Molecular systems biology of ErbB1 signaling: bridging the gap through multiscale modeling and high-performance computing

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The complexity in intracellular signaling mechanisms relevant for the conquest of many diseases resides at different levels of organization with scales ranging from the subatomic realm relevant to catalytic functions of enzymes to the mesoscopic realm relevant to the cooperative association of molecular assemblies and membrane processes. Consequently, the challenge of representing and quantifying functional or dysfunctional modules within the networks remains due to the current limitations in our understanding of mesoscopic biology, i.e., how the components assemble into functional molecular ensembles. A multiscale approach is necessary to treat a hierarchy of interactions ranging from molecular (nm, ns) to signaling (μm, ms) length and time scales, which necessitates the development and application of specialized modeling tools. Complementary to multiscale experimentation (encompassing structural biology, mechanistic enzymology, cell biology, and single molecule studies) multiscale modeling offers a powerful and quantitative alternative for the study of functional intracellular signaling modules. Here, we describe the application of a multiscale approach to signaling mediated by the ErbB1 receptor which constitutes a network hub for the cell's proliferative, migratory, and survival programs. Through our multiscale model, we mechanistically describe how point-mutations in the ErbB1 receptor can profoundly alter signaling characteristics leading to the onset of oncogenic transformations. Specifically, we describe how the point mutations induce cascading fragility mechanisms at the molecular scale as well as at the scale of the signaling network to preferentially activate the survival factor Akt. We provide a quantitative explanation for how the hallmark of preferential Akt activation in cell-lines harboring the constitutively active mutant ErbB1 receptors causes these cell-lines to be addicted to ErbB1-mediated generation of survival signals. Consequently, inhibition of ErbB1 activity leads to a remarkable therapeutic response in the addicted cell lines.

Introduction

ErbB family receptors (named because of their homology to the erythroleukemia viral gene product, v-erbB, and consisting of the epidermal growth factor receptor or EGFR/ErbB1/HER1, ErbB2/HER2, ErbB3, and ErbB4) signal by activating crucial pathways1 in response to activation by ligands such as the epidermal growth factor (EGF) and other related peptide growth factors. Through ligand-stimulated formation of various homodimeric and heterodimeric complexes, ErbB receptors are activated, leading to the phosphorylation of multiple tyrosine residues on the C-terminal tail of the receptors as well as on other substrate proteins. Through specific interactions of the phospho-tyrosine sites to binding domains, the receptors bind to cytosolic partners that are responsible for the recruitment and activation of multiple downstream cascades.2–7 Activation through the mitogen-activated protein kinase (MAPK) cascades of the extracellular signal-regulated kinases (ERKs) is functionally linked to proliferation. The phosphoinositide 3-kinase (PI3K) pathway leads to the activation of the serine/threonine protein kinase Akt (cellular homologue of the viral oncogene v-Akt) which is linked to survival. Other significant pathways mediated by ErbB signaling include activation and nuclear translocation of signal transducers and activators of transcription proteins.
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ErbB1 kinase stable in an inactive conformation which is maintained by a network of stabilizing interactions. This stark contrast of 27 to 13 stabilizing interactions surrounding the activation-loop and the N-terminal tail (Fig. 2d, the inset to Fig. 2d shows the shift in the C-helix position: see Fig. 2a and b) suggests a potential allosteric interaction between the N-terminal tail and the C-terminal tail.

In order to consider the effect of ErbB1 kinase dimerization on the network of stabilizing interactions, we have computed the root-mean-squared deviation (RMSD) as a measure of the overall structural change upon dimerization of ErbB1 kinase (Fig. 1a). The RMSD is calculated as the square root of the average of the squared differences between the positions of corresponding atoms in the two structures. The RMSD is a useful measure of the structural similarity between two protein structures. A smaller RMSD indicates a higher degree of similarity, while a larger RMSD indicates a greater degree of structural difference.

The RMSD is defined as:

\[ \text{RMSD} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (x_{i1} - x_{i2})^2} \]

where \( x_{i1} \) and \( x_{i2} \) are the coordinates of the same atom in the two structures, and \( N \) is the number of atoms.

In our study, we have computed the RMSD between the inactive and active conformations of ErbB1 kinase. The results show that the RMSD is significantly lower in the active state compared to the inactive state, indicating a conformational change induced by dimerization. This conformational change is further supported by the analysis of the hydrogen bonds and the interactions between the proteins.

Overall, our study provides insights into the structural changes induced by dimerization in ErbB1 kinase, highlighting the role of stabilizing interactions in the regulation of receptor tyrosine kinases in cancer biology.
accompanied by several changes in the stabilizing network consistent with the predicted bond-patterns in Fig. 2a–c. In particular, in the inactive conformation, the bonds between Y740–S744, H846–R865, K851–R812 surrounding the activation loop and the αC-helix were severed. Thus, already in the wildtype dimer, due to the re-configuration of the αC-helix, the bond pattern is found to be shifting significantly toward that observed in the active kinase. The key bonds stabilizing the wildtype inactive ErbB1 kinase are the E738–K836 and E848–R865 salt bridge interactions, as well as the L834–D813 hydrogen bond. In our dimer simulations, the E738–K836 salt bridge has considerably weakened: the fraction of the time this bond was present decreased from >90% in the monomer trajectory to ~60% in the dimer trajectory; moreover this bond has undergone considerable stretching allowing E738 to hydrogen bond to F832, which is one of the bonds seen in the active kinase. The L834–D813 interaction is at the threshold of still being considered a stabilizing hydrogen bond. The residue K851 is hydrogen bonded to E725, moving away from the inactive bond K851–R812 and towards the active salt bridge K851–E734. The E848–R865 salt bridge is not perturbed significantly due to the formation of the asymmetric dimer interface. Thus, we hypothesize that a few specific bonds act as gatekeepers for each step of the conformational change, namely the E738–K836 salt guards against the αC-helix movement and the E848–R865 salt bridge guards against the activation loop rearrangement. Our dimer simulations reaffirm our notion that the stabilizing network is susceptible to perturbation in the inactive conformation of the kinase, and that formation of the asymmetric dimer will have the effect of directly breaking the network of interactions around the αC-helix, thereby destabilizing the inactive state. The loss of these interactions and the shift of the αC-helix conformation towards the active state will provide the impetus for kinase domain activation. Intriguingly, several of the clinically identified mutations that have been reported to constitutively activate the kinase also directly perturb the stabilizing network by breaking key stabilizing bonds: these are marked by the symbol M in Fig. 2c. In addition, the deletion of residues L723 to P729 in the del L723–P729 ins S mutant re-configures the αC-helix in the inactive state to a conformation closer to the active state (data not shown). Thus, our delineation of the stabilizing hydrogen bond network provides molecular-level insight into the possible mechanisms by which activating mutations of ErbB1 kinase such as L834R and del L723–P729 ins S destabilize the inactive conformation. This preferential destabilization of the inactive conformation renders the receptor kinase constitutively active even as a monomer, producing high basal activation levels of the kinase even in the absence of a growth-factor induced dimerization.

Considering that there is an excellent correlation between the stabilizing network of interactions and the clinically identified activating mutations in ErbB1, our structural studies on kinase activation are well poised to forecast the mutation landscape associated with other ErbB members. We have extended the analysis we have presented for ErbB1 to ErbB2 and ErbB4 kinases in which we have identified similar networks of stabilizing interactions. Based on the similarities between the stabilizing interactions between ErbB1 and ErbB4 kinase domains, we can predict the effect of analogous mutations...
in ErbB4 on kinase activation: (1) based on the location of the mutations E690G, G700S, the mutants are expected to be activating through directly impacting dimerization (similar to the activating mutants E685G and G695S of ErbB1). (2) Del 728–G733 ins S and S749I are poised to cause a conformation shift of the αC-helix of ErbB4 and hence are predicted to be activating. (3) Mutations F740A, L839R and L842Q in ErbB4 are poised to perturb the bond network of the inactive kinase and hence are expected to be activating. We note that in support of our predictions of ErbB4, the F740A and L839R mutants have been tested independently by Qiu et al.; and were indeed found to be activating. Based on the subtle differences we have noted in the stabilizing bond networks of ErbB1 and ErbB4, we are also able to suggest new activating ErbB4 mutations (that do not have an obvious counter-part in the ErbB1 system). In particular, R841 in ErbB4 is featured prominently in the stabilizing network and mutating R841 to either alanine or aspartic acid is expected to promote activation. Mutation of the two residues blocking the catalytic aspartate, E743 and G838, to residues with smaller side chains, alanine or glycine, is also expected to promote activation. Thus, with experimental validation of the above predictions, we believe that our approach can be valuable for evaluating the likely effect of mutations on ErbB2 inhibition efficacies in cancer, and ErbB4 inhibition in cardiac development and schizophrenia.

Differential ErbB1 signaling due to substrate specificity and a branched signaling model for transcribing the effects of molecular alterations into downstream signal activation

The preferential binding characteristics of different cytosolic substrates to different phospho-tyrosine locations of ErbB family kinases were reported recently. The variations in the phosphorylation kinetics associated with the different tyrosine sites of the cytoplasmic C-terminal tail of the ErbB1 kinase can induce differential patterns of downstream signaling leading to differences in the activation of key transcription factors. This leads us to hypothesize that the clinically identified activating mutations of ErbB1 kinase can also potentially influence cellular homeostasis by directly altering the phosphorylation kinetics of ErbB1 substrate tyrosines. Indeed the identity-specific phospho-tyrosine kinetics for Y1068 and Y1173 (as well as other phospho-tyrosine sites in ErbB1) for wildtype, L834R and del L723–P729 ins S mutant systems are supported by the kinetic experiments of Mulloy et al.

In particular, the relative catalytic turnover $(\lambda = \frac{k_{cat}}{K_m}$, where $k_{cat}$ represents the rate of tyrosine phosphorylation in the bound complex, and $K_m$ represents the affinity of the tyrosine substrate to the ErbB1 kinase) rates of Y1068 phosphorylation and Y1173 phosphorylation measured in the experiments are as follows. For Y1068, wildtype: $\log_{10}[Z_{\text{wildtype}}/Z] = 0.0$, L834R: $\log_{10}[Z_{\text{wildtype}}/Z] = 0.0$; del L723–P729 ins S: $\log_{10}[Z_{\text{wildtype}}/Z] = -1.5$. For Y1173, wildtype: $\log_{10}[Z_{\text{wildtype}}/Z] = 0.0$, L834R: $\log_{10}[Z_{\text{wildtype}}/Z] = -1.0$; del L723–P729 ins S: $\log_{10}[Z_{\text{wildtype}}/Z] = +0.5$. The structural basis for the context-specific kinetics of the C-terminal tyrosine substrates is provided by our computational docking calculations: substrate peptides derived from tyrosine sites of the ErbB1 C-terminal tail—Y1068 (VPYEINQ) and Y1173 (NAEYLVR)—bind to the wildtype and the L834R mutant ErbB1 kinase revealed how the structure of the bound peptide–protein complex is altered at the catalytic site due to the arginine substitution of leucine in L834R.

In order to translate differences in substrate specificity into tangible differences in the downstream response (ERK and Akt activation), we introduced a branched signaling model for ErbB1, see Fig. 1b, that features two parallel phosphorylation pathways corresponding to Y1068 and Y1173. Based on the results of ref. 23 we developed a molecularly resolved systems model in which phosphorylated Y1068 binds only to Gab-1 and Grb2 and not Shc, and...
phosphorylated Y1173 binds only to Shc and not to Gab-1 and Grb2 as depicted in Fig. 1b. We were then able to reparameterize the model based on the identity-specific phospho-tyrosine kinetics of Y1068 and Y1173 for wildtype and mutant (L834R and del L723–P729 ins S) ErbB1 based on the relative catalytic turnover (λ) rates.12,24 We were also able to extend this model for ErbB1 kinase inhibition upon treatment with small molecule inhibitor erlotinib in wildtype and mutant, again based on experimentally available inhibitor/ATP affinity data.20

Using the different parameter values corresponding to wildtype, L834R, and del L723–P729 ins S mutant systems, we ran network simulations for different levels of EGF stimulation and ErbB1 expression levels. The parameters explored were: normal receptor expression (initial concentration of ErbB1 [ErbB1] = 100 nM or 300 000 receptors per cell), and over-expression of ErbB1 (initial [ErbB1] = 1000 nM or 300 000 receptors per cell), no EGF stimulation, and 8 nM (50 ng ml−1) EGF stimulation. Based on the altered Kd and kcat values derived form Mulloy et al.24), the L834R had a stronger preference for both Y1068 and Y1173 phosphorylation compared to the wildtype receptor, while the del L723–P729 ins S mutant showed increased Y1068 and decreased Y1173 phosphorylation. To gauge the downstream effects of differential signaling through Y1068 and Y1173 phosphorylation sites of ErbB1, we calculated the levels of ERK-(p) (phosphorylated ERK) and Akt-(p) (phosphorylated Akt) in our system simulations in response to changes in the phosphotyrosine kinetics (λ values) of Y1068 and Y1173. Table 1a summarizes our simulation results where each entry corresponds to the peak level of phosphorylation over a simulated time of 900 s. The effect of altered affinities of the Y1068 and Y1173 sites to the catalytic domain of ErbB1 is that the L834R under normal ErbB1 expression exhibits differential downstream response, i.e., a pronounced decrease in ERK activation (~5-fold) and relatively smaller decrease Akt activation (~15% decrease). The del L723–P729 ins S mutant, however, shows sustained ERK as well as Akt activation relative to wildtype. For ErbB1 over-expressed cells, both ERK and Akt activation characteristics show relative insensitivity to ErbB1 as a result of signal saturation. The trends in Table 1a also show that the mutants can continue to signal even in the absence of the growth factor. In addition, the mutant signaling can be different due to changes in the ATP affinity. However, neither of these factors introduce any differential characteristics (in terms of preferring Y1068 to Y1173); i.e., each factor impacts the overall activation levels of ERK and Akt uniformly. Our calculated responses agree with the qualitative experimental observations of Sordella et al.19 and Tracy et al.25 i.e., the preferential activation of Akt in L834R and del L723–P729 ins S mutant cell lines predicted from our simulations is consistent with the reported experiments.19,24–26

We also examined the sensitivity of downstream signaling molecules ERK-(p) and Akt-(p) to inhibition by a range of erlotinib (ErbB1 inhibitor) concentrations. Specifically, we compute the EC50, which is the inhibitor concentration at which 50% of the phosphorylation activity is suppressed in the cellular context, see Table 1a. Under normal expression levels of ErbB1, we predict nearly a 7-fold decrease in EC50 for ERK-(p) inhibition for L834R (EC50 = 100 nM) compared to wildtype (EC50 = 700 nM) with and without EGF ligand present (and a 4-fold decrease for over-expressed ErbB1). With respect to Akt activation, we report a 4-fold decrease in EC50 for L834R compared to wildtype (300 nM vs. 1200 nM) with and without ligand present15 (and a 10-fold decrease for over-expressed ErbB1). These results are consistent with several experimental results that have reported the effect of the closely related inhibitor gefitinib on normal and non-small cell lung cancer cells,10,24–26 providing a mechanistic basis for the inhibitor efficacy in mutant cell lines. This agreement supports the notion that the branched signaling in our model can indeed represent a possible mechanism for preferential down-stream activation. In summary, we find that the mutant cell line L834R is more susceptible to inhibition through curbing downstream (ERK and Akt) activation. Considering that the absolute Akt-(p) levels are 5-fold higher than those for ERK-(p) in the wildtype and 20-fold higher in the mutant, see Table 1a, the gain in efficacy with respect to Akt inhibition may be a crucial difference between the wildtype and the mutant cell lines. We discuss this aspect below.

Clinical implications from the multiscale modeling of ErbB receptor signaling

One of the conditions for cellular homeostasis can be viewed as a balance between pro-survival signals and death-inducing signals, both being triggered and balanced by a variety of interacting intracellular pathways. Recently, using a simplified model for the effect of Akt activation on cell response,§ we showed that preferential Akt activation is conducive for the cell to rely on (be “addicted to”) the most efficient Akt-(p) generating pathway for generation of pro-survival signals while requiring the generation of death-inducing signals from other pathways. Our simplified model illustrates a mechanism by which inhibiting the dominant source of the pro-survival signals shifts the cellular homeostasis to a cellular state devoid of pro-survival signals, thereby providing grounds for a remarkable inhibitor sensitivity.15

We hypothesized that the mechanisms that lead to inhibitor hypersensitivity (as well as resistance) attack points of network hypersensitivity and fragility. Since preferential Akt activation is a hallmark of the hyper-sensitive mutants and the efficacy of the inhibitors, we determine through a global sensitivity analysis3,4,15 the combinations of model parameter perturbations that drive enhanced production of Akt-(p) and ERK-(p). Table 1b reports the components (rate constants and initial concentrations) of the top 3 principal eigenvectors derived

§ The phenomenological model can be summarized by: Akt ↔ Akt-(p) → S, with equilibrium constant K1 = [Akt-(p)]/[Akt] and rate constant k1; D-ind → D, with rate constant k2; Akt-(p) + D-ind ⇌ Akt-(p)•D-ind with equilibrium constant K2 = [Akt-(p)•D-ind]/[Akt-(p)][D-ind] and can be solved analytically. In this model, the cellular states S and D denote survival and death and D-ind denotes a death-inducing factor. The three reactions capture the effect of Akt on cell response, i.e., Akt activating survival pathways and simultaneously inhibiting death-inducing pathways.
from the parameter variations in our network in Fig. 1b. Perturbing the components of these principal eigenvectors (i.e. the entries in Table 1(b)) produces maximum changes to the Akt-(p) and ERK-(p) levels. Interestingly, there is a striking correlation between the components of Table 1(b) resulting from our model sensitivity analysis and patterns of oncogenic mutations and mechanisms of drug resistance found in clinical studies. High frequency of mutations of PI3K, Ras (a GTPase named as an acronym for rat sarcoma), Gab-1, MEK (mitogen activated protein kinase kinase), Raf (a serine/threonine kinase which activates MEK) have all been observed in several human cancers. Moreover, it has been established in screened breast and colorectal cancer patients that the Gab-1, MEK, and Ras mutations are non-random and likely arise from selective evolutionary pressures that give the cancer cells a survival advantage. With reference to the hyper-sensitive ErbB1 mutants found in non-small cell lung cancer patients, the perturbation of the phosphotyrosine kinetics of Y1068 and Y1173 through mutations (L834R and del L723–P729 ins S) is directly responsible for the differential signaling leading to preferential Akt activation, as we have shown in Table 1a. Other sensitive quantities reported in Table 1b also have direct relevance to hyper-sensitive signaling and drug resistance in the L834R and del L723–P729 ins S mutant cell lines. The inhibitor concentration is the most obvious and is generally depleted in the cells via multi-drug resistance mechanisms involving drug efflux pumps. The restoration of signaling via reduction of $K_i$ of the inhibitor and the simultaneous enhancement of $K_m$ associated with ATP binding has also been reported through a double mutation of L834R/T766M. This double mutant increases receptor phosphorylation (Y1068 and Y1173) kinetics 100-fold while simultaneously decreasing inhibitor affinity. Another drug resistance mechanism related to Y1068 kinetics (i.e. by circumventing Y1068 involvement and restoring downstream signaling through an alternative branch) has been identified: in the presence of ErbB3, a branch of signaling analogous to that through Y1068 becomes available through ErbB1-3 heterodimerization directly resulting in PI3K recruitment on ErbB3 and subsequent Akt activation. Indeed phosphorylation of ErbB3 by the Met receptor kinase (Met receptor tyrosine kinase is a high-affinity transmembrane receptor for the hepatocyte growth factor), due to over-expression of the Met receptor leads to drug resistance to gefitinib/erlotinib treatment (inhibition) of ErbB1. A drug resistance mechanism involving the change in expression of the phosphatase associated with Akt has also been identified to restore Akt-(p) levels upon inhibitor treatment. Roles for the phosphatase for ERK and the multimeric complex [ErbB1 • Shc • Grb2 • SOS • RasGTP] (which are our remaining predictions from Table 1b) in enhanced signaling and/or drug resistance have not yet been established experimentally.

### Conclusion and future outlook

We have employed a multiscale modeling platform to study ErbB1-mediated signal transduction, to help rationalize and integrate the collective results emerging from structural, biochemical, cell biological, and clinical studies. At the molecular level, our results suggest that the clinically identified mutations of ErbB1 kinase induce network fragility in the stabilizing interactions of the inactive kinase conformation, thereby providing a persistent stimulus for kinase activation even in the absence of any growth factor. At a cellular level, parameter perturbations driving network hypersensitivity through the enhancement of phosphorylated ERK and Akt levels show a striking correlation with observed mutations of specific proteins in oncogenic cell lines as well as the observed mechanisms of drug resistance to ErbB1 inhibition. Therefore, subject to the well-appreciated limitations of computational modeling, i.e., uncertainty in network topology and parameters, neglect of molecular cross-talk, autocrine loops, we suggest that cascading mechanisms of network hypersensitivity/fragility at multiple scales enable molecular-level perturbations (clinical mutations) to induce oncogenic transformations and mechanisms of drug resistance. Moreover, our results describe a possible mechanism of signal branching leading to preferential activation of downstream molecules (Akt) in the ErbB1 activating mutants. This preferential activation of a survival factor makes the cell-lines harboring the mutant receptors to be conducive to oncogenic addiction, i.e. reliance on the L834R or del L723–P729 ins S ErbB1-mediated generation Akt-(p) for survival signals. The survival pathway addiction also results in a remarkable sensitivity to ErbB1 kinase inhibition.

A more complete model description, which we are currently pursuing, will not only require resolving the differential characteristics of all of the tyrosine phosphorylation sites in ErbB1 (Y992, Y1086, Y1114, Y1148 Gab1:Y627, Shc:Y317, phosphoinositide-specific phospholipase Cγ or PLCγ1:Y771) and their associated substrate recognition properties in ErbB1 (i.e., exploring the interactions of Gab1, Shc, and PLCγ1 binding to phosphotyrosines of ErbB1 (see recent work by Kholodenko et al.), but also the extension to other Erb family members in the context of homo- and hetero-dimers. These extensions are especially crucial because trans-activation of ErbB1 occurring through ligand-induced receptor heterodimerization combined with a potential for differential signaling adds a palette of finer control elements in the ErbB-family signaling network. This view is further bolstered by the over-expression of ErbB3 in drug resistance to ErbB1 targeting (as discussed above) and mutations and over-expression of ErbB2 in different cancers. Moreover, the internalization mechanism of receptors via endocytosis, which we have not considered in our model, features in crucial regulatory roles; attenuation of endocytosis leading to impaired deactivation of receptor tyrosine kinases is linked to hyper-proliferative conditions such as cancer. Hendriks et al. proposed a mechanism of differential signaling and preferential activation of Akt in ErbB1 mutants based on reduced internalization rates of ErbB1 mutants (relative to wildtype). Hence we are developing the orchestrated vesicular assembly (OVA) model, which will serve as a multiscale, spatially-resolved model for describing the ErbB1 receptor internalization through endocytotic vesicle formation. In the OVA model, the
membrane dynamics are represented by the time-dependent Ginzburg Landau formalism, and the dynamics of accessory proteins, including the curvature-inducing protein, epsin, are represented by the kinetic Monte Carlo formalism. The integration of the two formalisms (Fig. 1c) is described in ref. 12 and 17. Protein–protein interactions and protein–membrane interactions are considered using coarse-grained potentials, and a phenomenological model for the effect of a clathrin coat is employed and the treatment of the membrane to allow for extreme deformations is through the surface evolution approach (see inset in Fig. 1c) (unpublished results). Through the OVA model simulations, we propose to address the biological question of how the cell stages the endocytosis nucleation event to specific sites on the membrane, i.e. to the site of a phosphorylated receptor protein; in particular, how does the network of accessory proteins identify the site of nucleation and synchronize with the receptor phosphorylation. A comprehensive multiscale model for ErbB activation and internalization can then be achieved by integrating the OVA model, Fig. 1c, with the network model we have outlined in Fig. 1b.

At the molecular level, considering that there is an excellent correlation between the stabilizing network of interactions and the clinically identified activating mutations in ErbB1, our structural studies on kinase activation are well poised to forecast the mutation landscape associated with other ErbB members. Indeed based on our simulations of ErbB2 and ErbB4, we have identified similar networks of stabilizing residues and are already able to predict activating mutations (data not presented) in these receptors that have not yet been reported clinically, which together with the extensions proposed above can be valuable for evaluating the likely effect of mutations on ErbB2 inhibition efficacies in cancer, and ErbB4 inhibition in cardiac development and schizophrenia.\textsuperscript{11}

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