High throughput screening for chemical inhibitors of mammalian adenylyl cyclases expressed in fission yeast

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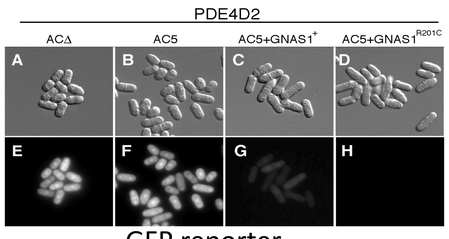
Abstract

The fission yeast *Schizosaccharomyces pombe* is an ideal host for high throughput screens (HTSs) to identify inhibitors of heterologously-expressed mammalian proteins involved in cyclic nucleotide metabolism. This is due to the following: 1) PKA is not essential in *S. pombe*, 2) PKA activity dramatically affects growth, mating and transcription of the *fbp1* gene, for which several reporters have been developed, 3) phenotypic screens in *S. pombe* are inexpensive and readily identify compounds that are both active against the target protein and are permeable to mammalian cells, 4) target identification is relatively straightforward using strains that express the fission yeast homolog of the target protein. Our previous HTSs using *S. pombe* strains expressing mammalian phosphodiesterases, identified compounds that are biologically active in mammalian cell culture and can be used as tool compounds or lead compounds for drug development. We have now successful deployed both GFP- and luciferase-based screens for mammalian adenylyl cyclase (AC) inhibitors using *S. pombe* strains that express mammalian ACs together with a mutationally-activated human GNAS G_S protein [1]. These screens were carried out in 1536-well microtiter dishes, allowing for quantitative HTSs in which compounds are tested at multiple concentrations. By recording GFP signals with an Acumen laser cytometer, we avoided the significant background generated by soluble fluorescent compounds. Successful screens of 100,000 to 125,000 compounds (the NCATS Genesis library) were completed with strains expressing human AC4, AC7, and AC9. This library is composed of collections of molecules with shared scaffolds to aid in the identification of lead compounds that are amenable to medicinal chemistry to develop more effective and drug-like molecules. Follow-up assays of cAMP production in response to compound treatment have identified two functional scaffolds that are likely to act as direct inhibitors of mammalian transmembrane ACs. Given the challenge of using biochemical approaches to inhibitor development for these integral membrane proteins, this work represents a significant break-through in the discovery of AC inhibitors that are likely to be effective in mammalian cells.

Introduction

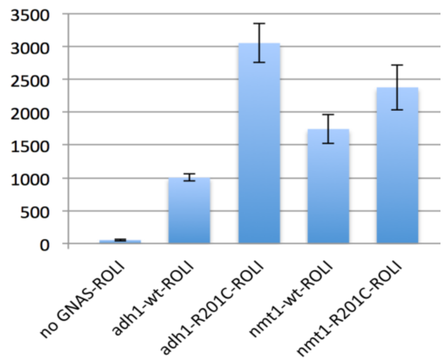
S. pombe strains expressing each of the ten mammalian ACs and lacking the *S. pombe git2/cyr1* AC were constructed. PKA-repressed *fbp1-GFP* and *fbp1-luciferase* reporters were used to detect both basal and $GNAS^{R201C}$-stimulated AC activity. In addition, cAMP levels could be measured directly by mass spectrometry as seen below.



GFP reporter

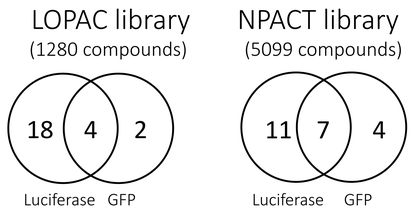
cyclase	AC7	AC7	AC8	AC8	AC9	AC9
GNAS	+	-	+	-	+	-
PDE	PDE1C4	PDE1C4	PDE1C4	PDE1C4	PDE4D2	PDE4D2
	51.3	779.5	20.5	2026.4	20.5	353.1
	57.1	1542.9	14.7	1657.1	14.7	590.5
Fold effect		21.4		104.7		26.8

Luciferase reporter



cAMP by mass spec

Pilot screens suggest use of the GFP screen as primary and luciferase as orthogonal screen



Fewer artifacts with GFP assay (using Acumen laser cytometer)

HTSs of NCATS Genesis Library

HsAC9- 100,000 compounds
Rn AC4- 125,000 compounds
HsAC7- 125,000 compounds

Strains also express $HsGNAS^{R201C}$, HsPDE4D2, and *fbp1-GFP* reporter

cAMP assays identify two active scaffolds

The Genesis library is composed of compounds obtained from a variety of vendors with the purpose of representing chemical scaffolds. cAMP assays identified two scaffolds that include multiple active compounds.

Most BCAC51 scaffold compounds are active

	5μM							
	AC4	SD	AC5	SD	AC7	SD	AC9	SD
BCAC51	0.31	0.05	0.29	0.05	0.68	0.24	0.32	0.17
BCAC52	0.27	0.21	0.43	0.29	0.52	0.21	0.62	0.34
BCAC53	0.15	0.13	0.20	0.17	0.73	0.28	0.28	0.11
BCAC54	0.26	0.31	0.45	0.28	0.82	0.44	0.37	0.15
BCAC57	0.31	0.23	0.69	0.34	0.67	0.21	0.37	0.15
BCAC61	0.11	0.05	0.54	0.38	0.35	0.33	0.63	0.57
BCAC62	0.07	0.05	0.28	0.07	0.30	0.14	0.30	0.13
BCAC63	0.24	0.22	0.45	0.38	0.52	0.35	0.43	0.05
BCAC64	0.36	0.10	0.66	0.23	0.80	0.41	0.53	0.13
BCAC65	0.27	0.19	0.71	0.59	0.60	0.23	0.42	0.10
BCAC66	0.19	0.14	0.65	0.62	0.60	0.15	0.42	0.07
BCAC67	0.17	0.14	0.48	0.26	0.37	0.36	0.61	0.14
BCAC69	0.62	0.23	1.40	0.61	1.05	0.57	1.22	0.62
BCAC71	0.82	0.33	0.84	0.24	0.93	0.40	0.74	0.11
BCAC72	1.08	1.08	1.08	0.53	1.17	0.56	0.72	0.17

cAMP levels were measured by mass spec 30 minutes after co-administration of test compound (5μM final) and 40μM Rolipram (to repress PDE4D2 activity). Values presented as a proportion of cAMP levels in DMSO + Rolipram controls and are the averages (and SD) from three independent experiments.

Fewer BCAC55 scaffold compounds are active

	5μM							
	AC4	SD	AC7	SD	AC5	SD	AC9	SD
BCAC55	0.26	0.10	0.57	0.20	ND	ND	ND	ND
BCAC58	0.26	0.16	0.61	0.35	0.34	0.04	0.47	0.22
BCAC59	1.30	0.35	0.97	0.60	1.89	1.97	0.59	0.25
BCAC60	1.01	0.44	1.56	0.87	0.86	0.35	0.67	0.28
BCAC68	0.68	0.08	0.64	0.39	1.02	0.23	0.99	0.45
BCAC73	0.89	0.54	1.30	0.43	1.16	0.66	1.09	0.30
BCAC74	0.89	0.64	0.96	0.49	1.42	0.72	0.77	0.06
BCAC75	1.20	0.31	0.98	0.55	1.17	0.21	1.00	0.53
BCAC76	0.98	0.34	0.91	0.58	1.16	0.73	0.97	0.42
BCAC77	1.13	0.34	0.75	0.29	1.21	0.57	1.04	0.66
BCAC78	1.30	0.45	1.44	0.70	0.74	0.29	1.89	1.01
BCAC79	1.46	0.77	1.05	0.60	1.02	0.11	1.24	0.53
BCAC80	0.34	0.14	1.04	0.29	0.49	0.19	1.45	0.69
BCAC81	0.53	0.33	0.56	0.34	0.31	0.28	0.47	0.12
BCAC82	0.94	0.29	0.91	0.48	0.55	0.32	1.62	0.77
BCAC83	0.73	0.16	1.11	0.49	0.66	0.29	2.14	1.24
BCAC84	1.65	0.33	1.03	0.39	0.85	0.51	1.79	0.54
BCAC85	0.84	0.69	0.68	0.44	0.80	0.04	1.74	1.06
BCAC86	1.68	0.41	1.65	1.00	0.98	0.10	1.06	0.34

Activity on two human cells lines is consistent with yeast-based assay data

HEK293T cells or CRL-11372 osteoblasts were treated with forskolin and rolipram for two hours to produce a ~25 to 40-fold elevation in cAMP levels. The reduction due to co-administration of the BCAC compounds (5μM) is shown as an average of six independent wells. Each cell line expresses multiple ACs. It is interesting to note the difference in the effect for some compounds when comparing the two cell lines.

BCAC51 series	HEK293T	CRL osteoblasts
BCAC51	0.57	0.87
BCAC52	0.74	0.79
BCAC53	0.69	0.70
BCAC54	0.42	0.87
BCAC57	0.74	0.74
BCAC61	1.07	0.49
BCAC62	0.26	0.46
BCAC63	0.26	0.37
BCAC64	1.07	0.68
BCAC65	0.61	0.36
BCAC66	0.50	0.29
BCAC67	0.56	0.27
BCAC88	ND	0.56
BCAC89	ND	0.52
BCAC55 series	HEK293T	CRL osteoblasts
BCAC55	1.43	ND
BCAC58	0.79	0.62
BCAC80	0.80	0.74
BCAC81	0.64	0.69
BCAC68	1.01	0.74

Conclusions:

- Mammalian ACs can be used to replace the *S. pombe* AC to regulate *fbp1* transcription
- HTSs in 1536 well plates (4μl cultures) can detect cell permeable AC inhibitors
- The BCAC51 scaffold appears to tolerate more substitutions than the BCAC55 scaffold

[1] Getz, R.A. et al. Cell Signal, 2019. 60: p. 114-121.