)$ when choosing $u = h(\bar{x}(p))$. This input is denoted as $\bar{u}(p) = h(\bar{x}(p))$.

Since our main interest is in stability properties of the equilibrium point $\bar{x}(p)$, we can restrict the analysis to the linear approximation of the systems (1) and (3) around the equilibrium point. By using Laplace transformation, the linear approximation of the open loop system (3) can be represented by a linear parameter-dependent transfer function

$$G(p, s) = \frac{k(p)q(p, s)}{r(p, s)}, \tag{4}$$

where q and r are polynomials in the complex variable s with coefficients depending on p. As a technical restriction, we assume that the open loop system has no poles or zeros on the imaginary axis, i.e. $r(p, \cdot)$ and $q(p, \cdot)$ are assumed to have no roots on the imaginary axis for any value of $p \in \mathcal{P}$ throughout this section.

2.2 Properties of the closed and open loop systems

Stability of an equilibrium point of the closed loop system depends on the position of the eigenvalues of the Jacobian $\frac{\partial F}{\partial x}(\bar{x}(p), p)$. To characterize these eigenvalues from conditions on the open loop system, we have the following theorem.

Theorem 1. Let $A(p) = \frac{\partial f}{\partial x}(\bar{x}(p), \bar{u}(p), p)$ and $A_{cl}(p) = \frac{\partial F}{\partial x}(\bar{x}(p), p)$. Assume that $s_0 \in \mathbb{C}$ is not an eigenvalue of $A(p)$. Then s_0 is an eigenvalue of $A_{cl}(p)$, if and only if $G(p, s_0) = 1$.

The proof of Theorem 1 is based on a representation of G as

$$G(p, s) = \frac{\det(sI - A_{cl}(p))}{\det(sI - A(p))} + 1.$$

Parameter values on the border of stability are characterised by the matrix $A_{cl}(p)$ having eigenvalues on the imaginary axis. To study the corresponding property in the frequency domain representation of the open loop system, we introduce the notation of critical frequencies and gains.

Definition 2. We say that $\omega_c \in \mathbb{R}$ is a *critical frequency* and $k_c \in \mathbb{R}$ a corresponding *critical gain* for the transfer function $G(p, \cdot)$ (4), if

$$\frac{k_c q(p, j\omega_c)}{r(p, j\omega_c)} = 1. \tag{5}$$

In general, different critical frequencies and gains will be obtained for different values of p.

The critical frequencies can be characterized independently of the critical gains. This result follows from (5), because the transfer function value at the critical frequency has to be a real number.

Proposition 1. ω_c is a critical frequency for $G(p, \cdot)$, if and only if

$$\text{Im}(q(p, j\omega_c)r(p, -j\omega_c)) = 0. \tag{6}$$

There exists a unique corresponding critical gain for any critical frequency ω_c, which is given by

$$k_c(p, \omega_c) = \frac{r(p, j\omega_c)}{q(p, j\omega_c)}. \tag{7}$$

The equation (6) is a polynomial in ω_c, thus all critical frequencies can be computed numerically for fixed parameters p. The set of all critical frequencies for the transfer function $G(p, \cdot)$ is given by

$$\Omega_c(p) = \{\omega \in \mathbb{R} \mid \text{Im}(q(p, j\omega)r(p, -j\omega)) = 0\} \tag{8}$$

The concept of critical frequencies and critical gains can be understood intuitively when considering the Nyquist plot of the transfer function $G(p, j\omega)$. A critical frequency is any value ω at which the Nyquist plot crosses the real axis. The corresponding critical gain is the value $k(p) = k_c$ which scales the Nyquist plot in such a way that the crossing point at the critical frequency is mapped to 1 in the complex plane. However, this intuitive way of scaling the Nyquist plot would require to keep all critical frequencies in $\Omega_c(p)$ constant when varying parameters, which would be a strong restriction. The next section presents an approach to overcome this restriction.

2.3 A minimal set of critical frequencies

The number of critical frequencies that exist for a given open loop system is often predefined by the position of the open loop poles and zeros in the left or right half complex plane. The following proposition guarantees the existence of a minimal number of critical frequencies.

Proposition 2. Let $\alpha = |p_+ - p_- + z_- - z_+|$, where p_+ (p_-) is the number of poles of $G(p, \cdot)$ in the right (left) half complex plane and z_+ (z_-) is the number of zeros of $G(p, \cdot)$ in the right (left) half complex plane. Then, for any $p \in \mathcal{P}$, $\Omega_c(p)$ has at least α distinct elements, if α is odd, and at least $\alpha - 1$ distinct elements, if α is even.

Since we assumed that $G(p, \cdot)$ has no poles or zeros on the imaginary axis, the number α is the same

for all parameters $p \in \mathcal{P}$. Thus, it can be used to characterise a set of critical frequencies as being minimal.

Definition 3. Under the assumptions of Prop. 2, the set of critical frequencies $\Omega_c(p)$ is called *minimal*, if it contains exactly the minimal number of elements according to Prop. 2.

If $\Omega_c(p)$ is minimal, we can label the roots of (6) in a consistent way, and write $\Omega_c(p) = \{\omega_c^1(p), \omega_c^2(p), \ldots, \omega_c^\alpha(p)\}$, where the ω_c^i can be identified with different solution branches of the polynomial equation (6).

2.4 Existence of critical parameter values

The concept of critical frequencies and gains is now applied to the problem of how stability depends on parameters. We study the problem of finding critical parameters $p_c \in \mathcal{P}$ on the border of stability, i.e. such that the eigenvalues of the Jacobian $A_{cl}(p_c)$ are located on the imaginary axis. Then there exist typically parameters p_0 and p_1 in a neighborhood of p_c such that the equilibrium $\bar{x}(p_1)$ is stable and $\bar{x}(p_2)$ is unstable.

The following theorem uses the loop–breaking approach and the concept of critical frequencies to characterise the existence of critical parameters.

Theorem 2. Assume that $\Omega_c(p)$ is minimal for all $p \in \mathcal{P}$. Then there exists $p_c \in \mathcal{P}$ such that $j\omega_c^i(p_c)$ is an eigenvalue of $A_{cl}(p_c)$, if and only if there exist $p_0, p_1 \in \mathcal{P}$ such that $G(p_0, j\omega_c^i(p_0)) \leq 1$ and $G(p_1, j\omega_c^i(p_1)) \geq 1$, for $\omega_c^i(\cdot) \in \Omega_c(\cdot)$ and for any $i \in \{1, 2, \ldots, \alpha\}$.

Thus, instead of having to look at how the n eigenvalues of the closed–loop system change with parameters, we have reduced this to one number, given by $G(p, j\omega_c^i(p))$, which contains all information about whether the system changes its stability properties when changing parameters. The result is global in the sense that the parameters p_0 and p_1 can be arbitrarily far apart from each other, still under the given conditions existence of critical parameters p_c is guaranteed.

2.5 Searching for critical parameter values

The theoretical approach outlined above can be used to search for parameter values such that the equilibrium point $\bar{x}(p)$ of the closed loop system (1) changes its stability. For a biochemical system, there are often nominal parameters p_0, giving rise to the equilibrium point $\bar{x}(p_0)$. We want

to find parameters p_1 such that $\bar{x}(p_0)$ and $\bar{x}(p_1)$ have different stability properties.

In view of the methods presented in this paper, given the open loop transfer function (4), one first needs to identify the critical frequency that is to be considered. This choice depends on the type of stability change one is looking for. When taking $\omega_c = 0$, it is possible to search for zero eigenvalues, and if $\omega_c > 0$, nonzero imaginary eigenvalues may be encountered, typically giving rise to a Hopf bifurcation in the closed loop system. The nominal transfer function value at the critical frequency is $G(p_0, j\omega_c(p_0))$. To change stability properties, we will then define a value γ as either $\gamma > 1$, if $G(p_0, j\omega_c(p_0)) < 1$, or as $\gamma < 1$ otherwise. Then, any solution to the nonlinear equation

$$G(p_1, j\omega_c(p_1)) = \gamma \qquad (9)$$

gives parameters p_1 such that $\bar{x}(p_0)$ and $\bar{x}(p_1)$ have different stability properties as indicated by the chosen critical frequency ω_c. This method has been implemented using a nonlinear constrained optimization algorithm from the MATLAB Optimization Toolbox (The MathWorks Inc., 2006). It allows to efficiently compute parameter values for the desired transfer function value for medium sized systems, as the example presented in the following section illustrates.

Once a parameter p_1 is known, we can use a straight line going from p_0 to p_1, defined as $p_\mu = p_0 + \mu(p_1 - p_0)$. The change in dynamical behaviour along this line can then be studied using classical bifurcation analysis with respect to μ, implemented usually via continuation methods (Kuznetsov, 1995). In this study, the software AUTO (Doedel *et al.*, 2006) has been used for the bifurcation analysis along the parameter line p_μ.

3. APPLICATION TO A MAPK SIGNALING MODULE

3.1 Model description

The method presented in Section 2 has been applied to an ODE model of a mitogen activated protein kinase (MAPK) signaling module. MAPK signaling is a recurring motif in cellular signaling pathways, and typically appears in a cascade involving three levels (Pearson *et al.*, 2001).

For this study, we consider a mathematical model for the Ras/Raf signaling pathway similar to the one presented by Kholodenko (2000). The inhibition of the upstream molecule SOS by activated MAPK, the lowest level in the cascade, constitutes a negative feedback circuit around the cascade. Via the loop–breaking approach, the influence of this feedback connection on existence of sustained oscillations in kinase activity is analysed.
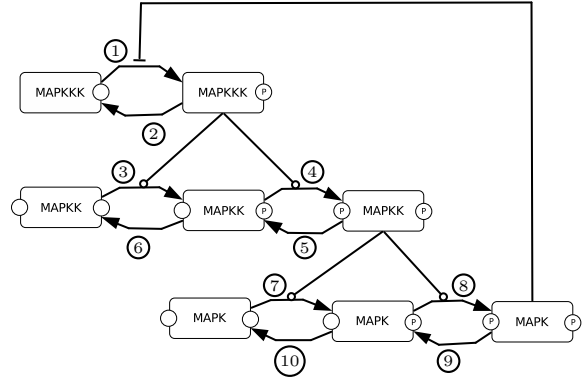

Fig. 1. Illustration of the MAPK cascade model.

Reaction	Rate
v_1	$V_1 \frac{x_{1t} - x_{11}}{(1 + x_{32}/K_i)(K_{m1} + x_{1t} - x_{11})}$
v_2	$V_2 \frac{x_{11}}{K_{m2} + x_{11}}$
v_3	$k_3 x_{11}(x_{2t} - x_{21} - x_{22})$
v_4	$k_4 x_{11} x_{21}$
v_5	$V_5 \frac{x_{22}}{K_{m5} + x_{22}}$
v_6	$V_6 \frac{x_{21}}{K_{m6} + x_{21}}$
v_7	$k_7 x_{22}(x_{3t} - x_{31} - x_{32})$
v_8	$k_8 x_{22} x_{31}$
v_9	$V_9 \frac{x_{32}}{K_{m9} + x_{32}}$
v_{10}	$V_{10} \frac{x_{31}}{K_{m10} + x_{31}}$

Table 1. Reaction rates in the MAPK cascade model

The structure of the model is illustrated in Fig. 1. The reaction rates as labeled in the figure are displayed in Table 1. The concentrations have been denoted as x_{11} = [MAPKKK*], x_{21} = [MAPKK*], x_{22} = [MAPKK**], x_{31} = [MAPK*] and x_{32} = [MAPK**]. The concentrations of unphosphorylated kinases can be computed by conservation laws and the three parameters x_{1t}, x_{2t} and x_{3t} for the total concentrations of the three kinases. The difference to the model from Kholodenko (2000) is that the phosphorylation reactions 3, 4, 7 and 8 are assumed to follow mass action rather than Michaelis-Menten kinetics. This is reasonable since the Michaelis-Menten kinetics assumes low enzyme concentration compared to the substrate, whereas the concentrations of the kinases are in a comparable range here. Nominal parameter values have been adopted from Kholodenko (2000), and are shown in Table 2 as p_0.

Using the reaction rates from Table 1, the model can be written as a system of five ODEs with 20 parameters:

$$\begin{aligned}
\dot{x}_{11} &= v_1 - v_2 \\
\dot{x}_{21} &= v_3 + v_5 - v_4 - v_6 \\
\dot{x}_{22} &= v_4 - v_5 \\
\dot{x}_{31} &= v_7 + v_9 - v_8 - v_{10} \\
\dot{x}_{32} &= v_8 - v_9
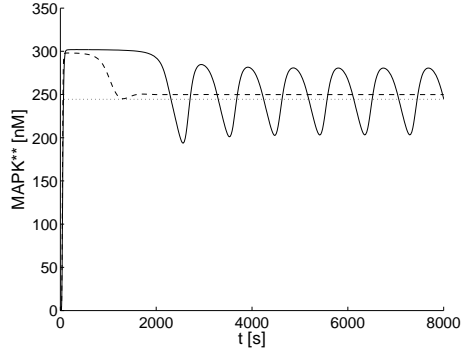\end{aligned} \qquad (10)$$

Fig. 2. Convergence to steady state for parameters p_0 (dashed line) and sustained oscillations for parameters p_1 (solid line). The oscillations coexist with an unstable equilibrium (dotted line).

For the nominal parameters p_0, the model has a stable equilibrium $\bar{x}(p_0)$. Solutions of the model converge quickly to the steady state, as depicted in Fig. 2.

3.2 Parameters for a change in stability properties

This section describes the application of the method presented in Section 2 to the problem of finding destabilizing parameters for the MAPK cascade model (10).

The first step is to choose a suitable loop breaking. For the MAPK cascade, an intuitive approach is to break the loop at the feedback inhibition of reaction v_1 by MAPK**. Thus we choose $h(x) = x_{32}$, to select [MAPK**] as an output, and replace x_{32} by the input u in the reaction rate v_1 to obtain the dynamics of the open loop system $f(x, u, p)$.

It can be shown that there is a unique equilibrium of (10) for any parameters in the biologically meaningful range. The equilibrium can easily be computed numerically. A linearisation of the open loop system around this equilibrium point and a Laplace transformation gives the transfer function $G(p, s)$, whose graph is shown in Fig. 3. The set of critical frequencies is minimal with $\alpha = 3$, which can be seen from Fig. 3 by the observation that the graph of $G(p_0, j\omega)$ encircles the origin monotonically. The only positive critical frequency is $\omega_c(p_0) = 0.017 s^{-1}$, and we will consider this frequency in the search for destabilizing parameters. The corresponding transfer function value is $G(p_0, j\omega_c(p_0)) = 0.12$, corresponding to the equilibrium $\bar{x}(p_0)$ being stable in the closed loop system.

For the computational approach described in Section 2.5, we chose $\gamma = 1.5$, such that the value of the transfer function would have to pass the point 1 when going from its inital value of 0.12 to γ. The optimization method converges to the parameters

Param.	p_0	p_1	Unit	rel. change
V_1	2.5	2.4	nM/s	1.05^{-1}
K_i	9	10.6	nM	1.18
K_{m1}	10	9.4	nM	1.06^{-1}
V_2	0.25	0.11	nM/s	2.24^{-1}
K_{m2}	8	1.6	nM	4.9^{-1}
k_3	0.001	0.0026	1/(s nM)	2.6
k_4	0.001	$3.5 \cdot 10^{-4}$	1/(s nM)	2.8^{-1}
V_5	0.75	0.32	nM/s	2.35^{-1}
K_{m5}	15	3.9	nM	3.8^{-1}
V_6	0.75	3.7	nM/s	5.0
K_{m6}	15	13.3	nM	1.12^{-1}
k_7	0.001	0.0033	1/(s nM)	3.3
k_8	0.001	$5.0 \cdot 10^{-4}$	1/(s nM)	2.00^{-1}
V_9	0.5	0.26	nM/s	1.92^{-1}
K_{m9}	15	14.9	nM	1.01^{-1}
V_{10}	0.5	2.5	nM/s	5.0
K_{m10}	15	15.0	nM	1.00
x_{1t}	100	100.0	nM	1.00
x_{2t}	300	300.8	nM	1.00
x_{3t}	300	304.2	nM	1.01

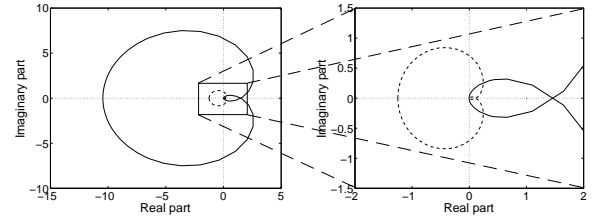Table 2. Reference parameters p_0 and parameters for instability p_1 in the MAPK cascade model.

Fig. 3. Nyquist plots of open–loop MAPK model for parameters p_0 (dashed line) and p_1 (solid line).

p_1, which give the desired value $G(p_1, j\omega_c(p_1)) = 1.5$ at a critical frequency $\omega_c(p_1) = 0.0065 s^{-1}$. The parameter values in p_1 are shown in Table 2. The maximal single parameter change from p_0 to p_1 has been restricted in the numerical implementation to be not more than a factor of 5. Even with this restriction, parameters leading to sustained oscillations have been found. However, 11 out of the 20 parameters have been changed by more than 20 % to achieve this.

The graph of $G(p_1, j\omega)$ is shown in Figure 3. For the new parameters p_1, the graph now encircles the point 1. By the argument principle, we see that the linearisation of the closed–loop system around the equilibrium has some eigenvalues in the right half complex plane and is thus unstable. The sustained oscillations that appear in this case are shown in Fig. 2.

In conclusion, our method is able to compute parameters which render the stable equilibrium unstable and thus lead to the emergence of sustained oscillations. About half of the parameters are varied by a non–negligible amount, but all variations are within the physiological range.
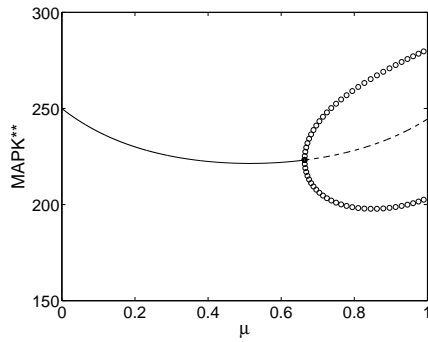
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Fig. 4. Bifurcation diagram along the line p_μ, showing stable equilibrium (solid line), unstable equilibrium (dashed line) and amplitude of oscillations (circles).

3.3 Bifurcation analysis along a line

Let us now consider the line $p_\mu = p_0 + \mu(p_1 - p_0)$. By classical bifurcation analysis with μ as bifurcation parameter, we can see how the system changes from the stable to the unstable equilibrium. The resulting bifurcation diagram is shown in Figure 4. As expected, there is a Hopf bifurcation between p_0 and p_1, at $\mu = 0.664$. The evolution of the limit cycle producing the sustained oscillations along the line in parameter space is obtained from the bifurcation diagram.

4. CONCLUSIONS

We introduced some theoretical tools to investigate the existence of parameters for which a bifurcation can occur in a dynamical system with a feedback circuit. These tools gave rise to a new computational method which allows to search for parameter values such that the stability properties of an equilibrium change in a specific way compared to the nominal parameter values. Our approach is particularly useful if there are many parameters in the system which can be varied simultaneously, and if the contribution of individual parameters to stability properties is not obvious. The ability to directly handle multiparametric variations is a clear advantage compared to using only classical bifurcation analysis.

We have shown the application of the proposed method to a model of a MAPK cascade. Using relatively small changes to most of the 20 parameters in the model leads to a change from a stable equilibrium to an unstable equilibrium with a stable limit cycle, producing sustained oscillations.

Note: Matlab and AUTO scripts for the described bifurcation analysis are available from the authors on request.

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