Engineering GPCR signaling pathways with RASSLs

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May. 28, 2015
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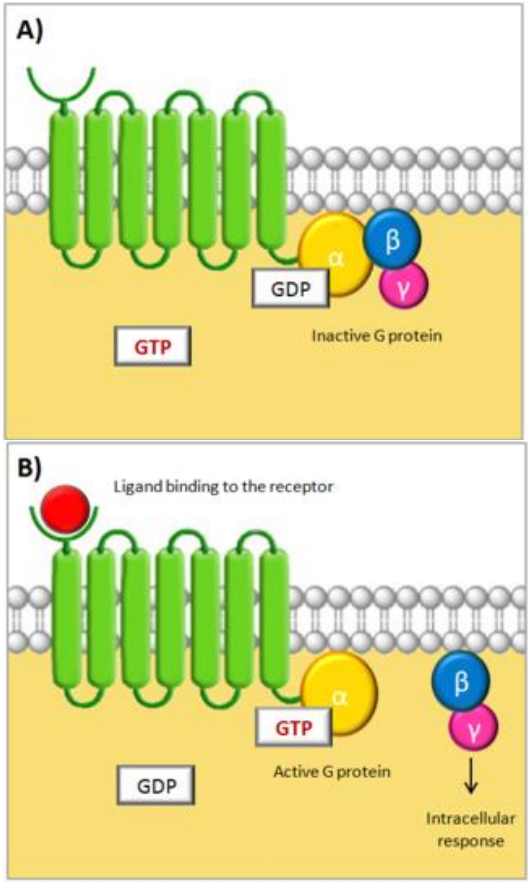
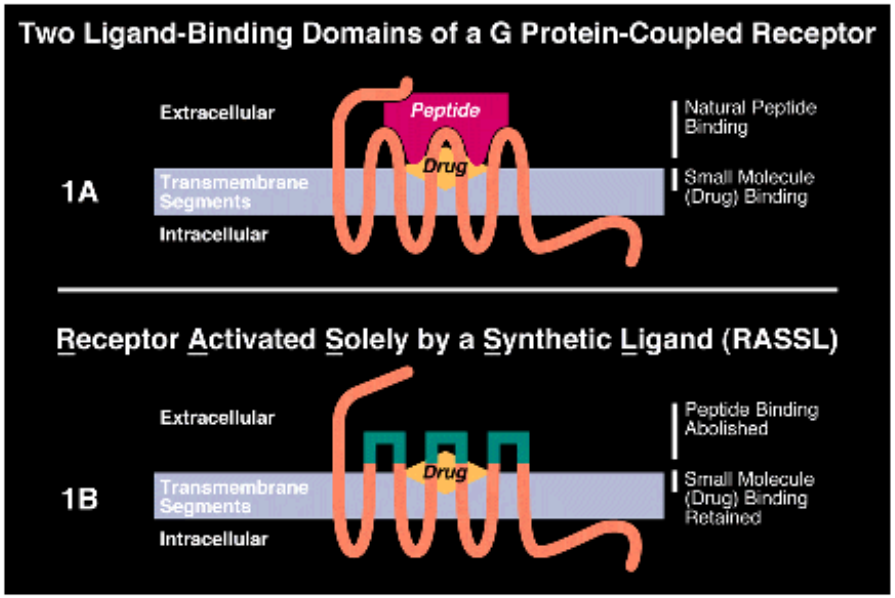
Receptors activated solely by synthetic ligands (RASSLs)

- G-protein-coupled receptors (GPCRs)
- RASSLs are engineered GPCRs
- Unresponsive to endogenous ligands but can be activated by nanomolar concentrations of synthetic small molecules
- Exists for three major GPCR signaling pathways – G_s, G_i , G_q
- Different names
  - Therapeutic receptor-effector complexes (TRECs)
  - Neoceptors
  - Designer receptors exclusively activated by designer drugs (DREADDs)





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NATURE METHODS | VOL.5 NO.8 | AUGUST 2008 | 673

Engineering GPCR signaling pathways with RASSLs

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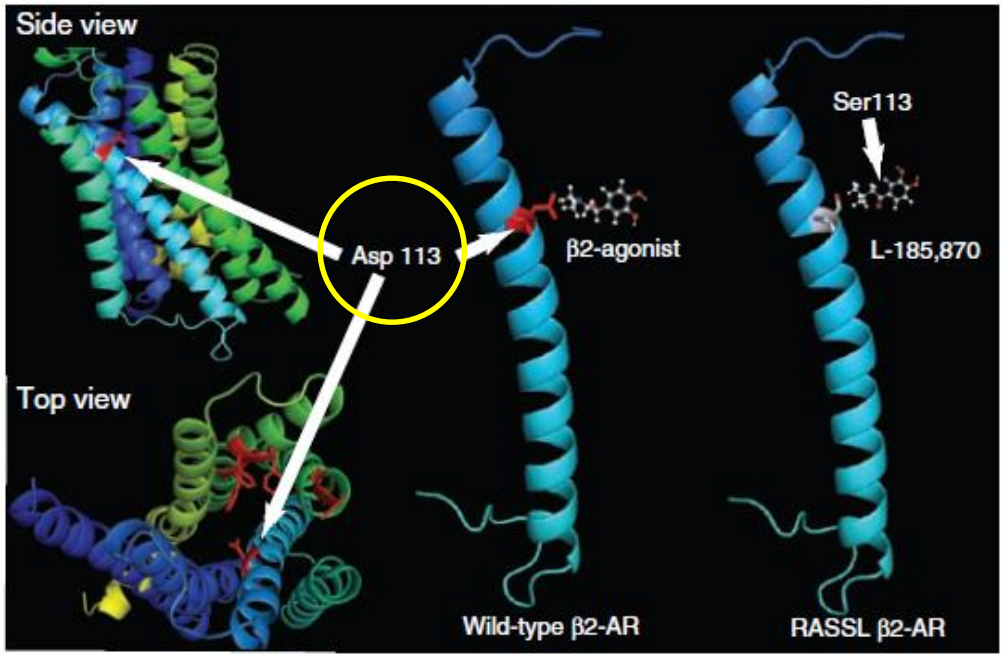
Research Interests

- Chemical-Genetics: uses directed molecular evolution to create GPCRs which are suitable for remotely controlling cellular signaling
- Chemical biology and the receptorome: massively-parallel physical screening of the GPCR

First attempt of designer GPCR – directed mutagenesis (1)

1. β2-adrenergic receptor (β2-AR) mutant



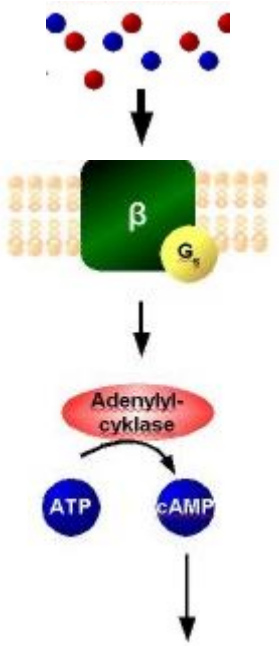
Asp113: critical for binding terminal amine groups conserved among endogenous GPCR

- Targeted mutagenesis of aspartic acid to serine greatly reduced activation by endogenous amines (Strader et al., 1991, J Biol Chem)
- But enabled butanone derivative (L-185,870) to activate the mutant receptor

However,

Low potency (EC_{50}=118μM)
Low affinity of synthetic agonist → Impractical for *in vivo* use
Unknown pharmacokinetics

Adrenalin, Noradrenalin



Heart muscle contraction, smooth muscle relaxation, glycogenolysis

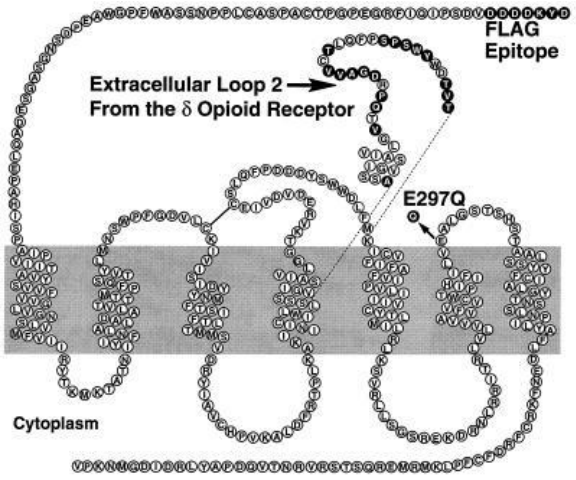
(Conklin et al., 2008, Nat Methods)

First attempt of designer GPCR – directed mutagenesis (2)

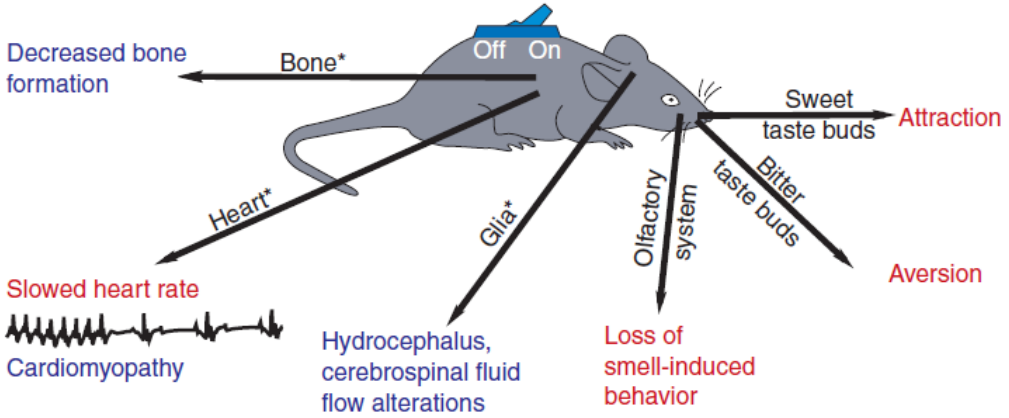
2. Using potent synthetic drugs developed

K-opioid receptor (KOR) agonists (e.g., spiradoline)

: Mediates effects that include altering the perception of pain, consciousness, motor control, and mood



(Coward et al., 1998, PNAS)



- First RASSL activated by agonist with nanomolar affinity
- Diverse phenotypes induced in transgenic animals
  - ligand-dependent: heart-rate modulation, bitter and sweet taste sensations
  - ligand-independent: cardiomyopathy, hydrocephalus, osteopenia
- RASSLs for existing drugs emerged:
  5-HT_4 serotonin, β2-adrenergic, H_1-histamine, A3 adenosine, 5-HT_{2A}-serotonin, MC4-melanocortin receptors

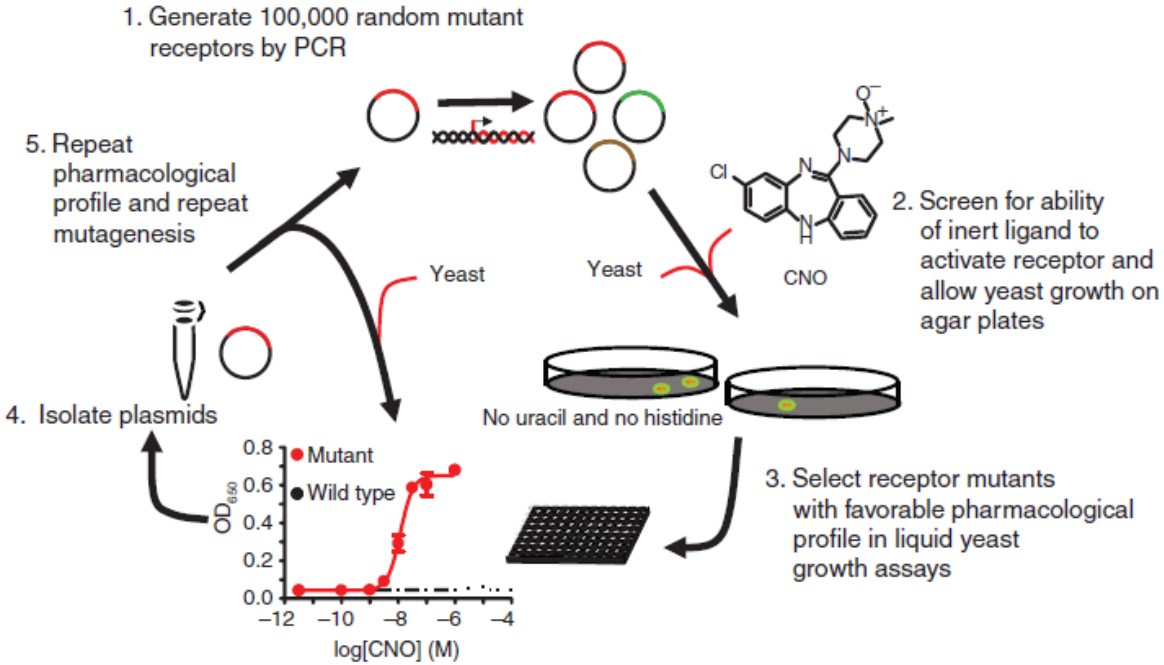
(Conklin et al., 2008, Nat Methods)

*** Limitations**

- Low affinity
- Profound phenotypes induced by constitutive activity upon RASSL overexpression *in vivo*
- Repetition of directed mutagenesis did not regularly yield receptors with ideal agonist affinities

Second generation RASSLs by yeast mutagenesis system (DREADD)

- Creating new RASSLs



- Screening for mutant receptors in yeasts
- Selecting mutants that lost the ability to respond to the natural ligand acetylcholine
- But gained the ability to respond with nanomolar potency to clozapine-N-oxide (CNO)
- DREADDs

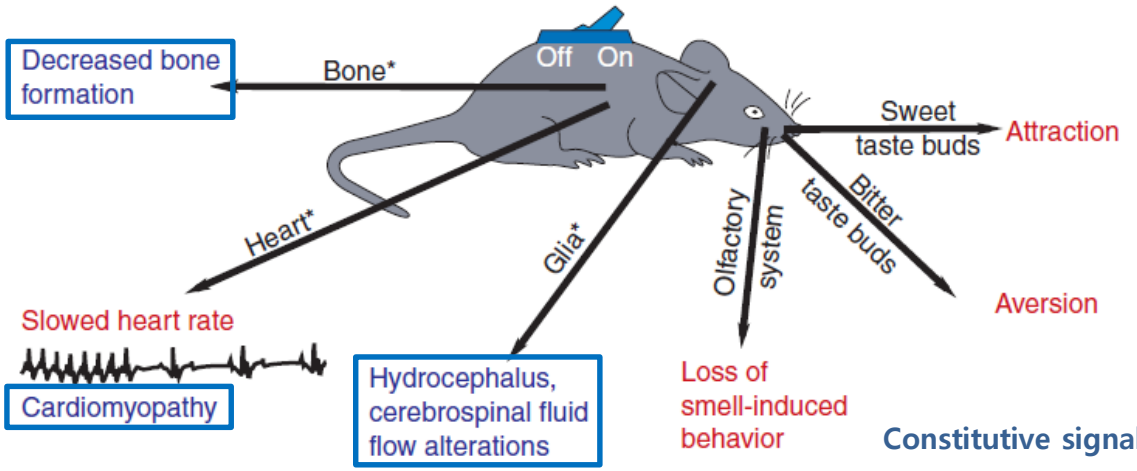
- G_i-coupled RASSLs led to hippocampal neuron silencing by Gβγ-mediated activation of G-protein inwardly rectivying K^+ (GIRK) channels

Name	Alternative names: notable versions	Signaling notes	Agonists (relative affinity)	Antagonists (relative affinity)	*In vivo* phenotypes
hRMD-q	DREADD hM3D: human M_3 muscarinic receptor with point mutations	G_q signaling	CNO (low nanomolar)	Atropine (reduced affinity versus wild type; high nanomolar)	None known
hRMD-i	DREADD hM4D: human M_4 muscarinic receptor with point mutations; M_2 receptor RASSL is also Gi coupled	G_i signaling	CNO (low nanomolar)	Atropine (reduced affinity versus wild type; high nanomolar)	Inhibits electrical signaling in brain slices
rRMD-s	DREADD RASSL2: rat M_3 receptor with point mutations and intracellular loops from the turkey β1-AR	G_s signaling	CNO (low nanomolar)	Atropine (reduced affinity versus wild type; high nanomolar)	None known

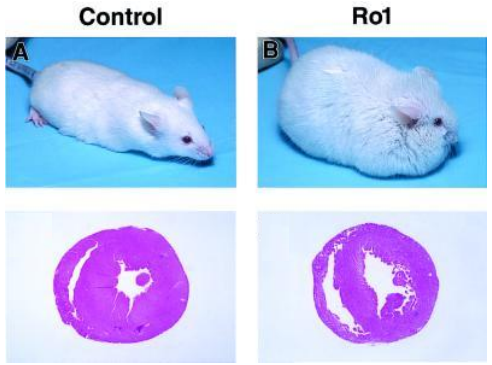
(Conklin et al., 2008, Nat Methods)

Constitutive signaling of RASSLs

First generation RASSLs with constitutive signaling have profound effects



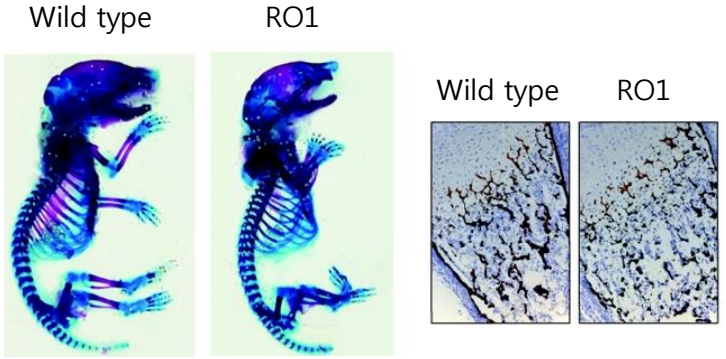
- Chronic expression of RASSLs in cardiomyocytes causes cardiomyopathy



(Redfern et al., 2000, PNAS)

- Overexpression of RASSLs in osteoblasts reduced skeletal mineralization, especially evident in the ribs and vertebrae.

RO1: modified Gi-coupled GPCR



(Peng et al., 2008, Endocrinology)

- RASSLs with different levels of constitutive activity will be required to recapitulate normal GPCR functions
- Second generation RASSLs lack constitutive activity → useful to study ligand-dependent effects

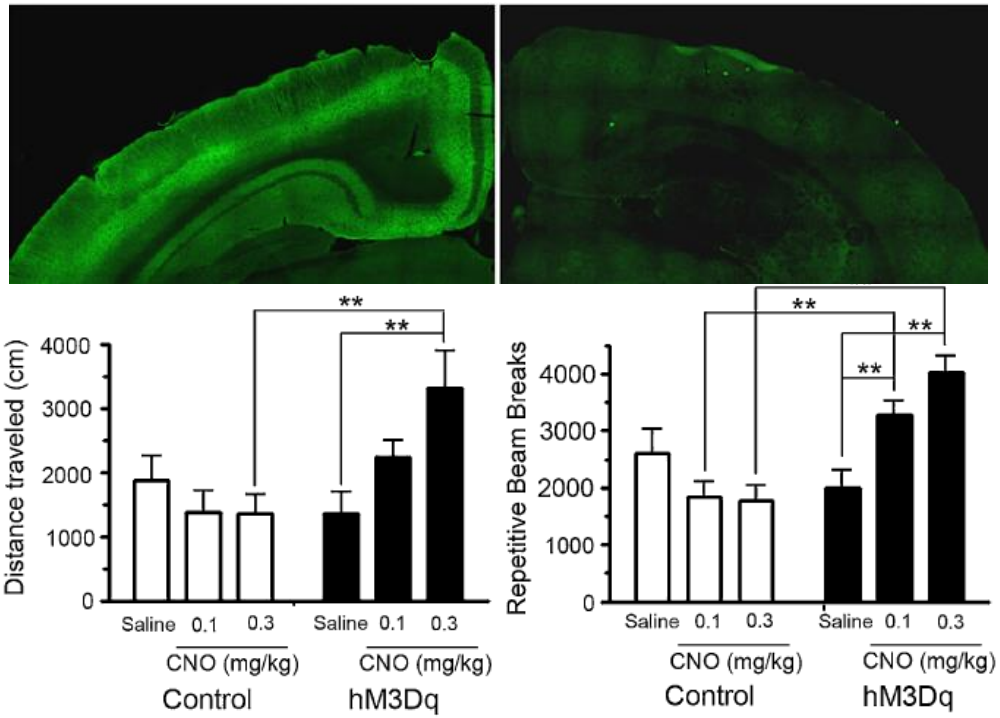
(Conklin et al., 2008, Nat Methods)

Future directions of RASSLs

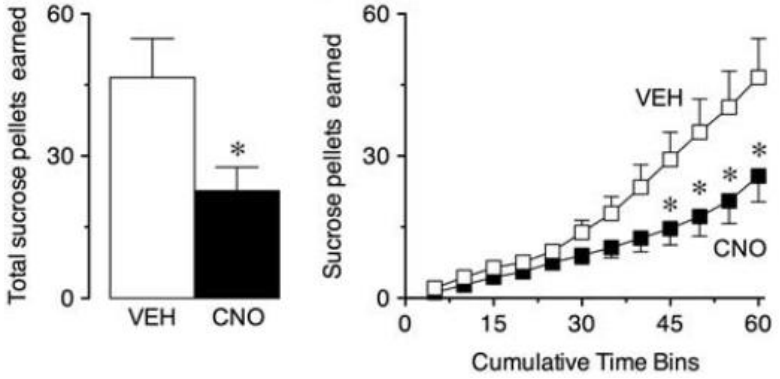
◆ Challenges to ideal RASSLs

- RASSLs that respond to a clinically approved, biologically inert drug
- RASSLs coupled to each of the GPCR pathways (noncanonical pathways including arrestins, GRKs, and intracellular kinases)
- RASSLs with different constitutive responses and desensitization properties

◆ *In vivo* applications



(Alexander et al., 2009, Neuron)



(Ferguson and Neumaier, 2012, Neurophycopharmacology)

- Activation of G_q-coupled receptor by CNO in transgenic mice increased locomotion, stereotypy, and limbic seizures
- Activation of virally injected G_i-coupled RASSL by CNO in the dorsal striatum of rats impaired action-outcome learning
  → Dysregulation of striatal circuits may contribute to the reinforcement learning and aberrant reward

Summary and Discussion

Summary

- RASSLs are engineered GPCRs that are unresponsive to endogenous ligands but can be activated by nanomolar concentrations of synthetic small molecules

	Name	Alternative names: notable versions	Signaling notes	Agonists (relative affinity)	Antagonists (relative affinity)	*In vivo* phenotypes
1st generation	hRO-i	Ro1: human κ-opioid receptor with δ-opioid extracellular loop and N-terminal Flag tag hRO3-i (RO3 or Rog): same as Ro1, but with point mutation and N-terminal GFP tag	G_i signaling, constitutive in heart but no apparent constitutive signaling in cultured cells	Spiradoline (low nanomolar) Salvinorin A (low nanomolar)	Nor-binaltorphimine-dilhydrochloride; Nor-BNI (low nanomolar)	Decreased heart rate, cardiomyopathy, decreased bone formation, induced sweet and bitter taste
	hRS-s	Rs1: human serotonin 4 receptor with point mutation and N-terminal Flag tag hRS2-s (Rs2): same as Rs1 but has N-terminal GFP tag	G_s-signaling with high constitutive activity	RS67333, RS39604, cisapride, ML10375, GR113808 (nanomolar)	None known	Massive increase in bone formation
	hRMC-s	RM1: human melanocortin 4 receptor with point mutations	G_s constitutive signaling essential for native receptor function	Tetrahydroisoquinoline (low nanomolar)	None known	None known
2nd generation	hRMD-q	DREADD hM3D: human M_3 muscarinic receptor with point mutations	G_q signaling	CNO (low nanomolar)	Atropine (reduced affinity versus wild type; high nanomolar)	None known
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(Conklin et al., 2008, Nat Methods)

Discussion

- GPR54, a G_q-coupled GPCR, is the familiar GPCR in GnRH neurons as kisspeptin receptor
- Mutations of GPR54 disturb puberty and cause infertility in rodents and humans.
- In a GPR54 knockout condition, using G_q-coupled RASSLs (hRMD-q) and CNO in GnRH neurons may replace the activity of GPR54 and kisspeptin

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