Rational selection of small molecules that increase transcription through the GAA repeats found in Friedreich's ataxia

LaKechia Grant[a], Jun Sun[b], Hongzhi Xu[a], S.H. Subramony[b], Jonathan B. Chaires[c], Michael D. Hebert[a],*

[a] *Department of Biochemistry, 2500 North State Street, The University of Mississippi Medical Center, Jackson, MS 39216-4505, USA*
[b] *Department of Neurology, 2500 North State Street, The University of Mississippi Medical Center, Jackson, MS 39216-4505, USA*
[c] *James Graham Brown Cancer Center, Department of Medicine, Health Sciences Center, 529 South Jackson Street, University of Louisville, KY 40202, USA*

Abstract **Friedreich's ataxia (FRDA) is an autosomal recessive trinucleotide repeat disease with no effective therapy. Expanded GAA repeats in the first intron of the FRDA gene are thought to form unusual non-B DNA conformations that decrease transcription and subsequently reduce levels of the encoded protein, frataxin. Frataxin plays a crucial role in iron metabolism and detoxification. To discover small molecules that increase transcription through the GAA repeat region in FRDA, we have made stable cell lines containing a portion of expanded intron 1 fused to a GFP reporter. Small molecules identified using the competition dialysis method were found to increase FRDA-intron 1-reporter gene expression. One of these compounds, pentamidine, increases frataxin levels in patient cells. Thus our approach can be used to detect small molecules of potential therapeutic value in FRDA.**
© 2006 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Neurodegenerative; Trinucleotide repeat; Competition dialysis; GFP

1. Introduction

Friedreich's ataxia (FRDA) is the most common inherited ataxia, affecting approximately 1 in 50 000 people [1]. Most patients with FRDA have a pathogenic expansion of a trinucleotide repeat (GAA) within the first intron of the *X25* gene (also known as *FRDA* or *FXN*). The expanded GAA repeats are thought to form unusual non-B DNA conformations that decrease transcription and subsequently reduce levels of the encoded protein, frataxin [2,3]. Frataxin is a highly conserved mitochondrial protein that plays a crucial role in providing iron for heme synthesis and the formation of Fe–S clusters [4]. Other work has shown that frataxin is important for iron detoxification [5]. Currently there are no effective treatments for FRDA patients, whose cardinal clinical features include progressive gait and limb ataxia, lower limb muscle weakness and dysarthria [4,6,7]. Other symptoms include hypertrophic cardiomyopathy, diabetes and vision problems [4,6,7]. Given

the putative role of frataxin, idebenone antioxidative therapy is being evaluated in clinical trials [8,9]. This compound has been shown to have cardioprotective effects in a mouse model of FRDA [10], although conflicting data regarding the role of oxidative damage in this disease exist [11]. Other approaches to treat FRDA are centered upon increasing the expression of frataxin. For example, a recent study has found that recombinant human erythropoietin increases frataxin levels, by an unknown mechanism, in cultured cells from FRDA patients [12]. Another strategy to increase frataxin involves using reagents that can attenuate the recalcitrant conformations (triplex or sticky DNA) expanded GAA repeats are thought to take, thereby increasing transcription and elevating frataxin levels. Recently, Gottesfeld and colleagues have shown in elegant work that polyamides that bind specifically to GAA repeats can increase frataxin mRNA and protein levels in an FRDA lymphoid cell line [13].

Competition dialysis is a very powerful technique that can be used to discover ligands that bind nucleic acids with structural- or sequence-selectivity [14]. As such, this method can conceivably be used to identify small molecules that bind selectively to the duplex form of the GAA repeat sequence over the triplex form. Such molecules will shift the equilibrium to reverse triplex formation, thereby inhibiting the unfavorable biological effects of triplex formation. Information gathered from the first-generation competition dialysis assay, in which binding data was gathered for the interaction of 126 compounds with 13 different nucleic acid structures and sequences [14], was used to identify small molecules that have the potential to increase transcription through the GAA repeats found in FRDA. We describe in this report the initial characterization of a subset of these compounds.

2. Materials and methods

2.1. Stable cell line construction
Normal (GAA_{15}) and expanded (GAA_{148}) genomic DNA was used as a template in a PCR reaction using previously described primers and conditions to amplify a fragment of the *X25/FRDA/FXN* intron 1 (note 28 in [15]). These primers generate a ~1.4 kb normal fragment that was subsequently digested with *Bam*HI and *Xma*I and cloned into pEGFP-N3 (Clontech, Palo Alto, CA) cut with *Bgl*II and *Xma*I. In so doing, the partial intron 1 sequence containing the GAA repeats is positioned upstream of the GFP coding sequence. Thus alterations in the ability to transcribe through this repeat will impact GFP expression. HeLa

*Corresponding author. Fax: +1 601 984 1501.
E-mail address: mhebert@biochem.umsmed.edu (M.D. Hebert).

cells were stably transfected with GAA$_{15}$-GFP or GAA$_{148}$-GFP plasmids using G418 selection per the manufacturer's instructions (Clontech). Three independently isolated cell lines were obtained for each construct.

2.2. Cell culture and small molecule treatment

GAA$_{15}$-GFP or GAA$_{148}$-GFP cell lines were cultured (37 °C, 5% CO$_2$) in DMEM medium (Invitrogen; Carlsbad, CA) supplemented with 10% fetal calf serum and penicillin/streptomycin. To monitor the effects of small molecules on the expression of GFP, GAA$_{148}$-GFP cells were seeded into six T25 flasks such that the flasks were 30% confluent on the day of the experiment. Specific compounds were added to three of the flasks at the indicated final concentrations, the remaining three flasks received vehicle only. All small molecules were purchased from Sigma (St. Louis, MO). After 48 h of incubation, cells were harvested and vortexed in 20 μl of high salt lysis buffer (50 mM Tris, pH 7.5, 0.5 M NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 2 mM EDTA), followed by a brief spin in a microcentrifuge and the addition of 20 μl of low salt lysis buffer (same as high salt, except 150 mM NaCl). The cell suspension was vortexed briefly and centrifuged for 5 min at full speed in a microcentrifuge at 4 °C. Protein concentration was determined using the BioRad protein assay (BioRad Laboratories, Hercules, CA). For immunofluoresence experiments, cells were grown in chambered slides, treated with the indicated compound and processed as described previously [16] after 48 h of incubation.

2.3. Western blotting and immunofluoresence analysis

Equal amounts of protein were added to 5× SDS loading buffer, boiled and subject to SDS–PAGE (12% gel) and Western blotting as previously described [17]. Each gel contained three independently isolated treated and untreated samples. The blot was probed with monoclonal anti-GFP antibodies (Roche, Indianapolis, IN), followed by the detection of SMN using a monoclonal antibody from BD Biosciences (Palo Alto, CA). Chemiluminescent signals were captured by X-ray film and quantified using NIH Image 1.63 image analysis software. The GFP signals of some of the untreated samples in Fig. 2 are faintly detectable due to short exposure times. GAA$_{15}$-GFP or GAA$_{148}$-GFP cell images were captured using a Nikon Eclipse E600 epifluorescent microscope with a Coolsnap fx digital camera (Roper Scientific, Tucscon, AZ) controlled by MetaView (Series 5) image capturing and processing software (Universal Imaging Corporation, Downingtown, PA). Pixel intensities were obtained using this software. Adobe Photoshop 7.0 (San Jose, CA) was used for the final processing of the images and labeling.

2.4. FRDA lymphocyte isolation and pentamidine treatment

Four patients with FRDA (GAA repeats in the range from 107 to 1305) were included in this study after the University of Mississippi Medical Center Institutional Review Board approved the protocol. Lymphocytes from fresh blood samples were isolated using Ficoll-Paque density gradients and cultured for 48 h in RPMI supplemented with 10% fetal calf serum, 2 mM L-glutamine and antibiotics. Where indicated, pentamidine was added to a final concentration of 30 μM. After incubation, lymphocytes were harvested, lysed and subjected to SDS–PAGE and Western transfer, followed by the detection of frataxin and SMN. A polyclonal antibody to frataxin (H-155, Santa Cruz Biotechnology, Santa Cruz, CA) was used for these experiments. All samples, except one, were subject to technical duplicates (in which the lysates were subjected to SDS–PAGE and frataxin and SMN detection twice). Limited material precluded a technical duplicate for the sample with 165 GAA repeats on one allele and 107 repeats on the other allele.

2.5. Statistical analysis

The ratio of GFP to SMN or frataxin to SMN is used to determine if a small molecule significantly increases GFP or frataxin expression through GAA repeats. For example, the GFP (or frataxin) signal for a certain drug at a certain concentration was quantified and divided by the SMN signal at this same condition. This ratio was compared to the ratio found in cells that did not receive treatment. Drugs which increase GFP (or frataxin) expression relative to SMN will yield numbers greater than 1. Significant differences were determined using Student's *t*-test (a *P*-value of less than 0.05 is considered significant).

To calculate differences in the fluorescence signal between cell lines and treatments, pixel intensities were determined followed by the application of Student's *t*-test (*P*-value of less than 0.05 is considered significant).

3. Results and discussion

Previous studies have shown that expanded GAA tracts reduce the expression of a reporter gene when transiently transfected into COS-7 cells [18]. To facilitate the identification of small molecules that increase transcription through expanded GAA repeats in living cells, we made stable cell lines in which a fragment of the *X25* intron 1 that contains the GAA repeats was transcriptionally fused upstream of a GFP reporter (Fig. 1). Two lines were made, one with a normal number of repeats (15) and another with an expanded tract (148). To demonstrate that these cell lines could be used for screening small molecules with therapeutic value to FRDA patients, we monitored the GFP signal for both cell lines by fluorescence microscopy and Western blotting. As can be observed in Fig. 1B, there is a statistically significant decrease in the GFP signal of cells with 148 repeats compared to the signal in cells with 15 repeats ($P = 1.21^{-13}$, compare panel a to panel c). Importantly, when cells are grown in the presence of 4′,6-diamidino-2-phenylindole (DAPI), a known binder of duplex DNA, the level of GFP expression increases in the GAA$_{148}$-GFP cell line ($P = 0.00018$) but remains essentially unchanged in the GAA$_{15}$-GFP line ($P = 0.6$, compare d–c and b–a). The decrease in GFP expression as a consequence of expanded GAA repeats was also verified by Western blotting (Fig. 1C). Consistent with the microscopic observations, the level of GFP in GAA$_{148}$-GFP cells, relative to SMN, is increased when cells are incubated with DAPI (Fig. 1C). SMN, the survival of motor neurons protein, serves an essential housekeeping function in the assembly of the spliceosomal machinery [19] and can therefore be used as a general transcription monitor and loading control. In contrast to the GAA$_{148}$-GFP results, DAPI treatment does not affect the GFP/SMN ratio in GAA$_{15}$-GFP cells. From these results we conclude that the GAA$_{148}$-GFP cell line represents an appropriate model to test the effects of small molecules on the transcription through GAA repeats.

Small molecules with the potential to bind selectively to the duplex form of the GAA repeat sequence were identified by analysis of data obtained from the first generation competition dialysis screening assay in which the interactions of 126 compounds with 13 nucleic acid structures were studied [20,14]. Classic groove binding agents were identified by the competition dialysis to prefer duplex over triplex forms, and would in fact displace the third strand to form a drug-duplex DNA complex [21]. The compounds DAPI, Hoechst 33258, distamycin, diminazene, netropsin and chromomycin were selected for the reporter assay by this approach. Pentamidine, while not tested by competition dialysis, was also selected based on its known properties as a groove-binding agent [22].

We next wanted to test if the compounds identified by the competition dialysis method can increase expression of the GFP reporter. Ideally, small molecules can be identified that upregulate GFP expression but do not affect the transcription of other genes or alter cell viability. The compounds were initially tested on the GAA$_{148}$-GFP cell line using 5 μM and 50 μM final concentrations. At 5 μM, chromomycin had severe
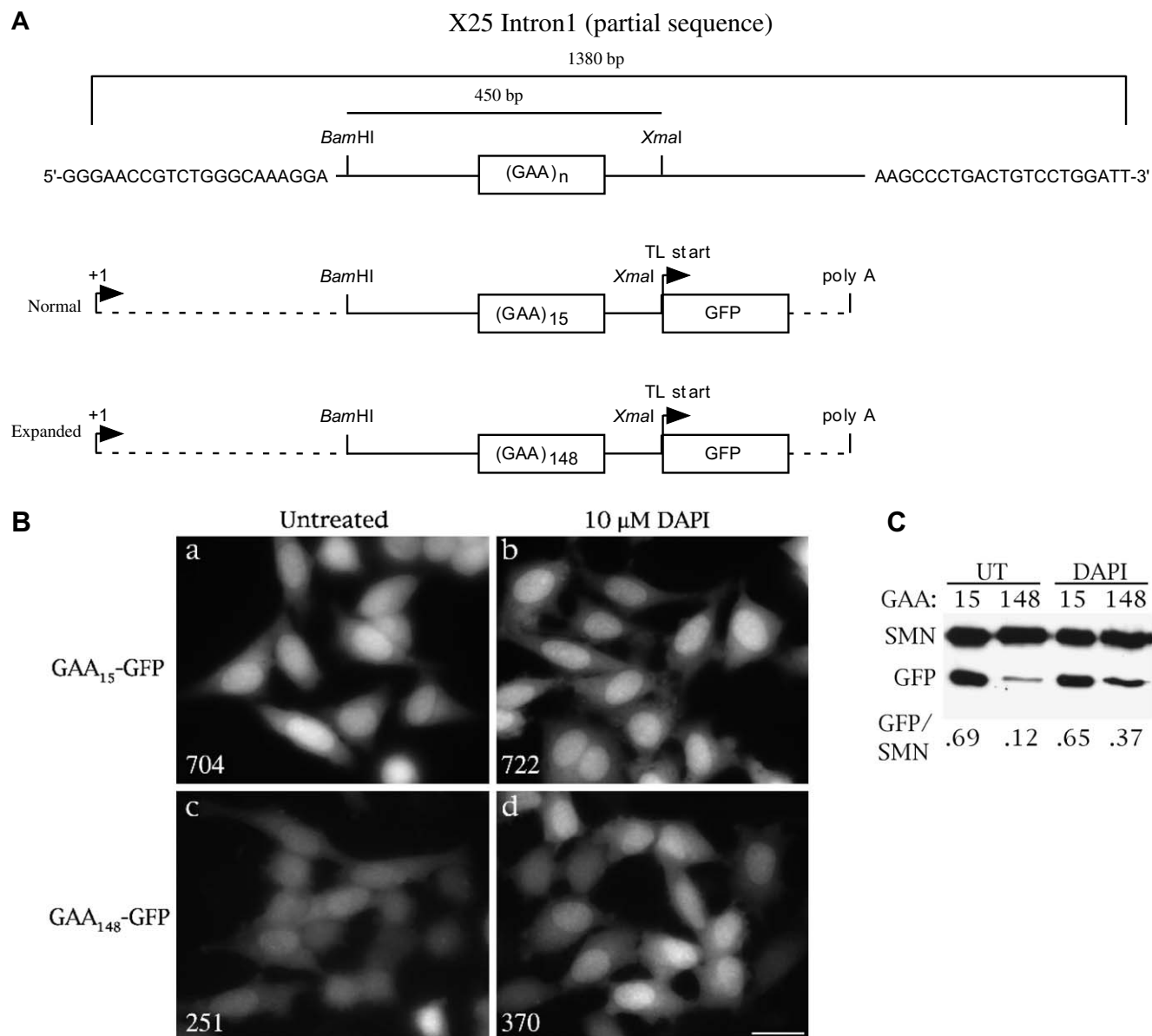
Fig. 1. The number of GAA repeats alters reporter gene expression. (A) Schematic of the stable cell lines used in this study. (B) GAA_{15}-GFP (line A) or GAA_{148}-GFP (line A) cells were untreated or treated with 10 μM DAPI for 48 h and images were obtained using fluorescence microscopy (1 s exposure). The pixel intensity for each cell was determined and the average pixel intensity for each field is shown at the bottom left of each panel. Scale bar = 20 μm. (C) Equal amounts of protein from untreated or DAPI treated GAA_{15}-GFP (line A) or GAA_{148}-GFP (line A) cells were run on a SDS–PAGE gel, followed by Western transfer and detection of the GFP reporter by anti-GFP antibodies. The blot was reprobed with anti-SMN to verify equal protein loading. In untreated cells, the expression of GFP is reduced relative to SMN in cells with 148 GAA repeats compared to 15 GAA repeats. DAPI treatment increases the ratio of GFP to SMN in GAA_{148}-GFP cells.

toxicity affects and was not explored further. Pentamidine was toxic at 50 μM final concentration, so additional assays were conducted on this compound using 10, 20 and 30 μM. For each assay, GAA_{148}-GFP cells were treated with small molecules at the indicated concentration for 48 h, followed by cell lysis, total protein isolation and detection of GFP by anti-GFP antibodies. The same blot was reprobed with antibodies to SMN to monitor the effects the small molecule may have on the transcription of other genes. As shown in Fig. 2, all of the compounds except netropsin elicit a statistically significant increase in the expression of the GFP reporter at a 5 μM concentration. For example, treatment of the GAA_{148}-GFP

cell line with 5 μM Hoechst 33258 increases the ratio of GFP to SMN by 24.2-fold compared to the GFP/SMN ratio in untreated cells (Fig. 2, panel D (top), panel G). 5 μM DAPI and pentamidine treatment yielded smaller, yet statistically significant increases in the expression of GFP (Fig. 2, panel B (top), panel E (top), panel G). Specifically, there was a 2.6-fold increase ($P = .015$) in GFP expression in DAPI treated cells, and a 1.65-fold increase ($P = .0086$) in pentamidine treated cells. Higher compound concentrations produced correspondingly higher levels of GFP reporter induction relative to SMN. However, we observed a decrease in the expression of SMN at higher small molecule concentrations, indicating
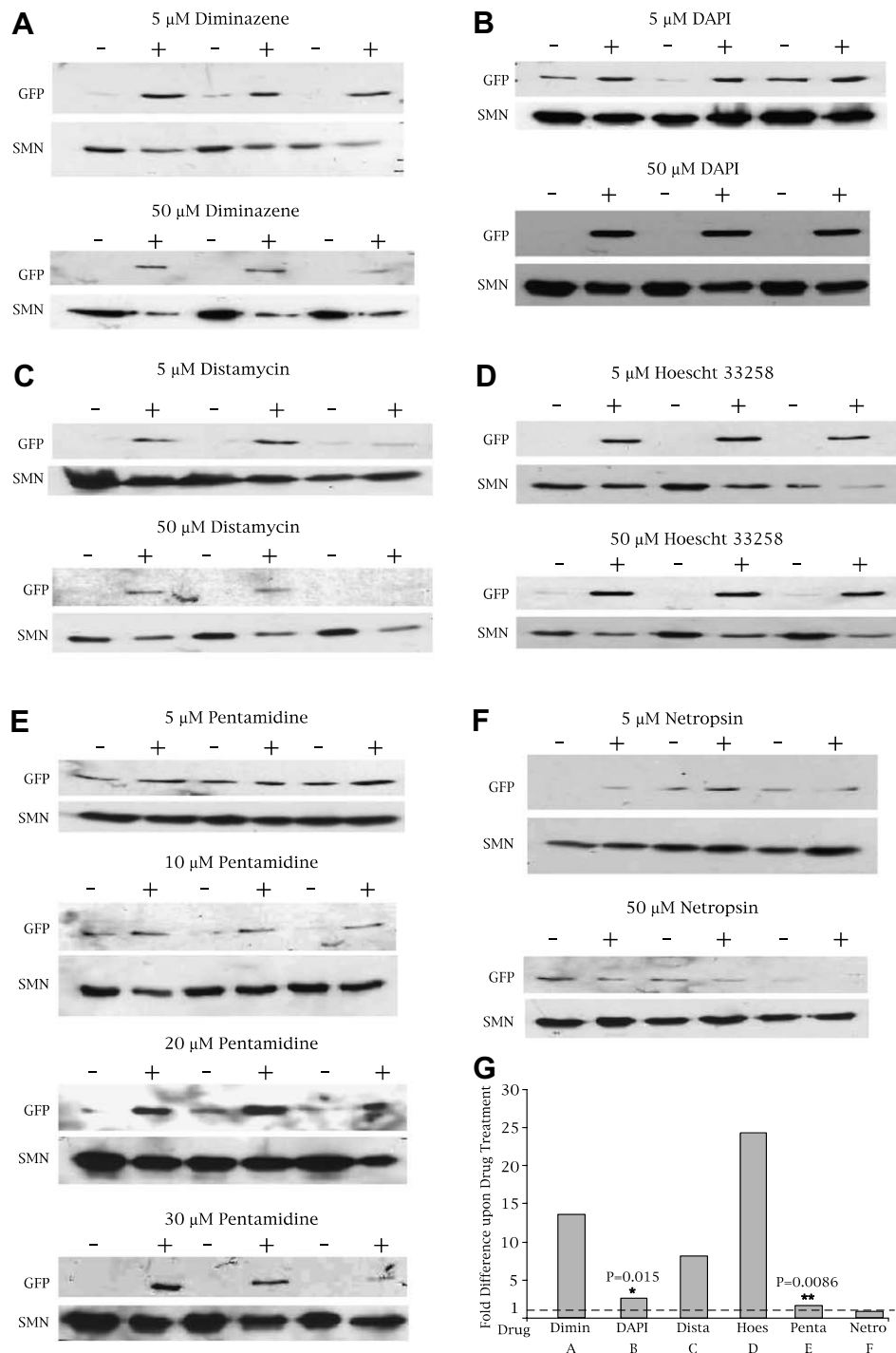
Fig. 2. Some small molecules identified by competition dialysis increase reporter gene expression. GAA$_{148}$-GFP (line A) cells were untreated (−) or treated (+) with the indicated small molecule for 48 h, followed by detection of GFP and SMN by Western blotting. The results using 5 μM and 50 μM final concentrations of the small molecules are shown, with the exception of pentamidine (panel E) in which 5 μM, 10 μM, 20 μM and 30 μM results are displayed. The histogram (panel G) shows the fold difference of the ratio of GFP to SMN in 5 μM treated GAA$_{148}$-GFP (line A) cells compared to untreated cells. No change in the ratio will yield 1 (denoted by dashed horizontal line). At this concentration, all compounds except netropsin produce significant increases in the expression of GFP relative to SMN. The P-values for DAPI and pentamidine are shown (P < 0.05 is considered significant).

that these compounds can perturb global transcription at high doses.

To generalize these results, we tested additional cell lines containing 15 or 148 GAA repeats with 5 μM of the small molecules. We do not observe statistically significant changes

in the ratio of GFP to SMN in cell lines with 15 GAA repeats upon treatment with the various small molecules (Fig. 3, panels A–C). In contrast, additional cell lines with 148 GAA repeats respond similarly to the different small molecules as found for the line used previously in Fig. 2 (Fig. 3. panels D
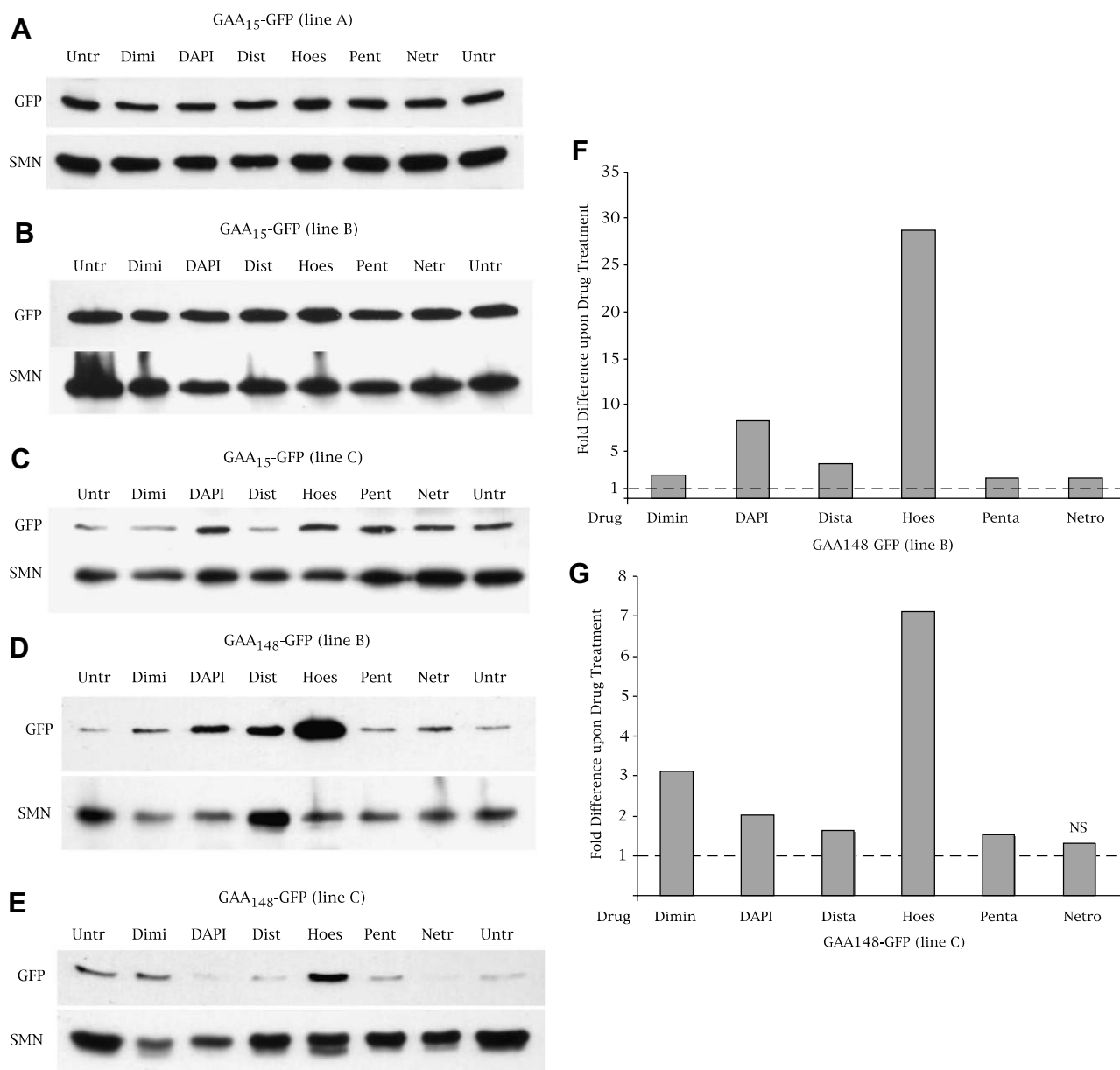
A GAA$_{15}$-GFP (line A)

Untr   Dimi   DAPI   Dist   Hoes   Pent   Netr   Untr

GFP

SMN

B GAA$_{15}$-GFP (line B)

Untr   Dimi   DAPI   Dist   Hoes   Pent   Netr   Untr

GFP

SMN

C GAA$_{15}$-GFP (line C)

Untr   Dimi   DAPI   Dist   Hoes   Pent   Netr   Untr

GFP

SMN

D GAA$_{148}$-GFP (line B)

Untr   Dimi   DAPI   Dist   Hoes   Pent   Netr   Untr

GFP

SMN

E GAA$_{148}$-GFP (line C)

Untr   Dimi   DAPI   Dist   Hoes   Pent   Netr   Untr

GFP

SMN

F GAA148-GFP (line B)

G GAA148-GFP (line C)

Fig. 3. Small molecules increase the expression of the GFP reporter in additional cell lines with 148 repeats but do not alter GFP expression in lines with 15 GAA repeats. (A–C) Three independently derived GAA$_{15}$-GFP cell lines (lines A, B and C) were untreated or treated with 5 μM of the indicated small molecule, followed by total protein isolation and Western blotting for GFP and SMN. No significant increase in the expression of GFP was detected upon small molecule treatment for any of the cell lines. (D and E) Two additional GAA$_{148}$-GFP cell lines (lines B and C) were untreated or treated with 5 μM of the indicated small molecule and processed as described above. At this concentration, all compounds produce statistically significant increases in the expression of GFP relative to SMN (F and G), with the exception of netropsin in GAA$_{148}$-GFP (line C). NS – not significant.

and E). In particular, Hoechst 33258 shows a striking increase in the expression of GFP relative to SMN in all three GAA$_{148}$-GFP cell lines (Figs. 2, panel G and 3 panels F and G). It is not known why Hoechst 33258 produces such a dramatic increase in reporter expression. Studies on Hoechst 33258 binding in vitro have shown that it shows a strong sequence preference for runs of 4–6 A–T bp, where it binds in the minor groove. G–C bp are thought to disfavor binding because of an amino group that protrudes into the minor groove, hindering the stereochemical fit [23,24]. Other work has shown that Hoechst 33258 can bind RNA [25] and exhibit antimicrobial activity

[26]. There are no explicit data for the preferential binding of Hoechst 33258 to GAA repeats, yet our cell reporter studies clearly show that this compound is a very effective inducer of GFP expression. One possibility is that Hoechst 33258 promotes GAA duplex formation and induces an open chromatin complex that facilitates transcription and translation.

Netropsin was the only tested compound that did not significantly induce reporter expression in all three cell lines (Figs. 2, panel G and 3 panels F and G). Interestingly, GAA$_{148}$-GFP (line B) showed only a modest increase in GFP expression upon diminazene treatment compared to that found in

GAA$_{148}$-GFP (line A), yet Hoechst 33258 yielded an almost identical fold-induction in both cell lines. The effects of some small molecules in GAA$_{148}$-GFP (line C) seem muted compared to that found in the other two lines. Hoechst 33258, for example, increases GFP reporter expression 7.1-fold in GAA$_{148}$-GFP (line C) but 24.2-fold in GAA$_{148}$-GFP (line A) and 29-fold in GAA$_{148}$-GFP (line B). It is possible that the observed differences in GFP induction are a consequence of different chromatin contexts into which the reporter construct was integrated in each cell line. Nevertheless, the fact that all three GAA$_{148}$-GFP cell lines showed significant increases in GFP reporter expression upon treatment with the same five compounds demonstrates their utility as a resource to identify molecules that promote transcription through GAA repeats.

To obtain information about the longevity of GFP upregulation in response to small molecules, we conducted experiments in which cells were treated with compounds followed by incubation in medium without the compound. In brief, GAA$_{148}$-GFP cells were treated for 48 h with 10 μM pentamidine or distamycin, followed by PBS wash and 48 h incubation in medium without the small molecule. As shown in Fig. 4, the amount of GFP signal relative to SMN is greatly increased in cells subject to PBS wash and subsequent 48 h incubation compared to cells treated for 48 h only (compare GFP signal in lanes 2 and 3 to that found in lanes 5 and 7). These findings demonstrate that small molecules identified by competition dialysis bind with high specificity and continue to exert benefi-
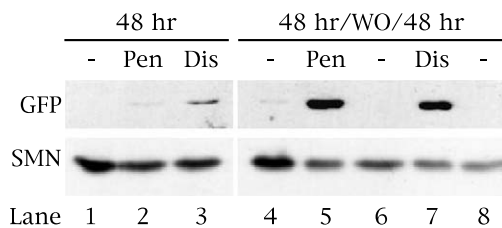
cial effects on transcription through GAA repeats even after excess compound has been removed.

In order to examine if small molecules identified by competition dialysis and screened by the cell reporter system also increase endogenous frataxin levels in patient cells, we cultured patient lymphocytes with 30 μM pentamidine. Although this compound does not increase GFP reporter expression to the same extent as other tested molecules such as DAPI or Hoechst 33258 (Figs. 2 and 3), pentamidine was chosen for this assay because it is approved for the treatment of infections in patients with HIV and thus can be easily tried in FRDA patients. Four different patients with differing GAA repeat lengths were tested. In all cases, we find that frataxin levels are increased relative to SMN upon pentamidine treatment (Fig. 5). As expected, samples with the highest repeat numbers have the least amount of induction. A previous report has shown that healthy FRDA carriers have around 40% of the normal frataxin mRNA level [27]. Consequently, a modest increase in frataxin levels, such as that induced by pentamidine, may be sufficient to moderate the symptoms of FRDA.

Here we report the generation of stable cell lines that can be used to screen compounds for their ability to promote transcription through expanded GAA repeats found in Friedreich's ataxia. We note that the GFP signal from all three lines with 148 GAA repeats is significantly decreased compared to the signal from the three lines with 15 GAA repeats (Figs. 1 and 3). This finding strongly supports, but does not unequivocally prove, that the reduced expression of GFP in the 148 repeat cell lines is the result of the expanded GAA repeats and not a consequence of other factors such as a transgene integration site that depresses GFP expression. We also note that our use of the housekeeping protein SMN as a loading control is valid given that its expression does not significantly change relative to GFP upon different treatments in the cell lines with 15 GAA repeats (Fig. 3). Although not conducted in this study, these cell lines can be adapted for high throughout drug screening using a plate reader format. Small molecules identified using the competition dialysis method were found to significantly increase cellular FRDA-reporter gene expression. These studies therefore demonstrate the feasibility and validity of this approach to uncover small molecules of potential therapeutic value in FRDA. Notably, we show in this report that pentamidine, which is approved for the treatment of infections in patients with HIV, can increase frataxin levels in cultured patient lymphocytes. We are currently testing

Fig. 4. Small molecules can increase reporter gene expression after exposure and incubation in medium lacking the small molecule. GAA$_{148}$-GFP (line A) cells were untreated (lane 1) or treated (10 μM) for 48 h with the indicated small molecule (lanes 2 and 3), followed by PBS wash and incubation a further 48 h. Extract from untreated cells subject to the wash and additional incubation was loaded in lanes 4, 6 and 8. Lanes 5 and 7 were initially incubated with pentamidine and distamycin, respectively. GFP and SMN were detected by immunoblotting.
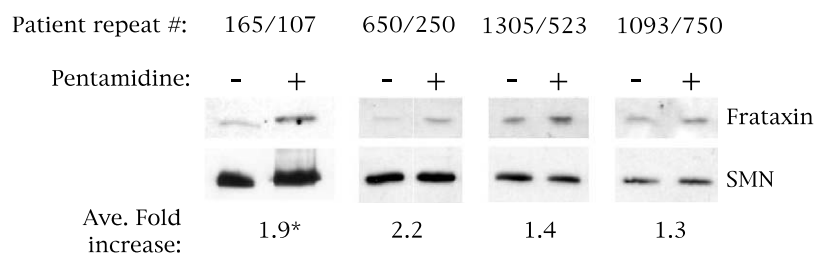

Fig. 5. Pentamidine increases the expression of frataxin in patient tissue. White blood cells from FRDA patients with the indicated GAA repeat numbers were isolated and cultured in the presence (+) or absence (−) of 30 μM pentamidine for 48 h. Total protein was isolated followed by the detection of frataxin (top panel) and SMN (bottom panel) by immunoblotting. Signals were quantified and the ratio of frataxin to SMN was determined. For each patient sample, the average fold increase of frataxin relative to SMN upon pentamidine treatment is shown. Technical duplicates were conducted for all samples except where indicated by an asterisk (this patient has 165 repeats on one allele and 107 repeats on the other allele).

if other small molecules identified in this report can also increase frataxin levels in patient cells.

References

[1] Harding, A.E. (1983) Classification of the hereditary ataxias and paraplegias. Lancet 1, 1151–1155.

[2] Wells, R.D., Dere, R., Hebert, M.L., Napierala, M. and Son, L.S. (2005) Advances in mechanisms of genetic instability related to hereditary neurological diseases. Nucleic Acids Res. 33, 3785–3798.

[3] Campuzano, V. et al. (1997) Frataxin is reduced in Friedreich ataxia patients and is associated with mitochondrial membranes. Hum. Mol. Genet. 6, 1771–1780.

[4] Gatchel, J.R. and Zoghbi, H.Y. (2005) Diseases of unstable repeat expansion: mechanisms and common principles. Nat. Rev. Genet. 6, 743–755.

[5] Gakh, O., Park, S., Liu, G., Macomber, L., Imlay, J.A., Ferreira, G.C. and Isaya, G. (2006) Mitochondrial iron detoxification is a primary function of frataxin that limits oxidative damage and preserves cell longevity. Hum. Mol. Genet. 15, 467–479.

[6] Wilson, R.B. (2003) Frataxin and frataxin deficiency in Friedreich's ataxia. J. Neurol. Sci. 207, 103–105.

[7] Delatycki, M.B., Williamson, R. and Forrest, S.M. (2000) Friedreich ataxia: an overview. J. Med. Genet. 37, 1–8.

[8] Schols, L., Meyer, C., Schmid, G., Wilhelms, I. and Przuntek, H. (2004) Therapeutic strategies in Friedreich's ataxia. J. Neural Transm. Suppl., 135–145.

[9] Rustin, P., Bonnet, D., Rotig, A., Munnich, A. and Sidi, D. (2004) Idebenone treatment in Friedreich patients: one-year-long randomized placebo-controlled trial. Neurology 62, 524–525, (author reply 525; discussion 525).

[10] Seznec, H., Simon, D., Monassier, L., Criqui-Filipe, P., Gansmuller, A., Rustin, P., Koenig, M. and Puccio, H. (2004) Idebenone delays the onset of cardiac functional alteration without correction of Fe–S enzymes deficit in a mouse model for Friedreich ataxia. Hum. Mol. Genet. 13, 1017–1024.

[11] Seznec, H. et al. (2005) Friedreich ataxia: the oxidative stress paradox. Hum. Mol. Genet. 14, 463–474.

[12] Sturm, B., Stupphann, D., Kaun, C., Boesch, S., Schranzhofer, M., Wojta, J., Goldenberg, H. and Scheiber-Mojdehkar, B. (2005) Recombinant human erythropoietin: effects on frataxin expression in vitro. Eur. J. Clin. Invest. 35, 711–717.

[13] Burnett, R., Melander, C., Puckett, J.W., Son, L.S., Wells, R.D., Dervan, P.B. and Gottesfeld, J.M. (2006) DNA sequence-specific polyamides alleviate transcription inhibition associated with long GAA · TTC repeats in Friedreich's ataxia. Proc. Natl. Acad. Sci. USA 103, 11497–11502.

[14] Chaires, J.B. (2005) Competition dialysis: an assay to measure the structural selectivity of drug–nucleic acid interactions. Curr. Med. Chem. Anticancer Agents 5, 339–352.

[15] Campuzano, V. et al. (1996) Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science 271, 1423–1427.

[16] Sun, J., Xu, H., Subramony, S.H. and Hebert, M.D. (2005) Interactions between Coilin and PIASy partially link Cajal bodies to PML bodies. J. Cell Sci. 118, 4995–5003.

[17] Hebert, M.D. and Matera, A.G. (2000) Self-association of coilin reveals a common theme in nuclear body localization. Mol. Biol. Cell 11, 4159–4171.

[18] Ohshima, K., Montermini, L., Wells, R.D. and Pandolfo, M. (1998) Inhibitory effects of expanded GAA.TTC triplet repeats from intron I of the Friedreich ataxia gene on transcription and replication in vivo. J. Biol. Chem. 273, 14588–14595.

[19] Meister, G., Eggert, C. and Fischer, U. (2002) SMN-mediated assembly of RNPs: a complex story. Trends Cell Biol. 12, 472–478.

[20] Chaires, J.B. (2002) (Beaucage, S.L., Bergstrom, D.E., Glick, G.D. and Jones, R.A., Eds.), Current Protocols in Nucleic Acid Chemistry, Vol. 1, pp. 8.3.1–8.3.8, John Wiley and Sons, Inc., New York.

[21] Ren, J. and Chaires, J.B. (1999) Sequence and structural selectivity of nucleic acid binding ligands. Biochemistry 38, 16067–16075.

[22] Neidle, S. (2001) DNA minor-groove recognition by small molecules. Nat. Prod. Rep. 18, 291–309.

[23] Pjura, P.E., Grzeskowiak, K. and Dickerson, R.E. (1987) Binding of Hoechst 33258 to the minor groove of B-DNA. J. Mol. Biol. 197, 257–271.

[24] Searle, M.S. and Embrey, K.J. (1990) Sequence-specific interaction of Hoechst 33258 with the minor groove of an adenine-tract DNA duplex studied in solution by 1H NMR spectroscopy. Nucleic Acids Res. 18, 3753–3762.

[25] Cho, J. and Rando, R.R. (2000) Specific binding of Hoechst 33258 to site 1 thymidylate synthase mRNA. Nucleic Acids Res. 28, 2158–2163.

[26] Disney, M.D., Stephenson, R., Wright, T.W., Haidaris, C.G., Turner, D.H. and Gigliotti, F. (2005) Activity of Hoechst 33258 against *Pneumocystis carinii* f. sp. muris, *Candida albicans*, and *Candida dubliniensis*. Antimicrob. Agents Chemother. 49, 1326–1330.

[27] Pianese, L. et al. (2004) Real time PCR quantification of frataxin mRNA in the peripheral blood leucocytes of Friedreich ataxia patients and carriers. J. Neurol. Neurosurg. Psychiatry 75, 1061–1063.