Biochemistry News

The newsletter of the Department of Biochemistry at the University of Otago

editor: Bronwyn Carlisle

August 2014

In This Issue

View from the Corner

It's mid-August and we are deep into the second term of classes. A lot has changed this year, and even more change is coming. Seems like a good time to get caught up with the first Biochemistry newsletter for 2014! This year our School Dean, Helen Nicholson, became the PVC International, and we have had an Acting Dean, A/Prof Pat Cragg for many months. Recently we have learned that Professor Vernon Ward, current HOD Microbiology has been selected to become the official new Dean of the Otago School of Medical Sciences. Vernon has been an excellent HOD and is an outstanding choice for Dean. We should all wish him well in this challenge, just as we should thank Pat for all of her hard work over the past year in her role as Acting Dean. Vernon's elevation to the Dean position has created a vacancy in Microbiology to fill and we should soon be hearing who their new HOD will be. The rumour mill is suggesting that it will be… oops, not supposed to say anything yet!

The changes taking place are not just outside our department, but we have been pretty busy inside Biochemistry as well. Just last month Frances Mosley left us after 15 years of service to join Human Nutrition as their Financial Administrator. We had a morning tea to help send her off and she then enjoyed a European holiday before making the transition to her new position. Frances' replacement is Debbie Acreman. She has joined us from Pharmacy and is getting settled in, learning about all of our many and varied accounts. She is preparing our draft 2015 budget. It's a huge job but Debbie is both meticulous and assiduous, the perfect combination for finances, and we are so happy to have her. Debbie was the second person added to the financial group this year, after David Scobie joined us in February. David came in as our Purchasing Officer to replace Chelsea Ivey who is now having an OE year.

Last but not least, at the end of this year it is my turn to stand down as the HOD of Biochemistry and the process to choose our new HOD is well underway. It has been my huge privilege to serve in this capacity and I am grateful for the opportunity. I am now planning to spend much of next year on sabbatical and hoping to avoid staff and other committee meetings, at least for a while!

We have only scratched the surface of all the change that has occurred in the department since the last newsletter, so please look inside to read about what we have all been up to which should include some new faces, some farewells, some awards, some surprises, some weddings and new additions, and finally at least one fire!

All my best,

Kurt Krause

Conference report

Origins 2014 and OQOL 2014, Nara and Kyoto, Japan

Recently I was lucky enough to be able to attend two origin of life conferences in Nara and Kyoto, two former capitals of Japan. Nara was the capital for ~70 years in the 8th century, and the large number of temples and shrines reflects a period in which Buddhism was dominant both as a religion and politically. At the centre of Nara is a park in which deer roam freely, and vendors sell plain biscuits to feed them – like we would feed ducks in the gardens! The conference centre was surrounded by picturesque bush-clad hills, similar to some of the nicer parts of New Zealand. The 40-minute bus trip from Osaka to Nara was fascinating, built-up all the way with houses interspersed with small paddies within a very urban setting.

The first conference, Origins 2014, was the second combined international conference between ISSOL (the International Society for the Study of the Origin of Life) and the Bioastronomy Society. As someone who grew up in the 1960s and whose childhood dream was to be an astronaut(!), the combination of origin of life talks with bioastronomy was brilliant, and I enjoyed learning lots of cool new things about the universe For example, did you know that stars form in groups, and that our sun is thought to have between 1,000 and 10,000 solar siblings that formed round about the same time and from out of the same material? This fascinating gem was from a talk by Eric Petigula (from the University of California) about the search for exoplanets. From his work he estimates that as many as ~22% of all stars possess an Earth-sized planet in the inhabitable zone; such small objects can't be directly observed from the Earth however, and require a space-based telescope such as the Hubble. The first exoplanet discovered was an example of a 'hot Jupiter', possessing ~2x Jupiter's mass but with a period of only 2-4 days, in other words extremely close to the parent star. Apparently, early evidence from indirect detection methods failed to convince some of the existence of exoplanets, with minds being changed only with the direct imaging of such planets passing in front of the star by infrared detection.

The second conference, OQOL (Open questions on the Origin of Life) 2014, was held following Origins over two days on the outskirts of nearby Kyoto, and was focused on the outstanding problems that face the field – the ones nobody talks about! A range of problems were posted on the website, of which five were chosen by popular vote. These formed the basis of the sessions, with participants/speakers instructed to address the particular question rather than trot out their standard research abstract, a directive that was definitely more honoured in the breach!

Highlights of the trip for me were:

- meeting with collaborators in Japan and the US (in a side trip to Vermont), and having a chance to discuss some of my ideas with experienced people in the field
- Ram Krishnamurthy speaking about his group's extensive work with RNA analogues containing alternative nucleobases, backbones etc
- learning about the stability of ribose when absorbed to a silica surface
- learning of a new potential source of phosphate on the early Earth, through solubilisation and upward transport by supercritical CO_2 formed at high pressure within the Earth's crust
- talking with a US researcher who has been studying RNA activity at acidic pH
- Hiroaki Suga talking about his proposal for tRNA aminoacylation being originally catalyzed by ancestral tRNA 5'-leader sequences
- trying some of the local cuisine: eggplant and (strong) wasabi, and fresh soybeans!

Harold Bernhardt

Introducing …

Debbie Acreman

I am originally from the City of Bath in the South West of England. My career in the UK was based around charity finance, I worked as a Finance Controller for several years and was very privileged to be in a role in which I spent a lot of time working for the Duchess of Cornwall raising both awareness of Osteoporosis and much needed funds, a job which I adored as it enabled me to meet many famous names and also raise vital funds to keep the charity operating. I also worked as a Financial Accountant for the largest charity based in the South West, another very enjoyable role ensuring compliance with legislation and carrying out audits (yes, I am afraid that some of us do enjoy this type of work).

After several years of talking about "starting a new life" I knew that if I was ever going to emigrate I had to stop talking and start acting. With that in mind, my husband applied for a job and within 6 months we had packed up our lives and boarded a plane to a destination we had never previously been to …… were we mad? I arrived in New Zealand in March 2011 with very mixed emotions, missing my family, friends, job, normality and worrying about an uncertain future, which may or may not turn out the way we had hoped for. The only thing we knew for certain was that this would be what we made it and there would be no turning back.

I started working for the University in September 2011 as a Campus Temp. This enabled me to network within the University and work within several departments. I secured my first permanent position with the School of Pharmacy in August 2012 and it is from that role that I have now joined the Department of Biochemistry.

Outside my working life I have many interests - most of my friends would say it is wine (and I would probably agree with them). My main hobby is building miniature 1/12th scale dolls houses and this takes up a lot of my time (and money), I also adore animals and am the proud mumma of 2 Ragdoll cats (which travelled with me from the UK), 2 Chihuahua dogs and a sheep.

An experience from my childhood which changed my life and I believe gave me determination to succeed happened when I was 9 years old. During a family holiday in Germany I got into difficulty whilst in a swimming pool and was very close to drowning. This trauma left me very scared and weary of water. I hated living with this fear, so in 2004 I booked a holiday to Egypt and enrolled in swimming lessons – I always refer to this as my "holiday from hell" but not only did I concur my fear of water, I also qualified as a Scuba Diver. That sense of achievement never left me and 6 months later I qualified as an Open Water Diver and then went on to become an Advanced Diver.

Introducing …

David Scobie

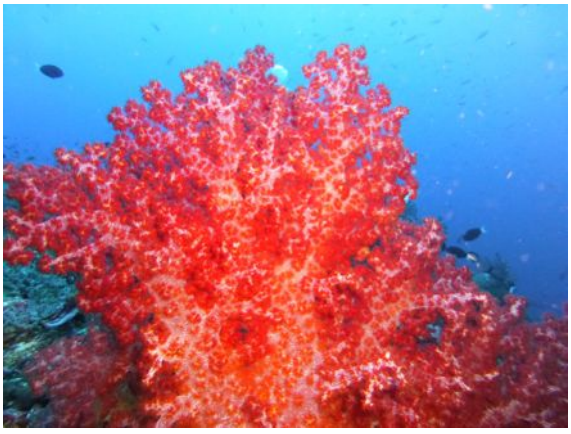
My name is David and I have now lived in Dunedin for just over 8yrs 6 months. I came up from Invercargill for a job, which lasted 8 yrs before I was made redundant yet again.

I am a diver, like walking (at times), reading and attempting to learn the Guitar. I also dabble in genealogy.

My diving has taking to several countries around the Pacific and into the Indian Ocean as well as into Milford , Doubtful & Marlborough Sounds, the Poor Knight and Foveaux Strait – Stewart Island as well around Dunedin Harbour and Moeraki. I have seen many varieties of fish and corals, fed sharks, taken heaps of photos, dived wrecks in New Zealand, the Solomon Islands, Chuuk Lagoon and PNG.

My experience with sharks is that generally they are not interested in you, although occasionally you get an inquisitive shark that will come close to us divers have a look then swim away. Although last year in PNG a small White tip reef shark rub against my leg.

Diving has allowed me to see places I probably would not have gone to otherwise and has pushed me to do things outside my comfort zone, which includes diving itself and diving on wrecks forty to fifty metres under the water.



Happy Hour

The Greek inventor of the centre-parted beard, Herodotus, once said that too much seriousness without any playtime leads slowly to madness.

That was true back then and it is still true now. Consequently, the aim of Happy Hour is to minimise the progression of insanity.

This has not been easy. Because of the change in licensing law (*Sale and Supply of Alcohol Act*, 2012), there are only twelve Happy Hours this year. Despite this, the eight Happy Hours so far have been outstanding.

Additionally, this year saw the return of the *Unofficial Biochem Pub Crawl*. Costumed teams of four set out on a dreary Dunedin day on an inwards pilgrimage from Saint Claire to the Octagon (Fig. 1). Several challenges had to be faced (Fig. 2): from selfies with strangers to continuously holding hands with a teammate while in the Kensington. However, a televised game of cricket-rugby resulted in a schism and most of the teams went to *The Asian* without completing the crawl.

After the Happy Hour of 4th July, yet another Jeff Goldblum movie was shown. Aliens, incessant chess metaphores, explosions and bad 90s fashion all met in *Independence day*. Parenthetically, *The race for the double helix* will not be shown this year even though it has Jeff in it.

Even though there may be special events or a fancy selection of Belgian beers brewed by silent Trappist monks, the thing that makes a Happy Hour event great are the people. After all, Happy Hour is for unwinding after work, meeting new colleagues and chatting to old friends about Ebola, convoluted interdepartmental politics, the Kardashians, bicycles and much more. This year the turnout of people has been spectacular and, on some occasions, it has verged close the 80-person fire safety limit. In fact, on 7th March, the fridge shut before the legal closing time because only Coca-cola cans, dusty 2% beers (a requirement by law) and a bottle of foul Aussie bubbles were left unsold.

Consequently, Happy Hour is the place to be and luckily there are still a few events in store. Upcoming Happy Hour events include the highly anticipated brewing competition on 19th September, the Halloween special on 31st October and the end of the year quiz on 28th November. So do come along, and, with a bit of luck, you'll manage to preserve your remaining sanity.

PS. I highly recommend joining the Facebook page (URL: goo.gl/VTlkAC; Fig. 3) for all up to date news and gossip.

Figure 1. The San Diego Newsteam. From left to right: Nathan Wakefield as Brian Fantana, Tom Wiggins as Ron Burgundy, Natasha Lousie as Veronica Cornigstone, Adam Graham as Champ Kind and Mike Fairhurst as Brick Tamland.

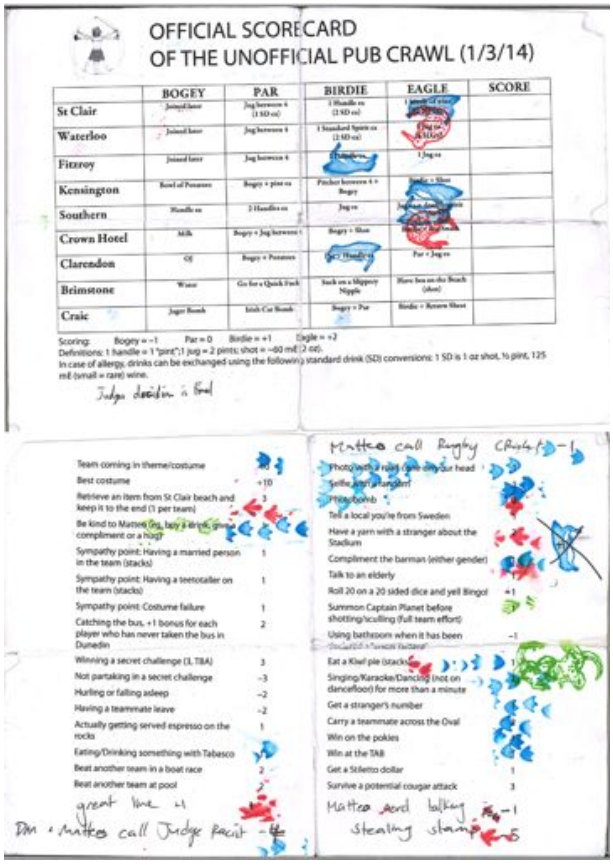


Figure 2. The scorecard of The Bad shirt Team (Matteo Ferla, Danielle Maddock, Vaughn Rankin and Matilda Newton). Despite eating five pies the team still lost.

Figure 3. QR code to the Happy Hour Facebook page.

News from Around the Department

Engineering and Evolving Enzymes

...because we've never had it so good and we're out of the woods, that's why.

My, what a busy 2014 we're having in the E^3 Lab. New(ish) arrivals in 2014 have included Tom Wiggins (Honours with Monica), Cecilia Chambers (Honours with Wayne), Shereen Asha Murugayah (back for a PhD with Monica, after Honours with her and Sigurd), and Miguel Ramirez (post-doc with Monica and Wayne). Suffice to say, Lab 114 hasn't been this packed since…ever? Monica and Wayne aren't helping matters, having both been spotted at the bench recently…

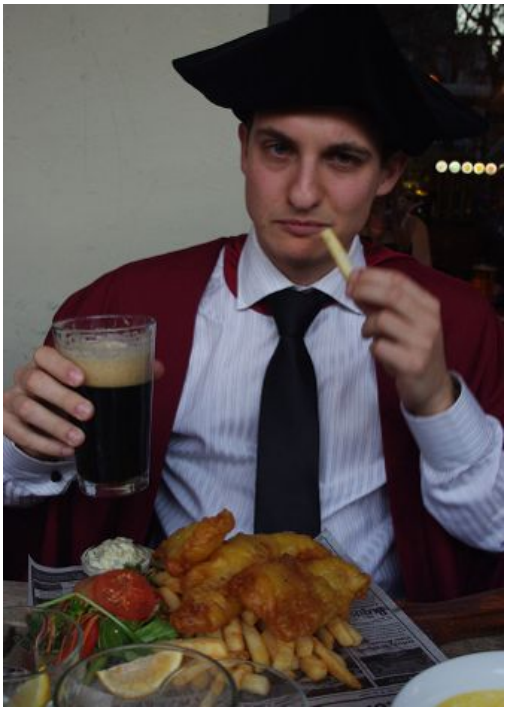
On the bright side, the pace of research has been hot. Among other highlights, Matteo and Wayne published their unequivocally-entitled article, "Bacterial methionine biosynthesis" in *Microbiology*; Danni, Wayne and Monica published the first paper with their LanzaTech collaborators (in *Applied and Environmental Microbiology*); and Monica and Wayne published the definitive DIY guide to everyone's favourite protein engineering technique, ITCHY (in *Methods in Molecular Biology*). Most recently, James, Jordan and Monica had their chemoreceptor paper accepted by *Molecular Microbiology*, pending some pesky revisions involving colony counts that may or may not drive them all crazy.

In between the usual lab shenanigans (double-D mutants, anyone?), the group went in force to the NZ Structural Biology meeting at Hanmer Springs. Congratulations to Tom for winning a poster prize, James for licking the face of a Professor, and Yoshio for showing Miguel the ancient Japanese-Kiwi tradition of skinny dipping in frigid duck ponds.

The other big news was, of course, Matteo finishing his PhD. After a marathon oral exam (who knew Julian Eaton-Rye would only start asking his hard questions in the 5th hour?) and some reasonably straightforward corrections…it was done! Congratulations to Matteo on earning the inaugural "Golden coli" thesis award, and good luck for his postdoctoral fellowship applications. Next up are Jordan and Matilda, who are busy finishing up experiments while also beginning to write their MSc and PhD theses, respectively.

This year is truly proving that time and tide wait for no ~~man~~ person. Nothing a Happy Hour or two won't fix, we're sure…

It has been an exhausting year.



Squiddies no more! Dr. Matteo Ferla enjoys a better class of fish and chips.

E^3 in Hanmer (two minutes before the blizzard started…).

News from lab JER

As I think about what to write in this newsletter, I glance outside and see the sleet and rain hitting the window and hear the wind blowing like crazy … when I get an email from Julian telling me that the weather is lovely and warm in the US where he is attending the Gordon conference! Great!

So 308 is bursting at the seams at the moment, with our 4th years Lauren Nicol, Matt Prouse and Duncan Ross gearing up to present their talks next week. I'm really looking forward to hearing them.

Tim Crawford gave a great talk in Botany last week, and is finalising some bench work with Western Blots, before hiding away in a room somewhere to churn out his PhD thesis.

Shiny Varghese is also hoping that Julian will stop giving her extra experiments so she can also write up her Masters thesis.

Harvinder Singh is plowing through data collection and collation as all good PhD students should.

We welcomed Sandeep Biswas a few months ago, who started his PhD with us. He has settled in well and getting the hang of things in the lab.

Most recently, we have welcomed Chris Williams who is visiting from Norway for 6 months. He has come from Martin Hohmann-Marriott's lab in Trondheim (also where 308's Jake Lamb is doing his PhD). It's fair to say that Dunedin's "snow day" last Friday was somewhat amusing for him.

We are still waiting on one more arrival in the JER group, although not sure where he will sit.

Plans are afoot for our mid-year social in the next few weeks, and we have decided to get our competitive game on and go ten-pin bowling followed by a meal somewhere afterwards. Should be a fun night!!

That's all from us.

Keep warm!

McCormick Lab comings and goings

The last few months in the McCormick lab have been busy as usual. Some of us have been away to exotic places and some of us have been left behind to continue experiments.

Commander and Chief Sally travelled to the other side of the world for a quick trip to a conference in Paris at the World Anti Oxidant Congress getting a wee bit of time to see the sights with her daughter before heading home.

Aimee had a wonderful trip to Bali with some interesting encounters with some monkeys particularly an overprotective mother who took exception to Aimee paying her baby too much attention. All caught on video too.

Ampz managed to get a trip home in mid semester break for a catch up with her family in Napier but now it's all busy getting experiments finished, preparing for her 4th year talk, getting assignments done. The list goes on.

Tanjina is frantically getting the last of the experiments done for the paper after a couple of comments from the reviewers with a little help from Carolyn on the western front. Once this paper is done Tanjina is off home to Bangladesh to see family who haven't see her for way too long. It will be nearly two years since she's been home. Time to put the pipettes down Tanjina and go see the folks. The lab will still be here when you get back we promise.

Monika is also busy getting experiments done and getting data together for a paper. Sometimes she makes it home to get some sleep.

Anne has spent the last while writing a review and says she never wants to do another review. Don't tell me you're missing the FPLC.

Carolyn is busy with her much loved western blots and counting down the days till she's lounging in the sun in Rarotonga and drinking cocktails. Carolyn will be returning however as Sally was successful in getting HRC funding for another three years for her western running research assistant.

Cancer Genetics

Bryony, Henry and Tyler have been to Germany, Melbourne and Singapore in the last wee while, learning awe inspiring and brilliant things about castles, roof top bars, steamed buns, oh and some science too. Anita has been winning yet more awards and even got profiled in the ODT! Mik has officially joined the CGL stronghold since coming back from Houston, I don't think we've scared him off yet, although seemingly the door name-plates are waiting to celebrate at Prof Reeve's retirement party before swapping.

Adrian, a local Doctor is joining us in the lab a few days a week to see if he enjoys science - so far he's still smiling and determined to get some positive results soon. Augustine et al. have published some of their recent positive results and the ensuing celebration continued regardless of snow day madness. Congrats!

We seemingly missed welcoming Adelaide as our super special Hons student after summer and now we're 6 weeks from waving her goodbye - we'll miss you! We'll try and take good care of Anna, (Anita's new masters student) for you too - any tips we need? What's her favourite chocolate?

Ledgerwood Lab

The Ledgerwood Lab welcomes new PhD candidate Rinky Parakra, from Gujarat, India. Rinky achieved a Masters degree in Biotechnology from the National Institute of Pharmaceutical Education and Research in Mohali, India in 2012, and subsequently carried out an internship at the Center of Advanced European Studies and Research in Germany. Rinky is investigating the peroxidase activity of cytochrome *c* and is being co-supervised by Guy Jameson in the Department of Chemistry.

Rinky, Kirstin, Matt and Liz went to The Oxygen Theme Meeting in Christchurch in July to present posters and discuss their research. Matt's poster detailed the role of peroxiredoxin 1 in H_20_2 signaling, while Kirstin's poster explained the phenomena of naturally occurring cytochrome *c* variants and their impact on human health, and Rinky's covered their peroxidase activity. The conference was thoroughly enjoyable and informative. Rewi has been carrying on research on cytochrome *c* variants caspase activation. Lily is working hard to finish her experimental work for her PhD.

Mace Lab

In this edition we welcome the newest member of the team, Abhishek who transitioned from first floor to second floor to start his new position as an ARF.

The talk of many of the newsletters is probably about the 2014 structural biology conference held in Hanmer Springs organised by Peter. The conference itself was a huge success with around 110 attendees and prominent New Zealand and international speakers. It was sad to leave the warm and relaxed conference atmosphere, as well as the beautiful scenery complete with fresh dumping of snow!

Until next time,
Adam, Abhishek and Peter.

Recent Proof That Money Can Buy Happiness... or at least good food.

News from Lab 216

Greetings, dear reader. What news from Lab 216? First of all, two new arrivals this year. Priya Philem has joined us from India and is well underway with her PhD, looking at protein-protein interactions in a biosynthetic pathway in *Pseudomonas aeruginosa*. She is coping well with the Dunedin winter though a bit more warmth would be welcome. Megan Styles is carrying out a BSc (Hons) project, investigating iron uptake genes in the PSA bacteria that are notorious for infecting kiwifruit. She has been generating lots of data and thought-provoking findings. Welcome both! Balancing the arrivals, after doing a great job in Dunedin Annabelle Watts has returned to Brisbane to take up a new position and be closer to family.

Welcome back to Astra and Jess who are both returnees after completing BSc (Hons) to carry out PhD study. Astra has been frequenting Dunedin Hospital, having first been stood on by her horse and then injuring her knee playing ice hockey. She is looking forward to a trip to South Africa to visit relatives though recent history suggests that she would be wise to take a lucky rabbit's foot (if not a horseshoe). In between this incident-packed existence and getting her PhD underway she has been taking the lead in trialing a plate reader in the lab. Jess is (mainly) coping with the noise of the plate reader just behind her bench, though as compensation she does now have a nice view of the carpark. At the other end of the PhD spectrum, congratulations to Leo! who became Dr Leo in May. Leo has carried out some contract work for a company since completing his PhD, and is working on a paper describing some of his PhD research. Becky Edgar, who has carried out much of her PhD at David Ackerley's lab in Wellington, has also handed in her thesis with an oral exam and Departmental seminar happening soon. Natasha Forester who has been carrying out her PhD at AgResearch in Palmerston North will be submitting her thesis in the next week or two and she and her husband Pete has found time to buy a new house. In between the starters and the finishers, Georgi has been pushing on with her PhD project, as well as making submissions on the Dunedin City Council Annual plan which attracted significant media attention. Of the older hands, Lois is getting to grips with the magic of RT-qPCR as well as providing the lab with samples of her excellent baking. Iain has been kept busy by all the above, but made time for a trip to the USA where as well as taking part in a conference he visited long-term collaborator Marv Miller and his wife Patty.

Three conferences and a wedding for lab 118

Actually, we are not as commitment adverse as the title suggests. Rachel, Casey and Matthias all committed to displaying posters at the OSMS poster evening, while Egor, Matthias, Malcolm, and Gabriel took their commitment all the way to the structural biology meeting at Hanmer Springs, where Wilbanks lab collaborations on MIF, CDO and peroxidases featured in talks. Congratulations are due to Peter Mace for organising a stimulating and enjoyable meeting. The very scenic snowstorm during the concluding session was just icing on the cake, or roads. Matthias showed great commitment, talking both at Hanmer Springs, and two days later at the Oxygen Theme meeting in Christchurch where he won the student speaker prize for a talk based on his recently published new assay for thiol dioxygenases. With Bronwyn's assistance, Matthias's paper also featured on the cover of *Analytical Biochemistry.*

Rachel is committed to the education of young minds, stepping up to supervise BIOC 192 labs. At the same time she is persuading first years to master pipetting, she is also persuading HEK293 cells to accept fluorescent Hsc70, most recently checking results with FACS, a new technique for our lab.

Malcolm and Gabriel are taking on their own challenge, extending the single molecule work on Hsp70. By the time you read this, you should have heard Gabriel describe his progress at the 38th departmental research meeting. Malcolm will miss that, as he is committed to spending that afternoon deep in the Australian Synchrotron with a selection or protein crystals. Based on previous synchrotron trips, he and Aimée are waiting to see if the Journal of Medicinal Chemistry will commit to their crystal structures of inhibitor:MIF complexes, reported in a manuscript written with Joel Tyndall and Mark Hampton, as well as Nina DIckerhof, erstwhile student of this department.

Egor has traveled furthest this winter, leaving immediately after the Hanmer Springs conference to visit family in Moscow and Turkey. Ask him how Moscow public opinion differs from Dunedin on recent events in the Ukraine. By the time you read this he will have given a talk in Chemistry and, perhaps, submitted his characterisation of the *P. aeruginosa* homologue of CDO (with assistance from Matthias, Lois, Iain, Torsten, *et al.*).

Jess and Aimée are both so close to submitting their theses, a very committing act. Aimée is planning a celebratory Canadian OE with Simon, while Jess has already committed to training and working as an EMT. She is cleared for driving the ambulance, but is not yet allowed to use the wig-wags.

In contrast, Casey does get to use the lights and siren on "the appliance", and was recently promoted to qualified fire-fighter. While not extinguishing chimney fires in

Ravensbourne or attending the OSMS and ultimate Oxygen theme meeting with Matthias, he published work on kinetics of oxygen transfer in the Journal of the *American Chemical Society*.

Our biggest story, of course, is that last month Antonia married Gio, her high school sweetheart, in Germany before they both returned to Dunedin. Just to tidy things up beforehand, she had her lactoperoxidase paper accepted in *The Journal of Biological Chemistry*. Congratulations and best wishes to bride and groom!

Excellent commitment and commitment to excellence all round!

The Merriman Lab

Since the last newsletter, the Merriman lab, have had an extraordinarily busy time with both work and play. Food, travel and publishing, feature highly in our main activities.

We welcome Callum Tanner as an ARF to our group, working as part of our Bioresources team. Callum previously completed his Honour project here in Biochemistry a short while ago. We farewelled Mansour who managed to sneak out the door to visit home (Iran) for a family wedding, enroute to taking up a position in a Diagnostic Laboratory in Wellington. Mansour is also finishing off his PhD thesis write-up. We wish him and his family all the best and hope they will visit Dunedin. Sara Altaf also visited us with her family when she graduated recently.

Marilyn, Ruth, Murray and Tanya recently visited a collaborator 's laboratory in San Diego for 3 weeks to process. nearly 3000 DNA preps from blood samples (~96000 tube lid opening and closings = blisters on fingers territory!). Tony, Mandy and Callum ensured that the lab work remained ticking over down-under. Tony kept busy writing papers and planning upcoming work. Mandy prepped and aliquoted 4197 gout and control DNA samples to send for the first stage of the Merriman Gout Genome-Wide Association Study. Callum, after a few weeks of training, took responsibility for the daily sample bench work along with Edana. We managed in their three-week absence without any issues, but we really missed their presence and are glad to have our lab team back together now. Also, they brought back large quantities of American confectionary and chocolate, the supplies of which are only becoming exhausted now.

Whilst in San Diego, the team managed to have a look around at some of the tourist and non-tourist sites. Some of our favourite places included: the San Diego Zoo, which has an open-air gondola to get from one end to the other, the USS Midway Aircraft Carrier, which is now a Museum housing a number of aircraft used in the past; and Balboa Park, which is a large park housing art galleries, museums, gardens and more. Of course it was warm there and the beaches were nice too. Lastly, we mustn't forget Ralph's our local supermarket and the small café, 'Zumba' which roasts their own coffee beans and actually knew what a flat white was! This place was a godsend (thanks Ruth) after having to stop drinking coffee because it tasted so terrible. Thanks to Ardea Biosciences for hosting us. We are still waiting for the DNA to arrive so we can quantify them and add them to our other samples, which is our largest and most expensive experiment to date (a GWAS study).

Ramadan has ended, and Humaira and Tahzeeb treated the lab to an Eid celebration morning tea (a.k.a feast). After this we were all so full that no lunch was required. Tahzeeb is sporting a lovely navy blue cast on her wrist thanks to an icy path, and looking forward to wedding celebrations on the 23rd September. Humaira is in the process of writing up (and hoping to beat Hoang's record write up). She is also looking forward to presenting at the American College of Rheumatology conference in Boston in November, along with a short side trip to visit

to see extended family whilst there.

Anna is off at the end of this week to the 6th International Symposium on Biomolecular Archaeology conference in Basel (which is "going to be awesome"), followed by a visit to Italy and Germany. Meanwhile, Tanya is going back to San Diego to the American Society of Human Genetics Society meeting in October and will briefly visit our collaborator site. Tanya is also checking out the local property market.

Next door, Hoang is learning that his six year old son is a very good negotiator after he explained to his school teacher he would oblige with cleaning up as long as he could earn a certificate. The result: mess cleaned up and one happy child with a certificate for helping. Jarrod is busy writing up his Master's thesis.

Murray has a new puppy called Widget, and who is keeping him and Hana busy. So far, Widget has escaped from "getting a swipe" from their cats, and has begun to get them out walking in the morning. James says he is "being consumed by the material" he is preparing for Queenstown Molecular Biology week during the semester break.

Ruth's daughter Charlotte turned 3 years old, and celebrated with an amazing Peppa Pig cake which Ruth created. She has now turned her craft skills to sewing a fancy dress for Charlotte. Mandy's sons Liam and James turned 4 years old and had a huge party in their tiny house. Anya turned 6 years old, as has Crawford Merriman. The 'terrific twos' have begun for Cushla and her husband.

Tony got to look after their kids for part of the time whilst Marilyn was in San Diego. I am not sure what the kids ate other than the mentioned Baked Potatoes and ham sandwiches, however they were still alive. Kara did make Tony buy some groceries at a decent sized supermarket before I got back, and he said he found there was quite a few items in the trolley not on his list. Marilyn enjoyed her time away with the others and reckons it would be a good place for sabbatical.July is our highest laboratory birthday month….. namely Cushla, Tony, Callum and Hoang; so we celebrated at the Staff Club with their curry buffet lunch (which was very good). Next we look forward to a spring pot luck at the Merriman's place.

Krause Lab

As the sleet hammers against the windows, we have been really appreciating our nice warm lab to hide out in until the mercury rises again.

So we have had a productive couple of months.

Emily solved a structure: alanine racemase from *Acinetobacter baumannii* (1.9 Å resolution) and she, Emma, Yoshio and Kurt published a paper about it.

Sinothai, Ashley, Yoshio and Kurt headed out to the Structural Biology meeting at Hanmer Springs, where Ashley won a prize for her beautiful poster, and Yoshio gashed his head open in a pool.

Emma passed her PhD oral exam and will graduate this month, then embark on a long journey to many countries, culminating in Germany to start a postdoc.

Olly and Miriam also got the travel bug and headed to Uppsala, Sweden for the International Symposium for Bioluminescence and Chemiluminescence, where they both gave talks about glowworm bioluminescence.

After failing once, Roman has just passed his full driver's licence test, and proved yet again that failure is the pillar of success!

Kurt also has the travel bug and is in the process of using up another passport, heading to conferences in Montreal, Korea and Washington DC over the next two months. Roman and Sinothai will join him on the trip to Washington DC, hoping to flex their networking muscles and find some future postdoc positions.

And Yoshio was millimetres away from being wiped out by an out of control car while slipping down the hill on the big snow day.

Keep warm everyone!

Miriam in front of Skokloster Castle on the conference excursion

Sinothai in the snow in Hanmer

Olly with other conference goers at café Linné in Uppsala

Recent Publications

Overexpression of *Medicago* SVP genes causes floral defects and delayed flowering in *Arabidopsis* but only affects floral development in *Medicago*.

M. Jaudal, J. Monash, L. Zhang, J. Wen, K. S. Mysore, R. Macknight, and J. Putterill

Journal of Experimental Botany, 2014 vol. 65 (2) pp. 429-442

Genetic analyses of bolting in bulb onion (*Allium cepa L.*).

Samantha Baldwin, Roopashree Revanna, Meeghan Pither-Joyce, Martin Shaw, Kathryn Wright, Susan Thomson, Leire Moya, Robyn Lee, Richard Macknight, and John McCallum

Theoretical And Applied Genetics, 2014 vol. 127 (3) pp. 535-547

Physical Interventions to Manipulate Texture and Tenderness of Fresh Meat: a Review.

Alaa El-Din A Bekhit, Alan Carne, Minh Ha, and Philip Franks

International Journal of Food Properties, 2014 vol. 17 (2) pp. 433-453

Genetic code evolution started with the incorporation of glycine, followed by other small hydrophilic amino acids.

Harold S Bernhardt and Wayne M Patrick

We propose that glycine was the first amino acid to be incorporated into the genetic code, followed by serine, aspartic and/or glutamic acid-small hydrophilic amino acids that all have codons in the bottom right-hand corner of the standard genetic code table. Because primordial ribosomal synthesis is presumed to have been rudimentary, this stage would have been characterized by the synthesis of short, water-soluble peptides, the first of which would have comprised polyglycine. Evolution of the code is proposed to have occurred by the duplication and mutation of tRNA sequences, which produced a radiation of codon assignment outwards from the bottom right-hand corner. As a result of this expansion, we propose a trend from small hydrophilic to hydrophobic amino acids, with selection for longer polypeptides requiring a hydrophobic core for folding and stability driving the incorporation of hydrophobic amino acids into the code.

Journal of molecular evolution, 2014 vol. 78 (6) pp. 307-309

Accurate computational prediction of the transcribed strand of CRISPR noncoding RNAs.

Ambarish Biswas, Peter Fineran, and Chris Brown

MOTIVATION:CRISPR RNAs (crRNAs) are a type of small noncoding RNA that form a key part of an acquired immune system in prokaryotes. Specific prediction methods find crRNA-encoding loci in nearly half of sequenced bacterial, and three quarters of archaeal, species. These CRISPR arrays consist of repeat elements alternating with specific spacers. Generally one strand is transcribed, producing long pre-crRNAs, which are processed to short crRNAs that base pair with invading nucleic acids to facilitate their destruction. No current software for the discovery of CRISPR loci predicts the direction of crRNA transcription.

RESULTS:We have developed an algorithm that accurately predicts the strand of the resulting crRNAs. The method uses as input CRISPR repeat predictions. CRISPRDirection uses parameters that are calculated from the CRISPR repeat predictions and flanking sequences, which are combined by weighted voting. The prediction may utilise prior coding sequence annotation, but this is not required. CRISPRDirection correctly predicted the orientation of 94% of a reference set of arrays.

AVAILABILITY AND IMPLEMENTATION:The Perl source code is freely available from http://bioanalysis.otago.ac.nz/CRISPRDirection.

CONTACT:chris.brown@otago.ac.nz
SUPPLEMENTARY INFORMATION: Reference datasets and predictions, CRISPRDirection, additional figures and methods.

Bioinformatics, 2014

Scan for Motifs: a webserver for the analysis of post-transcriptional regulatory elements in the 3' untranslated regions (3' UTRs) of mRNAs.

Ambarish Biswas and Chris M Brown

BACKGROUND:Gene expression in vertebrate cells may be controlled post-transcriptionally through regulatory elements in mRNAs. These are usually located in the untranslated regions (UTRs) of mRNA sequences, particularly the 3'UTRs.

RESULTS:Scan for Motifs (SFM) simplifies the process of identifying a wide range of regulatory elements on alignments of vertebrate 3'UTRs. SFM includes identification of both RNA Binding Protein (RBP) sites and targets of miRNAs. In addition to searching pre-computed alignments, the tool provides users the flexibility to search their own sequences or alignments. The regulatory elements may be filtered by expected

value cutoffs and are cross-referenced back to their respective sources and literature. The output is an interactive graphical representation, highlighting potential regulatory elements and overlaps between them. The output also provides simple statistics and links to related resources for complementary analyses. The overall process is intuitive and fast. As SFM is a free web-application, the user does not need to install any software or databases.

CONCLUSIONS:Visualisation of the binding sites of different classes of effectors that bind to 3'UTRs will facilitate the study of regulatory elements in 3' UTRs.

BMC *Bioinformatics*, 2014 vol. 15 p. 174

A genome-wide association study of rheumatoid arthritis without antibodies against citrullinated peptides.

L Bossini-Castillo, C de Kovel, H Kallberg, R van't Slot, A Italiaander, M Coenen, P P Tak, M D Posthumus, C Wijmenga, T Huizinga, A H M van der Helm-van Mil, G Stoeken-Rijsbergen, L Rodriguez-Rodriguez, A Balsa, I González-álvaro, M A Gonzalez-Gay, C Gómez-Vaquero, B Franke, S Vermeulen, I E van der Horst-Bruinsma, B A C Dijkmans, G J Wolbink, R A Ophoff, M T Maehlen, P van Riel, M Merriman, L Klareskog, B A Lie, T Merriman, J B A Crusius, E Brouwer, J Martín, N de Vries, R Toes, L Padyukov, and B P C Koeleman

Annals of the Rheumatic Diseases, 14 February 2014

Biosynthesis of novel pyoverdines by domain substitution in a non-ribosomal peptide synthetase of *Pseudomonas aeruginosa*.

Mark J Calcott, Jeremy G Owen, Iain L Lamont, and David F Ackerley

Pyoverdine is a fluorescent non-ribosomal peptide siderophore made by fluorescent pseudomonads. The Pseudomonas aeruginosa non-ribosomal peptide synthetase (NRPS) PvdD contains two modules that each incorporate an L-threonine residue at the C-terminal end of pyoverdine. In an attempt to generate modified pyoverdine peptides, we substituted alternative substrate-specifying adenylation (A) and peptide bond-catalyzing condensation (C) domains into the second module of PvdD. When just the A domain was substituted, the resulting strains produced only wild type pyoverdine - at high levels if the introduced A domain specified threonine, or at trace levels otherwise. The high levels of pyoverdine synthesis observed whenever the introduced A domain specified threonine indicated that these non-native A domains were able to communicate effectively with the PvdD C domain. Moreover, the unexpected observation that non-threonine specifying A domains were nevertheless incorporating threonine into pyoverdine suggests that the native PvdD C domain was exhibiting stronger selectivity than these A domains for the incorporated amino acid substrate (i.e., mis-activation of a threonine residue by the introduced A domains was more frequent than mis-incorporation of a non-threonine residue by the PvdD C domain). In contrast, substitution of both the C and A domains of PvdD generated high yields of rationally modified pyoverdines in two instances, these pyoverdines having a lysine or a serine residue in place of the terminal threonine. However, C-A domain substitution more commonly yielded a truncated peptide product, likely due to stalling of synthesis on a non-functional recombinant NRPS template.

Applied and environmental microbiology, 2014

Prospects for inhibiting the post-transcriptional regulation of gene expression in hepatitis B virus.

Augustine Chen, Nattanan Panjaworayan T-Thienprasert, and Chris M Brown

There is a continuing need for novel antivirals to treat hepatitis B virus (HBV) infection, as it remains a major health problem worldwide. Ideally new classes of antivirals would target multiple steps in the viral lifecycle. In this review, we consider the steps in which HBV RNAs are processed, exported from the nucleus and translated. These are often overlooked steps in the HBV life-cycle. HBV, like retroviruses, incorporates a number of unusual steps in these processes, which use a combination of viral and host cellular machinery. Some of these unusual steps deserve a closer scrutiny. They may provide alternative targets to existing antiviral therapies, which are associated with increasing drug resistance. The RNA post-transcriptional regulatory element identified 20 years ago promotes nucleocytoplasmic export of all unspliced HBV RNAs. There is evidence that inhibition of this step is part of the antiviral action of interferon. Similarly, the structured RNA epsilon element situated at the 5' end of the polycistronic HBV pregenomic RNA also performs key roles during HBV replication. The pregenomic RNA, which is the template for translation of both the viral core and polymerase proteins, is also encapsidated and used in replication. This complex process, regulated at the epsilon element, also presents an attractive antiviral target. These RNA elements that mediate and regulate gene expression are highly conserved and could be targeted using novel strategies employing RNAi, miRNAs or aptamers. Such approaches targeting these functionally constrained genomic regions should avoid escape mutations. Therefore understanding these regulatory elements, along with providing potential targets, may also facilitate the development of other new classes of antiviral drugs.

World journal of gastroenterology : WJG, 2014 vol. 20 (25) pp. 7993-8004

E-cadherin loss alters cytoskeletal organization and adhesion in non-malignant breast cells but is insufficient to induce an epithelial-mesenchymal transition.

Augustine Chen, Henry Beetham, Michael A Black, Rashmi Priya, Bryony J Telford, Joanne Guest, George A R Wiggins, Tanis D Godwin, Alpha S Yap, and Parry J Guilford

BACKGROUND:E-cadherin is an adherens junction protein that forms homophilic intercellular contacts in epithelial cells while also interacting with the intracellular cytoskeletal networks. It has roles including establishment and maintenance of cell polarity, differentiation, migration and signalling in cell proliferation pathways. Its downregulation is commonly observed in epithelial tumours and is a hallmark of the epithelial to mesenchymal transition (EMT).

METHODS:To improve our understanding of how E-cadherin loss contributes to tumorigenicity, we investigated the impact of its elimination from the non-tumorigenic breast cell line MCF10A. We performed cell-based assays and whole genome RNAseq to characterize an isogenic MCF10A cell line that is devoid of CDH1 expression due to an engineered homozygous 4 bp deletion in CDH1 exon 11.

RESULTS:The E-cadherin-deficient line, MCF10A CDH1-/- showed subtle morphological changes, weaker cell-substrate adhesion, delayed migration, but retained cell-cell contact, contact growth inhibition and anchorage-dependent growth. Within the cytoskeleton, the apical microtubule network in the CDH1-deficient cells lacked the radial pattern of organization present in the MCF10A cells and F-actin formed thicker, more numerous stress fibres in the basal part of the cell. Whole genome RNAseq identified compensatory changes in the genes involved in cell-cell adhesion while genes involved in cell-substrate adhesion, notably ITGA1, COL8A1, COL4A2 and COL12A1, were significantly downregulated. Key EMT markers including CDH2, FN1, VIM and VTN were not upregulated although increased expression of proteolytic matrix metalloprotease and kallikrein genes was observed.

CONCLUSIONS:Overall, our results demonstrated that E-cadherin loss alone was insufficient to induce an EMT or enhance transforming potential in the non-tumorigenic MCF10A cells but was associated with broad transcriptional changes associated with tissue remodelling.

BMC cancer, 2014 vol. 14 p. 552

Culture, law, ethics, and social implications: Is society ready for advanced genomic medicine?

Jon Cornwall, Tania Slatter, Parry Guilford, Cristin G. Print, Mark Henaghan, and Richman Wee

The Australasian medical journal, 2014 vol. 7 (4) pp. 200-202

Influence of the ABCG2 gout risk 141 K allele on urate metabolism during a fructose challenge.

N Dalbeth, M E House, G D Gamble, B Pool, A Horne, L Purvis, A Stewart, M Merriman, M Cadzow, A Phipps-Green, and T R Merriman

Arthritis research & therapy, 2014 vol. 16 (1)

Estimation of post-mortem interval using biochemical markers.

Andrea Evelyn Donaldson and Iain L Lamont

Australian Journal of Forensic Sciences, 2014 vol. 46 (1) pp. 8-26

Epigenetics, plasticity, and evolution: How do we link epigenetic change to phenotype?

Elizabeth J Duncan, Peter D Gluckman, and Peter K Dearden

Epigenetic mechanisms are proposed as an important way in which the genome responds to the environment. Epigenetic marks, including DNA methylation and Histone modifications, can be triggered by environmental effects, and lead to permanent changes in gene expression, affecting the phenotype of an organism. Epigenetic mechanisms have been proposed as key in plasticity, allowing environmental exposure to shape future gene expression. While we are beginning to understand how these mechanisms have roles in human biology and disease, we have little understanding of their roles and impacts on ecology and evolution. In this review, we discuss different types of epigenetic marks, their roles in gene expression and plasticity, methods for assaying epigenetic changes, and point out the future advances we require to understand fully the impact of this field. J. Exp. Zool. (Mol. Dev. Evol.) 9999B: 1-13, 2014. © 2014 Wiley Periodicals, Inc.

Journal of experimental zoology Part B, Molecular and developmental evolution, 2014

Genomic characterisation of Felis catus papillomavirus 4, a novel papillomavirus detected in the oral cavity of a domestic cat.

Magdalena Dunowska, John S Munday, Rebecca E Laurie, and Simon F K Hills

Virus Genes, 2014 vol. 48 (1) pp. 111-119

Algal and cyanobacterial bioenergy and diversity

Julian J Eaton-Rye, Michael A Packer, Tina C Summerfield, and Susanna A Wood

New Zealand Journal Of Botany, 2014 vol. 52 (1) pp. 1-5

A chromogenic assay of substrate depletion by thiol dioxygenases.

Matthias Fellner, Laura M Doughty, Guy N L Jameson, and Sigurd M Wilbanks

A fast and easy method for enzyme activity assays using the chromogenic Ellman's reagent, 5,5'-dithiobis-(2-nitrobenzoic acid), was developed. The method was used to measure the activity of the non-heme mono-iron enzyme cysteine dioxygenase. Quantifying the depletion of the substrate, cysteine, allowed standard kinetic parameters to be determined for the enzyme from Rattus norvegicus. The assay was also used to quickly test the effects of ionic strength, pH, enzyme storage conditions and potential inhibitors and activators. This assay facilitates a higher throughput than available HPLC-based assays, as it enjoys the advantages of fewer sample handling steps, implementation in a 96-well format, and speed. In addition, the relative specificity of Ellman's reagent coupled with its reaction with a wide range of thiols, means that this assay is applicable to many enzymes. Finally, the use of readily available reagents and instrumentation means that this assay can be used by practically any research group to compare results with those of other groups.

Analytical biochemistry, 2014

Bacterial methionine biosynthesis.

M P Ferla and W M Patrick

Methionine is essential in all organisms, as it is both a proteinogenic amino acid and a component of the cofactor, S-adenosyl methionine. The metabolic pathway for its biosynthesis has been extensively characterized in Escherichia coli; however, it is becoming apparent that most bacterial species do not use the E. coli pathway. Instead, studies on other organisms and genome sequencing data are uncovering significant diversity in the enzymes and metabolic intermediates that are used for methionine biosynthesis. In this review, we have summarized the different biochemical strategies that are employed in the three key steps for methionine biosynthesis from homoserine (i.e. acylation, sulfurylation and methylation). We have surveyed the presence and absence of the various biosynthetic enzymes in 1,593 representative bacterial species, shedding light on the non-canonical nature of the E. coli pathway. We have also highlighted ways in which knowledge of methionine biosynthesis can be utilized for biotechnological applications. Finally, we have noted gaps in the current understanding of bacterial methionine biosynthesis. For example, we discuss the presence of one gene (metC) in a large number of species that appear to lack the gene encoding the enzyme for the preceding step in the pathway (metB), as it is understood in E. coli. Therefore, this review aims to move the focus away from E. coli, in order to better reflect the true diversity of bacterial pathways for methionine biosynthesis.

Microbiology, 2014

Association analysis of the SLC22A11 (organic anion transporter 4) and SLC22A12 (urate transporter 1) urate transporter locus with gout in New Zealand case-control sample sets reveals multiple ancestral-specific effects.

Tanya J Flynn, Amanda Phipps-Green, Jade E Hollis-Moffatt, Marilyn E Merriman, Ruth Topless, Grant Montgomery, Brett Chapman, Lisa K Stamp, Nicola Dalbeth, and Tony R Merriman

INTRODUCTION:There is inconsistent association between urate transporters SLC22A11 (organic anion transporter 4 (OAT4)) and SLC22A12 (urate transporter 1 (URAT1)) and risk of gout. New Zealand (NZ) Māori and Pacific Island people have higher serum urate and more severe gout than European people. The aim of this study was to test genetic variation across the SLC22A11/SLC22A12 locus for association with risk of gout in NZ sample sets.

METHODS:A total of 12 single nucleotide polymorphism (SNP) variants in four haplotype blocks were genotyped using TaqMan® and Sequenom MassArray in 1003 gout cases and 1156 controls. All cases had gout according to the 1977 American Rheumatism Association criteria. Association analysis of single markers and haplotypes was performed using PLINK and Stata.

RESULTS:A haplotype block 1 SNP (rs17299124) (upstream of SLC22A11) was associated with gout in less admixed Polynesian sample sets, but not European Caucasian (odds ratio; OR = 3.38, P = 6.1 × 10-4; OR = 0.91, P = 0.40, respectively) sample sets. A protective block 1 haplotype caused the rs17299124 association (OR = 0.28, P = 6.0 × 10-4). Within haplotype block 2 (SLC22A11) we could not replicate previous reports of association of rs2078267 with gout in

European Caucasian (OR = 0.98, P = 0.82) sample sets, however this SNP was associated with gout in Polynesian (OR = 1.51, P = 0.022) sample sets. Within haplotype block 3 (including SLC22A12) analysis of haplotypes revealed a haplotype with trans-ancestral protective effects (OR = 0.80, P = 0.004), and a second haplotype conferring protection in less admixed Polynesian sample sets (OR = 0.63, P = 0.028) but risk in European Caucasian samples (OR = 1.33, P = 0.039).

CONCLUSIONS:Our analysis provides evidence for multiple ancestral-specific effects across the SLC22A11/SLC22A12 locus that presumably influence the activity of OAT4 and URAT1 and risk of gout. Further fine mapping of the association signal is needed using trans-ancestral re-sequence data.

Arthritis research & therapy, 2013 vol. 15 (6) pp. -R220

Effect of Aromatase Inhibition on Functional Gene Modules in Estrogen Receptor-Positive Breast Cancer and Their Relationship with Antiproliferative Response.

Qiong Gao, Neill Patani, Anita K Dunbier, Zara Ghazoui, Marketa Zvelebil, Lesley-Ann Martin, and Mitch Dowsett

To investigate potential associations between gene modules representing key biologic processes and response to aromatase inhibitors (AI) in estrogen receptor-positive (ER(+)) breast cancer.Patients and Methods: Paired gene expression and Ki67 protein expression were available from 69 postmenopausal women with ER(+) early breast cancer, at baseline and 2 weeks post-anastrozole treatment, in the presurgical setting. Functional gene modules (n = 26) were retrieved from published studies and their module scores were computed before and after elimination of proliferation-associated genes (PAG). Ki67 and module scores were assessed at baseline and 2 weeks post-anastrozole. Unsupervised clustering was used to assess associations between modules and Ki67.RESULTS: Proliferation-based modules were highly correlated with Ki67 expression both pretreatment and on-treatment. At baseline with and without PAGs, Ki67 expression was significantly inversely correlated with ERG, ESR1.2, SET, and PIK3CA modules. Modules measuring estrogen signaling strongly predicted antiproliferative response to therapy with and without PAGs. Baseline expression of insulin-like growth factor-1 (IGF-I) module predicted a poor change in Ki67-implicating genes within the module as involved in de novo resistance to AIs. High expression of Immune.2.STAT1 module pretreatment predicted poor antiproliferative response to therapy. A significant association between estrogen-regulated genes modules (ESR1, ESR1-2, SET, and ERG) was evident post AI.CONCLUSIONS: Multiple processes and pathways are affected by AI treatment in ER(+) breast cancer. Modules closely associated with ESR1 expression were predictive of good antiproliferative response to AIs, but modules representing immune activity and IGF-I/MAPK were predictive of poor Ki67 response, supporting their therapeutic targeting in combination with AIs. Clin Cancer Res; 1-10. ©2014 AACR.

Clinical cancer research : an official journal of the American Association for Cancer Research, 2014

Identification and characterization of a bacitracin resistance network in *Enterococcus faecalis*.

S Gebhard, C Fang, A Shaaly, D J Leslie, M R Weimar, F. Kalamorz, A Carne, and G M Cook

Antimicrobial Agents and Chemotherapy, 2014 vol. 58 (3) pp. 1425-1433

The transcriptome of the NZ endemic sea urchin Kina (*Evechinus chloroticus*).

Gareth B Gillard, Daniel J Garama, and Chris M Brown

BACKGROUND:Sea urchins are studied as model organisms for developmental and systems biology and also produce highly valued food products. Evechinus chloroticus (Kina) is a sea urchin species that is indigenous to New Zealand. It is the type member of the Evechinus genus based on its morphological characteristics. Previous research has focused on identifying physical factors affecting commercial roe quality of E. chloroticus, but there is almost no genetic information available for E. chloroticus. E. chloroticus is the only species in its genus and has yet to be subject to molecular phylogenetic analysis.

RESULTS:In this study we performed a de novo transcriptome assembly of Illumina sequencing data. A total of 123 million 100 base length paired-end reads were generated using RNA-Seq libraries from a range of E. chloroticus tissues from two individuals obtained from Fiordland, New Zealand. The assembly resulted in a set of 75,002 transcripts with an accepted read coverage and length, of which 24,655 transcripts could be functionally annotated using protein similarity. Transcripts could be further annotated with Gene Ontology, KEGG Orthology and InterPro terms. With this sequence data we could perform the first phylogenetic analysis of E. chloroticus to other species of its family using multiple genes. When sequences for the mitochondrial nitrogen dehydrogenase genes were compared, E. chloroticus remained outside of a family level clade, which indicated E. chloroticus is indeed a genetically distinct genus within its family.

CONCLUSIONS:This study has produced a large set of E. chloroticus transcripts/proteins along with functional annotations, vastly increasing the amount of genomic data available for this species. This provides a resource for current and future studies on E. chloroticus, either

to increase its commercial value, or its use as a model organism. The phylogenetic results provide a basis for further analysis of relationships between E. chloroticus, its family members, and its evolutionary history.

BMC Genomics, 2014 vol. 15 (1) p. 45

Gout in mori: Modern affliction or ancestral trait?

A L Gosling, E Matisoo-smith, and T R Merriman

Rheumatology (United Kingdom), 2014 vol. 53 (5) pp. 773-774

Hyperuricaemia in the Pacific: why the elevated serum urate levels?

Anna L Gosling, Elizabeth Matisoo-Smith, and Tony R Merriman

Rheumatology International, 2014 vol. 34 (6) pp. 743-757

Fractionation of whey proteins from red deer (Cervus elaphus) milk and comparison with whey proteins from cow, sheep and goat milks.

M. Ha, A E D Bekhit, M McConnell, S Mason, and A Carne

Small Ruminant Research, 2014 vol. 120 (1) pp. 125-134

A 9kDa antifreeze protein from the Antarctic springtail, *Gomphiocephalus hodgsoni*.

T. C. Hawes, C J Marshall, and D A Wharton

A 9kDA antifreeze protein (AFP) was isolated and purified from the Antarctic springtail, Gomphiocephalus hodgsoni. By combining selective sampling procedures and a modified ice affinity purification protocol it was possible to directly isolate a single AFP protein without recourse to chromatographic separation techniques. Mass spectrometry identified a single 9kDa component in the purified ice fraction. Intramolecular disulphide bonding was suggested by the presence of 12 cysteine residues. The specific amino acid composition is unique, particularly with regard to the presence of histidine (11.5%). But it also shows noticeable commonalities with insect AFPs in the abundance of cysteine (13.8%), while simultaneously hinting, through the presence of glycine (11.5%), that the metabolic building blocks of AFPs in Collembola may have a phylogenetically-determined component.

Cryobiology, 2014

Structure of the bacterial type II NADH dehydrogenase: a monotopic membrane protein with an essential role in energy generation.

Adam Heikal, Yoshio Nakatani, Elyse Dunn, Marion R Weimar, Catherine L Day, Edward N Baker, J Shaun Lott, Leonid A Sazanov, and Gregory M Cook

Molecular Microbiology, 2014 vol. 91 (5) pp. 950-964

The role of the MCM2-7 helicase complex during Arabidopsis seed development.

Rowan P Herridge, Robert C Day, and Richard C Macknight

The MINICHROMOSOME MAINTENANCE 2-7 (MCM2-7) complex, a ring-shaped heterohexamer, unwinds the DNA double helix ahead of the other replication machinery. Although there is evidence that individual components might have other roles, the essential nature of the MCM2-7 complex in DNA replication has made it difficult to uncover these. Here, we present a detailed analysis of Arabidopsis thaliana mcm2-7 mutants and reveal phenotypic differences. The MCM2-7 genes are coordinately expressed during development, although MCM7 is expressed at a higher level in the egg cell. Consistent with a role in the egg cell, heterozygous mcm7 mutants resulted in frequent ovule abortion, a phenotype that does not occur in other mcm mutants. All mutants showed a maternal effect, whereby seeds inheriting a maternal mutant allele occasionally aborted later in seed development with defects in embryo patterning, endosperm nuclear size, and cellularization, a phenotype that is variable between subunit mutants. We provide evidence that this maternal effect is due to the necessity of a maternal store of MCM protein in the central cell that is sufficient for maintaining seed viability and size in the absence of de novo MCM transcription. Reducing MCM levels using endosperm-specific RNAi constructs resulted in the up-regulation of DNA repair transcripts, consistent with the current hypothesis that excess MCM2-7 complexes are loaded during G1 phase, and are required during S phase to overcome replicative stress or DNA damage. Overall, this study demonstrates the importance of the MCM2-7 subunits during seed development and suggests that there are functional differences between the subunits.

Plant Molecular Biology, 2014

Plasmid Construction by SLIC or Sequence and Ligation-Independent Cloning.

Ryan E Hill and Julian J Eaton-Rye

Sequence and ligation-independent cloning (Nat Methods 4:251-256, 2007) is a powerful tool for the construction of multi-fragment complex plasmids in a simple and efficient manner. Plasmids consisting of 6-7

DNA fragments can be assembled in a single day, with additional 2 days for screening and extraction. SLIC requires PCR products with overlapping regions of 30-40 bp at the 5' and 3' ends, T4 DNA polymerase, and an optional RecA protein for construction.

Methods in molecular biology (Clifton, NJ), 2014 vol. 1116 pp. 25-36

Inhibition of storage pathology in prenatal CLN5-deficient sheep neural cultures by lentiviral gene therapy.

Stephanie M Hughes, Katie M Hope, Janet Boyu Xu, Nadia L Mitchell, and David N Palmer

Neurobiology of Disease, 2014 vol. 62 pp. 543-550

Enhancing the peroxidase activity of cytochrome c by mutation of residue 41: implications for the peroxidase mechanism and cytochrome c release.

Tracy M Josephs, Ian M Morison, Catherine L Day, Sigurd M Wilbanks, and Elizabeth C Ledgerwood

The peroxidase activity of cytochrome c may play a key role in the release of cytochrome c from the mitochondrial intermembrane space in the intrinsic apoptosis pathway. Induction of the peroxidase activity of cytochrome c is ascribed to partial unfolding and loss of axial co-ordination between the haem Fe and Met80, and is thought to be triggered by interaction of cytochrome c with cardiolipin (diphosphatidylglycerol) in vivo. However, the reaction mechanism for the peroxidase activity of either native or cardiolipin-bound cytochrome c is uncertain. In the present study we analyse the peroxidase activity of human and mouse cytochrome c residue 41 variants and demonstrate that stimulation of peroxidase activity can occur without prior loss of Fe-Met80 co-ordination or partial unfolding. The effects of cardiolipin and mutation of residue 41 are not additive, suggesting that cardiolipin stimulates peroxidase activity by the same mechanism as residue 41 mutation. Consistent with this, mutation of residue 41 did not enhance apoptotic release of cytochrome c from mitochondria. We propose that mutation of residue 41, and interaction with cardiolipin, increase peroxidase activity by altering the 40-57 Ω loop and its hydrogen bond network with the propionate of haem ring A. These changes enhance access of hydrogen peroxide and substrate to the haem.

The Biochemical journal, 2014 vol. 458 (2) pp. 259-265

Early Pregnancy Prediction of Preeclampsia in Nulliparous Women, Combining Clinical Risk and Biomarkers: The Screening for Pregnancy Endpoints (SCOPE) International Cohort Study.

Louise C Kenny, Michael A Black, Lucilla Poston, Rennae Taylor, Jenny E Myers, Philip N Baker, Lesley M McCowan, Nigel A B Simpson, Gus A Dekker, Claire T Roberts, Kelline Rodems, Brian Noland, Michael Raymundo, James J Walker, and Robyn A North

More than half of all cases of preeclampsia occur in healthy first-time pregnant women. Our aim was to develop a method to predict those at risk by combining clinical factors and measurements of biomarkers in women recruited to the Screening for Pregnancy Endpoints (SCOPE) study of low-risk nulliparous women. Forty-seven biomarkers identified on the basis of (1) association with preeclampsia, (2) a biological role in placentation, or (3) a role in cellular mechanisms involved in the pathogenesis of preeclampsia were measured in plasma sampled at 14 to 16 weeks' gestation from 5623 women. The cohort was randomly divided into training (n=3747) and validation (n=1876) cohorts. Preeclampsia developed in 278 (4.9%) women, of whom 28 (0.5%) developed early-onset preeclampsia. The final model for the prediction of preeclampsia included placental growth factor, mean arterial pressure, and body mass index at 14 to 16 weeks' gestation, the consumption of ≥3 pieces of fruit per day, and mean uterine artery resistance index. The area under the receiver operator curve (95% confidence interval) for this model in training and validation cohorts was 0.73 (0.70-0.77) and 0.68 (0.63-0.74), respectively. A predictive model of early-onset preeclampsia included angiogenin/placental growth factor as a ratio, mean arterial pressure, any pregnancy loss <10 weeks, and mean uterine artery resistance index (area under the receiver operator curve [95% confidence interval] in training and validation cohorts, 0.89 [0.78-1.0] and 0.78 [0.58-0.99], respectively). Neither model included pregnancy-associated plasma protein A, previously reported to predict preeclampsia in populations of mixed parity and risk. In nulliparous women, combining multiple biomarkers and clinical data provided modest prediction of preeclampsia.

Hypertension, 2014 vol. 64 (3) pp. 644-652

Reconstruction of an acetogenic 2,3-butanediol pathway involving a novel NADPH-dependent primary-secondary alcohol dehydrogenase.

Michael Köpke, Monica L Gerth, Danielle J Maddock, Alexander P Mueller, FungMin Liew, Séan D Simpson, and Wayne M Patrick

Acetogenic bacteria use CO and/or CO2 plus H2 as their sole carbon and energy sources. Fermentation processes with these organisms hold promise for producing chemicals and biofuels from abundant waste gas feedstocks while simultaneously reducing industrial greenhouse gas emissions. The acetogen Clostridium autoethanogenum is known to synthesize the pyruvate-derived metabolites lactate and 2,3-butanediol during gas fermentation. Industrially, 2,3-butanediol is valuable for chemical production. Here we identify and characterize the C. autoethanogenum enzymes for lactate and 2,3-butanediol biosynthesis. The putative C. autoethanogenum lactate dehydrogenase was active when expressed in Escherichia coli. The 2,3-butanediol pathway was reconstituted in E. coli by cloning and expressing the candidate genes for acetolactate synthase, acetolactate decarboxylase, and 2,3-butanediol dehydrogenase. Under anaerobic conditions, the resulting E. coli strain produced 1.1 ± 0.2 mM 2R,3R-butanediol (23 μM h(-1) optical density unit(-1)), which is comparable to the level produced by C. autoethanogenum during growth on CO-containing waste gases. In addition to the 2,3-butanediol dehydrogenase, we identified a strictly NADPH-dependent primary-secondary alcohol dehydrogenase (CaADH) that could reduce acetoin to 2,3-butanediol. Detailed kinetic analysis revealed that CaADH accepts a range of 2-, 3-, and 4-carbon substrates, including the nonphysiological ketones acetone and butanone. The high activity of CaADH toward acetone led us to predict, and confirm experimentally, that C. autoethanogenum can act as a whole-cell biocatalyst for converting exogenous acetone to isopropanol. Together, our results functionally validate the 2,3-butanediol pathway from C. autoethanogenum, identify CaADH as a target for further engineering, and demonstrate the potential of C. autoethanogenum as a platform for sustainable chemical production.

Applied and environmental microbiology, 2014 vol. 80 (11) pp. 3394-3403

Cell-surface signaling in *Pseudomonas*: Stress responses, iron transport, and pathogenicity.

M A Llamas, F Imperi, P Visca, and I L Lamont

FEMS Microbiology Reviews, 2014 vol. 38 (4) pp. 569-597

The Importance of the Hydrophilic Region of PsbL for the Plastoquinone Electron Acceptor Complex of Photosystem II.

Hao Luo, Simon A Jackson, Robert D Fagerlund, Tina C Summerfield, and Julian J Eaton-Rye

The PsbL protein is a 4.5kDa subunit at the monomer-monomer interface of Photosystem II (PS II) consisting of a single membrane-spanning domain and a hydrophilic stretch of ~15 residues facing the cytosolic (or stromal) side of the photosystem. Deletion of conserved residues in the N-terminal region has been used to investigate the importance of this hydrophilic extension. Using Synechocystis sp. PCC 6803, three deletion strains: Δ(N6-N8), Δ(P11-V12) and Δ(E13-N15), have been created. The Δ(N6-N8) and Δ(P11-V12) strains remained photoautotrophic but were more susceptible to photodamage than wild type; however, Δ(E13-N15) cells had the most severe phenotype. The Δ(E13-N15) mutant showed decreased photoautotrophic growth, a reduced number of PS II centers, impaired oxygen evolution in the presence of PS II-specific electron acceptors, and was highly susceptible to photodamage. The decay kinetics of chlorophyll a variable fluorescence after a single turnover saturating flash and the sensitivity to low concentrations of PS II-directed herbicides in the Δ(E13-N15) strain indicate the binding of plastoquinone to the QB-binding site had been altered such that the affinity of QB is reduced. In addition, the PS II-specific electron acceptor 2,5-dimethyl-p-benzoquinone was found to inhibit electron transfer through the quinone-acceptor complex of the Δ(E13-N15) strain. The PsbL Y20A mutant was also investigated and exhibited increased susceptibility to photodamage and increased herbicide sensitivity. Our data suggest the N-terminal hydrophilic region of PsbL influences forward electron transfer from QA through indirect interactions with the D-E loop of the D1 reaction center protein. Our results further indicate that disruption of interactions between the N-terminal region of PsbL and other PS II subunits or lipids destabilizes PS II dimer formation. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: Keys to Produce Clean Energy.

Biochimica et biophysica acta, 2014

Structure and function of the hydrophilic Photosystem II assembly proteins: Psb27, Psb28 and Ycf48.

Peter D Mabbitt, Sigurd M Wilbanks, and Julian J Eaton-Rye

Photosystem II (PS II) is a macromolecular complex responsible for light-driven oxidation of water and reduction of plastoquinone as part of the photosynthetic electron transport chain found in thylakoid membranes. Each PS II complex is composed of at least 20 protein subunits and over 80 cofactors. The biogenesis of PS II

requires further hydrophilic and membrane-spanning proteins which are not part of the active holoenzyme. Many of these biogenesis proteins make transient interactions with specific PS II assembly intermediates: sometimes these are essential for biogenesis while in other examples they are required for optimizing assembly of the mature complex. In this review the function and structure of the Psb27, Psb28 and Ycf48 hydrophilic assembly factors is discussed by combining structural, biochemical and physiological information. Each of these assembly factors has homologues in all oxygenic photosynthetic organisms. We provide a simple overview for the roles of these protein factors in cyanobacterial PS II assembly emphasizing their participation in both photosystem biogenesis and recovery from photodamage.

Plant physiology and biochemistry : PPB / Societe francaise de physiologie vegetale, 2014

Caspase Enzymology and Activation Mechanisms.

Peter D Mace, Stefan J Riedl, and Guy S Salvesen

Apical caspases 8, 9, and 10 are only active as dimers. These dimers are unstable, and to characterize their activity they need to be maintained in vitro in a dimeric state. We provide updated methods for those looking to characterize various aspects of caspase function. We describe full methods for those looking to activate caspases in vitro using kosmotropic reagents, an essential step in characterizing upstream (apical) caspases. We detail methods for fusion of caspase domains to engineered dimerization domains as an alternative method to trigger regulated dimerization of caspases. We also describe methods to determine caspase activity profiles in cells and provide methods for studying the ability of SMAC-mimetic reagents to release inhibition of caspases by IAPs.

Methods in enzymology, 2014 vol. 544C pp. 161-178

Validating Antibodies to the Cannabinoid CB2 Receptor: Antibody Sensitivity Is Not Evidence of Antibody Specificity.

Yannick Marchalant, Philip W Brownjohn, Amandine Bonnet, Torsten Kleffmann, and John C Ashton

Antibody-based methods for the detection and quantification of membrane integral proteins, in particular, the G protein-coupled receptors (GPCRs), have been plagued with issues of primary antibody specificity. In this report, we investigate one of the most commonly utilized commercial antibodies for the cannabinoid CB2 receptor, a GPCR, using immunoblotting in combination with mass spectrometry. In this way, we were able to develop powerful negative and novel positive controls. By doing this, we are able to demonstrate that it is possible for an antibody to be sensitive for a protein of interest-in this case CB2-but still cross-react with other proteins and therefore lack specificity. Specifically, we were able to use western blotting combined with mass spectrometry to unequivocally identify CB2 protein in over-expressing cell lines. This shows that a common practice of validating antibodies with positive controls only is insufficient to ensure antibody reliability. In addition, our work is the first to develop a label-free method of protein detection using mass spectrometry that, with further refinement, could provide unequivocal identification of CB2 receptor protein in native tissues.

The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society, 2014 vol. 62 (6) pp. 395-404

The Genetic Basis of Gout.

Tony R Merriman, Hyon K Choi, and Nicola Dalbeth

Gout results from deposition of monosodium urate (MSU) crystals. Elevated serum urate concentrations (hyperuricemia) are not sufficient for the development of disease. Genome-wide association studies (GWAS) have identified 28 loci controlling serum urate levels. The largest genetic effects are seen in genes involved in the renal excretion of uric acid, with others being involved in glycolysis. Whereas much is understood about the genetic control of serum urate levels, little is known about the genetic control of inflammatory responses to MSU crystals. Extending knowledge in this area depends on recruitment of large, clinically ascertained gout sample sets suitable for GWAS.

Rheumatic diseases clinics of North America, 2014 vol. 40 (2) pp. 279-290

Association of Autoimmune Addison's Disease with Alleles of STAT4 and GATA3 in European Cohorts.

Anna L Mitchell, Katie D R Macarthur, Earn H Gan, Lucy E Baggott, Anette S B Wolff, Beate Skinningsrud, Hazel Platt, Andrea Short, Anna Lobell, Olle Kämpe, Sophie Bensing, Corrado Betterle, Anna Kasperlik-Zaluska, Magdalena Zurawek, Marta Fichna, Ingrid Kockum, Gabriel Nordling Eriksson, Olov Ekwall, Jeanette Wahlberg, Per Dahlqvist, Anna-Lena Hulting, Marissa Penna-Martinez, Gesine Meyer, Heinrich Kahles, Klaus Badenhoop, Stephanie Hahner, Marcus Quinkler, Alberto Falorni, Amanda Phipps-Green, Tony R Merriman, William Ollier, Heather J Cordell, Dag Undlien, Barbara Czarnocka, Eystein Husebye, and Simon H S Pearce

BACKGROUND:Gene variants known to contribute to Autoimmune Addison's disease (AAD) susceptibility include those at the MHC, MICA, CIITA, CTLA4, PTPN22, CYP27B1, NLRP-1 and CD274 loci. The majority of the genetic component to disease

susceptibility has yet to be accounted for.

AIM:To investigate the role of 19 candidate genes in AAD susceptibility in six European case-control cohorts.

METHODS:A sequential association study design was employed with genotyping using Sequenom iPlex technology. In phase one, 85 SNPs in 19 genes were genotyped in UK and Norwegian AAD cohorts (691 AAD, 715 controls). In phase two, 21 SNPs in 11 genes were genotyped in German, Swedish, Italian and Polish cohorts (1264 AAD, 1221 controls). In phase three, to explore association of GATA3 polymorphisms with AAD and to determine if this association extended to other autoimmune conditions, 15 SNPs in GATA3 were studied in UK and Norwegian AAD cohorts, 1195 type 1 diabetes patients from Norway, 650 rheumatoid arthritis patients from New Zealand and in 283 UK Graves' disease patients. Meta-analysis was used to compare genotype frequencies between the participating centres, allowing for heterogeneity.

RESULTS:We report significant association with alleles of two STAT4 markers in AAD cohorts (rs4274624: P=0.00016; rs10931481: P=0.0007). In addition, nominal association of AAD with alleles at GATA3 was found in 3 patient cohorts and supported by meta-analysis. Association of AAD with CYP27B1 alleles was also confirmed, which replicates previous published data. Finally, nominal association was found at SNPs in both the NF-κB1 and IL23A genes in the UK and Italian cohorts respectively.

CONCLUSIONS:Variants in the STAT4 gene, previously associated with other autoimmune conditions, confer susceptibility to AAD. Additionally, we report association of GATA3 variants with AAD: this adds to the recent report of association of GATA3 variants with rheumatoid arthritis.

PLoS ONE, 2014 vol. 9 (3) p. e88991

Whole genome re-sequencing of two 'wild-type' strains of the model cyanobacterium Synechocystis sp. PCC 6803.

J N Morris, T S Crawford, A Jeffs, P A Stockwell, J. J. Eaton-Rye, and T C Summerfield

New Zealand Journal Of Botany, 2014 vol. 52 (1) pp. 36-47

Nano-sized manganese-calcium cluster in photosystem II.

M M Najafpour, M Z Ghobadi, B Haghighi, J. J. Eaton-Rye, T Tomo, J R Shen, and S I Allakhverdiev

Biochemistry-Moscow, 2014 vol. 79 (4) pp. 324-336

Water exchange rate in manganese-based water-oxidizing catalysts in photosynthetic systems: From the water-oxidizing complex in Photosystem II to nano-sized manganese oxides.

Mohammad Mahdi Najafpour, Mohsen Abbasi Isaloo, Julian J Eaton-Rye, Tatsuya Tomo, Hiroshi Nishihara, Kimiyuki Satoh, Robert Carpentier, Jian-Ren Shen, and Suleyman I Allakhverdiev

The water-oxidizing complex (WOC), also known as the oxygen-evolving complex (OEC), of Photosystem II in oxygenic photosynthetic organisms efficiently catalyses water oxidation. It is, therefore, responsible for the presence of oxygen in the Earth's atmosphere. The WOC is a manganese-calcium (Mn4CaO5(H2O)4) cluster housed in a protein complex. In this review, we focus on water exchange chemistry of metal hydrates and discuss the mechanisms and factors affecting this chemical process. Further, water exchange rates for both the biological cofactor and synthetic manganese water splitting are discussed. The importance of fully unveiling the water exchange mechanism to understand the chemistry of water oxidation is also emphasized here.

Biochimica et biophysica acta, 2014

Major Change in Regiospecificity for the Exo-1,3-β-glucanase from *Candida albicans* following Its Conversion to a Glycosynthase.

Y Nakatani, D S Larsen, S.M. Cutfield, and J.F. Cutfield

The exo-1,3-β-glucanase (Exg) from Candida albicans is involved in cell wall β-d-glucan metabolism and morphogenesis through its hydrolase and transglycosidase activities. Previous work has shown that both these activities strongly favor β-1,3-linkages. The E292S Exg variant displayed modest glycosynthase activity using α-d-glucopyranosyl fluoride (α-GlcF) as the donor and pNP-β-d-glucopyranoside (pNPGlc) as the acceptor but surprisingly showed a marked preference for synthesizing β-1,6-linked over β-1,3- and β-1,4-linked disaccharide products. With pNPXyl as the acceptor, the preference became β-1,4 over β-1,3. The crystal structure of the glycosynthase bound to both of its substrates, α-GlcF and pNPGlc, is the first such ternary complex structure to be determined. The results revealed that the donor bound in the -1 subsite, as expected, while the acceptor was oriented in the +1 subsite to facilitate β-1,6-linkage, thereby supporting the results from solution studies. A second crystal structure containing the major product of glycosynthesis, pNP-gentiobiose, showed that the -1 subsite allows another docking position for the terminal sugar; i.e., one position is set up for catalysis, whereas the other is an intermediate stage prior to the displacement of water from the active site by the incoming sugar hydroxyls. The +1 subsite, an aromatic "clamp", permits several different sugar positions and orientations, including a 180° flip that explains the observed variable

regiospecificity. The p-nitrophenyl group on the acceptor most likely influences the unexpectedly observed β-1,6-specificity through its interaction with F229. These results demonstrate that tailoring the specificity of a particular glycosynthase depends not only on the chemical structure of the acceptor but also on understanding the structural basis of the promiscuity of the native enzyme.

Biochemistry, 2014 vol. 53 (20) pp. 3318-3326

Adaptation of Iron Homeostasis Pathways by a *Pseudomonas aeruginosa* Pyoverdine Mutant in the Cystic Fibrosis Lung.

Angela T Nguyen, Maura J O'Neill, Annabelle M Watts, Cynthia L Robson, Iain L Lamont, Angela Wilks, and Amanda G Oglesby-Sherrouse

Cystic fibrosis (CF) patients suffer from chronic bacterial lung infections, most notably by Pseudomonas aeruginosa, which persists for decades in the lungs and undergoes extensive evolution. P. aeruginosa requires iron for virulence and uses the fluorescent siderophore pyoverdine to scavenge and solubilize ferric iron during acute infections. Pyoverdine mutants accumulate in the lungs of some CF patients, however, suggesting that the heme and ferrous iron acquisition pathways of P. aeruginosa are more important in this environment. Here, we sought to determine how evolution of P. aeruginosa in the CF lung affects iron acquisition and regulatory pathways through the use of longitudinal CF isolates. These analyses demonstrated a significant reduction of siderophore production during the course of CF lung infection in nearly all strains tested. Mass spectrometry analysis of one of these strains showed that the later CF isolate has streamlined the metabolic flux of extracellular heme through the HemO heme oxygenase, resulting in more-efficient heme utilization. Moreover, gene expression analysis shows that iron regulation via the PrrF small RNAs (sRNAs) is enhanced in the later CF isolate. Finally, analysis of P. aeruginosa gene expression in the lungs of various CF patients demonstrates that both PrrF and HemO are consistently expressed in the CF lung environment. Combined, these results suggest that heme is a critical source of iron during prolonged infection of the CF lung and that changes in iron and heme regulatory pathways play a crucial role in adaptation of P. aeruginosa to this ever-changing host environment.

Journal of Bacteriology, 2014 vol. 196 (12) pp. 2265-2276

Epigenetics and the Maternal Germline.

A J Osborne, E J Duncan, A G Cridge, and P K Dearden

Transgenerational Epigenetics

Differences In The Transcriptional Response To Fulvestrant And Oestrogen Deprivation In Er-Positive Breast Cancer.

Neill Patani, Anita K Dunbier, Helen Anderson, Zara Ghazoui, Ricardo Ribas, Elizabeth Anderson, Qiong Gao, Roger A'hern, Alan Mackay, Justin Lindemann, Robert Wellings, Jill Walker, Irene Kuter, Lesley-Ann Martin, and Mitch Dowsett

Purpose:Endocrine therapies include aromatase inhibitors and the selective oestrogen receptor (ER) down-regulator fulvestrant. This study aimed to determine if the reported efficacy of fulvestrant over anastrozole, and high- over low-dose fulvestrant, reflect distinct transcriptional responses. Experimental design:Global gene expression profiles from ERα-positive breast carcinomas before and during pre-surgical treatment with fulvestrant (n=22) or anastrozole (n=81), and corresponding in vitro models, were compared. Transcripts responding differently to fulvestrant and oestrogen (E) deprivation were integrated using gene ontology (GO), pathway and network analyses to evaluate their potential significance. Results:The overall transcriptional response to fulvestrant and E-deprivation was correlated (r=0.61 in pre-surgical studies, r=0.87 in vitro), involving down-regulation of E-regulated and proliferation-associated genes. The transcriptional response to fulvestrant was of greater magnitude than E-deprivation (slope=0.62 in pre-surgical studies, slope=0.63 in vitro). Comparative analyses identified 28 genes and 40 GO categories affected specifically by fulvestrant. Seventeen fulvestrant-specific genes, including CAV1/2, SNAI2 and NRP1, associated with ERα, androgen receptor (AR) and TP53, in networks regulating cell cycle, death, survival, and tumour morphology. Eighteen genes responding differently to fulvestrant specifically predicted anti-proliferative response to fulvestrant, but not anastrozole. Transcriptional effects of low-dose fulvestrant correlated with high-dose treatment, but were of lower magnitude (ratio=0.29). Conclusions:The transcriptional response to fulvestrant has much in common with E-deprivation, but is stronger with distinctions potentially attributable to arrest of E-independent ERα activity and involvement of AR signalling. Genes responding differently to fulvestrant may have predictive utility. These data are consistent with the clinical efficacy of fulvestrant versus anastrozole and higher dosing regimens.

Clinical cancer research : an official journal of the American Association for Cancer Research, 2014

Identification of reference genes for RT-qPCR in ovine mammary tissue during late pregnancy and lactation and in response to maternal nutritional programming.

A M Paten, S J Pain, S W Peterson, H T Blair, P R Kenyon, P K Dearden, and E J Duncan

The mammary gland is a complex tissue consisting of multiple cell types which, over the lifetime of an animal, go through repeated cycles of development associated with pregnancy, lactation and involution. The mammary gland is also known to be sensitive to maternal programming by environmental stimuli such as nutrition. The molecular basis of these adaptations is of significant interest, but requires robust methods to measure gene expression. Reverse-transcription quantitative PCR (RT-qPCR) is commonly used to measure gene expression, and is currently the method of choice for validating genome-wide expression studies. RT-qPCR requires the selection of reference genes that are stably expressed over physiological states and treatments. In this study we identify suitable reference genes to normalize RT-qPCR data for the ovine mammary gland in two physiological states; late pregnancy and lactation. Biopsies were collected from offspring of ewes that had been subjected to different nutritional paradigms during pregnancy to examine effects of maternal programming on the mammary gland of the offspring. We evaluated eight candidate reference genes and found that two reference genes (PRPF3 and CUL1) are required for normalising RT-qPCR data from pooled RNA samples, but five reference genes are required for analyzing gene expression in individual animals (SENP2, EIF6, MRPL39, ATP1A1, CUL1). Using these stable reference genes, we showed that TET1, a key regulator of DNA methylation, is responsive to maternal programming and physiological state. The identification of these novel reference genes will be of utility to future studies of gene expression in the ovine mammary gland.

Physiological Genomics, 2014 vol. 46 (15) pp. 560-570

CNP signal peptide fragments are present in the human circulation.

Chris J Pemberton, Maithri Siriwardena, Torsten Kleffmann, and A Mark Richards

BACKGROUND:Signal peptides may be novel biomarkers in cardiovascular diseases.

METHODS:We developed a novel immunoassay to the signal peptide of preproCNP (CNPsp) and used this to document circulating venous concentrations of CNPsp in normal healthy volunteers (n=109), regional plasma CNPsp concentrations in patients undergoing clinically indicated catheterisation (n=24) and temporal CNPsp concentrations in patients with ST-elevation myocardial infarction (STEMI) <4hrs after symptom onset (n=8). The structure/sequence of circulating CNPsp was confirmed by tandem mass spectrometry (MS/MS).

RESULTS:In normal human plasma, CNPsp was detectable at levels higher than NT-proCNP (74±17 vs. 20.±5.5pmol/L). There was no correlation between NTproCNP and CNPsp, but plasma concentrations of sibling signal peptides - CNPsp and BNPsp - were strongly correlated (r=0.532, P<0.001). In patients undergoing catheterisation, there were significant arterio-venous step-ups in CNPsp concentrations across the heart (P<0.01) and kidney (P<0.01). Arterial concentrations of CNPsp significantly correlated with heart rate (r=0.446, P<0.05). In STEMI patients, plasma concentrations of CNPsp showed a biphasic elevation pattern between 6 and 12 hours after symptom onset, with 12 hour values significantly elevated (3-fold) compared with levels at presentation (P<0.05). MS/MS verified circulating CNPsp to be preproCNP(14-23) and preproCNP(16-23) peptides.

CONCLUSIONS:This is the first report of a circulating preproCNP derived signal peptide. Given the clear cardiac and renal secretion profiles of CNPsp and its response in STEMI patients, further studies on potential biological functions and biomarker applications of CNPsp in cardiovascular disease are warranted.

Biochemical and Biophysical Research Communications, 2014

Nematodes from the Victoria Land coast, Antarctica and comparisons with cultured *Panagrolaimus davidi*.

M R Raymond, D A Wharton, and C J Marshall

Antarctic Science, 2014 vol. 26 (1) pp. 15-22

Frequency of CYP2C9 polymorphisms in polynesian people and potential relevance to management of gout with benzbromarone.

Rebecca L Roberts, Mary C Wallace, Daniel F B Wright, Murray Cadzow, Nicola Dalbeth, Peter B Jones, Lisa K Stamp, Andrew A Harrison, Michael A Black, and Tony R Merriman

OBJECTIVES:Gout is a major health problem in Polynesians and allopurinol, the drug of choice for the management gout, appears to be less effective in Polynesian patients. The uricosuric drug benzbromarone is an alternative treatment but CYP2C9 poor metabolisers (PMs) may be at a heightened risk of benzbromarone-induced hepatotoxicity. The objectives of this study were to determine the frequency of the PM alleles CYP2C9*2 and CYP2C9*3 in New Zealand (NZ) Caucasian and Polynesian gout cohorts; and then to test for novel CYP2C9 polymorphisms in Polynesians.

METHODS:Eight hundred and fifty-two Caucasians

(537 controls, 315 gout patients) and 1072 Māori and Pacific Island (Polynesian) people (620 controls, 452 gout patients) were genotyped for CYP2C9*2 and CYP2C9*3. Forty Polynesians were screened for novel CYP2C9 polymorphisms using whole genome sequencing.

RESULTS:Frequency of CYP2C9 PM alleles was significantly higher in Caucasians compared to Polynesians (CYP2C9*2: 13.5% versus 3.1%; CYP2C9*3: 5.5% versus 1.6%, P<1.2E-11). Within Polynesians, CYP2C9 PM alleles were rarer in Western Polynesians (Samoa, Tonga) than Eastern Polynesians (NZ and Cook Island Maori; CYP2C9*2: 0.6% versus 2.5%; CYP2C9*3: 0.4% versus 2.0%; P<0.03). A total of 152 SNPs were found by sequencing. None of these variants were predicted by in silico analysis to significantly impact on CYP2C9 expression or activity.

CONCLUSION:Prospective CYP2C9 genotyping of Caucasian gout patients may be warranted for benzbromarone, whereas the low frequencies of CYP2C9 PM alleles in Polynesians suggests that the CYP2C9 polymorphism may be of little or no relevance to benzbromarone prescribing in this population.

Joint, bone, spine : revue du rhumatisme, 2014 vol. 81 (2) pp. 160-163

Urate as a Potential Physiological Substrate for Lactoperoxidase.

A Seidel, G Jameson, R Turner, A Kettle, and S Wilbanks

Journal of Biological Inorganic Chemistry, 2014 vol. 19 pp. S272-S272

Uric Acid and thiocyanate as competing substrates of lactoperoxidase.

Antonia Seidel, Heather Parker, Rufus Turner, Nina Dickerhof, Irada S Khalilova, Sigurd M Wilbanks, Anthony J Kettle, and Guy N L Jameson

The physiological function of urate is poorly understood. It may act as a danger signal, an antioxidant, or a substrate for heme peroxidases. Whether it reacts sufficiently rapidly with lactoperoxidase (LPO) to act as a physiological substrate remains unknown. LPO is a mammalian peroxidase that plays a key role in the innate immune defense by oxidizing thiocyanate to the bactericidal and fungicidal agent hypothiocyanite. We now demonstrate that urate is a good substrate for bovine LPO. Urate was oxidized by LPO to produce the electrophilic intermediates dehydrourate and 5-hydroxyisourate, which decayed to allantoin. In the presence of superoxide, high yields of hydroperoxides were formed by LPO and urate. Using stopped-flow spectroscopy, we determined rate constants for the reaction of urate with compound I ($k1 = 1.1 \times 10(7)$ m(-1) s(-1)) and compound II ($k2 = 8.5 \times 10(3)$ m(-1) s(-1)). During urate oxidation, LPO was diverted from its peroxidase cycle because hydrogen peroxide reacted with compound II to give compound III. At physiologically relevant concentrations, urate competed effectively with thiocyanate, the main substrate of LPO for oxidation, and inhibited production of hypothiocyanite. Similarly, hypothiocyanite-dependent killing of Pseudomonas aeruginosa was inhibited by urate. Allantoin was present in human saliva and associated with the concentration of LPO. When hydrogen peroxide was added to saliva, oxidation of urate was dependent on its concentration and peroxidase activity. Our findings establish urate as a likely physiological substrate for LPO that will influence host defense and give rise to reactive electrophilic metabolites.

The Journal of biological chemistry, 2014 vol. 289 (32) pp. 21937-21949

Transcriptome of the New Zealand glowworm, *Arachnocampa luminosa*.

Miriam Sharpe, Peter Dearden, Gregory Gimenez, and Kurt Krause

Luminescence, 2014 vol. 29 pp. 45-46

Impaired response or insufficient dosage?-Examining the potential causes of "inadequate response" to allopurinol in the treatment of gout.

Lisa K Stamp, Tony R Merriman, Murray L Barclay, Jasvinder A Singh, Rebecca L Roberts, Daniel F B Wright, and Nicola Dalbeth

OBJECTIVES:Gout is one of the most common forms of arthritis. It is well established that urate-lowering therapy that aims for a serum urate less than at least 0.36mmol/l (6mg/dl) is required for the successful management of gout. Allopurinol, a xanthine oxidase (XO) inhibitor, is the most commonly used urate-lowering therapy. However, many patients fail to achieve the target serum urate on allopurinol; these patients can be considered to have "inadequate response" to allopurinol. Herein, we examine the potential mechanisms and implications of inadequate response to allopurinol.

METHODS:The literature was reviewed for potential causes for failure to reach target serum urate in patients receiving allopurinol.

RESULTS:The two most common causes of inadequate response to allopurinol are poor adherence and under-dosing of allopurinol. Adherent patients who fail to achieve target serum urate on standard doses of allopurinol form a group that could be considered to be "partially resistant" to allopurinol. There are four

potential mechanisms for partial allopurinol resistance: decreased conversion of allopurinol to oxypurinol; increased renal excretion of oxypurinol; abnormality in XO structure and/or function such that oxypurinol is rendered less effective and/or drug interactions.

CONCLUSIONS:It is important to determine the reasons for failure to achieve treatment targets with allopurinol, particularly as newer agents become available. The knowledge of the mechanisms for inadequate response may help guide the clinician towards making a therapeutic choice that is more likely to result in achieving the serum urate target.

Seminars in arthritis and rheumatism, 2014

Bioinformatic Methods to Discover Cis-regulatory Elements in mRNAs.

Stewart G Stevens and Chris M Brown

Bioinformatic Methods to Discover Cis-regulatory Elements in mRNAs.

Chapter 10, 151-169. Berlin, Heidelberg

DMAP: Differential Methylation Analysis Package for RRBS and WGBS data.

Peter A Stockwell, Aniruddha Chatterjee, Euan J Rodger, and Ian M Morison

MOTIVATION:The rapid development of high-throughput sequencing technologies has enabled epigeneticists to quantify DNA methylation on a massive scale. Progressive increases in sequencing capacity present challenges in terms of processing analysis and the interpretation of the large amount of data, investigating differential methylation between genome-scale data from multiple samples highlights this challenge.

RESULTS:We have developed a differential methylation analysis package (DMAP) to generate coverage-filtered reference methylomes and identify differentially methylated regions across multiple samples from reduced representation (RRBS) and whole genome bisulphite sequencing (WGBS) experiments. We introduce a novel fragment-based approach for investigating DNA methylation patterns for RRBS data. Further, DMAP provides the identity of gene and CpG features and distances to the differentially methylated regions in a format that is easily analysed with limited bioinformatics knowledge.

AVAILABILITY AND IMPLEMENTATION:The software has been implemented in C and has been written to ensure portability between different platforms. The source code and documentation is freely available (DMAP: as compressed TAR archive folder) from http://biochem.otago.ac.nz/research/databases-software/. Two test datasets are also available for download from the website. Test dataset 1 contains reads from chromosome 1 of a patient and a control, which is used, for comparative analysis in the current article. Test dataset 2 contains reads from a part of chromosome 21 of three disease and three control samples for testing the operation of DMAP, especially for the analysis of variance (ANOVA). Example commands for the analyses are included.Contact: peter.stockwell@otago.ac.nz; aniruddha.chatterjee@otago.ac.nzSupplementary Information: Supplementary data are available at Bioinformatics online.

Bioinformatics, 2014

Altered transcription of murine genes induced in the small bowel by administration of probiotic strain *Lactobacillus rhasus* HN001.

G. W. Tannock, C Taylor, B Lawley, D Loach, M Gould, A.C. Dunn, A.D. McLellan, M A Black, L McNoe, J Dekker, P Gopal, and M A Collett

Applied and environmental microbiology, 2014 vol. 80 (9) pp. 2851-2859

Expression of the developmental transcription factor fezf2 identifies a distinct subpopulation of layer 5 intratelencephalic-projection neurons in mature mouse motor cortex.

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The transcription factor encoded by Fez family zinc finger 2 (Fezf2) is necessary for normal development of the cerebral cortex. However, Fezf2 continues to be expressed in the mature brain, indicating that it might also be necessary for cortical function throughout life. Here, we show a unique identity of Fezf2-expressing intratelencephalic-projection neurons (IT-PNs) in layer 5 of the mature mouse motor cortex, using a Fezf2-Gfp reporter mouse, in vivo retrograde labeling, whole-cell electrophysiology with morphology reconstruction, and cluster analysis. Fezf2-expressing IT-PNs occupy layer 5A and display an apical dendritic tuft; functionally, they fire broad, adapting action potentials and exhibit an Ih-mediated voltage sag that influences their synaptic properties. In contrast, IT-PNs without Fezf2 expression mainly occupy layer 5B, do not display a tuft, and exhibit regular action potential firing and little sag. Both groups of IT-PNs demonstrated distinct frequency-selective synaptic responses to commissural inputs, indicating unique contributions within the cortical microcircuitry. Our findings establish a new, distinct physiological identity of Fezf2-expressing neurons within mature motor cortex.

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Effect of the defatting process, acid and alkali extraction on the physicochemical and functional properties of hemp, flax and canola seed cake protein isolates.

S S Teh, A E D Bekhit, A Carne, and J Birch

Journal of Food Measurement and Characterization, 2014 vol. 8 (2) pp. 92-104

The Use of Microwave and Pulsed Electric Field as a Pretreatment Step in Ultrasonic Extraction of Polyphenols from Defatted Hemp Seed Cake (*Cannabis sativa*) Using Response Surface Methodology.

S S Teh, B E Niven, A.E.-D.A. Bekhit, A Carne, and E. J. Birch

Food and Bioprocess Technology, 2014

Mutations in the zinc finger protein gene, ZNF469, contribute to the pathogenesis of keratoconus.

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Purpose: Mutations in the zinc finger protein gene ZNF469 cause recessive Brittle Cornea syndrome, characterised by spontaneous corneal perforations. Genome-wide association studies (GWAS) have implicated common variants in this gene as a determinant for central corneal thickness (CCT). We investigated the contribution of ZNF469 in a sample set of keratoconus patients. Methods: Forty-three patients with keratoconus, (49% Māori or Pacific (Polynesian)), were recruited. If a family history was present, family members were recruited. Participants underwent comprehensive examination and a DNA sample collected. Mutational analysis of ZNF469 was undertaken using Sanger sequencing, including an ancestrally-matched Polynesian control population. Bioinformatic databases of exome variation, and protein prediction software were used to determine presence and frequency, and pathogenicity for each observed change. Results: Fourteen non-synonymous missense SNPs were observed in ZNF469. Of the 43 probands, at least one probable disease causing variant was detected in 20 (46%) (16/32 sporadic, 4/11 familial) and two variants in 5, (11.6%) (3/32 sporadic, 2/11 familial). Only heterozygous changes segregated with disease. Three "deleterious" changes observed in the Polynesian controls were removed from analysis, therefore pathogenic variants occurred in 10/43 (23.3%). Conclusions: Rare missense mutations in ZNF469, predicted to be pathogenic, occurred heterozygously, at a frequency of 23% in a keratoconus population. ZNF469 is associated with CCT in GWAS, and therefore likely to play a role in the synthesis and/or organization of corneal collagen fibres. The pathogenic changes observed either genetically predispose towards a "thin" cornea, which then becomes keratoconic, or are directly pathogenic.

Investigative ophthalmology & visual science, 2014

A qualitative and quantitative analysis of the New Zealand media portrayal of Down syndrome.

S Wardell, R P Fitzgerald, M Legge, and K Clift

Disability and Health Journal, 2014 vol. 7 (2) pp. 242-250

Abundant local interactions in the 4p16.1 region suggest functional mechanisms underlying SLC2A9 associations with human serum uric acid.

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Human serum uric acid concentration (SUA) is a complex trait. A recent meta-analysis of multiple genome-wide association studies (GWAS) identified 28 loci associated with SUA jointly explaining only 7.7% of the SUA variance, with 3.4% explained by two major loci (SLC2A9 and ABCG2). Here we examined whether gene-gene interactions had any roles in regulating SUA using two large GWAS cohorts included in the meta-analysis [the Atherosclerosis Risk in Communities study cohort (ARIC) and the Framingham Heart Study cohort (FHS)]. We found abundant genome-wide significant local interactions in ARIC in the 4p16.1 region located mostly in an intergenic area near SLC2A9 that were not driven by linkage disequilibrium and were replicated in FHS. Taking the forward selection approach, we constructed a model of five SNPs with marginal effects and three epistatic SNP pairs in ARIC-three marginal SNPs were located within SLC2A9 and the remaining SNPs were all located in the nearby intergenic area. The full model explained 1.5% more SUA variance than that explained by the lead SNP alone, but only 0.3% was contributed by the marginal and epistatic effects of the SNPs in the intergenic area. Functional analysis revealed strong evidence that the epistatically interacting SNPs in the intergenic area were unusually enriched at enhancers active in ENCODE hepatic (HepG2, P = 4.7E-05) and precursor red blood (K562, P = 5.0E-06) cells, putatively regulating transcription of WDR1 and SLC2A9. These results suggest that exploring epistatic interactions is valuable in uncovering the complex functional mechanisms underlying the 4p16.1 region.

Human Molecular Genetics, 2014

Components of the dorsal-ventral pathway also contribute to anterior-posterior patterning in honeybee embryos (*Apis mellifera*).

Megan J Wilson, Nathan J Kenny, and Peter K Dearden

EvoDevo, 2014 vol. 5 (1) p.

Mannose-binding lectin 2 gene polymorphism and susceptibility to upper respiratory tract infection among endurance athletes.

F Zehsaz, N Farhangi, and M Legge

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Absolute quantification of apolipoproteins and associated proteins on human plasma lipoproteins.

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Lipoprotein-associated proteins form an intrinsic part of the major plasma lipoprotein classes. There is increasing evidence that the quantity of these proteins per lipoprotein particle determines lipoprotein function including redox, inflammatory and thrombotic properties and may impact on lipoprotein-related risks for developing heart disease. However, only limited information on the relative quantity of these proteins has been published and no comprehensive absolute quantitative data providing the stoichiometry of proteins associated with lipoproteins is available to date. To address this, we performed extensive absolute quantification by mass spectrometry of 17 lipoprotein-associated proteins on VLDL, LDL, Lp(a) and HDL from healthy subjects. For the first time we show the exact stoichiometry of apolipoproteins on different lipoprotein classes. The most distinct differences were seen in the abundance of all apoCs, apoE and apoF. We further revealed strong variations between individual samples, which indicates the complexity of the protein complement of lipoproteins and can provide additional insights into lipoprotein-related risk factors. This approach has the potential to determine alterations in the protein profiles of lipoproteins in disease states such as CVD or diabetes and, if performed on large cohorts, to translate into a tool for identifying new candidate biomarkers for risk of disease.

BIOLOGICAL SIGNIFICANCE:A more comprehensive picture about the protein complement on individual lipoprotein classes is the goal of lipoprotein proteomics analyses. Despite many such studies, there is a lack of absolute quantitative data on lipoproteins isolated from individual subjects. The stoichiometry of lipoprotein-associated proteins rather than their presence or absence could provide insights into an individual's predisposition for disease such as heart disease or diabetes. Our study provides a comprehensive overview of the absolute quantity of proteins on the major apolipoprotein classes VLDL, LDL, Lp(a) and HDL.

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