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Activation Free Energies in Partial Steps of the Sarcoplasmic Reticulum Ca-ATPase Cycle during the Transient States of the Reaction

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Abstract

The step-by-step free energy (ΔG) profile of the sarcoplasmic reticulum Ca-ATPase cycle during the transient state of simulated reactions was analyzed previously by Alonso and Hecht (1990). ΔG was negative at all the steps. Here we calculated the step-by-step activation energies ($\Delta G^{\pm}$) in the same model according the transition state theory. Transient species were introduced as chemical intermediates at each step; they react with enzyme species accordingly with the mass law, decaying at 6.56×10^{12} s^{-1}. Other rate constants are as in the referenced paper. The transient evolution of the model was followed with a computer program. The evolution of the ligands concentrations agree with experimental results. Enzymatic species concentrations lie within 10^{-10}–10^{-6}M, transient species lie within 10^{-19}-10^{-17}M, at 0.1 s, when the reaction is in a cuasi-steady-state. These differences preclude the use of the standard (ΔG^{o}) or basic (ΔG_{basic}) free energies, to describe ΔG fall along the cycle. Forward and backward $\Delta G^{\pm}$ are shown for 10 and 100 ms of simulated reaction; both are negative in forward cycles, at any reaction time, and their sum equals the partial ΔG. The results show the absence of any activation energy hill to be surmounted upon the reaction advance. Similar results were obtained for the successive binding of 2 Ca ions to the enzyme through (+)cooperative reactions.

Keywords: SERCA, Transition intermediates, Energy transduction, Enzyme energetics

1 Introduction

Several thermodynamic profiles of the sarcoplasmic reticulum (SR) Ca-ATPase cycle have been described. Some of them analyzed the standard free energy changes (ΔG^o) along the cycle [9, 12]; others analyzed the system under steady state conditions, using the concept of basic-free-energy (ΔG_{basic}) [10-12]. The report from Pickart and Jencks [12] also includes calculations of the step-by-step activation free-energies ($\Delta G^{\pm}$) according the transition state theory. Alonso and Hecht [1] described the step-by step Gibbs' free-energy profile (ΔG) of the cycle during the transient state of the transport reaction. All these reports pointed to describe the free energy fall along the ordered steps of the transport cycle.

The present work aimed to join the results from Pickart and Jecks [12] with those from Alonso and Hecht [1] to obtain the activation energies of the partial steps of the SR Ca-ATPase cycle during transient states of the reaction. Additionally, the activation energy of the successive binding of 2 Ca ions to the cytoplasmic side of the enzyme [9] is also analyzed as an example of a more simple reaction.

2 Methods

Scheme I shows the SR Ca-ATPase reaction cycle. This model was used in previous reports [1, 10-12]. Seven enzymatic species are linked by seven partial steps (J). Scheme II shows the successive binding of 2 Ca ions to the cytoplasmic side of the enzyme in a (+) cooperative reaction.

Partial steps are characterized by a direct (k_j) and a reverse (k_{-j}) rate constant; their values are taken from [1] or [2], for schemes I or II respectively. In turn, they were taken from [12] and [14] with slight modifications. The equilibrium constants (K_J) of the partial steps are defined as $K_J = k_j / k_{-j}$.

The standard free energy change at each step is:

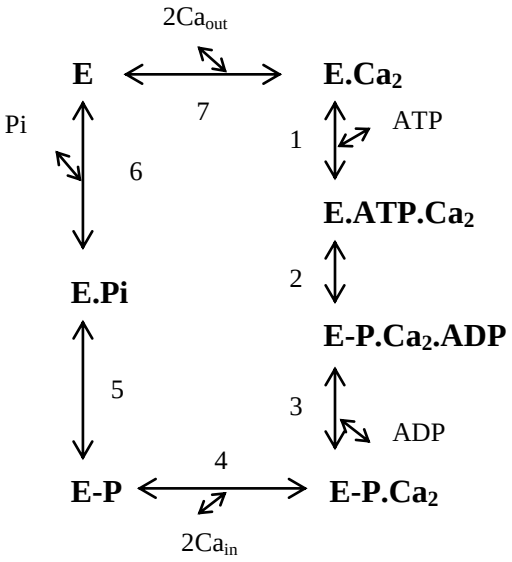
$$\Delta G^o_J = -RT \ln K_J \qquad \text{(Eq. 1)}$$

where R is the gas constant ($1.987 cal.K^{-1}.M^{-1}$) and T the thermodynamic temperature (310 K).

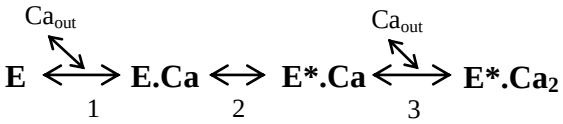
The free energy change for step J, at any concentration of reactants (r) and products (p), is:

$$\Delta G_J = \Delta G^o{}_J + \ln \frac{\pi_{p,J}}{\pi_{r,J}} \qquad \text{(Eq. 2)}$$

where $\pi_{p,J}$ and $\pi_{r,J}$ are the productory of the concentrations of ligands and enzyme species participating in partial reaction J as products and reactants, respectively.



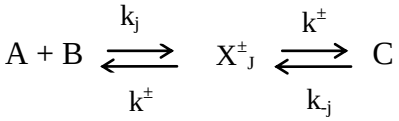
(Scheme I)



(Scheme II)

The scheme I cycle is fully reversible; 2 Ca ions are transported clockwise into the SR lumen at the expense of free energy of hydrolysis of 1 ATP molecule; counterclockwise, ATP is synthesized from ADP and Pi at the expense of free energy of a Ca gradient. The analyzed system simulates a suspension of vesicular SR membrane fragments with 1/1000 intra-/extra-vesicular volume ratio [1]. The model includes a permeability constant (k_p) linking Ca_{out} and Ca_{in} (not shown in the scheme); k_p=0 or 1 s^{-1} applies to Ca impermeable or permeable membranes respectively.

The transition state theory assumes that in any chemical reaction the reactant(s) needs additional energy -*activation energy*- to reach a particular intermediate state – the *activated complex* ($X^{\pm}$) - prior to become transformed in product(s), as shown in scheme III for a generalized reaction (J).



(Scheme III)

In both analyzed models (Schemes I and II) the activated complexes ($X^{\pm}_J$) are included as intermediates in each step, with the format exemplified in scheme III (they are not shown in schemes I and II for the sake of clearness). Their kinetic behavior is described in the Appendix, accordingly with the proposals of the original theory of the activated complex [4].

Applying Eq. 2 to the transient state ($X^{\pm}_J$) of the Scheme III reaction (Appendix, Eq. A8), the activation free energy for the forward $X^{\pm}$ formation is given by:

$$\Delta G^{\pm}_{\ j} = \Delta G^{\pm o}_{\ j} + RT \ln \frac{[X^{\pm}]}{[A][B]} \qquad \text{(Eq. 3a)}$$

Where $\Delta G^{\pm o}_j$ is the standard activation free energy change for step j (Appendix, Eq. A8). Eqs. 3a and 3b are equivalent:

$$\Delta G^{\pm}_{\ j} = -RT \ln \frac{v_j h}{k_B T} + RT \ln[X^{\pm}_{\ J}] \qquad \text{(Eq. 3b)}$$

Eq. 3 shows the dependence of $\Delta G^{\pm}_j$ on both the rate limiting velocity of the partial step (v_j), and the concentration of the activated complex ($[X^{\pm}_J]$). Both vary during the reactions transient states.

Step-by-step $\Delta G^{\pm}_j$ and $\Delta G^{\pm}_{-j}$ were calculated with Eq. 3b and free energy changes of the partial steps (ΔG_J) were calculated with Eqs. 2 or 4, indistinctly.

$$\Delta G_J = \Delta G^{\pm}_j - \Delta G^{\pm}_{-j} \qquad \text{(Eq. 4)}$$

The kinetic behavior of the models was simulated with a computer program based on the Runge-Kutta algorithm [7] implemented in Borland-Delphi software. The simulated reaction of the Scheme I model, with either $k_p = 0$ or 1 s^{-1}, starts by addition of 25 µM ATP to 1 µM Ca-ATPase equilibrated with 50 µM Ca. The Scheme II model starts by mixing 1 µM Ca-ATPase and 50 µM Ca. The program calculates the concentrations of all the species, including $X^{\pm}_J$ species, as a function of time.

3 Results

The evolution of the concentrations of the 7 enzymatic species (E_J) and those of the 7 activated complexes ($X^{\pm}_J$), (scheme I, $k_p = 0$), are shown in Fig. 1, A and B respectively, up to 0.1 s. Pi production reached linearity, at an approximate rate of 1 µMol . mg protein^{-1} . min^{-1} calculated as in [1], [Ca_i] reached 1.6 mM and ATP consumption was 4.2 %; ADP production and [Ca_o] decrease were also followed along the reaction (detailed results not shown). From the above results, activation

energies were calculated with Eq. 3b applied to both forward (j) and backward (-j) directions of the partial steps. Results are shown in Fig. 2A. Forward activation energies ($\Delta G^{\pm}_{j}$) are negative for all the steps, at any time. It implies the absence of free energy barriers to be surmounted during the reaction. Fig. 2B reproduces Fig 2A for permeable vesicles. The break imposed to some steps by Ca_{in} accumulation in sealed vesicles accounts for their differences. Backward activation energies ($\Delta G^{\pm}_{-j}$) are always positive but they are also shown with negative slopes because Fig 2 represents the forward energy flow of the cycle.

ΔG_J of the successive partial reactions were calculated with Eqs. 2 or 4, indisctinctly. Their values are shown in Figs. 2 as solid straight lines whose slopes represent $\Delta G_{J;}$ the energy level of the activated complex ($X^{\pm}_{J}$) always lies above the free energy fall straight line. Figs. 2 also show that free energy fall of the whole cycle and that of the partial steps reduce its negative value with the reaction time, approaching zero towards equilibrium, as previously reported [1]. The exceptions in steps 7 are due to the fact that simulations began assuming equilibrium between Ca_{out} and the free enzyme.

Fig. 3 compares (A and B) the step-by-step ΔG^{o} and $\Delta G^{\pm}$ values in the (+) cooperative binding of 2 Ca_{out} to the free enzyme (Scheme II). Results agree with those in Figs. 2

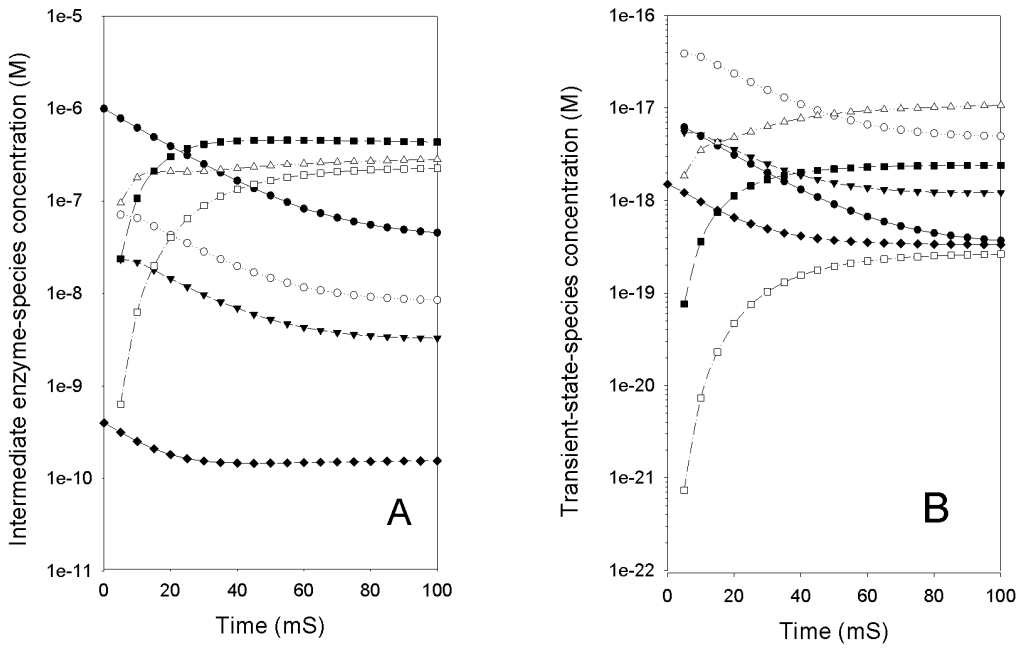


Figure 1. Time course of the Scheme I reaction including the activated complexes at each partial step. The reaction was simulated up to 0.1 s as described under Methods. **A**. Evolution of the enzymatic species: $E.Ca_2$ (●), $E.ATP.Ca_2$ (○), E-

$P.Ca_2.ADP$ (▼), $E\text{-}P.Ca_2$ (Δ), E-P (■), E.Pi (□) and E (◆). At zero time E and $E.Ca_2$ species are equilibrated with 50 µM Ca, and the other enzyme species equal zero. **B**. Evolution of the activated complexes ($X^{\pm}_J$) with time. $X^{\pm}_1$ (●), $X^{\pm}_2$ (○), $X^{\pm}_3$ (▼), $X^{\pm}_4$ (Δ), $X^{\pm}_5$ (■), $X^{\pm}_6$ (□) and $X^{\pm}_7$ (◆). Subindexes refer to the partial steps identified in Scheme I. At zero time $X^{\pm}_7$ is in equilibrium with E and $E.Ca_2$ and the other activated complexes equal zero.

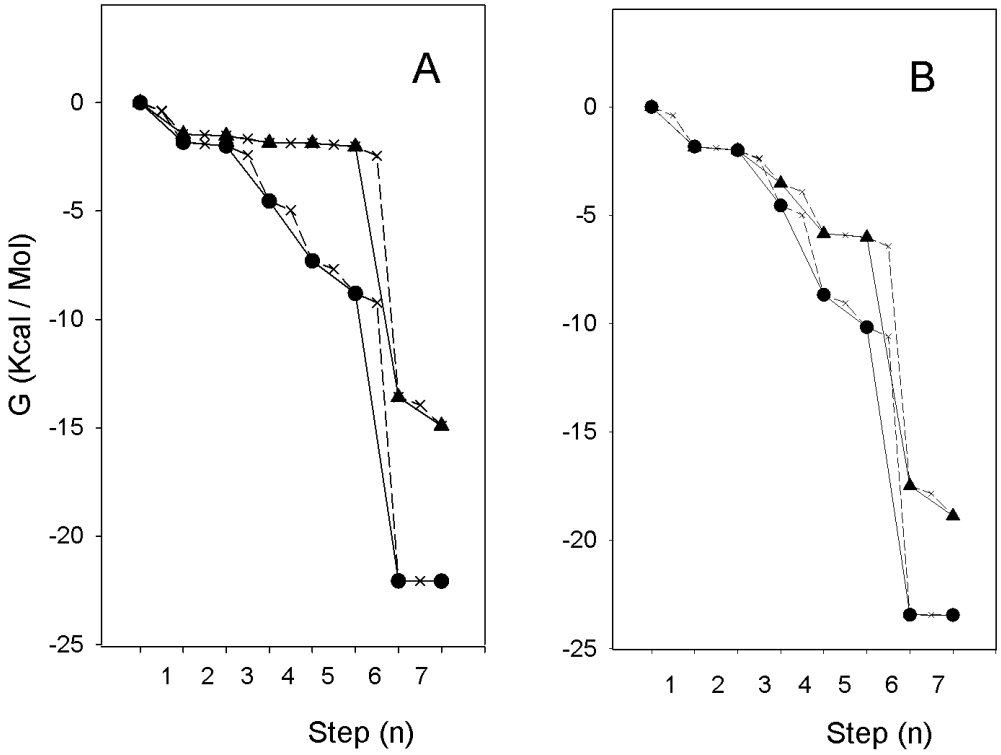


Figure 2. Gibbs' free energy fall and activation energies in the partial steps of the SR Ca-ATPase cycle, in Ca impermeable (**A**) or permeable (**B**) membranes, at 0.01 (●) and 0.1 (▲) s of simulated reactions. The solid lines show the free energy decay along the cycle; the solid symbols show the values of the sum of the free energy falls of the successive reaction steps up to that shown in the abscissa, expressed with reference to free energy of the $E.Ca_2$ species. Free energy falls at each partial step (ΔG_J) are the slopes of the solid lines at the step shown in the abscissa. Free energy levels of the activated complexes (×) are included as midway intermediates in each step. The slopes of the dashed straight lines before and after these energy levels are the forward and backward activation energies of the partial reaction, respectively.

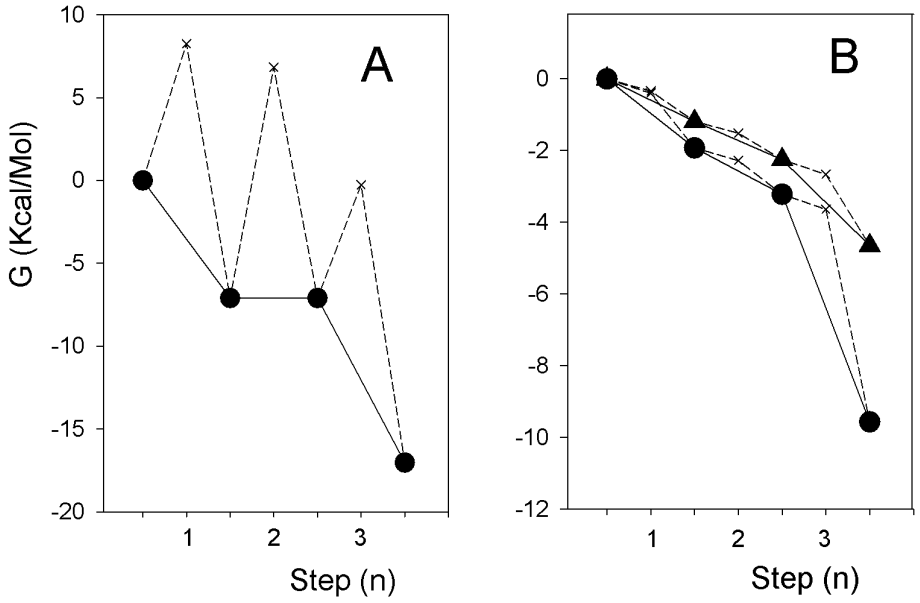


Figure 3. Step-by-step standard and transient free energy changes in Ca binding to the SR Ca-ATPase, accordingly to Scheme II. Solid lines: **A**. Step-by-step standard free energy changes (ΔG^{o}_{J}). B. Transient free energy changes at 1 (●) and 5 (▲) ms (ΔG_{J}). Dashed lines: A. Forward ($\Delta G^{\pm o}_{j}$) and backward ($\Delta G^{\pm o}_{-j}$) standard activation energy changes. B. Forward ($\Delta G^{\pm}_{j}$) and backward ($\Delta G^{\pm}_{-j}$) transient activation free energies, at 0.01 (●) and 0.1 (▲) s.

4 Discussion

The decrease in activation energy is primarily responsible for the increased rate of the catalyzed reactions [15, 5, 6]. According the generalized transition theory, the catalytic power of enzymes is due to the lowering of the activation free energy and any increase in the generalized transmission coefficient, as compared to that of the uncatalyzed reaction. The generalized transmission coefficient relates the actual rate for the reaction to that obtained from simple transition state theory, where coefficient equals to unity. We used rate constant values (k_j) obtained from enzyme catalyzed reactions to calculate phenomenological activation free energies that include the contribution of transmission coefficients.

Diagrams showing the step-by-step standard free energy changes of the Ca-ATPase cycle, calculated with Eq. 1, were previously reported (1, 2). The sum of these values (-7.3 Kcal/Mol) is the standard free energy change of ATP hydrolysis. We obtained comparable results (not shown) for our Scheme I model.

Since standard free energy only applies to 1 M concentrations of reactants and products, the results are not representative of the free energy fall along the cycle under physiological or *in vitro* experimental conditions. The concept of basic-free-energy [13] was developed to adequate calculations to different and constant ligand concentrations. It expresses the free energy change to drive standard concentrations of the enzyme species involved in the partial step, through first order reactions, towards their equilibrium with the specified ligand concentration. Basic free energy changes (ΔG_{basic}) were calculated for partial steps of the Ca-ATPase [9-13] and the Na/K-ATPase [3, 16] cycles, under steady-state conditions. Since the concentrations of the enzymatic species during the transient state of the reaction are far from standard values (Fig. 1A), ΔG_{basic} is not appropriate to describe the free energy fall at the transient partial steps of the reaction. Additionally, calculations of the forward and backward transient $\Delta G^{\pm}_{basic}$ values at each partial step assume a standard concentration for the activated complex. Fig. 1B shows how far are these transient concentrations from the standard ones. Pickart and Jencks [12] included the physiological values of the ligands as a factor of the k_j values in Eq. A8, to obtain the step-by-step forward and backward $\Delta G^{\pm}_{basic}$ of the Ca-ATPase cycle, and later ΔG_{basic} with Eq. 4. The diagram showed energy barriers to be surmounted along the cycle.

Alonso and Hecht [1] calculated the step-by-step free energy fall of the scheme I Ca-ATPase cycle, here reproduced in Fig. 2 by the solid lines slopes. The introduction of the activated complexes as intermediates in the partial steps, and the determinations of their concentrations, during the transport reaction allowed us to calculate the forward and backward $\Delta G^{\pm}$ values (dashed lines in Fig. 2). The striking feature is that all $\Delta G^{\pm}_{f}$ values are negative. These free energy falls are always followed by the further decay of the activated complexes. No energy hills to be surmounted appear in the spontaneous reactions advancements. In contrast, calculations of $\Delta G^{\pm o}_{f}$ and $\Delta G^{\pm o}_{b}$ always gave some positive values [1, 12]. Activation energies calculated for transient states of the cyclic model (Scheme I) with permeable membranes (Fig. 2B) also show the absence of energy hills to be surmounted along the cycle. Ca binding activation energies to free enzyme (Scheme II) agree with the above results (Fig 3).

5 Conclusions

5.1. All steps of the Ca-ATPase transiently running are accompanied by free energy decrease (Figs 2), while the same steps under standard conditions may have either negative or positive free energy changes [9-12].
5.2. A parallel result is observed for activation free energies in transient reactions, which are always negative, before and after the formation of the transient intermediate complex (Figs. 2 and 3B), while those obtained under standard conditions (ΔG^{o} or ΔG_{basic}) always have some positive free energy changes and hills along the cycle [9-12].

5.3. The results point to indicate that any exergonic chemical reaction which proceeds along several sequentially ordered partial steps has negative ΔG values at all their steps, including those of formation and decay of transient intermediate complexes.

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Appendix: Scheme III kinetics.

The activated complex ($X^{\pm}$) decay with very large and first order rate constant ($k^{\pm}$) into reactants or products with the same probability, at velocity:

$$v^{\pm} = k^{\pm} [X^{\pm}] \qquad \text{(Eq. A1)}$$

The $k^{\pm}$ value was deduced by Eyring (4) on the basis of statistical mechanic arguments:

$$k^{\pm} = \frac{k_B T}{h} \qquad \text{(Eq. A2)}$$

where k_B is the Boltzmann's constant (1.38exp-23 $J.K^{-1}$), and h the Planck's constant (6.626exp-34 J.s). From Eqs. A1 an A2, the decay velocity of the activated complex is:

$$v^{\pm} = \frac{k_B T}{h}[X^{\pm}] \qquad \text{(Eq. A3)}$$

k_j and k_{-j} are the limiting rate constants of the forward and backward scheme III reactions, respectively. Thus, the forward velocity is:

$$v_j = k_j [A] [B] \qquad \text{(Eq. A4)}$$

In equilibrium $v_j = v^{\pm}$ (Eqs. A3 and A4), then:

$$k_j = \frac{k_B T}{h}\left(\frac{[X^{\pm}]}{[A][B]}\right)_{eq} \qquad \text{(Eq. A5)}$$

Defining $K^{\pm}$ as the equilibrium constant of the activated complex with reactants in scheme III: $K^{\pm} = ([X^{\pm}]/([A][B]))_{eq}$,

Eq. A5 becomes $k_j = \frac{k_B T}{h} K^{\pm}$ (Eq. A6)

Applying Eq. 1 to calculate the free energy change for the formation of the activated complex, we obtain:

$$\Delta G^{\pm o} = -RT \ln K^{\pm} \qquad \text{(Eq. A7)}$$

which only applies to standard states. The standard free energy change of the forward formation of the activated complex derives from Eqs. A6 and A7:

$$\Delta G^{\pm o}{}_{j} = -RT \ln \frac{k_j h}{k_B T} \qquad \text{(Eq. A8)}$$

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