

Feedback regulation by Atf3 in the endothelin-1-responsive transcriptome of cardiomyocytes: Egr1 is a principal Atf3 target

Article

Accepted Version

Supplemental information

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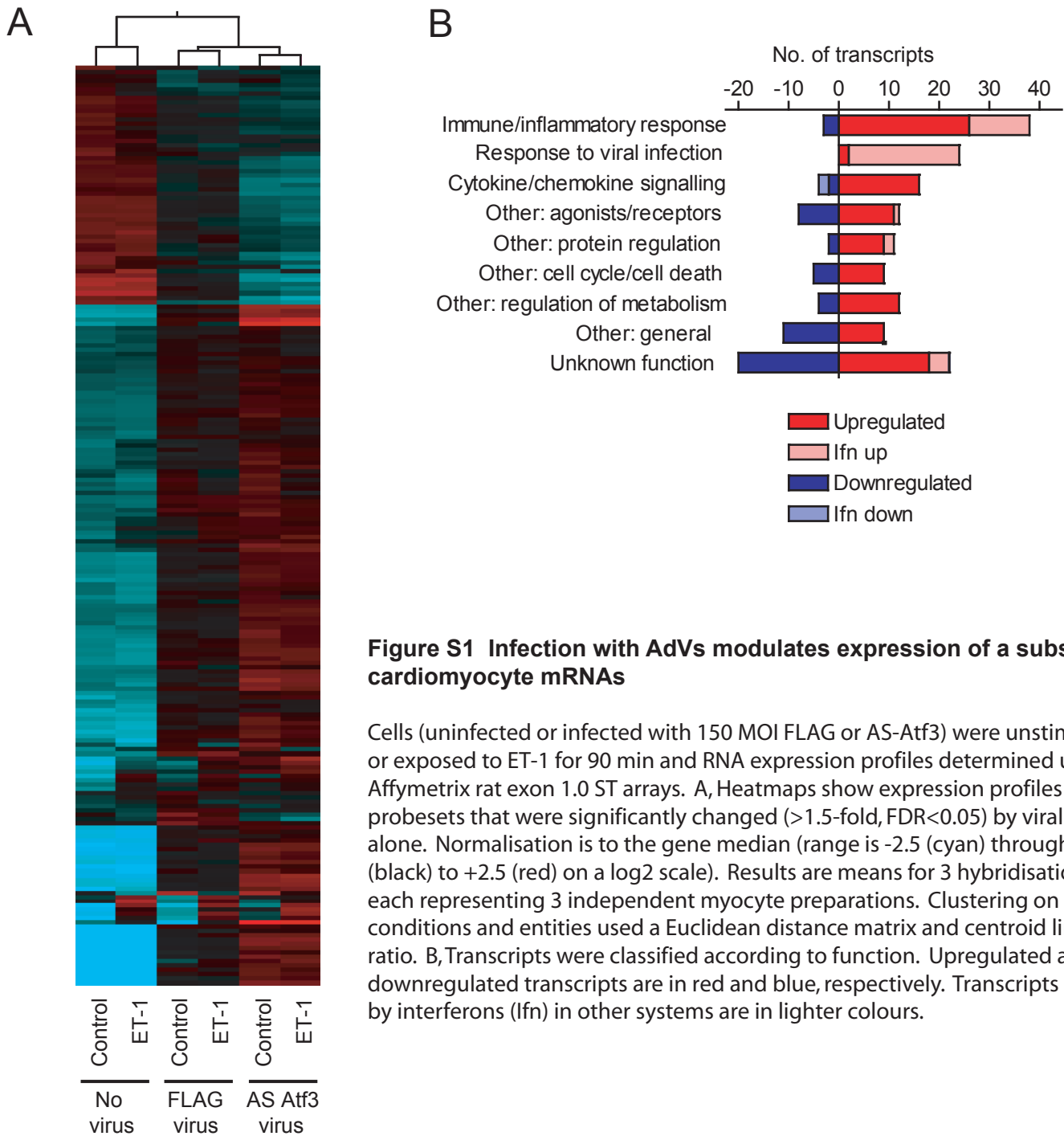


Figure S1 Infection with AdVs modulates expression of a subset of cardiomyocyte mRNAs

Cells (uninfected or infected with 150 MOI FLAG or AS-Atf3) were unstimulated or exposed to ET-1 for 90 min and RNA expression profiles determined using Affymetrix rat exon 1.0 ST arrays. A, Heatmaps show expression profiles for probesets that were significantly changed (>1.5 -fold, $FDR < 0.05$) by viral infection alone. Normalisation is to the gene median (range is -2.5 (cyan) through 0 (black) to $+2.5$ (red) on a \log_2 scale). Results are means for 3 hybridisations each representing 3 independent myocyte preparations. Clustering on conditions and entities used a Euclidean distance matrix and centroid linkage ratio. B, Transcripts were classified according to function. Upregulated and downregulated transcripts are in red and blue, respectively. Transcripts regulated by interferons (Ifn) in other systems are in lighter colours.

Supplemental Table 1 Response of cardiomyocyte transcriptome to adenoviral infection

Cardiomyocytes were uninfected (no virus) or infected with empty adenoviruses and gene expression profiles were examined using Affymetrix microarrays. The data were analysed using GeneSpring to identify transcripts that were significantly changed (>1.5-fold; FDR<0.05) by adenovirus infection. Transcripts are clustered according to known or probable function and are listed alphabetically with upregulated transcripts listed first. Results are means for n=3 independent hybridisations each representing 3 separate preparations of cardiomyocytes. * Transcripts identified as part of an interferon response in other systems.

Probeset	Genesymbol	Genedescription	Raw values (No virus)	Fold change induced by virus	
Antiviral response					
7196285	Adar	Adenosine deaminase, RNA-specific	204	1.80	*
7315869	Ddit3	DNA-damage inducible transcript 3	273	2.27	*
7301235	Ifi27	interferon, alpha-inducible protein 27	1785	1.98	*
7072322	Ifi35	Interferon-induced protein 35	91	1.97	*
7215722	Ifi44	Interferon-induced protein 44	126	3.96	*
7067089	Ifi47	Interferon gamma inducible protein 47	141	7.07	*
7041465	Ifit2	Interferon-induced protein with tetratricopeptide repeats 2	89	1.89	*
7041467	Ifit3	Interferon-induced protein with tetratricopeptide repeats 3	57	3.38	*
7170909	Iigp1	Interferon inducible GTPase 1	106	4.47	*
7067595	Irf1	Interferon regulatory factor 1	154	1.72	*
7131960	Irf9	Interferon regulatory factor 9	443	2.28	*
7092176	Mx1 2	Myxovirus (influenza virus) resistance 1/2	55	19.37	*
7087207	Mx2 1	Myxovirus (influenza virus) resistance 2/1	122	19.05	*
7098547	Oas1a/k	2'-5' oligoadenylate synthetase 1A/K	91	12.05	*
7102451	Oas1b/i	2-5 oligoadenylate synthetase 1B/i	55	6.03	*
7098544	Oas1i	2' -5' oligoadenylate synthetase 1I	73	5.81	*
7102456	Oas1k/a	2' -5' oligoadenylate synthetase 1K/A	97	1.80	*
7103432	Oas2	2'-5' oligoadenylate synthetase 2	41	3.58	*
7102992	Oasl	2'-5'-oligoadenylate synthetase-like	101	11.64	*
7103001	Oasl2	2'-5' oligoadenylate synthetase-like 2	84	9.95	*
7339147	Plscr1	Phospholipid scramblase 1	104	1.69	*
7305174	Rsad2	Radical S-adenosyl methionine domain containing 2	61	12.13	*
7055354	Trim5	Tripartite motif-containing 5	835	1.52	
7263822	Zc3hav1	Zinc finger CCCH type, antiviral 1	124	2.05	
Cytokine/chemokine signalling					
7073869	C1qtnf1	C1q and tumor necrosis factor related protein 1	189	1.58	
7356847	Ccl20	Chemokine (C-C motif) ligand 20	146	1.77	
7070340	Ccl7	Chemokine (C-C motif) ligand 7	1918	2.00	
7212626	Csf1	Colony stimulating factor 1 (macrophage)	122	1.84	
7071906	Csf3	Colony stimulating factor 3 (granulocyte)	220	2.80	
7116933	Cxcl10	Chemokine (C-X-C motif) ligand 10	37	5.21	
7116931	Cxcl11	Chemokine (C-X-C motif) ligand 11	472	1.81	
7123570	Cxcl13	Chemokine (C-X-C motif) ligand 13	132	1.69	
7055111	Il18bp	Interleukin 18 binding protein	155	1.92	
7260080	Il6	Interleukin 6	149	5.13	
7138335	Ripk3	Receptor-interacting serine-threonine kinase 3	151	1.71	
7362008	Stat1/4	Signal transducer and activator of transcription 1/4	906	2.26	
7311784	Stat2	Signal transducer and activator of transcription 2	157	3.36	
7216733	Tnf	Tumor necrosis factor (TNF superfamily, member 2)	94	2.19	
7327525	Tnfrsf11b	Tumor necrosis factor receptor superfamily, member 11b	1001	1.93	
7098502	Trafd1	TRAF type zinc finger domain containing 1	192	1.50	
7351276	Ccr1	Chemokine (C-C motif) receptor 1	123	0.53	
7041124	Il33	Interleukin 33	100	0.64	
7123902	Pf4	Platelet factor 4	704	0.59	
Immune/inflammatory response					
7149693	Bst2	Bone marrow stromal cell antigen 2	165	3.21	*
7093567	Cd80	CD80 molecule	80	2.20	
7093779	Cd86	CD86 molecule	106	1.63	
7216827	Cfb	Complement factor B	66	2.85	
7296860	Cmpk2	Cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial	72	7.20	*
7327552	Enpp2	Ectonucleotide pyrophosphatase/phosphodiesterase 2	127	2.31	
7107717	Fcgr3a	Fc fragment of IgG, low affinity IIIa, receptor	160	2.86	
7200128	Gbp2	Guanylate binding protein 2	101	7.94	*
7076940	Irgm	Immunity-related GTPase family, M	240	6.43	
7293880	Isg15	ISG15 ubiquitin-like modifier	64	14.88	*
7033289	Isg20	Interferon stimulated exonuclease gene 20	84	2.56	*
7270067	Klrl1	Killer cell lectin-like receptor subfamily K, member 1	28	1.72	
7084895	Lgals3bp	Lectin, galactoside-binding, soluble, 3 binding protein	319	3.79	*

7080131	Lgals5/9	Lectin, galactose binding, soluble 5/9	116	2.19	*
7080134	Lgals9/5	Lectin, galactoside-binding, soluble, 9/5	162	4.47	*
7069999	Nos2	Nitric oxide synthase 2, inducible	65	1.87	
7266324	Reg3g	Regenerating islet-derived 3 gamma	1758	1.57	
7216994	RT1 class Ia, locus A2/A1 locus A3 RT1 class Ib, locus EC2 MHC class I RT1.Aa alpha-chain		289	2.02	
7220575	RT1 class I, locus1 RT1 class I, locus CE12/CE14		271	2.24	
7216676	RT1 class I, locus CE10/CE7/CE11		164	1.72	
7224452	RT1 class I, locus CE11/CE7 RT1 class Ib, locus EC2		271	2.00	
7224458	RT1 class I, locus CE12/14 RT1 class I, locus1		244	2.31	
7224429	RT1 class I, locus CE13/CE14		102	1.78	
7224511	RT1 class I, locus CE15		422	1.76	
7220557	RT1 class I, locus CE3/A3 RT1 class Ia, locus A1/A2 RT1 class Ib, locus EC2		116	1.75	
7220541	RT1 class I, locus CE5/CE4 RT1 class Ib, locus EC2 MHC class I RT1.Aa alpha-chain mature alpha chain of MHC class Ib protein-like		327	2.04	
7216505	RT1 class Ib, locus N2/N1/N3		103	1.66	
7216519	RT1 class Ib, locus N3/N1/N2		189	2.17	
7216562	RT1 class I, locus T24, gene 1/4 MHC class I RT1.O type 149 processed pseudogene		198	2.09	
7216540	RT1 class I, locus T24, gene 1/1/4 MHC class I RT1.O type 149 processed pseudogene RT1 class Ib, locus EC2		210	3.82	
7220923	Tap1	Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	120	3.12	
7220903	Tap2	Transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	106	1.77	
7221026	Tapbp	TAP binding protein	239	2.78	
7269637	Tapbp/Vamp1	TAP binding protein-like vesicle-associated membrane protein 1	113	1.59	
7145662	Tlr3	Toll-like receptor 3	80	1.83	
7226106	Tor1b	Torsin family 1, member B	154	1.51	*
7112701	Tor3a	Torsin family 3, member A	162	1.91	*
7071036	Trim25	Tripartite motif-containing 25	194	2.01	*
7295338	Xdh	Xanthine dehydrogenase	155	1.56	*
7226238	Aif1 Lamc3	Allograft inflammatory factor 1-like laminin gamma 3	920	0.65	*
7111837	Cfh	Complement factor H	122	0.60	
7325371	Lyz2 Lyc2	Lysozyme 2 lysozyme C type 2	350	0.59	
7321134	Mir196a	MicroRNA mir-196a	72	0.66	*

Agonists/Receptors

7035427	Adm	Adrenomedullin	210	1.52	
7322881	Angptl4	Angiopoietin-like 4	186	1.74	
7301401	Bdkrb1	Bradykinin receptor B1	53	1.81	*
7232862	Bmp2	Bone morphogenetic protein 2	40	1.97	
7123853	Ereg	Epiregulin	55	2.55	
7204090	Fst	Follistatin	83	1.93	
7144218	Gdf15	Growth differentiation factor 15	168	2.89	
7261075	Hgf	Hepatocyte growth factor	155	1.57	
7348035	Htr1b	5-hydroxytryptamine (serotonin) receptor 1B	33	1.60	
7318105	Ly6e	Lymphocyte antigen 6 complex, locus E	127	2.89	
7216279	Olr1730	Olfactory receptor 1730	42	1.64	
7201157	Ptger3	Prostaglandin E receptor 3 (subtype EP3)	64	1.59	
7055094	Folr2	Folate receptor 2 (fetal)	174	0.66	
7364798	Htr2b	5-hydroxytryptamine (serotonin) receptor 2B	99	0.65	
7313468	Igf1	Insulin-like growth factor 1	2690	0.36	
7071991	Igfbp4	Insulin-like growth factor binding protein 4	494	0.63	
7146031	Msr1	Macrophage scavenger receptor 1	640	0.57	
7055209	Olr63	Olfactory receptor 63	180	0.61	
7263730	Ptn	Pleiotrophin	825	0.37	
7094158	Tfrc	Transferrin receptor	1300	0.58	

Cell adhesion/extracellular matrix

7296103	Sdc1	Syndecan 1	184	1.78	
7247754	Sdc4	Syndecan 4	1059	1.57	
7213140	Vcam1	Vascular cell adhesion molecule 1	140	1.78	
7202072	Vcan	Versican	618	1.62	
7317088	Col14a1	Collagen, type XIV, alpha 1	271	0.64	
7319496	Fbln1	Fibulin 1	299	0.65	
7260805	Fgl2	Fibrinogen-like 2	254	0.60	
7169581	Pcdhb21	Protocadherin beta 21	88	0.65	

Cell cycle/cell death

7341999	Birc3	Baculoviral IAP repeat-containing 3	70	1.55	
7321430	Cdk2	Cyclin dependent kinase 2	229	1.57	
7217282	Cdkn1a	Cyclin-dependent kinase inhibitor 1A	1072	2.49	
7114725	Ephx1	Epoxide hydrolase 1, microsomal	124	2.25	
7041442	Fas	Fas (TNF receptor superfamily member 6)	275	1.62	

7055435	Hpx	Hemopexin	93	2.43
7325416	Mdm2	Mdm2 p53 binding protein homolog (mouse)	678	1.64
7211447	Mllt11	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 11	121	1.62
7192589	Tnfsf10	Tumor necrosis factor (ligand) superfamily, member 10	53	2.00
7207681	Ccna2	Cyclin A2	261	0.56
7203074	Ccnb1	Cyclin B1	354	0.66
7218195	Cdc2	Cell division cycle 2, G1 to S and G2 to M	234	0.55
7132836	Pbk	PDZ binding kinase	164	0.57
7338657	Ttk	TTK protein kinase	88	0.64

Protein synthesis/modification/folding/degradation

7088621	Dtx3l	Deltex 3-like (Drosophila)	55	2.16
7114108	Eef1g Slamf7	Eukaryotic translation elongation factor 1 gamma SLAM family member 7	302	1.56
7260038	Mettl20	Methyltransferase like 20	114	1.52
7361254	Mitd1	MIT, microtubule interacting and transport, domain containing	1236	2.46
7220914	Psmb8	Proteasome (prosome, macropain) subunit, beta type 8 (large multifunctional peptidase 7)	62	1.76
7216935	Psmb9	Proteasome (prosome, macropain) subunit, beta type 9 (large multifunctional peptidase 2)	170	3.44
7138231	Psme2	Proteasome (prosome, macropain) activator subunit 2	1159	1.61
7103916	Serpnb2	Serine (or cysteine) peptidase inhibitor, clade B, member 2	180	7.32
7101220	Serpine1	Serine (or cysteine) peptidase inhibitor, clade E, member 1	214	1.98
7241130	Serping1	Serine (or cysteine) peptidase inhibitor, clade G, member 1	597	1.55
7195163	Serpini1	Serine (or cysteine) peptidase inhibitor, clade I, member 1	113	2.12
7247744	Slpi Slpil2	Secretory leukocyte peptidase inhibitor antileukoproteinase-like 2	1043	2.21
7374050	Capn6	Calpain 6	107	0.57
7332657	Mmp12	Matrix metalloproteinase 12	1365	0.44

Regulation of metabolism/signalling/transcription

7250403	Abcb1b/1a	ATP-binding cassette, sub-family B (MDR/TAP), member 1B/1A	175	3.24
7318515	Apol3	Apolipoprotein L, 3	168	1.53
7226167	Ass1	Argininosuccinate synthetase 1	216	1.85
7356120	Cyp27a1	Cytochrome P450, family 27, subfamily a, polypeptide 1	218	1.90
7352252	Enpp4	Ectonucleotide pyrophosphatase/phosphodiesterase 4	243	1.72
7363922	Glb1l	Galactosidase, beta 1-like	150	1.54
7327675	Has2	Hyaluronan synthase 2	256	1.78
7037881	Mgmt	O-6-methylguanine-DNA methyltransferase	158	1.54
7185182	Nqo1	NAD(P)H dehydrogenase, quinone 1	241	1.52
7093806	Parp9	Poly (ADP-ribose) polymerase family, member 9	80	1.81
7329573	Pvalb	Parvalbumin	57	1.56
7237508	Ptges	Prostaglandin E synthase	252	3.02
7106132	Ptgs2	Prostaglandin-endoperoxide synthase 2	136	1.92
7339559	Rbp2	Retinol binding protein 2, cellular	93	2.28
7041029	Rcl1	RNA terminal phosphate cyclase-like 1	391	1.57
7244077	Slc28a2	Solute carrier family 28 (sodium-coupled nucleoside transporter), member 2	174	1.57
7250503	Steap1	Six transmembrane epithelial antigen of the prostate 1	419	1.81
7184185	Asf1b	ASF1 anti-silencing function 1 homolog B (S. cerevisiae)	176	0.65
7115455	Atf3	Activating transcription factor 3	183	0.45
7197511	Casq2	Calsequestrin 2 (cardiac muscle)	1048	0.58
7156835	F13a1	Coagulation factor XIII, A1 polypeptide	237	0.34
7250763	Gng11	Guanine nucleotide binding protein (G protein), gamma 11	826	0.65
7164749	Hist1h2ai	Histone cluster 1, H2ai-like	1978	0.62
7034549	Kcne3	Potassium voltage-gated channel, Isk-related subfamily, gene 3	548	0.61
7240783	Pde1a	Phosphodiesterase 1A, calmodulin-dependent	343	0.61
7261911	Pdk4	Pyruvate dehydrogenase kinase, isozyme 4	219	0.66
7288397	Ptplad2	Protein tyrosine phosphatase-like A domain containing 2	141	0.66
7362066	Sdpr	Serum deprivation response	835	0.56
7219202	Smpdl3a	Sphingomyelin phosphodiesterase, acid-like 3A	618	0.65
7025936	Tcf21	Transcription factor 21	724	0.63

Proteins with no known function/hypothetical proteins

7329479	Apol9a	Apolipoprotein L 9a	147	4.07
7220127	Ascc3	Activating signal cointegrator 1 complex subunit 3	215	1.77
7070512	LOC360228	WDNM1 homolog	139	1.57
7333434	LOC500956	Unknown	150	1.53
7138718	Phf11/11l	PHD finger protein 11/11-like	46	2.81
7260055	RGD1309621	Similar to hypothetical protein FLJ10652	155	2.46
7065377	RGD1561157	Unknown	117	1.71
7235527	Rnf114	Ring finger protein 114	951	1.70

7073915	Rnf213	Ring finger protein 213	51	2.54	
7073928	Rnf213	Ring finger protein 213	65	1.99	
7094771	Rtp4 Ctdsp1	Receptor (chemosensory) transporter protein 4 CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1	139	11.46	*
7070419	Slfn3	Schlafen 3	131	5.04	*
7070393	Slfn5	Schlafen family member 5	98	1.85	*
7072336	Tmem106a	Transmembrane protein 106A	78	1.85	
7252491	Tmem140	Transmembrane protein 140	206	1.83	
7047575	Unknown	Unknown	55	1.57	
7088625	Unknown	Unknown	88	2.72	
7116103	Unknown	Unknown	71	2.16	
7129836	Unknown	Unknown	38	1.54	
7153410	Unknown	Unknown	96	1.74	
7254427	Unknown	Unknown	119	1.55	
7328454	Unknown	Unknown	46	1.64	
7371101	Unknown	Unknown	54	2.67	
7126637	Cd38	CD38 molecule	608	0.61	
7191192	Fam134b	Family with sequence similarity 134, member B	1202	0.62	
7372873	Fam70a	Family with sequence similarity 70, member A	534	0.51	
7133474	Lcp1	Lymphocyte cytosolic protein 1	545	0.57	
7164764	LOC680097	Similar to germinal histone H4 gene	6612	0.58	
7367891	LOC680166	Unknown	204	0.61	
7157871	LOC682649	Similar to Histone H2A type 1	647	0.53	
7260779	Lrrc17	Leucine rich repeat containing 17	298	0.38	
7060488	Ms4a6b/11	Membrane-spanning 4-domains, subfamily A, member 6B/11	215	0.52	
7337370	Ns5atp9	NS5A (hepatitis C virus) transactivated protein 9	307	0.66	
7035206	Olfml1	Olfactomedin-like 1	355	0.48	
7328472	Tmem71	Transmembrane protein 71	128	0.59	
7077262	Unknown	Unknown	100	0.66	
7135004	Unknown	Unknown	194	0.62	
7164849	Unknown	Unknown	845	0.66	
7176278	Unknown	Unknown	208	0.65	
7367340	Unknown	Unknown	67	0.57	
7368283	Unknown	Unknown	409	0.63	
7369003	Unknown	Unknown	1284	0.63	
7370879	Unknown	Unknown	86	0.63	

Table 2 Transcripts upregulated by ET-1 that are regulated by Atf3 but are unaffected by FLAG virus infection *, IEG; **, non-IEG

Transcript Cluster	Gene symbol	Raw values	Control			ET-1		
			No virus	FLAG	AS-Atf3	No virus	FLAG	AS-Atf3
AS Atf3 enhances response to ET-1								
Cluster A1								
7169197	Egr1 *	289	1	0.95	1.02	1.29	1.24	4.73
7222466	Egr2 *	85	1	0.92	0.87	1.32	1.59	2.90
7288744	Jun *	425	1	0.93	1.44	1.21	1.31	2.10
7320920	Nr4a1 *	64	1	0.95	1.12	3.78	4.29	6.63
Cluster A2								
7308330	Actn1 *	152	1	0.94	2.61	2.72	2.22	3.56
7185572	Bcar1 *	154	1	1.05	1.94	1.56	1.41	2.23
7231595	Chac1 **	142	1	1.01	2.04	0.96	1.08	3.03
7114850	Enah *	130	1	0.98	2.28	2.00	1.60	2.86
7265600	Gadd45a **	854	1	1.00	2.94	1.98	1.93	3.09
7243216	Grem1 **	52	1	1.13	1.81	1.83	1.49	2.68
7179800	Ier2 *	368	1	0.96	2.16	1.67	1.79	4.56
7354973	Nop58	195	1	0.95	2.86	1.93	1.62	3.39
7124933	Rasl11b *	500	1	1.13	2.22	1.73	1.91	3.02
7096358	Slc7a1 **	88	1	1.10	2.80	2.18	1.74	3.54
7115195	Tgfb2 **	181	1	1.06	2.77	1.79	1.67	2.99
AS Atf3 inhibits response to ET-1								
Cluster B1								
7260283	Insig1	2796	1	0.94	0.62	1.54	1.50	0.95
7144691	Sc4mol **	1376	1	0.99	0.60	1.72	1.72	0.89
7133039	Stc1 *	560	1	1.17	0.58	1.21	1.23	0.59
Cluster B2								
7123129	Agpat9	65	1	0.96	1.07	2.82	2.22	1.40
7123848	Areg **	127	1	1.07	1.20	5.63	7.01	2.09
7043230	Dusp5 **	178	1	0.93	0.87	3.03	2.99	1.54
7345585	Fdx1 **	459	1	0.87	0.93	2.94	3.05	1.98
7187674	Glx1	191	1	0.94	0.82	1.66	1.92	1.14
7202670	Hmgcr *	527	1	1.00	0.72	1.62	1.71	1.04
7150663	Mfap3l	116	1	0.98	0.91	1.66	1.34	0.88
7235566	Pard6b **	62	1	1.06	1.19	3.20	3.62	1.48
7204067	Pelo **	863	1	1.12	1.19	1.76	1.90	1.24
7104906	RGD1562617	127	1	0.90	0.88	2.36	1.41	0.89
7214260	Sgms2	77	1	1.03	1.04	4.42	3.27	1.28
7141508	Spry2 **	513	1	0.99	1.20	2.87	2.79	1.74
7297329	Twist1 **	126	1	1.14	0.96	2.83	3.77	2.24
7350922	Xirp1 **	217	1	1.02	1.40	4.09	3.53	1.70
7297247	Unknown	121	1	1.12	1.14	2.21	2.22	1.41

Table 3 Transcripts unaffected by FLAG virus infection that are upregulated by ET-1 with significantly increased expression in control cells by Atf3 knockdown. *, IEG; **, non-IEG.

Transcript Cluster	Gene symbol	Raw values	Control			ET-1		
			No virus	FLAG	AS Atf3	No virus	FLAG	AS Atf3
Cluster C								
7305953	Arl4a	482	1	1.07	1.69	1.60	1.99	1.61
7301400	Bdkrb2 **	104	1	1.09	1.77	1.74	1.98	2.21
7111272	Btg2 *	311	1	0.93	1.87	3.66	4.22	3.28
7209338	Ccnl1 *	249	1	0.99	1.77	1.69	1.54	2.47
7045969	Cnksr3	166	1	1.14	1.96	1.61	1.63	2.21
7350918	Csrnp1	146	1	1.17	2.42	3.63	3.82	4.59
7044959	Ctgf *	342	1	0.91	3.04	3.85	4.13	5.22
7215359	Cyr61 *	407	1	1.04	1.74	5.22	5.40	6.22
7143265	Eaf1	130	1	1.13	1.87	1.56	1.57	2.12
7163221	Edn1 *	71	1	1.11	1.78	1.19	1.26	2.42
7147985	Efnb2 **	71	1	0.99	1.74	1.42	1.45	2.15
7281135	Epha2 *	90	1	1.16	3.52	2.89	3.29	4.32
7297136	Fam110c	55	1	1.09	1.92	3.70	4.75	3.76
7242382	Fjx1	149	1	1.07	1.74	1.74	1.71	2.10
7252051	Finc **	143	1	0.94	1.47	2.86	2.31	1.80
7162363	Gadd45g *	357	1	0.99	3.33	4.72	4.19	5.92
7174562	Hbegf *	100	1	0.88	2.73	3.90	4.33	3.84
7193413	Hspa4l	63	1	0.93	2.29	1.94	1.48	2.00
7165193	Inhba **	88	1	1.09	2.09	6.95	6.30	6.29
7257290	Lmcd1 *	283	1	1.17	4.41	5.84	6.64	5.84
7100653	Mafk *	187	1	1.00	2.06	2.23	2.25	2.36
7068314	Map2k3 **	106	1	1.00	1.65	1.91	1.78	1.96
7194351	Mei1 Tsc22d2	149	1	0.99	1.84	2.45	2.43	2.43
7317471	Myc *	156	1	1.02	2.04	2.28	2.34	2.92
7155813	Nfil3 *	67	1	1.04	2.71	2.57	2.83	3.71
7189518	Plk2 *	1457	1	1.15	2.79	2.14	2.65	3.58
7051029	Ppp1r15a	115	1	1.02	1.93	1.57	1.57	2.26
7360736	Ptp4a1	638	1	1.05	2.14	2.39	2.27	2.36
7048738	PVR *	430	1	1.16	2.58	3.64	3.77	3.48
7340175	Rassf1 *	163	1	0.99	1.90	1.62	1.68	2.04
7091811	Rcan1	2101	1	1.12	1.77	1.90	1.95	1.95
7331581	Rnd1 *	203	1	1.20	3.24	4.78	4.76	3.58
7238766	Rnd3 *	311	1	1.01	1.96	1.82	1.56	2.00
7029999	Sertad1 *	217	1	1.09	1.86	1.95	2.50	2.39
7279509	Stk40	144	1	1.00	1.64	1.62	1.57	1.75
7075088	Tnfrsf12a *	750	1	1.09	2.75	3.17	3.26	3.77
7106964	Tnfsf18	80	1	0.87	2.02	9.32	6.92	9.29
7199859	Tspan5 **	217	1	1.08	1.69	2.30	2.02	1.95
7120783	Unknown	93	1	0.92	1.47	1.64	1.52	1.55
7339862	Unknown	185	1	0.84	1.53	1.62	1.14	1.60
7085544	Vgll3	133	1	0.96	1.59	2.87	2.52	2.78
7228236	Xirp2	124	1	0.93	2.14	3.84	2.73	2.17

Table 4 Transcripts affected by FLAG virus infection that are upregulated by ET-1 and regulated by Atf3.

Transcript Cluster	Gene symbol	Raw values	Control			ET-1		
			No virus	FLAG	AS Atf3	No virus	FLAG	AS Atf3
AS Atf3 enhances response to ET-1								
Cluster D								
7120521	Lif *	82	1	1.75	3.70	2.88	3.62	7.30
7106132	Ptgs2 *	136	1	1.92	3.93	4.94	4.44	9.84
7237652	Slc25a25 *	94	1	1.22	1.71	1.41	1.64	2.19
7028549	Il11	143	1	1.20	1.74	2.88	2.82	3.87
7284153	Ripk2 **	238	1	1.31	2.79	2.53	2.72	3.58
7261019	Sema3c	208	1	1.22	2.16	1.67	1.80	2.21
7035407	Wee1	303	1	1.21	1.89	1.74	2.23	2.26
7096947	Zfand2a *	288	1	1.23	1.60	1.73	2.17	2.39
AS Atf3 inhibits response to ET-1								
Cluster E								
7259100	Apold1	533	1	0.82	0.63	1.64	1.43	1.02
7105894	B3galt2	505	1	0.79	1.12	2.31	1.97	1.07
7051386	Csrp3	1062	1	0.75	0.72	1.53	1.37	0.72
7084788	Socs3	229	1	1.25	1.01	1.75	1.64	0.77
7370997	Unknown	313	1	0.69	0.81	1.83	1.43	0.80
7135004	Unknown	194	1	0.62	1.24	2.47	2.47	1.40
7305661	Unknown	81	1	0.80	1.25	3.46	3.56	2.12

Table 5 Transcripts that are downregulated by ET-1 and regulated by Atf3

Transcript Cluster	Gene symbol	Raw values	Control			ET-1		
			No virus	FLAG	AS Atf3	No virus	FLAG	AS Atf3
AS Atf3 enhances response to ET-1								
Cluster F								
7279127	Cited4	231	1	0.91	0.48	0.52	0.59	0.47
7180333	Ednra	2175	1	0.99	0.60	0.58	0.64	0.41
7115625	G0s2	2545	1	0.86	0.49	0.30	0.26	0.12
7190436	Lifr	390	1	0.88	0.55	0.60	0.54	0.37
7269707	Ntf3	250	1	1.18	0.54	0.52	0.65	0.37
AS Atf3 inhibits response to ET-1								
Cluster G								
7222734	Ddit4	1553	1	1.16	2.04	0.46	0.55	0.79
7120384	Pik3ip1	339	1	0.83	0.57	0.29	0.34	0.46
7346730	Smad6	266	1	1.05	1.41	0.50	0.57	1.03
7197113	Txnip	2024	1	0.90	1.10	0.32	0.39	0.96
7173133	Unknown	690	1	0.74	0.15	0.23	0.16	0.18

Table 6 Primers used for qPCR and sqPCR

Nucleotide positions in transcripts are shown in parentheses for each primer. mRNA sequences (gene symbols are given with accession numbers in parentheses) were from the Rat Genome Database viewed at <http://www.ncbi.nlm.nih.gov/entrez>.

qPCR Primers			
Gene symbol (accession no.)	Size (bp)	Forward primer	Reverse primer
Atf3 (NM_012912.1)	108	TCGCCATCCAGAACAAGCA (140-158)	GGGCCACCTCAGACTTGGT (229-247)
Egr1 (NM_012551.2)	98	CTACGAGCACCTGACCACAGAGTC (204-227)	GCAACCGGGTAGTTTGGCT (283-301)
Gapdh (NM_017008.3)	93	CCAAGGTCATCCATGACAACCTT (476-497)	AGGGGCCATCCACAGTCTT (550-568)
Ptgs2 (NM_017232.3)	90	GAAGAACTTACAGGAGAGAAAGAAATGG (1393-1420)	CAGCAGGGCGGGATACAGT (1464-1482)
Dusp1 (NM_053769.3)	62	GCGCGCTCCACTCAAGTC (337-354)	GGGCAGGAAGCCGAAAAC (381-398)
Dusp5 (NM_133578.1)	70	CGACATTAGCTCCCACCTTCAA (882-903)	AGGACCTTGCCTCCCTCTTC (934-953)
Areg (NM_017123.1)	108	CTGCTGGTCTTAGGCTCAGG (218-237)	CACAAGTCCACCAGCACTGT (306-325)
Il6 (NM_012589.1)	157	GAGTTGTGCAATGGCAATTC (202-221)	ACTCCAGAAGACCAGAGCAG (339-358)
Il1r1 (NM_013037.1)	100	GCCCTTCATCTGGGCTACACT (68-88)	GCAATGGCACAGGAAGGTAAC (147-167)
sqPCR PRIMERS			
Atf3 (NM_012912.1)	331	GCTGCCAAGTGTCGAAACAAG (298-318)	CAGTTTTCCAATGGCTTCAGG (608-628)
Gapdh (NM_017008.3)	452	ACCACAGTCCATGCCATCAC (520-539)	TCCACCACCCTGTTGCTGTA (952-971)

Supplementary Methods - Mathematical Model

1 Reaction equations

The reaction equations governing the expression of Egr1 and Atf3 mRNA, Atf3 protein and subsequent suppression of Egr1 mRNA expression by Atf3 protein are described as follows. Each of the following processes occur at the rate indicated. Further details on these can be found in Table 6.

The phosphorylation of MKK by ET-1 is denoted by



which subsequently phosphorylates the unphosphorylated ERK



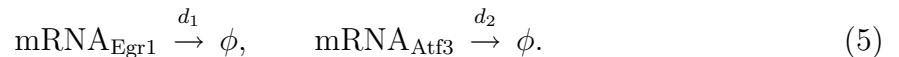
ERK-P is now free to transcribe both Egr1 and Atf3 mRNA such that



and



which are both degraded



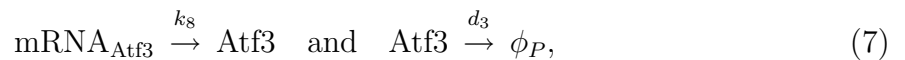
Here \cdot denotes a complex and ϕ the degraded mRNA.

The suppression of Egr1 mRNA transcription by Atf3 is described by



where the concentration of ERK-P is considered to be in excess.

Finally the translation of Atf3 mRNA to Atf3 protein and subsequent degradation of the protein are denoted by



respectively, where ϕ_P denotes degraded protein. In this work we do not explicitly account for the degraded mRNAs or Atf3 protein.

2 Mathematical Model

The Law of Mass Action (6) was applied to equations (1)-(6). This led to the following system of nonlinear ordinary differential equations

$$\frac{dm}{dt} = -k_1 e_t m, \quad (8)$$

$$\frac{dm_P}{dt} = k_1 e_t m, \quad (9)$$

$$\frac{dE}{dt} = -k_2 m_P E, \quad (10)$$

$$\frac{dE_P}{dt} = k_2 m_P E - k_3 E_P D_E + k_{-3} T_E - k_5 E_P D_A + k_{-5} T_A, \quad (11)$$

$$\frac{dD_E}{dt} = -k_3 E_P D_E + k_{-3} T_E, \quad (12)$$

$$\frac{dT_E}{dt} = k_3 E_P D_E - k_{-3} T_E - k_7 T_E A, \quad (13)$$

$$\frac{dM_E}{dt} = k_4 T_E - d_1 M_E, \quad (14)$$

$$\frac{dD_A}{dt} = -k_5 E_P D_A + k_{-5} T_A, \quad (15)$$

$$\frac{dT_A}{dt} = k_5 E_P D_A - k_{-5} T_A, \quad (16)$$

$$\frac{dM_A}{dt} = k_6 T_A - d_2 M_A, \quad (17)$$

$$\frac{dA}{dt} = k_8 M_A - k_7 T_E A - d_3 A, \quad (18)$$

$$\frac{dS}{dt} = k_7 T_E A. \quad (19)$$

Each of the variables is defined as follows: e_T represents the concentration of ET-1 (denoted e_T =[ET-1]), m =[MKK], m_P =[MKK-P], E =[ERK], E_P =[ERK-P], D_E =[DNA_{Egr1}], D_A =[DNA_{Atf3}], T_E =ERK-P·DNA_{Egr1}, T_A =ERK-P·DNA_{Atf3}, M_E =[mRNA_{Egr1}], M_A =[mRNA_{Atf3}], S =[Atf3·DNA_{Egr1}] and A =[Atf3]. Here the concentration of ET-1 is assumed to be constant. The rate of Atf3 protein to Egr1 DNA binding is assumed to be immediate and no delays are incurred in this process.

The system is closed with the initial conditions

$$\begin{aligned} m &= m_0, & m_P &= 0, & E &= E_0, & E_P &= 0, & D_E &= D_{E0}, & T_E &= 0, \\ M_E &= M_{E0}, & D_A &= D_{A0}, & T_A &= 0, & M_A &= 0, & A &= 0 & \text{and } S &= 0, \end{aligned} \quad (20)$$

which state that MKK and ERK are initially assumed to be unphosphorylated, the concentration of Egr1 mRNA is non-zero whilst no Atf3 mRNA, Atf3 protein or any of the complexes have been created.

The governing system of equations can be simplified as follows. Addition of equations (8) and (9), integration with respect to time and application of the respective initial conditions yields the conservation relation

$$m + m_P = m_0. \quad (21)$$

Substituting for m into equation(9), integrating and applying the initial condition yields

$$m_P(t) = m_0 (1 - e^{-k_1 e_T t}). \quad (22)$$

Addition of equations (12), (13) and (19), integration with respect to t and application of the initial conditions yields

$$D_E + T_E + S = D_{E0}. \quad (23)$$

Likewise for equations (15) and (16)

$$D_A + T_A = D_{A0}. \quad (24)$$

Assuming equation (16) is quasi-steady and substituting for D_A using equation (24) leads to

$$T_A \simeq \frac{D_{A0} E_P}{E_P + K_5}, \quad (25)$$

where $K_5 = k_{-5}/k_5$.

Bringing all these results together gives

$$m_P(t) = m_0 (1 - e^{-k_1 e_T t}), \quad (26)$$

$$\frac{dE}{dt} = -k_2 m_P E, \quad (27)$$

$$\frac{dE_P}{dt} = k_2 m_P E - k_3 E_P (D_{E0} - T_E - S) + k_{-3} T_E, \quad (28)$$

$$\frac{dT_E}{dt} = k_3 E_P (D_{E0} - T_E - S) - k_{-3} T_E - k_7 T_E A, \quad (29)$$

$$\frac{dM_E}{dt} = k_4 T_E - d_1 M_E, \quad (30)$$

$$\frac{dM_A}{dt} = \frac{k_6^* E_P}{E_P + K_5} - d_2 M_A, \quad (31)$$

$$\frac{dA}{dt} = k_8 M_A - k_7 T_E A - d_3 A, \quad (32)$$

$$\frac{dS}{dt} = k_7 T_E A, \quad (33)$$

with the initial conditions

$$E = E_0, \quad E_P = 0, \quad T_E = 0, \quad M_E = M_{E0}, \quad M_A = 0, \quad A = 0 \quad \text{and} \quad S = 0, \quad (34)$$

where $k_6^* = k_6 D_{A0}$. When Egr1 transcription is not suppressed by Atf3 protein we have $k_7 = 0$.

3 Parameterisation

The mathematical model has been informed with data available within the literature, from our own previous studies as well as work undertaken here. A complete list of the parameter values used can be found in Table 6. In cases where parameter values have been derived these are explained as follows.

Estimation of the activation rate of MKK and ERK: The time course for activation of ERK1 was determined previously (9) and maximal activation was at 3 minutes. The time course for activation of MKK was determined by immunoblotting with antibodies to phosphorylated (i.e. activated) MKK using primary antibodies from Cell Signaling Technology Inc. A representative blot is shown in Figure 1. The time course for activation by a range of agonists (epidermal growth factor, ET-1, phorbol 12-myristate 13-acetate or platelet-derived growth factor) all showed maximal activation by 2-3 minutes. We therefore assumed the time to maximal activation of MKK to be 2 min with a further 1 min for maximal activation of ERK.

The concentration of MKK in cardiomyocytes was estimated by immunoblotting cardiomyocyte extracts from a known number of cells alongside known concentrations of recombinant MKK1. Antibodies to total MKK were from Cell Signaling Technology Inc. Following densitometric analysis, a standard curve was constructed from which the amount of MKK in the myocyte extract was estimated. The concentration was calculated on the basis of the estimated volume of a neonatal myocyte. The concentration of ERK was assumed to be similar to MKK given that this lies within the range seen in other cells (4).

$k_1 e_t$ - *Rate of MKK activation by ET-1:* The time taken for maximal activation of MKK by ET-1 is 2 minutes, thus

$$k_1 e_t = \frac{1}{120s} = 8.30 \times 10^{-3} s^{-1}.$$

k_2 - *Rate of ERK activation by MKK:* The time taken to activate ERK by MKK is 60 seconds, so

$$k_2 E = \frac{1}{60s} = 1.67 \times 10^{-2} s^{-1}.$$

The total ERK concentration is 130nM such that

$$k_2 = 1.28 \times 10^5 (\text{Ms})^{-1}.$$

k_4^* , k_6^* - *Egr1 and Atf3 transcription rates:* The size of the Atf3 and Egr1 genes, mRNAs and proteins were for mouse (for rat, the 5' untranslated region was not defined for Egr1 and the rat genome is not well sequenced in the intronic regions for Atf3). Sequences were obtained from NCBI. For Atf3 (Gene ID: 11910), this gives a total gene length of

13038 base pairs (bp), a 5' untranslated region of 62 nucleotides and protein of 181 amino acids. For Egr1 (Gene ID: 13653), this gives a total gene length of 3750 bp. To estimate the rate of transcription, the total length of the gene was used allowing for an additional 200 nucleotides to be transcribed before termination. The maximum rate of transcription has been estimated recently to range from 55 b/s to greater than 800 b/s (1; 5). Thus to transcribe one molecule of mRNA from one gene, assuming a rate of 55 bases per second, takes

$$\frac{3950\text{bases}}{55\text{bases/s}} = 71.82\text{s}$$

Per gene this equates to 1.39×10^{-2} molecules mRNA s^{-1} . Since a cell contains two genes, we have 2.78×10^{-2} molecules of Egr1 mRNA being synthesized per cell per second. Taking the cell volume of 6.7pl we obtain

$$k_4^* = \frac{2.78 \times 10^{-2} \text{molecules s}^{-1}}{6.710^{-9} \text{ml}} = 4.15 \times 10^5 \text{molecules ml}^{-1} \text{s}^{-1} = 6.89 \times 10^{-16} \text{Ms}^{-1}. \quad (35)$$

We can undertake a similar calculation for Atf3 transcription to obtain

$$k_6^* = 5.15 \times 10^{-15} \text{Ms}^{-1}.$$

k_8 - *Atf3 translation rate*: The rate of translation of Atf3 was estimated on the basis of scanning of the 5' untranslated region at a rate of 6 nucleotides per second (11), translation of the coding sequence at a rate of 20 amino acids per second (N.B. the reported rate of translation is in the range of 4 - 20 amino acids per second (10; 12) and we presume translation of IEGs is efficient) with 5 ribosomes attached simultaneously to each mRNA (N.B. the predicted occupancy is 1 ribosome per 32 amino acids)(7).

K_5 and k_3, k_{-3} - *ERK-P dissociation rates for Egr1 and Atf3 DNA*: The model is based on the assumption that phospho-ERK bind to transcription factors that are pre-bound to the Atf3 and Egr1 promoters and this drives transcription. We presume that the ERK binding is mediated through a DEF motif with an estimated dissociation rate of $0.5 \mu\text{M}$ (the dissociation rate for Elk1 is $0.25 \mu\text{M}$; that for Fos is $1 \mu\text{M}$) (2). Given that $K_3 = k_{-3}/k_3$ we use an initial estimate of $k_{-3} = 5 \times 10^{-2}/\text{s}$ to obtain $k_3 = 1 \times 10^5 (\text{Ms})^{-1}$.

d_1, d_2, d_3 - *Degradation rates of Egr1 mRNA, Atf3 mRNA and Atf3 protein*: To estimate the half-life of Egr1 and Atf3 mRNA, cardiomyocytes were exposed to ET-1 for 30 min then incubated without or with actinomycin D ($4 \mu\text{M}$, added directly to the culture dish). Cells were harvested at the indicated times following addition of actinomycin D and mRNA expression was measured by qPCR. GraphPad Prism 4 was used to fit a one phase exponential decay curve to the data shown in Figure 1, giving a half-life of 13 min for Egr1 and 49 min for Atf3. The rate of degradation is defined by

$$d = \frac{\ln 2}{t_{1/2}}.$$

Parameter	Definition	Value
m_0	Total MKK.	130nM
E_0	Total ERK.	130nM
M_{E0}	Initial Egr1 mRNA concentration.	1pM
D_{e0}	Egr1 DNA concentration.	33.2pM
$k_1 e_t$	Rate of MKK activation by ET-1.	$8.30 \times 10^{-3} \text{ s}^{-1}$
k_2	Rate of ERK activation by MKK.	$1.28 \times 10^5 (\text{Ms})^{-1}$
k_3	Rate of ERK-P activation of Egr1 DNA.	$1.00 \times 10^5 (\text{Ms})^{-1}$
k_{-3}	Rate of ERK-P reverse activation of Egr1 DNA.	$5.00 \times 10^{-2} (\text{Ms})^{-1}$
k_4^*	Egr1 mRNA transcription rate.	$6.89 \times 10^{-15} \text{ M/s}$
k_6^*	Atf3 mRNA transcription rate.	$1.03 \times 10^{-15} \text{ M/s}$
k_7	Atf3 supression rate.	To be determined.
k_8	Atf3 translation rate.	0.25 s^{-1}
K_5	ERK-P and Atf3 DNA dissociation rate.	$0.5 \times 10^{-6} \text{ M}$
d_1	Degradation rate of Atf3 mRNA.	$8.89 \times 10^{-4} \text{ s}^{-1}$
d_2	Degradation rate of Egr1 mRNA.	$2.36 \times 10^{-4} \text{ s}^{-1}$
d_3	Degradation rate of Atf3 protein.	$2.36 \times 10^{-4} \text{ s}^{-1}$

Table 6: Model parameter values.

which leads to

$$d_1 = 8.89 \times 10^{-4} \text{ s}^{-1} \quad \text{and} \quad d_2 = \frac{\ln 2}{2580\text{s}} = 2.36 \times 10^{-4} \text{ s}^{-1}. \quad (36)$$

We assume Atf3 protein degrades at the same rate as Atf3 mRNA.

Egr1 DNA concentration: We assume there are 2 molecules of DNA per cell. The volume of a neonatal myocyte was estimated given that an adult myocyte has a volume of 34 pl with a capacitance of 66pF (3; 8) and the capacitance of a neonatal myocyte is 13pF (8). This gives 6.7pl per cell leading to a concentration of 33.2pM.

4 Results

The governing system of nonlinear ordinary differential equations (ODEs) (27)-(33) was solved using Gear's method available in Matlab (The Mathworks, Version 7.11) via the solver ode15, with $m_P(t)$ given by equation (26).

Using the parameter values detailed in Table 6 we found the 20-fold change in Egr1 mRNA determined experimentally could not be re-produced using these values (using an initial estimate of $k_7 = 1 \times 10^5 (\text{Ms})^{-1}$). As such we undertook a sensitivity analysis in which we varied the rates of Egr1 and Atf3 mRNA transcription (k_4^*, k_6^*), ERK-P reverse

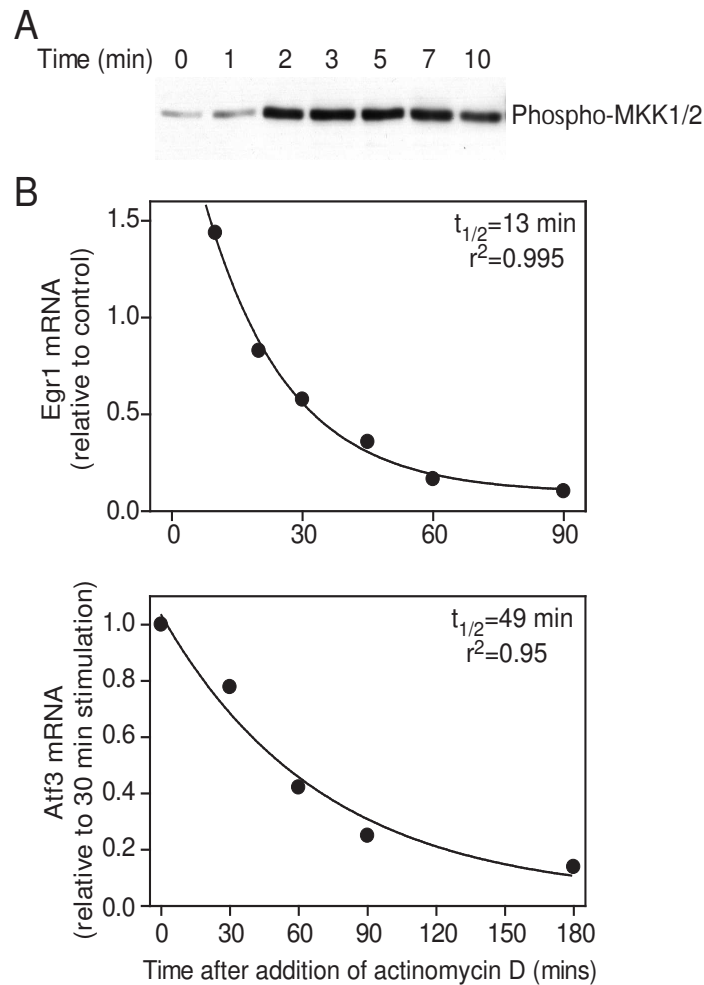


Figure 1: A, Cardiomyocytes were exposed to ET-1 for the times indicated. Protein extracts were immunoblotted with antibodies to phospho-MKK. A representative image is shown. B, Cardiomyocytes were stimulated for 30 min before addition of actinomycin D ($4\mu\text{M}$) to inhibit transcription. Expression of Egr1 (upper panel) or Atf3 (lower panel) mRNAs were measured by qPCR at the indicated times after actinomycin D addition. A one phase exponential curve was fitted using GraphPad Prism 4.

activation of Egr1 DNA (k_{-3}) and the ERK-P association rate for Atf3 DNA (K_3). The most appropriate variation in these values which gave a good fit to the data was found to be a 5-fold increase in both the transcription rates of Egr1 and Atf3 mRNA ($5 \times k_4^*, 5 \times k_6^*$), a 10-fold decrease in the rate of ERK-P dissociation for Egr1 DNA ($k_{-3}/10$) and a 50-fold decrease in the ERK-P association rate for Atf3 DNA ($K_3/50$). Such a variation in the rates of Egr1 and Atf3 transcription is equivalent to a rate of 275 bases/s rather than the original assumption of 55 bases/s. Such a variation lies within the range recently reported by (1) and (5).

This led to a very good fit to the experimental data in terms of the magnitude variation in Egr1 mRNA observed experimentally and a relatively good fit (qualitatively) to the suppression of Egr1 mRNA by Atf3. To further improve this model-data fit we adjusted the rate of Atf3 suppression (k_7). Good fits to the data we obtained for the range of values $1.00 \times 10^5(\text{Ms})^{-1} \leq k_7 \leq 6 \times 10^5(\text{Ms})^{-1}$.

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